# Captopril Attenuates Cardiovascular and Renal Disease in a Rat Model of Heart Failure With Preserved Ejection Fraction

*Eman M. Salah, MD,*† Sheldon I. Bastacky, MD,† Edwin K. Jackson, PhD,‡ and Stevan P. Tofovic, MD, PhD*§*

**Abstract:** Heart failure with preserved ejection fraction (HFpEF), a prevalent form of heart failure, is frequently accompanied by the metabolic syndrome and kidney disease. Because current treatment options of HFpEF are limited, evaluation of therapies in experimental models of HFpEF with the metabolic syndrome and kidney disease is needed. In this study, we evaluated the effects of captopril, furosemide, and their combination in aged, obese $ZSF_1$ rats, an animal model of HFpEF with the metabolic syndrome and chronic kidney disease as comorbidities. Captopril (100 mg/kg), furosemide (50 mg/kg), or their combination was administered orally to obese $ZSF_1$ rats aged 20 to 44 weeks. Untreated $ZSF_1$ rats served as controls. After 24 weeks of treatment, captopril significantly lowered systemic blood pressure and attenuated HFpEF as evidenced by significantly reduced left ventricular end diastolic pressures (10.5 ± 1.4 vs. 4.9 ± 1.3 mm Hg in Control vs. Captopril, respectively) and significantly lower left ventricular relaxation time constants (28.1 ± 2.9 vs. 18.3 ± 3.1 ms in Control vs. Captopril, respectively). The captopril-induced improvement in left ventricular function was associated with reduced cardiac hypertrophy, ischemia, necrosis, and vasculitis. Captopril also increased renal blood flow and glomerular filtration rate, reduced renal vascular resistance and proteinuria, and improved renal histology (ie, reduced renal hypertrophy, glomerulosclerosis, and tubular atrophy/dilation). Furosemide alone provided little benefit; moreover, furosemide did not augment the therapeutic benefits of captopril. This study suggests that chronic administration of captopril, but not furosemide, could be beneficial in patients with HFpEF, particularly in those with comorbidities such as obesity, diabetes, and dyslipidemias.

**Key Words:** metabolic syndrome, HFpEF, kidney disease, captopril, furosemide

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From the Departments of *Medicine; †Pathology; and ‡Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA. Dr. Salah is now with the Sohag Faculty of Medicine, Sohag University, Egypt; and §Vascular Medicine Institute, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA.

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Reprints: Stevan P. Tofovic, MD, PhD, Division of Pulmonary, Allergy and Critical Care Medicine, Vascular Medicine Institute, Department of Medicine, University of Pittsburgh School of Medicine, 100 Technology Drive, 542 Pittsburgh, PA 15219 (e-mail: tofovic@pitt.edu).



## INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) is detected in more than 50% of all heart failure patients, and the prevalence of HFpEF is growing by 1% per year, relative to heart failure with reduced ejection fraction (HFrEF).[1,2] Comorbidities are much more prevalent in HFpEF patients compared with HFrEF patients, and patients with HFpEF tend to be older and more likely to be hypertensive, obese, and have the metabolic syndrome/diabetes, and/or renal dysfunction.[3,4] In contrast to significant progress in the management of HFrEF, little or no progress has been made in the treatment of HFpEF; this is mainly because of the lack of specific therapies.[5,6]

To evaluate specific therapies for HFpEF, novel animal models of HFpEF associated with hypertension, diabetes, and kidney disease are needed. In this regard, our previous studies,[7–10] as well as recent reports by other groups[11,12] indicate that the $ZSF_1$ rat model shares many features and has most common comorbidities seen in patients with HFpEF. We were first to report that $ZSF_1$ rats, in addition to expressing the metabolic syndrome and hypertension, exhibit marked cardiac hypertrophy and diastolic dysfunction.[8,9] Moreover, our previous work demonstrates that the $ZSF_1$ rat model replicates all features of chronic diabetic kidney disease in humans[7] and meets the 6 validation criteria for rodent models of progressive diabetic nephropathy proposed by the NIH-created Animal Models of Diabetic Complications Consortium: (1) >50% decline in glomerular filtration rate (GFR) over the lifetime of the animal; (2) >10-fold increase in albuminuria compared with controls; (3) advanced mesangial expansion with or without nodular sclerosis and mesangiolysis; (4) any degree of arteriolar hyalinosis; (5) glomerular basement membrane thickening by >50% over baseline; and (6) tubulointerstitial fibrosis.[13] Finally, our work also shows that the $ZSF_1$ rat model is useful for studying novel therapeutics for treating HFpEF. For example, our studies demonstrate that in adult male $ZSF_1$ rats, chronic treatment with BG9928, a dual adenosine $A_1$ and $A_{2B}$ receptor antagonist, improves glucose homeostasis and this is associated with improved LV and renal function and histopathology.[10]

The renin–angiotensin–aldosterone system (RAAS) also plays a critical role in regulation of blood pressure, and development and progression of heart and renal disease and inhibitors of RAAS have evolved into a cornerstone pharmacotherapy of hypertension and cardiac and renal disease, in

particular in patients with metabolic syndrome and diabetes.[14] RAAS inhibitors are the first class of drugs proved to alter the natural history of heart failure and lead to long-term benefit and are cornerstone therapy for all stages of systolic heart failure.[15] However, the overall benefit of RAAS inhibitors in HFpEF are poorly defined. Yet, a recent meta-analysis of observational cohort studies suggests potential mortality benefit of RAAS inhibitors in HFpEF and emphasizes the importance of a new well-designed randomized clinical trial to be conducted.[16]

Reduced renal function and cardiorenal interaction may contribute to development of HFpEF through complex mechanisms that may include, but are not limited to, volume overload because of inadequate renal salt and fluid handling, hypertension of renal origin, as well as oxidative stress and inflammation associated with advanced kidney disease.[17] The current standard therapy for correction of volume overload in heart failure patients includes the use of loop diuretics. Although loop diuretics improve symptoms in most patients, their acute and chronic use may have detrimental effects on renal function. Clinical outcomes in patients with heart failure are adversely affected by decreases in kidney function, with impaired renal function being associated with increased mortality in both acute and chronic heart failure patients.[18,19]

Clinicians most commonly use furosemide among loop diuretics for patients with heart failure. Unfortunately, no outcome study on the long-term use of loop diuretics in HFpEF is available.[20] However, recent studies on animals and clinical studies suggest that (in particular, long-acting) loop diuretics may have beneficial effects in patients with HFpEF.[21,22] In this regard, a novel extended release torsemide formulation has been recently developed and it is currently being studied in patients with heart failure.[23]

In this study, we examined the effects of captopril, an angiotensin-converting enzyme inhibitor, with and without co-administration of furosemide, a loop diuretic, on HFpEF, kidney disease, and cardiac and renal histopathology in aged, obese, male $ZSF_1$ rats.

## METHODS

### Animals and Treatments

A total of 38, 20-week-old, male, obese (body weight 579 ± 6 g), $ZSF_1$ rats were obtained from Genetic Models (Indianapolis, IN). Animals were randomly assigned to 1 of the 4 experimental groups: 1. Control (n = 8); obese male $ZSF_1$ rats receiving water and food (Purina 5008 rodent diet, Purina Mills, Land O'Lakes, St. Louis, MO) ad libitum. 2. CAPTO group (n = 10), animals receiving 100 $mg \cdot kg^{-1} \cdot d^{-1}$ captopril in drinking water. 3. FURO group (n = 10), animals receiving furosemide (50 $mg \cdot kg^{-1} \cdot d^{-1}$) in drinking water. 4. CAPTO + FURO group (n = 10). Treatments were continued for 24 weeks. The experimental procedures and protocols used in this study were reviewed and approved by the University of Pittsburgh Animal Care and Use Committee. Additional 5–8 aged, male lean (Ln) and 8–10 obese (Ob) $ZSF_1$ rats were used for baseline metabolic, cardiovascular, and renal function measurements and for evaluation of renal, cardiac, and hepatic histopathology.

### Metabolism Cage Studies

Before, and 12 and 24 weeks into treatment, rats were placed in metabolic cages. Body weight, 24-hour food and water intake, and urine output were determined. After 24-hour measurement of metabolic parameters, rats were anesthetized using halothane and a 32-gauge needle was inserted into an exposed jugular vein for the collection of 2 mL of blood for analysis of creatinine, plasma renin activity (PRA), triglycerides, and cholesterol.

Plasma samples were analyzed in duplicate for creatinine levels (Beckman creatinine analyzer, Beckman Instruments, Fullerton, CA), triglycerides, cholesterol (Sigma-Aldrich, St. Louis, MO), PRA (radioimmunoassay; New England Nuclear, Waltham, MA), and aldosterone activity [Rat Aldosterone (ALD) ELISA; Kamiya Biomedical Company, Seattle, WA]. Protein levels in urine were determined by modification of the Lowry method.

### Measurements of Blood Pressure, Left Ventricle Performance, and Renal Hemodynamics and Excretory Function

At 44 weeks of age, animals were anesthetized using pentobarbital (45 mg/kg, intraperitoneal) and instrumented for measurements of blood pressure, renal hemodynamics, and urine collection. Briefly, a PE-240 polyethylene catheter was inserted into the trachea to facilitate breathing. The left femoral artery was exposed and a polyethylene cannula (PE-50) connected to a digital blood pressure analyzer (BPA 200; Micro-Med Inc, Louisville, KY) was inserted for continuous measurement of systolic blood pressure, diastolic blood pressure (DBP), mean arterial blood pressure, and heart rate (HR). The right carotid artery was exposed and cannulated with a short section of PE-50 tubing, which was advanced into the left ventricle and connected to a high sensitive, ultra-low volume pressure transducer (0.023-nL displacement per mm Hg) and to a heart-performance analyzer (HPA 400; Micro-Med Inc, Louisville, KY). The following pressure–time parameters of left ventricular (LV) function were measured: HR, left ventricular peak systolic pressure, left ventricular end diastolic pressure (LVEDP), left ventricular minimum diastolic pressure, maximum dP/dt during ventricular contraction (+dP/dt), maximum dP/dt during ventricular relaxation (−dP/dt), and time constant of ventricular relaxation (Tau). The pressure–time parameters were collected electronically over 60 minutes and stored in digital (Excel) format.

Next, 2 PE-50 cannulas were placed in the left jugular vein for infusion of $^{14}C$-inulin and supplemental anesthetic, respectively. Finally, through a midline abdominal incision, the left kidney was exposed, and a PE-10 catheter was inserted into the left ureter to facilitate the collection of urine. A flow probe (Model 1RB; Transonic Systems, Inc, Ithaca, NY) was placed on the left renal artery for the determination of renal blood flow, and intravenous infusion of $^{14}C$-inulin (0.035 μCi/50 μL saline/min) was initiated. A 45-minute

stabilization period was permitted before two 30-minute clearance periods were conducted. Blood pressure and renal blood flow were recorded at 5-minute intervals and averaged during a 30-minute urine collection. A midpoint blood sample (100 μL) for measurement of radioactivity and hematocrit was collected. Plasma and urine $^{14}$C-inulin radioactivity were measured (liquid scintillation analyzer, Model 2500 TR; Packard Instrument Company, Downers Grove, IL) and urine volume was determined gravimetrically. Inulin clearance (an estimate of GFR) and renal vascular resistance were calculated. No differences (time effects) between 2 clearance periods were detected and therefore, the data represent the (60-minute) average of the 2 clearance periods.

## Histological Evaluation

Rats were euthanized with an overdose of pentobarbital. Heart, kidneys, and liver were removed and stored in 10% formalin buffer. Tissues were processed into paraffin blocks for light microscopy. Two histological sections (3–5-μm thick) were cut and stained using hematoxylin and eosin (H&E; kidney, heart, and liver), methenamine silver–trichrome (MST; kidney), or picrosirius red and trichrome (kidney). Samples were analyzed in blinded fashion by histopathologists (E.M.S. and S.I.B). Histopathology scores were assessed semiquantitatively as 0 (absent), 0.5 (trace), 1 (mild), 2 (moderate), and 3 (severe) for renal histopathology, including focal general glomerular sclerosis, focal segmental glomerular sclerosis, interstitial fibrosis, tubular atrophy/dilation, proteinaceous material, other casts, glomerular and interstitial inflammation, vascular inflammation, and vascular hypertrophy. The assessment of cardiac histopathology was conducted using the same scale and by looking for vascular, pericardial, and endocardial inflammation, ischemic and necrotic degeneration, and vascular thickening and vasculitis. The evaluation of liver histopathology included examination for steatosis (microvesicular and macrovesicular), inflammation (perivascular, portal tract, and parenchyma), and degenerative changes (perivascular and zonal apoptotic bodies).

## Statistical Analyses

Data are expressed as mean ± standard error of the mean. Statistical analyses were performed using the Number Cruncher statistical software program (Kaysville, UT). Group comparison for data from metabolic studies (repeated measurements) were performed by 2-factor, hierarchical analysis of variance (2F-ANOVA), followed by Fisher's LSD test for post hoc comparisons. The probability value of $P < 0.05$ was considered statistically significant. One-factor ANOVA was used to compare renal and cardiac histology and renal hemodynamics and excretory function data among all 4 groups, followed by Fisher's LSD test for post hoc comparisons.

# RESULTS

Baseline comparison of metabolic, renal, and cardiac parameters of control adult obese $ZSF_1$ rats (Ob) and their lean homozygote and heterozygote (Ln+/+ and Ln+/fa) littermates are presented in Table 1 and Figure 1. The male obese $ZSF_1$ rat model exhibits the metabolic syndrome, with adult animals developing overt and severe diabetes. Furthermore, adult obese $ZSF_1$ rats have reduced renal function, and renal histopathology closely resembles changes seen in humans with diabetic nephropathy. Compared with lean control $ZSF_1$ rats (Fig. 1G), obese $ZSF_1$ animals have thickening of the glomerular basement membrane (Fig. 1H). Also, compared with lean littermates, the adult obese animals develop severe cardiac hypertrophy and LV diastolic dysfunction, but have preserved ejection fraction and cardiac output (Table 1), and aging is associated with more pronounced cardiac fibrosis in obese $ZSF_1$ animals (Fig. 1B) compared with lean controls (Fig. 1A). Compared with nondiabetic lean littermates, the aged obese $ZSF_1$ rats have increased LVEDP (10.6 ± 2.3 mm Hg vs. 18.5 ± 1.95 mm Hg, Ln vs. Ob) and LV-Tau (12.1 ± 1.07 vs. 18.8 ± 2.5 ms).

**TABLE 1.** Metabolic Parameters, Renal and Cardiac Functions, and Weights of Organs in Aged Lean and Obese $ZSF_1$ Rats

| Parameter | Lean (Ln) $ZSF_1$ | Obese (Ob) $ZSF_1$ |
|---|---|---|
| n | 9–12 | 9–12 |
| Age (wk) | 38–44 | 38–44 |
| Body weight (g) | 559 ± 8 | 696 ± 18* |
| MABP (mm Hg) | 156 ± 5 | 178 ± 9* |
| Food ($g \cdot kg^{-1} \cdot d^{-1}$) | 39.91 ± 0.9 | 55.2 ± 2.7* |
| Water ($mL \cdot kg^{-1} \cdot d^{-1}$) | 52.0 ± 1.6 | 165 ± 22* |
| Urine volume ($mL \cdot kg^{-1} \cdot d^{-1}$) | 30.0 ± 1.2 | 133 ± 19.7* |
| Blood glucose (mg/dL) | 150 ± 9 | 407 ± 30* |
| Glycosylated hemoglobin (%) | 5.1 ± 01 | 12.2 ± 0.0.3* |
| Insulin (ng/mL) | 0.79 ± 0.14 | 3.18 ± 0.35* |
| Triglycerides (mg/dL) | 70.3 ± 17 | 2523 ± 511* |
| Cholesterol (mg/dL) | 53 ± 14 | 370 ± 27* |
| Fatty acids (nMoL) | 0.15 ± 0.09 | 1.08 ± 0.12* |
| Kidney (g) | 1.73 ± 0.04 | 3.06 ± 0.13* |
| Kidney/body weight (mg/g) | 3.19 ± | 5.46 ± 0.23* |
| RBF ($mL \cdot min^{-1} \cdot g^{-1}$ kidney) | 4.47 ± 0.21 | 3.03 ± 0.22 |
| RPF ($mL \cdot min^{-1} \cdot g^{-1}$ kidney) | 2.35 ± 0.11 | 1.78 ± 0.14 |
| RVR (mm $Hg \cdot mL^{-1} \cdot g^{-1}$ kidney) | 35.9 ± 1.52 | 54.3 ± 4.73* |
| GFR/($mL \cdot min^{-1} \cdot g^{-1}$ kid) | 1.31 ± 0.10 | 0.87 ± 0.7* |
| UPE ($mg \cdot kg^{-1} \cdot d^{-1}$) | 84.1 ± 10.6 | 443.1 ± 52.3* |
| UPE/Cr ratio (mg/mg) | 2.5 ± 0.1 | 8.1 ± 0.9* |
| UAE ($mg \cdot kg^{-1} \cdot d^{-1}$) | 1.0 ± 0.1 | 17.2 ± 7.8* |
| UAE/Cr Ratio (mg/mg) | 0.027 ± 0.004 | 0.341 ± 0.19* |
| NAG/Cr (mU/mg) | 10.1 ± 0.3 | 22.1 ± 3.9* |
| Heart (g) | 1.37 ± 0.03 | 1.69 ± 0.03* |
| Heart/tibia (mg/mm) | 30.2 ± 0.6 | 40.5 ± 0.7* |
| LVPSP (mm Hg) | 173.3 ± 10.2 | 183.5 ± 12.2 |
| LVEDP (mm Hg) | 10.6 ± 2.3 | 18.5 ± 1.95* |
| LV-Tau (ms) | 12 ± 1 | 18.8 ± 2.5* |
| Cardiac output (mL/min) | 106.1 ± 16.1 | 95.5 ± 9.0 |
| Ejection fraction | 73.4 ± 1.8 | 79.3 ± 6.2 |
| Contractility index (1/s) | 131 ± 4 | 107 ± 17 |
| Liver (g) | 13.3 ± 0.41 | 25.6 ± 0.62* |
| Liver/body weight ratio (g/kg) | 40.3 ± 1.34 | 60.1 ± 2.05* |

*$p<0.05$.

Cr, creatinine; LVPSP, left ventricular peak systolic pressure; MABP, mean arterial blood pressure; NAG, excretion and activity of N-acetyl-β-D-glucosaminidase; RBF, renal blood flow; RPF, renal plasma flow; RVR, renal vascular resistance; Tau, left ventricular time constant of isovolumetric relaxation; UAE, urinary albumin excretion; UPE, urinary protein excretion.

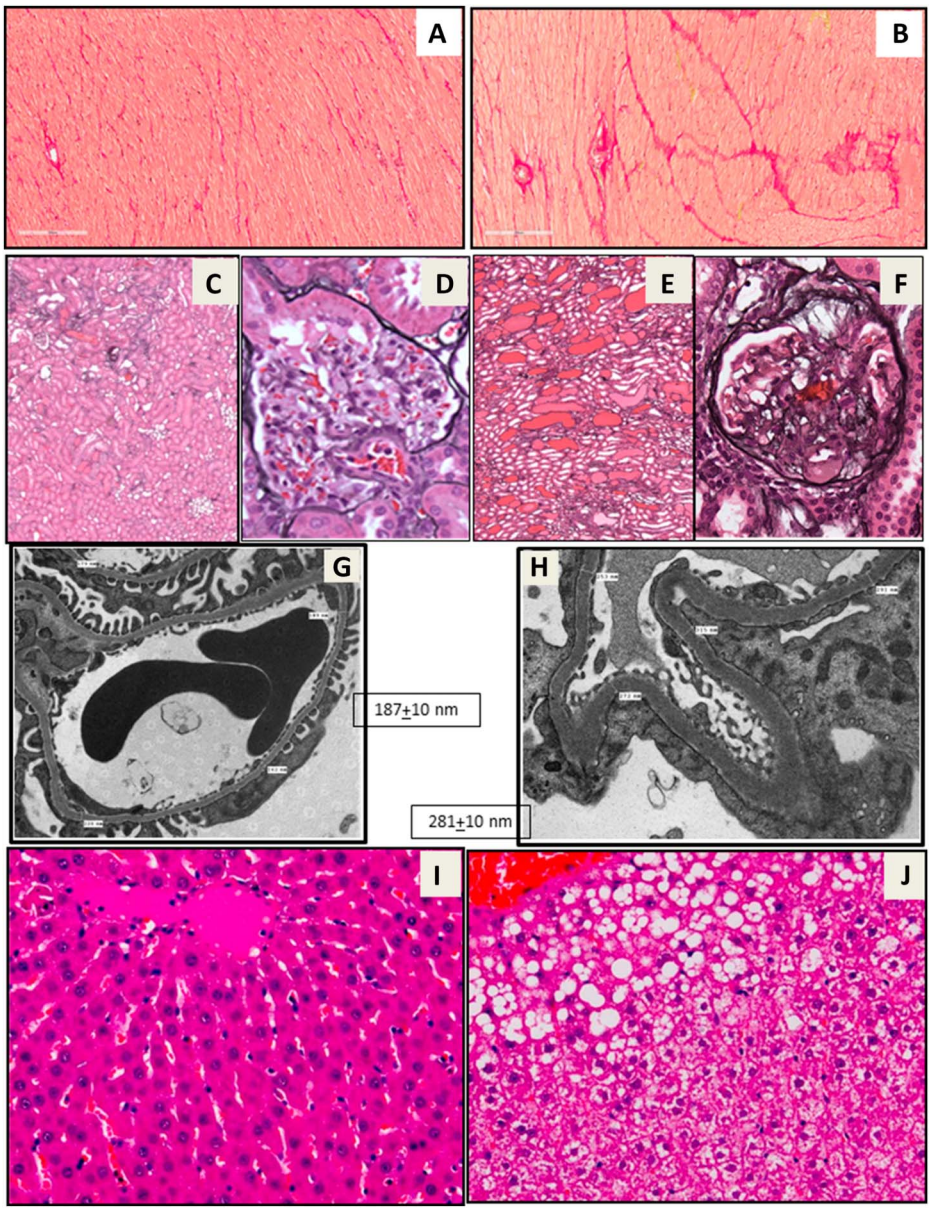


**FIGURE 1.** Picrosirius red stain of myocardium illustrates mild (A) and marked (B) fibrosis in hearts from aged lean and obese $ZSF_1$ rats, respectively; near-normal glomerular and tubulointerstitial histopathology in lean $ZSF_1$ rats (C, D); significant tubulointerstitial changes (E) and glomerulosclerosis (F) in obese $ZSF_1$ rats; glomerular basement membrane thickness in lean (G) and obese (H) $ZSF_1$ rats; absence of hepatocellular steatosis in lean (I) and moderate diffuse hepatocellular steatosis in obese $ZSF_1$ rats (J).

Chronic treatment using captopril or captopril plus furosemide reduced systolic blood pressure, diastolic blood pressure, and left ventricular peak systolic pressure, but had no effect on HR (Fig. 2). By contrast, furosemide per se did not affect these variables. Notably, captopril and captopril plus furosemide, but not furosemide per se, reduced LVEDP (Fig. 2). Also, captopril reduced LV-Tau, whereas neither furosemide nor furosemide plus captopril affected LV-Tau. Captopril, without and with furosemide, significantly reduced cardiac hypertrophy and cardiac necrosis, whereas furosemide per se did not (Fig. 3 and see **Fig. 1S**, **Supplemental Digital Content 1**, http://links.lww.com/JCVP/A287). All 3 treatments attenuated histological scores for cardiac ischemia and cardiac vasculitis.

The evaluation of renal excretory function in obese $ZSF_1$ rats at 20, 32, and 44 weeks of age (ie, 0, 12, and 24 weeks of treatment) revealed progressive renal disease, as evidenced by time-related increases in plasma creatinine and proteinuria and decreases in creatinine clearance (Fig. 4). As expected, captopril and captopril plus furosemide, but not furosemide per se, prevented further decline in renal function and reduced proteinuria (Fig. 4). After 24 weeks of treatment, acute measurements indicated improved renal hemodynamics, increased excretory function, and reduced proteinuria in animals treated using captopril and captopril plus furosemide compared with aged controls, whereas furosemide per se did not affect these variables (Fig. 5). However, all 3 treatments reduced glomerulosclerosis, tubular atrophy, and the amount of intratubular proteinaceous material (Fig. 6 and see **Fig. 2S**, **Supplemental Digital Content 1**, http://links.lww.com/JCVP/A287).

Time-related changes in PRA and plasma aldosterone levels in all 4 groups are shown in Figure 7. At 12 and 24

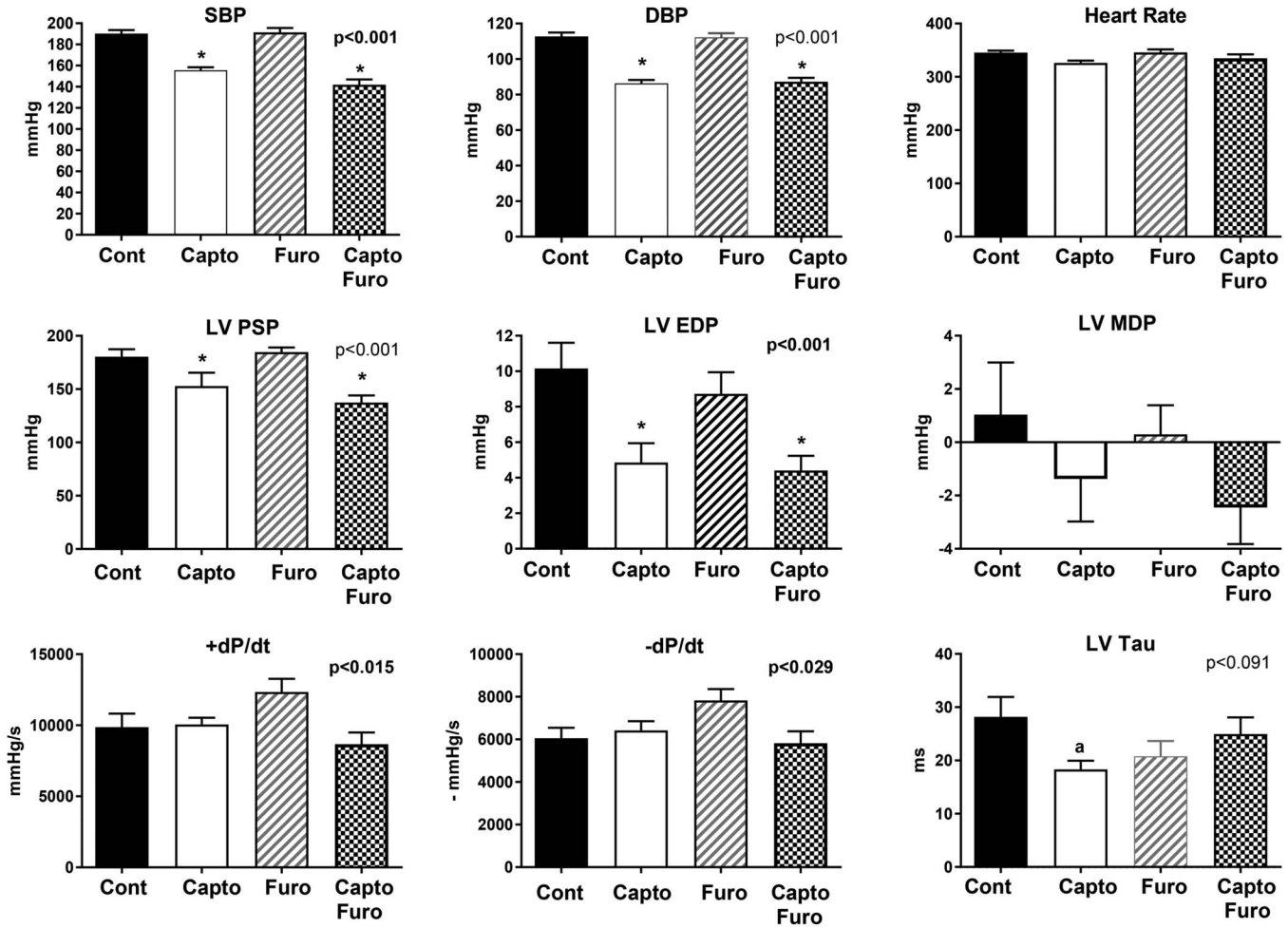


**FIGURE 2.** Bar graphs show the effects of captopril, furosemide, and their combination on SBP, DBP, HR, LVPSP, LVEDP, LVMDP, +dP/dt, −dP/dt, and the constant of isovolumetric left ventricular relaxation (LV-Tau) in obese aged $ZSF_1$ rats under pentobarbital anesthesia. Values represent the mean ± SEM. (n = 8–10); *P*-values in bar graphs from 1 to F ANOVA; *$P < 0.5$ versus control and furosemide; a—$P < 0.5$ versus control. LVMDP, left ventricular minimum diastolic pressure; LVPSP, left ventricular peak systolic pressure; SBP, systolic blood pressure.

weeks into the study, the aging control $ZSF_1$ rats had lower PRA activity compared with baseline values. As expected, 12 weeks into treatments, captopril alone or in combination with furosemide induced a 4–5-fold increase in PRA, whereas furosemide per se induced a more modest increase in PRA. At 24 weeks into these treatments, the well-described PRA escape phenomenon (ie, time-related reductions in elevated PRA) was detected in all 3 treatment groups. These findings suggest that the obese $ZSF_1$ model shares another feature of aging in humans, ie, reduced RAAS activity. Aging was also associated with reduced plasma aldosterone levels regardless of treatment (Fig. 7).

In the control group, plasma levels of triglycerides increased over the 24-week period from 1982 to 4351 mg/dL ($P = 0.004$) (Fig. 8). Similar time-related increases in total cholesterol levels were detected in aging control ZSF1 rats. (Fig. 8). It is noteworthy that captopril alone or in combination with furosemide attenuated age-related increases in plasma triglycerides and cholesterol, whereas furosemide per se did not (Fig. 8). Notably, 24-week treatment using captopril, furosemide, or their combination had no effect on body weight, food or fluid consumption, and urine volume (data not shown).

Obese, aged $ZSF_1$ rats exhibited moderate hepatic steatosis, inflammation, and zonal degenerative changes with presence of apoptotic bodies (data not shown). Treatment using captopril alone or in combination with furosemide had no effects on microvesicular and macrovesicular steatosis, but tended to reduce portal tract, parenchymal, and perivascular inflammation, and number of apoptotic bodies (data not shown).

## DISCUSSION

Chronic pressure overload induced by hypertension causes ventricular hypertrophy and LV dysfunction leading in some patients to HFrEF (previously known systolic heart failure) and in others to HFpEF (previously called diastolic

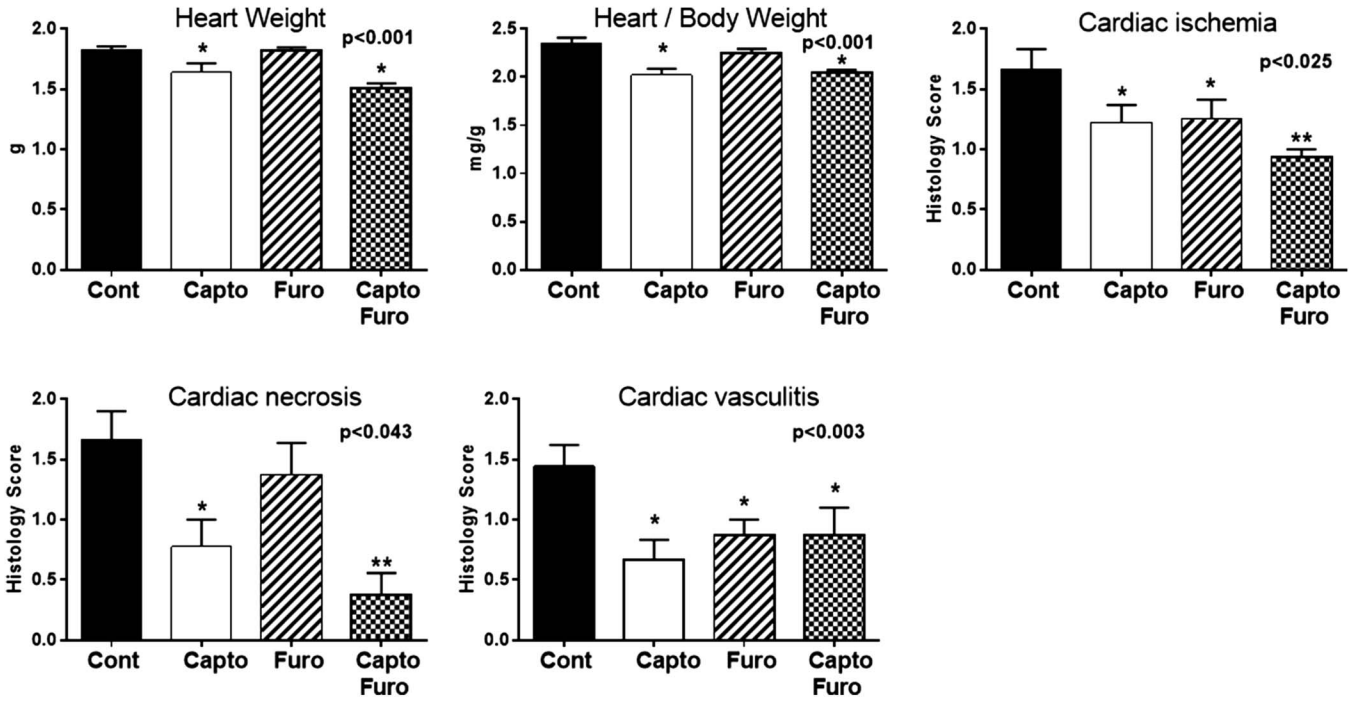


**FIGURE 3.** Bar graphs show the effects of chronic treatment using captopril, furosemide, or their combination on heart weight and cardiac histopathology scores (0 = absent; 0.5 = trace; 1 = mild; 2 = moderate; and 3 = severe) in obese aged $ZSF_1$ rats. Values represent the mean ± SEM. (n = 8–10); *P*-values in bar graphs from 1–F ANOVA; $*P < 0.05$ versus control group; $**P < 0.05$ versus captopril group.

heart failure). HFpEF is more common in patients with the metabolic syndrome and renal dysfunction and therefore, in preclinical studies, animal models with these characteristics are required to evaluate potentially beneficial therapies.

The male obese $ZSF_1$ rat model exhibits the metabolic syndrome, ie, obese animals are hypertensive, have severely increased plasma lipids and marked hyperinsulinemia, and develop overt diabetes. In most adult obese $ZSF_1$ rats, fed plasma glucose levels exceed 500 mg/dL and glycosylated hemoglobin levels exceed 13%. Notably, at an age early compared with lean littermates, young 8-week-old obese $ZSF_1$ rats have 10-fold elevations in plasma insulin levels.[7] With aging, plasma insulin levels decline by 60%; yet, at 38 weeks of age, obese animals do not develop failure of beta-cells and still have a 4-fold increase in plasma insulin (Table 1). This model also develops severe kidney disease and hemodynamically and histopathologically meets the NIH-proposed valuation criteria for rodent models of progressive diabetic nephropathy.[13] Clearly, this is a rodent model of severe metabolic syndrome with renal dysfunction and

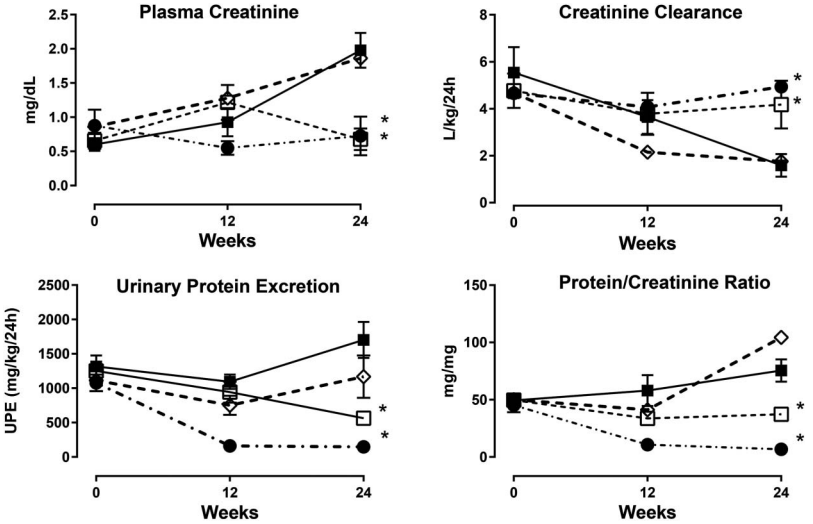


**FIGURE 4.** Line graphs show the effects of chronic treatment using captopril, furosemide, or their combination on plasma creatinine, creatinine clearance, UPE, and protein excretion normalized by creatinine excretion in conscious obese $ZSF_1$ rats. Values represent the mean ± SEM. (n = 8–10); $*P < 0.05$ versus control group; (■ - Control, □- Captopril, ◇ - Furosemide, ● - Capto+Furo). UPE, urinary protein excretion.

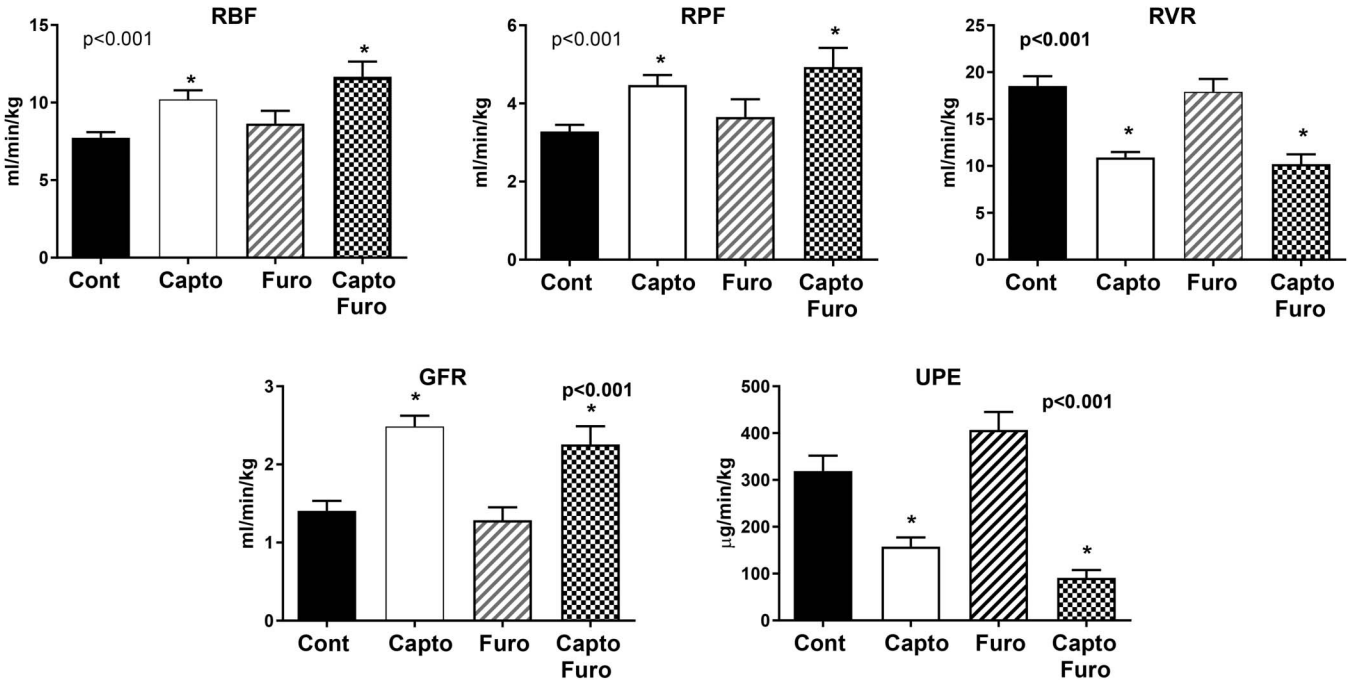


**FIGURE 5.** Bar graphs show the effects of chronic treatment using captopril, furosemide, or their combination on RBF, RPF, RVR, GFR, and UPE in obese $ZSF_1$ rats under pentobarbital anesthesia. Values represent the mean ± SEM. (n = 8–10); *P*-values in bar graphs from 1 to F ANOVA; $*P < 0.05$ versus control group. RBF, renal blood flow; RPF, renal plasma flow; RVR, renal vascular resistance; UPE, urinary protein excretion.

HFpEF, and thus represents a promising preclinical model to study drugs that might be effective in reducing morbidity and mortality in HFpEF patients.

RAAS plays a central role in electrolyte homeostasis, in regulation of blood pressure, and in the development and progression of renal and heart disease. Accordingly, RAAS inhibitors are cornerstone therapy for cardiovascular and renal diseases associated with the metabolic syndrome and diabetes. An important finding of this study is that the obese $ZSF_1$ rat, a model that shares many features of metabolic syndrome-related cardiovascular and renal diseases in humans, responds to standard treatment for heart failure. In this regard, the aged obese $ZSF_1$ rat responds to captopril treatment with improved LV diastolic function (ie, improved HFpEF), renal hemodynamics, excretory function, and lipid status. In this study, we used 20-week-old obese male $ZSF_1$ rats. At this age, animals already have severe hypertension, extremely elevated plasma lipids, and fully developed diabetic kidney disease.[7–9] Importantly, at 20 weeks of age, $ZSF_1$ rats exhibit HEpEF as evidenced by an unchanged ejection fraction; yet, an increased LVEDP and LV-Tau.[8–12,24] This makes even more remarkable the detected beneficial effects of captopril in aging $ZSF_1$ rats with fully developed target organ damage associated with the metabolic syndrome and diabetes. Captopril retards the progression of kidney disease in aged $ZSF_1$ rats as evidenced by reduced proteinuria, greater GFR, and improved renal histopathology. Similar beneficial effects in terms of renal hemodynamics, excretory function, and renal histopathology occur in aged $ZSF_1$ rats treated using captopril and furosemide, suggesting that on the long-term basis, furosemide does not compromise the renoprotective effects of captopril. However, furosemide alone does not have any beneficial effects on renal function and structure in aged obese $ZSF_1$ rats.

Notably, captopril improves LV diastolic function, ie, 24-week captopril treatment reduces LVEDP and the isovolumetric relaxation time constant (Tau). Captopril also improves cardiac histopathology including lessened cardiac vasculitis, degenerative ischemic changes, and necrosis. The beneficial changes on LV diastolic function and LV structure are also seen with captopril plus furosemide treatment, with exception that LV-Tau is not significantly improved by the combination.

There are some limitations regarding the analysis of heart performance in this study. In the current study, we used a highly sensitive fluid catheter to measure LV pressure–time variables to evaluate the alterations in LV performance. No measurements of LV volumes, cardiac output, and ejection fraction were conducted. The mechanisms that control LV function during diastole are complex and are affected by LV active relaxation[25] as well as by viscoelastic properties of the left ventricle.[26] LVEDP and Tau are mostly measures of active relaxation that may be affected by changes in compliance (ie, passive stiffness of the left ventricle); yet, these parameters do not provide a direct measure of LV compliance. It is noteworthy that a recent study in which both invasive pressure–volume analysis and noninvasive echocardiography were used showed significant correlations between invasively measured LVEDP and echocardiographic parameters of LV stiffness in adult $ZSF_1$ rats.[12] This suggests that the decreases in LVEDP by captopril in this study were mediated in part by

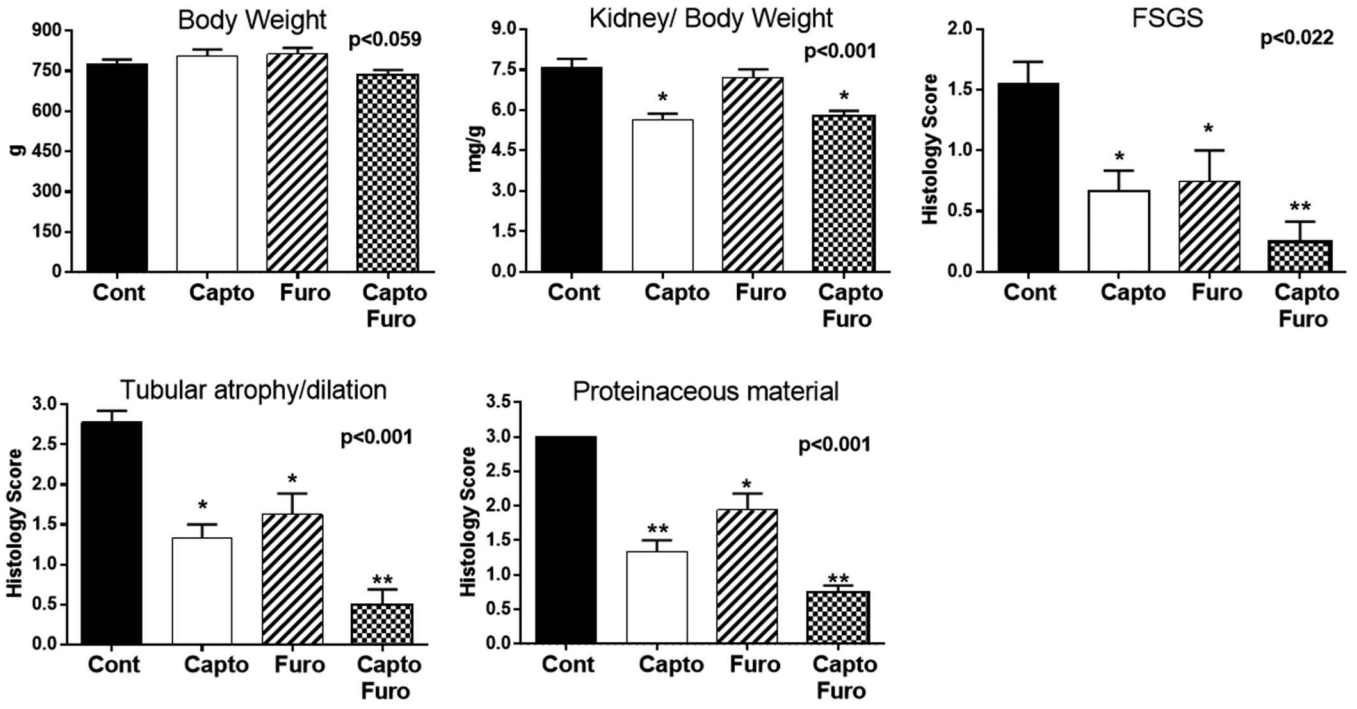


**FIGURE 6.** Bar graphs show the effects of chronic treatment using captopril, furosemide, or their combination on kidney weight and renal histopathology scores (0 = absent; 0.5 = trace; 1 = mild; 2 = moderate; and 3 = severe; FSGS, focal segmental glomerular sclerosis) in aged obese $ZSF_1$ rats. Values represent the mean ± SEM. (n = 8–10); *P*-values in bar graphs from 1-F ANOVA; $*P < 0.05$ versus control group, $**P < 0.05$ versus captopril group.

improvements in LV compliance. Furthermore, another recent study[24] demonstrated that in obese $ZSF_1$ rats, LV stiffness was accompanied by a prolonged Tau. Importantly, Tau was sufficiently prolonged such that the predicted time for complete relaxation exceeded the effective filling time and thus contributed to the rise in LVEDP. Therefore, captopril-induced reduction of Tau may have contributed to the reduction of LVEDP and eventually improved the symptoms of HFpEF. Inasmuch as HFpEF in humans is due both to abnormalities in active relaxation and passive stiffness

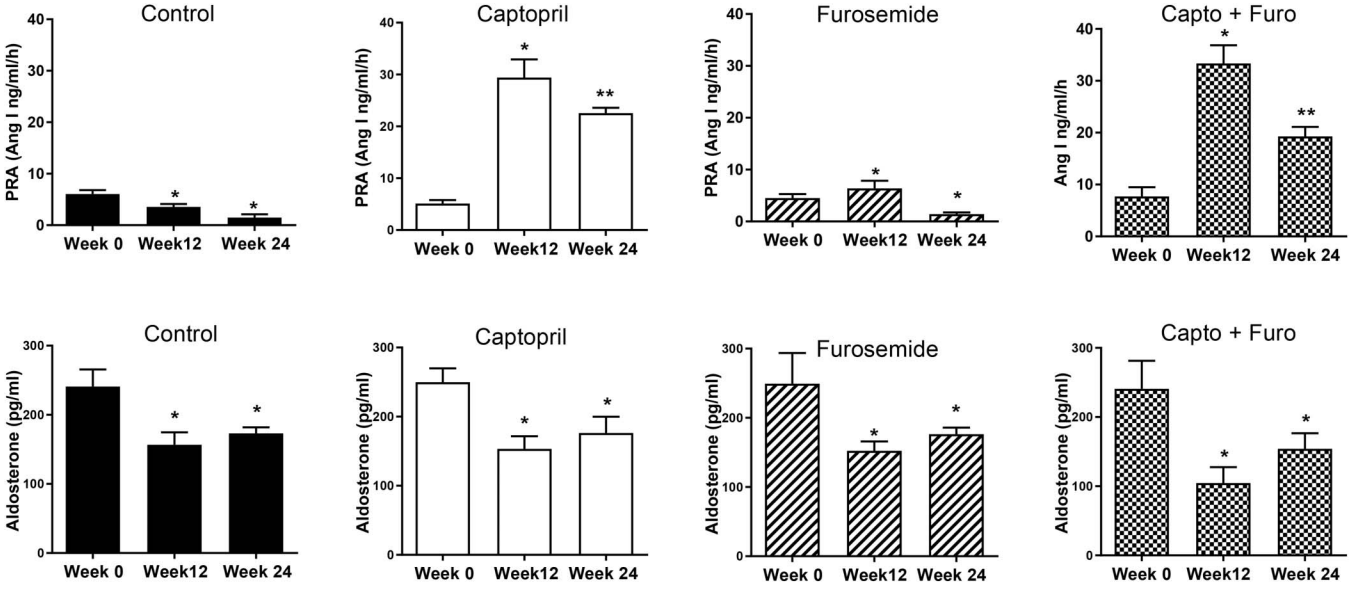


**FIGURE 7.** Bar graphs show the effects of chronic treatment using captopril, furosemide, or their combination on renin-angiotensin-aldosterone system in conscious aged obese $ZSF_1$ rats. Values represent the mean ± SEM. (n = 8–10); $*P < 0.5$ versus week 0; $**P < 0.05$ versus week 0 and week 12.

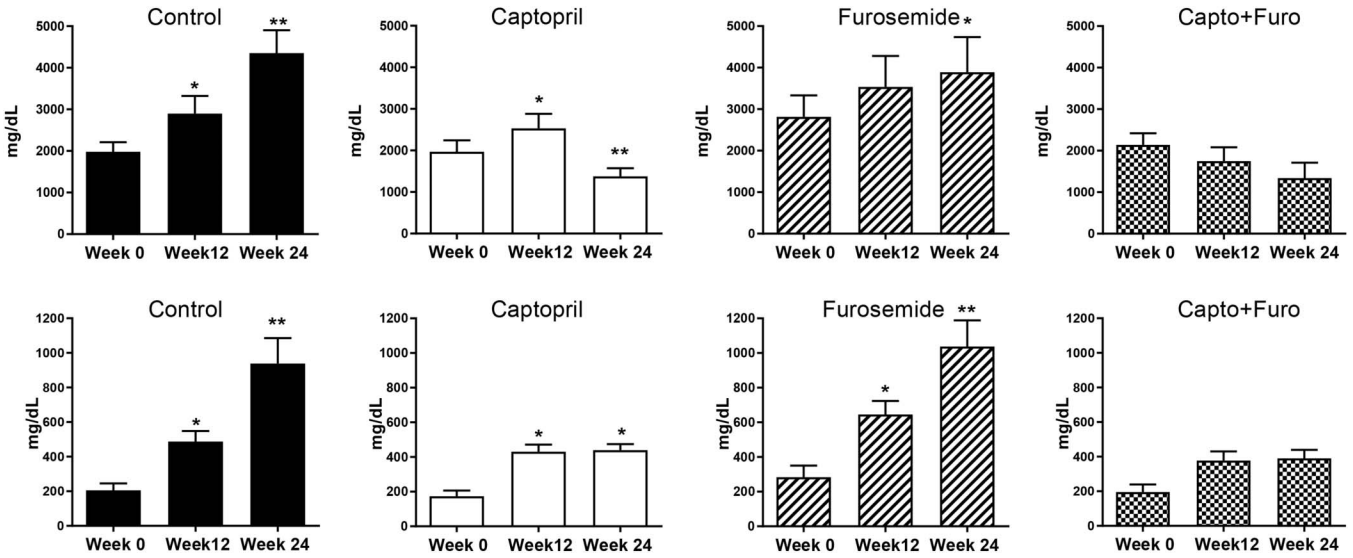


**FIGURE 8.** Bar graphs show the effects of chronic treatment using captopril, furosemide, or their combination on plasma triglycerides (upper panel) and cholesterol (lower panel) in conscious aged obese $ZSF_1$ rats. Values represent the mean ± SEM. (n = 8–10). *$P < 0.5$ versus week 0; **$P < 0.05$ versus week 0 and week 12.

of the left ventricle,[27] the changes in LVEDP and Tau observed using captopril (due to improvements in active and/or passive properties of the left ventricle) would be of benefit in patients with HFpEF.

The optimal dosing of RAAS may be critical in successful treatment of heart failure.[15] In this study, we used a relatively high dose of captopril (100 mg/kg) that may question the translation of findings of this study to the clinical arena. Notably, use of high dose of ACE inhibitors was reported to be well tolerated, and to provide additional benefit over low dose in patients with heart failure.[28]

Our cardiac findings using captopril are consistent with several other studies. In this regard, in streptozotocin diabetic rats, 2 weeks of treatment using captopril attenuated diastolic dysfunction and reduced apoptosis of cardiomyocytes.[29] Furthermore, captopril attenuated the ischemia/reperfusion injury–induced diastolic dysfunction and prompted faster recovery related to diastolic dysfunction.[30] Captopril also attenuated cardiac fibrosis and ameliorated radiation-induced LV diastolic dysfunction in rats.[31] In hypertensive patients, captopril reduced blood pressure and LV hypertrophy and improved diastolic function.[32]

Our recent study in young $ZSF_1$ rats[7] and unpublished data in adult male $ZSF_1$ rats suggest that 24-week treatment using enalapril, an alternative ACE inhibitor, is not as effective regarding improving cardiac hypertrophy and histopathology as is captopril. Notably, in patients with myocardial infarction, captopril is more efficacious than losartan in improving systolic and overall LV function.[33] This raises the question regarding whether captopril has unique effects in HFpEF independent of RAAS inhibition. Although captopril was the first clinically used RAAS inhibitor, currently it is less frequently used in clinical practice. It should be mentioned that captopril, unlike other RAAS inhibitors, is a sulfhydryl-containing drug and therefore acts as a scavenger of free radicals that protects against tissue injury from oxidative and nitrosative damage.[34,35] Furthermore, captopril may have beneficial effects on deregulated calcium homeostasis in myocardium with diastolic dysfunction. In this regard, myocardial ischemia induces numerous changes in calcium homeostasis including elevated free cytosol calcium during diastole which is associated with ischemia-induced diastolic dysfunction.[36] Furthermore, oxidative stress and endoplasmic reticulum stress decrease calcium uptake by sarcoplasmic reticulum, which subsequently leads to diabetic cardiomyopathy and diastolic dysfunction.[37] It is noteworthy that in canine and human ischemic myocardium, captopril, but not enalapril a nonsulfhydryl ACE inhibitor, acts as a reducing agent and improves calcium uptake.[38–41] Taken together, the evidence invokes the hypothesis that the unique structure of captopril entails additional beneficial effects in the falling myocardium. This hypothesis warrants further studies of comparative efficacy of captopril vis-a-vis other ACE inhibitors in experimental HFpEF.

Another important finding is that the $ZSF_1$ rat model mimics the changes in RAAS activity associated with aging in humans. Aging in $ZSF_1$ rats is associated with reduced activity of RAAS, ie, time-related decreases in PRA and aldosterone levels. This is consistent with reported data in humans, in which human senescence is associated with decreased PRA.[39–41] The decline in PRA with age is attributed to age-related glomerulosclerosis[42] that in aged $ZSF_1$ rats reaches 50%–60%.[7–9] Plasma aldosterone is also reduced with age in humans, resulting in a greater risk for hyperkalemia in older individuals, especially when coupled with the age-associated decline in GFR.[43] In aged $ZSF_1$ rats, there is also an age-related decrease in aldosterone levels. Compared with young animals, the aged $ZSF_1$ rats have reduced GFR by 50%–60%; yet, in this study, captopril did not alter plasma potassium levels (data not shown). The fact that aged $ZSF_1$ rats have low RAAS activity makes even more remarkable the beneficial cardiac and renal effects of captopril.

In conclusion, this work supports 2 main conclusions: (1) Aged, male, obese $ZSF_1$ rats represent a useful preclinical model for investigating potential therapeutics for HFpEF; and (2) captopril, either alone or in combination with furosemide, improves diastolic function, cardiac histopathology, plasma lipids, and kidney structure and function in this animal model of HFpEF. Thus, this study indicates that the obese $ZSF_1$ model is responsive to standard therapy for heart failure and raises the novel concept that captopril may have additional beneficial effects in HFpEF over other RAAS inhibitors.

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