Angiotensin-Converting Enzyme Inhibition Prolongs Survival and Modifies the Transition to Heart Failure in Rats With Pressure Overload Hypertrophy Due to Ascending Aortic Stenosis

Ellen O. Weinberg, PhD; Frederick J. Schoen, MD, PhD; Dorinda George, BA; Yutaka Kagaya, MD; Pamela S. Douglas, MD; Sheldon E. Litwin, MD; Heribert Schunkert, MD; Claude R. Benedict, MD, PhD; Beverly H. Lorell, MD

Background We tested the hypotheses that long-term administration of the angiotensin-converting enzyme (ACE) inhibitor fosinopril will regress hypertrophy, modify the transition to heart failure, and prolong survival in rats with chronic left ventricular (LV) pressure overload due to ascending aortic stenosis.

Methods and Results Aortic stenosis was created in weaning male Wistar rats by a stainless steel clip placed on the ascending aorta. Age-matched control animals underwent a sham operation (Sham group, n=57). Six weeks after surgery, rats with aortic stenosis were randomized to receive either oral fosinopril 50 mg·kg⁻¹·d⁻¹ (Fos/LVH group, n=38) or no drug (LVH group, n=36) for 15 weeks. Pilot studies confirmed that this dosage produced significant inhibition of LV tissue ACE in vivo. Animals were monitored daily, and survival during the 15-week treatment period was assessed by actuarial analysis. At 15 weeks, in vivo LV systolic and diastolic pressures and heart rate were measured. To assess contractile function, the force-calcium relation was evaluated by use of the isovolumic buffer-perfused, balloon-in-LV heart preparation at comparable coronary flow rates per gram LV weight. Quantitative morphometry was performed. Mortality during the 15-week trial was significantly less in the Fos/LVH group than in the LVH group (3% versus 31%, P<.005). No deaths occurred in the Sham group. In vivo LV systolic pressure was similar between Fos/LVH and LVH hearts (223±10 versus 232±9 mm Hg) and significantly higher than the Sham group (99±3 mm Hg, P<.05). In vivo LV diastolic pressure was significantly lower in Fos/LVH hearts than in LVH hearts (10±2 versus 15±2 mm Hg), and both were significantly higher than in the Sham group (5±1 mm Hg, P<.05). Heart rate was similar among all groups. Despite equivalent elevation of LV systolic pressure, fosinopril resulted in regression of myocyte hypertrophy in Fos/LVH versus LVH (myocyte cell width, 14.8±0.5 versus 20.8±2.2 μm, P<.05) to normal levels (Sham, 16.3±0.9 μm). Quantitative morphometry demonstrated that the regression of LV myocyte hypertrophy in the Fos/LVH group was associated with a relative increase in the fractional volume of fibrillar collagen and noncollagen interstitium. In the isolated heart experiments, LV systolic developed pressure relative to perfusate [Ca²⁺] was significantly higher in Fos/LVH hearts than in LVH hearts. The improvement in systolic function was not related to any difference in myocardial high-energy phosphate levels, since LV ATP and creatine phosphate levels were similar in Fos/LVH and LVH hearts.

Conclusions In rats with ascending aortic stenosis, chronic ACE inhibition with fosinopril improved survival, decreased the extent of LV hypertrophy, and improved cardiac function despite persistent elevation of LV systolic pressure. The favorable effects of fosinopril may be related in part to inhibition of the effects of cardiac ACE on myocyte hypertrophy rather than to systemic hemodynamic mechanisms.

Key Words • angiotensin • enzymes • fosinopril • hypertrophy

Angiotensin-converting enzyme (ACE) inhibition has been shown to improve survival in patients with advanced heart failure,¹ delay the development of heart failure in patients with asymptomatic left ventricular (LV) dysfunction,² and attenuate the progression of LV dilatation and dysfunction in patients surviving myocardial infarction.³ Animal studies have shown that chronic ACE inhibition can attenuate ventricular remodeling⁴ and improve survival after myocardial infarction in rats⁵-⁹ and delay the progression of LV failure and prolong survival in the cardiomyopathic hamster.¹⁰ Regression of LV hypertrophy has also been obtained by ACE inhibition both in humans with hypertension¹¹,¹² and in experimental animals with pressure overload.¹³-¹⁵

The favorable effects of ACE inhibitors on survival and regression of hypertrophy in heart failure and hypertension can often be attributed to interference with the systemic renin-angiotensin system, resulting in a reduction in systemic blood pressure and total periph-
eral resistance, thereby unloading the LV.\textsuperscript{16} However, recent studies from our laboratory and others have suggested that local cardiac tissue ACE and the renin-angiotensin system are activated in animal models of heart failure\textsuperscript{17} and cardiac hypertrophy.\textsuperscript{18-20} Although the efficacy of ACE inhibitors in prolonging survival in heart failure and reversing cardiac hypertrophy has been recognized, it has been difficult to dissociate the systemic hemodynamic effects of ACE inhibition on left systolic pressure from the effects of blockade of cardiac tissue ACE. Furthermore, whereas prior studies have suggested that ACE inhibition prevents or regresses LV hypertension in rats with aortic banding, it is unclear whether interference with cardiac hypertrophy in the presence of persistent pressure overload has a beneficial or adverse effect on long-term survival and cardiac function.\textsuperscript{14,15,19,21}

To address these issues, we used male Wistar rats with pressure-overload LV hypertension caused by experimental ascending aortic stenosis. We have shown that this model, which is characterized by the absence of systemic hypertension, is associated with a stage of compensated concentric LV hypertrophy 6 to 8 weeks after banding that is followed by later decompensation, with premature death 20 weeks after banding.\textsuperscript{22} Furthermore, at the early stage of compensated hypertrophy, we have shown that LV cardiac ACE activity and mRNA levels are elevated and associated with an increased local intracardiac generation of angiotensin II that is blocked by specific ACE inhibition.\textsuperscript{23,24} We hypothesized that if the activation of the cardiac tissue renin-angiotensin system contributed to the development of hypertrophy and subsequent failure, then chronic administration of the ACE inhibitor fosinopril would decrease the extent of hypertrophy and favorably modify the transition to cardiac failure in rats with established LV hypertension despite persistent severe elevation of LV systolic pressure overload due to ascending aortic constriction. Six weeks after aortic banding, rats were randomized to 15 weeks of oral fosinopril therapy or no drug, with age-matched sham-operated controls. We studied the effects of chronic fosinopril treatment on survival, organ and cellular hypertrophic remodeling, and LV systolic and diastolic function assessed in vivo and in the isolated heart preparation.

We demonstrate in this study for the first time that the effects of ACE inhibition on LV hypertension can be dissociated from the elevation of LV systolic pressure and that chronic treatment with the ACE inhibitor fosinopril improves survival and improves cardiac function despite severe persistent elevation of LV systolic pressure.

**Methods**

**Preparation of Animals**

Male Wistar rats were obtained from Charles River Breeding Laboratories. After induction of anesthesia with intraperitoneal pentobarbital, aortic stenosis was created in weanling rats (body weight, 60 to 70 g; age, 3 to 4 weeks) by use of a tantalum clip (Weck, Inc.) of 0.58-mm internal diameter placed on the ascending aorta via a thoracic incision. Additional animals underwent a left thoracotomy without placement of the clip to serve as age-matched controls. All rats were housed in a viral-antigen–free facility and were fed normal rat chow (Purina) and water ad libitum.

**Drug Randomization**

Six weeks after surgery, rats with aortic stenosis were randomized to receive either treatment with fosinopril (Bristol-Myers Squibb) 50 mg·kg\textsuperscript{1}·d\textsuperscript{-1} added to the drinking water or no treatment. This dosage of fosinopril was chosen because a pilot study showed that this dosage resulted in 72.5\% inhibition of rabbit lung ACE activity by homogenates of LVs from rats dosed orally for 3 days with fosinopril.\textsuperscript{23} The study cohort consisted of three groups: fosinopril-treated rats with aortic stenosis (Fos LVH, n = 38), untreated rats with aortic stenosis (LVH, n = 36), and untreated sham-operated rats as age-matched controls (Sham, n = 57). Drug or no drug treatment continued for 15 weeks (weeks 6 to 21 after banding).

**Survival and In Vivo LV Measurements**

Animals were monitored daily, and deaths were recorded. Survival over the 15-week trial was analyzed by the standard Kaplan-Meier analysis with the log rank test and \( x^2 \) analysis.\textsuperscript{25} Body weights and in vivo blood pressures were measured biweekly with the tail-cuff method (Narco Biosystems). At the end of the 15-week treatment period, rats from each group were randomly selected for measurements of in vivo LV pressure (Sham, n = 11; LVH, n = 9; Fos/LVH, n = 7). Under inotrperitoneal sodium pentobarbital anesthesia and supplemental nasal oxygen, the LV apex was punctured with an 18-gauge needle connected to a Statham transducer via a short length of polyethylene tubing. A sample of blood was then collected from the vena cava for determination of plasma renin and catecholamine levels.

**Serial Echocardiographic Assessment**

Serial echocardiograms were obtained as previously reported in detail from our laboratory.\textsuperscript{26} Briefly, animals were lightly anesthetized with ketamine HCl 50 mg/kg IP and xylazine 10 mg/kg IP. The chest was shaved, and the rats were imaged prone from below. All echocardiograms were done with a commercially available ultrasonograph with a 7.5-MHz phased-array transducer (Hewlett-Packard). M-mode images were obtained from an optimized two-dimensional short-axis view of the LV and recorded at a paper speed of 100 mm/s. Anterior and posterior wall thicknesses and LV internal dimensions were measured with the standard ASE leading-edge method on three consecutive cycles and averaged by a single observer in a blinded fashion. Measurements obtained in this way have shown excellent interobserver and intraobserver variability \((r = .989 \text{ and } .995, \text{ respectively})\) and good correlations between calculated LV mass and postmortem LV weight \((r = .78, \text{ SEM} = .124, \text{ } P < .0001; \text{ echocardiographic mass} = 1.027 \text{[LV weight]} + .006 \text{~g}).\textsuperscript{26}

**Perfusion Technique**

At the end of the 15-week treatment period (21 weeks after aortic banding), eight Fos/LVH, six LVH, and six Sham rats were killed, and the isolated hearts were subjected to hemodynamic evaluation using the isovolumic buffer-perfused rat heart preparation previously described in detail.\textsuperscript{27,28} Body weight was recorded. The rats were anesthetized with intraperitoneal sodium pentobarbital, and the thorax was rapidly opened. Within 20 seconds, the heart was removed and perfused by a constant-flow pump (MasterFlex; Cole-Parmer Instrument Co) through a short cannula inserted into the aortic root just below the level of the aortic clip. The hearts were placed in a constant-temperature chamber and maintained at 36°C. The perfusate consisted of modified Krebs-Henseleit buffer of the following composition (in mmol/L): NaCl 118, KCl 4.7, CaCl\textsubscript{2} 1.2, KH\textsubscript{2}PO\textsubscript{4} 1.2, MgSO\textsubscript{4} 1.2, NaHCO\textsubscript{3} 25, lactate 1.0, and glucose 5.5. The perfusate was equilibrated with a 5% CO\textsubscript{2}/95% O\textsubscript{2} gas mixture that achieved a PO\textsubscript{2} of 500 mm Hg and a pH of 7.35 to 7.40. A small cannula was inserted through the LV apex to vent any thebesian
drainage. A thermistor and a pacing electrode were inserted into the right ventricle via the right atrium and tied in place. A collapsed latex balloon, slightly larger than the LV chamber such that no measurable pressure was generated by the balloon itself over the range of volumes used, was placed in the LV, and LV pressure was measured via a Statham P23Db pressure transducer (Gould Instruments) connected to the balloon via a short length of stiff polyethylene tubing. Coronary perfusion pressure was measured from a side arm of the aortic perfusion cannula connected to a second Statham P23Db pressure transducer. The coronary flow rate was measured by timed collection of the samples of coronary venous effluent.

The hearts were perfused for a stabilization period of 20 minutes at a paced heart rate of 240 beats per minute, which was continued throughout the experiment. In hearts from rats with aortic stenosis, coronary flow rate was adjusted to achieve a mean coronary perfusion pressure of 110 mm Hg and was then held constant throughout the experiment. In sham-operated control animals, coronary flow rate was adjusted to achieve a mean coronary perfusion pressure of 75 mm Hg and was then held constant throughout the experiment. These differing levels of flow and perfusion pressures were chosen in recognition of the difference between the in vivo coronary perfusion pressure to which the control and aortic stenosis groups were chronically exposed and because prior experience with these hearts showed that this approach would achieve comparable myocardial perfusion flow rates per gram of LV weight.22–24

At the end of the stabilization period, LV filling curves were generated. The balloon was emptied, and balloon volume was then increased in increments of 0.05 mL to a maximum end-diastolic pressure of approximately 30 mm Hg. At each increment of balloon volume, LV systolic and diastolic pressures were recorded. A filling curve was generated in this manner at three different perfuse calcium concentrations: 0.6, 1.2, and 3.0 mmol/L. After hemodynamic evaluation, hearts were rapidly removed from the perfusion apparatus, and the left and right ventricles were dissected and weighed (Sham, n=6; LVH, n=6; Fos/LVH, n=8). When the animals were killed, body weights were recorded and tibial lengths were obtained as an index of growth.

**Morphological Examination and Quantitative Tissue Morphometry**

Tissue morphometry was done by a modification of the methods of Anversa et al27 previously published by our laboratory.24 In brief, hearts from 13 Sham, 4 LVH, and 5 Fos/LVH rats were studied. Hearts were retrograde-perfused through the aorta at a perfusion pressure of 80 to 100 mm Hg with Karnovsky fixative solution (containing 2% paraformaldehyde and 2.5% glutaraldehyde) for 3 to 5 minutes. Transverse midventricular slices of the hearts were conventionally embedded in paraffin, cut at 6 μm, stained with hematoxylin and eosin and Masson’s trichrome stain, examined by light microscopy, and semiquantitatively graded for extent and character of necrosis, inflammation, and fibrosis. Three tissue blocks from the lateral mid free wall of each ventricle were embedded in Poly/Bed 812 (Polysciences) and trimmed for thin sectioning to obtain areas of tissue with transversely sectioned myofibers. Five electron photomicrographs of random areas of ultrathin sections of each tissue block were printed at ×5000, calibrated with a diffraction grating replica magnification standard (EF Fulliam, Inc.) and analyzed morphometrically with a superimposed rectangular grid consisting of 221 sampling points distributed over a total area of 2169 μm² per electron micrograph. Quantitative determination of the fractional volumes (Vr) of myocardium composed of myocytes, capillary lumen, capillary endothelial cells, interstitial cells, extracellular matrix, and fibrillar collagen was done by counting the fraction of sampling points (Pv) overlaying each of these components (Vr=PVv). Mean myocyte width was determined by measurement of transnuclear widths of random, longitudinally oriented myocytes in the circular midwall muscle bundles with a calibrated eyepiece reticle (25 cells for each sample) on routinely prepared histological sections embedded in paraffin, cut at 6 μm, and stained with hematoxylin and eosin. Results are presented as mean±SEM values computed from the average measurements obtained from each heart.

To estimate cell length, additional hearts were collagenase-perfused, and isolated, dissociated myocytes were obtained as previously described28 for assessment of diastolic cell length using an inverted-stage microscope (Nikon). Diastolic cell length was measured at a superfused [Ca2+]i of 1.2 mmol/L in the presence of a paced rate of 3 Hz at 36°C to 37°C. Nine Fox/LVH, 9 LVH, and 10 Sham hearts underwent this protocol.

**Plasma Norepinephrine Levels, Renin Activity, and ACE Activity**

Plasma norepinephrine, renin activity, and ACE activity were determined in 8 Fos/LVH, 7 LVH, and 8 Sham animals. Plasma norepinephrine was determined by a radioenzymatic assay using the enzyme carbobachol-O-methyl transferase (COT)29. Four milliliters of blood was obtained from tubules containing reduced glutathione and ethylene-bis(oxyethylenenitrile)-tetraacetic acid and placed on ice. Within 60 minutes of collection, blood was centrifuged at 30,000g at 4°C for 20 minutes to separate the plasma. Plasma was stored at −80°C and was assayed within 12 weeks of collection. Plasma was deproteinized with perchloric acid and then incubated with COMT and [3H]methyl-S-adenosyl-L-methionine to convert norepinephrine to [3H]normetanephrine. This was subsequently extracted and separated by thin-layer chromatography and quantified by liquid scintillation counting. The intra-assay coefficient of variation for norepinephrine in this assay was 4%, whereas interassay coefficient of variation was 5.14%.

Plasma renin activity was measured by the method described by Sealy and Laragh.30 Blood was collected into chilled tubes containing EDTA, was kept on ice, and was processed and stored as for plasma norepinephrine assay. To measure plasma renin activity, one aliquot of the sample was incubated at 37°C for 30 minutes and another for 3 hours. After this, the samples were plunged into an ice bath to stop the renin activity. Then, a standard radioimmunossay was done using 125I-angiotensin I. The results were calculated with the standard curve that runs concurrently with each assay. ACE activity was measured with a radiometric assay (Ventrex Laboratories) that uses radiolabeled [3H]Hip-Gly-Gly-Gly, which is converted by ACE to [3H]hippuric acid.31

**Measurements of LV High-Energy Phosphate and Hydroxyproline Levels**

Myocardial ATP and creatine phosphate contents of six Fos/LVH, seven LVH, and six Sham hearts were analyzed by standard enzymatic methods.32 At the end of a 30-minute control period of oxygenated perfusion, the heart was trimmed of atria and right ventricle, and, while still being perfused, the LV was rapidly frozen with aluminum clamps that had been cooled in liquid nitrogen. Frozen samples of LV myocardium were stored in liquid nitrogen. The frozen myocardial sample was rapidly weighed and pulverized in a mortar under liquid nitrogen. A portion of the frozen powder was weighed and subsequently dried at 37°C for 48 hours to determine the ratio of frozen to dry weight; the remainder of the frozen pulverized specimen was ground with 0.6N perchloric acid and homogenized with a glass tissue homogenizer. The homogenate was centrifuged, and the supernatant was neutralized with 5 mol/L K2CO3. Aliquots of the neutralized homogenate were then analyzed for ATP by the method of Adams32 by use of preweighed reagent vials (catalog number 336 UV, Sigma Chemical Co). The creatine phosphate was measured by adding an excess of creatine kinase to the ATP assay reaction.
mixture after the ATP assay reaction had come to completion. Tissue high-energy phosphate is expressed as micromoles per gram dry weight.

For the determination of hydroxyproline content, LV tissue from six Fos/LVH, six LVH, and six Sham hearts was homogenized in buffered saline and lyophilized. Lyophilized tissue was hydrolyzed in 6 mol/L hydrochloric acid at 105°C overnight and then neutralized with sodium hydroxide. Hydroxyproline content, an index of collagen, was measured colorimetrically with chloramine-T and Ehrlich’s B reagent. Total protein was measured by the method of Lowry et al. Hydroxyproline is expressed in micrograms per milligram dry weight and micrograms per milligram protein.

**Statistical Analysis**

All values are expressed as the mean±SEM. The statistical analysis of differences observed between LVH, Fos/LVH, and Sham groups was done by ANOVA comparison or ANOVA for repeated measures where appropriate and Fisher’s exact test for post hoc analyses. Statistical significance was accepted at the level of *P*<.05.

**Results**

**Survival**

Chronic ACE inhibition with fosinopril treatment significantly improved survival in rats with ascending aortic stenosis. During the 15-week treatment period, 11 of 36 animals died in the untreated LVH group (31%) compared with 1 of 38 animals in the fosinopril-treated LVH group (3%). The Kaplan-Meier survival analysis (Fig 1) demonstrated a significant improvement in survival in the Fos/LVH group compared with the LVH group (*P*<.005). None of the rats in the Sham group died.

**Blood Pressure, Body Weight, and Tibial Length**

Shown in Fig 2 are the biweekly tail-cuff blood pressure measurements for all groups. Fosinopril treatment resulted in a modest and statistically significant reduction in systolic tail-cuff blood pressure of approximately 12 mm Hg compared with untreated LVH rats, which was sustained during the treatment period. Diastolic pressure was approximately 8 mm Hg lower in the Fos/LVH group than in the LVH group throughout the treatment period. Heart rates were similar in the Fos/LVH, LVH, and Sham groups (344±24, 344±28, 354±26 beats per minute, respectively, NS). Body weight was slightly but significantly lower in the Fos/LVH group than in both the LVH group and the Sham group at the end of the 15-week treatment period, as shown in Table 1. The lower body weight in the Fos/LVH rats was not due to decreased food consumption in this group compared with LVH rats. Food consumption was measured for 1 week at week 12 of the treatment period and was similar between Fos/LVH (n=10) and LVH (n=10) rats (25.4±0.6 versus 25.9±0.4 grams per rat per day, respectively, *P*=NS). Tibial length, an index of growth independent of body fat or fluid homeostasis, was similar among all groups (Table 1).

**In Vivo LV Function**

Measurements of in vivo LV pressure are shown in Fig 3. In vivo LV systolic pressure measured after 15 weeks of fosinopril treatment was 223±10 mm Hg in Fos/LVH rats versus 232±9 mm Hg in LVH rats (*P*<NS). LV systolic pressure in both aortic stenosis groups was higher than in the Sham group (99±3 mm Hg, *P*<.05). In vivo LV diastolic pressure was lower in Fos/LVH than in LVH rats (10±2 versus 15±2 mm Hg, *P*<.05). LV diastolic pressure in both aortic stenosis groups was higher than in the Sham group (5±1 mm Hg, *P*<.05). Heart rate was similar in Fos/LVH (207±23), LVH (235±28), and Sham rats (267±31 beats per minute, *P*=NS).
TABLE 1.  Left Ventricular and Right Ventricular Hypertrophy

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>Tibial Length, mm</th>
<th>LV Wt, g</th>
<th>RV Wt, g</th>
<th>RV Wt/LV, mg/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>631±12</td>
<td>45.5±0.2</td>
<td>1.127±0.056</td>
<td>25±1</td>
<td>0.607±0.033</td>
</tr>
<tr>
<td>LVH</td>
<td>601±22†</td>
<td>45.2±0.4</td>
<td>1.642±0.068</td>
<td>37±1*</td>
<td>0.700±0.101</td>
</tr>
<tr>
<td>Fos/LVH</td>
<td>548±9*</td>
<td>45.6±0.3</td>
<td>1.465±0.038</td>
<td>32±1*</td>
<td>0.565±0.025</td>
</tr>
</tbody>
</table>

LV Wt indicates left ventricular weight; LV Wt/LV, left ventricular weight/tibial length; RV Wt, right ventricular weight; RV Wt/LV, right ventricular weight/tibial length; Sham, sham-operated untreated control group; LVH, untreated rats with aortic stenosis; Fos/LVH, fosinopril-treated rats with aortic stenosis. P value indicates result of data analysis by ANOVA. Values are mean±SEM.

*Significantly different from Sham; †significantly different from Fos/LVH.

Echocardiographic Measurements

In vivo echocardiographic measurements were obtained in 10 untreated aortic-banded LVH rats, 12 Fos/LVH rats, and 6 Sham rats at 6 weeks after banding before initiation of the trial and at the conclusion of the treatment trial. At baseline, the three groups exhibited similar LV diastolic cavity dimensions. The aortic-banded groups exhibited similarly increased LV mass, and LV fractional shortening was not depressed relative to the Sham group. At the end of the treatment period, the Fos/LVH group showed a 36% reduction in the increment in calculated LV mass observed in the untreated LVH group relative to the Sham group. At the end of the treatment period, compared with baseline, the untreated LVH rats showed a serial increase in LV mass (1.67±0.10 to 2.14±0.25 g, P<.05) and systolic LV dimension (4.3±0.2 to 5.8±0.3 mm, P<.05), with a concomitant reduction in fractional shortening (46±2% to 39±2%, P<.05). In contrast, the fosinopril-treated rats did not show any serial change in LV mass (1.65±0.12 to 1.76±0.07 g, NS), systolic dimension (3.8±0.2 to 4.4±0.3 mm, NS), or fractional shortening (51±2% to 49±2%, NS).

Effects of Fosinopril on LV Hypertrophy

The magnitude of LV hypertrophy is shown in Table 1. LV weight was significantly greater in LVH rats than in Sham rats. Fosinopril treatment was associated with a 43% reduction in the increment of LV weight and a 42% reduction in the increment of LV weight/tibial length observed in the LVH group relative to the Sham group (Table 1). LV myocyte cell width was significantly greater in myocytes from LVH hearts than in Sham hearts; in contrast, LV myocyte width in Fos/LVH rats was significantly less than in myocytes from LVH rats (14.8±0.5 versus 20.8±2.2 µm, P<.05) and similar to myocytes from the Sham group (16.3±0.9 µm). Diastolic myocyte lengths were similar between the LVH and Fos/LVH groups (171±7 versus 165±5 µm, P=NS), and both groups were significantly longer than in the Sham group (143±5 µm, P<.05).

Right ventricular weight and right ventricular weight/tibial length tended to be higher in LVH hearts than in both Sham and Fos/LVH hearts, but because of heterogeneity in the LVH group, the differences were not significant (Table 1). Right ventricular myocyte cell width was significantly greater in myocytes from LVH hearts than in Sham hearts. Right ventricular myocyte width in Fos/LVH rats was less than in myocytes from LVH rats (15.4±0.1 versus 20.5±3.4 µm, P<.05) and similar to myocytes from the Sham group (14.6±1.7 µm). Right ventricular myocyte length was not measured.

Morphology and Quantitative Morphometry

Routine light microscopy did not reveal consistent differences among the Sham, LVH, and Fos/LVH groups in overall morphological features, including necrosis, inflammation, or fibrosis.

Quantitative determination of the fractional volumes (%) of LV myocardium composed of myocytes, capillary...
lumen, capillary endothelial cells, noncollagen interstitial matrix, and interstitial fibrillar collagen was performed (Table 2). Representative electron micrographs are shown in Fig 4. In LV tissue from the Fos/LVH group compared with the two other groups, there was a reduction in fractional myocyte volume consistent with regression of hypertrophy in this group. The fractional capillary volume was significantly increased in the LVH and Fos/LVH groups compared with the Sham group. The total interstitial volume fraction was significantly increased in the Fos/LVH group compared with the Sham and LVH groups. This change in composition of the LV myocardium in the Fos/LVH rats was characterized by a relative increase in the fractional volume of both fibrillar collagen and noncollagen interstitial matrix. Endothelial and interstitial cell volume fractions were similar among all groups.

Hydroxyproline content, an index of collagen content, was not statistically different among all groups (Table 3). To control for potential changes in total protein among groups, tissue total protein was also used as a reference for expression of hydroxyproline content. Hydroxyproline content expressed in micromgrams per milligram protein was not different among groups.

Isolated Heart Studies
In the isolated perfused hearts (Sham, n=6; LVH, n=6; Fos/LVH, n=8), coronary flow rate per gram LV was similar in all groups by study design (Sham, 17.5±0.6; LVH, 16.2±0.3; Fos/LVH 17.1±0.3 mL·min⁻¹·g⁻¹, P=NS).

Diastolic Pressure–Volume Relations
The relation of LV end-diastolic pressure versus balloon volume for all groups is shown in Fig 5. The diastolic pressure–volume relation was significantly shifted upward and to the left in LVH hearts compared with Shams, indicating a decrease in LV diastolic chamber distensibility. Diastolic function was significantly improved in the Fos/LVH hearts relative to the LVH hearts, as shown by the rightward shift of the pressure-volume relation compared with the LVH hearts, but was not restored to the level of normal Sham hearts. Note that the in vitro measurements of diastolic function corroborated differences in the in vivo measurements of LV diastolic pressure.

LV Contractile Function
The dose-response relation between LV systolic developed pressure per gram LV and perfusate calcium concentration at identical LV diastolic balloon volumes was measured as a surrogate for the force-calcium relation, which is studied in isolated muscle (Fig 6). The LVH hearts showed significantly less capacity to increase systolic pressure in response to an increase in perfusate calcium compared with the Fos/LVH hearts. The relation between LV systolic developed pressure and perfusate calcium was greater in the Fos/LVH group relative to the LVH group.

Plasma Norepinephrine Levels and Renin Activity
Plasma norepinephrine levels were not significantly different among all groups, although there was a trend for plasma norepinephrine levels to be lower in the Fos/LVH group relative to the LVH group (Table 4). Plasma renin activity was similar between Sham and LVH groups, and both were significantly lower than the Fos/LVH group (Table 4). Plasma ACE activity was similar between Sham and LVH groups, and both were significantly higher than in the Fos/LVH group (Table 4).

ATP and Creatine Phosphate Contents
There was no difference in LV ATP content between the groups (Table 5). There was a trend, which did not achieve statistical significance, for creatine phosphate content to be depressed in the LVH group compared with the Fos/LVH and Sham groups (P=.07).

Discussion
The present study demonstrates that chronic administration of the ACE inhibitor fosinopril to rats with pressure-overload LV hypertrophy due to ascending aortic stenosis resulted in a marked survival benefit, a reduction in the extent of LV hypertrophy, normalization of LV myocyte cell width, and the preservation of systolic and diastolic function. A novel feature of this study is that the beneficial effects of chronic ACE inhibition occurred in the presence of a sustained elevation of LV systolic pressure due to the surgically placed clip around the ascending aorta. These results suggest that specific inhibition of ACE favorably alters the hypertrophic remodeling process and the transition to heart failure and that this effect can be dissociated from the magnitude of elevation of LV systolic pressure.

The ascending aortic banding model of LV hypertrophy is an ideal model in which to dissociate the effects of inhibition of ACE from the effects of the systemic renin-angiotensin system on LV systolic pressure regarding hypertrophic remodeling. Work from our laboratory has previously shown that in hearts with compen-
satory hypertrophy due to ascending aortic banding, the cardiac tissue ACE message levels and activity and the capacity for local angiotensin II generation are increased in the absence of activation of the systemic renin-angiotensin system.\textsuperscript{23,24} The results from the present study extend these findings to show that in this model, in which systemic hypertension is not present, norepinephrine levels and plasma renin activity are similar to those in age-matched controls. Fosinopril treatment, which was begun 6 weeks after aortic banding and continued for 15 weeks, caused a mild reduction in systemic arterial tail-cuff blood pressure that was sustained for the treatment period in association with an increase in plasma renin activity and decrease in ACE activity consistent with prior studies of chronic ACE inhibition.\textsuperscript{11,36}

**Hemodynamic Effects of ACE Inhibition**

In the clinical setting of heart failure due to dilated cardiomyopathy, ACE inhibition is aimed at modifying the hemodynamic responses to the underlying LV systolic dysfunction and may cause a reduction of LV systolic load by the mechanisms of arterial dilation and a fall in LV systolic pressure, a reduction in LV cavity dilatation via changes in venous capacitance and blood volume, and a fall in heart rate and contractility after withdrawal of sympathetic stimulation.\textsuperscript{16,20} From the viewpoint of myocardial mechanics, LV systolic load can
be estimated by the calculation of LV systolic wall stress, which is directly related to the magnitude of LV pressure and cavity radius and indirectly related to wall thickness and mass. In this prospective survival trial, animals were not chronically instrumented or withdrawn for experimentation during the trial, so serial calculations of wall stress are not available. Nonetheless, the in vivo LV pressure data and serial echocardiographic data afford insights into the changes in load in this model. In contrast with dilated cardiomyopathy, the predominant hemodynamic perturbation in chronic valvular aortic stenosis or ascending aortic banding is the elevation of LV systolic pressure in the absence of cavity dilatation or depression of systolic fiber shortening. This stimulates the hypertrophic response of the parallel addition of myofibrils and an increase in wall thickness that tends to restore wall stress to normal.37 In this study, in vivo echocardiographic data obtained 6 weeks after banding before initiation of the treatment trial showed that the rats with ascending aortic stenosis exhibited these hallmarks of LV pressure overload: LV end-systolic and end-diastolic dimensions were not increased and fractional shortening was not impaired relative to age-matched controls, whereas LV mass was increased. Thus, in contrast to patients with dilated cardiomyopathy, it is highly unlikely that the effects of ACE inhibition in the aortic stenosis rats were mediated by the correction of an initial LV volume overload via changes in venous capacitance or blood volume. At the conclusion of the 15-week treatment period (21 weeks after banding), in vivo LV systolic pressures measured proximal to the ascending aortic band were severely elevated and equivalent between the aortic stenosis rats that were treated with fosinopril and the untreated rats. Thus, this model differs from dilated cardiomyopathy or hypertension, in which changes in systolic arterial pressure caused by ACE inhibition cause parallel changes in LV systolic pressure. Furthermore, the preliminary in vivo echocardiographic studies obtained at the end of the 15-week treatment period indicate that no serial changes in LV end-systolic or end-diastolic dimension or fractional shortening occurred in the fosinopril-treated animals. In contrast,
Table 4. Plasma Norepinephrine Levels, Plasma Renin Activity, and ACE Activity

<table>
<thead>
<tr>
<th>Group</th>
<th>PNE, pg/mL</th>
<th>PRA, ng·mL⁻¹·h⁻¹</th>
<th>ACE Activity, U/mL</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>909±82</td>
<td>26±4</td>
<td>52.3±3.9</td>
<td>8</td>
</tr>
<tr>
<td>LVH</td>
<td>2357±904</td>
<td>23±5</td>
<td>63.1±4.7</td>
<td>7</td>
</tr>
<tr>
<td>Fos/LVH</td>
<td>1285±196</td>
<td>124±32*</td>
<td>28.9±2.9*</td>
<td>8</td>
</tr>
<tr>
<td>( P )</td>
<td>.13</td>
<td>.002</td>
<td>.0001</td>
<td></td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; PNE, plasma norepinephrine levels; PRA, plasma renin activity; Sham, sham-operated untreated control group; LVH, untreated rats with aortic stenosis; and Fos/LVH, fosinopril-treated rats with aortic stenosis. Data were analyzed by ANOVA. Values are mean±SEM. *Significantly different from Sham group.

Fosinopril therapy appeared to prevent the development of the depression of LV fractional shortening and an increase in systolic cavity dimension, which occurred in the untreated aortic stenosis animals. Heart rate was similar in all groups. Thus, taken together, these data support the hypothesis that the beneficial effects of ACE inhibition on the regression of hypertrophy and survival in the fosinopril-treated rats occurred in the absence of the reduction of LV systolic pressure or serial changes in LV cavity dimensions, contractility, or heart rate.

Regression of LV Hypertrophy

Chronic ACE inhibition significantly reduced the extent of LV hypertrophy and altered the composition of the myocardium. At the cellular level, ACE inhibition resulted in a significant reduction in myocyte width of aortic-banded rats such that myocyte width was similar to that of sham-operated normal hearts. Cell length was similar in LV myocytes from aortic-banded rats treated with ACE inhibition versus no drug, suggesting that ACE-induced changes in myocyte remodeling occurred predominantly via alterations in the parallel addition of myofibrils, which characterizes pressure-overload hypertrophy.\(^{11}\) Quantitative morphometry showed that the regression of myocyte hypertrophy was not accompanied by proportional changes in other components of the myocardium. Although this model is not associated with the severe fibrosis and elevation of collagen content that is detected in some patients with advanced hypertrophy\(^{12}\) and in some animal models of experimental pressure overload, the untreated rats with aortic stenosis exhibited a modest increase in the volume fraction of fibrillar collagen relative to the sham-operated control rats. In the aortic stenosis rats treated with ACE inhibition, the reduction of myocyte width and myocyte volume fraction was associated with a relative increase in total interstitial volume fraction comprised largely of a twofold increase in the volume fraction of fibrillar collagen. In this study, we do not know whether a qualitative change in collagen type also occurred.

The relative increase in interstitial fibrillar collagen may be related in part to the notion proposed by Weber et al\(^{13}\) and Mukherjee and Sen\(^{14}\) that angiotensin II and aldosterone may have disparate effects on myocyte growth versus collagen deposition. Alternatively, similar disproportionate changes in the relative composition of the myocardium have been reported by Krayenbuehl et al\(^{15}\) in patients with aortic stenosis who underwent aortic valve replacement. In that study, morphometric analysis performed within 2 years of valve replacement demonstrated a reduction in myocyte and muscle fiber diameter and nearly a twofold increase in percent interstitial fibrosis. In the present study, hydroxyproline content, a measure of total collagen content, was similar among all groups. Thus, it is feasible that the relative increase in the interstitium and collagen volume fractions observed in our experiment may reflect "filling in" of myocardial volume previously occupied by myofibrils or differences in the time course of remodeling of the myofibrils in comparison with the collagen and interstitial matrix. The disproportionate changes in composition of the myocardium may also explain why LV mass in the rats treated with ACE inhibition did not completely regress to the level of the sham-operated normal rats.

Mechanisms of Cellular Remodeling

Our finding that regression of myocyte hypertrophy occurred in aortic-banded rats treated with ACE inhibition is remarkable in light of the fact that aortic-banded rats treated with ACE inhibitor versus no drug showed equivalent severe elevation of LV systolic pressure in the range of approximately 225 mm Hg. This observation is consistent with accumulating evidence that neurohormonal stimuli as well as hemodynamic stress can induce myocardial hypertrophy.\(^{16,17}\) Our findings support the notion that the hemodynamic stimulus of the elevation of ventricular systolic pressure alone is insufficient to maintain myocellular hypertrophy and that an interplay of angiotensin II stimulation and load may be involved. The octapeptide angiotensin II can directly promote protein synthesis and growth of myocytes.\(^{18,19}\) The critical signaling pathways responsible for the direct trophic effect of angiotensin II are not yet characterized and may involve receptor-mediated activation of protein kinase C, resulting in mobilization of calcium, and stimulation of Na⁺-H⁺ exchange, resulting in intracellular alkalosis.\(^{20}\) In addition, angiotensin II derived from internalization or intracellular production may also directly bind to target sites within the cell nucleus.\(^{20}\) Preliminary studies in isolated cultured neonatal rat myocytes suggest that mechanical stretch itself may stimulate myocyte angiotensin II release and that the presence of angiotensin II is mandatory for stimulation of cultured myocytes to occur.\(^{21}\) Much further work is needed to clarify the interplay of load and the

Table 5. Left Ventricular ATP and Creatine Phosphate Measurements

<table>
<thead>
<tr>
<th>Group</th>
<th>ATP, ( \mu mol/g ) DW</th>
<th>CP, ( \mu mol/g ) DW</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>18.43±0.83</td>
<td>19.92±2.32</td>
<td>6</td>
</tr>
<tr>
<td>LVH</td>
<td>18.00±1.19</td>
<td>14.15±1.40</td>
<td>7</td>
</tr>
<tr>
<td>Fos/LVH</td>
<td>17.18±0.87</td>
<td>18.04±1.23</td>
<td>6</td>
</tr>
<tr>
<td>( P )</td>
<td>.69</td>
<td>.07</td>
<td></td>
</tr>
</tbody>
</table>

CP indicates creatine phosphate; DW, left ventricular dry weight; Sham, sham-operated untreated control group; LVH, untreated rats with aortic stenosis; and Fos/LVH, fosinopril-treated rats with aortic stenosis. Data were analyzed by ANOVA. Values are mean±SEM.
renin-angiotensin system in both the initiation of myocyte growth and the later maintenance of hypertrophy.

In this study, it is attractive to infer that chronic fosinopril therapy inhibited both systemic and cardiac tissue ACE and blunted the trophic effect of local cardiac angiotensin II production on LV myocytes. We have demonstrated that our model is characterized by increased LV ACE activation and capacity for local angiotensin II synthesis, and we and others recently documented the presence of angiotensin II receptors in rat myocardium and myocytes. The identification of subtypes of type 1 angiotensin II receptors suggests the potential for diverse pathways of angiotensin-mediated signal transduction in myocyte and nonmyocyte cells. However, other neurohormonal mechanisms could account for the regression of hypertrophy in this study. Angiotensin II enhances sympathetic neurotransmitter release, and the regression of hypertrophy in animals treated with ACE inhibitor could be related in part to blunting of the trophic effect of sympathetic stimulation on myocellular growth. In addition, ACE inhibitors also limit the degradation of the vasoactive peptide bradykinin and the release of endothelin, which also may modify cardiac hypertrophy. Thus, it will be of interest in future trials to examine the effects of direct angiotensin II receptor antagonists in this model. Furthermore, although there was no difference in the magnitude of LV systolic pressure elevation in the LVH and Fos/LVH groups, fosinopril treatment did cause a modest but significant reduction in tail-cuff pressure. Further trials using other vasodilators to simulate the modest reduction in tail-cuff pressure seen in this study would be useful to confirm the hypothesis that the effects of fosinopril on the regression of hypertrophy and survival are specific to the intervention of ACE inhibition.

Effects of ACE Inhibition on Survival

We observed that fosinopril improved survival and function in aortic stenosis rats with persistent elevation of LV systolic pressure despite the regression of hypertrophy. This challenges the traditional notion that the initiation and maintenance of myocyte hypertrophy are fundamental and mandatory adaptations required for survival and the preservation of pump function in animals subjected to chronic systolic pressure overload. A limitation of this study is that the analysis of survival was confined to the 15-week treatment period from 6 to 21 weeks after aortic banding. Thus, we do not yet know whether the striking survival benefit would be sustained during very late follow-up. We selected this drug treatment interval for this trial because prior studies in our laboratory had shown that this time interval was associated with a consistent mortality of 30% to 35% in aortic stenosis rats relative to sham-operated controls and because this period embraces the critical time window in which animals undergo a transition from compensated hypertrophy to early failure with associated changes in cardiac gene reprogramming. Several mechanisms could account for the enhanced survival seen in the ACE inhibitor–treated animals in this trial. First, it is possible that premature death in the untreated aortic-banded animals may have been associated with development of severe LV dysfunction and dilatation relative to the untreated animals that survived. Precise histological analyses could not be performed in animals that died prematurely. However, gross inspection of the hearts from the dead animals in comparison with those of animals killed electively at conclusion of the trial did not support this hypothesis. A more likely possibility is that ACE inhibition prevented premature deaths from cardiac arrhythmia, possibly triggered by myocardial ischemia. We have demonstrated that coronary vascular reserve in this model is severely impaired, with an increased susceptibility to severe ischemic diastolic dysfunction, which is favorably modified by acute administration of ACE inhibitor. Thus, it is feasible that chronic ACE inhibition modified coronary vascular reserve and prevented transient ischemic elevations in diastolic pressure. Alternatively, there is evidence that angiotensin II aggravates, whereas ACE inhibitors protect against, reperfusion arrhythmias after transient ischemia in the rat.

Effects of ACE Inhibition on LV Function

Distinct from its effects on survival, chronic ACE inhibition also resulted in improved systolic and diastolic function in comparison with untreated aortic stenosis rats. LV hypertrophy is regarded as an initial adaptation to an increase in systolic load whereby the parallel addition of sarcomeres restores systolic wall stress to normal and preserves systolic function. In both human patients and experimental models, this "adaptive" phase is followed by a transition to decompensation and failure characterized by reduced contractile force and alterations in cross-bridge cycling, impaired diastolic function related to impaired relaxation and altered passive myocardial properties, and changes in contractile protein and calcium regulatory gene expression. In the present study, we observed that an equivalent level of LV systolic pressure generation was maintained in vivo in the aortic-banded rats treated with ACE inhibitor relative to untreated rats in the presence of a lower in vivo LV end-diastolic pressure and the regression of myocyte hypertrophy. Serial echocardiographic studies performed in vivo also suggest that chronic ACE inhibition prevented the late development of LV systolic dilatation and depression of ejection fraction seen in untreated animals. To examine the mechanism of the apparent preservation of in vivo systolic function despite partial loss of the adaptive mechanism of myocyte hypertrophy, we studied cardiac function in isolated perfused hearts in the absence of confounding factors of systemic neurohumoral activation and pericardial constraint. The isovolumic hearts were studied under conditions of equivalent coronary flow per gram, normothermia, and physiologically paced heart rate. The relation between LV systolic developed pressure per gram over a range of perfusate calcium concentrations at identical LV balloon volume was determined in the intact hearts as a surrogate for the force-calcium relation, which can be measured in isolated muscle preparations. This analysis demonstrated that the developed pressure-calcium relation was shifted upward and to the left in the ACE inhibitor–treated aortic stenosis rats and significantly different from the untreated aortic stenosis rats. These observations suggest that in vivo systolic function was preserved in the ACE inhibitor–treated aortic stenosis rats by the mechanism of improved contractile response.
to extracellular calcium, which compensated for the regression of hypertrophy and loss of force-bearing units.

Corroborating our results, prior studies in muscles from rats with postinfarction failure have shown that chronic treatment with the ACE inhibitor captopril also resulted in partial normalization of myocardial contractility and improvement in the contractile response to increasing extracellular calcium.60 Further, preliminary studies performed in isolated unloaded collagenase-dissociated myocytes from our model indicate that the calcium-shortening relation is significantly enhanced in myocytes from fosinopril-treated rats compared with untreated aortic stenosis rats. LV high-energy phosphate levels were similar in the treated and untreated animals, suggesting that differences in myocardial energy charge are unlikely to explain the difference in the force-calcium relation. Further studies in skinned muscle fibers will be needed to determine whether the differences in systolic function seen in the intact heart in vivo and in the isolated heart preparation are related to differences in calcium responsiveness at the level of the myocyte or myofibrillar calcium sensitivity.

Diastolic function was also modestly improved but not normalized by chronic ACE inhibition in this study. In the fosinopril-treated aortic stenosis rats, in vivo LV end-diastolic pressure was lower than in untreated animals but higher than levels in sham-operated rats. Studies of the LV diastolic pressure-volume relation in the isolated perfused hearts indicated that the elevation of LV end-diastolic pressure in the aortic stenosis rats was related to an upward and leftward shift in the diastolic pressure-volume relation compared with sham-operated control hearts, consistent with the impairment of LV diastolic distensibility observed in aortic stenosis patients with advanced hypertrophy and early heart failure.38,49,60 The diastolic pressure-volume relation in the ACE inhibitor–treated hearts was partially restored toward normal over a physiological range of diastolic pressure, indicating an improvement in diastolic distensibility. The improvement in diastolic function in ACE inhibitor–treated rats may be partially related to structural changes in geometry and composition of the LV wall. In this regard, we speculate that the incomplete improvement in diastolic distensibility relative to the control animals may be related in part to the disproportionate regression of myocyte hypertrophy relative to interstitial collagen.

Alternatively, the improvement in diastolic function may be due in part to effects of blockade of locally produced angiotensin II. We have previously shown that the intracellular activation of angiotensin I to II caused a marked increase in diastolic pressure and slowing of relaxation in hypertrophied hearts from aortic-banded rats that were modified by acute intracardiac ACE inhibition with enalaprilat.24 In hypertrophied myocytes from spontaneously hypertensive rats, angiotensin II has been reported to delay and slow relaxation.40 In patients with LV dysfunction, intravenous benazeprilat improved LV diastolic distensibility, as shown by a downward shift of the diastolic pressure-volume relation.70 Recently, Friedrich et al71 demonstrated that acute intracardiac ACE inhibition with intracoronary enalaprilat infusion improved LV distensibility and relaxation in patients with advanced hypertrophy due to aortic stenosis. Further studies in isolated myocytes from this model may be helpful to elucidate the effects of chronic ACE inhibition on diastolic function at the level of the myocyte independent of changes in myocardial geometry and composition.

Summary

In summary, we demonstrated that chronic treatment with the ACE inhibitor fosinopril markedly improved survival, regressed myocyte hypertrophy, and modified the transition to failure in rats with ascending aortic stenosis despite persistent severe elevation of LV systolic pressure. These data provide support for the concept that the renin-angiotensin system contributes to the transition from compensated hypertrophy to failure and premature death distinct from the stimulus of the elevation of LV systolic pressure.

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References


Angiotensin-converting enzyme inhibition prolongs survival and modifies the transition to heart failure in rats with pressure overload hypertrophy due to ascending aortic stenosis.

E O Weinberg, F J Schoen, D George, Y Kagaya, P S Douglas, S E Litwin, H Schunkert, C R Benedict and B H Lorell

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