Lisinopril-Mediated Regression of Myocardial Fibrosis in Patients With Hypertensive Heart Disease

Christian G. Brilla, MD, PhD; Reinhard C. Funck, MD; Heinz Rupp, PhD

Background—In arterial hypertension, left ventricular hypertrophy (LVH) includes myocyte hypertrophy and fibrosis, which leads to LV diastolic dysfunction and, finally, heart failure. In spontaneously hypertensive rats, myocardial fibrosis was regressed and LV diastolic function was improved by treatment with the angiotensin-converting enzyme inhibitor lisinopril. Whether this holds true for patients with hypertensive heart disease was addressed in this prospective, randomized, double-blind trial.

Methods and Results—A total of 35 patients with primary hypertension, LVH, and LV diastolic dysfunction were treated with either lisinopril (n=18) or hydrochlorothiazide (HCTZ; n=17). At baseline and after 6 months, LV catheterization with endomyocardial biopsy, Doppler echocardiography with measurements of LV peak flow velocities during early filling and atrial contraction and isovolumic relaxation time, and 24-hour blood pressure monitoring were performed. Myocardial fibrosis was measured by LV collagen volume fraction and myocardial hydroxyproline concentration. With lisinopril, collagen volume fraction decreased from 6.9±0.6% to 6.3±0.6% (P<0.05 versus HCTZ) and myocardial hydroxyproline concentration from 9.9±0.3 to 8.3±0.4 μg/mg of LV dry weight (P<0.0001 versus HCTZ); this was associated with an increase in the early filling and atrial contraction LV peak flow velocity ratio from 0.72±0.04 to 0.91±0.06 (P<0.05 versus HCTZ) and a decrease in isovolumic relaxation time from 123±9 to 81±5 ms (P<0.0002 versus HCTZ). Normalized blood pressure did not significantly change in either group. No LVH regression occurred in lisinopril-treated patients, whereas with HCTZ, myocyte diameter was reduced from 22.1±0.6 to 20.7±0.7 μm (P<0.01 versus lisinopril).

Conclusions—In patients with hypertensive heart disease, angiotensin-converting enzyme inhibition with lisinopril can regress myocardial fibrosis, irrespective of LVH regression, and it is accompanied by improved LV diastolic function.

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Key Words: hypertension ■ hypertrophy ■ fibrosis ■ diastole

In experimental models of arterial hypertension, previous studies have demonstrated that in addition to left ventricular hypertrophy (LVH), which is mediated by myocyte hypertrophy, progressive myocardial fibrosis occurs.1–4 This fibrosis accounts for the development of LV diastolic dysfunction.1,4,5 In vivo and in vitro experiments have shown that the effector hormones of the renin-angiotensin-aldosterone system, angiotensin II and aldosterone,3,6,7 stimulate fibroblast-mediated collagen synthesis. Angiotensin II also suppresses the collagenase activity7 that synergistically leads to progressive collagen accumulation within the cardiac interstitium. In spontaneously hypertensive rats, LVH and myocardial fibrosis could be regressed and LV diastolic function could be improved by treatment with the angiotensin-converting enzyme (ACE) inhibitor lisinopril.8,9 These improvements occurred independently of the drug’s antihypertensive effect.5 Whether myocardial fibrosis can also be regressed in patients with hypertensive heart disease, which is defined as arterial hypertension with LVH, myocardial fibrosis, and LV diastolic dysfunction, was the primary objective of the present study.

Overall Study Design

This study, which was approved by the ethics committee of the University of Marburg, was designed as a prospective, double-blind, randomized, parallel-group trial evaluating antihypertensive treatment with lisinopril versus hydrochlorothiazide (HCTZ) in patients with primary arterial hypertension who were analyzed by intention to treat. The primary end point was myocardial fibrosis. This was evaluated by 2 independent methods: morphometrically by collagen volume fraction (CVF) and biochemically by hydroxyproline concentration (HPro) of LV endomyocardial biopsies. Secondary end points were as follows: (1) systolic and diastolic blood pressures; (2) LVH determined by LV mass index (LVMI) and myocyte diameter (MyoD); and (3) LV diastolic function, which was evaluated for relaxation by the ratio of peak flow velocities during early filling and atrial contraction (E/A) and isovolumic relaxation time (IVRT) and for myocardial stiffness by the stiffness constant k and the A-wave duration of transmitral flow during atrial contraction (AWD). Because of a lack of human data, the target population size (n=35) was...
TABLE 1. Baseline Characteristics of Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Lisinopril All</th>
<th>Lisinopril Without Dropouts</th>
<th>HCTZ All</th>
<th>HCTZ Without Dropouts</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
<td>11</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Random blocks</td>
<td>6/9/0/3</td>
<td>3/6/0/2</td>
<td>6/5/2/4</td>
<td>5/5/1/3</td>
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<tr>
<td>Age, y</td>
<td>57±2</td>
<td>57±3</td>
<td>57±2</td>
<td>59±2</td>
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<tr>
<td>Sex, M/F</td>
<td>16/2</td>
<td>11/0</td>
<td>11/6</td>
<td>8/6*</td>
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<tr>
<td>Mean 24-hr SBP, mm Hg</td>
<td>138±4</td>
<td>137±5</td>
<td>139±4</td>
<td>136±4</td>
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<tr>
<td>Mean 24-hr DBP, mm Hg</td>
<td>85±3</td>
<td>86±4</td>
<td>83±3</td>
<td>80±2</td>
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<tr>
<td>LVMI, g/m²</td>
<td>175.6±14.6</td>
<td>170.3±15.7</td>
<td>176.1±16.5</td>
<td>160.0±13.9</td>
</tr>
<tr>
<td>CVF, %</td>
<td>6.3±0.6</td>
<td>6.9±0.6</td>
<td>7.0±0.9</td>
<td>6.4±0.8</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>17.0±1.3</td>
<td>15.9±1.2</td>
<td>17.1±1.6</td>
<td>17.1±1.7</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>0.76±0.04</td>
<td>0.77±0.04</td>
<td>0.78±0.06</td>
<td>0.82±0.03</td>
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<tr>
<td>EF, %</td>
<td>78±2</td>
<td>78±1</td>
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<td>78±3</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. LVEDP indicates LV end-diastolic pressure; EF, LV ejection fraction.

*P<0.01 vs lisinopril.

Patient Population

A total of 35 white patients of either sex were enrolled according to the following inclusion criteria: symptomatic patients (dyspnea or angina pectoris) with primary hypertension in whom coronary artery disease was excluded by coronary angiography, LVH with LVMI>$134 \text{ g/m}^2$ in men or >$110 \text{ g/m}^2$ in women, LV diastolic dysfunction with an E/A ratio <1, and an age of 18 to 70 years. Patients were excluded from the study if 1 of the following criteria were met: ACE inhibitor or HCTZ therapy in the past year; LV systolic dysfunction; malignant hypertension; presence of any other cardiovascular disease or coexisting systemic disorder; allergy to ACE inhibitors, HCTZ, or prazosin; history of angioneurotic edema; history of severe renal disease; history of cancer; life expectancy of <6 months; drop-out phase was performed; participation in other treatment trials within the previous 3 months.

Randomization

No wash-out phase was performed to assure continuous antihypertensive treatment (required by the ethics committee). Therefore, patients were randomized in a blocked manner according to their antihypertensive pretreatment by computer-generated random numbers as follows: block I: antiadrenergic agents; block II: calcium-channel blockers; block III: combined treatment with antiadrenergic agents and calcium-channel blockers; and block IV: other antihypertensive pretreatment. The code was kept at the monitoring center and was not broken until all data were attained. After randomization, 18 patients were assigned to lisinopril and 17 to HCTZ treatment. Both groups did not differ with respect to their baseline characteristics, except for sex distribution (more female patients were assigned to HCTZ treatment in patient cohorts who finished the study; Table 1).

Study Protocol

At baseline, informed consent was obtained and the following examinations were performed: physical and laboratory examination, 24-hour blood pressure monitoring, Doppler echocardiography (2D, pulse-wave Doppler), and LV catheterization, including endomyocardial biopsies of the inferior wall.

Thereafter, previous antihypertensive drug therapy was substituted in a double-blind fashion by either 5 mg of oral lisinopril or 25 mg of HCTZ once daily; study drugs were identical in appearance. Dosages were adjusted to an optimal blood pressure response (maximal dosages of lisinopril or HCTZ, 20 and 50 mg/d, respectively), with follow-up checks after 1, 2, 4, 8, 12, 18, and 24 weeks. If diastolic blood pressure (DBP) was not <90 mm Hg or systolic blood pressure (SBP) was not ≤160 mm Hg, prazosin could be added in an open manner up to a maximal dosage of 15 mg/d. To check patients’ compliance, tablet counts were made at each visit. After 6 months, the same procedures done at baseline were performed.

Laboratory Measurements

Serum creatinine and potassium were determined at each visit using standard methods. At baseline and at the end of study, plasma renin and aldosterone concentrations were measured. Samples were collected in a supine position after a 15-minute resting period. Plasma renin concentration was measured by immunoradiometric assay (Nichols Institute) using monoclonal antibodies against human renin with 0.2% cross-reactivity with prorenin and a normal range of 5 to 47 mU/L. Plasma aldosterone concentrations were determined by radioimmunoassay (Serope Diagnostics) with magnetic separation (normal range, 42 to 416 pmol/L; cross-reactivity with other steroids, ≤0.004%).

Blood Pressure Monitoring and Doppler Echocardiography

SBP and DBP were monitored in a sitting position at each visit using standard cuff equipment. At baseline and at the end of the study, 24-hour blood pressure recordings with readings in 15-minute intervals were obtained. The end-diastolic thickness of the interventricular septum and LV posterolateral wall, as well as the LV end-diastolic diameter, were measured by 2D echocardiography to calculate LVMI, as reported elsewhere. E/A ratio and AWD were determined using pulse-wave Doppler in which the sample volume was placed at the tips of mitral valve leaflets in the 4-chamber view while all patients were in sinus rhythm. IVRT was measured as the time interval between the end of...
TABLE 2. Blood Chemistry

<table>
<thead>
<tr>
<th></th>
<th>Lisinopril</th>
<th>HCTZ</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 mo</td>
</tr>
<tr>
<td>Renin, mU/L</td>
<td>21.8±5.6</td>
<td>78.5±21.8*</td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
<td>624.2±77.7</td>
<td>344.0±36.1*</td>
</tr>
<tr>
<td>K⁺, mmol/L</td>
<td>4.3±0.1</td>
<td>4.3±0.1</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>86.6±2.7</td>
<td>85.8±3.5</td>
</tr>
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</table>

Data are expressed as mean±SEM. *P<0.05 vs baseline.

LV outflow and the start of LV inflow using simultaneous registrations of outflow and inflow signals by high-pulse repetition frequency pulse-wave Doppler.

Hemodynamics
LV pressure was measured by a 7-French pigtail catheter connected to a Statham pressure transducer (placed at right atrial level). Immediately after LV end-diastolic pressure was measured, standard LV angiography was performed. After a blood withdrawal of 200 mL, a second LV end-diastolic pressure measurement and LV angiogram were performed to construct end-diastolic pressure-volume diagrams for each patient.12 LV end-diastolic and end-systolic volumes were determined by Simpson’s rule and computerized planimetry of the LV cavity. LV ejection fraction was calculated. End-diastolic wall thickness was measured13 and myocardial diastolic stiffness was calculated for the midwall at the LV equator, assuming spherical geometry by stiffness constant k, as reported elsewhere,13,14 using an exponential curve fit program for end-diastolic pressure volume data (Statistica 6.0, StatSoft).

Quantitative Morphometry
LV endomyocardial biopsies were obtained using a 7-French biop­tome.15 LV endomyocardial samples were either immediately frozen in liquid nitrogen (3 samples) or placed in 5% formalin (3 samples) until biochemical and histological analysis. CVF was determined by morphometry of LV endomyocardial biopsies sections stained with the collagen-specific dye Sirius red using an automated image analyzer (CUE 3, Olympus), as previously reported,5,8,9 in which all fields of all samples were analyzed. Three well-sized samples (5 to 10 mg) were considered representative of the whole LV myocardium because we focused on reactive fibrosis, which presents as a diffuse accumulation of fibrillar collagen within the cardiac interstitium.16 MyoD was measured perpendicularly to the outer cell contour using hematoxylin- and eosin-stained sections. For each section, 15 representative myocytes were examined. The normal range of CVF and MyoD of LV tissue samples of explanted hearts designated for heart transplantation is 0.1% to 1.1% and 15.2 to 17.3 μm, respectively.

Hydroxyproline Assay
Frozen endomyocardial biopsies were thawed and minced. After samples were made into derivatives with phenylisothiocyanate, the phenylthiocarbamyl-hydroxyproline concentration was determined using high-pressure liquid chromatography, as previously reported.17 Using this technique, highly reproducible results can be obtained (±8% SEM for the whole procedure). The hydroxyproline concentration was calculated as the HPro content per milligram of dry tissue, where tissue dry weight was determined as previously reported.17

Statistical Analysis
Data were obtained in a double-blind manner and expressed as mean±SEM. To compare baseline characteristics of the 2 groups, data were analyzed by Student’s t test for unpaired data; χ² analysis was used for categorical data. To analyze treatment effects of lisinopril versus HCTZ over 6 months, the following hierarchical testing18 was applied: (1) 2 (between 2 treatment groups) by 2 (repeated measures with 2 levels, at baseline and 6 months) ANOVA and (2) a Student’s t test for paired data. The latter was performed if a significant treatment effect (treatment/time interaction) was found and was done to analyze changes within patient groups (baseline versus 6 months). A 2-sided P<0.05 was required for statistical significance.

Results
Dosages of Study Drugs and Patients’ Compliance
After titration, mean dosages of lisinopril and HCTZ were 11.4±7.2 and 45.6±9.8 mg, respectively. In 5 of 18 lisinopril-treated patients and in 10 of 17 HCTZ-treated patients (P=NS), additional treatment with prazosin with a mean final dosage of 4.8±2.2 and 7.5±4.1 mg, respectively, was required to normalize blood pressure.

Four lisinopril-treated patients did not want to complete the study, and an additional 3 patients stopped the study early due to adverse effects (dizziness, dry mouth). In the HCTZ group, 1 patient had to be excluded because blood pressure could not be controlled by study drugs, and 2 patients did not wish to complete the study. In 1 HCTZ-treated patient, hypokalemia of 2.8 mmol/L occurred, which could be controlled by potassium supplements. No other adverse effects or complications occurred.

In the lisinopril group, plasma renin concentrations increased after the 6-month period (P<0.05); this increase was associated with a decrease in plasma aldosterone concentrations (P<0.05; Table 2). The typical rise in plasma renin concentration after ACE inhibition proved the compliance of patients in regularly taking their study drugs. The same seems to be true for HCTZ-treated patients, in whom a decrease in serum potassium was found (P<0.05). Serum creatinine remained unchanged in both groups.

Myocardial Fibrosis
At baseline, all patients had morphological evidence of myocardial fibrosis (Figure 1). After 6 months, treatment with lisinopril resulted in the regression of myocardial fibrosis, as measured by CVF (P<0.05) and myocardial HPro concentration (P<0.00001; treatment effect versus HCTZ). With lisinopril, CVF was reduced from 6.9±0.6 to 6.3±0.6% (P<0.01) and HPro from 9.9±0.3 to 8.3±0.4 μg/mg LV dry weight (P<0.00001), whereas no regression of myocardial fibrosis occurred with HCTZ (CVF and HPro at baseline: 6.4±0.8% and 9.5±0.5 μg/mg LV dry weight, respectively; 6 months: 6.5±0.8% and 10.4±0.6 μg/mg, respectively).
Blood Pressure and LVH

In both groups, SBP and DBP from 24-hour blood pressure recordings did not significantly change throughout the study because all patients were pretreated with antihypertensive agents and no washout phase was performed (Table 3). However, all patients had a DBP $\geq 100$ mm Hg when antihypertensive agents were withdrawn for baseline LV catheterization. Likewise, in both groups, the percentage of patients with a SBP $\geq 160$ mm Hg or a DBP $\geq 90$ mm Hg did not significantly change, although in both groups blood pressure tended to be better controlled at the end of the study.

No significant treatment effect on LVMI was found with lisinopril (baseline, 170±16 g/m²; 6 months, 177±15 g/m²). LVMI tended to be decreased by HCTZ treatment (baseline, 176±17 g/m²; 6 months, 145±11 g/m²; $P=0.08$). More specifically, the treatment effect of HCTZ on MyoD was significant ($P<0.01$) compared with lisinopril (Figure 2). In HCTZ-treated patients, MyoD decreased from 22.1±0.6 to 20.7±0.7 mm ($P<0.005$), whereas no change occurred in lisinopril-treated patients (baseline, 22.5±0.4 mm; 6 months, 22.8±0.3 mm).

LV Diastolic Function

Treatment with lisinopril resulted in an improvement of LV diastolic function, as evidenced by a significant rise of the E/A ratio in lisinopril-treated patients compared with HCTZ-treated patients ($P<0.05$; Table 3). In addition, the treatment effect of lisinopril was significant for both IVRT ($P<0.0002$) and AWD ($P<0.002$). With lisinopril, IVRT was reduced from 123±9 to 81±5 ms ($P<0.0005$), whereas AWD increased from 149±16 to 247±16 ms ($P<0.001$; Figure 3). With HCTZ, IVRT (baseline, 117±8 ms; 6 months, 121±9 ms) and AWD (baseline, 154±19 ms; 6 months, 144±16 ms) were not affected. In both groups, the stiffness constant $k$ remained unchanged (Table 3).

Discussion

The present study represents the first clinical trial evaluating the effects of ACE inhibition on myocardial fibrosis in patients with hypertensive heart disease in which the primary end point (ie, myocardial fibrosis) was measured directly by 2 independent methods. We chose a treatment period just long enough to remove a significant amount of collagen from the myocardium, in keeping with the half-life of fibrillar collagen. In a 1-year study, we would have expected greater effects. However, we did not want to lose too many patients as dropouts because of the invasive nature of the study. Indeed, even during 6 months, we observed a significant dropout of patients, mainly at their own request.

TABLE 3. Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Lisinopril</th>
<th>HCTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 mo</td>
</tr>
<tr>
<td>Mean 24-hr SBP, mm Hg</td>
<td>137±5</td>
<td>136±6</td>
</tr>
<tr>
<td>Mean 24-hr DBP, mm Hg</td>
<td>86±4</td>
<td>84±4</td>
</tr>
<tr>
<td>24-hr SBP, % $\geq$ 160 mm Hg</td>
<td>49.2±3.9</td>
<td>30.3±10.6</td>
</tr>
<tr>
<td>24-hr DBP, % $\geq$ 90 mm Hg</td>
<td>34.9±6.9</td>
<td>26.5±9.4</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>0.72±0.04</td>
<td>0.91±0.06*†</td>
</tr>
<tr>
<td>k</td>
<td>14.4±1.5</td>
<td>14.3±1.1</td>
</tr>
</tbody>
</table>

*Data are expressed as mean±SEM.
†$P<0.05$ for significant treatment effect over 6 months vs HCTZ; †$P<0.01$ vs baseline.
Baseline characteristics were compared for all patients in either treatment group and for patients who completed the study. Overall, both groups seemed to be comparable. A significant difference was found only in the sex distribution in patient cohorts without dropouts: more female patients were assigned to HCTZ treatment. However, no effect of sex on any of the examined end points is known. Indeed, no difference in the response of male and female patients was evident. Because patients were randomized in a blocked manner according to their antihypertensive pretreatment and no significant difference of random blocks was found in either treatment group, any influence of pretreatment on results of the present study can be ruled out.

After 6 months of treatment with the ACE inhibitor lisinopril, a significant reduction in myocardial HPro concentration and CVF was found, indicating that regression of myocardial fibrosis can be achieved in patients with hypertensive heart disease by specific antihypertensive treatment that counteracts the adverse trophic effects of angiotensin II.5,7,9 These results, obtained by the direct measurement of myocardial collagen concentration, confirmed the findings of Diez et al,20 who found in patients with primary hypertension that treatment with the ACE inhibitor lisinopril resulted in a decrease in serum concentrations of procollagens type I carboxy and type III amino terminal peptides, which are markers of tissue collagen synthesis. However, in the present study under conditions of advanced hypertensive heart disease with LVH and diastolic LV dysfunction, CVF remained elevated after the rather short controlled treatment period. In addition, because reactive fibrosis is primarily mediated via the circulating and local renin-angiotensin-aldosterone system whereas replacement fibrosis is under separate controls,16 it can be assumed that reactive fibrosis was affected by long-term ACE inhibition with lisinopril whereas replacement fibrosis due to microscopic scars may contribute to the still-elevated CVF values at the end of the study.

LVH measured by LVMI and MyoD did not further regress with lisinopril treatment because blood pressure was already controlled at baseline. In contrast, with HCTZ treatment, a significant decrease in myocyte diameter was found, presumably due to a predominant reduction of LV preload and the fact that blood pressure was controlled as well as with lisinopril. This is in accordance with the perception that hemodynamics are the most relevant determinants of LVH21 and does not support the view that ACE inhibitors have more power to regress LVH compared with diuretics, as suggested by a retrospective meta-analysis of antihypertensive treatment studies.22 Indeed, a large-scale prospective clinical trial, the treatment of mild hypertension study, has also shown that LVH could be significantly more regressed by a diuretic agent compared with the ACE inhibitor enalapril.23

Regression of myocardial fibrosis in lisinopril-treated patients was associated with a significant improvement of LV diastolic function, as measured by an increase in the Doppler echocardiographically–determined E/A ratio; in contrast, no change occurred with HCTZ. Because a reduced E/A ratio predominantly reveals impaired relaxation,11 it can be concluded that LV relaxation in particular could be improved by lisinopril treatment. This has been confirmed by measure-
ments of IVRT, which revealed a prolonged relaxation at baseline in both groups (IVRT >100 ms) that was decreased to almost-normal values in lisinopril-treated patients only.11 Myocardial fibrosis impairs the relengthening of cardiac myocytes during the relaxation phase of the cardiac cycle24 and thereby leads to relaxation abnormalities of the myocardium. In addition, lisinopril treatment increased AWD, indicating improvement of LV compliance.25 In contrast, no significant change in the myocardial stiffness constant was found in either treatment group. We chose a well-established determination of the stiffness constant.12,14 However, potential errors exist in the calculation of myocardial stiffness in that we assumed homogeneity in myocardial structure and isotropic behavior. In addition, the pressure recordings obtained by routinely used fluid-filled catheter systems seemed to be inadequate to detect small changes within the short observation period.

Prevention of the progression of heart failure by long-term ACE inhibition was confirmed in several large-scale clinical trials. However, the underlying mechanism for these beneficial effects of ACE inhibition in heart failure is not well understood. The influence of the renin-angiotensin-aldosterone system on myocardial fibrosis3,5,7,20,26 and its counteraction by ACE inhibition could be one important determinant, particularly because myocardial fibrosis is progressive over time and associated with the progressive deterioration of cardiac function.2,4,9 In the present study, we showed for the first time that in patients with hypertensive heart disease, myocardial fibrosis can be regressed by long-term ACE inhibition, leading to an improvement of LV diastolic function. This study sets the stage for large-scale clinical trials using invasive or noninvasive measurements of myocardial fibrosis in patients with hypertensive heart disease. These trials will be of major clinical importance because arterial hypertension is still one of the most frequent causes of heart failure in Western countries, and any prevention of the appearance of symptomatic heart failure is of the utmost socioeconomic relevance.

Acknowledgments

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References
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