Matrix Metalloproteinases/Tissue Inhibitors of Metalloproteinases

Relationship Between Changes in Proteolytic Determinants of Matrix Composition and Structural, Functional, and Clinical Manifestations of Hypertensive Heart Disease

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Background—Chronic hypertension may cause left ventricular (LV) remodeling, alterations in cardiac function, and the development of chronic heart failure (CHF). Changes in the composition of the extracellular matrix (ECM) known to occur in hypertension are believed to be causally related to these structural, functional, and clinical outcomes. However, whether the determinants of ECM composition, such as the balance between ECM proteases (matrix metalloproteinases [MMPs]) and their tissue inhibitors [TIMPs]), are altered in hypertensive heart disease is unknown.

Methods and Results—Plasma MMP-2, -9, and -13 values, TIMP-1 and -2 values, and Doppler echocardiography images were obtained for 103 subjects divided into 4 groups: (1) reference subjects (CTL) with no evidence of cardiovascular disease, (2) hypertensive (HTN) subjects with controlled blood pressure and no LV hypertrophy, (3) hypertensive (HTN) subjects with controlled blood pressure and with LV hypertrophy (HTN/LVH) but no CHF, and (4) hypertensive subjects with controlled blood pressure, LVH, and CHF (HTN/LVH/CHF). Compared with CTL, patients with HTN had no significant changes in any MMP or TIMP. Patients with HTN/LVH had decreased MMP-2 and MMP-13 values and increased MMP-9 values. Only patients with HTN+LVH+CHF had increased TIMP-1 values. A TIMP-1 level >1200 ng/mL was predictive of CHF.

Conclusions—Patients with hypertension but normal LV structure and function had normal MMP/TIMP profiles. Changes in MMP profiles that favor decreased ECM degradation were associated with LVH and diastolic dysfunction. An increased TIMP-1 level predicted the presence of CHF. Although these findings should be confirmed in a larger prospective study, these data do suggest that changes in the MMP/TIMP balance may play an important role in the structural, functional, and clinical manifestations of hypertensive heart disease. (Circulation. 2006;113:2089-2096.)

Key Words: heart failure ⊗ hypertension ⊗ hypertrophy ⊗ matrix metalloproteinases

Epidemiological studies have shown that chronic arterial hypertension causes left ventricular (LV) structural remodeling, significant alterations in cardiac function, and the development of chronic heart failure (CHF). The structural and functional manifestations of hypertensive LV remodeling have been associated with significant changes in the extracellular matrix (ECM) composition. For example, experimental and clinical studies have shown that hypertensive heart disease can result in increased fibrillar collagen content, altered fibrillar collagen geometry, and an increased collagen I to III isotype ratio. However, the specific molecular and biochemical determinants that contribute to this ECM remodeling process in patients with hypertensive heart dis-

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ease have not been completely defined. Two of the major determinants that alter collagen homeostasis are the rate and extent of collagen degradation. Collagen degradation is under the control of matrix metalloproteinases (MMPs), a family of zinc-dependent interstitial enzymes, and their tissue inhibitors (TIMPs). In hypertensive heart disease, if TIMPs are increased and MMPs are decreased, this would favor decreased collagen degradation and increased collagen accumulation. The present study focused on one pathophysiological determinant of ECM composition, the regulatory role played by changes in the ECM proteolytic system, ie, the balance between MMPs and TIMPs. The purpose of this study was to...
TABLE 1. Demographic, LV Structure/Function, and MMP/TIMP Data

<table>
<thead>
<tr>
<th></th>
<th>Reference Controls (n=53)</th>
<th>LVH Patients (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>20/33</td>
<td>24/26</td>
</tr>
<tr>
<td>Age, y</td>
<td>59±1</td>
<td>60±2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>127±2</td>
<td>137±3*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>75±1</td>
<td>76±2</td>
</tr>
<tr>
<td>EDV, mL/m²</td>
<td>51±2</td>
<td>52±2</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>66±1</td>
<td>72±2*</td>
</tr>
<tr>
<td>LV mass, g/m²</td>
<td>99±3</td>
<td>162±6*</td>
</tr>
<tr>
<td>Volume/mass, mL/g</td>
<td>0.54±0.02</td>
<td>0.32±0.01*</td>
</tr>
<tr>
<td>Mitral E/A</td>
<td>0.95±0.04</td>
<td>0.91±0.05</td>
</tr>
<tr>
<td>Isovolumic relaxation time, ms</td>
<td>83±2</td>
<td>91±3*</td>
</tr>
<tr>
<td>E-wave deceleration time, ms</td>
<td>208±8</td>
<td>234±10*</td>
</tr>
<tr>
<td>Tissue doppler E', cm/s</td>
<td>10.1±0.4</td>
<td>7.4±0.4*</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>10±1</td>
<td>16±1*</td>
</tr>
<tr>
<td>MMP-2, ng/mL</td>
<td>1387±39</td>
<td>1205±44*</td>
</tr>
<tr>
<td>MMP-9, ng/mL</td>
<td>13±3</td>
<td>26±3*</td>
</tr>
<tr>
<td>TIMP-1, ng/mL</td>
<td>997±36</td>
<td>1291±70*</td>
</tr>
<tr>
<td>TIMP-2, ng/mL</td>
<td>44±4</td>
<td>58±7</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

*P<0.05 compared with reference controls.

Methods

Subjects
Two groups of subjects were recruited into this study: reference controls and patients with LV hypertrophy (LVH). Reference controls were identified from locally sponsored health fairs and volunteers from the Medical University of South Carolina staff. Of the reference controls screened, 35% were enrolled, 50% had 1 of the exclusion criteria listed next, and 15% declined participation. LVH patients were identified from echocardiographic studies. Of the patient echocardiograms screened, 10% were enrolled, 75% had 1 of the exclusion criteria listed below, and 15% declined participation.

There were some exclusion criteria common to both groups: (1) history of myocardial infarction; (2) regional wall-motion abnormality; (3) coronary revascularization surgery; (4) amyloidosis, sarcoidosis, HIV, hypertrophic obstructive cardiomyopathy, or valvular heart disease; (5) ejection fraction <50%; (6) malignancy; (7) significant renal or hepatic dysfunction; (8) rheumatological disease; or (9) blood pressure >140/90 mm Hg.

One hundred three subjects were enrolled in this study: 53 reference control subjects and 50 subjects with evidence of LVH (LV wall thickness >1.2 cm and/or LV mass index ≥125 gm/m²; Table 1). The reference control subjects were subdivided into 2 groups on the basis of the presence or absence of hypertension; 39 control subjects (referred to as “reference controls without hypertension”) had no history of hypertension, no evidence of cardiovascular disease, no symptoms or physical evidence of cardiovascular disease, no cardiovascular medication use, and all echocardiographic measurements within the normal range (Table 2); and 14 patients (referred to as “reference controls with hypertension”) had a history of arterial hypertension, controlled blood pressure (pharmacologically treated to meet JNC 7 criteria, ie, <140/90 mm Hg), no LVH, and all echocardiographic measurements within the normal range (Table 2). LVH patients were subdivided into 2 groups on the basis of the presence or absence of CHF. Twenty-three patients with hyperten-

TABLE 2. Reference Controls With and Without Hypertension and LVH Patients With and Without CHF

<table>
<thead>
<tr>
<th></th>
<th>Reference Control Without Hypertension (n=39)</th>
<th>Reference Control With Hypertension (n=14)</th>
<th>LVH Without CHF (n=23)</th>
<th>LVH With CHF (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>126±3</td>
<td>131±4</td>
<td>138±3*</td>
<td>133±4</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>74±2</td>
<td>77±2</td>
<td>82±2*</td>
<td>72±2$</td>
</tr>
<tr>
<td>EDV, mL</td>
<td>97±3</td>
<td>94±5</td>
<td>98±6</td>
<td>104±5</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>65±1</td>
<td>66±1</td>
<td>70±2*</td>
<td>73±2*</td>
</tr>
<tr>
<td>LV mass, g/m²</td>
<td>94±5</td>
<td>101±3</td>
<td>160±7*†</td>
<td>164±7†</td>
</tr>
<tr>
<td>Mitral E/A</td>
<td>0.98±0.05</td>
<td>0.85±0.05</td>
<td>0.80±0.09*</td>
<td>0.97±0.07†</td>
</tr>
<tr>
<td>Tissue Doppler E', cm/s</td>
<td>10.0±0.4</td>
<td>9.8±0.5</td>
<td>8.4±0.4†</td>
<td>7.2±0.5‡</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>10±1</td>
<td>11±1</td>
<td>13±2</td>
<td>17±2‡</td>
</tr>
<tr>
<td>PCWP/EDV, mm Hg/mL</td>
<td>0.09±0.01</td>
<td>0.11±0.01</td>
<td>0.12±0.01</td>
<td>0.17±0.01‡</td>
</tr>
<tr>
<td>Ea, mm Hg/mL</td>
<td>1.50±0.05</td>
<td>1.61±0.09</td>
<td>1.67±0.10*</td>
<td>1.45±0.11‡</td>
</tr>
<tr>
<td>MMP-2, ng/mL</td>
<td>1383±44</td>
<td>1399±84</td>
<td>1119±48*†</td>
<td>1286±73</td>
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<tr>
<td>MMP-9, ng/mL</td>
<td>13±4</td>
<td>14±5</td>
<td>27±3*†</td>
<td>24±4†</td>
</tr>
<tr>
<td>TIMP-1, ng/mL</td>
<td>1000±42</td>
<td>988±76</td>
<td>1092±77</td>
<td>1364±86‡†</td>
</tr>
<tr>
<td>TIMP-2, ng/mL</td>
<td>42±4</td>
<td>48±7</td>
<td>58±11</td>
<td>59±9</td>
</tr>
</tbody>
</table>

Ea indicates effective arterial elastance. Data are mean±SEM.

Significant differences among all 4 groups were analyzed by ANOVA and Tukey’s multiple comparison tests:

*P<0.05 vs reference controls without hypertension; †P<0.05 vs reference controls with hypertension; ‡P<0.05 vs LVH without CHF.
tion, controlled blood pressure, and LVH but no CHF were referred to as “LVH without CHF” (Table 2). The second subgroup consisted of 26 patients with hypertension, controlled blood pressure, LVH, and CHF and were referred to as “LVH with CHF.” All of these patients had evidence of CHF as defined according to Framingham criteria, evidence of abnormal relaxation (decreased E’), increased stiffness (increased pulmonary capillary wedge pressure [PCWP] and increased PCWP-end-diastolic volume [EDV] ratio), a markedly reduced 6-minute walk distance (979 ± 86 feet in the LVH group; 1839 ± 60 feet in the LVH+CHF group compared with 1839 ± 60 feet in the LVH—CHF group; P < 0.05), ejection fraction ≥50%, and therefore, had diastolic heart failure.

The medications used to treat hypertension were chosen and monitored by the patient’s primary physician and not the investigators. These included diuretics, renin-angiotensin-aldosterone antagonists (angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and aldosterone blockers), direct vasodilators (nitrates and hydralazine), α-adrenergic blockers, central nervous system blockers, aspirin, β-adrenergic receptor blockers, and calcium channel blockers. The mean duration of antihypertensive treatment was 6.4 ± 1.5 years.

**Echocardiographic Methods**

Echocardiography was performed with a Sonos 5500 system (Agilent Technologies, Andover, Mass) with a 4-MHz transducer. Measurements were made according to American Society of Echocardiography criteria. LV and left atrial volumes were calculated with the method of discs. LV mass was calculated according to the formula of Devereux et al. Doppler measurements of LV structure and function. A Mantel Haenszel technique was used to examine the relation between MMP and TIMP levels and plasma data were examined first by a univariate and then by a multivariate regression analysis. The structure, function, MMP, or TIMP data were analyzed by ANOVA and Tukey multiple-comparison tests. A value of P < 0.05 was considered significant. Simple linear regression was used to examine the relation between MMP and TIMP levels and measurements of LV structure and function. A Mantel Haenszel χ² and receiver operating curves were used to evaluate the association between MMP-13 and TIMP-1 levels and the presence of LVH and CHF. The potential effects of the medications on structure, function, or plasma data were examined first by a univariate and then by a multivariate regression analysis. The structure, function, MMP, or TIMP measurement was the dependent variable, with the medication entered as a dummy variable. A single drug was examined, and then drugs in combination were examined.

The research protocol used in this study was reviewed and approved by the institutional review board at the Medical University of South Carolina. Written, informed consent was obtained from all participants. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

**Reference Control Versus LVH**

**Structure/Function Data**

The reference control subjects had an age and sex distribution similar to the LVH subjects (Table 1). Compared with reference controls, LVH subjects had higher systolic blood pressures and lower diastolic pressures.

**TABLE 3. Plasma MMP/TIMP in Published Studies of Patients With Hypertension**

<table>
<thead>
<tr>
<th>Author (Reference No.)</th>
<th>MMP-2</th>
<th>MMP-9</th>
<th>TIMP-1</th>
<th>TIMP-2</th>
<th>MMP-13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present Study or First</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unreated hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yasmin (22)</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td></td>
<td>↔</td>
</tr>
<tr>
<td>Tayebjee (23-25)</td>
<td>↑</td>
<td>↓</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Li-Saw-Hee (29)</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Zervoudaki (30)</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Timms (28)</td>
<td>↑</td>
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<tr>
<td>Lindsay (27)</td>
<td>↑</td>
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<tr>
<td>Lavidi (26)</td>
<td>↑</td>
<td>↑</td>
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<td>↑</td>
</tr>
<tr>
<td>Treated hypertension</td>
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<td></td>
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<tr>
<td>Tayebjee (24, 25)</td>
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<tr>
<td>Li-Saw-Hee (29)</td>
<td>↔</td>
<td>↑</td>
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<tr>
<td>Zervoudaki (30)</td>
<td>↔</td>
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<tr>
<td>Lavidi (26)</td>
<td>↓</td>
<td>↑</td>
<td></td>
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</tr>
</tbody>
</table>

Present study (c/w PreTx (c/w Ctl) c/w PreTx (c/w Ctl) c/w PreTx (c/w Ctl))

Present study (c/w PreTx indicates compared with pretreatment value (ie, before institution of antihypertensive medications; c/w Ctl, compared with normal age- and sex-matched reference control values.

**MMP/TIMP Plasma Measurements**

Gelatinases (MMP-2 and -9), collagenase (MMP-13), and tissue inhibitors of MMPs (TIMP-1 and -2) were examined with the use of 2-site ELISA kits (Amersham Pharmacia Biotech, Buckinghamshire, UK). Plasma and the respective MMP standards were added to precoated wells containing the antibody to the MMP or TIMP of interest and washed. The resultant reaction was read at a wavelength of 450 nm (Labsystems Multiskan MCC/340, Helsinki, Finland). Because MMP-13 was found in very low levels in plasma, the MMP-13 results were categorized as either detectable or nondetectable.

**Statistical Analysis**

MMPs and TIMPs were measured every 2 hours for 6 hours to calculate a coefficient of variance for MMP/TIMP measurements between and within individual subjects in a subgroup of reference control subjects (n = 20) with a 1-way random-effects ANOVA. The coefficient was calculated as the square root of the within-person mean square error x 100. The intrapatient coefficient of variation for MMP-2 was 11.2 ± 1.1%; for TIMP-1, 8.5 ± 2.2%; and for TIMP-2, 14.3 ± 1.7%. An intra-assay coefficient of variation that quantified variation in the assay technique itself was < 6% for all MMPs and TIMPs.

Initially, comparisons between reference controls versus LVH subjects were made with a 2-tailed Student t test. Subsequently, comparisons between all 4 groups (reference control with versus without hypertension versus LVH with versus without CHF) were analyzed by ANOVA and Tukey multiple-comparison tests. A value of P < 0.05 was considered significant. Simple linear regression was used to examine the relation between MMP and TIMP levels and measurements of LV structure and function. A Mantel Haenszel χ² and receiver operating curves were used to evaluate the association between MMP-13 and TIMP-1 levels and the presence of LVH and CHF. The potential effects of the medications on structure, function, or plasma data were examined first by a univariate and then by a multivariate regression analysis. The structure, function, MMP, or TIMP measurement was the dependent variable, with the medication entered as a dummy variable. A single drug was examined, and then drugs in combination were examined.

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pressures, significant concentric remodeling as evidenced by a 60% greater LV mass index, no difference in EDV, and a 40% lower LV EDV–versus–mass ratio. Compared with reference controls, LVH subjects had significant abnormalities in the indices of LV diastolic relaxation and LV diastolic stiffness: increased isovolumic relaxation time, increased E-wave deceleration time, decreased E’, increased PCWP, and an increased PCWP–versus–LV EDV ratio (0.16±0.01 mm Hg/mL in LVH) compared with reference controls (0.09±0.01 mm Hg/mL, P<0.05), suggesting that there was an increase in the LV instantaneous end-diastolic operating stiffness.

MMP and TIMP Plasma Profiles

Compared with reference controls, MMP-2 was decreased and MMP-9 was increased in LVH subjects. Significant differences were found in MMP-13 detectability (Figure 1). Forty-seven percent of the reference control subjects had a detectable level of MMP-13, whereas MMP-13 was detectable in only 15% of the LVH subjects (χ²=17.89, P<0.001; odds ratio, 0.24). The plasma TIMP-1 value was significantly increased in LVH compared with reference control subjects. TIMP-2 and the MMP-9–TIMP-1 and MMP-2–TIMP-2 ratios were not different between reference control and LVH subjects.

Reference Controls Without Hypertension Versus Reference Controls With Hypertension

Structure/Function Data

Reference control subjects without hypertension served as the age- and sex-matched reference control group for comparison with the reference control with hypertension, the LVH without CHF, and the LVH with CHF groups. There were no significant differences in any demographic parameter or any echocardiographic measurement of LV structure or function between reference controls without hypertension versus reference controls with hypertension (Table 2). Left atrial maximum volume (LAMV) and emptying fraction (LAEF) were similar in reference controls without hypertension (LAMV, 40±2 mL and LAEF, 42±3%) compared with reference controls with hypertension (LAMV, 42±4 mL and LAEF, 43±2%).

MMP and TIMP Plasma Profiles

There were no significant differences in any MMP or TIMP plasma level between reference control subjects without hypertension versus reference controls with hypertension.

LVH Without CHF Versus LVH With CHF

Structure/Function Data

There were no significant differences in systolic blood pressure, LV volume, or mass between LVH without CHF and LVH with CHF subjects (Table 2). However, diastolic function was significantly more impaired in those with LVH with CHF compared with LVH without CHF. Indices of diastolic relaxation were slower, diastolic stiffness was greater, and filling pressures were higher in LVH with CHF compared with LVH without CHF. In particular, in the LVH without CHF patients, tissue Doppler E’ was decreased (8.4±0.4 cm/s with 95% confidence intervals [CIs] of 7.4 to 9.3) compared with reference controls without hypertension (10±0.4 cm/s; 95% CI, 9.3 to 11) and reference controls with hypertension (9.8±0.5 cm/s; 95% CI, 8.1 to 11). E’ fell further in LVH with CHF (7.2±0.5 cm/s; 95% CI, 6.2 to 8.3). In the LVH without CHF patients, PCWP was unchanged (13±2 mm Hg; 95% CI, 10.5 to 15.2) compared with reference controls without hypertension (10±1 mm Hg; 95% CI, 9.3 to 10.6) and reference controls with hypertension (11±1 mm Hg; 95% CI, 9.1 to 12.2) but was increased in LVH with CHF (17±2 mm Hg; 95% CI, 15.2 to 17.7). The PCWP–versus–LV EDV ratio was unchanged in the LVH without CHF patients but was significantly increased in the LVH with CHF patients. Effective arterial elastance was increased in LVH without CHF and was decreased in LVH with CHF groups. LAMV was increased in LVH without CHF subjects (LAMV, 53±4 mL; P<0.05, compared with reference controls) and increased further in LVH with CHF subjects (LAMV, 70±5 mL; P<0.05, compared with LVH without CHF). LAEF was unchanged in the LVH with CHF group (LAEF, 42±3%; P<0.05, compared with reference controls) but was increased in LVH with CHF (LAEF, 48±2% compared with LVH without CHF).

MMP and TIMP Plasma Profiles

There were no significant differences in the values of MMP-2, -9, -13; TIMP-2; or MMP-TIMP ratios in LVH without CHF compared with LVH with CHF (Figure 1). However, TIMP-1 was significantly increased in LVH with CHF (1364±86 ng/mL; 95% CI, 1185 to 1543) compared with LVH without CHF (1092±77 ng/mL; 95% CI, 933 to 1252). In fact, TIMP-1 was elevated only in subjects with CHF. TIMP-1 was unchanged in the LVH without CHF patients compared with reference controls without hypertension (1000±42 ng/mL; 95% CI, 915 to 1085) and reference controls with hypertension (988±76 ng/mL; 95% CI, 824 to 1152).
There was a significant relation between TIMP-1 and the extent of LV remodeling. As TIMP-1 increased, LV mass increased ($r = 0.56, P < 0.05$), and the volume-mass ratio fell ($r = -0.56, P = 0.005$; Figure 2A). There was a significant relation between TIMP-1 and TDI E’. Higher levels of TIMP-1 were associated with lower values of E’, indicating a slower LV diastolic relaxation rate. $r = -0.41, P < 0.05$. TDI indicates tissue Doppler imaging; E’, peak early diastolic velocity of mitral annulus.

Relationship Between MMP and TIMP Plasma Profiles and LV Structure and Function

There was a significant relation between TIMP-1 and the extent of LV remodeling. As TIMP-1 increased, LV mass increased ($r = 0.30, P = 0.005$), and the volume-mass ratio fell ($r = -0.56, P = 0.001$; Figure 2A). There was a significant relation between TIMP-1 and the extent of diastolic dysfunction. As TIMP-1 increased, the mitral E-A ratio decreased ($r = -0.22, P < 0.027$), E’ fell ($r = -0.62, P = 0.001$; Figure 2B), and PCWP increased ($r = 0.28, P = 0.013$). Finally, there was a significant relation between the extent of CHF and TIMP-1 levels. The mean value of TIMP-1 was higher in LVH subjects with CHF who were in New York Heart Association class III versus class II. Having a TIMP-1 level >1200 ng/mL was predictive of having LVH with CHF ($\chi^2 = 4.6, P = 0.03$; specificity, 88%; positive predictive value, 94%; odds ratio, 3.54; 95% CI, 1.08 to 11.50). The area under the receiver operator curve was 0.71.

There was no relation between the use of a specific medication and differences in LV structure, function, or plasma MMP/TIMP profiles between groups. Specifically, there were no differences in any MMP or TIMP level between patients grouped by any medication or combination of medications. Nonetheless, it is recognized that this study was not powered sufficiently to completely address the effects of drugs on LV structure, function, or plasma MMP/TIMP profiles. Therefore, these data and analyses must be interpreted with appropriate caution.

Discussion

There are 3 unique findings in this study: (1) Patients with hypertension but normal LV structure and function had a normal MMP/TIMP profile, (2) changes in MMP and TIMP profiles that favor decreased ECM degradation (decreased MMP-2 and -13 and increased TIMP-1) were associated with LVH and diastolic dysfunction, and (3) increased TIMP-1 predicted the presence of CHF.

Although pleiotropic in their substrates and actions, changes in myocardial MMPs and TIMPs have predictable effects on the ECM. For example, MMP-2 (a gelatinase) degrades basement membrane proteins, fibrillar collagen peptides, and newly synthesized collagen fibers. In the present study, MMP-2 was significantly decreased in patients with hypertensive LVH. MMP-9 (a gelatinase) has the same structural protein substrates as MMP-2 but has a much lower level of activity. However, MMP-9 has significant effects on important biologically active proteins and peptides, such as transforming growth factor-β, and other “profibrotic” proteins and pathways. Activation of profibrotic pathways by increased MMP-9 would be expected to increase ECM accumulation. Thus, the decreased MMP-2 and increased MMP-9 levels found in LVH patients in the present study may be one factor contributing to the observed structural and functional changes seen in hypertensive heart disease.

MMP-13 is a collagenease that is found at very low levels in plasma and is difficult to quantify accurately, even with a high-sensitivity assay. Therefore, in the present study, rather than reporting MMP-13 as a measured value, the results were dichotomized. Detectable MMP-13 in the plasma of patients with LVH was greatly reduced and was further reduced in patients with LVH and CHF. The reduction in this collagenolytic enzyme would be expected to cause reduced fibrillar collagen turnover, reduced degradation, and increased ECM accumulation.

MMP activity is regulated at several levels that include not only transcriptional regulation but also posttranslational modification, such as TIMP binding. The TIMPs bind to active MMPs in a 1:1 relationship, inhibit MMP enzymatic activity, and thereby form an important control point with respect to net ECM proteolytic activity. The present study showed that plasma levels of TIMP-1 increased in patients with LVH and CHF. As a result, the balance between MMPs and TIMPs was altered in favor of reduced ECM proteolytic activity, which would therefore facilitate ECM accumulation. There are known TIMPs, and the transcriptional regulation of these molecules is not homogeneous. Discordant levels of TIMPs have been observed both in animal models of heart failure and in patients with cardiomyopathic disease. In the present study, a robust increase in TIMP-1 was observed in LVH patients with CHF. In contrast, only a small increase in TIMP-2 was observed in LVH patients with or without CHF. These observations likely underscore the different functions and regulatory pathways for TIMPs in the LV.
remodeling process. A unique finding of the present study was that a specific type of TIMP, TIMP-1, was strongly associated with the development of CHF. In patients with LVH and CHF, it is not clear whether the increased TIMP-1 levels contributed to the development of CHF or were the result of its development. What is clear, however, is that increased TIMP-1 was uniquely present in patients with LVH and CHF, and a plasma TIMP-1 value >1200 ng/mL was predictive for CHF. Therefore, this plasma analyte should be considered in the development of diagnostic criteria for heart failure with a normal ejection fraction (diastolic heart failure) and for the design of novel therapeutic management strategies for diastolic heart failure. However, it is recognized that the partition value of TIMP-1 at 1200 ng/mL was chosen in a post hoc rather than a prospective fashion. Therefore, the validity of its predictive value must be interpreted with appropriate caution and confirmed in additional studies that use a large, prospective, serial study design.

The changes in MMP/TIMPs that occur in patients with hypertensive heart disease may effect growth regulation in both the extracellular and cardiomyocyte compartments, which together result in concentric LVH and increased collagen content. Collagen homeostasis is determined by the balance between synthesis, posttranslational modification, and degradation. In hypertensive heart disease, Diz et al10 and Lopez et al11,12 have shown that increased collagen content was associated with increased plasma markers of collagen synthesis, decreased collagen degradation, and decreased collagen turnover. Changes in the MMP/TIMP profiles found in the present study suggest potential mechanisms by which changes in synthesis, degradation, and turnover may take place.

Although there are many determinants of LV structural remodeling, blood pressure is one of the most important. However, data from the present study suggest that even after blood pressure has been adequately controlled, ongoing changes in MMPs and TIMPs may predict, probably determine, and are certainly associated with persistent concentric remodeling, LVH, and diastolic heart failure. Regression of LVH requires appropriate remodeling of the ECM, including degradation and turnover of ECM components (particularly the basement membrane proteins) and alterations in cardiomyocyte–matrix interactions. The present study has shown that patients with hypertensive LVH had persistent abnormalities in specific MMP (decreased MMP-2) and TIMP (increased TIMP-1) profiles, which would be expected to favor continued cardiomyocyte–basement membrane–matrix connections and not the ECM turnover necessary to accommodate LV mass regression. It seems likely, therefore, that the ongoing changes in MMPs and TIMPs seen in the present study contribute to the phenotypic and structural changes present in hypertensive heart disease.

Table 3 summarizes some of the previous clinical studies that have examined changes in plasma MMP and TIMP profiles in patients with hypertension.22–30 There does not appear to be any consistent pattern of changes in MMP/TIMPs across these studies. Some of this variability may be based on the methods used to make the plasma measurements. For example, some studies used ELISA methods, whereas other studies used zymography and reverse zymography to measure MMP/TIMP plasma levels. Zymography has been successfully used in tissue extracts, but there are some limitations to this approach, particularly for plasma samples. First, a number of binding proteins exist within the plasma, which covalently bind MMPs and make their extraction from plasma samples difficult. Second, plasma protein binding of MMPs and TIMPs will yield multiple bands on zymography, which make quantification and identification of specific MMP and TIMP types problematic. Third, the zymographic approach does not readily yield an absolute MMP or TIMP value but rather yields relative comparative values. In the present study, a validated and calibrated high-sensitivity ELISA provided quantitative assessment of specific MMPs and TIMPs. In addition to these technical considerations, MMP/TIMP levels may change, dependent on ambient blood pressure, the antihypertensive medications used to treat hypertension, the development of LVH, changes in LV afterload and preload, and the development of clinical heart failure.31,32 Because changes in each of these factors may alter MMPs and TIMPs, each was assessed in the present study. Most of the previous studies, however, did not specifically measure the changes in LV structure, LV function, LV load, or clinical heart failure status in their patients. As a consequence of these differences in experimental design, direct comparisons between the present and previous studies with regard to MMP and TIMP levels must be done with caution. Nevertheless, Table 3 attempts to place previous studies in context with the present study.

The present study used plasma levels of MMPs and TIMPs as surrogate markers to reflect changes in myocardial levels of these enzymes and peptides. However, there are several limitations to this approach that must be acknowledged. First, MMP activation and TIMP binding are compartmentalized processes that occur within the myocardial interstitium.13,14 Thus, plasma levels do not necessarily reflect the net ECM proteolytic activity that occurs within the myocardium. Fortunately, data from previous clinical and experimental studies suggest that the differences in plasma MMP and TIMP levels observed between reference controls and patients with hypertensive heart disease in the present study are likely to reflect differences at the myocardial level.33–35 A second limitation to plasma sampling of MMPs and TIMPs is that it is possible that the myocardium is not the only source of MMPs and TIMPs in LVH patients. Therefore, measurements of plasma MMP and TIMP levels represent the summation of MMPs and TIMPs released from both cardiac as well as noncardiac sources. However, the specific exclusion criteria in the present study helped eliminate significant changes in the major noncardiac sources of MMPs and TIMPs. Nevertheless, it must be recognized that patients with hypertension and LVH, with or without CHF, may have changes in other noncardiac tissues, such as the kidneys and vasculature, that may contribute to MMP and TIMP release into the plasma. We clearly recognize, however, that the findings of the present study that demonstrate differences in plasma MMP and TIMP levels between reference controls and LVH patients are associative findings. Whether these changes are
mechanistically related to myocardial ECM accumulation warrants further study.

Conclusions
A specific pattern of changes in the ECM proteolytic system was associated with each structural, functional, and/or clinical manifestation of hypertensive heart disease. Subjects with adequately controlled blood pressure with no structural or functional changes in the LV did not have any changes in the MMP/TIMP signature. However, patients with LVH despite adequate blood pressure control had decreased MMP-2 and -13 values. Increases in TIMP-1 were found in patients with LVH and CHF. In particular, the transition from hypertensive LVH to the development of CHF may be heralded by changes in MMPs and TIMPs, such as an increase in TIMP-1 >1200 ng/mL or the absence of MMP-13. However, the present study had a limited sample size, used a cross-sectional design, and did not perform serial studies over time. These limitations mandate that our observations be further tested and confirmed by guest on May 1, 2017 http://circ.ahajournals.org/ Downloaded from

Acknowledgments
This study was supported by a Heart Failure Society of America Fellowship Award (S.H. Ahmed) and grants from the Research Service of the Department of Veterans Affairs (M.R. Zile, F.G. Spinale) and the National Heart, Lung, and Blood Institute (PO1-HL-48788: M.R. Zile, F.G. Spinale; RO1-HL-59165: F.G. Spinale; MO1-RR-01070-251: M.R. Zile, F.G. Spinale).

Disclosures
None.

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**CLINICAL PERSPECTIVE**

Chronic arterial hypertension is a common cause of left ventricular (LV) concentric hypertrophy, decreased relaxation rate, and increased stiffness. The structural and functional changes caused by hypertension result from changes to both of the principle constituents of the myocardium, the cardiomyocyte and particularly the extracellular matrix (ECM). These LV structural and functional changes create the substrate necessary for the development of diastolic heart failure. However, what controls these changes in the ECM, whether blood pressure control alone can prevent or reverse these changes, and whether knowledge of the ECM control mechanisms would aid the diagnosis or treatment of hypertensive heart disease are unknown. The present study showed that changes in the pattern of specific ECM proteolytic proteins/peptides (matrix metalloproteinases [MMPs]) and their tissue inhibitors [TIMPs]) were associated with each structural, functional, and clinical manifestation of hypertensive heart disease. Subjects with adequately controlled blood pressure and no LV structural or functional changes did not have any changes in the MMP/TIMP signature. Therefore, treatment of hypertension can prevent changes in the ECM and its proteolytic system. However, patients with residual or resistant LV hypertrophy, despite adequate blood pressure control, had abnormal MMPs. The development of diastolic heart failure was heralded by an increase in TIMP-1 to >1200 ng/mL. These data suggest that regression of LV hypertrophy and prevention of diastolic heart failure are dependent on more than just changes in blood pressure alone, and the need to target and normalize changes in MMP/TIMPs may be warranted. Understanding this ECM-dependent pathophysiology may lead to improved diagnosis and treatment of patients with hypertensive heart disease.
Matrix Metalloproteinases/Tissue Inhibitors of Metalloproteinases: Relationship Between Changes in Proteolytic Determinants of Matrix Composition and Structural, Functional, and Clinical Manifestations of Hypertensive Heart Disease


_Circulation_. 2006;113:2089-2096; originally published online April 24, 2006;
doi: 10.1161/CIRCULATIONAHA.110.573865

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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