Hyperhomocysteinemia leads to pathological ventricular hypertrophy in normotensive rats

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Hyperhomocysteinemia (Hhe) leads to pathological ventricular hypertrophy in normotensive rats. Am J Physiol Heart Circ Physiol 285: H679–H686, 2003. First published May 1, 2003; 10.1152/ajpheart.00145.2003.—A recent report indicated that hyperhomocysteinemia (Hhe), in addition to its atherothrombotic effects, exacerbates the adverse cardiac remodeling seen in response to hypertension, a powerful stimulus for pathological ventricular hypertrophy. The present study was undertaken to determine whether Hhe has a direct effect on ventricular remodeling and function in the absence of other hypertrophic stimuli. Male Wistar-Kyoto rats were fed either an amino acid-defined control diet or an intermediate Hhe-inducing diet. After 10 wk of dietary treatment, rats were subjected to echocardiographic assessment of left ventricular (LV) dimensions and systolic function. Subsequently, blood was collected for plasma homocysteine measurements, and the rats were killed for histomorphometric and biochemical assessment of cardiac remodeling and for in vitro cardiac function studies. Significant LV hypertrophy was detected by echocardiographic measurements, and in vitro results showed hypertrophy with significantly increased myocyte size in the LV and right ventricle (RV). LV and RV remodeling was characterized by a disproportionate increase in perivascular and interstitial collagen, coronary arteriolar wall thickening, and myocardial mast cell infiltration. In vitro study of LV function demonstrated abnormal diastolic function secondary to decreased compliance because the rate of relaxation did not differ between groups. LV systolic function did not vary between groups in vitro. In summary, in the absence of other hypertrophic stimuli short-term intermediate Hhe caused pathological hypertrophy and remodeling of both ventricles with diastolic dysfunction of the LV. These results demonstrate that Hhe has direct adverse effects on cardiac structure and function, which may represent a novel pathological hypertrophy (19) that imposes an abnormal hemodynamic load on the ventricles, including pressure-overload conditions such as hypertension and volume-overload conditions such as regurgitant valvular disease and arteriovenous fistulas. This pathological hypertrophy is the result of adverse cardiac remodeling, which can be defined as a change in the relationship between the normal components of the myocardium. Specifically, it is characterized by a combination of myocyte hypertrophy and nonmyocyte cell proliferation with increased collagen deposition, resulting in increased fibrosis disproportionate to myocyte hypertrophy (42). Disproportionate accumulation of collagen can initially lead to increased stiffness of the myocardium and diastolic dysfunction, whereas severe myocardial fibrosis may result in impaired contractile function. Coronary arteriolar changes with medial thickening and perivascular fibrosis may accompany cardiac remodeling, especially in hypertension (28).

We recently reported (19) that Hhe exacerbates the adverse myocardial and coronary arteriolar remodel-
ing seen in hypertension, a powerful hemodynamic stimulus for cardiac hypertrophy. The results of that study showed that Hhe enhanced hypertension-induced myocardial fibrosis and worsened the associated diastolic dysfunction (19). In the present study, we examined the direct effects of Hhe on cardiac structure and function independent of any hemodynamic stimulus for remodeling. We report here the novel finding that short-term intermediate Hhe directly leads to pathological hypertrophy of both the LV and right ventricle (RV) with myocardial fibrosis and LV diastolic dysfunction. These findings suggest that Hhe may contribute to the high prevalence of heart failure secondary to diastolic dysfunction, independent of traditional risk factors such as hypertension.

MATERIALS AND METHODS

Animals. All procedures in this study were approved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences. Three-month-old male Wistar-Kyoto (WKY) rats (300–325 g) were purchased from Harlan Sprague Dawley (Indianapolis, IN) and maintained in our institutional Division of Laboratory Animal Medicine on a 12:12-h light-dark cycle with free access to chow and water. The animals were randomized into two groups after 7- to 10-day acclimatization to this facility. Animals in each group were then allowed free access to one of two purified amino acid diets (Harlan Teklad, Indianapolis, IN) for 10 wk: control or intermediate Hhe inducing (IH). The IH diet was designed by supplementing the amino acid-defined control diet with 9 g/kg homocysteine (the disulfide form of homocysteine) to produce intermediate (30–100 μmol/l) Hhe in the rats. These diets were described in our recent report (19).

Body weight, chow consumption, and fluid intake were monitored weekly. Echocardiographic measurements were done at the end of the 10-wk treatment period while animals were lightly anesthetized with a volatile agent. Arterial blood pressure was subsequently measured in anesthetized animals by carotid artery cannulation, followed by isolation of the hearts for in vitro studies. Blood was obtained for plasma homocysteine measurement at the end of the study.

Echocardiographic assessment. Echocardiographic measurements were done with a HP Sonos 5500 system with a S12 (5–12 MHz) probe. Parasternal short-axis views were obtained after carefully aligning the imaging plane to the short axis of the heart, and the images were transferred to a digital echocardiographic analysis system (ProSolve). The thickness of the interventricular septum and posterior LV free wall were measured at end diastole. Diastolic and systolic dimensions and areas of the LV were also measured in the short-axis view. Fractional area change (FAC), a measure of systolic function, was calculated as follows: FAC = (LV end-diastolic area – LV end-systolic area)/LV end-diastolic area.

Langendorff-perfused hearts. Hearts were isolated from rats in each of the treatment groups after 10 wk of dietary intervention and perfused via the aorta with an oxygenated Krebs-Henseleit solution (37°C) of the following composition (in mM): 118.0 NaCl, 27.1 NaHCO3, 3.7 KCl, 1.8 CaCl2, 1.2 MgCl2, 1.0 KH2PO4, and 11.1 glucose. The flow rate was set at 7.0 ml·g heart−1·min−1, a value similar to that observed when flow is examined at a constant pressure of 60 mmHg; coronary pressure was monitored continuously by a Statham pressure transducer. Both atria were removed, and the ventricles were paced electrically at 250 beats/min by platinum contact electrodes positioned on the interventricular septum. A fluid-filled balloon catheter (connected to a pressure transducer) was placed in the LV to measure intraventricular pressure, and the heart was enclosed in a humidified, temperature-controlled chamber. Contractile function was monitored by measuring peak pressure, maximum rate of contraction (+dP/dt max), and maximum rate of relaxation (−dP/ d t max) at various preload balloon volumes (10–80 μl, a range that elicited maximum contractility in all preparations). In addition to a polygraph recording, all data were digitized and analyzed with the use of acquisition and analysis software (CODAS; DataQ Instruments, Akron, OH). The response of coronary pressure to infusion of adenosine was also determined as a measure of coronary flow reserve.

Morphometric measurement of collagen volume fraction, coronary arteriolar remodeling, and cardiomyocyte size. Morphometric measurements were done as previously described (19). Five-micrometer sections of formalin-fixed, paraffin-embedded tissue were stained with picrosiris red, which is specific for fibrillar collagen (21). Digital images of stained sections were acquired with a digitizing camera (Hamamatsu, Bridgewater, NJ) attached to a Zeiss Axioskop microscope (Carl Zeiss, Thornwood, NY). The integrated image analysis software packages Axiovision 1 and KS300 V3.0 (Carl Zeiss) were used for morphometric analysis. Perivascular collagen was measured in 50- to 200-μm vessels (10–15 per LV per section; 5–8 per RV per section) and expressed relative to vessel luminal area. Interstitial collagen was analyzed in 30–40 microscopic fields per ventricle per section, and interstitial collagen volume fraction was calculated as the percentage of collagen-stained interstitial regions relative to total area. Arteriolar wall-to-lumen ratio was analyzed in 50- to 200-μm vessels (10–15 per LV per section; 5–8 per RV per section). Myocyte size was determined in hematoxylin and eosin-stained sections. The transverse diameter of ~100 myocytes per animal was measured.

Assessment of total collagen by Sirius red-fast green spectrophotometry. The relative content of total collagen was measured spectrophotometrically by a method adapted from Junqueira and coworkers (20). The collagen content determined by this method correlates well with fibrosis as determined by morphometry (21, 22). Paraffin-embedded tissue was cut in 15-μm sections, and three sections were deparaffinized in xylol, xylol-ethanol (1:1, vol/vol), ethanol-water (1:1, vol/vol), and water. The sections were then placed in small test tubes (10 × 75 mm) and covered with 0.2 ml of a saturated solution of picric acid in distilled water containing 0.1% fast green FCF and 0.1% Sirius red F3BA. The tubes were covered with aluminum foil and incubated at room temperature for 30 min in a rotary shaker. The fluid was carefully withdrawn, and the sections were repeatedly rinsed with distilled water until the rinse fluid was colorless. One milliliter of 0.1 N NaOH in absolute methanol (1:1, vol/vol) was then added, and each tube was gently mixed until all color was eluted from the specimen. The eluted color was read in a spectrophotometer at 540 and 605 nm (corresponding to the maximum absorbances of Sirius red and fast green, respectively). The corrected absorbance at 540 nm (corrected for the contribution of fast green to the absorbance at 540 nm) was then calculated by subtracting from the absorbance at 540 nm the value corresponding to 29.1% of the optical density at 605 nm. The absorbances at 540 and 605 nm were then divided by their respective color equivalents (2.08 and 38.4) to obtain the amount of collagen and noncollagenous protein in the specimen. An estimate of collagen content, expressed as a percentage of total protein, was thus obtained.
Estimation of mast cell number. Five-micrometer sections of each heart were stained with toluidine blue to identify mast cells (33). The total number of mast cells in each section was counted in a blinded fashion.

Statistical analysis. Data were evaluated by ANOVA with a Student-Newman-Keuls post hoc test or by t-test as appropriate with Sigmastat (SPSS, Chicago, IL). The criterion for significance was a P value <0.05. Data are reported as means ± SE.

RESULTS

Animal model of Hhe. Table 1 shows data obtained after 10 wk of treatment with control and IH diets. Plasma homocysteine levels in animals fed the control diet were in the normal range, whereas the IH diet increased plasma homocysteine into the intermediate range (30–100 μmol/l). Body weight and chow intake (data not shown) were similar in the two groups; however, the ventricular weight-to-body weight ratio was significantly greater in the IH group compared with controls. Diastolic and systolic blood pressure did not differ significantly between groups.

Echocardiographic data. After 10 wk of dietary intervention, LV structure and function were evaluated in vivo by echocardiographic analysis. Table 2 shows average values obtained from the two dietary groups. As indicated by values for interventricular septum and posterior wall thickness, intermediate Hhe was associated with a significant concentric hypertrophy of the LV. LV diastolic area did not differ significantly when compared between the two groups; however, FAC was greater in the IH group compared with controls.

Effects of Hhe on cardiac remodeling. Table 3 shows the effects of Hhe on the myocyte and nonmyocyte compartments of LV and RV myocardium. Mean myocyte diameter was significantly greater in the IH group in both LV and RV. Figure 1, A and B, shows representative hematoxylin and eosin-stained sections from control and IH LV, demonstrating the increase in myocyte size and the presence of an increased number of boxcar nuclei in the IH group. Perivascular collagen deposition was measured in picrosirius-stained sections and normalized to luminal area. As shown in Table 3, perivascular collagen was increased significantly in both ventricles of the IH group compared with controls. Figure 1, C and D, shows representative picrosirius-stained sections from the two groups that demonstrate the increased perivascular collagen deposition. Intersitial collagen (measured in 30–40 fields/slide) was also increased significantly in the IH group compared with the control group (Table 3). Figure 1, E and F, shows representative sections of LV from control and IH groups, demonstrating this increased interstitial fibrosis. We also measured the ratio of collagen to noncollagenous protein in 15-μm sections (including LV and RV) with the Sirius red dye elution method. Results of this assay showed a significant increase in collagen in the IH group (Table 3), thereby supporting data obtained by morphometric analysis. The coronary arteriolar wall-to-lumen ratio was also increased significantly in both ventricles in the IH group, as shown in Table 3 and in the representative LV sections (Fig. 1, C and D). We also examined the relationship of mast cells to Hhe-induced cardiac remodeling because of the purported association between mast cell infiltration and myocardial collagen accumulation (31). Figure 1, G and H, shows representative toluidine blue-stained LV sections from each group. There was a statistically significant increase in total mast cell number with intermediate Hhe (see Table 3). Interestingly, mast cells were seen primarily in the perivascular areas and in areas of fibrous tissue accumulation.

Effects of Hhe on basal LV function. Basal LV systolic and diastolic function was assessed with the isolated Langendorff-perfused heart preparation. The results of these experiments are shown in Table 3.

Table 1. Parameter values in WKY rats after 10-wk treatment with control or intermediate hyperhomocysteinemia-inducing diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>IH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>404 ± 11</td>
<td>383 ± 7</td>
</tr>
<tr>
<td>Ventricular weight, g</td>
<td>1.38 ± 0.04</td>
<td>1.52 ± 0.08</td>
</tr>
<tr>
<td>Ventricular weight to body weight, mg/g</td>
<td>3.36 ± 0.06</td>
<td>3.99 ± 0.28*</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>132 ± 8</td>
<td>126 ± 6</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>88 ± 6</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>Plasma homocysteine, μmol/l</td>
<td>6.0 ± 0.4</td>
<td>32.7 ± 1.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8/group. IH, intermediate hyperhomocysteinemia inducing; WKY, Wistar-Kyoto. *Significantly different from corresponding values in the control group.

Table 2. Effects of control or IH diet on echocardiographic parameters in normotensive WKY rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>IH</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV diastolic area, cm²</td>
<td>0.52 ± 0.03</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>Fractional area change, %</td>
<td>0.67 ± 0.02</td>
<td>0.76 ± 0.02*</td>
</tr>
<tr>
<td>Interventricular septum thickness, cm</td>
<td>0.17 ± 0.01</td>
<td>0.21 ± 0.01*</td>
</tr>
<tr>
<td>Posterior wall thickness, cm</td>
<td>0.16 ± 0.01</td>
<td>0.20 ± 0.01*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8/group. LV, left ventricular. *Significantly different from corresponding values in the control group.

Table 3. Effects of intermediate hyperhomocysteinemia on cardiac remodeling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>IH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cardiomyocyte diameter, μm</td>
<td>15.75 ± 0.94</td>
<td>21.56 ± 0.97*</td>
</tr>
<tr>
<td>LV</td>
<td>14.37 ± 1.72</td>
<td>21.19 ± 1.19*</td>
</tr>
<tr>
<td>Perivascular collagen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV</td>
<td>1.73 ± 0.27</td>
<td>3.39 ± 0.90*</td>
</tr>
<tr>
<td>RV</td>
<td>0.52 ± 0.08</td>
<td>1.25 ± 0.18*</td>
</tr>
<tr>
<td>Intersitial Collagen, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV</td>
<td>1.11 ± 0.06</td>
<td>1.74 ± 0.09*</td>
</tr>
<tr>
<td>RV</td>
<td>1.30 ± 0.07</td>
<td>1.82 ± 0.10*</td>
</tr>
<tr>
<td>Collagen to total protein, %</td>
<td>3.49 ± 0.26</td>
<td>4.53 ± 0.41*</td>
</tr>
<tr>
<td>Coronary arteriolar wall to lumen ratio</td>
<td>1.55 ± 0.24</td>
<td>2.83 ± 0.50*</td>
</tr>
<tr>
<td>LV</td>
<td>1.20 ± 0.12</td>
<td>1.76 ± 0.23*</td>
</tr>
<tr>
<td>RV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total mast cell number per section</td>
<td>11 ± 5</td>
<td>29.6 ± 3.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8/group. Perivascular collagen values are ratio of collagen to luminal area. RV, right ventricular. *Significantly different from corresponding control values.
volume in the LV balloon was increased in 10-μl increments (balloon volume when empty was 90 μl) while intraventricular pressure was monitored for diastolic pressure, peak systolic pressure, +dP/dt_max, and −dP/dt_max. Figure 2A shows the LV diastolic pressure-volume relationship. There was a statistically significant upward shift of the curve in the IH group, indicating diastolic dysfunction.

Effects of increasing balloon volume on developed pressure (peak systolic − diastolic pressure) in each of the two groups are shown in Fig. 2B; no significant differences were observed at any balloon volume. Maximum values for developed pressure were 81.3 ± 8.1 and 81.9 ± 3.7 mmHg in the control and IH groups, respectively. Figure 2, C and D, shows the response of +dP/dt_max and −dP/dt_max to increasing balloon volumes. As with developed pressure, there were no significant differences between the two groups, indicating that the myocardial contractile and relaxation responses were not altered by Hhe. Maximum observed values for +dP/dt_max were 2,704 ± 271 mmHg/s for control rats and 2,685 ± 123 mmHg/s for IH rats, whereas corresponding maximum values for −dP/dt_max were 1,477 ± 113 and 1,456 ± 48 mmHg/s.

Fig. 1. Representative left ventricular (LV) sections. Hematoxylin and eosin-stained sections of control (A) and intermediate hyperhomocysteinemia (Hhe)-inducing (IH) (B) diet groups show Hhe-induced cardiomyocyte hypertrophy with boxcar nuclei. Picrosirius-stained sections from control (C) and IH (D) groups show Hhe-induced perivascular collagen accumulation and arteriolar wall thickening. Similarly stained sections from the control (E) and IH (F) groups show an increase in interstitial collagen in the IH group compared with control. Toluidine blue-stained sections of LV from control (G) and IH (H) groups show an Hhe-associated increase in mast cells. Arrows indicate mast cells. Original magnification: A and B, ×200; C–F, ×100; G and H, ×400.
Coronary artery perfusion pressure was monitored at a constant flow rate of 7.0 ml·g heart⁻¹·min⁻¹, and the maximum adenosine-induced decline in coronary perfusion pressure (obtained at 10⁻⁶-10⁻⁵ M adenosine) was used as a marker of coronary flow reserve. There were no differences in baseline coronary perfusion pressure (control 59.8 ± 6.3 mmHg; IH 53.3 ± 4.6 mmHg) or in the adenosine-induced decline in coronary perfusion pressure (control 22.0 ± 4.5 mmHg; IH 21.4 ± 8.2 mmHg).

In summary, these results show that 10 wk of Hhe leads to a diastolic dysfunction in normotensive rats that is mediated primarily by increased fibrosis and myocardial stiffness; -dP/dt_max did not vary significantly. There were no significant changes in basal coronary perfusion pressure or its maximum response to adenosine. Similarly, basal cardiac systolic function, as measured by developed pressure and +dP/dt_max, was not affected by Hhe.

DISCUSSION

This study, which examined effects of Hhe on the structure and function of normal myocardium in the absence of other adverse stimuli, showed that intermediate Hhe leads to pathological hypertrophy of both cardiac ventricles with 1) a disproportionate increase in collagen relative to myocyte hypertrophy, 2) mast cell infiltration, 3) thickening of the coronary arteries, and 4) LV diastolic dysfunction. These results suggest that Hhe may contribute to cardiovascular morbidity and mortality via actions on the myocardium independent of other stimuli for remodeling.

The prevalence of elevated plasma homocysteine levels increases from an estimated 5% in the general population to 13–47% in patients with symptomatic vascular disease (26, 29, 30), and numerous clinical and epidemiologic studies have shown an association between Hhe and CVD, especially myocardial infarction and stroke (12, 16). In addition, a clinical study by Blacher and coworkers (2) reported a correlation between Hhe and altered cardiac structure. These authors measured LV mass and plasma homocysteine levels in 75 patients with end-stage renal disease undergoing hemodialysis. Multivariate analysis showed a positive correlation between echocardiographically measured LV mass index and plasma homocysteine, even after adjustment for age, sex, systolic blood pressure, and hematocrit. Results of the present study support this clinical report.

The normal myocardium is composed of cardiomyocytes and the nonmyocyte compartment (13, 18, 43), with type I and III fibrillar collagens forming a major structural component (18). Pathological conditions, such as hypertension and valvular disease, lead to myocyte hypertrophy and nonmyocyte cell proliferation with an increased concentration of collagen. This abnormal or pathological LVH, which is characterized by disproportionately increased fibrosis in the perivascular and interstitial regions, leads initially to deleterious effects on diastolic function (increased myocardial stiffness) and subsequently to depressed systolic function due to interference with coordinated myocyte contraction. Hence, the amount of collagen in the myocardium seems to be a major
determinant of cardiac dysfunction in various pathological states (42).

Several pathogenic mechanisms postulated for Hhe-induced vascular disease, including endothelial dysfunction and smooth muscle cell proliferation, may also contribute to its direct cardiac effects. These include oxidative stress (1, 23, 40, 41), activation of protein kinase C (6, 35), and altered collagen metabolism (24, 38–40). Thus, on the basis of these known pathogenic mechanisms, we postulated that Hhe may cause pathological cardiac hypertrophy by direct actions that elicit cardiomyocyte hypertrophy and myocardial fibrosis. Because the human heart is limited in its ability to metabolize homocysteine (4), it may be particularly susceptible to these proposed direct effects. Alternatively, adverse effects of Hhe on cardiac structure and function may result from indirect actions mediated via initial insult at other target sites such as the endothelium (11). Future studies are required to delineate both the site and cellular mechanisms underlying the adverse cardiac remodeling.

Results of our current experiments in the male WKY rat showed that intermediate Hhe is associated with a LVH that is detectable in vivo by echocardiography; increases in both posterior wall and septal thicknesses were observed. These findings were confirmed by ventricle-to-body weight ratios, which showed a significant increase in the IH group (18.75% increase) compared with controls. There was no significant difference in diastolic chamber dimensions, indicating that the hypertrophy was concentric rather than eccentric with cavity dilatation (14).

Histomorphometric studies showed that the pathological concentric hypertrophy observed in current experiments was characterized by an increase in myocyte diameter with a disproportionate increase in collagen relative to myocyte hypertrophy. Coronary arteriolar changes were characterized by significant increases in wall thickness and the amount of perivascular collagen. There were also significant increases in interstitial collagen and the ratio of collagen to noncollagenous protein in the IH group. All of these changes were observed in both LV and RV, suggesting that they may be mediated humorally or by direct effects of homocysteine on the heart. Nonetheless, observed changes in LV diastolic function could have imposed a hemodynamic stress on the RV that contributed to changes in that chamber. In contrast, observed alterations in the LV were not the result of changes in hemodynamic load because blood pressure and LV cavity dimension, a major determinant of wall stress and remodeling, did not vary between the groups. Interestingly, these Hhe-associated changes were similar in nature to the adverse remodeling seen initially in response to hypertension (28). Thus the current results indicate that Hhe is an independent stimulus of pathological LVH, which mimics the actions of known humoral mediators of adverse cardiac remodeling such as angiotensin II and aldosterone that initiate myocardial collagen deposition in the perivascular region followed by interstitial distribution (42). The interstitial collagen deposition could be replacement fibrosis after myocyte loss or an independent increase in production and/or decrease in degradation (42), and the hypertrophic response of cardiomyocytes may be a compensatory response to myocyte loss. Myocyte loss could also be the result of arteriolar changes and consequent hypoxia due to an increased diffusion distance (34). Long-term studies are addressing the issue of myocyte loss and potential systolic dysfunction with prolonged Hhe.

The pathological LVH induced by Hhe in this study led to a diastolic dysfunction that was characterized by an upward shift in the pressure-volume relationship. Diastolic dysfunction can result from either an increase in passive chamber stiffness or impaired myocardial relaxation (5). In our model, the dysfunction was likely the result of altered compliance due to observed structural changes, because there was no significant difference in the rate of relaxation (−dP/dtmax). In vitro measurements have been used extensively to estimate changes in diastolic function (3, 8, 10, 17), and in the absence of changes in chamber diameter (as demonstrated by echocardiographic analysis) and −dP/dtmax, an upward shift in the diastolic pressure-volume relationship is strongly suggestive of diminished compliance. Systolic function, as measured by FAC, was found to be increased in vivo, whereas in vitro measurements in the Langendorff preparation showed no change in systolic function as measured by peak developed pressure and +dP/dtmax. Ventricular hypertrophy can be associated with normal or supranormal systolic function (15), and these somewhat conflicting results suggest that the enhanced systolic function observed in vivo in our model was secondary to changes in neurohumoral control or hemodynamic load.

A potential mechanism for the cardiac remodeling elicited by Hhe is through recruitment of inflammatory cells, which may secrete mediators of myocyte hypertrophy, cell death, or fibrosis. Mast cells secrete several mediators of cardiomyocyte and fibroblast function and are thought to play a role in the remodeling of various tissues including the myocardium (31, 32). In our model of Hhe-induced cardiac remodeling, there was a significant increase in myocardial mast cell numbers, suggesting a potential causative role of mast cells. A previous study in our laboratories (19) showed that Hhe worsens the adverse cardiac and coronary arteriolar remodeling and diastolic dysfunction observed in male spontaneously hypertensive rats. However, these changes were most evident with severe Hhe. The present study demonstrates pathological ventricular hypertrophy in response to more clinically relevant intermediate Hhe in the absence of any other hypertrophic stimulus.

In conclusion, the results of the current study suggest a novel mechanism for the contribution of Hhe to cardiovascular morbidity and mortality. Hhe, which is reported to be present in up to 5% of the general population (29, 30), may lead to adverse remodeling of the myocardium, pathological LVH, and diastolic dysfunction. This potential mechanism for Hhe-associated
CVD may prove to be quite significant in light of the high prevalence of heart failure secondary to diastolic dysfunction (7).

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DISCLOSURES

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