Serial Echocardiographic Assessment of Left Ventricular Geometry and Function After Large Myocardial Infarction in the Rat

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Background The development of heart failure after acute myocardial infarction (MI) may be related to alterations of left ventricular (LV) structure and function. Pathological LV remodeling may exacerbate systolic and diastolic dysfunction because increased cavity dimensions tend to increase LV wall stress. Unfortunately, many complicating factors have made it difficult to clearly define the time course of LV remodeling after myocardial infarction in patients, and the contribution of structural changes to altered function has not been fully explored.

Methods and Results To determine the type, magnitude, and time course of changes in LV geometry and function, we performed transthoracic Doppler echocardiographic examinations in rats before and 1 and 6 weeks after transmural MI induced by coronary ligation. LV internal diastolic dimension was greater in infarcted than in sham-operated rats at 1 and 6 weeks after MI (9.4±0.6 versus 8.0±0.5 and 10.1±0.9 versus 8.5±0.9 mm, respectively; P<.05 compared with sham-operated rats). There was significant thinning of the infarcted anterior wall at 1 and 6 weeks (0.99±0.2 versus 1.33±0.19 and 0.96±0.22 versus 1.51±0.18 mm, P<.05), while the thickness of the noninfarcted posterior wall increased but was not different from normal growth in sham-operated rats. Six weeks after surgery, fractional shortening was impaired (11±5% versus 35±5%, P<.05), and systolic thickening of the noninfarcted posterior wall was depressed (38±9% versus 67±18%, P<.05) in infarcted rats compared with shams. These changes in structure and systolic function were accompanied by progressive alterations in LV diastolic filling. Peak early filling velocity increased at 1 and 6 weeks in MI rats (91±9 versus 79±9 and 100±14 versus 74±11 cm/s, P<.05), and the deceleration rate of the early filling wave was more rapid in rats with MI (21.6±5.0 versus 15.6±3.1 and 26.1±9.8 versus 11.2±2.7 m/s², P<.05). Late filling velocity was decreased (16±15 versus 33±7 and 15±18 versus 34±5 cm/s, P<.05), resulting in a marked increase in the ratio of early to late filling. The peak velocity and the velocity-time integral of LV outflow did not change after MI.

Conclusions Postinfarction LV remodeling in the rat is characterized by progressive cavity dilatation, inadequate hypertrophy of the surviving myocardium, the gradual development of regional contractile dysfunction in noninfarcted segments, and marked abnormalities of diastolic filling. These changes can be tracked longitudinally with transthoracic echocardiography.

Key Words • myocardial infarction • echocardiography • diastole • spectroscopy • hypertrophy

I nflammatory heart disease is the leading cause of congestive heart failure in most Western countries. Transmural myocardial infarction (MI) may initiate a cascade of progressive structural and geometric changes in the left ventricle that is commonly referred to as "pathological remodeling." The remodeling process is believed to serve initially as a compensatory mechanism to maintain cardiac output. However, the architectural changes that occur may contribute to the development of congestive symptoms by creating afterload mismatch and by exacerbating left ventricular (LV) diastolic dysfunction. Transthoracic Doppler echocardiographic (echo-Doppler) examination of the heart is an excellent noninvasive method for assessing LV structure and function after MI. However, defining the natural history of postinfarction heart failure with this technique is limited because most patients do not have a preinfarction study, many have preexisting cardiovascular disease, and most patients receive treatments in the immediate post-MI period that alter LV loading conditions. Therefore, the use of an appropriate animal model may allow a better understanding of the processes that lead to post-MI heart failure.

Previous studies have used two-dimensional echocardiography to characterize LV geometry in a canine model of MI. The utility of these investigations in helping to delineate the contribution of pathological LV remodeling to the development of heart failure has been limited by the difficulty in producing large transmural infarctions in the dog. The characteristics of the infarction are important because it is commonly appreciated that significant LV dilatation in humans and animals occurs almost exclusively after transmural myocardial necrosis. In addition, infarction of <20% of the left ventricle may result in minimal hemodynamic aberrations or ventricular dilatation, since hypertrophy of the surviving myocardium alone may be adequate to normalize LV wall stress. Previous investigators have demonstrated that ligation of the left coronary artery in the rat results in transmural anterior infarction that is followed by marked hemodynamic alterations and progressive increases in...
LV volume similar to those seen in patients with anterior MI.\textsuperscript{8-11,14} Unfortunately, longitudinal studies in the rat model have been limited because of the small size of the animals and the rapid heart rate. Recently, high-frequency ultrasound transducers capable of imaging small hearts have become available. In this study, we demonstrate the technical feasibility of using serial transthoracic echocardiography to follow postinfarction LV remodeling in the rat. We also demonstrate the rapid appearance of chamber dilatation, systolic dysfunction, and a "restrictive" LV diastolic filling pattern after MI.

**Methods**

**Production of Myocardial Infarction**

All studies described in this manuscript were performed according to the guidelines of the American Physiological Society. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Ind) weighing 220 to 250 g were anesthetized with methohexital (Eli Lilly and Co, Indianapolis, Ind) 60 mg/kg IP. Rats were intubated and ventilated with a volume-cycled small-animal ventilator. An anterior thoracotomy was performed, the heart was rapidly exteriorized, and a 6-0 silk suture was tightened around the proximal left anterior descending coronary artery. Positive end-expiratory pressure was applied to fully inflate the lungs, and the muscle layer and skin were closed separately. Sham-operated rats underwent identical surgery but did not sustain a myocardial infarction.

**Echocardiographic Studies**

**Validation study.** To date, few published articles have described the use of transthoracic echocardiography in rats to measure LV size.\textsuperscript{15} Therefore, we performed a preliminary validation study to ascertain the accuracy and reproducibility of the technique in normal rats before attempting to do studies

![Diagram showing echocardiograms](image)

**Fig. 2.** Examples of M-mode echocardiograms obtained with two-dimensional guidance from a short-axis midventricular view from a sham-operated rat and a rat with a 6-week-old anterior infarction. AW indicates anterior wall; PW, posterior wall; and MI, myocardial infarction. Note increased left ventricular cavity dimensions, akinesia and thinning of anterior wall, and hypokinesis of posterior wall in rat with MI. In addition, heart rate is comparable in the sham-operated and infarcted rats, as can be seen from the ECG.
on rats with MI. In this study, 10 adult rats (500 to 800 g) each underwent transthoracic echo-Doppler examinations on 2 different days, not more than 72 hours apart. Rats were anesthetized with ketamine HCl (Parke Davis, Morris Plains, NJ) 50 mg/kg and xylazine (Lloyd Laboratories, Shenandoah, Iowa) 10 mg/kg IP. The chest was shaved, and the rats were placed prone on a specially designed apparatus. Images were obtained by placing the transducer against the chest from below. Echocardiograms were performed with a commercially available echocardiographic system equipped with a 7.0- or 7.5-MHz phased-array transducer (Acuson, Mountain View, Calif, or Hewlett Packard, Andover, Mass).

Fig 3. Comparison of left ventricular (LV) mass calculated from M-mode echocardiography and postmortem LV mass in rats. The symbol * indicates mass calculations from the first validation study; △, mass calculations from the repeat study; and ◆, mass calculations from the sham-operated rats in the infarction study. LV mass was calculated from an anatomically corrected cube formula with American Society for Echocardiography dimensions (see "Methods").

A two-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscles (Fig 1A and 1B). In general, the best views were obtained with the transducer lightly applied to the mid upper left anterior chest wall. The transducer was then gently moved cephalad or caudal and angled until desirable images were obtained. After it was ensured that the image was on axis (based on roundness of the ventricular cavity), two-dimensional targeted M-mode tracings were recorded through the anterior and posterior LV walls at a paper speed of 100 mm/s (Fig 2). This orientation was chosen to allow delineation of wall thickness and motion in infarcted and noninfarcted territories. Anterior and posterior end-diastolic and end-systolic wall thicknesses and LV internal dimensions were measured by the American Society for Echocardiography (ASE) leading-edge method from at least three consecutive cardiac cycles on the M-mode tracings. These were analyzed with a commercially available off-line analysis system (Cardiac Workstation, Freeland Systems, Louisville, Colo) by a single observer (S.E.L.) blinded to prior results.

LV mass was calculated from a standard cube formula:

\[
\text{LV mass} = 1.04 \times [(\text{EDD} + \text{PWT} + \text{AWT})^3 - \text{EDD}^3]
\]

where 1.04 is the specific gravity of muscle, EDD is LV end-diastolic dimension, PWT is diastolic posterior wall thickness, and AWT is diastolic anterior wall thickness. A regression equation relating calculated LV mass to postmortem LV mass was developed. We found that multiplying the calculated mass by a constant similar to that used in the anatomically validated Devereux correction for ASE dimensions in humans allowed us to predict postmortem LV mass more accurately. The modified equation

\[
\text{LV mass} = (\text{cubeformula}) \times 0.8 + 0.14
\]

was then prospectively applied to subsequent echoes performed in rats without MI (Fig 3). LV mass was not calculated in rats with MI because of the marked alterations in LV symmetry and shape. LV outflow tract diameter was measured at the base of the aortic leaflets in a parasternal long-axis view.

Moving the transducer toward the cardiac apex (caudal) and angling anteriorly allowed the acquisition of an apical four-chamber view showing the mitral and tricuspid valves simultaneously. Pulsed-wave Doppler spectra of mitral inflow were recorded from the apical four-chamber view, with the sample volume placed near the tips of the mitral leaflets and adjusted to the position at which velocity was maximal and the flow pattern was laminar (Fig 4). Sample volume was set at the smallest size available. The left atrium was then interrogated with pulsed-wave Doppler for the presence of mitral regurgitation. LV outflow velocity was recorded from the apical five-chamber view achieved by further anterior angulation of the transducer. All Doppler spectra were recorded on paper at 100 mm/s and analyzed off-line as previously described. Numbers represent mean of at least three consecutive cardiac cycles. Cardiac output was calculated as

\[
\text{CO} = \text{Aortic VTI} \times \left( \frac{\pi (\text{LV outflow diameter}/2)^2}{2} \right) \times \text{heart rate}
\]

where VTI is velocity-time integral. An entire study typically required 5 to 15 minutes of imaging time.

Reproducibility. To determine interobserver variability, M-mode tracings from all 20 of the validation studies were photocopied and marked (left ventricular epicardial and endocardial surfaces at the beginning of the R wave and at peak posterior wall thickening) independently by two different observers (S.E.L. and P.S.D.), and a third set was marked by S.E.L. for intraobserver variability. All the marked tracings were analyzed as previously described by another observer in a blinded fashion.

Postinfarction study. Transthoracic echo-Doppler studies were performed 1 or 2 days before and 1 and 6 weeks after MI (n = 11) or sham surgery (n = 16). All studies were analyzed as
described for the validation study. In addition, infarct size was estimated from the 6-week short-axis echoes by observing the akinetic region in real time and measuring the percentage of the LV endocardial circumference that was akinetic on a freeze-frame image at end diastole. In 8 of 11 rats with large MI, no A waves could be seen above the filter cutoff on the mitral inflow Doppler spectra. In these cases, peak A-wave velocity was considered to be 5 cm/s (the lowest limit of the high-pass wall filter cutoff on the Doppler spectral tracing).

### Hemodynamic Studies

One or 2 days after the final echocardiogram, rats were lightly anesthetized with ether. The right carotid artery was isolated by cut-down, and a 1-mm micromanometer-tipped catheter (Millar Instruments, Houston, Tex) was passed retrogradely into the left ventricle under constant pressure monitoring. The analog signal was passed through an electronic differentiator to record dP/dt.

### Pathological Studies

After completion of hemodynamic measurements, rats were deeply anesthetized with ether and killed by rapid excision of the heart. The atria were trimmed from the ventricles, and the right ventricle and left ventricle plus septum were separated and weighed. The tissues were then immersion fixed in 10% buffered formalin. Each heart was cut in cross section at four levels from apex to base and prepared for routine histology. Thin sections from each level were stained with Masson's trichrome. The section corresponding to the short-axis echo (mid ventricle, papillary muscle level) was projected, and infarct size was estimated by measuring the percentage of the total endocardial circumference replaced by scar tissue.

### Statistical Analysis

All values are shown as mean±SD except where specified. Assessment of agreement between the two examinations in the validation study was performed according to the method of Bland and Altman. Briefly, a coefficient of repeatability was calculated as

\[
2 \times (SD \text{ of the differences between studies 1 and 2})
\]

If the studies are reasonably reproducible, the mean difference should be zero, and the bounds defined by 2 SD (coefficient of repeatability), which can be expected to contain 95% of the differences, should be small enough that repeat studies can be considered accurate enough to be useful. In the postinfarction study, comparisons were done with a two-factor ANOVA with one grouping factor (sham versus infarct) and a repeated-measures factor (time). Where appropriate, comparisons to determine the significance of changes within the same group over time and between groups at each time interval were performed with Scheffe’s test for multiple comparisons. A probability of \( P < .05 \) was considered to be significant.

## Results

### Validation Study

Measurements of LV size from the two consecutive studies in normal rats are shown in Table 1. There were no significant differences in any parameter between the two studies. The agreement between the studies is shown by the coefficient of repeatability. There was a significant correlation between calculated LV mass and postmortem LV mass in an additional group of rats (\( r = .78, P < .0001, \text{SEE} = 0.12 \) g) (Fig 3), confirming the accuracy of the measurements. Measurements of interobserver and intraobserver variability are shown in Table 2. LV diastolic dimension and posterior wall thickening measured on sequential studies are shown for individual rats in Fig 5. Measurements of mitral inflow and LV outflow velocities are shown in Table 3. Calculated cardiac outputs were similar to those reported with other techniques.

### Postinfarction Study

Body weights and postmortem cardiac chamber weights for the two groups of rats are shown in Table 4. Rats with MI gained less weight than the sham-operated rats. LV weight and LV/body weight were not different in sham-operated and MI rats; however, there was prominent right ventricular hypertrophy in the MI rats as indicated by an increase in right ventricular weight and right ventricular/body weight.
Hemodynamic parameters measured under ether anesthesia for the two groups of rats are shown in Table 5. Heart rate was comparable in the two groups. Rats with MI had significant systolic dysfunction, as evidenced by decreased LV systolic pressure, mean arterial pressure, and peak rate of LV pressure rise. Rats with MI also had severe diastolic dysfunction, as defined by a decrease in peak rate of LV pressure fall and a marked elevation of LV end-diastolic pressure.

Examples of freeze-frame two-dimensional echocardiographic short-axis images at end systole and end diastole from a sham-operated rat and a rat with a large MI are shown in Fig 1. M-mode images of the left ventricle from a sham-operated rat and a rat with a large anterior infarction are shown in Fig 2. Echocardiographic assessments of LV geometry and function for both groups of rats at each of the three time points are shown in Table 6. Compared with sham-operated rats, rats with large MI exhibited progressive LV remodeling. Structural changes observed included thinning of the infarcted anterior wall and increased LV systolic and diastolic dimensions at 1 week, with additional changes by 6 weeks (Fig 6A). There was an increase in thickness of the noninfarcted posterior wall with time; however, posterior wall thickness also increased in the sham-operated rats over the course of the 6-week study period because of normal growth. The wall-thinning ratio (anterior wall thickness/posterior wall thickness) progressively decreased in rats with MI (Fig 6B). Relative wall thickness (2×posterior wall thickness/LV internal dimension) showed a strong trend toward being decreased (P=.06) in the MI rats at 6 weeks (Fig 6C). Thus, the increase in LV cavity size appears to be disproportionate to the increase in thickness of the surviving myocardium, suggesting inadequate hypertrophy and continued afterload mismatch.

Functional abnormalities accompanied the architectural remodeling of the left ventricle (Table 6). Systolic thickening of the anterior wall was severely depressed immediately after MI and remained so at 6 weeks (Fig 7A). Function of the noninfarcted posterior wall showed more gradual deterioration, with a significant decrease in systolic thickening compared with sham by 6 weeks after MI (Fig 7B). The combination of the impaired regional function and LV cavity enlargement resulted in a dramatic decrease in fractional shortening (Fig 7C).

Examples of pulsed-wave Doppler recordings of mitral inflow from a sham-operated rat and a rat with an anterior myocardial infarction are shown in Fig 4. Doppler-derived indexes of systolic and diastolic function are shown in Table 7. MI caused profound and progressive alterations of LV diastolic filling characterized by increased early filling velocity (E), rapid deceleration of the early filling wave (E-F slope), and decreased atrial (A) filling velocity (Fig 8). Mitral regurgitation was observed in 3 of 11 rats with MI at 6 weeks. In all 3 rats, pulsed Doppler interrogation of the left atrium showed that the regurgitant flow was detected only in the region immediately behind the valve, suggesting a mild degree of regurgitation. Only 1 of 16 sham-operated rats had detectable mitral regurgitation.

### Table 3. Doppler Measurements of Mitral Inflow and Aortic Outflow Recorded on Sequential Transthoracic Echocardiographic Examinations in Rats (n=10) Performed 48 Hours Apart

<table>
<thead>
<tr>
<th></th>
<th>E Velocity, Velocity, Velocity, cm/s</th>
<th>LVOT VTI, cm/s</th>
<th>LVOT Output, mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>72±16 38±10 90±9</td>
<td>0.054±0.008 150±37</td>
<td></td>
</tr>
<tr>
<td>Study 2</td>
<td>79±12 38±9 92±18</td>
<td>0.059±0.011 171±71</td>
<td></td>
</tr>
</tbody>
</table>

All values are mean±SD. E indicates early filling; A, atrial filling; LVOT, left ventricular outflow tract; and VTI, velocity-time integral. Cardiac output calculated from the product of aortic velocity-time integral, aortic root cross-sectional area, and heart rate. Coefficient of repeatability calculated as described in "Methods" section.

### Table 4. Body and Postmortem Ventricular Wall Weights in Sham-Operated and Infarcted Rats

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Infarcted</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>420±14</td>
<td>394±15</td>
</tr>
<tr>
<td>RV, mg</td>
<td>240±6</td>
<td>425±37*</td>
</tr>
<tr>
<td>LV, mg</td>
<td>856±26</td>
<td>843±25</td>
</tr>
<tr>
<td>RV/BW, mg/g</td>
<td>0.58±0.02</td>
<td>1.08±0.10*</td>
</tr>
<tr>
<td>LV/BW, mg/g</td>
<td>2.06±0.0</td>
<td>2.14±0.04</td>
</tr>
</tbody>
</table>

All results are mean±SEM. BW indicates body weight; RV, right ventricular mass; and LV, left ventricular mass. *P<.05 compared with sham.
TABLE 5. Hemodynamic Measurements and Infarct Sizes in Sham-Operated Rats and Rats With Myocardial Infarction 6 Weeks Previously

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=16)</th>
<th>MI (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR, bpm</strong></td>
<td>347±15</td>
<td>356±8</td>
</tr>
<tr>
<td><strong>MAP, mm Hg</strong></td>
<td>111±4</td>
<td>98±3*</td>
</tr>
<tr>
<td><strong>LVSP, mm Hg</strong></td>
<td>128±3</td>
<td>111±2*</td>
</tr>
<tr>
<td><strong>LVEDP, mm Hg</strong></td>
<td>6±1</td>
<td>27±3*</td>
</tr>
<tr>
<td>+dP/dt, mm Hg/s</td>
<td>638±95</td>
<td>383±156*</td>
</tr>
<tr>
<td>−dP/dt, mm Hg/s</td>
<td>4683±149</td>
<td>3010±145*</td>
</tr>
<tr>
<td>Infarct size, echo, %</td>
<td>. . .</td>
<td>50±2</td>
</tr>
<tr>
<td>Infarct size, histological, %</td>
<td>. . .</td>
<td>51±2</td>
</tr>
</tbody>
</table>

Results are mean±SEM. MI indicates myocardial infarction; HR, heart rate; bpm, beats per minute; MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt, peak rate of left ventricular pressure rise (+) or fall (−); and infarct size, % LV endocardial circumference.

*P<.05 compared with sham.

regurgitation, which was similarly very mild in severity. Peak LV outflow velocity and the velocity-time integral were not different among the sham and MI rats (Table 7).

Discussion

Transmural myocardial necrosis, particularly of the anterior wall of the heart, may lead to progressive LV remodeling and eventual heart failure.5,22 Because of many complicating factors in patients with coronary artery disease (eg, coexisting cardiovascular disease, interruption of the ischemic process by reperfusion or revascularization, and use of load-modifying pharmacological treatments in the peri-infarct period), it has been difficult to clearly delineate the sequence and time course of changes in LV geometry and function after MI. In this study, we show the technical feasibility of using serial transthoracic echocardiography to characterize the remodeling process in a small-animal model of post-MI heart failure. In this model, significant LV dilatation occurs in the first week after MI and progresses over the subsequent 6-week period. There is a progressive reduction in systolic thickening of myocardium remote from the infarcted territory. In addition, the development of a “restrictive” LV filling pattern, as measured by pulsed-wave Doppler analysis of mitral inflow, occurs within 1 week after MI but substantially worsens by week 6.

Postinfarction LV Remodeling

This is the first study to longitudinally document the sequence of changes in LV geometry in vivo in rats with transmural anterior MI. Our findings complement previous ex vivo studies of infarcted rat hearts.8,11-14 We found a significant increase in LV end-diastolic dimension by 1 week after infarction (Fig 6). Such early changes in LV size have not always been observed in patients. The very early cavity dilatation may reflect a more rapid evolution of necrosis and healing in the rat compared with humans.23 However, since baseline studies of LV structure in patients are frequently not obtained until days to weeks after the infarction, it is possible that a significant amount of early remodeling may not have been appreciated in previous human studies. Some studies have shown decreases in LV volume and “infarct regression” over weeks to months after infarction in subgroups of patients.5 This phenomenon was observed exclusively in patients with inferior infarctions. In contrast, no rats with MI showed a decrease in LV dimension between baseline and 1 week after infarction, and only 2 of 11 rats showed a small decrease in LV diastolic dimension between 1 and 6 weeks after MI. The inexorable increase in LV cavity size may be attributed to the fact that all rats had transmural necrosis of a large territory in the anterolateral wall without reperfusion or salvage of infarct border zones. Furthermore, previous findings in patients may be due to revascularization, pharmacological unloading, or res-

TABLE 6. Left Ventricular Size and Function in Rats Assessed With M-Mode Echocardiography Before and 1 and 6 Weeks After Sham Surgery or Ligation of the Left Coronary Artery

<table>
<thead>
<tr>
<th></th>
<th>Anterior Wall Thickness Diastole, mm</th>
<th>Anterior Wall Thickness Systole, mm</th>
<th>Anterior Wall Thickening, %</th>
<th>Posterior Wall Thickness Diastole, mm</th>
<th>Posterior Wall Thickness Systole, mm</th>
<th>Posterior Wall Thickening, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham pre</td>
<td>1.26±0.22</td>
<td>2.20±0.42</td>
<td>76±28</td>
<td>1.31±0.23</td>
<td>2.28±0.28</td>
<td>77±12</td>
</tr>
<tr>
<td>MI pre</td>
<td>1.14±0.05</td>
<td>2.20±0.27</td>
<td>94±28</td>
<td>1.32±0.15</td>
<td>2.22±0.45</td>
<td>70±9</td>
</tr>
<tr>
<td>Sham 1 week</td>
<td>1.33±0.19</td>
<td>2.09±0.43</td>
<td>60±41</td>
<td>1.34±0.22</td>
<td>2.26±0.26</td>
<td>72±10</td>
</tr>
<tr>
<td>MI 1 week</td>
<td>0.99±0.20*</td>
<td>1.07±0.22*</td>
<td>9±15†</td>
<td>1.45±0.29</td>
<td>2.19±0.32</td>
<td>40±9†</td>
</tr>
<tr>
<td>Sham 6 weeks</td>
<td>1.51±0.18</td>
<td>2.55±0.25†</td>
<td>70±21</td>
<td>1.53±0.25†</td>
<td>2.52±0.32</td>
<td>67±18</td>
</tr>
<tr>
<td>MI 6 weeks</td>
<td>0.96±0.22*</td>
<td>1.07±0.33**</td>
<td>13±30†</td>
<td>1.68±0.32</td>
<td>2.31±0.42</td>
<td>38±9†</td>
</tr>
</tbody>
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ANOVA

<table>
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<td>.1824</td>
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</tr>
<tr>
<td></td>
<td>.0062</td>
<td>.0544</td>
<td>.312</td>
</tr>
</tbody>
</table>

All results are mean±SD. AW indicates anterior wall; PW, posterior wall; LV, left ventricular; pre, before procedure; MI, ligation of left coronary artery (myocardial infarction); and ANOVA, P values by two-factor repeated-measures ANOVA (see "Methods"). Relative wall thickness was defined as 2×PW/LV inner diameter.

*P<.05 compared with sham; †P<.05 compared with preoperative baseline; ‡P<.05 compared with 1 week sham; §P<.05 compared with 1 week MI.
solution of ischemia in noninfarcted regions. The suggestion of very early architectural changes in the rat ventricle after MI may have ramifications with regard to the most appropriate time to initiate afterload-reducing therapy or to attempt opening of an occluded artery.

Another interesting aspect of the present work was the finding of a strong trend toward a decrease in relative thickness of the noninfarcted myocardium compared with LV diameter. This trend occurred despite previously documented increases in cross-sectional area of myocytes from noninfarcted territories in this model\(^{10,13,24}\) and an observed increase in posterior wall thickness. The decrease in relative wall thickness in rats with MI implies that the limits of compensation have been reached and there is "inadequate hypertrophy" of the surviving myocardium. This in turn contributes to continued afterload mismatch and provides an extrinsic mechanism for impairment of systolic function in noninfarcted myocardium (see next paragraph).\(^{13,20,21}\)

### Systolic Function

Postinfarction heart failure is certainly related in part to the loss of contractile tissue. In addition, ongoing afterload mismatch would be expected to impair shortening of surviving myocardium.\(^{20,21}\) Our data are compatible with this hypothesis; however, we were not able to assess any load-independent measures of contractility. Previous in vitro work from our laboratory suggests that the gradual development of depressed intrinsic contractile function in regions distant from the infarction may also contribute to global systolic dysfunction.\(^{25}\)

In that study, maximal force development in isolated noninfarcted papillary muscles from rats with postinfarction heart failure was depressed even though levels of free ionized calcium in the cytoplasm of myocytes were not different between tissues from sham-operated and infarcted rats.

### Diastolic Function

Many aspects of diastolic function have been inferred from the pattern of transmitral flow seen on pulsed-wave Doppler. Although the significance of abnormal mitral inflow patterns in different disease states has been controversial and many variables may influence mitral filling, recent work strongly supports the notion that mitral inflow patterns reflect certain hemodynamic alterations that may evolve over time in various cardiac
conditions.26 The "restrictive" filling pattern characterized by increased peak E velocity, decreased peak A velocity (or absent A wave), and rapid E-wave deceleration seen in our rats is similar to the transmural flow profiles observed in patients with advanced cardiac amyloidosis and patients with end-stage heart failure of various causes who were being evaluated for cardiac transplantation.27,28 This mitral inflow pattern was not caused by heart rate–related “fusion” of the E and A waves, since heart rates were not different in the two groups of rats. Furthermore, all rats appeared to be in sinus rhythm, with easily discernible p waves on the ECGs.

Increased peak E wave velocity probably results from increased left atrial pressure, which accentuates the early left atrial–LV pressure gradient and causes the mitral valve to open earlier and at a higher crossover pressure.26,29 The rapid E wave deceleration appears to be most strongly associated with an increase in operating chamber stiffness.29 Although we were not able to directly measure left atrial pressure in our rats, we did find a marked increase in LV end-diastolic pressure in all the rats with infarctions. In addition, we have previously examined the passive-elastic properties of the infarcted left ventricle ex vivo and found significant increases in operating LV chamber stiffness.13 Operating chamber stiffness, or the slope of the LV pressure-volume relation, reflects the mechanical properties of both the infarcted and noninfarcted regions of the ventricle, as well as the distending pressure. We also have previously demonstrated increased stiffness of the surviving, noninfarcted myocardium that may be due to accumulation of interstitial collagen.24

The progressive increases in the E/A ratio and the rate of E-wave deceleration parallel the gradual increases in LV dimensions and the slow deterioration of contractile performance in the noninfarcted myocardium. Although our data do not allow firm conclusions

**TABLE 7.** Pulsed-Wave Doppler Measurements of Mitral Inflow and Aortic Outflow in Rats Before and 1 and 6 Weeks After Sham Surgery or Myocardial Infarction

<table>
<thead>
<tr>
<th></th>
<th>E Velocity, cm/s</th>
<th>A Velocity, cm/s</th>
<th>E/A</th>
<th>E Deceleration, m/s²</th>
<th>LVOT Velocity, cm/s</th>
<th>LVOT VTI, cm</th>
<th>Heart Rate, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham pre</td>
<td>77±12</td>
<td>34±7</td>
<td>2.4±0.9</td>
<td>12.4±3.6</td>
<td>95±11</td>
<td>0.056±0.008</td>
<td>253±30</td>
</tr>
<tr>
<td>MI pre</td>
<td>79±13</td>
<td>36±6</td>
<td>2.3±0.5</td>
<td>13.9±3.2</td>
<td>106±7</td>
<td>0.064±0.005</td>
<td>235±15</td>
</tr>
<tr>
<td>Sham 1 week</td>
<td>79±9</td>
<td>33±7</td>
<td>2.5±0.60</td>
<td>15.6±3.1</td>
<td>86±8</td>
<td>0.049±0.007</td>
<td>254±20</td>
</tr>
<tr>
<td>MI 1 week</td>
<td>91±9*</td>
<td>16±15*</td>
<td>10.7±7.5*</td>
<td>21.6±5.0*</td>
<td>86±16</td>
<td>0.050±0.011</td>
<td>267±20</td>
</tr>
<tr>
<td>Sham 6 weeks</td>
<td>74±11</td>
<td>34±5</td>
<td>2.2±0.5</td>
<td>11.2±2.7†</td>
<td>95±14</td>
<td>0.055±0.009</td>
<td>236±26</td>
</tr>
<tr>
<td>MI 6 weeks</td>
<td>100±14*</td>
<td>15±18*</td>
<td>15.6±8.6*</td>
<td>26.1±9.8*†</td>
<td>92±27</td>
<td>0.050±0.013</td>
<td>245±18</td>
</tr>
</tbody>
</table>

**ANOVA**

*Group* .0001 .0002 .0001 .0001 .5423 .567 .2722
*Time* .0774 .0066 .0002 .0026 .0256 .0058 .0257
*Interaction* .0077 .0145 .0002 .0003 .374 .0976 .7404

All results are mean±SD. E indicates early filling; A, atrial filling; LVOT, left ventricular outflow tract; VTI, velocity-time integral; bpm, beats per minute; pre, before procedure; MI, ligation of left coronary artery (myocardial infarction); and ANOVA, P values by two-factor repeated-measures ANOVA (see "Methods"). *P<.05 compared with sham at same point in time; †P<.05 compared with preoperative baseline; ‡P<.05 compared with 1 week sham.
regarding the precise cause of the diastolic abnormalities, the restrictive LV filling pattern characteristic of end-stage heart failure was observed in our rats at a time when there are known elevations of LV filling pressures and increased operating LV chamber stiffness. These results are consistent with previous studies showing that the atrial contribution to cardiac output is diminished when LV filling pressures are elevated.30,31 Furthermore, we did not find evidence in any rats of significant mitral regurgitation that might cause an increased early filling velocity. Thus, our data are very compatible with the leading contemporary theories about the genesis of different mitral inflow profiles.26,29 To the best of our knowledge, this is the first study to report mitral inflow patterns in rats.

Technical Considerations

The high-frequency neonatal transducers used in this study allowed us to obtain good-quality transthoracic images of the beating rat heart. The resolution of this system is calculated to be approximately 0.2 mm. With a small sector size and shallow imaging depth, sampling rates for two-dimensional imaging averaged 40 to 60 Hz. Given a heart rate of 250 to 300 beats per minute in the anesthetized rats, 8 to 15 frames would be recorded per cardiac cycle. At the lower end of this spectrum, it would be difficult to precisely identify end systole and end diastole. Therefore, calculations of LV size and function based on the clear identification of end-systolic or end-diastolic frames on two-dimensional echocardiograms are difficult. Because of this, we chose to assess LV size and function from the M-mode echocardiograms, which have a much higher sampling rate.

The accuracy of the M-mode measurements is supported by the similarity to recently reported ex vivo morphometric measurements in sham-operated and infarcted rat hearts.32 In addition, our findings show excellent interobserver and intraobserver reproducibility (Table 2). However, there was more variability in sequential studies of individual rats. This variability probably results from differences in transfuser placement and/or alignment. Nonetheless, the magnitude of change in certain parameters (eg, LV diastolic dimension) after large MI was substantially greater than the variability associated with the technique. Post hoc power calculations suggest that 10 to 12 rats in each group would need to be studied to have ≥0.8 chance of detecting a 1.0-mm increase in LV dimension (assuming α=0.05). Thus, this technique appears to be adequate to assess changes in LV structure and function with reasonable sample sizes.

Unfortunately, it is difficult to quantify global systolic function from M-mode recordings, especially in a segmentally infarcted ventricle. Another factor limiting volumetric assessments of global systolic performance was the inability to visualize the true cardiac apex and thereby obtain an accurate long-axis dimension or to consistently obtain short-axis views of the left ventricle at multiple levels. Both of these are necessary to calculate volumes with Simpson's rule (the most accurate way to calculate volume in an asymmetric chamber). Hence, our assessment of LV systolic performance could reflect only regional function. However, evaluation of regional function of the surviving myocardium is significant. The gradual development of contractile dysfunction in nonischemic regions has not been widely appreciated in the past.

Conclusions

The studies reported here validate the application of a relatively new technology to an established small-animal model of postinfarction heart failure. Using this technique, we have documented the time course of changes in LV structure and function after transmural anterior MI. Important findings include the early and progressive development of LV chamber enlargement and impaired diastolic filling, followed by the more gradual appearance of systolic dysfunction in the noninfarcted myocardium and the evolution of a restrictive LV filling pattern indicating severe diastolic dysfunction. We believe that the application of transthoracic echo-Doppler examination in the rat will be a very valuable and cost-effective method for evaluating the effects of agents intended to attenuate the postinfarction remodeling process.

Acknowledgments

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