Echocardiographic changes after myocardial infarction in a model of left ventricular diastolic dysfunction

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Echocardiographic changes after myocardial infarction in a model of left ventricular diastolic dysfunction. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2018–H2029, 1997.—To determine the early and late effects of myocardial infarction on left ventricular (LV) diastolic function in the rabbit postinfarction model, male New Zealand White rabbits were randomly assigned to ligation of the circumflex artery or sham operation. Serial echocardiographic and Doppler studies were performed on both groups of animals at baseline and 1 h and 3 wk after surgery (n = 10 for each group) after verification of the reproducibility and repeatability of the measurements. At 1 h postinfarction, decreases in early mitral inflow velocity (E wave) and mitral inflow velocity with atrial contraction (A wave) and increases in the mean pulmonary venous systolic-to-diastolic ratio and A wave reversal velocities were observed, without changes in LV geometry. By 3 wk postinfarction, increases in the mitral E-to-A ratio (1.1 ± 0.3 vs. 2.9 ± 0.9, P < 0.001) and left atrial area (131 ± 23 vs. 510 ± 72 mm², P < 0.001) and decreases in the pulmonary venous systolic-to-diastolic ratio (0.56 ± 0.20 vs. 0.79 ± 0.14, P = 0.008) were consistent with severe diastolic abnormalities (restricted physiology). The findings of this study demonstrate that coronary artery ligation in the rabbit provides a reproducible echocardiographic and Doppler model of LV diastolic dysfunction that is consistent with abnormalities found in humans with previous myocardial infarction, symptoms of heart failure, and preserved LV systolic function.

Although overall cardiovascular mortality in the United States is decreasing, the incidence and prevalence of heart failure are increasing. The largest group of patients with heart failure is composed of those with coronary artery disease and previous myocardial infarctions (30). Two-dimensional echocardiography and Doppler imaging (ED) have emerged as important noninvasive clinical tools for the assessment of left ventricular (LV) systolic and diastolic function after myocardial infarction. For example, in the echocardiographic substudy from the Survival and Ventricular Enlargement (SAVE) Trial, increases in end-systolic and end-diastolic LV cavity areas provided independent prognostic information in patients with postinfarction remodeling (29). With acute myocardial infarction, the transmirtal early mitral inflow velocity-to-mitral inflow velocity with atrial contraction ratio (E/A) and early mitral deceleration time are Doppler predictors of heart failure that are independent of systolic function (20). Clinical studies of patients with myocardial infarction are limited by the difficulties of controlling for infarct size, infarct location, loading conditions, and multiple pharmacological interventions. Postinfarction animal models may be more useful for defining the time course and pathophysiology of heart failure by providing data from a relatively homogeneous infarct population with a known onset and etiology of LV dysfunction (14). In contrast to invasive LV pressure measurements, echocardiography offers a mechanism for defining specific subgroups of LV dysfunction and for defining the transition between compensated ventricular hypertrophy and decompensated heart failure. For example, ED evidence of ventricular decompensation in a pressure-overload model of hypertrophy is predictive of abnormal contraction and relaxation velocities in myocytes isolated from these hearts (22). Inasmuch as molecular studies of myocardial tissue define subcellular changes that occur with the progression of heart failure, ED variables may prove to be more sensitive and practical phenotypic markers of LV changes associated with the altered expression of contractile and noncontractile proteins. Recently, increases in LV mass determined by M-mode echocardiography were found in mice overexpressing the myf 5 transgene compared with wild-type FVB/NJ mice (7), and increases in early mitral and aortic flow velocities were reported in phospholamban-deficient transgenic mice (9).

Despite the utility of measuring LV systolic and diastolic dysfunction in small rodent models using standard, commercially available echocardiographic equipment, these studies are limited by rapid heart rates, short diastolic filling periods, and small chamber dimensions. Difficulties in measuring left atrial size and pulmonary venous flow velocities prohibit a complete assessment of LV diastolic function. Recently, we described coronary artery ligation in the rabbit as a model for the study of heart failure after myocardial infarction (15). Compared with small rodent models, this model offers several advantages for the study of LV dysfunction, including lower operative mortality, direct visualization of the infarct artery, greater consistency...
of infarct size, larger chamber dimensions, and slower intrinsic heart rates. Also, more LV tissue (~3.5–5 g) is available for postmortem molecular and cellular studies than can be obtained from rats or mice. The purpose of this study was to 1) evaluate the rabbit postinfarction model for the serial ED study of LV diastolic dysfunction, 2) determine the early and late effects of myocardial infarction on LV function and geometry using ED measurements, and 3) serve as a benchmark for future integrative studies.

METHODS

Animals. New Zealand White rabbits (3–5 kg) were kept in separate cages in an environmentally controlled animal research facility. The animals were subjected to circadian light-dark cycles, maintained on standard rabbit chow (Ralston-Purina, St. Louis, MO), and given water ad libitum. All animal studies were performed following institutional care and use regulations developed by the Tucson Veterans Affairs Medical Center and the University of Arizona. These regulations are in accordance with guidelines set forth by the National Institutes of Health (17a) and the American Physiological Society.

Experimental infarction. Coronary artery ligation was performed as described earlier with minor modifications (15). Briefly, animals were anesthetized with an intramuscular injection of 5 mg/kg xylazine, 16 mg/kg ketamine, and 0.6 mg/kg acepromazine. After intubation, the animals were ventilated (model 683, Harvard Apparatus, S. Natik, MA) at a tidal volume of 15 ml and minute ventilation rate of 80. Isoflurane (2%) anesthetic was administered through an anesthesia apparatus (series 1000, Ohio-Heidbrink, Madison, WI). A left thoracotomy was performed through the fourth intercostal space, and the large marginal branch of the circumflex artery was ligated in its proximal portion with 5-0 Ti-cron suture. In contrast to our earlier protocol, ischemic preconditioning was not performed. Using this modified protocol, we observed no increase in the frequency of perioperative ventricular fibrillation. For sham-operated animals, the pericardium was incised, but coronary ligation was not performed. Before the incision was closed with contiguous tissues, residual air was evacuated from the thorax using silicont tubing attached to a 10-ml syringe. Lidocaine (1 mg/kg iv) was administered at the completion of surgery. During the postoperative recovery period, a single dose of penicillin (300,000 U sc) was administered, and buprenorphine (0.2 mg/kg sc) was injected for analgesia.

Echocardiographic measurements. Commercially available echocardiographic equipment (model 750, VingMed, Milpitas, CA), including a 7.5-MHz pediatric transducer, was used for all studies. Echocardiographic studies were performed during continuous facemask or endotracheal administration of 2% isofluorane. All M-mode, two-dimensional, and Doppler measurements were recorded on 1/2-in. S-VHS videotape, and portions of each study were recorded on paper at a speed of 100 mm/s. Off-line measurements were performed in duplicate using system software or an image analysis system (EchoPac 4.2.4, VingMed) with digitally acquired data. Two-dimensional echocardiographic measurements were made from the apical four-chamber, basal parasternal short-axis, and midventricular parasternal short-axis views (Fig. 1).

From the right lateral decubitus position, an apical four-chamber view was obtained. Mitral inflow velocities were recorded using a 1.0-mm sample volume placed at the tips of the mitral valve leaflets (Fig. 2). The position of the sample volume was adjusted until narrow-bandwidth, laminar flow was visualized and peak velocities were maximized by audio and video. Early mitral acceleration time was measured as the time interval from baseline to peak early filling wave (E wave), and deceleration time was measured from peak E wave to baseline. LV isovolumic relaxation time was measured from the aortic closure signal to the mitral valve opening signal. After anterior angulation of the transducer, peak LV outflow velocities and time-velocity integrals were recorded from the apical five-chamber view at the level of the aortic valve (Fig. 3A). Peak pulmonary venous inflow velocities during ventricular systole and diastole and at atrial contraction were measured, typically from the right superior pulmonary vein in an apical five-chamber view (Fig. 4).

From the left lateral decubitus position, pulsed Doppler spectra of peak pulmonic valve velocities were recorded in a parasternal short-axis view (Fig. 3B). Right atrioventricular (a bicuspid valve) inflow velocities were measured at the
leaflet tips in a modified right ventricular (RV) inflow view. For right and left heart measurements, maximal peak velocities were measured to account for changes in peak velocities during the respiratory cycle. M-mode measurements of chamber dimensions and wall thicknesses were recorded from a midventricular, short-axis view at the papillary muscle level (Fig. 5). The LV was bisected between papillary muscles at the largest diameter of the ventricle. Interventricular septal and posterior end-diastolic and end-systolic wall thicknesses and LV internal dimensions were measured according to the American Society of Echocardiography leading-edge method (27).

Color M-mode Doppler measurements of mitral inflow were performed from the right lateral decubitus position and a four-chamber view (28). Transducer position was adjusted to maximize mitral velocities by pulsed Doppler. Zoomed color M-mode tracings were initiated to depict a continuous column of early mitral inflow. Nyquist limit, LV reject, and color gain settings were standardized for all recordings. The time delay and distance of color between mitral leaflet tips and apex were measured from each end of the color column, and propagation rate was reported as the distance-to-time ratio.

Interpretive variability and reproducibility. Each of the reported measurements from 10 separate studies \((n = 10)\) was analyzed by two different observers (DDY and PGA) to assess interobserver repeatability. A single observer (DDY) also analyzed the same 10 studies 1 wk later (intraobserver repeatability). To determine reproducibility, five normal animals were studied in the same manner on different days. All analyses were blinded to the other observer and to tabulated data. For each study, up to 150 beats were recorded for any given parameter. All measurements were made from beats chosen at the discretion of each observer, not from predetermined beats.

Hemodynamic measurements. At the end of the 3-wk period, hemodynamic measurements were obtained immediately after ED measurements. A 5-Fr micromanometer-tipped catheter (Millar Instruments, Houston, TX) was inserted into the right carotid artery. The catheter was advanced into the aorta and then into the LV under constant pressure monitoring. For measurement of aortic pressures, a second Millar catheter was advanced into the descending aorta through a femoral artery. The zero-pressure baselines for these two catheters were obtained by placing the pressure sensors in 37°C saline. A 3.5-Fr thermistor microprobe was advanced from the contralateral femoral artery to the descending aorta. The right internal jugular vein was cannulated with PE-200 tubing that was advanced into the right atrium and used for pressure measurement and cold saline infusion.

After the animals recovered and stabilized, LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), and heart rate were recorded on a physiological recorder (model ES 1000, Gould Instrument, Cleveland, OH). The maximal rate of LV pressure development and decline (peak \(+\)dP/dt and \(-\)dP/dt, respectively) was obtained from a differentiating circuit in the recorder with the high-filter frequency cutoff set at 100 Hz. LV pressures from 15 to 25 consecutive cardiac cycles were digitized using an IBM PC. For each parameter, duplicate measurements were performed 5 min apart to ensure reproducibility, and averaged data were reported. Right atrial pressures and aortic pressures were recorded. At least five thermodilution cardiac outputs were measured at core body temperatures of 38–39°C using a cardiac output computer (Cardiotherm 500, Columbus Instruments, Columbus, OH). Next, a median sternotomy was used to place a 2-0 silk tie around the ascending aorta. A piece of polyethylene tubing 2–3 cm long (1.75 mm ID) was placed over the tie and used as an occlusion device. The aorta was occluded for \(5\) s to produce isovolumic contractions. Peak \(+\)dP/dt was determined by measuring the difference between peak systolic pressure and LVEDP after five stable beats and averaged for two separate aortic occlusions.

Calculations. Relative wall thickness was defined as \([(\text{IVS}_d + \text{PW}_d)/\text{LVID}_d] \times 100\%\), where \(\text{IVS}_d\) is interventricular septal thickness during diastole, \(\text{PW}_d\) is posterior wall thickness during diastole, and \(\text{LVID}_d\) is LV internal dimension...
Stroke volume index was determined from each of three methods: 1) \( V_{\text{LVOT}} \times (D/2)^2/BW \), where \( V_{\text{LVOT}} \) is the velocity-time integral of the Doppler signal measured at the level of the aortic valve, \( D \) is the diameter of the aortic valve measured by two-dimensional echocardiography, and \( BW \) is body weight; 2) \( \text{LVESVI} - \text{LVEDVI} \), where \( \text{LVESVI} \) and \( \text{LVEDVI} \) are the end-diastolic and end-systolic volume indexes, respectively, calculated from volume measurements; and 3) CI/HR, where CI is the cardiac index measured with a thermodilution probe and HR is heart rate.

Peak + dP/dt and –dP/dt and time constant of isovolumic relaxation (\( \tau \)) were determined using customized software, as reported earlier (21, 24).

Meridional end-systolic wall stress (ESS) was calculated as

\[
\text{ESS} = \left[ 0.334 \text{LVSP}(\text{LVID}_{d}) / \text{PW}_{s} (1 + \text{PW}_{s} / \text{LVID}_{d}) \right] \times 10^3 \text{ dyn/cm}^2
\]

(25), and meridional end-diastolic wall stress (EDS) was calculated as

\[
\text{EDS} = \left[ 0.334 \text{LVEDP}(\text{LVID}_{d}) / \text{PW}_{d} (1 + \text{PW}_{d} / \text{LVID}_{d}) \right] \times 10^3 \text{ dyn/cm}^2
\]

where \( \text{PW}_{s} \) is posterior wall thickness during systole.

Statistical analysis. Measurements are reported as means \( \pm \) SD unless otherwise indicated. A two-factor analysis of variance was performed using a grouping factor (sham operation vs. infarction) and a repeated-measures time factor (baseline, 1 h, 3 wk). The analysis was performed on each variable to determine the effects of time, the effects of infarction, and any interaction between the two factors. Where appropriate, comparisons to determine the significance of changes within the same group over time were performed with the Student-Newman-Keuls test for multiple comparisons. Sham and infarction groups were compared at a given time point separately by unpaired \( t \)-test. Relations between parameters were evaluated by least-squares linear regression analysis. Significance was defined at \( P < 0.05 \).

Agreement between measurements in the validation study was determined according to the methods of Bland and Altman (4) and expressed as the mean of the differences between observations \( \pm 2 \text{SD} \) of the differences (coefficient of repeatability or variation). With low variability, the mean of the differences should approach zero, and the coefficient of variation should be small. Variation between two measurements also was calculated as the mean difference between observations divided by the mean of the observations and expressed as a percent error.

**RESULTS**

Intraobserver variability and reproducibility. Technically high-quality ED data were obtained from all animals. Table 1 shows the interobserver variability, intraobserver variability, and reproducibility of the ED measurements. There was excellent agreement between observers for M-mode, two-dimensional, and Doppler measurements. Interobserver variability was highest for the color M-mode time delay and E wave acceleration times. As expected, the intraobserver percent error was generally lower than the interobserver percent error and was <10% for all measurements except the color M-mode time delay. Reproducibility between measurements obtained on separate days also was \( \leq 15\% \) for most values.
Baseline data. Twenty-four-hour survival was 11 of 14 (79%) for the infarction group and 11 of 11 (100%) for the sham-operated group. Three-week survival was 100% for those animals that survived the first 24-h postoperative period. Two animals died during instrumentation at 3 wk (1 in each group), and all data from these animals were excluded. Baseline weights were similar between the sham and infarcted groups (3.9 ± 0.7 and 4.1 ± 0.6 kg, respectively, P = 0.501). Baseline M-mode, two-dimensional, pulsed-wave Doppler and color M-mode measurements also were equivalent between the two groups (P > 0.05 for each measurement).

Effects of myocardial infarction on LV systolic function and geometry. One hour after circumflex artery ligation, severe lateral wall and apical hypokinesis or akinesis was uniformly observed. There were no changes

Fig. 4. Pulmonary venous flow velocities recorded from right superior pulmonary vein in an apical 5-chamber view (1 h postinfarction).

Fig. 5. M-mode recording of left ventricle 3 wk postinfarction. Image was obtained from a midventricular, short-axis view at level of papillary muscles. Note thinned, echodense (scarred), and akinetic posterior wall.
in the systolic or diastolic septal wall thicknesses, posterior wall thicknesses, or percent septal wall thickening early after infarction (P > 0.05 for each). The percent thickening of the posterior wall, however, was decreased in the infarcted group compared with the control group (19 ± 14 vs. 40 ± 25%, P = 0.032). Also, there was a time-dependent effect of infarction on the percentage of posterior wall thickening (P = 0.026).

At 3 wk, posterolateral wall thinning and severe hypokinesis or akinesis were observed. Apical thinning and hypokinesis or akinesis also were typically observed. Compared with measurements in the sham-operated group, however, septal and posterior wall thicknesses and percent thickening were unchanged (P > 0.05 for each). After infarction, there was enlargement of LV internal dimensions during systole and diastole (Table 2; P < 0.001), resulting in a decrease in wall thickness relative to chamber dimension compared with the sham-operated group (P = 0.026). Increases in end-systolic and end-diastolic volume indexes derived from M-mode measurements were observed at 3 wk postinfarction (Fig. 6). Furthermore, a fourfold increase in mean left atrial area was seen in the infarction group at 3 wk (P < 0.001).

Myocardial infarction did not affect percent fractional shortening in the anteroposterior plane. In contrast, mediolateral fractional shortening, V_{cd} in orthogonal planes, and LV ejection fraction were each decreased by 25–37% at 1 h compared with the sham-operated group (Table 2). Fractional shortening, V_{cd}, and LV ejection fraction were unchanged 3 wk postinfarction compared with the 3-wk control group.

Transmitral flow. Transmitral inflow velocities were markedly altered by myocardial infarction (Table 3). Time-dependent effects of infarction were observed in peak E wave velocities, peak velocities with atrial contraction (A wave), E/A, and deceleration times. Acutely, decreases in peak E and A wave velocities were observed in the infarction group compared with the sham-operated group, without a change in heart rate (R-R interval = 365 ± 16 and 370 ± 17 ms, respectively, P > 0.05). The mitral E/A, however, remained relatively constant. No changes were observed in mean E wave acceleration time, deceleration time, or isovolumic relaxation time 1 h postinfarction.

Three weeks after infarction, peak E wave velocities in the infarcted group were increased by 44% and peak A wave velocities were decreased by 43% compared with measurements in the sham-operated group. These changes resulted in a >2.5-fold increase in the E/A after myocardial infarction (Fig. 7A; P < 0.001). Decreases in early mitral acceleration time were observed at 3 wk compared with the sham-operated group (P = 0.041), and there was a trend toward a decrease in early mitral deceleration time for the postinfarction group (P = 0.053).

Pulmonary venous flow velocities. Infarction resulted in changes in pulmonary venous systolic and diastolic flow velocities (Table 3). A time-dependent effect of infarction was observed for peak pulmonary venous flow velocities.
systolic flow velocities. Early after infarction, the mean systolic flow velocity was unchanged in the infarcted group, but the mean diastolic flow velocity decreased by 35% (P < 0.001), resulting in an increased systolic-to-diastolic ratio (P = 0.035). A 75% increase in peak reversal velocity with atrial contraction was observed compared with the sham-operated group (P = 0.004). Consistent with abnormalities in LV diastolic function at 3 wk, there was a 47% reduction in peak pulmonary venous systolic flow velocity (P < 0.001) and a 29% decrease in the systolic-to-diastolic ratio compared with the control group (Fig. 7B; P = 0.008). The difference between mitral and pulmonary venous A wave duration (MVa - PVa), however, did not reach statistical significance in the infarcted group compared with the sham-operated group at 3 wk (−2 ± 25 and 16 ± 15 ms, respectively, P = 0.067).

LV outflow velocities. Myocardial infarction alone had no effect on LV outflow velocities, velocity-time integrals, or systolic ejection periods. There was, however, a time-dependent effect for each of these parameters (P < 0.001). Also, an interaction between time and infarction was observed for the LV systolic ejection period (P < 0.001). Compared with the sham-operated group, the systolic ejection period was increased 1 h after infarction (167 ± 12 and 194 ± 18 ms, respectively, P < 0.001), whereas there was no difference between the two groups at 3 wk (129 ± 18 and 124 ± 13 ms, respectively, P = 0.486).

RV inflow and outflow velocities. Measurement of RV outflow velocities through the pulmonic valve revealed neither a time nor an infarction dependence (P = 0.139 and P = 0.470, respectively). At 1 h postinfarction, RV outflow velocities were unchanged in the infarction group compared with the control group (51 ± 10 and 57 ± 10 cm/s, respectively, P = 0.196). Likewise, the early atrioventricular inflow velocity was unchanged in the acute postinfarction period (35 ± 7 and 40 ± 10 cm/s, respectively, P = 0.212). There was, however, a time dependence for early atrioventricular inflow velocities (P = 0.029) and an interaction between time and infarction over the 3-wk period (P = 0.015). The mean early filling velocity was increased in the postinfarction group compared with the sham-operated group at 3 wk (50 ± 10 vs. 41 ± 9 cm/s, P = 0.049).

Color Doppler flow and color M mode. Color Doppler flow proved useful in determining regions of maximal flow. Depiction of mitral inflow, right atrioventricular inflow, LV outflow, and RV outflow using color Doppler was readily obtained in a typical examination. Mitral regurgitation was detected by color Doppler or pulsed

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Table 2. Chamber dimensions and LV systolic function

<table>
<thead>
<tr>
<th>Time</th>
<th>LVIDs, mm</th>
<th>LVIDd, mm</th>
<th>RWT, %</th>
<th>LA size, mm²</th>
<th>%FS</th>
<th>Vcf, s⁻¹</th>
<th>EFvol, %</th>
<th>EFw, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pre-Sham</td>
<td>10.9 ± 0.8</td>
<td>15.4 ± 1.3</td>
<td>33 ± 7</td>
<td>139 ± 23</td>
<td>29 ± 4</td>
<td>25 ± 9</td>
<td>1.90 ± 0.28</td>
<td>1.73 ± 0.75</td>
</tr>
<tr>
<td>Pre-MI</td>
<td>11.4 ± 1.0</td>
<td>15.3 ± 1.7</td>
<td>33 ± 5</td>
<td>127 ± 15</td>
<td>26 ± 6</td>
<td>23 ± 7</td>
<td>1.80 ± 0.37</td>
<td>1.78 ± 0.49</td>
</tr>
<tr>
<td>Postoperatively</td>
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<td>1 h</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>9.7 ± 1.3⁶</td>
<td>14.0 ± 1.6</td>
<td>41 ± 8⁶</td>
<td>123 ± 20</td>
<td>31 ± 5</td>
<td>24 ± 6</td>
<td>1.85 ± 0.43</td>
<td>1.41 ± 0.36</td>
</tr>
<tr>
<td>MI</td>
<td>10.5 ± 1.3</td>
<td>14.7 ± 1.7</td>
<td>37 ± 8</td>
<td>125 ± 18</td>
<td>28 ± 9</td>
<td>17 ± 4⁶</td>
<td>1.38 ± 0.43</td>
<td>0.89 ± 0.29</td>
</tr>
<tr>
<td>3 wk</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>12.6 ± 1.5⁷</td>
<td>15.5 ± 1.3</td>
<td>34 ± 6⁷</td>
<td>131 ± 17</td>
<td>19 ± 7⁷</td>
<td>20 ± 9</td>
<td>1.70 ± 0.60</td>
<td>1.67 ± 0.73</td>
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<tr>
<td>MI</td>
<td>15.4 ± 1.2⁸</td>
<td>19.2 ± 1.7⁸</td>
<td>28 ± 5⁸</td>
<td>510 ± 72⁸</td>
<td>20 ± 6⁸</td>
<td>14 ± 8⁸</td>
<td>1.62 ± 0.57</td>
<td>1.12 ± 0.66⁸</td>
</tr>
</tbody>
</table>

Values are means ± SD. Midventricular M-mode measurements of left ventricular (LV) chamber dimensions and systolic function. Measurements were obtained in anteroposterior (A-P) and mediolateral (M-L) planes using a parasternal short-axis view at level of papillary muscles. RWT, relative wall thickness; LA size, area of left atrium during atrial diastole measured from an apical 4-chamber view; %FS, percent fractional shortening; Vcf, velocity of circumferential fiber shortening; EFvol, ejection fraction by volume calculations; MI, myocardial infarction; see Table 1 for definition of other abbreviations. Significantly different from sham-operated (Sham): ⁶P < 0.05; ⁷P < 0.01; ⁸P < 0.001. Significantly different from baseline: ⁹P < 0.05; ¹⁰P < 0.01. Significantly different from 1 h: ¹¹P < 0.05; ¹²P < 0.01.

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Fig. 6. Changes in left ventricular end-systolic and end-diastolic volume indexes (ESVI and EDVI) at 1 h and 3 wk postinfarction in sham-operated and myocardial infarcted (MI) rabbits.
Doppler in only one animal (infarct group), whereas tricuspid regurgitation was noted in four animals (infarct group). Color M-mode measurements revealed an increased mitral inflow time delay between leaflet tips and apex at 3 wk (19 ± 9 vs. 39 ± 19 ms, P = 0.008), but not at 1 h postinfarction (19 ± 7 vs. 25 ± 8 ms, P = 0.091). The propagation rate of early mitral inflow also was decreased at 3 wk (99 ± 42 vs. 43 ± 17 cm/s, P = 0.001), but not at 1 h postinfarction (101 ± 55 vs. 89 ± 62 cm/s, P = 0.653).

Hemodynamic measurements. LV pressure measurements were obtained at 3 wk, immediately after echocardiographic measurements (Table 4). No change in mean heart rate was observed between the two groups. Also, no differences were observed in LVSP, peak +dP/dt, stroke volume index, or cardiac index 3 wk postinfarction. In contrast, LVEDP and + were increased, and peak developed LV pressure was decreased in the postinfarction group. End-diastolic, but not end-systolic, wall stress was increased in the infarcted group.

Comparison of echo-Doppler and hemodynamic indexes. The linear correlation between stroke volume indexes calculated from echo-Doppler measurements and those calculated from thermodilution measurements is shown in Fig. 8. Compared with the thermodilution determinations (x), Doppler-derived values (y1) overestimated the measurement (y1 = 0.23 + 1.34x), whereas volume-derived values (y2) underestimated the measurement (y2 = 0.026 + 0.79x). For sham-operated animals, Doppler- and volume-derived measurements correlated well with thermodilution measurements (r = 0.90, P < 0.001 and r = 0.70, P = 0.02, respectively), whereas for infarcted animals the correlation was not as close (r = 0.23, P = 0.524 and r = 0.43, P = 0.184, respectively).

In addition to stroke volume, we examined the relationships between the E/A, the ratio of peak velocity of pulmonary venous flow during ventricular systole to that during diastole (PV_s/PV_d), and LVEDP at 3 wk postinfarction. There was a strong positive correlation...
Table 4. Hemodynamic measurements at 3 wk

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>220±9</td>
<td>213±7</td>
<td>0.67</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>63±14</td>
<td>65±9</td>
<td>0.60</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>4±2</td>
<td>12±6</td>
<td>0.0037</td>
</tr>
<tr>
<td>Peak +dP/dt, mmHg/s</td>
<td>1.926±0.507</td>
<td>1.166±0.245</td>
<td>0.12</td>
</tr>
<tr>
<td>Peak −dP/dt, mmHg/s</td>
<td>1.929±0.474</td>
<td>1.584±0.298</td>
<td>0.087</td>
</tr>
<tr>
<td>t, ms</td>
<td>18.4±1.8</td>
<td>24.9±4.9</td>
<td>0.0024</td>
</tr>
<tr>
<td>SVI, ml/kg</td>
<td>0.62±0.18</td>
<td>0.69±0.20</td>
<td>0.42</td>
</tr>
<tr>
<td>CI, ml·min⁻¹·kg⁻¹</td>
<td>132±22</td>
<td>124±12</td>
<td>0.36</td>
</tr>
<tr>
<td>TPRI, mmHg·min⁻¹·kg⁻¹</td>
<td>0.42±0.07</td>
<td>0.40±0.11</td>
<td>0.63</td>
</tr>
<tr>
<td>Peak DP, mmHg</td>
<td>133±26</td>
<td>99±24</td>
<td>0.018</td>
</tr>
<tr>
<td>ESS, dyn/cm²</td>
<td>66±26</td>
<td>84±25</td>
<td>0.132</td>
</tr>
<tr>
<td>EDS, dyn/cm²</td>
<td>10±9</td>
<td>26±17</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Values are means ± SD. HR, heart rate; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; peak +dP/dt, maximal rate of LV pressure development; peak −dP/dt, maximal rate of LV pressure decline; t, time constant of LV relaxation; SVI, stroke volume index; CI, cardiac index; TPRI, total peripheral resistance index; peak DP, peak developed LV pressure; ESS, end-systolic wall stress; EDS, end-diastolic wall stress.

between E/A (x₁) and LVEDP (y; y = 2.79 + 3.13x₁, r = 0.74, P < 0.001) and a strong inverse correlation between the PV₆/PV₄ (x₂) and LVEDP (y = 17.0 − 14.4x₂, r = 0.70, P < 0.001). As expected, there also was an inverse correlation between E/A and PV₆/PV₄ (x₂ = 0.87 − 0.10x₁, r = 0.64, P = 0.009).

Postmortem heart weights. Postmortem LV and RV weights are shown in Table 5. The mean LV weight (noninfarcted tissue) and LV/BW from the infarcted group were equal to the mean LV weight and LV/BW from the sham-operated group. These results are consistent with postinfarction hypertrophy of the noninfarcted myocardium after circumflex ligation. The mean RV weight and RV/BW were markedly increased in the infarcted group, which is suggestive of pulmonary hypertension. At 3 wk, no changes in mean body weight were observed between the two groups (3.9 ± 0.6 and 4.1 ± 0.5 kg, respectively, P = 0.429).

Table 5. Postmortem heart weights

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
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<tbody>
<tr>
<td>LV Wt, g</td>
<td>4.54±0.37</td>
<td>1.19±0.20</td>
</tr>
<tr>
<td>RV Wt, g</td>
<td>1.44±0.17</td>
<td>0.38±0.06</td>
</tr>
<tr>
<td>LV/BW</td>
<td>4.57±0.48</td>
<td>1.12±0.07</td>
</tr>
<tr>
<td>RV/BW</td>
<td>0.98±0.33</td>
<td>2.56±0.48</td>
</tr>
<tr>
<td>RV-to-body wt rt.</td>
<td>6.3±0.14*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. LV and right ventricular (RV) wet weights were obtained postmortem. LV/BW, LV-to-body wt ratio; RV/BW, RV-to-body wt ratio. LV and LV/BW in MI group do not include scar weights. *P < 0.001 vs. sham-operated group.

DISCUSSION

The findings of this study demonstrate that coronary artery ligation in the rabbit provides a reproducible ED model of LV diastolic dysfunction that is consistent with abnormalities of LV filling and pulmonary venous flow found in humans after myocardial infarction. Unlike smaller rodent models, the two-dimensional, M-mode, and Doppler views observed in this model are comparable to those described by the American Society of Echocardiography for human echocardiographic studies (27). We have shown that high-quality ED data can be obtained with low inter- and intraobserver variability and excellent reproducibility. Our repeatability data are particularly remarkable, in that the values represent comparisons of an unbiased selection of multiple beats from an entire recording (≥ 100 beats), rather than analyses of individual, predetermined beats.

Effects of myocardial infarction on wall motion and geometry. In this model the early and late effects of myocardial infarction on LV geometry and systolic and diastolic function were measured noninvasively and relatively free of confounding variables. One hour after myocardial infarction, measurements of fractional shortening and V₆ were more severely depressed in the mediolateral plane than in the anteroposterior plane. In addition, posterior wall hypokinesis was observed, without a change in anteroposterior fractional shortening. Taken together, these findings are reflective of severe lateral wall hypokinesis, lesser posterior wall involvement, and compensatory septal hyperkinesis. The two-dimensional findings are consistent with visual inspection of the large circumflex artery distribution in situ and the lateral and apical extent of the LV scar when examined postmortem. At 3 wk postinfarction, increases in the noninfarcted LV mass, increases in LV systolic and diastolic chamber dimensions, and decreases in relative wall thickness are consistent with eccentric LV remodeling and hypertrophy. Similar echocardiographic changes in LV geometry occur after myocardial infarction in the rat model (14) and in humans (5, 17, 29). Factors predisposing to infarct expansion in the rabbit model include moderate-to-large infarction, first infarction, transmural infarction, apical involvement, and persistent occlusion of the infarct-related vessel (17). Whereas others have reported infarct expansion only with anterior or anterolateral infarctions in humans (16), it is interesting that ventricular remodeling occurs with occlusion of the
large circumflex artery in the rabbit. The apical distribution of this artery in rabbits, however, is similar to the apical distribution of the left anterior descending coronary artery in humans.

Prominent changes in Doppler flow velocities were observed early and late after myocardial infarction. Acutely, coronary artery ligation produced decreases in peak E and A wave velocities, increases in the pulmonary venous systolic-to-diastolic ratio, and increases in the peak pulmonary venous reversal velocity with atrial contraction. The decrease in peak E and A wave velocities with infarction appeared to occur in the absence of a stroke volume reduction, since there were no differences in LV outflow tract velocity-time integrals. The diminished mitral A wave velocity and the increased pulmonary venous A wave reversal velocity early after infarction are indicative of reduced transmural anterograde flow and increased retrograde flow with atrial contraction. This increased impedance to transmural flow at atrial systole suggests an increase in operating LV chamber stiffness with preserved left atrial contractility.

Over the 3 wk after myocardial infarction, marked increases in left atrial size were observed, suggesting chronic elevations in left atrial pressure. In addition, left atrial function was decreased by 3 wk postinfarction, as suggested by a reduction in transmural and pulmonary venous peak A wave velocities and a reduction in the pulmonary venous systolic velocity. The systolic component of pulmonary venous flow is determined by downward mitral annular displacement during ventricular systole and by the fall in atrial pressure with atrial relaxation. An inverse relationship between the percentage of systolic filling and the mean left atrial pressure has been reported.

The rabbit as a model of LV diastolic dysfunction. The changes in transmural flow velocities, pulmonary venous flow velocities, and left atrial size observed in the rabbit are consistent with the natural history of LV diastolic filling abnormalities in humans. In the rabbit the observed pattern at baseline is similar to that observed in children and young adults (E > A, PVd > PVs, and normal left atrial size and function). This “juvenile” pattern was acutely altered after circumflex artery ligation (E > A, PVd = PVs, and normal atrial size and function). By 3 wk postinfarction, a pattern of restricted physiology was present (E ≫ A, PVd >> PVs, increased left atrial size, decreased left atrial function, and decreased mitral inflow propagation rate). Between baseline and 3 wk postinfarction, the pulmonary venous flow velocities exhibited a biphasic pattern of change, with increases in the PVd/PVs ratio early and decreases in the PVs/PVd ratio late after infarction.

The Doppler features of restricted physiology in this model are associated with increases in LVEDP, +dP/dt, fractional shortening, ejection fraction, or end-systolic wall stress. The marked abnormalities in LV diastolic filling observed after myocardial infarction, therefore, occurred without prominent LV systolic dysfunction. Peak developed LV pressure was the only measurement of LV contractility that was different between the sham-operated and infarcted groups at 3 wk. Thus, during the 3 wk analyzed in this study, the rabbit model best reflects severe LV diastolic dysfunction in the absence of severe LV systolic dysfunction. Adequate modeling of LV diastolic dysfunction has been lacking, even though it is now well established that LV diastolic abnormalities may predominate in a large proportion of patients with heart failure symptoms. The current model accurately represents this group of patients with previous myocardial infarction, preserved LV ejection fraction, and symptoms of heart failure.

Relationship to LV diastolic dysfunction in humans. Studies of Doppler flow velocities have established transmural flow velocities, pulmonary venous systolic and diastolic velocities, and left atrial size and function as indexes of LV diastolic function. Three altered patterns of transmural flow in humans have been characterized, resulting from abnormal LV relaxation and/or increases in chamber stiffness. These patterns have been classified as impaired relaxation, pseudonormalization, and restrictive physiology on the basis of the E/A, pulmonary venous patterns, and left atrial size and function. The most severe form of abnormal diastolic filling, restricted physiology, consists of an increase in peak E wave velocity, a decrease in peak A wave velocity, shortening of early mitral deceleration time, shortening of isovolumic relaxation time, a decrease in the pulmonary venous systolic-to-diastolic ratio, an increase in left atrial size, and markedly diminished left atrial function. Earlier work has shown that a restrictive transmural flow pattern is the single best predictor of cardiac death in patients with congestive heart failure. Also, the peak pulmonary venous reversal velocity with atrial contraction and the difference in duration between mitral and pulmonary venous atrial velocities have been correlated with LV filling pressures.

Technical considerations. Compared with small rodent models, the slower heart rates in rabbits allow for improved quantification of chamber dimensions by two-dimensional imaging. At an average heart rate of 220 beats/min and a frame rate of 12–13 frames per cardiac cycle. Slow heart rates also allow better visualization and separation of mitral E and A waves during diastole and more accurate measurements of systolic and diastolic time intervals. Also, the larger chamber dimensions of this model permit assessment of Doppler flow velocities in the LV outflow tract, pulmonary artery, right atrio-ventricular valve, and pulmonary veins. The ability to sample pulmonary venous flow into the left atrium adds important data for the assessment of LV diastolic function. Compared with small rodent or canine models of heart failure, the rabbit postinfarction model represents a new alternative for studying LV diastolic dysfunction.
Study limitations. In this study, LV dysfunction was demonstrated by ED early after myocardial infarction. LV hemodynamics, however, were not measured at the 1-h time point. Pressure measurements early postinfarction would have allowed hemodynamic correlation with Doppler measurements but would have required a larger number of animals. Also, hemodynamic and echocardiographic measurements were performed under mild isoflurane anesthesia, which may have influenced loading conditions and mean aortic pressures. Whereas identical anesthesia was used for the sham-operated and infarcted animals, we cannot rule out differential effects of isoflurane between groups.

Several of the echocardiographic measurements deserve special comment. Because of lung interference, the lateral wall infarct zone could not be perpendicularly bisected during the M-mode portion of the study. It was necessary for us, therefore, to use indirect measurements of fractional shortening in this plane. Also, because of lateral segmental wall motion abnormalities, measurements of volume derived from M-mode measurements in the anteroposterior plane may not account for changes in the mediolateral plane. This probably explains why stroke volume indexes derived from thermodilution cardiac outputs correlate with volume measurements in the sham-operated group, but not the infarcted group. Why Doppler-derived measurements of stroke volume index do not correlate well with thermodilution techniques in the infarct group is less clear but may be the result of right atrioventricular and/or pulmonic valvular regurgitation from pulmonary hypertension postinfarction.

Mitral deceleration time is an important prognostic indicator in patients with heart failure. Deceleration time, acceleration time, isovolumic relaxation time, and A wave durations were much shorter in the rabbit model than in humans. The shorter time intervals are explained by the shorter cycle length in the rabbit than in humans. In fact, the measured time intervals corrected for differences in cycle length are quite comparable. Striking differences between groups, however, were not observed in the rabbit. In part, this may be explained by increasing variability and decreasing accuracy of the measurements at the higher heart rates.

Finally, left atrial pressures were not measured in this study. Although the pattern of pulmonary venous flow may provide an estimate of left atrial pressure in the absence of myocardial contractile dysfunction, this relationship may not hold early after coronary artery ligation with acute myocardial depression (10). Other studies, however, have demonstrated that transmural E and A wave velocities, pulmonary venous flow velocities, and left atrial size and function correlate with left atrial pressures in chronic heart failure (1, 26).

In conclusion, this study demonstrates that the rabbit postinfarction model is a repeatable and reproducible model for the detailed echocardiographic and Doppler study of LV geometry and function after myocardial infarction. Marked segmental wall motion abnormalities 1 h postinfarction resulted in an acute reduction in ejection fraction and alterations in transmural and pulmonary venous flow velocities. Despite the limited effects of infarction on LV systolic parameters measured 3 wk postinfarction, Doppler measurements of transmural flow, pulmonary venous flow, and left atrial size are consistent with restricted LV physiology. It is anticipated that this model will have an important role in future integrative studies, particularly the study of LV diastolic dysfunction and its relation to human disease.

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