Echocardiographic assessment of cardiac function in conscious and anesthetized mice

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Yang, Xiao-Ping, Yun-He Liu, Nour-Eddine Rhaaleb, Nobutaka Kurihara, Henry E. Kim, and Oscar A. Carretero. Echocardiographic assessment of cardiac function in conscious and anesthetized mice. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1967–H1974, 1999.—Using a high-frequency linear transducer (15L8), we studied 1) the feasibility of performing echocardiography in nonanesthetized mice compared with mice given pentobarbital sodium (Pento) or a mixture of ketamine and xylazine and 2) the feasibility of echocardiographic evaluation of left ventricular (LV) hypertrophy, dilatation, and function in mice with two-kidney, one-clip hypertension or myocardial infarction (MI). Heart rate (HR) in awake mice was 658 ± 9 beats/min; Pento and ketamine plus xylazine reduced HR to 377 ± 11 and 293 ± 19 beats/min, respectively, associated with a significant decrease in shortening fraction (SF), ejection fraction (EF), and cardiac output (CO) and an increase in LV end-diastolic (LVEDD) and end-systolic dimensions (LVESD). Mice with 4 wk of two-kidney, one-clip hypertension had increased LV mass (15.62 ± 0.62 vs. 22.17 ± 1.79 mg) without altered LV dimensions, SF, EF, or CO. Mice studied 4 wk post-MI exhibited obvious LV dilatation and systolic dysfunction, as evidenced by increased LVEDD and LVESD and decreased SF, EF, and CO. Our findings clearly show the adverse impact of anesthesia on basal cardiac function and the difficulty in interpreting data obtained from anesthetized mice. We believe this is the first study to demonstrate the feasibility of using echocardiography to assess cardiovascular function in the nonanesthetized mouse.

two-kidney, one-clip hypertension; myocardial infarction; left ventricular dilatation; hypertrophy; dysfunction

WITH THE ADVENT OF transgenic technology, genetically altered mice with remarkable cardiovascular phenotypes are now available, which allows us to study the role of various genes in the regulation of hemodynamics and cardiac function in physiological as well as pathophysiological situations. To benefit from the full potential of these genetically engineered mice, it is critical to develop approaches for accurate and reproducible measurements of cardiac morphology and function in intact animals. Although classic methods of measuring cardiac hemodynamics and function by means of ventricular catheterization and radiolabeled microspheres, and thermolodization techniques are available in mice, they require instrumentation and microscopic techniques and are difficult to perform more than once in the same animal. Transthoracic echocardiography is an established noninvasive procedure and has frequently been used to assess cardiac morphology and performance in larger animals such as dogs and rats (3, 4). Recently, several investigators have shown that echocardiography is a valid approach to noninvasive evaluation of left ventricular (LV) mass and function in vivo in anesthetized mice (7, 10, 21, 25). However, it is well known that most anesthetics such as barbiturates, halothane, and isoflurane cause cardiovascular and respiratory system depression (5, 13, 18, 20). Although ketamine has been reported to have less of a cardiodepressor effect (6, 12), it is usually used in combination with xylazine or diazepam to improve muscle relaxation and prolong analgesia (6, 8). However, such a combination has been shown to produce hypothermia and to have negative inotropic and chronotropic effects (9, 24, 26).

To our knowledge, echocardiographic evaluation of LV function in anesthetized and nonanesthetized mice has not been compared. In the present study, we performed echocardiography in mice with and without anesthesia using a newly developed high-frequency linear transducer (15L8, Acuson, Mountain View, CA). Two commonly used anesthetics, pentobarbital sodium and a combination of ketamine and xylazine, were tested. We also assessed the feasibility of echocardiographic detection of LV hypertrophy and dilatation, as well as evaluation of LV function in conscious mice, using two experimental models established in our laboratory: 1) LV hypertrophy induced by two-kidney, one-clip renovascular hypertension and 2) chronic heart failure induced by coronary artery ligation. LV dimensions, mass, and systolic function were studied.

METHODS

Animals

Twelve-week-old male 129 SvEvTac mice weighing 23–28 g were purchased from Taconic Farms (Germantown, NY). Mice were housed in an air-conditioned room with a 12:12-h light-dark cycle and received standard mouse chow and tap water ad libitum. This study was approved by the Henry Ford Hospital Care of Experimental Animals Committee.

Surgical Induction of Two-Kidney, One-Clip Renovascular Hypertension

Mice were anesthetized with pentobarbital sodium (Pento; 50 mg/kg ip), an incision was made in the midabdomen, and the left renal artery was carefully dissected from the renal vein under aseptic conditions (27). A silver clip with an internal diameter of 0.102 mm was placed around the left renal artery, and the abdomen was closed. Mice were placed on a heating pad until they awoke.
Surgical Induction of Myocardial Infarction

Mice were anesthetized with pentobarbital (50 mg/kg ip), intubated, and ventilated with room air using a positive-pressure ventilator. A left thoracotomy was performed via the fourth intercostal space, the heart was exposed, and the pericardium was opened as described previously (28). The left anterior descending coronary artery (LAD) was ligated with a 9-0 silk suture near its origin between the pulmonary outflow tract and the edge of the left atrium. Our pilot studies showed that ligation at this site causes 40–50% infarction of the LV (unpublished observations). Acute myocardial ischemia was deemed successful when the anterior wall of the LV became pale. The lungs were then inflated by increasing positive end-expiratory pressure, and the thoracotomy site was closed. Animals were kept on a heating pad until they recovered.

Measurement of Systolic and Mean Blood Pressure

Systolic blood pressure (SBP) was measured in conscious mice using a noninvasive computerized tail-cuff system (BP-2000, Visitech, Apex, NC) as described previously (1, 11). Mean blood pressure (MBP) was measured in a separate group of mice. For catheterization, mice were anesthetized with pentobarbital (50 mg/kg ip) and a polyethylene catheter (PE-10 fused to PE-50) was inserted into the abdominal aorta with pentobarbital (50 mg/kg ip) and a polyethylene catheter (PE-10 fused to PE-50) was inserted into the abdominal aorta via the left femoral artery. The distal end of the catheter was subcutaneously brought out the back of the neck (22). Twenty-four hours later, mice were placed in plastic cages individually and MBP was measured continuously in nonrestrained awake mice and recorded on a four-channel polygraph (Brush 440, Gould, Cleveland, OH).

Echocardiographic Method

Before the study, the mice were trained on two to three separate occasions over a period of 3 days using the method described below. During the first training session, some mice developed bradycardia when they were held [heart rate (HR) reduced from ~600 to ~400 beats/min]. However, with repeated training the bradycardia disappeared and the mice remained calm. Images obtained during the training sessions were not recorded. To determine whether holding mice causes sympathetic stimulation that might increase HR, we also measured HR using the tail-cuff method in the same mice and compared these values with the values obtained by echocardiography.

For echocardiography in awake mice (29), we picked up the mouse by the nape of the neck and held it firmly in the palm of one hand in the supine position, with the tail held tightly between the last two fingers. The left hemithorax was carefully shaved, and a warmed ultrasound transmission gel (Parker Laboratory, Orange, NJ) was applied to the precordium. Transthoracic echocardiography was performed using an Acuson 256 equipped with a 15-MHz linear transducer (15L8) in a phased-array format, which offers 0.35-mm lateral resolution and 0.25-mm axial resolution, real-time digital acquisition, storage, and review capabilities. Generally, the heart was first imaged in the two-dimensional (2-D) mode in the parasternal long-axis view. From this view, an M-mode cursor was positioned perpendicular to the interventricular septum and posterior wall of the LV at the level of the papillary muscles, and M-mode images were obtained for measurement of wall thickness and chamber dimensions. The cursor was moved to the aortic root and the aortic dimension obtained, after which a 2-D short-axis view of the mid-LV was obtained at the chordal level by rotating the transducer clockwise 30–45°. Images from this view were used to measure LV cross-sectional area. Aortic flow velocity was measured with pulsed-wave Doppler on the parasternal long-axis view. The sample volume cursor was placed in the aortic root and the transducer angled slightly, which allowed aortic flow parallel to the interrogation beam so that maximum aortic flow velocity was obtained easily. Images were stored in digital format on a magnetic optical disk for review and analysis.

Image Analysis

LV chamber dimensions and wall thickness. End-diastolic (LVEDD) and end-systolic LV dimension (LVESD) and interventricular septum (IVST) and posterior wall thickness (PWT) were measured from the M-mode tracings. During diastole, LV dimension and wall thickness were measured from the maximum chamber cavity; during systole, they were measured during maximum anterior motion of the posterior wall. All measurements were done from leading edge to leading edge according to the American Society of Echocardiography guidelines (23).

LV mass. LV mass was calculated according to uncorrected cube assumptions using the equation (21)

\[ \text{LV mass} = 1.055([\text{IVST} + \text{LVEDD} + \text{PWT}]^3 - \text{LVEDD}^3) \]

where 1.055 is the specific gravity of the myocardium. The derived LV mass was normalized for body weight and expressed in milligrams per 10 g of body weight. Echocardiographic and postmortem LV mass were correlated.

LV shortening fraction. Shortening fraction (SF), a measure of LV systolic function, was calculated from the M-mode LV dimensions using the equation

\[ \text{SF} (\%) = \frac{[\text{LVEDD} - \text{LVESD}]/\text{LVEDD}}{100} \]

Ejection fraction. Ejection fraction (EF) was calculated from the LV cross-sectional area (2-D short-axis view) using the equation

\[ \text{EF} (\%) = \frac{[\text{LVDA} - \text{LVSA}]/\text{LVDA}}{100} \]

where LVDA is LV diastolic area and LVSA is LV systolic area.

Cardiac output. After aortic flow velocity, velocity-time integral (VTI), and aortic root dimension (AoD) were obtained, cardiac output (CO) was calculated from the following equations

\[ \text{CSA} = (\text{AoD}/2)^2 \times \pi \]

\[ \text{SV} = \text{CSA} \times \text{VTI} \]

\[ \text{CO} = \text{SV} \times \text{HR} \]

where SV is stroke volume and CSA is cross-sectional area. CO was normalized for body weight and expressed as milliliters per minute per 10 g of body weight.

All primary measurements such as LV wall thickness, dimensions, and cross-sectional areas were traced manually and digitized by goal-directed, diagnostically driven software installed within the echocardiograph. Three beats were averaged for each measurement.

Variability

Intraobserver variability was tested by 1) having the same person repeat the echocardiographic data analysis on different days (n = 6) and 2) performing echocardiography again in the same mice on different days (n = 9). Interobserver variability was determined by having two people measure the same data (n = 10). Intra- and interobserver errors were
calculated as the difference between the two observations divided by the mean and expressed as a percentage. The agreement for both intra- and interobserver variability was evaluated by intra- and interclass correlation coefficients (ICC). The ICC value is the ratio of the between-subject variance to the sum of the between-subject variance and the within-subject variance. The smaller the within-subject variance, the better the agreement and the higher the ICC.

Experimental Protocols

Protocol I: Effect of anesthesia on LV function. First, echocardiography was performed in 24 conscious mice. Twelve mice were then anesthetized with pentobarbital (50 mg/kg ip) and the other 12 with a mixture of ketamine (150 mg/kg ip) and xylazine (15 mg/kg ip). Mice were placed on a heating pad to maintain proper body temperature, and echocardiography was repeated. To determine the blood pressure response to anesthesia, MBP was measured before (n = 10) and after either pentobarbital (n = 5) or xylazine (n = 5).

Protocol II: Echocardiographic assessment of LV chamber dimensions, mass index, and function in mice with two-kidney, one-clip hypertension or myocardial infarction. Echocardiography was performed in conscious mice after 4 wk of two-kidney, one-clip hypertension (n = 12) or myocardial infarction (MI; n = 9) and compared with the 24 normotensive mice described in protocol I, which served as the controls.

Statistics

Statistical analysis was performed by the Department of Biostatistics and Research Epidemiology at our institution. Data were expressed as means ± SE. A nonparametric Kruskal-Wallis test or unpaired Student’s t-test was used to compare variables between groups. P ≤ 0.01 was considered significant. Variation was evaluated by determination of ICC. This measure indicates excellent agreement if >0.75, fair to good if between 0.4 and 0.75, and poor if <0.4.

RESULTS

Echocardiography in Nonanesthetized Versus Anesthetized Mice

We found that the anesthetics pentobarbital and ketamine plus xylazine had significant negative inotropic and chronotropic effects on the mouse heart (Fig. 1 and Table 1). The chronotropic effects were more profound when the mice were anesthetized with ketamine plus xylazine. In awake mice, HR was 658 ± 9 beats/min; pentobarbital and ketamine plus xylazine reduced HR to 377 ± 11 and 293 ± 19 beats/min, respectively. Associated with this was a significant reduction in SF (51.4 ± 1.1% in awake mice vs. 33.1 ± 1.8% with pento-
barbital and 34.6 ± 1.1% with ketamine + xylazine), EF (83.6 ± 1.3 vs. 52.6 ± 2.0 and 55.1 ± 1.7%), and CO (13 ± 0.6 vs. 8.6 ± 0.7 and 7.2 ± 3.8 ml/min·10 g⁻¹) (Fig. 2) and an increase in LV chamber dimensions (LVEDD and LVESD: 2.52 ± 0.06 and 1.22 ± 0.05 mm in awake mice vs. 3.06 ± 0.09 and 2.05 ± 0.08 mm with pentobarbital and 3.57 ± 0.08 and 2.34 ± 0.08 mm with ketamine + xylazine) (Table 1). In anesthetized mice, septal and LV posterior wall thickness were reduced during diastole but remained unchanged during systole. Anesthesia also caused a significant fall in MBP (121 ± 4 mmHg before anesthesia vs. 92 ± 6 mmHg with pentobarbital and 85 ± 8 mmHg with ketamine + xylazine).

Echocardiography in Mice with Two-Kidney, One-Clip Hypertension

SBP increased from 114 ± 3 mmHg before surgery to 137 ± 5 mmHg after 4 wk of two-kidney, one-clip manipulation (n = 12); this was accompanied by LV hypertrophy as indicated by increased wall thickness and LV mass (LV mass: 15.62 ± 0.62 mg in controls vs. 22.17 ± 1.79 mg in 2-kidney, 1-clip; P < 0.01) (Fig. 3 and Table 2), which correlated well with actual heart weight (r² = 0.88; P < 0.001) (Fig. 4). The LV chamber was smaller than in the controls, consistent with concentric hypertrophy. Despite existing LV hypertrophy, SF, EF, and CO in two-kidney, one-clip mice were not significantly different from in the controls (Fig. 5), which suggests that LV function was well compensated during this stage of two-kidney, one-clip hypertension (4 wk). There was no significant difference in HR measured by echocardiography compared with that measured using the tail-cuff method.
Four weeks after MI, there was significant LV chamber dilatation (LVEDD and LVESD: 2.52 ± 0.06 and 1.22 ± 0.05 mm in controls vs. 3.54 ± 0.31 and 2.05 ± 0.29 mm in mice with MI) associated with increased posterior wall thickness (Fig. 6 and Table 2). Most mice with MI developed a large aneurysm involving the apex and anterior wall, which resulted in a greater increase in LV mass (15.6 ± 0.62 vs. 29.8 ± 5.29 mg). The correlation between calculated LV mass and true heart weight was statistically significant ($r^2 = 0.77; P < 0.05$), although to a lesser degree than in the two-kidney, one-clip mice (Fig. 4). This is most likely explained by the altered LV geometry produced by MI. These mice also had obvious LV dysfunction, indicated by a decrease in SF (51.4 ± 1.0 vs. 31.5 ± 2.6%), EF (83.6 ± 1.3 vs. 42.7 ± 4.8%), and CO (13.0 ± 0.6 vs. 6.2 ± 0.5 ml·min⁻¹·10g⁻¹) (Fig. 4).

### Variability and Reproducibility

Intra- and interobserver variability are summarized in Table 3. Overall, agreement was good to excellent except for interobserver agreement for IVST and PWT. HR measured by echocardiography in awake mice was similar to that measured by tail cuff in the same mice (Tables 1 and 2), indicating that echocardiography did not cause significant stress when the mice were well trained.

### DISCUSSION

Recent advances in transgenic technology, particularly the development of genetically altered mice with remarkable cardiovascular phenotypes, have provided a means of studying the role of various genes in the regulation of hemodynamics and cardiac function in both physiological and pathophysiological conditions. Thus it is critical to develop approaches for accurate and repeated measurements of cardiac morphology and function in intact animals. Although echocardiographic evaluation of cardiac function has been employed in mice, it was carried out with the animals under anesthesia, which may alter cardiac function and thereby alter LV mass. The correlation between calculated LV mass and true heart weight was statistically significant ($r^2 = 0.77; P < 0.05$), although to a lesser degree than in the two-kidney, one-clip mice (Fig. 4). This is most likely explained by the altered LV geometry produced by MI. These mice also had obvious LV dysfunction, indicated by a decrease in SF (51.4 ± 1.0 vs. 31.5 ± 2.6%), EF (83.6 ± 1.3 vs. 42.7 ± 4.8%), and CO (13.0 ± 0.6 vs. 6.2 ± 0.5 ml·min⁻¹·10g⁻¹) (Fig. 4).

### Table 2. Heart rate, ventricular wall thickness, and chamber dimensions in mice with two-kidney, one-clip hypertension or myocardial infarction

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW, g</th>
<th>HR, beats/min</th>
<th>Systolic Dimensions, mm</th>
<th>Diastolic Dimensions, mm</th>
<th>Mass, mg/10g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Echo Tail cuff</td>
<td>IVST LVD PWT</td>
<td>IVST LVD PWT</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>24</td>
<td>24.0 ± 0.4</td>
<td>658 ± 9</td>
<td>622 ± 10</td>
<td>0.90 ± 0.02</td>
<td>1.22 ± 0.05</td>
</tr>
<tr>
<td>2K, 1C</td>
<td>12</td>
<td>22.5 ± 0.6</td>
<td>635 ± 20</td>
<td>639 ± 10</td>
<td>1.17 ± 0.04</td>
<td>1.08 ± 0.06</td>
</tr>
<tr>
<td>MI</td>
<td>9</td>
<td>28.5 ± 0.5</td>
<td>687 ± 11</td>
<td>655 ± 17</td>
<td>0.86 ± 0.11</td>
<td>2.62 ± 0.26†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of mice. Echo, HR measured by echocardiography; Tail cuff, HR measured by tail-cuff method; 2K, 1C, two-kidney, one-clip renovascular hypertension; MI, myocardial infarction. *P < 0.01; †P < 0.001 vs. controls.

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**Fig. 4.** Regression between left ventricular (LV) mass measured by echocardiography (Echo) and actual LV weight in controls and mice with 2K, 1C renovascular hypertension ($y = 1.05x + 9.16$) and myocardial infarction (MI; $y = 0.56x + 19.3$).

**Fig. 5.** SF (A), EF (B), and CO (C) in controls and mice with 2K, 1C renovascular hypertension and MI. *P < 0.05; **P < 0.01 vs. controls.
confound interpretation of the data (7, 10, 19, 21, 25). We believe that this is the first report to document the success of repeated echocardiography in nonanesthetized mice using a high-frequency linear transducer (15L8).

We found that it is important to train mice before echocardiography is performed. During the first training session, some mice developed bradycardia (HR decreased to ~400 beats/min) rather than tachycardia, probably due to vagal stimulation when the mouse was held in one hand. However, after two to three training sessions over a period of 3 days, the bradycardia disappeared and the mice remained calm. HR measured by echocardiography in awake mice is ~650 beats/min, which is comparable to that measured with a computerized tail-cuff system (550–650 beats/min), suggesting that echocardiography in awake mice may not cause considerable sympathetic stimulation compared with other widely accepted methods of physiological evaluation in the intact, nonanesthetized animal.

We also confirmed that echocardiography is a powerful tool for detecting changes in LV dimensions and function induced by hypertension or MI in mice. In particular, echocardiography provides information about patterns of LV hypertrophy (eccentric or concentric) and the status of LV remodeling and performance. We observed that LV dilatation and cardiac decompensation (as indicated by increased LV chamber dimensions and mass and decreased SF and CO) occurred more rapidly in mice with MI than in rats, although the LAD was ligated at the same level (near its origin between

![Fig. 6. M-mode (top) and Doppler echocardiograms (bottom) of mice with MI.](http://ajpheart.physiology.org/)

### Table 3. Intra- and interobserver class correlation coefficients

<table>
<thead>
<tr>
<th>Variable</th>
<th>%Error</th>
<th>ICC</th>
<th>%Error</th>
<th>ICC</th>
<th>%Error</th>
<th>ICC</th>
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</thead>
<tbody>
<tr>
<td>Systole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVST</td>
<td>1.3 ± 3.9</td>
<td>0.57</td>
<td>-0.3 ± 3.6</td>
<td>0.85</td>
<td>10.1 ± 6.6</td>
<td>0.33</td>
</tr>
<tr>
<td>LVD</td>
<td>5.3 ± 5.2</td>
<td>0.79</td>
<td>-1.7 ± 4.7</td>
<td>0.87</td>
<td>-0.8 ± 8.5</td>
<td>0.91</td>
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<tr>
<td>PWT</td>
<td>1.5 ± 3.9</td>
<td>0.81</td>
<td>-1.1 ± 1.8</td>
<td>0.93</td>
<td>4.5 ± 12.9</td>
<td>0.58</td>
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<td>Diastole</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVST</td>
<td>-0.6 ± 3.9</td>
<td>0.85</td>
<td>1.1 ± 5.6</td>
<td>0.58</td>
<td>-2.4 ± 6.1</td>
<td>0.97</td>
</tr>
<tr>
<td>LVD</td>
<td>-1.6 ± 3.7</td>
<td>0.90</td>
<td>-0.5 ± 2.2</td>
<td>0.71</td>
<td>4.4 ± 7.0</td>
<td>0.88</td>
</tr>
<tr>
<td>PWT</td>
<td>-2.7 ± 3.6</td>
<td>0.69</td>
<td>-0.8 ± 5.5</td>
<td>0.64</td>
<td>-1.9 ± 11.4</td>
<td>0.37</td>
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<td>AoD</td>
<td>-1.2 ± 3.5</td>
<td>0.79</td>
<td>0.8 ± 3.0</td>
<td>0.67</td>
<td>-0.8 ± 4.3</td>
<td>0.82</td>
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<td>LV mass</td>
<td>-5.1 ± 5.2</td>
<td>0.92</td>
<td>-0.3 ± 6.3</td>
<td>0.81</td>
<td>3.1 ± 17.5</td>
<td>0.88</td>
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<td>HR</td>
<td>-0.3 ± 1.4</td>
<td>0.94</td>
<td>1.0 ± 3.2</td>
<td>0.89</td>
<td>-0.3 ± 3.9</td>
<td>0.81</td>
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<tr>
<td>SF</td>
<td>-5.5 ± 6.8</td>
<td>0.48</td>
<td>3.3 ± 3.6</td>
<td>0.54</td>
<td>5.9 ± 10.7</td>
<td>0.54</td>
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<tr>
<td>EF</td>
<td>-0.4 ± 1.2</td>
<td>0.49</td>
<td>0.2 ± 3.0</td>
<td>0.57</td>
<td>-3.5 ± 3.6</td>
<td>0.49</td>
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<tr>
<td>CO</td>
<td>6.6 ± 16.3</td>
<td>0.79</td>
<td>-1.4 ± 11.6</td>
<td>0.84</td>
<td>-9.7 ± 12.8</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Values are means ± SE. ICC, intra- and interobserver class correlation coefficients; AoD, aortic root dimension; SF, shortening fraction; EF, ejection fraction; CO, cardiac output.

In general, anesthesia produces cardiac and respiratory depression as well as fluctuations in body temperature via either a direct or indirect action on the central nervous system and heart. For example, barbiturates have been reported to depress myocardial contractility (5, 17), with a resultant decrease in CO and increase in total peripheral resistance. Although ketamine reportedly has less of a negative inotropic effect on the heart, its negative chronotropic effect is not negligible, particularly when used in combination with xylazine (6, 25, 26). In animals with preexisting cardiac pathology, such as mice with MI or LV hypertrophy, inhibition of myocardial contractility and alteration of the autonomic nervous system by anesthetics may further jeopardize cardiovascular function, confound interpretation of hemodynamic data, or underestimate LV function. In the present study, we found that both pentobarbital and a mixture of ketamine and xylazine altered cardiac hemodynamics and performance significantly in mice, as demonstrated by decreased MBP, HR, SF, EF, and CO, probably due to inhibition of sympathetic activity and myocardial contractility. The negative chronotropic effect induced by a combination of ketamine and xylazine was more profound than that seen with pentobarbital. Thus it is much better to perform echocardiography in nonanesthetized mice, particularly when done frequently (in our case, once a week).
the pulmonary outflow tract and the edge of the left atrium) and infarct size was similar (~40–50% of the LV). We previously reported that in rats significant LV chamber dilatation and cardiac dysfunction occurred at least 8 wk after MI (14, 15), whereas in mice they became obvious by 4 wk. This rapid deterioration of LV function in mice after MI may be related to aggravated infarct expansion, aneurysm formation, and, therefore, increased LV wall stress. Indeed, we found that ~40% of mice died from cardiac rupture during the first week of MI (unpublished observations), and more than one-half of the survivors had a large LV aneurysm (1 wk after MI). Although we did not measure LV wall stress directly, we assume it was greatly increased in mice with MI; this would increase both LV workload and oxygen consumption, eventually leading to rapid decompensation of LV function. On the other hand, 4 wk of pressure overload (2-kidney, 1-clip hypertension) resulted in concentric LV hypertrophy, as characterized by an increase in wall thickness without a significant change in LV chamber size. LV function was also compensated during this stage of hypertension, because SF, EF, and CO were similar to control values. Increased wall thickness or LV hypertrophy is a compensatory response to systemic hypertension, which reduces wall stress and maintains normal cardiac function for a period of time.

We also found a good relationship between echocardiographic LV mass and actual heart weight, particularly in two-kidney, one-clip hypertensive mice, indicating that echocardiographic evaluation of LV mass is more sensitive in hearts with uniform geometry and wall motion and less sensitive in asymmetric enlarged hearts, such as those with MI.

In summary, we believe that echocardiography provides a powerful tool for studying cardiac morphology and function as well as the progression of LV remodeling and dysfunction in mice. However, our findings clearly show the adverse impact of anesthesia on basal cardiac function and the difficulty in interpreting data obtained from anesthetized mice. We believe this is the first study to demonstrate the feasibility of using echocardiography to assess cardiovascular function in the nonanesthetized mouse.

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