Visualization of the Heart and Determination of Left Ventricular Mass in Rats by Echocardiography

Takashi Nakamura, M.D., Kazutoshi Shimoo, M.D., Toshiro Kuribayashi, M.D.,* Kin-ya Matsubara, M.D., Masami Shima, M.D., Akiyoshi Matsumuro, M.D., Akihiro Azuma, M.D., Hiroshi Katsume, M.D., and Masao Nakagawa, M.D.

SUMMARY

A high-frequency transducer was used to determine the optimal parameters for visualizing the heart in 40 normal Wistar, 15 SHR, and 10 aorta-banded rats. The rats were 5 to 30 weeks old and weighed between 105 and 705 grams. Two-dimensional and M-mode views of the ventricles, atria, valves, and great arteries were obtained by placing the transducer beneath the rats through the left or right parasternal window in either the prone or the right decubitus positions, respectively. Left ventricular (LV) mass was determined on the basis of a spheroid model; these values correlated well with the LV weight for both the Wistar rats ($r=0.94$, $p<0.001$) and the rats with cardiac hypertrophy due to pressure load ($r=0.87$, $p<0.001$). These results were highly reproducible. This indicates that echocardiography is useful for obtaining quantitative measurements in rats.

Additional Indexing Words:
Ultrasound imaging Animal model Left ventricular hypertrophy Spontaneously hypertensive rat Aorta-banded rat

The rat is particularly useful as an experimental animal in cardiovascular research. However, echocardiographic measurements of cardiac wall dynamics have been limited in small animals because the method has inadequate spatial resolution. This study tested the utility of a new high-frequency transducer (7.5 MHz) in rats and determined optimal conditions for visualizing the rat heart. The accuracy of this method was then assessed by comparing determinations of the left ventricular wall mass and area with...
Materials and Methods

Animals: Forty normal male Wistar rats 5–30 weeks old (weight: 105–705 g), 15 male SHR rats 20–30 weeks old (weight: 315–445 g), and 10 aorta-banded male Wistar rats (ABR) 12–14 weeks old (weight: 350–450 g) were used. The 40 Wistar rats were divided into 2 groups: 20 rats weighing less than 320 g and 20 rats weighing more than 320 g. The suprarenal segment of the aorta was banded at 8 weeks of age for a duration of 4–6 weeks.

Echocardiography: Echocardiography was performed with a Hewlett-Packard Sonos 100 mechanical sector scanner, using a single element transducer with a frequency of 7.5 MHz. This system was capable of depicting pulsed Doppler flow mapping. The transducer was focused at a depth of 2.5 cm and had an axial resolution of 0.5 mm and a lateral resolution of 0.9 mm. Real-time, two-dimensional (2-D) imaging was performed at a maximal frame rate of 43 Hz on the narrowest scan angle. M-mode tracings were obtained using a 2-D reference sector and printed on video-imaging papers by video copy processor 77570 B (Hewlett-Packard Co.). Electrocardiograms were recorded simultaneously.

Under anesthesia with urethane (100 mg/100 g body wt, ip) or pentobarbital (3 mg/100 g body wt, ip), rats were placed in the prone or right lateral decubitus position so that the upwardly fixed transducer could find the left or right parasternal echo window, respectively (Fig. 1). This position provided better image quality than did the supine position. In addition, a rubber pouch filled with transmission gel was put on the transducer tip as an ultrasound coupler to visualize the heart within the optimal performance zone of the transducer. The long or short axis image of the heart was obtained by moving the rat and by rotating the transducer. This method allowed us to identify the great arteries, four cardiac chambers, and four cardiac valves in the 2-D image. The orientation of these images was confirmed by pulsed Doppler flow mapping and contrast echocardiography with a 0.2 ml dose of hand-agitated iopamidol injected into the cannulated jugular vein or the LV cavity.

Echocardiographic determination of LV mass: The leading edge method was used to measure the external (De) and internal (Di) diameters of the LV; these determinations were made at end-diastole from M-mode images of five successive beats, obtained from the left parasternal window. We selected the two recordings from each rat in which De showed the greatest values. Image quality was good under adequate gain settings and respiratory move-
ments were minimal.

On the basis of the assumption that the LV is a spheroid with homogeneous wall thickness, we calculated the LV mass as $1.05 \times (D_e^3 - D_i^3)$, where 1.05 is the specific gravity of the fixed myocardium determined from its volume and weight.6),7) Furthermore, the cross-sectional area (CSA) of the LV wall was calculated as $(\pi/4) \times (D_e^2 - D_i^2)$, which has been considered to be another index of myocardial mass.8)

Anatomic determination of the LV mass: After the sonographic examination, the chest was opened and the heart was arrested at end-diastole by injecting a saturated EDTA solution (0.1–0.2 ml) into the right atrium.2) After the right atrium was incised to drain the perfusate, the coronary artery was perfused with 10% formalin via the cannulated carotid artery at a perfusion pressure of 80 mmHg. The heart was removed and immersed in 10% formalin for several days.

After the atria and right ventricle (RV) were removed, the left ventricle was cut midway between the aortic root and apex, and weighed. The anatomic CSA of the LV wall was assessed by planimetry of a ×6 magnified picture of the transversely cut surface,2) excluding the papillary muscles and trabeculae carneae.

Test of accuracy of echocardiographic measurement: A multipurpose Tissue-equivalent Phantom (Nuclear Associates Co.) was used to confirm the actual performance of the imaging system. We also evaluated the accuracy of echocardiographic measurements using 20 formalin-perfused hearts, cut in 2-mm thick transverse slices. The De and Di were measured by M-mode echocardiography with the tissue sections suspended in a water bath. The data were compared with direct measurements obtained by planimetry as
described above. The 0.3-mm nylon strands were attached as a marker for the same measurement site to the anterior and posterior wall of each tissue section before the two measurements.

Statistical analysis: Data are presented as mean ± SD. Paired and unpaired t-tests were used as appropriate and differences were considered significant if the value was less than 0.05. Accuracy of echocardiographic measurements was evaluated by correlation coefficients obtained from least-squares linear regression and standard errors of the estimate between the anatomic and echocardiographic data. Comparisons of two correlation coefficients were performed using Fisher’s Z transformation followed by the test for difference of means.

Interobserver variability was evaluated by comparing the echocardiographic LV mass measurements made by two observers. Intraobserver variability was also evaluated by comparing repeat measurements of the LV mass by a single observer. The reproducibility of echocardiographic LV mass determinations from the same material was assessed by comparing the value from the two optimal tracings recorded and measured by a single echocardiographer. The variabilities of the values for different determinations were evaluated by one-way analysis of variance. Also evaluated were both
correlation coefficients and the standard errors of the estimate between the standard determination and these three variabilities.

**Results**

*Echocardiographic images:* Both the right and left parasternal windows permitted visualization of short and long axis views of the LV (Figs. 2 and 3). The long axis plane contained the LV, left atrium, and aortic root; the short axis view could be moved from the base to apex of the heart. The area from the great arteries to the papillary muscles was imaged without rim shadows in both views.

The cardiac image through the right window in the decubitus position was usually effaced by the lung. It was sometimes limited to the cardiac base and the image at the midventricular level was obscure. However, it was analogous to the image through the left parasternal window in man in both the 2-D and M-mode orientations. Through the right window in the rat, the M-mode beam passed through the RV free wall, ventricular septum, and LV free wall between two papillary muscles. Additionally, the beam was perpendicular to the left atrial wall, allowing the measurement of the
left atrial dimension (Fig. 2).

The left window in the prone position provided a different image orientation. At the level of the papillary muscles, the M-mode beam passed through the anterior and posterior LV wall (i.e., the left side of the anterior and posterior junctions), without visualizing the ventricular septum (Fig. 3). This caused overestimation of the LV posterior wall thickness due to the intervening posterior papillary muscle, which required careful adjustment of the beam direction and gain setting. Also, regions of the RV cavity other than the outflow tract were often obscured by the sternal shadow.

The circumferential wall motion of the LV could not be assessed on 2-D images. The spatial resolution and frame rate of 2-D images may be insufficient to resolve motion of the small LV at a high heart rate (300–400/min) in rats. However, the 2-D image was clear enough to obtain adequate M-mode tracings (Fig. 4). Furthermore, we could easily find the presence of LV hypertrophy or dilatation in 2-D images.

LV mass and CSA: The mean LV mass determinations from echocardiographic measurements were similar to the directly measured LV weight in each experimental group (Table I). However, the mean estimated CSA

Fig. 4. M-mode echocardiograms of the left ventricle through the left parasternal window.
A: Normal Wistar rats weighing 105 g (5-wk-old).
B: Normal Wistar rat weighing 380 g (14-wk-old).
C: SHR rat weighing 370 g (30-wk-old).
D: Aorta-banded rat weighing 380 g (14-wk-old).
Table I. Anatomic and Echocardiographic Data of LV Mass (LV wt) and CSA

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Normal</th>
<th>LV hypertrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td></td>
<td></td>
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<tr>
<td>Echocardiographic estimate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV mass (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSA (mm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>De (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Di (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatomic measurement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV wt (mg) (range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV wt/BW (mg/g)</td>
<td></td>
<td></td>
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<tr>
<td>CSA (mm²)</td>
<td></td>
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</table>

*p<0.001 vs anatomic CSA. § p<0.01 vs normal Wistar rats with body wt greater than 320 g, # p<0.01 vs ABR. Abbreviations: ABR=aorta-banded rat; BW=body weight; CSA=cross-sectional area of the LV wall; De and Di=external and internal diameters of the LV, respectively.

from the echocardiograms was significantly smaller than that of the directly measured CSA (Table I). There were high correlations between the echocardiographic LV mass and the LV weight, and between the echocardiographic CSA and anatomic CSA. There were no significant differences between correlation coefficients for the data from the small Wistar and large Wistar groups and between the SHR and ABR (Table II, Fig. 5). Standard

Table II. Correlation Coefficients and Standard Errors of Estimate between Echocardiographic and Anatomic Data

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>LV hypertrophy</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>&lt;320 g</td>
<td>&gt;320 g</td>
</tr>
<tr>
<td>Echo LV mass vs Anat. LV wt</td>
<td>r</td>
<td>0.93*</td>
</tr>
<tr>
<td></td>
<td>SEE (mg)</td>
<td>69</td>
</tr>
<tr>
<td>Echo CSA vs Anat. CSA</td>
<td>r</td>
<td>0.91*</td>
</tr>
<tr>
<td></td>
<td>SEE (cm²)</td>
<td>4.1</td>
</tr>
<tr>
<td>Echo CSA vs (LV wt)^2/3</td>
<td>r</td>
<td>0.95*</td>
</tr>
<tr>
<td></td>
<td>SEE (cm²)</td>
<td>3.9</td>
</tr>
</tbody>
</table>

*p<0.001. r=correlation coefficient; SEE=standard error of estimate. Other abbreviations as in Table I.
errors of the estimate between the anatomic and echocardiographic LV mass measurements were less than 10% of the mean anatomic value in every group. Additionally, the echocardiographic CSA was well correlated with the two-thirds power of the anatomic LV weight (Table II).

The SHR and ABR showed significantly higher ratios of LV weight to body weight than Wistar rats of a similar body weight, suggesting the development of LV hypertrophy (Table I). Although the echocardiographic and anatomic determinations of LV mass and CSA were similar, the internal diameter was significantly greater in the ABR than in the SHR (Table I). This difference probably reflects the response to an acute and progressive pressure load in the ABR in contrast to the mild, chronic load in the SHR.

The variations of the values for different LV mass determinations were not statistically significant between two observers, two observations, or two
Table IV. In-Vitro Echocardiographic Measurement of Tissue Specimens

<table>
<thead>
<tr>
<th></th>
<th>Echo</th>
<th>Anatomic</th>
</tr>
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<tbody>
<tr>
<td>De (mm)</td>
<td>12.4 ± 1.3</td>
<td>12.5 ± 1.4</td>
</tr>
<tr>
<td>(r, SEE)</td>
<td>(0.95*, 0.42)</td>
<td></td>
</tr>
<tr>
<td>Di (mm)</td>
<td>6.1 ± 1.0</td>
<td>6.4 ± 0.9</td>
</tr>
<tr>
<td>(r, SEE)</td>
<td>(0.91*, 0.43)</td>
<td></td>
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</table>

*p < 0.001. Abbreviations as in Tables I and II.

recordings in either normal Wistar rats or rats with LV hypertrophy (Table III). High reproducibility was also indicated by close correlations and small standard errors of the estimate of these different determinations.

Accuracy of in-vitro echocardiographic measurements: The mean measured diameters of the 10-, 6-, and 4-mm cylinders in the tissue phantom by M-mode echocardiograms were 9.947 ± 0.066, 5.949 ± 0.063, and 3.920 ± 0.060 mm, respectively. The external and internal dimensions of the LV tissue sections were 12.5 ± 1.4 and 6.4 ± 0.9 mm, respectively; the M-mode external and internal dimensions (12.4 ± 1.3 and 6.1 ± 1.0 mm) provided accurate estimates of these measurements (Table IV).

DISCUSSION

We have shown that ultrasound imaging allows in-situ visualization of the heart which can be used for LV mass determination in rats. Using a 5-MHz transducer, Young et al\(^9\) have accurately determined the LV mass in both normal rabbits and rabbits with aortic regurgitation, and LV weights of 0.8–6.8 g. Our results indicate that the method can be applied to animals with a LV weight of 0.4–2.0 g by employing a 7.5-MHz transducer. These results were not compromised significantly by limiting conditions which include transducer resolution, imaging quality, inter-subject variations and inter-observer differences.\(^{10}\)

The quality of the cardiac echo images in rats was superior in the prone or natural position than in the supine position. Furthermore, upright fixation of the transducer facilitates scanning because the rats can be moved with respect to the fixed beam direction. As a result, identical tomographic planes can be obtained. We did not employ the apical approach, which may facilitate both the measurement of the long axis and Doppler studies of flow velocities in the long axial direction,\(^3\) because it is difficult to fix the rat in positions for this approach. Furthermore, complete short axis images were not always obtained, particularly through the right parasternal window. A coupler on the transducer tip may in part be responsible for this, since it
may widen the unsurveyed area behind the sternum.

The limit of resolution of cardiac valves was about 0.2 mm in 2-D and M-mode images as evidenced by the fact that valves of this thickness were observed in relatively large rats. Thus, the resolution was appropriate for accurately measuring dimensions.

The LV mass was estimated from the M-mode image on the basis of a simple spheroid model with a uniform wall thickness.\(^6\),\(^7\) One source of error, then, was that the papillary muscles and the apical wall thickness were ignored. The actual shape of the LV is analogous to a spheroid with a thin apical wall and with a variable ratio of long and short axial distances.\(^2\) Nonetheless, these estimates yielded an accurate approximation of the anatomic measurements in each experimental group. This suggests that the model adequately approximates the LV mass. The measurements from ABR and SHR, further confirmed the validity of the approximation for varying LV geometries.

The CSA measurements were independent of geometric assumptions.\(^8\) The CSA estimates from echocardiograms were well correlated with the anatomic measurements, suggesting its utility in directly assessing the development of cardiac hypertrophy. However, the echocardiographic CSA tended to underestimate this parameter. This difference may not be due to recording or measurement errors (Table IV). Rather, it may reflect post-mortem swelling of the myocardial tissue during fixation.

The availability of the method in small animals such as rats will contribute to experimental cardiology, facilitating in particular the examination of normal and pathological cardiac growth and function. Furthermore, there are several strains of small animals available as potent disease models for human cardiac diseases, for example, Syrian hamsters for dilated cardiomyopathy,\(^11\) SHR rats for hypertensive heart disease,\(^12\) and WKY rats for congenital heart malformations.\(^13\) Since echocardiography can be applied to the estimation of ventricular wall motion and chamber size, this method is likely to provide important insights into mechanisms of heart disease in animal models.

**References**

3. Adams D, Mark DB, Kisslo J: The Doppler Examination in Basic Doppler Echocardiogra-