Accuracy of echocardiographic estimates of left ventricular mass in mice

KEITH A. COLLINS,1 CLAUDIA E. KORCARZ,1 SANJEEV G. SHROFF,2 JAMES E. BEDNARZ,1 RICHARD C. FENTZKE,1 HUA LIN,1 JEFFREY M. LEIDEN,1 AND ROBERTO M. LANG1

1Noninvasive Cardiac Imaging Laboratory, University of Chicago, Chicago, Illinois 60637; and 2Department of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

Received 25 August 2000; accepted in final form 27 November 2000

MURINE MODELS have been increasingly used to study a variety of cardiovascular disorders, such as dilated cardiomyopathy, left ventricular (LV) hypertrophy, myocardial infarction, and hypertension (3, 4, 10, 20). These transgenic and surgically modified mouse models require noninvasive imaging methods that would allow accurate assessment of structural and functional cardiac changes. LV mass (LVM) is a commonly used and important descriptor of cardiac status. Previous studies (2, 12, 22) have examined the accuracy of M-mode and two-dimensional LVM measurement methods in small cohorts of predominantly normal mice. Transthoracic two-dimensionally directed, M-mode echocardiography has yielded estimates of mouse LVM with relatively good correlation compared with necropsy values (6, 9, 20). However, M-mode echocardiography is limited in that images are obtained in only one plane, and thus LVM calculation is subject to greater error than formulas derived from multiplanar images (6).

Two-dimensional echocardiography has also been limited by low-frame rate-image acquisition relative to the high heart rate of the mouse and also by inappropriate frequency transducers for near-field imaging (12). Recent advances in echocardiographic technology enable better long- and short-axis views as well as more accurate M-mode measurements (2, 22, 23). The development of high-frame rate imaging and increased probe frequency has significantly enhanced murine imaging. Newly available probes also have smaller footprints that are more appropriate to the animal size. Finally, the creation of high-frequency linear transducers has improved near-field imaging, avoiding the use of acoustic standoffs with their inherent problems.

In humans, two-dimensional, area-length-based estimates of LVM have been shown to be more accurate than M-mode-based estimates (1, 17). We hypothesized this to be true in mice as well, particularly when taking advantage of enhanced imaging capabilities. Accordingly, we sought to compare the accuracy of known echocardiographic formulas for estimating LVM in a large number of mice relative to previous studies and to determine the sources of error in these estimates. Specifically, we compared the necropsy LV weights with determined LVM, using M-mode (cubed formula) as well as two-dimensional (area-length and truncated ellipsoid formulas) measurements over a wide range of LV sizes, cardiac geometries, and weights. Accordingly, our study was designed to include a chronic model of LV hypertrophy induced by aortic banding, a transgenic model of dilated cardiomyopathy, and normal CD-1 mice for control.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
METHODS

Animal preparation. A total of 91 adult CD-1 mice of both sexes with a mean body weight of 32.5 g (range 16.5–57.4 g) were studied. This included 38 CD-1 normal mice, 44 aortic-banded mice, and 9 transgenic cAMP response element binding protein (CREB)A133 mice (described below). Image quality was not a criterion for inclusion in this study. Anesthesia was induced by administering isoflurane in a closed chamber at 5% (Ohmeda Fluotec 3, Matrx Medical; Orchard Park, NY) in 80% room air-20% O₂, followed by 0.5–2.0% isoflurane through a nose cone throughout the experiment. Animals were then secured to a custom-made water bed in a shallow left lateral decubitus position to facilitate ultrasound imaging. The bed was connected to a circulating water bath set at 37°C to prevent hypothermia. Flat pieces of metal secured to the water bed underneath the paws served as electrocardiographic electrodes with Redux Créme (Hewlett-Packard; Andover, MA) to enhance conduction. All procedures were performed in accordance with the guidelines established by the American Physiological Society and the Animal Care and Use Committee of the University of Chicago.

Two to three weeks before noninvasive imaging, 49 CD-1 normal mice underwent aortic banding to induce LV hypertrophy. The mice were anesthetized, intubated with an 18-gauge angiocatheter, and ventilated with 1% isoflurane mixed with O₂ at 130–150 breaths/min with a 0.8–1.2 ml tidal volume. After a midsternotomy was performed along the upper third of the sternum, the ascending aorta was isolated and constricted at midarch with a 7-0 nylon suture to the size of a 27-gauge needle placed along the vessel. Two weeks after banding, the aortic pressure gradient was assessed by acquiring Doppler velocities in the ascending and descending aorta. If the pressure gradient failed to exceed 15 mmHg, the animal was not included in the study. Animals that prematurely died before acquiring all images necessary for comparative analysis were also discarded from the study for a final count of 44 mice for this subgroup.

The transgenic mice (CREB)A133 were generated and genetically confirmed, as previously described in detail (3). Briefly, transgenic mice were produced that over express a dominant-negative form of CREB (CREB)A133 under the transcriptional control of the cardiac-specific α-myosin heavy chain promoter. Presence of the CREB)A133 transgene was confirmed by Southern blot analysis of purified tail DNA using the Fast Track 2.0 kit (Invitrogen; San Diego, CA) in accordance with the manufacturer’s instructions described previously (3). Although the adult CREB)A133 mice were over 16 wk old and already displayed phenotypic characteristics of congestive heart failure, such as marked lung congestion and peripheral edema, changes in cardiac dimensions and function were not necessarily maximal at the time of imaging.

After imaging was complete, mice were immediately euthanized and their hearts removed. The left ventricles were carefully isolated by trimming the atria, the valves, and the right ventricular wall, and then blotted of excess fluid and weighed (Denver XP-1500 portable balance).

Data acquisition. Cardiac ultrasound imaging was performed using a high-frequency 15-MHz linear transducer (Sonos 5500, Agilent; Andover, MA) at a frame rate of 120 frames/s. Parasternal long- and short-axis views were obtained after adjusting gain settings for optimal epicardial and endocardial wall visualization. Two-dimensional echocardiographic loops of at least 20 cardiac cycles and M-mode tracings were stored digitally on magneto-optical disk for offline analysis.

Measurements. From the short-axis view, epicardial and endocardial LV areas were measured offline at end systole and end diastole. Images were considered adequate for measurement when >75% of the epicardial and endocardial contour could be adequately visualized. In accordance with the American Society of Echocardiography recommendation, the short-axis endocardial border was traced on the innermost endocardial edge, and the epicardial border was traced along the first bright pixel immediately adjacent to the darker...
myocardium (Fig. 1). The LV length, defined as the distance between the apex and the mitral annular plane, was obtained from parasternal long-axis views in which the mitral annular plane and the apex were well defined. LV measurements were made from at least three cardiac cycles at both end systole and end diastole.

Two-dimensionally targeted M-mode echocardiographic images were obtained at the level of the papillary muscles from the parasternal short-axis view and recorded at a speed of 150 cm/s. LV internal diameters and wall thicknesses (leading edge to trailing edge) were obtained at end systole and end diastole from cross-sectional short-axis views. Heart rate was measured and shortening fraction was calculated from the M-mode tracing. For both two-dimensional and M-mode methods, LV end-diastolic measurements were obtained at the peak of the R wave, whereas end-systolic measurements were obtained at the time of minimal chamber area.

LVM was calculated using the following three formulas. The first formula is the two-dimensional, area-length method: $LVM = 1.05 \left[ \frac{(IVS + LVID + LVFPW)^3}{3} - (LVID)^3 \right]$, where IVS and LVFPW are the interventricular septal and posterior wall thickness, respectively, and LVID is the LV internal diameter (1). The second formula consists of the M-mode (cubic) method: $LVM = 1.05 \left[ \frac{(IVS + LVID + LVFPW)^3}{3} - (LVID)^3 \right]$. The third formula is the truncated ellipsoid method: $LVM = \pi \left[ \frac{1}{2} \pi \frac{(b + T)^2}{2} - \frac{d^2}{4} \right] \frac{1}{3} (a + T)^3 - \frac{1}{4} \pi (a + T)^2 \frac{d^2}{4} (a - \frac{1}{3}a^3)$, where $b$ is the minor axis radius of the LV measured at the level of the papillary muscle tip. Its placement determines the division of the measured LV length ($L$) into a full major radius ($a$) and a truncated major radius ($d$). The average $T$ is calculated from $A_1$ and $A_2$ (18). This analysis was performed in a subgroup of 31 mice, representing the entire spectrum of LVM.

An experienced reader performed all measurements. A subgroup of 30 mice representing the entire spectrum of LVM was remeasured by the first reader (C) and by a blinded second reader (J). Intraobserver variability was calculated as $(C_1 - C_2)/(C_1 + C_2)/2$, where $C_1$ and $C_2$ are the two measurements performed by the first reader from the same images on different days. Similarly, interobserver variabilities were calculated as $(J_1 - C_1)/(J_1 + C_1)/2$, where $J_1$ represents the second observer’s measurements.

### Statistical analysis

All data are presented as means ± SD. Intergroup echocardiographic estimates of LVM at end systole and end diastole were compared by two-way ANOVA. Linear regression analyses were used to compare LVM estimates with true LV weights determined at necropsy. With the use of the Bland-Altman analysis, the agreement between echocardiographic LVM and necropsy weight was calculated as the mean (bias) ± 2 SD (error) of the differences between echocardiographic and necropsy LVM, and the bias and error for each method were determined. A $P \leq 0.05$ was considered statistically significant. Sample size computations were also performed for both M-mode and two-dimensional, area-length methods to determine the number of animals required to detect significant 15% difference of the mean LV weight between two or three groups of mice, with a statistical power of $\geq 0.8$ and an $\alpha$ of 0.05. The computation assumes that samples are taken from populations normally distributed with equal variance and is calculated using the SD of the end-diastolic LVM measured by either method for the normal and aortic-banded mice groups.

### RESULTS

Calculation of LVM by the area-length method was possible in 90/91 (99%) mice at end diastole and 89/91 (98%) at end systole. Two-dimensionally targeted M-mode images of the left ventricle were technically adequate in 83/91 (91%) and 84/91 (92%) mice for end-diastolic and end-systolic LVM measurements, respectively. The truncated ellipsoid formula was used in a subgroup of animals ($n = 31$) due to the difficulties in identifying the epicardial boundary in the long-axis view.

Measured chamber dimensions as well as heart rates and shortening fractions for all subgroups are presented in Table 1. Statistically significant increases in epicardial and endocardial areas, LV length, and posterior wall thickness were noted at end diastole in aortic-banded mice, compared with normals. CREB133 mice had significantly larger cavity sizes with a tendency toward decreased wall thickness, compared with normal mice.

### Table 1. Echocardiographic measurements of left ventricular dimensions for normal CD-1, aortic-banded, and transgenic CREB133 mice

<table>
<thead>
<tr>
<th></th>
<th>Normal CD-1</th>
<th>Banded</th>
<th>Transgenic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diastole</td>
<td>Systole</td>
<td>Diastole</td>
</tr>
<tr>
<td>$A_1$, mm$^2$</td>
<td>24.3 ± 3.9</td>
<td>20.6 ± 3.6</td>
<td>29.8 ± 4.4$^*$</td>
</tr>
<tr>
<td>$A_2$, mm$^2$</td>
<td>12.6 ± 2.7</td>
<td>7.8 ± 2.7</td>
<td>14.3 ± 2.6$^*$</td>
</tr>
<tr>
<td>$T$, mm</td>
<td>7.28 ± 0.52</td>
<td>6.48 ± 0.53</td>
<td>7.78 ± 0.69$^*$</td>
</tr>
<tr>
<td>T, mm</td>
<td>0.78 ± 0.10</td>
<td>1.01 ± 0.17</td>
<td>0.93 ± 0.20$^†$</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>0.80 ± 0.13</td>
<td>1.09 ± 0.20</td>
<td>0.96 ± 0.17$^†$</td>
</tr>
<tr>
<td>LVID, mm</td>
<td>4.23 ± 0.48</td>
<td>3.02 ± 0.61</td>
<td>4.41 ± 0.43$^*$</td>
</tr>
<tr>
<td>LVFPW, mm</td>
<td>0.78 ± 0.13</td>
<td>1.03 ± 0.16</td>
<td>0.92 ± 0.20$^†$</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>336 ± 98</td>
<td>22 ± 10</td>
<td>284 ± 110</td>
</tr>
<tr>
<td>SF, %</td>
<td>29 ± 9</td>
<td>28 ± 10</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>38</td>
<td>44</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; $n = $ number of mice. Two-dimensional measurements: $A_1$, epicardial area; $A_2$, endocardial area; $L$, parasternal long-axis length; $T$, short axis-derived thickness; M-mode measurements: IVS, interventricular septal thickness; LVID, left ventricular (LV) internal diameter; LVFPW, LV posterior wall thickness; HR, heart rate; SF, shortening fraction. CREB, cAMP response element binding protein. HR was measured and the shortening fraction was calculated from the M-mode tracing. *$P < 0.05$ †$P < 0.0001$, compared with the normal CD-1 group, at end diastole or end systole. $\ddagger P < 0.05$, compared with aortic-banded mice, at end diastole or end systole.
normal CD-1 mice. Because of the small sample size of the transgenic subgroup no significant differences were noted in either heart rate or shortening fraction.

End-diastolic and end-systolic echocardiography-based estimates of LVM and necropsy weights are presented in Table 2. The LV weights measured at necropsy for both aortic-banded and CREBA133 mice were significantly higher than the normal CD-1 mice. The use of the CREBA133 and the aortic-banded mice provided a wide range of LVM (62–210 mg) and end-diastolic chamber dimensions (3.1–5.5 mm). Peripheral edema characteristic of CREBA133 mice resulted in significantly higher body weight compared with normal controls (43.7 ± 5.8 vs. 29.2 ± 8.8 g, \(P < 0.0001\)). Thus measurements indexed to body mass are not shown for comparison between groups.

As seen in Fig. 2A, a good correlation existed between LVM determined by the area-length method at end diastole and necropsy LV weight for all mice (\(y = 0.97x + 3.89; r = 0.91\), standard error of estimate (SEE) = 14.1 mg, \(P < 0.0001\)). This correlation was stronger than that of the M-mode calculation of diastolic LVM and necropsy LV weight (\(y = 1.15x + 21.63; r = 0.81\), SEE = 26.3 mg, \(P < 0.0001\); Fig. 2C). Likewise, LV measured at end systole using the area-length method more strongly correlated with necropsy LV weights than systolic M-mode LVM (slope = 0.98 vs. 1.15 and \(r = 0.90\) vs. 0.85, respectively; Fig. 2).

The Bland-Altman analysis was used to compare the echocardiographic estimates of LV mass with the necropsy LV weights (Fig. 3). The bias (mean difference) between LVM calculated by the two-dimensional, area-length method and necropsy LV weight was significantly smaller than that obtained using the M-mode method (1.0 vs. 38.4 mg or 1.4% vs. 36.7%, \(P < 0.0001\); Fig. 3, A and B). Similarly, the error, defined as
2 SD above and below the mean difference between the echocardiographic LVM estimate and necropsy LV weight, was smaller for the two-dimensional, area-length method compared with the M-mode method (28.0 vs. 53.2 mg or 27.0% vs. 51.6%, \( P < 0.0001 \); Fig. 3, A and B). Results obtained using data from end systole are also presented in Fig. 3. The biases for both the area-length and M-mode methods were similar when measured at end diastole or end systole (mean: 1.4% vs. 1.7%, area length, and 36.7% vs. 23.0%, M mode, respectively). Similarly, the errors did not differ between end diastole and end systole (27.0% vs. 28.6%, for two-dimensional, area-length, and 51.6% vs. 42.1%, for M mode, respectively).

In a subgroup of 31 mice, spanning the full range of LVM, a further estimation of LVM by the truncated ellipsoid method was performed (Table 3). On average, only the area-length-derived LV estimates did not significantly differ from LV necropsy weights; M-mode and truncated ellipsoid methods overestimated and underestimated LVM, respectively. In linear regression analyses comparing estimated LVM to necropsy values, the correlation coefficients of the area-length (\( r = 0.94 \)) and truncated ellipsoid methods (\( r = 0.94 \)) were both greater than the M-mode correlation coefficient (\( r = 0.88, P < 0.0001 \)). The bias and error values derived in Bland-Altman analyses for area-length and truncated ellipsoid methods were also nearly identical, and both were significantly lower than the corresponding M-mode values (Table 3).

Sample size computations on the basis of the SDs of the normal CD-1 mice (Table 2) showed that to detect statistically significant differences (power \( \geq 0.8, \alpha = 0.05 \)) of 15% of the mean LV weight between two or three groups of mice, the area-length method would require 20 or 25 mice, respectively, whereas the M-mode method would require 49 or 60 mice. With the use of the larger SD of the banded mice (Table 2), sample size computation showed that both methods would require larger groups (62 or 77 mice for area-length, and 117 or 146 mice for M mode for two or three groups, respectively).

![Fig. 3. Bland-Altman analyses showing the agreement as a percent difference between necropsy LV weight and LVM calculated by the AL method (A and C) or by the two-dimensionally guided M-mode method (B and D) at end diastole (top) and end systole (bottom). Horizontal reference lines are zero percent difference (bold), the bias (center thin line) and 2 SD above and below the bias (outlying thin lines). Bias ± 2 SD for AL method were 1.4 ± 27.0% vs. 36.7 ± 51.6% for the M-mode method at end diastole and 1.7 ± 28.6% vs. 23.0 ± 42.1%, respectively, at end systole. \( n = 90, 89, 83, \) and 84 for A, B, C, and D, respectively. Closed circles, normal CD-1 mice; open circles, transgenic mice; and closed triangles, aortic-banded mice.](http://ajpheart.physiology.org/)

### Table 3. Comparison of necropsy- and LVM derived by two-dimensional, area-length, M-mode, and truncated ellipsoid methods

<table>
<thead>
<tr>
<th></th>
<th>Necropsy</th>
<th>Area-Length</th>
<th>M Mode</th>
<th>Truncated-Ellipsoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVM (diastole), mg</td>
<td>114 ± 40</td>
<td>115 ± 43</td>
<td>156 ± 57†</td>
<td>105 ± 39*</td>
</tr>
<tr>
<td>Slope</td>
<td>1.02</td>
<td>0.62</td>
<td>0.89</td>
<td>0.92</td>
</tr>
<tr>
<td>Intercept, mg</td>
<td>-0.3</td>
<td>16.8</td>
<td>34.6</td>
<td></td>
</tr>
<tr>
<td>( r )</td>
<td>0.94</td>
<td>0.89</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Bias ± 2 SD, %</td>
<td>11.1 ± 17.7‡</td>
<td>39.6 ± 45.2</td>
<td>-12.5 ± 16.0‡</td>
<td></td>
</tr>
</tbody>
</table>

\( n \), 31 mice. Slope, intercept, and correlation coefficient (\( r \)) determined by linear regression analyses; bias and error (2 SD) determined by Bland-Altman analyses. *\( P < 0.05 \), †\( P < 0.0001 \), compared with necropsy value; ‡\( P < 0.05 \), compared with M-mode value.
Although our study population was composed of a wide range of LV sizes, shapes, and weights, a single geometric construct was used to obtain two-dimensional, area-length-based LVM estimates (half ellipsoid and half cylinder) (13, 17). With the use of multiple linear regression analysis, we assessed whether differences in end-diastolic size [parasternal short-axis chamber diameter, i.e., LV internal diameter (LVID); parasternal long-axis length (L)], shape (LVID/L), and relative hypertrophy (parasternal short-axis thickness-to-radius ratio, 2T/LVID) could explain the error values (two-dimensional, area-length estimate of LVM – necropsy LV weight). This analysis was performed for each group of animals separately and for all animals combined. As seen in the APPENDIX, there was no difference in the error pattern among the three groups (normal, banded, and transgenic). Two covariates (2T/LVID and L) were identified as being significant, with the following regression equation: %Error = −5.7 + 6.5 (2T/LVID) + 4.1 (L); r = 0.46; P < 0.0001. Because both slope coefficients are positive, two-dimensional, area-length method overestimates LVM at high values of 2T/LVID (relative hypertrophy) and/or L (length) (APPENDIX). This regression relationship, although significant, could explain only 21% of the total variation in error values.

Intraobserver variability for both the area-length method and the M mode-based calculations was small (9.1 ± 8.7% vs. 11.9 ± 13.4%, respectively, end diastole, Fig. 4). No differences in intraobserver variabilities were noted when measuring at end systole versus end diastole. Interobserver variability was significantly larger with M-mode-based measurements than those obtained in area-length-based calculations at end diastole (20.9 ± 15.9% vs. 6.1 ± 6.3%, P < 0.0001) and at end systole (17.4 ± 15.4% vs. 8.7 ± 6.5%, P < 0.05).

DISCUSSION

Genetic modifications are frequently used to produce mice models to investigate the molecular basis of cardiac growth and development (3, 4, 7). These technologies have led to a proliferation of transgenic and knockout mice models displaying a variety of cardiovascular phenotypes. To noninvasively study these phenotypes in a serial manner, it is necessary to develop accurate and reproducible measurements of cardiac morphology and function. This study evaluated the accuracy and reproducibility of echocardiographic M-mode and two-dimensional, area-length estimates of LVM in mice over a wide range of chamber sizes and wall thicknesses. Our main findings were the following: 1) the M-mode method systematically overestimated LVM; 2) mass estimates by the two-dimensional, area-length method were better than those obtained by the M-mode method (significantly reduced bias and error and higher correlation); and 3) although the two-dimensional, area-length method yielded mass estimates that correlated highly with necropsy weights (r = 0.90) and had insignificant bias (<2%), the error of individual estimates was ~25% (from Bland-Altman analysis). Attempts to reduce this inaccuracy by using a more realistic geometric model (truncated ellipsoid) or by covariance analysis of individual errors were unsuccessful.

**M-mode estimates of LVM.** Although transthoracic M-mode echocardiography has been widely used for the assessment of LVM in a variety of animal models (8, 11, 19), it has important limitations that originate from the unidimensional nature of this technique. In our study, M-mode LVM measurements significantly overestimated necropsy weights in most cases (Table 2). Early studies using M-mode echocardiography in mice estimated LVM using the parasternal long-axis view (7, 12), resulting in frequently overestimated measurements, most likely due to the ultrasound beam not being perpendicular to the parasternal long-axis plane. Even with the use of two-dimensional guidance from the parasternal short-axis view at the level of the papillary muscles (2), investigators have reported either overestimation (9, 20) or underestimation of LVM (6), compared with necropsy values.

Overestimation of M-mode mass measurements might also be due to the severe geometric constraints imposed by the cubed formula: both endocardial and epicardial surfaces are full ellipsoids, each having a
long-to-short axis ratio of 2:1. In our study, the long-to-short axis ratio was —2.0 (1.75 ± 0.17, end diastole), consistent with a systematic overestimation of LVM. Similarly, Youn et al. (23) recently found a long-to-short axis ratio <2.1 in 10 normal mice. The assumption of equal long-to-short axis ratio for endocardial and epicardial surfaces results in increased wall thickness at the apex, inconsistent with the mouse LV morphology. This erroneous assumption also contributes to the overestimation of LVM.

Two-dimensional, area-length estimates of LVM. On the basis of previous comparative human and animal studies (7, 9, 17, 23), we anticipated that the two-dimensional, area-length method, in which the LVM is assumed to be bullet-shaped (half ellipsoid with a cylinder on the top), might be more accurate for determining LVM in murine hearts of various sizes and shapes. In this study, the LVM estimations by the two-dimensional, area-length method correlated more closely to necropsy weights than the M-mode calculations. In addition, compared with M-mode estimates, linear regression analysis of area-length LVM produced slopes much closer to one and intercepts closer to zero. Although the transgenic animal group was too small to make definitive conclusions specifically for this subgroup, our study provides data on a large number of animals over a wide range of LVM sizes, and the results are consistent with recent findings obtained in a small number of normal (23) and hypertrophied mice (2). Importantly, the bias for the two-dimensional, area-length method was significantly smaller than that of the M-mode measurements.

We hypothesized that because the endocardium of the interventricular septum and posterior walls is usually best visualized at end systole, wall thickness measurements performed at this time in the cardiac cycle could possibly result in improved accuracy. Moreover, any error in measuring the larger end-systolic thicknesses would be a smaller proportion of that measurement. However, irrespective of the method, LVM estimates were similar at end systole and end diastole.

In the present study, two-dimensional, area-length-based mass estimates were highly reproducible, as reflected by intraobserver variabilities around 10% and even lower interobserver variabilities, which were also less than one-half those of M-mode-based values. The M-mode variability for LVM estimation between readers in our study was similar to that found by Hoit et al. (7) and Tanaka et al. (20) who found an attributable error of 25–30%, predominantly as a result of wall thickness measurement error.

We suspected that recent technological advances contributed to the superior performance of the two-dimensional, area-length method. To obtain high-quality echocardiographic images in mice, it is necessary to use high-frequency ultrasound transducers that have higher spatial resolution than predecessor probes and are expected to result in reduced errors (17). The linear probe used in this study has the additional advantage of not requiring a standoff, which by itself may become a source of error (4). Finally, the higher frame rate obtained with this linear transducer (~120 Hz) allowed increased sampling throughout the cardiac cycle, which is critical in small animals with high heart rates. As our results show, these technological improvements provided an image quality suitable for LVM measurements in nearly all mice.

Attempts to improve the two-dimensional area-length estimates of LVM. Despite the fact that the two-dimensional, area-length method was more accurate than the M-mode method in a variety of mouse models, significant individual errors were still noted between necropsy weights and estimated LVM. We examined whether individual errors could be improved using two approaches. First, to allow more geometric flexibility, the truncated ellipsoid formula, which places the minor axis more basally, was applied. We found this method underestimated LVM and did not improve error values significantly (Table 3) thus providing no obvious advantage over the two-dimensional, area-length method and having the disadvantage of more complex data acquisition. Second, multiple linear regression was performed to identify covariates that could account for observed errors. Although we identified two significant covariates, they accounted for only 21% of the total variance in the error. In brief, both approaches to improve the performance of the two-dimensional, area-length method did not yield positive results, suggesting that the errors originate from basic measurement inaccuracies. Indeed, the theoretical analysis, described in detail in the Appendix, predicted an error of 20% due to measurement inaccuracy, in agreement with the errors we obtained.

Implications for future studies. On the basis of the above analysis of sources of error, the major implication of our results is that further improvement in accuracy would come from improvements in measurement instrumentation. Furthermore, our sample size calculations indicate that the lower levels of variability of the area-length method provide an additional advantage over the M-mode method, such that future studies would require smaller animal groups to detect significant differences. However, the specific group sizes should be interpreted cautiously and adjusted accordingly when the effects of surgical or genetic treatment on LVM may not be uniform in all animals, resulting in larger-than-natural variance.

Alternative methods. Noninvasive assessment of LVM, as well as cardiac function, can certainly be accomplished in mice by other means. Magnetic resonance imaging and ultrafast computed tomography scanning offer accurate estimation of LVM (5, 14, 21), but their use may be limited by expense and availability. Three-dimensional echocardiography, a fervent goal of cardiac imaging, offers a theoretical potential for assessing hearts of widely varying sizes and pathologies (16). Transesophageal imaging of the murine heart with an intracardiac probe has been used but is limited by low-frame rates (15).

The present study, performed on a large number of mice with a wide range of chamber sizes, demonstrates that transthoracic echocardiographic imaging of the
murine heart, using improved transducer technology, can be used to accurately and reproducibly estimate LVM with the two-dimensional, area-length formula. This methodology will allow serial assessment of cardiac phenotypic changes in a variety of mouse models. Our results suggest that even in animals with uniform ventricular geometry, the use of the two-dimensional, area-length technique should be positively considered in view of its increased accuracy, markedly decreased intra- and interobserver variabilities as well as potentially smaller sample sizes necessary to demonstrate differences between groups. Further improvements in accuracy are likely to come from modification in instrumentation rather than from refinement of geometric models.

APPENDIX

Predicted Uncertainty in LV Mass Estimates Due to Measurement Inaccuracies: A Theoretical Analysis

The two-dimensional, area-length formula (see text) uses three measured variables to calculate the LV mass (LVM) estimate: chamber length (L), epicardial short-axis area (A1), and endocardial short-axis area (A2). Thus the measurement inaccuracy in each of these measurements will contribute to the uncertainty in LVM estimate. As a first approximation, assume that the short-axis areas (A1 and A2) are related to linear dimensions (D1 and D2) via a circular geometry (i.e., A1 = \( \pi D_1^2 / 4 \) and A2 = \( \pi D_2^2 / 4 \)). With this assumption, the computed wall thickness (T), is equal to \( (D_1 - D_2)/2 \). The two-dimensional, area-length formula can now be written as follows

\[
LVM = 1.05 \left[ \frac{5}{6} \left( \frac{\pi D_1^2}{4} \right) \left( L + \frac{D_1 - D_2}{2} \right) - \frac{5}{6} \left( \frac{\pi D_2^2}{4} \right) \right] \quad (A1)
\]

Algebraic simplification of Eq. A1 yields

\[
LVM = 0.6872(D_L^2 + 0.5D_1^2 - 0.5D_2^2 - D_2^2L) \quad (A2)
\]

LVM is a function of three variables \((D_1, D_2, \text{ and } L)\), and therefore the predicted uncertainty in LVM (\( \Delta LVM \)) can be expressed in terms of the measurement inaccuracies of \( D_1, D_2, \) and \( L (\Delta D_1, \Delta D_2, \text{ and } \Delta L, \) respectively) as follows

\[
\Delta LVM = \left| \frac{\partial LVM}{\partial D_1} \right| \Delta D_1 + \left| \frac{\partial LVM}{\partial D_2} \right| \Delta D_2 + \left| \frac{\partial LVM}{\partial L} \right| \Delta L \quad (A3)
\]

where \( | \cdot | \) denotes absolute value and \( \delta LVM/\delta D_1, \delta LVM/\delta D_2, \text{ and } \delta LVM/\delta L \) are partial derivatives given by (from Eq. A2):

\[
\frac{\partial LVM}{\partial D_1} = 0.6872(2LD_1 + 1.5D_1^2 - D_1D_2) \quad (A4)
\]

\[
\frac{\partial LVM}{\partial D_2} = 0.6872(-0.5D_1^2 - 2D_2L) \quad (A5)
\]

\[
\frac{\partial LVM}{\partial L} = 0.6872(D_1^2 - D_2^2) \quad (A6)
\]

With the use of Eqs. A4–A5, one can calculate the partial derivatives at nominal values of \( L, A_1 \) (equivalently, \( D_1 \)), and \( A_2 \) (equivalently, \( D_2 \)) (Table 1). These derivative values can then be substituted in Eq. A3 to obtain the uncertainty in LVM. Typically, the lateral resolution in a two-dimensional image is much lower than the axial resolution, resulting in larger uncertainties in \( D_1 \) and \( D_2 \) (which are calculated from \( A_1 \) and \( A_2 \)) than that in \( L \). However, as a first approximation, we assume that uncertainties in all three variables are the same and equal to the axial resolution corresponding to the imaging frequency (\( \Delta D_1 = \Delta D_2 = \Delta L = \text{axial resolution} \)). This will yield an optimistic estimate of LVM uncertainty; the real value will be greater.

REFERENCES


