Accurate Volume Determination in the Isolated Ejecting Canine Left Ventricle by Two-dimensional Echocardiography

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SUMMARY Two-dimensional echocardiography can provide serial cross-sectional images of the left ventricular cavity. We examined whether such serial images from steady-state ejecting hearts would allow three-dimensional reconstruction and accurate volume estimation without major geometric assumptions. Cross-circulated, paced dog hearts were suspended in a blood-filled tank. Serial cross-sectional images were taken at 3-mm intervals along the vertical axis. Left ventricular cavity and muscle areas of each image were planimetered with a light-pen system and summated for volume: total volume = \( \Sigma \) (areas \( \times \) 3 mm). Direct left ventricular volume was measured through the cardiac cycle with a volumetric chamber connected to a balloon in the ejecting left ventricle. In six hearts, 67 separate direct volume measurements (range 9.5–54.7 ml) from various points in the cardiac cycle were compared with the simultaneous echo volume measurements. By least squares linear regression, echo volume = 1.01 (direct volume) – 0.44 ml; \( r = 0.972, \) SEE = 2.93 ml. Provided accurate cross-sectional localization is available, these studies suggest that extremely accurate steady-state left ventricular volume can be determined noninvasively in the ejecting heart from multiple cross-sectional images.

ACCURATE ASSESSMENT of cardiac ventricular volume has been found to be important in evaluating ventricular function. However, the methods of volume determination in the past have been invasive, have required major assumptions about ventricular geometry, and have altered ventricular function. Single-beam (M-mode) echocardiography provides a noninvasive technique that does not alter cardiac performance, but methods currently in use for echocardiographic determination of ventricular volume rely heavily on assumptions of simple geometry. Unfortunately, many cardiac diseases deform the heart so that the geometric assumptions used may not be valid. Two-dimensional echocardiography can overcome many of these difficulties because it has the potential to provide accurate volume measurements without reliance on major geometric assumptions.

Previous investigators have relied on postmortem hearts to validate angiographic methods, which in turn have been used for the comparison of echocardiographic methods. A more suitable standard for comparison is provided by the isolated ejecting canine heart preparation, which allows continuous direct intraventricular volume measurements under a variety of loading conditions.

By combining the two-dimensional echocardiogram with the knowledge of transducer position and orientation, it should be possible to reconstruct a three-dimensional representation of a chamber and thereby measure volume. In the present study, we test the feasibility and accuracy of two-dimensional echocardiographic volume determination without reliance on geometric assumptions, by comparing this technique with direct volume measurements, first in smooth-surfaced objects with simple geometry (thin-walled balloons), next with static but irregular images (formalin-fixed hearts) and, finally, in the isolated ejecting canine heart preparation. We show that accurate two-dimensional echocardiographic volume determination is feasible in both static and working preparations.

Materials and Methods

Ultrasonic Tank and Imaging

Ultrasonic images were obtained with a wide-angle, phased-array, two-dimensional echocardiograph (Varian V-3000) using a 2.25 MHz transducer. Images were recorded at 60 frames/sec on 1-inch video tape for further quantitative analysis.

A specially built tank was used for all ultrasonic recordings. This tank consisted of a plastic chamber 25 cm square by 30 cm deep (fig. 1). On one corner was mounted a 3-cm wide ultrasound-lucent window (Mylar) and an adjustable transducer mounting device that held the transducer in a horizontal position and allowed only vertical movement by means of a screw drive. The vertical position could be measured to the nearest 1 mm by calibration marks on the mounting device. Inside the plastic tank opposite the echo window was placed an L-shaped insert measuring 12.5 cm on a side with an ultrasound-lucent front. This insert was filled with mineral oil and served as an ultrasonic baffle to reduce reverberations from the rigid plastic
walls. This left a $12.5 \times 12.5 \times 30$ cm fluid-filled reservoir in front of the baffle in which suspended objects could be examined echocardiographically.

**Thin-walled Balloons**

Thin-walled latex balloons (Trojans #70) were filled with saline and closed at the open end with suture material. The balloons were then suspended in the ultrasonic chamber and horizontal echocardiographic slices were recorded at 3-mm intervals through the vertical length of the balloon (fig. 2). The fluid-filled balloon was then weighed, emptied of contents, dried, and reweighed. The difference in weights divided by the specific gravity (1.006 g/ml) gave the volume of saline in the balloon.

**Figure 1.** Ultrasonic imaging tank. The transducer is held against an ultrasound-lucent (Mylar) window upon which vertical motion of the echocardiographic transducer is regulated and quantified with a calibrated, screw-drive mount. Behind the Mylar window is a reservoir in which objects for echocardiographic imaging are submerged in blood or saline as appropriate. Behind this reservoir is a mineral oil-filled compartment that serves as an ultrasonic baffle.

**Figure 2.** Method for obtaining serial tomographic cross-sectional images for fluid-filled balloons. Echocardiographic images as shown in the three examples here are recorded every 3 mm along the vertical axis and digitized for reconstruction of balloon volume.
Formalin-fixed Canine Hearts

Four formalin-fixed canine left ventricles were trimmed at the atrioventricular groove, suspended in the tank, and imaged at 3-mm intervals over the vertical length as described above. These hearts were then suspended in air and ventricular size was measured directly by filling the ventricular cavity with a known amount of water from a graduated cylinder. Myocardial volume was measured by fluid displacement.

Isolated Ejecting Hearts

The preparation consisted of an isolated cross-circulated canine heart containing a balloon in the left ventricle attached to a volumetric chamber for direct volume determination (fig. 3). Six pairs of adult mongrel dogs (20-25 kg) were anesthetized with sodium pentobarbital (25 mg/kg i.v.). The chest of the first dog was opened under mechanical ventilation and cannulae were placed in the subclavian artery and right atrium. These cannulae were then connected to the femoral artery and femoral vein, respectively, of the second dog, which served as a support animal for the heart of the first dog. The aorta, brachiocephalic artery, superior and inferior venae cavae, ayzgos veins, and pulmonary hila were ligated and the heart was removed from the dog's chest. Coronary arteries were perfused by retrograde flow through the aorta. The right and left ventricles of the isolated heart were vented to air and the atria were opened. Coronary venous and thebesian blood was collected by a reservoir for return to the support dog.

A thin latex balloon (Qualatex #9) was attached at its base to a nylon mounting device, which was secured by a pursestring suture in the mitral annulus (fig. 3). The neck of the balloon was pulled through the apex. The balloon was connected through the mitral valve to the aortic valve. Changes in the volume of the intraventricular balloon resulted in changes in the fluid level in the volumetric chamber, and were sensed as a changed conductance between the electrodes. This system has been shown to be accurate to 0.5 ml over a 100-ml range and allows instantaneous left ventricular volume determination throughout the cardiac cycle. The loading conditions of the heart were determined by the pressure in the air column over the fluid level in the volumetric chamber. By altering this pressure, the heart could be made to eject over a wide range of volumes. The direct volume was monitored by a strip chart recorder (Brush Model #480), converted on-line to digital form at 5-msec intervals (Analogic A/D converter model) and stored on magnetic tape (Nova 1220 computer).

This isolated heart preparation was suspended in the ultrasonic chamber described above (fig. 1). The tank was filled with blood and served as a reservoir for venous return to the support dog. As the heart was paced at a constant rate, direct volume was continuously recorded while the echo transducer was moved vertically through the length of the ventricular chamber. Transducer movement was interrupted at 3-mm intervals, and the pacing stimulus used to synchronize simultaneous data on the echocardiographic and direct volume recordings. Using this method, a set of echocardiographic recordings over the entire length of the ejecting ventricle could be obtained in less than 2 minutes. Direct volume at any given time in the cardiac cycle varied less than 2%. After recording one set of echocardiographic and direct volume data over the length of the ventricle, the loading condition was changed and recordings over a different range of volumes were obtained.

Data Analysis and Statistical Methods

The direct volume measurement corresponding in time to each echocardiographic image could be iden-
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Figure 4. Method for serial reconstruction of left ventricular cavity and myocardial volume in the isolated ejecting heart. Serial tomographic images are recorded every 3 mm along the vertical axis of the stable beating preparation. Four representative images from different levels are shown here. Endocardium and epicardial margins are digitized to give planimetered area within the endocardial and epicardium, respectively. The 3-mm serial slices are summated to reconstruct ventricular volume and mass.

Two-variable, least squares linear regression analysis.10 The paired t test was used for comparison of group means.11

Results

Thin-walled Balloons

Eleven balloons ranging in volume from 50–215 ml (mean 119 ml) were studied for echocardiographic volume determination as described above for the isolated hearts. Over this range of volumes there was a close linear correlation (r = 0.999) between echo volume and directly measured volume (fig. 5). The least squares linear regression equation for echocardiographically determined volume on directly measured volume was $V_{echo} = 1.06 V_{direct} - 6.95$, where $V_{echo}$ is volume by echo, and $V_{direct}$ is volume by direct measurement (SEE = 2.36 ml).

Formalin-fixed Canine Hearts

Four canine left ventricles weighing 92–112 g (mean 96 g) had directly measured cavity volumes of 9.9–46 ml (mean 29.4 ml). The equation for the least squares linear regression of echo volume on directly measured volume was: $V_{echo} = 1.03 V_{direct} - 0.49$ (r = 0.998, SEE = 1.31 ml). Myocardial volume measured by fluid displacement for these four hearts ranged from 72–98 ml (mean 85.6 ml). The equation for the least squares linear regression of echocardiographically measured myocardial volume on directly measured myocardial volume was: $V_{echo} = 1.01 V_{direct} + 2.64$ (r = 0.978, SEE = 3.01 ml).

tified using the pacing stimulus as a marker. Direct volume was digitized at 200 Hz and the echocardiogram recorded at 60 Hz so that simultaneous data could be obtained at 50-msec intervals. The selected frames of the echocardiographic recordings from thin-walled balloons, formalin-fixed hearts, and ejecting hearts were then digitized using a computerized light-pen system (Varian Instruments, Palo Alto, California) programmed to give planimetered area (fig. 4). Ventricular volume of the ejecting hearts at the specific time in the cardiac cycle was equal to the sum of the planimetered area for each slice at that time $\times$ 0.03 cm, the distance between slices. Measurements of areas were made in duplicate or triplicate and averaged. Data were accepted for analysis only if the measured direct volume during the entire run did not vary more than 0.5 ml at any given time in the cardiac cycle. The direct volume measurements corresponding to each of the 16–21 echocardiographic slices were averaged for comparison to the volume of the summed slices.

Using the above method, echocardiographically determined volumes were calculated through the cardiac cycle at regular intervals of 50 or 100 msec, depending on heart rate, which was held constant at 80–160 beats/min. In each case the last volume determined in the cardiac cycle was within 50 or 100 msec, respectively, of the end of the cardiac cycle, defined here as the onset of the next pacing stimulus.

To disprove the null hypothesis that a significant correlation is lacking between volumes measured echocardiographically and directly, volumes determined by the two techniques were compared using
Isolated Ejecting Hearts

Volume determinations were made at six to eight points through the cardiac cycle on six isolated ejecting left ventricular preparations. In three of these preparations, loading conditions were changed as previously described and the determinations were repeated over the new volume range. This produced a total of 67 volume measurements over a range of ventricular cavity volumes of 9.4–54.7 ml (fig. 6). The correlation of echocardiographically determined volume with directly measured volume was high \( r = 0.972 \), and the slope of the equation for least squares linear regression approached unity, with \( V_{\text{echo}} = 1.01 V_{\text{direct}} - 0.44 \) (S.E.E = 2.93 ml). The ejection fraction of these hearts ranged from 13–52% (mean 31%).

Figure 7A shows the relationship between echocardiographically and directly measured cavity volume for one of these hearts. At each of the eight points in the cardiac cycle there was close agreement between the two measurements. To determine whether echocardiographically measured ventricular volume was likely to differ systematically from the directly measured volume during any part of the cardiac cycle, average difference between the two measurements for the six hearts was plotted vs percent time of the cardiac cycle (fig. 7B). During the time from 20–50% of the cardiac cycle, echocardiographic measurement of ventricular volume was less than direct volume measurement by 7.2% (2.33 ± 2.14 ml, mean ± SE), and at three of these times the differences were significant (fig. 7B). Maximal discrepancy between echocardiographic and direct volume measurement occurred during the maximal rate of change in cavity volume.

\[ V_{\text{echo}} = 1.01 V_{\text{direct}} - 0.44 \]


**Figure 7. A)**Comparison of simultaneous echocardiographic and direct measurements of ejecting ventricular volume throughout the cardiac cycle as percent of heart-time for a single, steady-state, ejecting canine heart. Volume (ordinate) is shown as a function of time (abscissa). The solid line indicates direct volume measurements, the broken line indicates echocardiographic measurements. **B** Difference between echocardiographic and direct volume measurements for six ejecting hearts (nine separate loading conditions). Measurements (mean ± SEM), given as a function of time in the cardiac cycle on the abscissa, are normalized to 100%. Paired *t* test.

Smaller differences were shown between values obtained at end-systole and end-diastole, and differences between ejection fractions calculated by the two methods were not statistically significant (28.8 ± 10.1% by echo vs 31.4 ± 11.5% direct). The graph of the equation for the least squares linear regression of ejection fraction by echo on directly measured ejection fraction shown in figure 8 shows the close correlation between values by the two methods (*r* = 0.921).

To validate echo measurement further, constancy of mass was tested by measuring left ventricular myocardial volume for each heart at various times in the cardiac cycle (fig. 9). As would be predicted, the measured myocardial volume of each ejecting heart remained relatively constant through the cardiac cycle. Maximal variation was 5.3 ± 2.1% (mean ± SD, NS). To determine the correlation between echocardiographically measured myocardial volume and myocardial mass, the product of the mean value for echocardiographically measured myocardial volume and the specific gravity of cardiac muscle (1.063 g/ml) was compared with the weight of the corresponding left ventricular free wall and septum for each of the six heart preparations. This gave a mean mass for the six hearts of 99.9 ± 16.6 g (mean ± sd) by echo vs 108.1 ± 24.4 g direct (NS).

**Discussion**

In this study, we determined the accuracy of volume measurements in the ejecting left ventricle with two-dimensional echocardiographic techniques. First, we defined the accuracy of two-dimensional echocardiography as a technique of volume measurement in
smooth-surfaced objects with simple geometry. This was done using thin-walled, fluid-filled balloons over a range of volumes representative of those expected in the normal human left ventricle. The close linear correlation (fig. 5) between echocardiographic volume and directly measured volume shows the accuracy of echocardiographic volume measurement under ideal conditions of smooth surfaces.

Second, we studied volume measurement of the formalin-fixed canine left ventricle, which required only static images but presented an irregular surface. Again, using the method of serial reconstruction of cross-sectional images taken at 3-mm intervals, a remarkably close correlation is obtained between echocardiographic volume and directly measured ventricular volume. The regression equation has a slope approaching unity and an intercept close to the origin.

Finally, because clinical research applications of two-dimensional echocardiography are dependent on assessing volume in the beating heart, we focused our major effort on the isolated ejecting heart preparation. We chose this preparation because it offers the most accurate model currently available in which simultaneous direct volume measurements of the ejecting left ventricle can be obtained as a standard against which to compare the measurements obtained echocardiographically. The accuracy of the direct volumetric chamber method of ventricular volume measurement has been shown previously to be 0.5 ml over a 100-ml range.

A technique allowing the heart to remain intact in the chest would be preferable; however, no such method that allows simultaneous direct volume measurement is currently available. Other methods, which depend upon direct measurement of postmortem ventricular volume or comparison with angiographically derived volumes that depend on assumptions of simple geometry, have clear limitations as a standard against which new noninvasive techniques may be compared.

Two-dimensional echocardiography provides accurate volume determinations in the working heart. The tedious method whereby volumes are calculated from serial cross-sectional images need not detract from its potential value, because with advances in both echocardiographic and computer technology much of this tedium may be minimized. Additionally, further studies should reveal if specific geometric assumptions and models may be applied to increase the ease of application without sacrificing accuracy. Although the ventricular volumes from ejecting canine preparations are smaller than the range expected in the adult human, the accuracy of volume determination in this study for fluid-filled balloons of 100–200 ml suggests that we can apply our conclusions to include ejecting ventricles with volumes in the human range.

Although echocardiographic measurements consistently underestimated direct volume by approximately 7% during the ejection phase of the cardiac cycle, when the rate of volume change was greatest, the accuracy of the echocardiographic method for measurement of volume at end-diastole and end-systole was high. This finding is consistent with the observation that during rapid ventricular contraction, endocardial margins of individual video frames often are blurred and more difficult to digitize than frames from end-diastolic and end-systolic parts of the cardiac cycle, when rates of volume change were less rapid. No statistically significant differences could be shown between ejection fractions determined simultaneously by the two methods.

In the present study, the echocardiographic measurement of myocardial volume was less accurate than measurement of chamber volume. Presumably, this is a result of either poor resolution of the epicardial margin or loss of certain areas of myocardium from the echocardiographic image. We do not know if accuracy in measurement of myocardial volume would be equally decreased in the closed-chest, intact model; major improvements may result from further advances in ultrasonic technology.

In conclusion, we have shown that, without reliance on geometric assumptions, two-dimensional echocardiographic determination of left ventricular volumes in the ejecting heart is feasible and remarkably accurate. An ejecting isolated heart preparation that measures volumes independent of geometric assumptions has been used as the standard for comparison. Provided enough sections can be obtained, and transducer position is known, accurate, noninvasive ventricular volume determinations can be made in man.

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