Fetal Ventricular Mass Determination on Three-Dimensional Echocardiography
Studies in Normal Fetuses and Validation Experiments

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**Background**—Estimation of ventricular volume and mass is important for baseline and serial evaluation of fetuses with normal or abnormal hearts. Direct measurement of chamber wall volumes and mass can be made without geometric assumptions by 3D fetal echocardiography. Our goals were to determine the feasibility of using fast nongated 3D echocardiography for fetal volumetric and mass assessments, to validate the accuracy of the ultrasound system and the measurement technique, and if satisfactory, to develop normal values for fetal ventricular mass during the second and third trimesters.

**Methods and Results**—This was a prospective outpatient study of 90 consecutive normal pregnancies during routine obstetric services at Oregon Health & Science University (Portland). Optimized 3D volumes of the fetal thorax and cardiac chambers were rapidly acquired and later analyzed for right and left ventricular mass by radial summation technique from manual epicardial and endocardial traces. Experiments to validate the ultrasound system and measurement technique were performed with modified small balloon models and in vivo and ex vivo small animal experiments. Our study established the feasibility of fetal ventricular mass measurements with 3D ultrasound technology and developed normal values for right and left ventricular mass from 15 weeks’ gestation to term.

**Conclusions**—Nongated fast 3D fetal echocardiography is an acceptable modality for determination of cardiac chamber volume and mass with good accuracy and acceptable interobserver variability. The method should be especially valuable as an objective serial measurement in clinical fetal studies with structurally or functionally abnormal hearts. (Circulation. 2004;110:1054-1060.)

**Key Words:** echocardiography, fetal ■ imaging ■ fetus ■ heart defects, congenital

Most congenital heart disease (CHD) is believed to be multifactorial in causation and occurs at an incidence of 8 of 1000 live births.1,2 Cardiac embryogenesis is a complex process with multiple genetic, epigenetic, and morphodynamic contributors. Fetal heart size could be affected by cardiac disorders of structure (eg, Ebstein’s anomaly), functional disorders (eg, fetal cardiomyopathy), or extracardiac factors (eg, extrinsic compression from a diaphragmatic hernia). Although structural and functional defects are often obvious prenatally and addressed postnatally, there are gaps in our understanding of the in utero development of many aspects of CHD. Cardiac dimensions and Doppler measurements have served as objective estimates that are commonly used to ascertain severity, prognosis, termination counseling, or the nature and timing of intervention. Subjective eyeball impressions often guide management, risk stratification, and prognosis-based counseling in a variety of CHDs such as hypoplastic right (RV) or left ventricle (LV), atrioventricular or ventriculoarterial valve stenosis, and complex CHD. Two-dimensional and M-mode–based studies have established a range of chamber dimensions in the normal5–7 and abnormal fetal heart.8 Simple 1D or 2D measurements, however, may appear within normal limits or pseudonormal in early and mid-gestation in fetuses with severe forms of CHD.9,10

The shape of the developing heart has been discussed in terms of morphodynamics and selective advantage of flow patterns in the asymmetry of cardiac looping.11 Logically, as the chamber shape evolves to improve mechanical advantage, the developing ventricular walls face differential strains and shear forces. Determination of normal ventricular mass and its comparison in abnormal hearts can give insight into myocardial response to such intracavitary events. Fetuses with intrauterine growth restriction may be predisposed to adult-onset hypertension and heart disease according to the concept of fetal programming through ill-understood persisting metabolic, physiological, and endocrine (mal)adapt-
tive mechanisms. Understanding fetal cardiac response to increased placental afterload could help in understanding the developmental origins of adult heart disease.

Three-dimensional ultrasound as a tool for volumetric assessments of fetal organs has previously shown good interobserver and intraobserver variability (≈2%), and cardiac mass measurements by reconstructed 3D cardiac ultrasound have been validated in an extensive in vitro experiment simulating adult-sized hearts. More recently, rapid-acquisition, nongated 3D fetal echocardiography has provided improved spatial understanding of the developing heart.

The goal of our study was to determine the feasibility of using fast nongated 3D echocardiography for fetal volumetric and mass assessments. Because measurements are in the range of fractions of milliliters and grams, we also sought to validate the accuracy of the ultrasound system and measurement technique applied. We intended to develop preliminary nomograms of normal values for fetal ventricular mass during the second and third trimesters after due validation experiments.

Methods

Fetal 3D Echocardiography

Patients

Ninety consecutive pregnant women attending the Perinatology Services of Oregon Health & Science University were studied after they provided informed consent. Indications for ultrasound were confirmation of gestational age and fetal growth. Other indications were advanced maternal age, abnormal screening markers, previous obstetric complications, history of siblings with anomalies, or suspicion of minor anomalies. Women with multiple gestation or obstetric complications, history of siblings with anomalies, or gestational diabetes and fetuses with major congenital or chromosomal anomalies were excluded. Routine obstetric ultrasound at the unit was followed by the 3D part of the study. This was a cross-sectional multiethnic study, and serial studies in the same patient were not obtained.

Gestational age was calculated from the first date of the last menstrual period and confirmed by ultrasonography. Estimated fetal weight was computed according to Hadlock’s formula on the basis of biparietal diameter, abdominal circumference, and femur length.

Ultrasound System

The ultrasound system used for all 3D and most 2D studies was the Voluson 730 (GE Medical Systems, Kretz Ultrasound). A 4- to 8-MHz variable-frequency wide-band motorized transducer with a convex array (65×55 mm) suitable for abdominal examinations was used. The maximum bandwidth (≈20 MHz) was 2 to 7 MHz. With 192 elements and a volume sweep radius of 27 mm, the maximum scanning angle was 80° to 75°. Thus, the maximum scanning volume was 0.360 L; the minimum was 0.025 L. The maximal voxel density was ≈1 million voxels per volume, and maximal frame rate was ≈20 Hz (average of 8 to 15 Hz for limited volume acquisitions). For single-sweep volume acquisition, the sweep is automatic, so the activated probe sweeps an arc encompassing the predetermined volume. This is recorded as a single data set in any plane. Secondary rotation or slicing was then feasible. In this way, the clearest possible views were obtained for analysis.

Postacquisition Image Processing

Image processing was performed offline with 3D View software, which is compatible with Microsoft-based computers. Each volume data set was imported into the application and manipulated by rotating, angulating, and slicing in any of the 3 displayed orthogonal planes, which would simultaneously reset views in the other 2 planes. A central reference point could be selected by moving through any plane. Secondary rotation or slicing was then feasible. In this way, the clearest possible views were obtained for analysis.

Virtual organ computer-aided analysis combines 3D ultrasound tissue, presented as voxels, and the geometric information embedded in a 3D data set to create a surface geometry by 3D triangulation of traced 2D contours. Volume measurements are performed by integration of polygon areas generated by rotation in incremental steps of 6° to 30° around a fixed axis for a complete 360°:

\[ \text{Volume} = \sum_{i=1}^{N} \frac{2N}{1} \cdot TA_i \cdot d_s \]

where \( N \) is number of marked contours; \( TA_i \) (i = N), either (1) the half-plane polygon area in plane i, or (2) i = N, half-plane polygon in area; and \( d_s \), the distance between the center of the mass of \( TA \) and rotation axis of the contour.

While tracing is being done in one plane, the software reconstructs the traced points in orthogonal planes, providing a 3D reference that improves accuracy. Hence, measurements are made on the ‘‘original’’ echo slices and not from a reconstructed model. Myocardial volume of each ventricular wall was the difference between epicardial and endocardial shells. Ventricular mass was equal to the volume multiplied by myocardial density, which was assumed to be 1.05 g/cm³ across all gestational ages. The 3D acquisition was not gated; hence, the cardiac phase could not be determined. However, rapid acquisition within fractions of a second virtually ‘‘froze’’ the fetal heart, so most images appeared to be in diastole. Although end-systolic and end-diastolic ventricular cavity volumes would differ substantially, wall mass should be nearly the same, so we could use unprocessed data for mass calculations. End-diastolic myocardial mass may exceed end-systolic myocardial mass because of diastolic intramyocardial sinusoidal blood, but this is likely to be a very small amount.

Clinical Cardiac Volume Acquisition

Once fetal lie was determined and an optimal view of the fetal thorax was established with high-resolution 2D imaging, the volume/region of interest was optimized in terms of scanning depth, angle, transmit focus, and line density. Acquisition was not gated, so maternal and fetal cardiorespiratory events did not influence transducer activation. The volume thus acquired was displayed on the screen as 3 orthogonal views, which most often showed little motion in the fetal heart walls or valves. Most often, the fetal heart appeared ‘‘frozen’’ in mid-diastole. Smaller volumes meant quicker acquisition and reduced the likelihood of temporal distortion from fetal cardiorespiratory motion. A maximum of 8 scans or 10 minutes was used to complete scanning.

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Validation Experiments

Balloon Model
In vitro double-walled customized miniature balloons were imaged in 2 series of volume subsets to simulate volumes and masses seen in middle to late gestation. An inner balloon containing a known "volume" was surrounded by an intervening premeasured layer of gel simulating "mass" within an outer balloon. The inner balloon was filled with 5 to 25 mL water (volume) and 4 to 10 mL gel (mass) inserted between the 2 balloons, attached to a pump mechanism (modified rodent ventilator), suspended in water in a Perspex bath, and imaged through a latex window in the side of the bath. This apparatus was run at 120 bpm. The same technique of region-of-interest optimization, image acquisition, and tracing was used in all validation methods on the same Voluson 730 ultrasound system. Tracings and measurements were performed by 3 blinded operators after they had been instructed by the principal investigator. Specifically, mass tracings were done from the outer edge of the inner balloon to the inner edge of the outer balloon, similar to the Penn convention of mass estimation.20 One reader was common to the balloon validation experiments, the mean percentage difference was calculated as [(observed volume – actual volume) / actual volume] × 100. Thus, a positive absolute value or percentage indicated an overestimation, whereas a negative value indicated an underestimation.

Animal Model
In vivo and ex vivo studies in small animal hearts were studied to introduce the elements of cardiorespiratory motion and variable tissue windows in the validation process. Adult rats were selected as a small animal model and rabbits as a midsized model to simulate the range of human fetal heart volumes through middle to late gestation, respectively. All animal experiments were conducted in accordance with institutional guidelines for live vertebrate studies. Eight rats were anesthetized with 2% isoflurane, followed by in vivo 3D transthoracic imaging after the chest was shaved and prepared. Higher preterminal isoflurane concentrations lowered heart rates from a baseline of ~250 bpm to the mid-100s and was then further increased to euthanize the animals. The atria were dissected along the atroventricular sulcus; great arteries were removed just below the semilunar valves. The resulting biventricular preparation was weighed in a chemical balance and then suspended in a saline bath at room temperature for 3D ex vivo imaging with the same Voluson 730 ultrasound system.

Results

Fetal 3D Echocardiography
Case Yield and Feasibility
There was no preselection of cases on the basis of 2D image quality; ie, even women with difficult or suboptimal 2D studies were accepted for 3D imaging and analysis. Of the 90 studies performed, 15 could not be quantitatively analyzed because the data sets could not be satisfactorily processed and traced. There were 3 studies (11%) in women at <20 weeks’ gestation (n=27), 7 studies (18%) in women at 20 to 28 weeks’ gestation (n=37), and 5 studies (18%) in women at 32 weeks’ gestation to term (n=26) that could not be analyzed. Thus, the measurable studies ranged in gestation from 15.5 to 37 weeks.

There was definitely a learning curve component to data manipulation, and considerably more time was spent in the volumes acquired early in the study period (~20 minutes versus 3 to 5 minutes later), which was attributable to familiarization with software and spatial reorientation. Likewise, in the second half of the study, the number of inadequate studies was halved through use of harmonic methods, particularly in spine-down primary windows. Internal comparison of our data showed a clear linear correlation between gestational age and estimated fetal weight, thus confirming a reliable distribution.
**Ventricular Myocardial Mass**

Both RV and LV masses were obtained from each adequate image set. Myocardial mass was compared with gestational age and estimated fetal weight. There was a linear correlation between RV mass and gestational age (r²=0.83) and weight (r²=0.79) and between LV mass and age (r²=0.81) and weight (r²=0.82) (Figure 2). There seemed to be an exponential increase in ventricular mass with age [RV mass=0.029⁰⁰⁰¹⁰⁰×age (r²=0.78); LV mass=0.02⁰⁰⁰⁰⁹⁸¹⁰⁰×age (r²=0.81)]. On comparing the exponential and quadratic polynomial curves of each ventricular mass estimation with gestational age, there seemed to be a change in slope at 27.5 weeks, which corresponded to 1300-g estimated fetal weight in our data. Unsure of the implications of this observation, we separately analyzed our data before and after 27.5 weeks. Intraobserver variability, when performed by the primary investigator on randomly selected clinical fetal studies (n=10), showed a variability of 2%. Interobserver variability on clinical data, after a second untrained observer (G.H.) was instructed, was <10%.

**Validation Experiments**

**Balloon Model**

There was good correlation of volume (r=0.90) and mass (r=0.87) assessments. For the balloon volumes, the mean percentage difference between observer calculations and the actual volume varied from 3.2% (observer 1) to 6.4% (observer 2) to 2.8% (observer 3) (Table 2 and Figure 3); positive percentages indicate observer overestimations. Minimum discrepancies by the more experienced third observer (G.H.) were 2.8±10.6%. Maximum overestimation errors were noted at the lowest volumes of 5 mL, and the fewest errors were seen in mid-volumes of 10 to 15 mL. Interobserver variability was between 1.7% and 4%.

**Animal Model**

There was no significant difference between measured and explanted biventricular mass, either statistically or on Bland-

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**TABLE 2. Mean Percentage Difference in Interobserver Mass and Volume Estimations**

<table>
<thead>
<tr>
<th>Observer</th>
<th>Actual mass, mg</th>
<th>Actual volume, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observer 1, %</td>
<td>Observer 2, %</td>
<td>Observer 3, %</td>
</tr>
<tr>
<td>4 (n=12)</td>
<td>13.6±30.6</td>
<td>−29.4±9.0</td>
</tr>
<tr>
<td>6 (n=9)</td>
<td>−15.7±6.6</td>
<td>−11.6±7.2</td>
</tr>
<tr>
<td>8 (n=18)</td>
<td>0.8±10.2</td>
<td>−1.1±12.5</td>
</tr>
<tr>
<td>10 (n=18)</td>
<td>−5.7±13</td>
<td>−14±9.1</td>
</tr>
<tr>
<td>5 (n=9)</td>
<td>−23.6±4.72</td>
<td>−36±36</td>
</tr>
<tr>
<td>7.5 (n=12)</td>
<td>−10.8±20.5</td>
<td>−4.5±30.7</td>
</tr>
<tr>
<td>10 (n=9)</td>
<td>14.3±19.6</td>
<td>10.7±12.7</td>
</tr>
<tr>
<td>10–15 (n=30)</td>
<td>−1.4±18.5</td>
<td>−5.2±13.8</td>
</tr>
<tr>
<td>15–20 (n=6)</td>
<td>−3.7±11</td>
<td>−5.6±6.7</td>
</tr>
</tbody>
</table>

Mass is defined as the amount of gel interposed between 2 balloon layers in the experimental model. Volume is defined as the amount of water contained within the innermost balloon in the experimental model. Mean percentage difference is calculated as [(observed−actual)/actual×100]. Positive values indicate overestimation by observer. Mean overall difference for mass by observer 1 is 4.6±20.5%; for observer 2, 14.4±14.4%; and for observer 3, 3.4±15.9%. Mean overall difference for volume by observer 1 is 3.2±18%, for observer 2, 6.4±20.3%; and for observer 3, 2.8±10.6%.

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**TABLE 1. Comparative Features and Measurements According to Gestational Age Intervals**

<table>
<thead>
<tr>
<th>Age Interval</th>
<th>15 to 19.5 Weeks</th>
<th>20 to 27.5 Weeks</th>
<th>28 Weeks to Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, wk</td>
<td>17.4±2.1 (24)</td>
<td>22.7±2.5 (30)</td>
<td>32.1±3 (21)</td>
</tr>
<tr>
<td>Maternal age, y</td>
<td>28.0±4.0 (21)</td>
<td>27.2±7.0 (25)</td>
<td>29.4±6.1 (17)</td>
</tr>
<tr>
<td>Estimated fetal weight, g*</td>
<td>224.0±53.7 (20)</td>
<td>597±262 (24)</td>
<td>1887±690 (17)</td>
</tr>
<tr>
<td>Heart volume, mL*</td>
<td>1.7±0.7 (18)</td>
<td>6.2±3.3 (25)</td>
<td>19±6 (10)</td>
</tr>
<tr>
<td>RV muscle mass, g*</td>
<td>0.56±0.39 (19)</td>
<td>1.64±2.7 (22)</td>
<td>3.89±1.37 (15)</td>
</tr>
<tr>
<td>LV muscle mass, g*</td>
<td>0.56±0.36 (19)</td>
<td>1.19±0.95 (21)</td>
<td>3.89±1.58 (16)</td>
</tr>
</tbody>
</table>

*Statistical significance between each of the gestational groups studied.
Altman plots. The earlier parts of each study with heart rates of ≈200 bpm were more likely to reflect motion artifacts, but the higher concentrations of isoflurane used preterminally served to decrease the heart rates of most rats to the mid-100s, with resulting improvement in resolution. The relatively large footprint of the transducer was challenging for transthoracic imaging. Ex vivo estimations tended to slightly overestimate mass (9%), whereas the in vivo ones tended to underestimate mass (7%); neither difference was statistically significant.

Discussion

3D Ultrasound as an Appropriate Modality

Chamber size quantification can be performed by M-mode or 2D studies using area-length calculations or the more accurate biplane Simpson’s method. Both modalities are restricted to monoplanes and assume a prolate ellipsoid shape of the LV with a ratio of long axis to short axis of 2:1. These assumptions may not hold for the developing heart, which can hardly be assumed to be a prolate ellipsoid from its earliest developmental stages. M mode is highly dependent on the incident plane and is inaccurate for volume estimation, with poor repeatability and high interobserver variability. The 2D-based calculations seemed to overestimate RV volumes by as much as 30% to 35% in an adult study comparing RV volumes. As many as 40% of normal fetuses can have artifactually increased cardiac dimensions on random 2D measurements compared with phase-specific M-mode dimensions. Thus, both M-mode and 2D modalities seem prone to overestimation of dimensions, volumes, and therefore mass. A fetal study comparing the accuracy of 2D and 3D ultrasound found that 2D significantly overestimated heart volume assessments compared with 3D by a factor of 45% and showed the greater reproducibility of 3D compared with 2D measurements.

Quantitative 3D echocardiography has been applied for ventricular volume and functions in children and adults, fewer studies have been done in fetal life. In addition, 3D quantifications of other fetal organs (eg, lungs, liver, kidneys, cerebellum) have been done. We believe that this is the first study of 3D myocardial mass assessments that spans gestational age from 15 to 37 weeks, along with detailed validation protocols.

Accuracy/Reproducibility Validation

Both intraobserver variability and interobserver variability (2% and <10%, respectively) are reassuring compared with system accuracy claimed by the manufacturer (∼9% for volumes). Volumetric assessments on 3D data can be done equally well by technicians or specialists. A learning curve seemed apparent and affected accuracy and time. A specific concern was the accuracy of the system for small volumes and masses; however, rat model validation studies showed reasonable accuracy at even these small volumes. The tendency for overestimation in the ex vivo limb of this experiment likely indicates postmortem tissue changes, osmotic uptake in a saline bath, and problems with epicardial hyperechogenicity.

Growth of Ventricular Volumes and Myocardial Mass

Changes in fetal ventricular volumes with progressing gestation have been previously established. Our data confirm this pattern with myocardial mass. An interesting observation is that there appeared to be a change in slope of discretely measured ventricular masses at ≈27.5 weeks, corresponding
to an estimated fetal weight of \(~1300\) g. Because this was a cross-sectional study and serial growth assessments were not done, a comment on change in growth velocity cannot be made, but this finding deserves closer attention in a longitudinal study. As a conjecture, the developmental period at \(~26\) to \(~28\) weeks may mark a shift from predominant cell differentiation to predominant cell growth.

Ventricular mass correlated with estimated fetal weight based on Hadlock’s formula. This was our best parameter to “normalize” biventricular mass to fetal size and is an important aspect because it alludes to a relative proportionate cardiac growth. Normalizing fetal measurements to estimated weight might, in fact, be more appropriate than making a simple comparison with gestation.

Ventricular Codominance

The fetal RV and LV face nearly the same systemic afterload. Similar to previous studies, the RV-to-LV mass ratio in our study was close to unity and decreased only insignificantly toward term. The interventricular septum was included in measurement of both the RV and LV. This may have diluted any obvious preponderance of one or the other ventricle. Separating out the septum while making measurements was found to result in such small volumes as to increase inaccuracy. The LV seemed to be minimally larger in volume and mass through all gestational ages. This may be in slight variance with previous literature in which 2D-estimated RV volume was slightly larger. This difference is likely to be due to the greater degree of overestimation that applies to 2D methods that assume similar shape for both cavities, resulting in more overestimation of the RV than the LV.

Study Difficulties and Limitations

Problems in the study were due mostly to difficult windows, fetal movement, and inadequate resolution. Limited acquisition time (10 minutes) and inclusion of all undertaken studies may have diminished the yield.

Spatial resolution is variable in the axial, lateral, and elevational planes, and at least 2 of these need to be adequate for a confident trace of 3D volumes. Issues of resolution decay and artifacts have been amply highlighted by Nelson et al. and we faced them in our study.

In the ex vivo limb of the animal experiment, exaggerated epicardial brightness causing difficulties in epicardial tracing might explain some overestimation. This hyperechoic appearance may have resulted from the angle of incident ultrasound beams and reverberation. In some fetal studies, the epicardial-pericardial rim was hyperechoic; suitable postprocessing was able to clarify the rim in most cases.

A 3D/4D mechanism that easily integrates fetal gating at high frame rates would have been ideal. This would have enabled an accurate 3D estimation of cardiac function and volumes/masses in specific cardiac phases. Also, a higher frame rate would be used so that the data would have higher resolution and a better yield of measurable studies. Both of these features are part of a recent system upgrade to the Voluson product, and 4D-based studies are ongoing in our laboratory, with encouraging preliminary results.

The papillary muscles and trabeculations were included in mass measurement because they are definitely a part of the ventricular musculature. Their accurate delineation required very clear images and was the most likely source of error.

Conclusions

Fast 3D ultrasound is a robust, accurate, and reproducible modality for fetal ventricular mass measurement. A nomogram for RV and LV masses from direct measurement of 3D multiplanar data has been developed and measurement accuracy has been validated in our study.

References

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