Right and left ventricular wall deformation patterns in normal and left heart hypoplasia chick embryos

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Tobita, Kimimasa, and Bradley B. Keller. Right and left ventricular wall deformation patterns in normal and left heart hypoplasia chick embryos. Am J Physiol Heart Circ Physiol 279: H959–H969, 2000.—The vertebrate embryonic ventricle transforms from a smooth-walled single tube to trabeculated right ventricular (RV) and left ventricular (LV) chambers during cardiovascular morphogenesis. We hypothesized that ventricular contraction patterns change from globally isotropic to chamber-specific anisotropic patterns during normal morphogenesis and that these deformation patterns are influenced by experimentally altered mechanical load produced by chronic left atrial ligation (LAL). We measured epicardial RV and LV wall strains during normal development and left heart hypoplasia produced by LAL in Hamburger-Hamilton stage 21, 24, 27, and 31 chick embryos. Normal RV contracted isotropically until stage 24 and then contracted preferentially in the circumferential direction. Normal LV contracted isotropically at stage 21, preferentially in the longitudinal direction at stages 24 and 27, and then in the circumferential direction at stage 31. LAL altered both RV and LV strain patterns, accelerated the onset of preferential RV circumferential strain patterns, and abolished preferential LV longitudinal strain (P < 0.05 vs. normal). Mature patterns of anisotropic RV and LV deformation develop coincidentally with morphogenesis, and changes in these deformation patterns reflect altered cardiovascular function and/or morphogenesis.

epicardial strain; embryonic heart; morphogenesis; hypoplastic left heart syndrome

Cardiac development depends on the dynamic interaction of morphogenetic and environmental conditions, and altered ventricular load may result in abnormal maturation of cardiac function and altered morphogenesis (4, 5, 10–13, 26). Several experimental models that change preload or afterload in the chick embryo reproducibly result in structural anomalies identical to those seen in patients (10, 11, 26). Most congenital cardiovascular malformations are due to errors in morphogenesis of right and/or left heart structures during these early stages, with varying degrees of functional adaptation. Therefore, experimental models that allow the investigation of right (RV) and left ventricular (LV) structural and functional maturation during early cardiac morphogenesis provide an opportunity to define adaptive mechanisms that regulate cardiac development.

Hypoplastic left heart syndrome (HLHS) is a relatively rare congenital heart defect that occurs in ~3.8% of patients with congenital heart disease. Despite the availability of in utero diagnosis and improved surgical strategies, HLHS contributes disproportionately to morbidity and mortality for congenital heart disease patients (8, 10, 26). One proposed etiology for left heart hypoplasia is inadequate LV filling due to altered intracardiac blood flow patterns during early cardiovascular morphogenesis (10, 26).

Many indexes of cardiovascular (CV) performance, including atrial and ventricular pressure, chamber dimensions, and ventricular filling and ejection velocities and volumes (3, 14, 15, 21, 28), can now be quantified during the embryonic period of CV development. These global measures of CV function are relatively insensitive to regional variations in ventricular function; however, measures of regional epicardial wall strain can be used to evaluate the regional myocardial function (7, 27, 29). Epicardial ventricular wall strain patterns are initially isotropic before ventricular trabeculation and then become anisotropic during ventricular morphogenesis (27).

In the present study, we hypothesized that embryonic ventricular contraction patterns change from globally isotropic to chamber-specific anisotropic patterns during normal CV morphogenesis, and that these deformation patterns are influenced by experimentally altered mechanical load produced by chronic left atrial ligation. We investigated developmental changes in RV and LV epicardial wall strains in normal and in the experimental model of HLHS in chick embryos. During normal development, wall deformation patterns changed from globally isotropic to RV- or LV-specific anisotropic patterns. Hypoplastic left heart embryos displayed altered ventricular filling patterns and altered epicardial strain patterns consistent with the paradigm that altered ventricular filling results in altered ventricular function and geometry during CV development.
MATERIAL AND METHODS

Embryo selection. White Leghorn chicken embryos were studied at Hamburger-Hamilton (HH) stage 21 (3.5 days), stage 24 (4 days), stage 27 (5 days), and stage 31 (7 days) of a 46-stage (21 days) incubation period as previously described (9). The selected stages represent doublings of embryo mass and represent a period of geometric increase in CV performance (3). Normal embryos were studied acutely at each stage. Embryos that were dysmorphic or exhibited overt bleeding were excluded. During this developmental period, the embryonic ventricles transform from a common pulsatile chamber to geometrically distinct, septated RV and LV chambers (1, 24, 25).

Production of left heart hypoplasia (left atrial ligation). Embryos were initially incubated to stage 21, when the primitive right and left atria become morphologically distinct (24). A 1-cm² hole was made in the shell, and the inner shell and extraembryonic membranes were removed to expose the developing embryo. The embryo was then gently positioned left side up, and a microforceps was used to make a slit-like opening in the thoracic wall. An loop of 10-0 nylon suture tied with an overhand knot was then placed across the primitive left atrium and tightened, decreasing the effective volume of the left atrium (26) (Fig. 1). Each embryo was then repositioned to its original right-side-up orientation, the opening in the thoracic wall. An egg shell was sealed with parafilm, and embryos were then reincubated until HH stages 24, 27, and 31. Sham-operated embryos underwent the same procedure with the exception of suture ligation.

The total number (n) of experimental embryos was as follows: n = 180 control embryos, n = 61 sham-operated embryos, and n = 344 left atrial ligation (LAL) embryos. Cumulative survival rates for LAL embryos were 85% to stage 24 (vs. 98% in sham embryos), 46% to stage 27 (vs. 87% in sham embryos), and 21% to stage 31 (vs. 78% in sham embryos; P < 0.01 by the Kaplan-Meier method).

Atrioventricular blood flow velocity. Atrioventricular (AV) blood flow velocity was measured with a 20-MHz pulsed Doppler velocimeter (model no. 202; Triton Technology, San Diego, CA) and a 0.5-mm-diameter piezoelectric Doppler crystal. The velocimeter has a pulse-repetitive frequency of 125 kHz with a focus range of 0.5–5.5 mm. The internal audio filter contains a four-pole Butterworth filter with a high-pass (wall filter) cutoff at 100 Hz and a low-pass cutoff at 17 kHz. The velocimetry system is linear up to 100 mm/s for steady flow and linear up to 50 mm/s for pulsatile flow (31). The Doppler crystal was positioned on the epicardium of the ventricle, aimed parallel to the direction of AV blood flow, and then adjusted with a micromanipulator to obtain the strongest velocity signal. Analog velocity waveforms were digitally sampled at 500 Hz by an analog-to-digital board (AT-MIO 16; National Instruments, Austin, TX) and then stored with the use of a custom-programmed data acquisition and analysis system (LabVIEW, National Instruments). Maximum and average AV inflow velocities and the passive-to-active inflow velocity-time integral ratio were calculated as average values from three consecutive waveforms for each embryo (Fig. 2).

Epicardial wall strains. Epicardial wall strains were measured by the tracking of triangular arrays of 10-μm-diameter microspheres attached to the embryonic epicardium (27). A microforceps was used to place microspheres on the epicardial surface of the developing RV or LV (Fig. 3). The average distance between microspheres was 70–100 μm. The embryo was then positioned for imaging on a photomicroscope stage so that the plane of the epicardial surface containing microspheres was perpendicular to the imaging axis. This orientation was confirmed by rotating the egg slightly until the microspheres changed size equally as they moved in and out of focus during the cardiac cycle.

Video images were acquired with the use of a photomicroscope (model M400; Wild Leitz, Rockleigh, NJ), a CCD video camera with a 1/250-s digital shutter (KP-D50U; Hitachi, Denshi, Japan), a frame grabber board (LG-3; Scion, Frederick, MD), and a custom-programmed 8-bit gray-scale analog-to-digital image acquisition system (LabVIEW, National Instruments). This custom acquisition system captured sequential video images at 60 Hz for 4-s intervals (28). After the segment capture, a 50-μm-division scribed glass standard was recorded in the plane of each embryo for software calibration (LabVIEW, National Instruments).
Individual video fields were analyzed to calculate the two-dimensional coordinates of the centroids of each microsphere through three cardiac cycles. End diastole was chosen as the reference for strain as previously described (27). The scribed image coordinate data sets (27) were transformed from image to cardiac coordinates by the following equation:

\[ e_{ij} = \sqrt{(x_{ij} - x_{ij,0})^2 + (y_{ij} - y_{ij,0})^2} \]

where \( e_{ij} \) is the strain component and \( x_{ij,0}, y_{ij,0} \) are the coordinate components of the triangular edges, with \( e_i \) and \( e_j \) being unit vectors along the respective axes. This transformation provides the epicaldial circumferential (\( e_{11} \)), longitudinal (\( e_{22} \)), and shear (\( e_{12} = e_{21} \)) strains relative to a short and approximately cylindrical section of the curved tube region containing the markers. The eigenvalue problem was solved on the basis of the matrix of strain components to compute the principal strains (27) \( e_1 \) and \( e_2 \).

Data from at least two consecutive contractions were analyzed for each embryo to calculate peak systolic epicardial strain components (circumferential, longitudinal, and shear strains) and peak systolic principal strains.

**External and transverse cross-sectional ventricular areas.**

After the completion of each physiological study, normal, sham, and LAL hearts at stages 27 and 31 were perfused fixed with 2% glutaraldehyde in isotonic chick Ringer solution by puncturing of the sinus venosus with a 31-gauge needle connected to a 1-ml syringe. After perfusion/fixation, RV and LV oblique epicardial views and transverse sections at midventricular level were recorded to determine ventricular dimensions. One pixel in the image in each embryo ranged from \( 5.2 \times 10^{-5} \) to \( 1.0 \times 10^{-4} \) mm².

We planimetered RV and LV epicardial borders from each oblique view after perfusion/fixation (Figs. 4 and 5) and cross-sectional areas from each transverse section at midventricular level (Fig. 6) and then calculated transverse cross-sectional areas at midventricular level at stages 27 and 31 as indexes of ventricular size. There was good linear correlation between in vivo end-diastolic and perfusion-fixed external ventricular epicardial areas (\( y = 0.90x + 0.11; r = 0.96, \text{SE} = 0.15 \text{ mm}^2 \)). RV and LV epicardial cross-sectional areas and LV-to-RV cross-sectional-area ratio from the transverse section were calculated for each embryo.
Statistical analysis. Data are presented as means ± SE. The mean values between circumferential and longitudinal strain components in each stage and developmental changes in ventricular areas in each group were compared with the use of the unpaired t-test. A weighted least squares linear regression analysis was performed to analyze the developmental changes in heart rate and AV blood flow velocities in each group. Single-factor ANOVA was performed to assess developmental changes in strain components in each group. Single-factor ANOVA was also used to compare the mean values of strain component, AV blood flow velocity, and ventricular dimensions among experimental groups in each developmental stage. When an assumption of either data normality or equal variance was violated, a nonparametric Kruskal-Wallis test was performed. Individual comparisons were performed with the use of a Duncan’s multiple range test. Mortality was assessed by the Kaplan-Meier method. Statistical significance was defined by a value of \( P < 0.05 \). All calculations were performed with the use of STATISTICA (Statsoft, Tulsa, OK).

RESULTS

Heart rate and AV inflow velocities. Heart rate increased linearly from stage 21 to stage 31 in each group of embryos (\( r = 0.93, 0.88, \) and 0.90, respectively; Table 1) and was similar among control, sham, and experimental groups at each stage. Thus LAL did not alter the cardiac rate or rhythm during development.

Maximum and average AV inflow velocities increased linearly from stage 21 to stage 31 in each group, and the ratio of passive to active AV flow decreased linearly with ventricular development, consistent with increased atrial contractile mass and function (\( r = 0.54–0.94 \); Fig. 2, Table 2). LAL decreased both maximum and average common AV inflow velocities acutely at stage 21, and this alteration in atrial contractile function persisted until stage 27 for the RV despite the redistribution of blood flow from the LV to the RV (ANOVA, \( P < 0.05 \) vs. normal). At stage 31, maximum RV inflow velocity was similar between LAL and normal groups; however, average RV inflow velocity was higher after LAL, consistent with increased RV filling volume (\( P < 0.05 \) vs. normal). At stage 31, maximum LV inflow velocity was reduced between LAL and normal groups; however, average LV inflow velocity was similar between LAL and normal groups.
Of note, the RV and LV passive-to-active AV flow ratio was similar to normal after LAL at stage 31 despite changes in RV and LV geometry and function.

Developmental epicardial strain patterns. Figures 7 and 8 show representative developmental changes in RV and LV strain-time curves during one cardiac cycle for normal and LAL experimental embryos. Table 3 summarizes the results of peak systolic epicardial strains in all groups. Across the developmental stage range, normal and sham RV circumferential (CIR) and principal strains increased at stages 27 and 31 ($P < 0.05$ vs. stage 21); however, longitudinal (LNG) and shear strains did not change significantly. Normal and sham LV CIR and principal strains increased by stage 31, LV LNG strains increased significantly at stage 27 ($P < 0.05$ vs. normal), and shear strains did not change significantly. In LAL, both RV and LV CIR, LNG, and principal strains increased significantly compared with stage 21 ($P < 0.05$); however, shear strains were similar to those in all developmental stages.

Normal and sham RV myocardium contracted isotropically until stage 24 and changed the contraction patterns preferentially in the CIR direction at stages 27 and 31 ($P < 0.05$ vs. LNG). LV myocardium contracted isotropically at stage 21, LNG strains predominated at stages 24 and 27 ($P < 0.05$ vs. CIR), and then CIR strains predominated at stage 31 ($P < 0.05$).

LAL reduced RV and LV epicardial strain patterns acutely at stage 21. LAL accelerated the onset of preferential RV CIR strain patterns at stage 24 and abolished preferential LV LNG strains at stages 24 and 27 ($P < 0.05$ vs. normal). Shear strain was much smaller than other strain components in all groups, and shear strain was not altered after LAL.

Ventricular dimensions and morphology. Figures 4–6 show representative photographs of developmental changes in the morphology and dimensions of the embryonic RV and LV viewed from the anterior epicardium, via transverse section, and from an apical view toward the right and left AV valves. Table 4 summarizes the results of ventricular transverse cross-sectional areas in all groups. By stage 31, RV area was significantly larger after LAL ($P < 0.05$ vs. normal), and LV area was reduced ($P < 0.05$). LV-to-RV transverse cross-sectional-area ratios (LV/RV ratios) were similar at stage 27; however, the LV/RV ratio of LAL was consistent with LV hypoplasia by stage 31 ($P <
0.05. After LAL, the left AV appeared smaller than the right AV valve at stage 31 (Fig. 6, E and F).

DISCUSSION

The present study expands on our previous investigation of embryonic ventricular epicardial wall strains during normal development (27) and is the first to examine selective embryonic RV and LV wall deformation patterns and function during normal and experimentally altered CV development. We tested the hypothesis that ventricular contraction patterns develop chamber-specific anisotropic patterns during normal morphogenesis and that these deformation patterns are influenced by experimentally altered mechanical load. We demonstrated two important findings of embryonic ventricular deformation patterns during normal and abnormal early CV morphogenesis. The first is that the development of unique RV and LV myoarchitecture is associated with changes in epicardial deformation patterns from a global, isotropic pattern to RV- and LV-specific anisotropic patterns. Second, epicardial deformation patterns and ventricular geometry change in response to the altered mechanical loading conditions produced by chronic LAL, resulting in left heart hypoplasia.

Measurement of epicardial strain. Numerous measures of embryonic ventricular function (e.g., pressure, dimension, stroke volume, and wall stress-strain rela-
Table 1. Heart rate

<table>
<thead>
<tr>
<th></th>
<th>Stage 21</th>
<th>Stage 24</th>
<th>Stage 27</th>
<th>Stage 31</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>146 ± 2</td>
<td>163 ± 2</td>
<td>180 ± 3</td>
<td>225 ± 3</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>(n = 19)</td>
<td>(n = 19)</td>
<td>(n = 17)</td>
<td>(n = 18)</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>149 ± 3</td>
<td>169 ± 2</td>
<td>184 ± 2</td>
<td>222 ± 3</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 14)</td>
<td>(n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAL</td>
<td>147 ± 2</td>
<td>167 ± 3</td>
<td>179 ± 2</td>
<td>226 ± 3</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>(n = 19)</td>
<td>(n = 18)</td>
<td>(n = 21)</td>
<td>(n = 10)</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SE (in beats/min); n = no. of experiments. LAL, left atrial ligation; r, regression coefficients of weighted least squares linear regression analysis among stages for each experimental group. There were no statistical differences among experimental groups at each stage.

tions) are adequate to describe global properties of the embryonic ventricular performance (3, 13, 14, 21, 28). However, these indexes are relatively insensitive to changes in regional ventricular function, especially after the onset of differentiation into RV and LV structures. In the mature ventricle, wall strain measurement has been useful to detect normal and abnormal regional ventricular function (29). During CV morphogenesis, we can measure both “morphogenetic” and “mechanical” strains. Morphogenetic strains are generated by cell growth, division, motion, shape change, and death. Lacktis and Manasek (16) measured morphogenetic strains by tracking epicardial markers during the process of cardiac looping of the chick embryonic ventricle. Morphogenetic strains reflect myocardial growth and remodeling and may reflect changes in both the dimensions and material properties of the developing myocardium. In contrast to the morphogenetic strains, mechanical strains are related directly to applied loads and short-term mechanical strains, or their associated stresses may regulate long-term morphogenetic strains during development (27).

The present study showed that normal ventricular wall deformation patterns changed from isotropic to RV- or LV-specific anisotropic patterns. Several studies have related wall deformation patterns and wall structure (fiber orientation) in the mature LV. Waldman et al. (30) found that the principal shortening direction and fiber orientation are almost parallel in outer myocardial wall (~50% of wall thickness) but are perpendicular in the inner wall. LeGrice et al. (17) proposed that maximum shear deformation is a result of relative sliding between myocardial laminae.

In the embryonic ventricle, myocardial architecture is quite different from the mature ventricle in that the compact layer (future ventricular wall) is much thinner than the mature ventricle and most of the embryonic myocardial wall is trabecular (1, 25). Sedmera et al. (25) described the normal developmental changes in embryonic chick ventricular myoarchitecture and showed that the process of ventricular trabeculation includes three stages that are similar to those in mammalian embryos. Type I (primary) trabeculation is characterized by trabeculae attached to the full length of the ventricular wall (HH stage 16 to 20). Type II (secondary) trabeculation includes an arrangement of secondary trabeculae oriented dorsoventral in the LV and radial in the RV (stage 21 to 29). Starting at stage 31, type III (tertiary) trabeculation patterns in the LV are longitudinal and slightly spiraled (counterclockwise, viewed from base toward the apex), with oblique connecting segments between trabeculae. Trabecular alignment is similar to the orientation of muscle fibers in the compact layer at later stages (after stage 35). RV tertiary trabeculae patterns are arranged in a counterclockwise spiral that resembles the arrangement of

Table 2. Atrioventricular inflow velocity

<table>
<thead>
<tr>
<th></th>
<th>Stage 21</th>
<th>Stage 24</th>
<th>Stage 27</th>
<th>Stage 31R</th>
<th>Stage 31L</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>7</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>62.1 ± 2.0</td>
<td>72.4 ± 2.5</td>
<td>103.6 ± 4.1</td>
<td>117.1 ± 1.4</td>
<td>122.8 ± 3.1</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>25.0 ± 0.8</td>
<td>29.5 ± 1.2</td>
<td>39.3 ± 1.1</td>
<td>44.3 ± 1.0</td>
<td>46.0 ± 1.7</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>0.29 ± 0.03</td>
<td>0.16 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.65</td>
</tr>
<tr>
<td>Sham</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>57.6 ± 1.8</td>
<td>73.0 ± 2.4</td>
<td>104.8 ± 2.3</td>
<td>119.1 ± 2.6</td>
<td>128.4 ± 3.0</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>25.5 ± 0.9</td>
<td>28.8 ± 0.5</td>
<td>40.6 ± 1.3</td>
<td>44.9 ± 1.9</td>
<td>47.6 ± 1.6</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>0.28 ± 0.06</td>
<td>0.17 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.63</td>
</tr>
<tr>
<td>LAL</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>39.0 ± 2.1*</td>
<td>53.4 ± 2.4*</td>
<td>77.6 ± 3.1*</td>
<td>124.6 ± 7.0</td>
<td>99.2 ± 5.0*</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>21.4 ± 1.0*</td>
<td>25.5 ± 1.2*</td>
<td>33.5 ± 1.4*</td>
<td>50.3 ± 2.6*</td>
<td>44.1 ± 1.6</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>0.51 ± 0.07*</td>
<td>0.31 ± 0.04*</td>
<td>0.23 ± 0.02*</td>
<td>0.08 ± 0.02</td>
<td>0.09 ± 0.01</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = no. of experiments. Stage 31R, stage 31 right ventricle; Stage 31L, stage 31 left ventricle; E/A ratio, passive-to-active filling ratio. *P < 0.05 vs. normal group.
muscle fibers in the adult. These embryonic trabecular patterns may reflect the orientation of wall stresses, because the compact layer at these stages is too thin to generate preferential contractile force. The critical role of trabecular contractile function on global CV performance is highlighted by the transgenic mouse embryos with embryo lethal CV phenotypes, including the failure of myocardial trabeculation and proliferation (20).

Changes in epicardial deformation patterns may also relate to developmental changes in the activation sequence of the developing ventricle. Chuck et al. (2) measured developmental changes in the activation sequence of embryonic chick ventricle and found that the embryonic RV apex always depolarized before the RV base, regardless of the embryonic stages. However, LV activation sequence changed from an initial base-to-apex pattern to an apex-to-base pattern at stages 29–31, suggesting the onset of a functioning His-Purkinje system. As has been suggested by numerous investigators (1, 24), they speculated that the embryonic circulation functions in series (atrium, LV, bulboventricular foramen, RV, and outflow tract) before the completion of ventricular septation and that a sequential activation sequence optimizes embryonic cardiac function. After the completion of ventricular septation, the circulation is a modified parallel circuit, and an apex-to-base ventricular activation pattern optimizes ventricular ejection. In the present study, normal LV deformation patterns changed from longitudinal, predominant at stages 24 and 27, to circumferential, predominant at stage 31, coincident with the changes in activation sequence noted by Chuck et al. (2). Thus we speculate that embryonic ventricular wall deformation patterns reflect both changes in myoarchitecture and

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**Fig. 7.** Representative developmental changes in strain-time curves for normal chick embryos. A: stage 21. B: stage 24. C: stage 27. D: stage 31. Solid, broken, and dashed lines indicate epicardial circumferential, longitudinal, and shear strains, respectively. The x-axis is time (in ms). The y-axis is normalized strain relative to end diastole. Strain patterns changed from isotropic to RV- or LV-specific anisotropic patterns.
transitions in ventricular activation sequence during CV development.

Left atrial ligation and ventricular deformation patterns. Our data of AV inflow patterns after LAL showed a reduced contribution of atrial contraction to ventricular filling from stage 21 to stage 27 and an increased average RV filling velocity with decreased LV peak velocity at stage 31. Harh et al. (10) initially produced a model of HLHS in the chick embryo by inserting a nylon filament into the left AV canal. They speculated that after interference with blood flow between the left atrium and LV, RV inflow volume would increase and LV inflow volume would decrease, resulting in RV hyperplasia and LV hypoplasia. Sedmera et al. (26) described the changes in myoarchitecture that occur after chronic LAL in the chick embryo. After LAL at stage 21, the principal LV trabecular sheets are more closely packed together, and they are thinner than normal by stage 29; however, the developmental process of trabecular compaction is accelerated at later stages. After LAL at stage 21, the RV is dilated, and the principal trabeculae are more widely spaced and thinner than normal until stage 34. At later stages after LAL, the compact layer becomes thicker than normal; however, the process of trabecular compaction is delayed and the dilatation persists. They suggested that decreased volume load to the LV results in shrinkage and accelerates morphogenesis and that increased volume load initially produces RV dilation followed by secondary trabecular proliferation and thickening of the compact myocardium.

Our data on epicardial strain patterns after LAL showed that both RV and LV wall strain patterns were markedly different from normal embryos.
ventricular wall is assumed to be an incompressible elastic material, all strains are deviatomic (because wall mass is preserved during the cardiac cycle), and thus the deviatomic stress is determined by the strain alone (18, 28). From this assumption, our results of acute and chronic altered wall deformation patterns in LAL may indicate that alterations in wall stress distribution (magnitude and direction) are critical determinants on normal and altered morphogenesis. Lin and Taber (18) speculated that normal cardiac growth is similar to volume-overload hypertrophy and that the embryonic heart grows and develops to adapt ventricular geometry and function to optimize mechanical efficiency. Several studies have demonstrated that CV adaptation occurs in response to changes in wall stress and/or strain (12, 18, 23). Furthermore, these mechanical environment effects can extend to the level of local gene expression, resulting in differential regulation of mRNA and changes in cardiomyocyte structure and function (19, 22). Thus biomechanical signal-transduction cascades that translate regional changes in embryonic ventricular deformations to changes in gene expression are likely candidate regulatory mechanisms for regulating the adaptive response of the developing heart to altered loading conditions.

Limitations. Limitations and sources of errors in the methods used to measure and analyze epicardial strain have been discussed previously (7, 27, 29). The two-dimensional strain measurements used in the present study have been validated in previous studies; yet, transmural three-dimensional strain distributions would provide a more complete picture of the embryonic deformation patterns. However, because of the relative thinness of the embryonic compact and trabecular myocardial layers, it is difficult to generate three-dimensional bead arrays in the embryonic ventricle. After LAL, the severity of LV hypoplasia is also influenced by the extent of delayed closure of the primary interventricular foramen (10, 26). Finally, it is important to interpret strain patterns in relationship to transmural myofiber alignment (6), and, in contrast to the mature heart, there is currently limited information on myofiber maturation in the developing myocardium.

In conclusion, our study provides the first detailed insight into RV and LV wall deformation patterns in normal developing embryonic chick hearts and in hearts with experimentally induced HLHS. Mature patterns of anisotropic RV and LV deformation develop coincidentally with morphogenesis of distinct RV and LV myocardium.
LV myoarchitecture, and changes in these deformation patterns reflect altered CV function and/or morphogenesis. Our results confirm the importance of adequate mechanical loading for normal cardiac morphogenesis.

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