End-systolic myocardial stiffness is a load-independent index of contractility in stage 24 chick embryonic heart

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Tobita, Kimimasa, and Bradley B. Keller. End-systolic myocardial stiffness is a load-independent index of contractility in stage 24 chick embryonic heart. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H2102–H2108, 1999.—Cardiac morphogenesis and function are interrelated during cardiovascular development. We evaluated the effects of acute alteration of loading condition to chick embryonic ventricular contractility using end-systolic myocardial stiffness based on the incremental elastic modulus concept. End-systolic stress-strain relations including geometric factor and end-systolic myocardial stiffness were determined from the simultaneous measurement of ventricular pressure and chamber dimension in the following four groups of stage 24 White Leghorn chick embryos: volume infusion (n = 9), conotruncal occlusion (n = 9), calcium suffusion (n = 10), and verapamil suffusion (n = 8). The end-systolic stress-strain relationship was linear in each embryo. There was no correlation between end-systolic myocardial stiffness and end-systolic stress. End-systolic myocardial stiffness increased with calcium suffusion (P < 0.05 vs. volume infusion). The geometric factor increased after verapamil suffusion (P < 0.05). End-systolic myocardial stiffness normalized by geometric factor was not changed by alteration of preload or afterload, increased after calcium suffusion, and decreased after verapamil administration (P < 0.05). These results suggest that normalized end-systolic myocardial stiffness is a load-independent index of ventricular contractility in the developing embryonic chick ventricle.

chick embryonic ventricle; end-systolic stress-strain relationship

THE DEVELOPING CARDIOVASCULAR system can acutely and chronically adapt to changes in preload, afterload, and contractility (11). Congenital cardiovascular anomalies occur as the result of abnormal maturation of cardiac function and altered morphogenesis (2, 5, 7). During the embryonic period of rapid cardiovascular growth and morphogenesis in avian and mammalian species, the heart transforms from a straight tube to a looped tube to a four-chamber heart, and there is dramatic maturation of the ventricular myocardium (17). Embryonic cardiovascular adaptation occurs at the tissue and cellular levels; however, there are limits to embryonic cardiovascular adaptation that result in a normal mature phenotype. Several experimental models in the chick embryo reproducibly result in structural anomalies identical to those seen in patients (1, 9, 22).

Many indexes of embryonic cardiovascular function such as ventricular pressure, cardiac output and arterial impedance are interdependent (4, 12, 13, 30). Despite the size and geometry of the embryonic heart, ventricular-vascular interactions can be determined using pressure-volume relations and arterial impedance (12, 30). These studies suggest that the embryonic cardiovascular system rapidly alters cardiac output and arterial impedance in response to altered loading conditions (30). It is less clear, however, whether ventricular “contractility” changes acutely or chronically because of the “load dependence” of standard indexes of cardiovascular function (12, 13).

Relatively “load-independent” measures of ventricular function include maximum end-systolic elastance (18) and systolic myocardial stiffness (16). The basic model of time-varying elastance assumes that arterial load is constant. Previous attempts to analyze embryonic end-systolic pressure-volume relations (ESPVR) have been complicated by extreme curvilinearity of these relations because of simultaneous changes in arterial tone (10, 13, 24). However, acute conotruncal occlusion isolates the ventricle from the arteries, allowing a new, accurate assessment of embryonic ventricular function (13).

We investigated ESPVR and maximum systolic stiffness during acute changes in ventricular preload, afterload, and myofilament activation. End-systolic myocardial stiffness normalized for changes in geometry reflected changes in contractility in the embryonic heart.

MATERIALS AND METHODS

Embryo preparation and developmental staging. Vertebrate cardiac morphogenesis follows a relatively similar process, although with different time lines, across a broad range of species (11). In the present study, we selected the Hamburger-Hamilton stage 24 chick embryo (8), because this developmental stage is representative of the embryonic heart containing a trabecular myocardium before cardiac septation (19). The stage 24 chick embryo is comparable to embryonic day 12.5 in the mouse and to Streeter horizon XV in humans (20).

Fertilized White Leghorn chicken eggs were incubated blunt end up in a forced-draft incubator to Hamburger-Hamilton stage 24 (4 days) of a 46-stage (21 days) incubation period (8). Each egg was positioned on a photomicroscope stage under radiant warmers to maintain ambient temperature between 37 and 38°C. An ∼1-cm² hole in the shell was made, and the inner shell and extraembryonic membranes were removed to expose the developing embryo. Embryos that were dysmorphic or exhibited overt bleeding were excluded from future study.

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Hemodynamic preparation. We simultaneously measured intraventricular pressure and ventricular dimensions using a custom-integrated physiological and morphometry workstation. A fluid-filled glass capillary pipette was positioned with the use of a micromanipulator (Leitz, Wetzlar, Germany) to puncture the developing right ventricle, and the intraventricular pressure was measured using a servo-null pressure system (model 900A, World Precision Instruments, Sarasota, FL). The servo-null pressure is linear (y = -0.995x - 0.23, r = 0.99, SE = 0.11 mmHg) to a standing water column over the range of 0–10 mmHg, and the rise time of the pressure system is <10 ms (3). Video images were acquired using a photomacroscope (model M400, Wild Leitz, Rockleigh, NJ), a video camera (model 70-new viscon tube, Dage-MTI, Michigan City, IN), a frame-grabber board (LG-3, Sdon, Frederick, MD), and a custom-programmed analog-digital image acquisition system (LabVIEW, National Instruments, Austin, TX). This custom acquisition system simultaneously captured video images at 60 Hz and intraventricular pressure at 600 Hz with an analog-digital board (AT-MIO 16; National Instruments) for 4 s. The pressure waveform was decimated from 600 to 60 Hz and interpolated with the image data. A 50-µm-division scribed glass standard was recorded in the plane of each embryo after imaging for calibration of image analysis software (LabVIEW). Baseline image and pressure data were recorded in each embryo before experimental interventions.

Ventricular preload alteration. We obtained ventricular pressure and dimension data during increasing ventricular preload by rapid volume injection. Rapid volume injection was performed using a 50-µl graduated syringe (Hamilton, Reno, NV) and a programmable microsyringe pump (SP210iw, World Precision Instruments). The syringe was connected by plastic tubing via a three-way stopcock to a reservoir of warmed, oxygenated Krebs-Henseleit buffer (KHB) and a 10-µm-tip diameter glass pipette inserted into the sinus venosus. Pressure and image data were acquired simultaneously during a single 2-µl-volume injection over 5 s (0.4 µl/s).

Ventricular afterload alteration. Our previous study of acute, near-complete conotruncal occlusion showed that embryonic ventricular peak systolic pressure and end-diastolic volume changed almost simultaneously in response to the near-complete conotruncal occlusion (13). This rapid preload response to alteration of afterload in the embryo confounded our attempt to change ventricular afterload without changing preload. Thus, in the present study, we altered only afterload without changing preload by gradual conotruncal occlusion.

The conotruncus was occluded using a microforceps mounted on a micromanipulator. The forceps was closed gradually over 5 s to narrow the conotruncus until end-diastolic volume visibly increased.

Alteration of contractility. The immature embryonic ventricular myocyte primarily regulates intracellular calcium via the sarcolemma (6, 28). We used KHB containing either 6 mM ionized calcium or 2 mM ionized calcium plus 4 × 10⁻⁵ mM verapamil to increase or decrease myofilament calcium availability, respectively. These buffer solutions reproducibly alter twitch force in the isolated embryonic myocardium (B. B. Keller, unpublished data).

Measurement of arterial impedance. We measured dorsal aortic blood pressure and flow velocity simultaneously as previously described (30) and then calculated arterial impedance using a three-element windkessel model before and during the rapid volume injection in the same-stage chick embryos.

Experimental protocols. We analyzed hemodynamic data in the following four groups of embryos. In the volume infusion protocol (n = 9), a rapid volume injection was performed. In the conotruncal occlusion protocol (n = 9), a gradual conotruncal occlusion was performed. In the calcium suffusion protocol (n = 10), 10 µl of KHB containing 6 mM ionized calcium was suffused onto the ventricle and a rapid volume injection was performed 30 s later. In the verapamil suffusion protocol (n = 8), 10 µl of KHB containing 4 × 10⁻⁵ mM verapamil was suffused onto the ventricle and a rapid volume injection was performed 30 s later. After the completion of data recording, maximal ventricular contraction and cavity obliteration were achieved using topical 2 M sodium chloride administration in each embryo to allow the calculation of myocardial wall volume (12).

Video image processing. We planimetered the ventricular epicardial border manually from each video field and measured epicardial ventricular cross-sectional area (A) (Fig. 1). Intra- and interobserver error of area measurement by planimetry is not significant (P > 0.29 and P > 0.96, respectively) (12). Ventricular volume was calculated using a simplified ellipsoidal geometric model. The ellipsoid equation is derived from equations for A of an ellipsoid, A = πDL, and the volume (V) of the ellipsoid of revolution, V = (4πD²L)/3, where D is the minor semiaxis and L is the major semiaxis. We measured epicardial ventricular L and D at maximum and minimum A in each embryo (Table 1) and assumed a fixed ventricular aspect ratio (L/D = 4/3) during the entire cardiac cycle; thus V = 0.65A³/2. Ventricular cavity volume (Vc) was calculated as total volume (Vt) minus myocardial wall volume (Vw). Ventricular internal minor semiaxis dimension (D,) and wall
Table 1. Epicardial ventricular major and minor axis dimension ratio

<table>
<thead>
<tr>
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<th>Infusion</th>
<th>Occlusion</th>
<th>Ca²⁺</th>
<th>Verapamil</th>
<th>P</th>
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<tr>
<td>n</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>L/D at max</td>
<td>1.31 ± 0.02</td>
<td>1.32 ± 0.03</td>
<td>1.32 ± 0.01</td>
<td>1.30 ± 0.02</td>
<td>0.87</td>
</tr>
<tr>
<td>L/D at min</td>
<td>1.32 ± 0.03</td>
<td>1.33 ± 0.03</td>
<td>1.33 ± 0.02</td>
<td>1.30 ± 0.03</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of experiments. Infusion, rapid volume infusion; occlusion, gradual conotruncal occlusion; Ca²⁺, calcium suffusion + rapid volume injection; verapamil, verapamil suffusion + rapid volume injection; L/D at max, major-to-minor axis dimension ratio at maximum cross-sectional area; L/D at min, major-to-minor axis dimension ratio at minimum cross-sectional area. Epicardial ventricular major and minor axis ratio were same at maximum and minimum areas. Ratio was not different between intervention groups.

Thickness (h) were calculated by the following equations:

\[ V_t = (\pi/6)(D_i + h)^2(L_i + h) = (\pi/6)\left[\left(\pi/4\right)D_i^3 + (\pi/4)D_i^2h + (\pi/12)D_ih^2 + h^3\right] \]

\[ V_c = V_t - V_m = (\pi/6)\pi D_i^2L_i = (\pi/2)\pi D_i^3 \]

\[ V_m = V_t - V_c = (\pi/6)\pi\left[\left(\pi/4\right)D_i^3 + (\pi/12)D_ih^2 + h^3\right] \]

where \( L_i \) is the internal major semiaxis dimension and \( L/D_i = 4/3 \). In addition, the material properties of the embryonic myocardium are assumed to be constant during acute change of the ventricular volume (24).

End-systolic myocardial stiffness. With the use of elasticity theory, an arbitrary state of stress or strain is expressible as the sum of hydrostatic and deviatomic stresses or strains. For an incompressible elastic material, all strains are deviatomic (because volumes are preserved), and thus the deviatomic stress alone is determined by the strain (27). The hydrostatic stress is determined from the boundary values of the stress. Thus in an \( r, \theta, \phi \) coordinate system, the total stress components \( \sigma_i \) may be expressed in terms of the strain components \( \varepsilon_i \) as

\[ \sigma_r = -P_0 + (\varepsilon_r)E_{r,r} \]
\[ \sigma_\theta = -P_0 + (\varepsilon_\theta)E_{\theta,\theta} \]
\[ \sigma_\phi = -P_0 + (\varepsilon_\phi)E_{\phi,\phi} \]

where \( P_0 \) is a uniform hydrostatic pressure, \( E \) is Young's modulus, and \( r, \theta, \phi \) are radial, circumferential, and meridional coordinates, respectively.

Strain difference \( \varepsilon \). Assuming the embryonic ventricle to be a thick-walled ellipsoidal shell, total strain difference \( \varepsilon \) is defined as the difference of the circumferential \( (\varepsilon_c) \) and radial \( (\varepsilon_r) \) strain components at the equator of the ellipsoid (16). Using the natural strain definition, the strain difference is expressed as

\[ \varepsilon_c = \ln \left(D_m/D_{0,m}\right) \]
\[ \varepsilon_r = \left(2 + D_m^2/L_m^2\right)\varepsilon_c \]
\[ \varepsilon = (\pi/6)(\varepsilon_c - \varepsilon_r) \]

Thus the total strain difference is calculated by

\[ \varepsilon = (\pi/6)(2 + D_m^2/L_m^2)\ln \left(D_m/D_{0,m}\right) \]

where \( D_m, L_m, \) and \( D_{0,m} \) are, respectively, minor semiaxis, major semiaxis, and zero-stress minor semiaxis midwall diameter at the equator.

Stress difference \( \sigma \). Stress difference \( \sigma \) is defined as the difference of the circumferential \( (\sigma_c) \) and radial \( (\sigma_r) \) stress components. Note that these stresses are averaged over the entire cross section at the equator (15, 16). Thus the average stress difference \( \sigma \) is calculated by

\[ \sigma = \sigma_{c,a} - \sigma_{r,a} = (PD_m/h)[1 - (D_m^2/2L_m^2) - (3h^2/8D_m^2)] \] (5)

where \( \sigma_{c,a} \) and \( \sigma_{r,a} \) are the average circumferential and radial stresses and \( P \) is left ventricular pressure. Average systolic myocardial stiffness. From Eq. 2, the average systolic myocardial stiffness \( (E_{av}) \) is calculated as

\[ \sigma = E_{av} \varepsilon = E_{av}[(2 + D_m^2/L_m^2)\ln (D_m/D_{0,m})] \] (6)

Note that Eq. 6 indicates that the stress-strain relationship is linear (18).

ESPVR based on end-systolic stress-strain relations. Ventricular midwall volume \( (V_m) \) based on the thick-walled ellipsoidal model is

\[ V_m = (\pi/6)\pi D_m^2L_m \] (7)

Assuming that \( L_m/D_m = 4/3 \), then

\[ D_m = \left(\frac{3}{V_m/k}\right) \] (8)

where \( k = (\pi/6)P_0 \).

We can then use \( V_m \) and \( V_{0,m} \) to calculate

\[ D_m/D_{0,m} = \left(\frac{V_m/V_{0,m}}{3}\right) \] (9)

where \( V_{0,m} \) is zero-stress midwall volume. Thus Eq. 6 is expressed as

\[ \sigma = (4/3)E_{av}\ln (V_m/V_{0,m}) \] (10)

We then converted end-systolic stress-strain relations to ESPVR using the equation

\[ \sigma_{es}/P_{es} = G = (D_m/h)[1 - (D_m^2/2L_m^2) - (3h^2/8D_m^2)] \]

\[ = \alpha + \beta V_{es} \] (11)

where \( \sigma_{es} \), \( P_{es} \), and \( V_{es} \) are end-systolic stress difference, end-systolic pressure, and end-systolic midwall volume, respectively. \( G, \alpha, \) and \( \beta \) are a geometric factor and regression coefficients, respectively.

Using Eqs. 10 and 11, ESPVR are expressed as

\[ P_{es} = \left(\frac{4/3}{G}\right)E_{av}\ln (V_m/V_{0,m}) \] (12)

Note that Eq. 12 indicates that ESPVR are curvilinear.

Determination of end-systolic stress-strain points. Each end-systolic stress-strain point is defined as the point where the \( \alpha \)-to-\( \varepsilon \) ratio reaches a maximum after the onset of systole. We calculated the end-systolic stress-strain point in the following iterative manner. \( D_{0,m} \) was first assumed. End-systolic stress-logarithmic midwall dimension points were then fit by linear-regression analysis. A new \( D_{0,m} \) was then obtained by extrapolation to zero stress. This iterative procedure was continued until the value for \( D_{0,m} \) converged.

Statistical analysis. Data are presented as means ± SE. Two-way repeated-measures ANOVA was used to assess the time course of the changes of arterial impedance during rapid
Table 2. Ventricular hemodynamic data after interventions

<table>
<thead>
<tr>
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<th>Infusion</th>
<th>Occlusion</th>
<th>Ca²⁺</th>
<th>Verapamil</th>
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<tbody>
<tr>
<td><strong>n</strong></td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td><strong>Heart rate, min⁻¹</strong></td>
<td>141 ± 4.1</td>
<td>136 ± 5.0</td>
<td>144 ± 1.1</td>
<td>141 ± 6.9</td>
</tr>
<tr>
<td><strong>Peak pressure, mmHg</strong></td>
<td>2.20 ± 0.08</td>
<td>2.17 ± 0.06</td>
<td>2.38 ± 0.08</td>
<td>2.16 ± 0.08</td>
</tr>
<tr>
<td><strong>End-diastolic volume, µl</strong></td>
<td>0.36 ± 0.01</td>
<td>0.37 ± 0.02</td>
<td>0.38 ± 0.02</td>
<td>0.46 ± 0.02*</td>
</tr>
</tbody>
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Values are means ± SE; n, no. of experiments. Data shown are before rapid volume injection or gradual conotruncal occlusion. *P < 0.05 vs. infusion.

volume injection between each group. The mean values for each group were analyzed by one-way ANOVA. When an assumption of either data normality or equal variance was violated, a nonparametric Kruskal-Wallis test was performed. Individual comparison was performed by a Duncan’s multiple-range test. Statistical significance was defined by a value of P < 0.05. Linear regression analysis with the minimum least square method was performed to analyze the end-systolic stress-dimension, stress-strain relations, and geometric factor. To evaluate linearity, we performed an F-test in each linear-regression analysis. All calculations were performed using Statistica (Statsoft, Tulsa, OK).

RESULTS

Hemodynamic data. Table 2 shows hemodynamic data after intervention in each group. Embryonic heart rate and peak pressure were similar among all groups (P > 0.50). End-diastolic volume increased after verapamil suffusion (P < 0.05 vs. volume infusion). Table 3 shows arterial impedance after volume infusion, Ca²⁺, and verapamil treatments. Peripheral resistance (P = 0.38), characteristic impedance (P = 0.08), and total arterial compliance (P = 0.22) were unaffected by these acute interventions.

End-systolic stress-strain relations. Figure 2 displays end-systolic stress-dimension and stress-strain relations for one representative embryo. F-test indicated that these relations showed no significant departure from linearity. Extrapolation of these relations yielded D₀,m. Figure 3 and Table 4 show that F-test demonstrated no significant departure from linearity in any group and that no statistical relationship was observed between end-systolic myocardial stiffness and stress. These results indicate that end-systolic myocardial stiffness is independent of end-systolic stress over a wide range of stresses.

End-systolic myocardial stiffness, G, and V₀,m. End-systolic myocardial stiffness (Eav,max) increased only after Ca²⁺ suffusion (P < 0.05). G increased only after verapamil suffusion (P < 0.05). End-systolic myocardial stiffness normalized by G (Eav,max/G) increased after Ca²⁺ suffusion and decreased after verapamil suffusion (P < 0.05). V₀,m increased after verapamil suffusion (P < 0.05, Table 5). Figure 4 shows that pressure-volume relations based on the stress-strain relationship are curvilinear and that Eav,max/G is the slope of these relations.

Table 3. Arterial hemodynamic parameters after interventions

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<th>Infusion</th>
<th>Ca²⁺</th>
<th>Verapamil</th>
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<tr>
<td><strong>n</strong></td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Rₚ, 10⁶ dyn·s/cm⁻⁵</strong></td>
<td>1.42 ± 0.05</td>
<td>1.44 ± 0.05</td>
<td>1.43 ± 0.04</td>
</tr>
<tr>
<td><strong>Rₙ, 10⁶ dyn·s/cm⁻⁵</strong></td>
<td>0.18 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td><strong>C, 10⁻⁶ cm³/dyn</strong></td>
<td>0.20 ± 0.01</td>
<td>0.28 ± 0.02</td>
<td>0.21 ± 0.01</td>
</tr>
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</table>

Values are means ± SE; n, no. of experiments. Rₚ, peripheral resistance; Rₙ, characteristic impedance; C, total arterial compliance. Arterial impedances were similar between intervention groups (P = 0.08–0.38).
DISCUSSION

End-systolic stress-strain relationship: systolic myocardial stiffness concept. In the original theory of maximum ventricular elastance, the ESPVR is linear and the maximum ventricular elastance is sensitive to the ventricular contractility and is independent of loading conditions (18). However, animal and clinical studies revealed curvilinearity of ESPVR, resulting in a revised curvilinear model with contractility and a geometric factor (10, 16, 21, 26). Taber et al. (24, 29) modeled curvilinear ESPVR in chick embryonic ventricle theoretically using the thick-walled cylindrical tube model and correlated experimental wall strains and ESPVR with a pseudostrain-energy density function of the myocardium. According to the model of Mirsky et al. (16), ventricular end-systolic stress-strain relations are linear and the slope of the relationship, \( E_{av,max} \), is independent of ventricular load and sensitive to altered ventricular contractility. The linear end-systolic stress-strain relationship implies a curvilinear ESPVR. The present study showed that the end-systolic stress-strain relations in chick embryonic ventricle were linear and that end-systolic myocardial stiffness was independent of end-systolic stress. These relations were not changed by the alterations of loading condition. The results of the present study were similar to those in the mature left ventricle and indicate that the systolic myocardial stiffness concept is useful to assess the contractility of the developing myocardium.

End-systolic myocardial stiffness, geometric factor, and normalized end-systolic myocardial stiffness. From Eq. 12, ESPVR is expressed as \( P_{es} = (41/72) \cdot (E_{av,max}/G) \cdot \ln(V_{es}/V_{0,m}) \). Note that the slope of the curve is determined by \( E_{av,max}/G \). In the mature left ventricle, changes of \( E_{av,max}/G \) directly reflect the alteration of contractility because \( G \) is close to unity during altered load (16). In the present study, \( G \) of the verapamil

\[ E_{av,max}, \text{ end-systolic myocardial stiffness, G, geometric factor; } E_{av/G}, \text{ end-systolic myocardial stiffness normalized by geometric factor; } V_{0,m}, \text{ zero-stress midwall volume. } * P < 0.05 \text{ vs. infusion.} \]
suffusion group was significantly higher than that of other groups.

Simply stated, G was described by Mirsky et al. (16) as a conversion factor between stress-strain relations and pressure-volume relations. We calculated this factor from each embryonic ventricle using the method of Mirsky et al. This factor is a constant of each heart, and it represents the effect of the ventricular dimension-wall thickness relation to wall stress. In the present study, we assumed the embryonic ventricle as a simplified ellipsoid, and the L-to-D ratio was not changed in any group (Table 1). Previous study of surficial epicardial strain in stage 24 chick embryo showed that the magnitudes of circumferential and longitudinal strains were similar to each other, implying that the embryonic ventricle contracts isotropically in both directions at this stage (25). Increased G in the verapamil group indicates dilatation of the ventricular cavity and thinning of the ventricular wall. Thus, in embryonic ventricle, end-systolic myocardial stiffness normalized by a geometric factor, $E_{av,max}/G$, should be used to evaluate alterations in ventricular contractility. $E_{av,max}/G$ was not changed in preload or afterload alterations, increased after Ca$^{2+}$ suffusion, and decreased after verapamil suffusion. In this stage of embryonic ventricle, the myocytes have a limited calcium reserve and poorly developed sarcoplasmic reticulum and T-tubule system (6, 28). Extracellular calcium directly regulates the force generation of contractile proteins (28). Thus, $E_{av,max}/G$ is load independent and reflects changes in ventricular contractility.

Ventricular zero-stress volume. Ventricular zero-stress volume increased after verapamil suffusion. Ventricular dimension and geometry at the zero-stress point depend on residual strain. Previous data on residual strain in the chick embryonic ventricle show that residual strain changes dramatically at the onset of trabeculation, suggesting that residual strain is sensitive to changes in ventricular structure (23). From this point of view, even in the same stage of the embryo, residual strain may be changed by conditions that alter ventricular geometry. Thus further experiments are needed to directly evaluate myocardial properties, including material properties and residual strain during changes in Ca$^{2+}$ flux.

Assumptions and limitations. There are several assumptions and limitations to the present study. First, the embryonic ventricle is assumed to be a thick-walled ellipsoidal shell with a fixed ratio of semiminor and semimajor axis diameter during systole. The actual ventricular shape in chick embryonic ventricle at this stage is more complex than a simple ellipsoidal shell. Thus our method of calculation of ventricular cavity volume and wall thickness may not accurately assess absolute volume and wall thickness changes during the cardiac cycle. In addition, this analysis is also influenced by changes in myocardial trabeculation. However, at present, there are no more accurate methods to assess absolute ventricular volumes and wall thickness in the embryonic heart.

Second, the calculation of embryonic myocardial stress and strain depends on the model formulations. Strain-time curves and peak strains in the present study were similar to previous data in the embryonic heart (25). To calculate the embryonic ventricular wall stress, we assumed that the embryonic myocardium is a freely deforming body composed of an isotropic, homogeneous, and incompressible elastic material. Several models can be used to quantify wall stress; however, it is difficult to compare our results of end-systolic wall stress with those obtained for the mature left ventricle. Yang et al. (29) measured epicardial Lagrangian strain in the stage 21 chick embryonic ventricle and computed circumferential wall stress distribution based on a thick-walled pseudoporoelastic cylindrical model. The end-systolic transmural stress gradient of chick embryonic ventricle was proposed to be smaller than that of mature left ventricle because of the strain-softening constitutive relations of embryonic myocardium. However, limited experimental data is available to support their assumptions regarding embryonic myocardial material properties (14).

Finally, the theoretical model of systolic stiffness concept assumes that stress is a function of strain alone. Viscous and inertial effects are excluded. Although these effects do not significantly influence end-systolic stiffness in the mature left ventricle, the embryonic myocardium differs markedly in ultrastructure, with a small volume fraction of organized extracellular matrix and less anisotropy (25). In addition, recent data of the passive myocardial stress-strain relations in stage 16 and stage 18 embryos show that the hysteresis loops are larger than mature myocardium, indicating different viscoelastic properties (14). Further study is needed to evaluate how changes in material properties influence embryonic ventricular end-systolic stress-strain relations and systolic myocardial stiffness during maturation of the embryonic myocardium.

The present study is the first evaluation of ventricular contractility using end-systolic stress-strain relations and maximum systolic myocardial stiffness in the embryonic ventricle. Our results showed that the end-systolic myocardial stiffness normalized by a geometric factor is a load-independent index of the embryonic ventricular contractility.

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