Transmural cardiac strains in the lateral wall of the ovine left ventricle


Transmural cardiac strains in the lateral wall of the ovine left ventricle. Am J Physiol Heart Circ Physiol 288: H1546–H1556, 2005. First published December 8, 2004; doi:10.1152/ajpheart.00716.2004.—The constant-volume property of contracting cardiac muscle has been invoked in models of heart wall mechanics that predict that systolic subendocardial left ventricular (LV) wall thickening must significantly exceed subepicardial thickening. To examine this prediction, we implanted arrays of radiopaque markers to measure lateral equatorial wall transmural strains and global and regional LV geometry in seven sheep and studied the four-dimensional dynamics of these arrays using biplane videofluoroscopy (60 Hz) in anesthetized intact animals 1 and 8 wk after surgery. A transmural gradient of systolic lateral wall thickening was observed at 1 wk ($P = 0.009$; linear regression) but was no longer present at 8 wk ($P = 0.243$). Referenced to end diastole, group mean ($ \pm $SD) end-systolic radial subepicardial, midwall, and subendocardial wall thickening strains were, respectively, 0.08 $ \pm $ 0.08, 0.14 $ \pm $ 0.08, and 0.22 $ \pm $ 0.12 at 1 wk and 0.19 $ \pm $ 0.07 ($P = 0.02$; 1 vs. 8 wk), 0.20 $ \pm $ 0.04, and 0.23 $ \pm $ 0.07 at 8 wk. With the exception of an 8-ml (7%) increase in end-diastolic volume ($P = 0.04$) from 1 to 8 wk, LV shape and hemodynamics were otherwise unchanged. We conclude that equivalent hemodynamics can be generated by the left ventricle with or without a transmural gradient of systolic wall thickening in this region; thus such a gradient is unlikely to be a fundamental property of the contracting LV myocardium. We discuss some implications of these findings regarding mechanisms involved in systolic wall thickening.

THE FINDING THAT MUSCLES MAINTAIN a constant volume during contraction has played an important role in the understanding of fundamental contractile mechanisms. In the mid-17th century, Jan Swammerdam and Jonathan Goddard first demonstrated this phenomenon in skeletal muscle (30) and in so doing disproved a theory of muscle contraction that had held sway for 1,600 years. Three centuries later, there is evidence that the volume of the left ventricular (LV) myocardium during systole is not entirely constant but, as a first approximation, can usefully be assumed to be so, because it varies by $\sim 2$–15% (9, 21, 34, 37, 44, 46).

In their influential theoretical model of the mechanics of the left ventricle, Arts et al. (1) used constant myocardial volume as a key assumption, and, using this assumption, they and others (11, 19, 25, 38) subsequently predicted transmural variations in LV systolic wall thickening. By constraining constant volumes within various straightforward geometries, all of these models predict that systolic subendocardial thickening will be significantly greater than subepicardial thickening. Although two studies appear to contradict this prediction in reporting near-uniform transmural thickening (14, 16), the overwhelming majority of experimental evidence, derived using a variety of approaches, has supported the view that systolic subendocardial thickening is substantially greater than subepicardial thickening, with reported systolic subendocardial-to-subepicardial thickening ratios of 1.4–11 (4, 6, 10–12, 18, 23–26, 29, 32–34, 43, 45).

We recently undertook a series of experiments involving surgical implantation of transmural bead sets in the lateral LV wall of sheep hearts. Without intervening procedures, 1 and 8 wk after surgery, four-dimensional (4D) data sets from stereoradiographic studies of the implanted beads were processed to yield transmural strains from each heart. With the above previous theoretical predictions and published experimental results in mind, we hypothesized that systolic radial strain (i.e., wall thickening) would exhibit a transmural gradient from subepicardium to subendocardium and, because previous work suggested it is a fundamental mechanism, that this gradient would be observed at both 1 and 8 wk postoperatively. As reported here, the 1-wk studies yielded the expected results. The 8-wk studies, however, were surprising in that the transmural wall thickening gradient had vanished, while LV hemodynamics were virtually the same as the 1-wk values. These findings require exploration in some depth because they comprise the only data of which we are aware from the same individual sites in the same hearts that exhibited both the presence and absence of the transmural gradients predicted by current LV models. Such an exploration may affect the understanding of fundamental mechanisms underlying the contractile dynamics of the heart wall.

METHODS

All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the Guide for Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (U.S. Department of Health and Human Services, NIH Publication 85-23, Revised 1985). This study was approved by the Stanford Medical Center Laboratory Research Animal Review Committee, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and conducted according to Stanford University policy.

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Surgical preparation. Seven Dorsett hybrid sheep (67 ± 10 kg) were premedicated with ketamine (25 mg/kg im) for venous and arterial catheter placement. Anesthesia was induced with thiopental sodium (6.8 mg/kg iv) and maintained with inhalational isoflurane (1–2.5%) in supplemental oxygen. Single doses of cefazolin sodium (1 g iv) and gentamicin sulfate (80 mg iv) were administered preoperatively, and antibiotic therapy was continued during the early postoperative period. Through a left thoracotomy, 13 miniature tantalum subepicardial radiopaque markers (outer diameter, 1.3 mm; length, 1.5–3.0 mm) were surgically implanted to silhouette the LV chamber along four equally spaced longitudinal meridians (Fig. 1, anterior, markers 1, 2, 3, and 4; septal, markers 1, 5, 6, and 7; posterior, markers 1, 8, 9, and 10; and lateral, markers 1, 11, 12, and 13) in three transverse planes (Fig. 1, apical, markers 2, 5, 8, and 11; equatorial, markers 3, 6, 9, and 12; and basal, markers 4, 7, 10, and 13). Epicardial echocardiography (Sonos 5500; Hewlett-Packard, Palo Alto, CA) was used to locate an equatorial segment of the lateral LV wall equally spaced between the papillary muscles for placement of a transmural bead set and to measure the diastolic wall thickness in this region (Fig. 2). Three transmural columns of beads (four beads each, shown immediately below marker 12 and, in expanded view, at right in Fig. 1) were then implanted into this region using techniques similar to those described previously (44). Briefly, a planar Plexiglas template was sutured to the epicardium to establish the epicardial tangent plane at this site. Three holes in this template were drilled normal to the plane of the template at the vertices of a 10-mm equatorial triangle to be used as guides for a depth-adjustable bead insertion trocar. Three transmural columns of three 0.7-mm-diameter gold beads were implanted, normal to the epicardial tangent plane and evenly spaced from endocardium to epicardium, using this trocar (Fig. 1, markers 16–18, 20–22, and 24–26). The deepest beads were implanted at 90% of the echocardiographically determined endocardial wall thickness. After these beads were inserted, larger beads (1.7-mm diameter; Fig. 1, markers 15, 19, and 23) were sewn onto the epicardial surface above each column. The chest was then closed, and the sheep was resuscitated.

Experimental protocol. At 1 wk and again at 8 wk postoperatively, each animal was taken to the cardiac catheterization laboratory, sedated with ketamine (1–4 mg·kg⁻¹·h⁻¹ iv infusion), placed in the right lateral decubitus position, intubated, mechanically ventilated, and maintained with inhalational isoflurane (1–2.5%) in 100% oxygen. A micromanometer-tipped pressure transducer (model MP-500; Millar Instruments, Houston, TX), previously calibrated in a water bath, was advanced into the left ventricle via a carotid artery catheter. Biplane videofluoroscopic images of all radiopaque markers (Fig. 1, markers 1–26) were acquired at 60 Hz with an Optimus 2000 biplane Lateral ARC 2/Poly Diagnost C2 system (Philips Medical Systems, Pleasanton, CA) with the image intensifiers in the 7-in. mode and orientations optimized to visualize each individual marker unambiguously. Analog LV pressure (LVP) and surface lead electrocardiographic signals were recorded in digital format on each video image. All measurements were taken during steady-state baseline conditions with the heart in normal sinus rhythm and ventilation transiently arrested at end expiration. After the 8-wk study, conventional 3.0-mm perfusion balloon catheters were placed in the proximal left anterior descending and left circumflex coronary arteries. The animals were euthanized by administration of Pentothal Sodium (1 g iv) followed by an intravenous bolus of KCl (80 meq) to depolarize and arrest the hearts at end diastole (ED). LVP was then adjusted via central venous known marker-to-marker 3D lengths, was previously shown to be reduced. The change in epicardial LVV is an accurate measurement of the LV chamber volume and LV muscle mass; our previous studies have shown that the change in epicardial LVV is an accurate measurement of LV volume.
of the change in LV chamber volume (28). For each cardiac cycle, ED was defined as the videofluoroscopic frame immediately before the upstroke of the LVP curve, defined by dLVP/dt > 120 mmHg/s. End systole (ES) was defined as the videofluoroscopic frame when dLVP/dt changed the sign from minus to plus, a definition that captures the onset of relaxation and is consistent with the definition associated with the concept of ES elastance (28). Stroke volume (SV) was computed as LVED volume (LVEDV) − LVES volume (LVESV), and ED compliance was approximated as LVEDV + LVEDP. Preload recruitable stroke work (PRSW) was computed from steady state and immediately after cava occlusion beats as the slope of the linear regression of SW on EDV, where SW was calculated as the integral of LVP (P) multiplied by change in volume (dV) as SW = ∫PdV over each cardiac cycle. Figure 3 shows ED and ES times so defined on three-beat LV pressure-volume (P-V) loops from a representative heart in this study in both the 1- and 8-wk studies. At each sample time in each study, additional volumes within the bead columns were computed by space-filling tetrahedra at the subepicardial level (using time in each study, additional volumes within the bead columns were heart in this study in both the 1- and 8-wk studies. At each sample time in each study, additional volumes within the bead columns were computed by space-filling tetrahedra at the subepicardial level (using markers 15, 16, 19, 20, 23, and 24; Fig. 1), midwall level (using markers 17, 18, 21, 22, 25, and 26), and in total (the sum of the subepicardial, midwall, and subendocardial volumes).

LV shape. The shape of the LV region containing the transmural bead set was defined as the videofluoroscopic frame immediately before the upstroke of the LVP curve, defined by dLVP/dt > 120 mmHg/s. End systole (ES) was defined as the videofluoroscopic frame when dLVP/dt changed the sign from minus to plus, a definition that captures the onset of relaxation and is consistent with the definition associated with the concept of ES elastance (28). Stroke volume (SV) was computed as LVED volume (LVEDV) − LVES volume (LVESV), and ED compliance was approximated as LVEDV + LVEDP. Preload recruitable stroke work (PRSW) was computed from steady state and immediately after cava occlusion beats as the slope of the linear regression of SW on EDV, where SW was calculated as the integral of LVP (P) multiplied by change in volume (dV) as SW = ∫PdV over each cardiac cycle. Figure 3 shows ED and ES times so defined on three-beat LV pressure-volume (P-V) loops from a representative heart in this study in both the 1- and 8-wk studies. At each sample time in each study, additional volumes within the bead columns were computed by space-filling tetrahedra at the subepicardial level (using markers 15, 16, 19, 20, 23, and 24; Fig. 1), midwall level (using markers 17, 18, 21, 22, 25, and 26), and in total (the sum of the subepicardial, midwall, and subendocardial volumes).

LV mobility. To estimate the potential constraining effect of post-surgical adhesions, the regional mobility of the LV in the relatively immobile thorax (anesthetized animal, respiration suspended) was defined for each beat as the 3D displacements in laboratory coordinates from ED to ES of the bead set origin (Fig. 1, O) and adjacent LV epicardial markers (Fig. 1, markers 3, 9, and 11-13).

Cardiac finite strains. Placement of the transmural bead set allowed assessment of transmural 3D myocardial deformations in the lateral equatorial region of the LV wall using an approach similar to that described by Costa et al. (6). At each sample time, 1) markers 15, 19, and 23 established a local epicardial tangent plane with a local origin at their center (Fig. 1, O, right); 2) a local radial axis (X1) was defined normal to this epicardial tangent plane with positive direction away from the LV chamber (Fig. 1, right); 3) a local long axis (X2) was defined, positive toward the LV base, at the intersection of the epicardial tangent plane with a plane containing X1 and a line from apex bead 1 through O [Fig. 1; this long axis aligns within 8° of the long axis defined by Streeter (40)] (Harrington K, personal communication); and 4) the local right-handed Euclidian “cardiac” coordinate system was completed with a circumferential axis (X3) normal to X2 and X1 (Fig. 1). The reference (undeformed) state was taken at ED for each beat, and the ensuing positions of the beads for that beat were defined by displacement from their positions at ED as characterized by a continuous polynomial position field with quadratic dependence in X3 and bilinear dependence in X1 and X2 using least-squares minimization. The material gradient of the position field is the local deformation gradient tensor (F) wherewith Green strain (E) calculated as E = (F T F − I)/2, where I is the identity tensor. In cardiac coordinates (X1, X2, X3), the three normal strain components measure the local myocardial stretch or shortening along the circumferential (E11), longitudinal (E22), and radial (E33) cardiac axes. The three shear strains (E12, E13, and E23) represent angle changes between pairs of the originally orthogonal coordinate axes. Strains were interpolated along the centroid of the bead columns at 1% increments of wall depth from the epicardium to the most subendocardial bead. For each beat, bead positions at ES (deformed configuration) at 20, 50, and 80% wall depth were compared with their positions at ED (reference configuration). Because the most subendocardial bead was consistently at ~90% of the wall depth from epicardium to endocardium as measured echocardiographically, the 20, 50, and 80% depth strains reported here are actually more nearly at 18, 45, and 72% transmural depths, but this small difference is not important in the context of the present study. Three-beat averages were used to characterize the data from each of the seven hearts at each study time (1 and 8 wk). Principal strains were computed as the eigenvalues of E and the angular relationship of the first principal strain (maximum positive eigenvector) to the radial (X1) axis computed from the appropriate vector dot product. ED remodeling strains were obtained for each heart by comparison of bead positions at ED from the 1-wk study (reference configuration) with the positions of these same beads in the same heart at ED from the 8-wk study (deformed configuration). ES remodeling strains were obtained for each heart by comparison of bead positions at ES from the 1-wk study (reference configuration) with the positions of the same beads in the same heart at ES from the 8-wk study (deformed configuration).

Statistical analysis. Data are reported as means ± SD unless otherwise stated. Changes in hemodynamics, volumes, and shapes were compared using Student’s t-test for paired observations. Cardiac strains were compared using two-way repeated-measures ANOVA with Student-Newman-Keuls pairwise multiple comparisons (SigmaStat, version 2.03; SPSS, Chicago, IL). ED and ES strains were compared with zero using a one-sample t-test. The slope of the linear regression of E33 versus depth was tested against zero to test for the existence of a transmural gradient of wall thickening. P < 0.05 was considered statistically significant.

RESULTS

Hemodynamic data for the group are summarized in Table 1. Beat-to-beat hemodynamic parameters for each three-beat sequence were highly reproducible (see, e.g., Fig. 3). EDV increased by 8 ml (7%) from 1 to 8 wk. Although the 10% fall in heart rate and 10% increase in the duration of systole over this interval were possibly significant, there were no statistically significant changes in EDP, EDV/EDP, maximal time derivative of LVP during contraction and relaxation (±dP/
The first principal radial strains for the group at the three transmural levels and two study times are summarized in Table 3. The direction of the first principal (maximum positive) strain relative to the radial \((R)\) axis was unchanged from 1 to 8 wk. Although the magnitude of the first principal strain was unchanged in the subendocardium over this interval, it increased significantly by 108% in the subepicardium and 32% in the midwall.

Cardiac ED remodeling strains from 1 to 8 wk are summarized in Table 4. Possibly significant subepicardial thinning of 17% and midwall thinning of 12% were noted, along with a transmural gradient of diastolic remodeling \(E_{33}\) strain. No significant cardiac ES remodeling strains were observed from 1 to 8 wk (Table 5), but a substantial transmural gradient of systolic remodeling \(E_{33}\) strain was found. At ED, overall wall thickness was unchanged from 1 wk (0.84 ± 0.07 cm) to 8 wk (0.81 ± 0.09 cm) \([P = \text{not significant (NS)}]\).

The group mean sphericization index of the LV chamber at ED was 1.08 ± 0.10 at 1 wk and 1.09 ± 0.13 \((P = \text{NS})\) at 8 wk. At ES, this index was 1.14 ± 0.11 at 1 wk and 1.13 ± 0.14 \((P = \text{NS})\) at 8 wk. Thus global LV shape was unchanged from 1 to 8 wk. At ED, the group mean \((\pm \text{SD})\) circumferential ROC of the region including the bead columns was 3.53 ± 0.18 cm at 1 wk and 3.59 ± 0.31 cm \((P = \text{NS})\) at 8 wk; at ES, this ROC was 3.10 ± 0.15 cm at 1 wk and 3.19 ± 0.30 cm \((P = \text{NS})\) at 8 wk. Thus the circumferential ROC was also unchanged from 1 to 8 wk.

While we could not reliably measure the LV long-axis ROC, the sphericization index is actually a surrogate for this parameter and did not change between the 1- and 8-wk studies.

Systolic wall thickening \((E_{33})\) data for each heart at each transmural depth are plotted in Fig. 5. Linear regression of these data yielded a gradient significantly different from zero at 1 wk \((P = 0.009)\) and not significantly different from zero at 8 wk \((P = 0.243)\).

Pressure, volume, and thickening data from a representative heart are shown in Fig. 6 (P-V loops from this heart are shown in Fig. 3; this is heart 4 in Fig. 4). At 1 wk (Fig. 6A), the transmural gradient of \(E_{33}\) at ES is clearly evident [note that this gradient increases during isovolumic relaxation (IVR)]; at 8 wk (Fig. 6B) the gradient at ES is gone. At both study times, \(E_{33}\) at all levels increased very little during the first half of systole. The major increase in \(E_{33}\) occurred in the last half of systole, with peak values occurring during IVR. Both graphs shown in Fig. 6 exhibit instances of wall thinning and thickening.

### Table 1. Hemodynamic data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 wk</th>
<th>8 wk</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>103±17</td>
<td>93±17</td>
<td>0.08</td>
</tr>
<tr>
<td>(T_{ED-ES}), ms</td>
<td>252±13</td>
<td>278±24</td>
<td>0.06</td>
</tr>
<tr>
<td>EDP, mmHg</td>
<td>17±7</td>
<td>8±6</td>
<td>0.27</td>
</tr>
<tr>
<td>EDV, ml</td>
<td>107±20</td>
<td>115±23*</td>
<td>0.04</td>
</tr>
<tr>
<td>EDP/EDV, ml/mmHg</td>
<td>9±4</td>
<td>23±15</td>
<td>0.11</td>
</tr>
<tr>
<td>(+dP/dt)max, mmHg/s</td>
<td>1,715±225</td>
<td>1,390±416</td>
<td>0.10</td>
</tr>
<tr>
<td>PRSW, mmHg</td>
<td>68±15</td>
<td>75±10</td>
<td>0.211</td>
</tr>
<tr>
<td>LVPmax, mmHg</td>
<td>118±20</td>
<td>113±10</td>
<td>0.57</td>
</tr>
<tr>
<td>ESP, mmHg</td>
<td>114±19</td>
<td>108±9</td>
<td>0.53</td>
</tr>
<tr>
<td>ESV, ml</td>
<td>87±14</td>
<td>91±17</td>
<td>0.13</td>
</tr>
<tr>
<td>(-dP/dt)max, mmHg/s</td>
<td>-1,880±462</td>
<td>-1,812±379</td>
<td>0.70</td>
</tr>
<tr>
<td>LVPVmin, ml</td>
<td>84±14</td>
<td>88±17</td>
<td>0.11</td>
</tr>
<tr>
<td>SV, ml</td>
<td>24±7</td>
<td>28±8</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Group mean \((\pm SD)\) data from ovine hearts (\(n = 7\)). HR, heart rate; \(T_{ED-ES}\), duration of systole from end diastole (ED) to end systole (ES); EDP, end-diastolic pressure; EDV, end-diastolic volume; \(+dP/dt\)max, maximum time derivative of left ventricular pressure (LVP) during isovolumic contraction; PRSW, preload recruitable stroke work; ESP, end-systolic pressure; ESV, end-systolic volume; \(-dP/dt\)max, maximum time derivative of LVP during isovolumic relaxation; LVPV, left ventricular pressure; SV, stroke volume. \(P\) values from two-sided paired \(t\)-test of 8-wk data compared with 1-wk data. *\(P = 0.04\) 1 vs. 8 wk, Student’s two-tailed paired \(t\)-test.

### Table 2. Transmural LV lateral wall systolic cardiac strains

<table>
<thead>
<tr>
<th>20% Depth</th>
<th>50% Depth</th>
<th>80% Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk</td>
<td>8 wk</td>
<td>1 wk</td>
</tr>
<tr>
<td>(E_{11})</td>
<td>-0.06±0.03</td>
<td>-0.09±0.04</td>
</tr>
<tr>
<td>(E_{22})</td>
<td>0.01±0.05</td>
<td>0.02±0.08</td>
</tr>
<tr>
<td>(E_{32})</td>
<td>0.08±0.08</td>
<td>0.19±0.07*</td>
</tr>
<tr>
<td>(E_{x2})</td>
<td>0.01±0.01</td>
<td>0.02±0.02</td>
</tr>
<tr>
<td>(E_{33})</td>
<td>0.05±0.06</td>
<td>0.11±0.09</td>
</tr>
<tr>
<td>(E_{33})</td>
<td>0.02±0.03</td>
<td>0.01±0.04</td>
</tr>
</tbody>
</table>

Group means \((\pm SD)\) from the 1- and 8-wk studies of ovine hearts (\(n = 7\)). \(E_{11}\), circumferential strain; \(E_{22}\), longitudinal strain; \(E_{33}\), radial strain; \(E_{32}\), circumferential-longitudinal shear; \(E_{x2}\), longitudinal-radial shear; \(E_{x3}\), circumferential-radial shear. *\(P = 0.02\) 1 vs. 8 wk, two-way repeated-measures ANOVA with Student-Newman-Keuls (SNK) pairwise multiple comparisons. †20% vs. 50%, §50% vs. 80%, and §20% vs. 80% SNK pairwise multiple comparisons \((P < 0.05)\) at same study time. Depth was measured as a percentage of the radial distance from the epicardial bead to the most subendocardial bead.
Fig. 4. Radial systolic strain \( E_{33} \); ED (reference configuration), ES (deformed configuration) at week 1 (A) and week 8 (B) for each of the seven hearts at 20% transmural depth from epicardium (filled bars), 50% depth (open bars), and 80% depth (shaded bars). Data from heart 4 are displayed in Figs. 3, 6, and 7.

Fig. 6B shows a gradient for the subendocardial volume, 4.2% at 1 wk and 5.7% at 8 wk \( P = 0.04, \) subendocardial systolic thickening remained virtually unchanged during this interval. These findings may have implications for mechanistic theories of heart wall dynamics. Before discussing these implications, however, we address whether the 8-wk data we report are anomalous, because, at first glance, our 8-wk data appear to contradict the results of many earlier studies.

The earliest measurements of systolic transmural wall thickening were conducted with implanted sonomicrometer crystals. With this approach, systolic subendocardial-to-subepicardial thickening ratios of 4.9 (34) and 2.5 (25) in the anterior and posterior regions were reported, respectively, in open-chest studies of dogs. Sonomicrometer studies in conscious dogs, typically 1–2 wk after surgery, reported ratios of 2.0 in the lateral wall (11), 3.6 in the posterolateral wall (18), and 1.2 in the anterior wall (16). Our finding of a thickening ratio of 2.75 at 1 wk after surgery is consonant with these findings. Interestingly, the 1.2 ratio reported by Hittinger et al. (16) is identical to our 8-wk results, which in our study proved not to describe a statistically significant gradient. The limitations of

### Table 3. First principal radial strains

<table>
<thead>
<tr>
<th>Depth</th>
<th>1 wk</th>
<th>8 wk</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Magnitude</td>
<td>Angle, °</td>
</tr>
<tr>
<td>20%</td>
<td>0.13 ± 0.07</td>
<td>30 ± 17</td>
</tr>
<tr>
<td>50%</td>
<td>0.19 ± 0.07</td>
<td>24 ± 14</td>
</tr>
<tr>
<td>80%</td>
<td>0.28 ± 0.10</td>
<td>23 ± 9</td>
</tr>
</tbody>
</table>

Group means (±SD) from the 1- and 8-wk studies of ovine hearts \( n = 7 \): * \( P < 0.04, \) two-tailed paired \( t \)-test comparing 8-wk with 1-wk values. Depth measured as a percentage of the radial distance from the epicardial bead to the most subendocardial bead. Magnitude, first principal eigenvector. Angle, angular value between first principal eigenvector and radial (X) axis.

### Table 4. Transmural LV lateral wall end-diastolic remodeling cardiac strains

<table>
<thead>
<tr>
<th>Depth</th>
<th>1–8 wk</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>20% P</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>( E_{11} )</td>
<td>-0.05 ± 0.13</td>
</tr>
<tr>
<td>( E_{22} )</td>
<td>0.01 ± 0.14</td>
</tr>
<tr>
<td>( E_{33} )</td>
<td>-0.17 ± 0.19</td>
</tr>
<tr>
<td>( E_{12} )</td>
<td>0.05 ± 0.11</td>
</tr>
<tr>
<td>( E_{23} )</td>
<td>-0.06 ± 0.15</td>
</tr>
<tr>
<td>( E_{13} )</td>
<td>-0.01 ± 0.15</td>
</tr>
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</table>

Group means (±SD) from the 1- and 8-wk studies of ovine hearts \( n = 7 \): \( P \) at 1 wk, reference configuration; ED at 8 wk, deformed configuration. \( P \) values from two-tailed paired \( t \)-test compared with zero. * \( P = 0.07, \) depth 80% vs. 20%, two-way repeated-measures ANOVA with SNK pairwise multiple comparisons. Depth measured as a percentage of the radial distance from the epicardial bead to the most subendocardial bead.

DISCUSSION

The principal finding of the present study is that equivalent hemodynamics could be generated by the left ventricle in the presence or absence of a transmural gradient of systolic wall thickening in the lateral equatorial region. This gradient, observed 1 wk after surgery, was no longer present at 8 wk. The means by which the gradient was abolished was an increase in subepicardial systolic thickening from 1 to 8 wk, with subendocardial thickening remaining virtually unchanged during this interval. These findings may have implications for mechanistic theories of heart wall dynamics. Before discussing these implications, however, we address whether the 8-wk data we report are anomalous, because, at first glance, our 8-wk data appear to contradict the results of many earlier studies.
studies using sonomicrometer crystals, however, are well known and widely admitted, and include 1) difficulty in separating wall shear from wall thickening, 2) difficulty in assessing the function of different myocardial layers at the same site, 3) a requirement to tunnel into the LV wall, and 4) a requirement for surgery to implant the crystals. Our approach in the present study overcomes the first two of these limitations but still suffers from the latter two, which we think may relate to the difference between our 1- and 8-wk data. In any event, our 1-wk postoperative data are not in conflict with the bulk of acute or early postoperative data from implanted sonomicrometers.

Because of the well-known limitations of studies using sonomicrometer crystals, other approaches were soon thereafter applied to this measurement. Myers et al. (29) used epicardial echocardiography to measure the distance from the epicardium to sutures in the LV wall and reported a thickening ratio of 2.0 in the anterior wall of open-chest dogs. Hartley et al. (14) sewed single crystals on the epicardium and, using pulsed Doppler, found no transmural wall thickening gradients in the posterolateral region of either open-chest dogs or 12-day postoperative chronic pigs. Other investigators, however, using this same pulsed-Doppler approach, reported systolic endocardial-to-epicardial thickening ratios in the anterior wall of open-chest dogs of 1.4 (12) and 2.1 (10) in the posterolateral wall of open-chest dogs of 1.7 (10) and, in 8-day postoperative conscious dogs, 1.7 in the anterior region and 2.3 in the posterolateral region (4). Epicardial echocardiography requires surgery and transmural tunneling and may not entirely avoid the confounding effect of transmural shearing. Furthermore, while studies using pulsed Doppler avoid having to tunnel into the myocardium, they still require surgery, require stitches in the epicardium, do not avoid the confounding effect of transmural shearing, and suffer from uncertainty as to whether the Doppler signals are returned from the same tissue targets throughout the cardiac cycle. In any event, our 1-wk data are not in conflict with either the acute or 1 wk postoperative studies, and, interestingly, our 8-wk data support the 2-wk data of Hartley et al. (14). All of these studies, however, including ours, suffer from the requirement for surgical interventions.

Waldman et al. (44) were first to use transmural columns of radiopaque beads to assess the function of different myocardial layers at the same site, avoiding the problem of separating wall

Table 5. Transmural LV lateral wall end-systolic remodeling cardiac strains

<table>
<thead>
<tr>
<th></th>
<th>20% Depth</th>
<th>50% Depth</th>
<th>80% Depth</th>
<th>20% Depth</th>
<th>50% Depth</th>
<th>80% Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>E₁₃</td>
<td>-0.06±0.14</td>
<td>0.30</td>
<td>-0.05±0.12</td>
<td>0.31</td>
<td>-0.06±0.17</td>
<td>0.41</td>
</tr>
<tr>
<td>E₂₂</td>
<td>-0.03±0.13</td>
<td>0.58</td>
<td>-0.03±0.13</td>
<td>0.60</td>
<td>-0.07±0.21</td>
<td>0.43</td>
</tr>
<tr>
<td>E₃₃</td>
<td>-0.14±0.23</td>
<td>0.16</td>
<td>-0.07±0.19</td>
<td>0.36</td>
<td>0.13±0.28**</td>
<td>0.27</td>
</tr>
<tr>
<td>E₁₂</td>
<td>0.05±0.09</td>
<td>0.20</td>
<td>0.04±0.10</td>
<td>0.35</td>
<td>-0.01±0.08</td>
<td>0.83</td>
</tr>
<tr>
<td>E₁₃</td>
<td>-0.03±0.12</td>
<td>0.48</td>
<td>-0.04±0.07</td>
<td>0.19</td>
<td>-0.01±0.15</td>
<td>0.88</td>
</tr>
<tr>
<td>E₂₃</td>
<td>-0.04±0.14</td>
<td>0.52</td>
<td>-0.03±0.09</td>
<td>0.43</td>
<td>-0.01±0.11</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Group means (±SD) from the 1- and 8-wk studies of ovine hearts (n = 7). ES at 1 wk, reference configuration; ES at 8 wk, deformed configuration. P values from two-tailed paired t-tests compared with zero. Depth 80% vs. 20% (*P = 0.02); 80% vs. 50% (†P = 0.04) from two-way repeated-measures ANOVA with SNK pairwise multiple comparisons. Depth measured as a percentage of the radial distance from the epicardial bead to the most subendocardial bead.
shear from wall thickening. Studies applying this approach to the anterior wall of open-chest dogs have reported systolic subendocardial-to-subepicardial thickening ratios of 1.8 (45), 2.3 (26), 2.9 (43), 3.0–3.8 (2, 6), and 11 (23). In closed-chest animals, Omens and Covell (32) found a systolic thickening ratio of 2.3 in anesthetized dogs 1 wk after surgery, and LeGrice et al. (24) found a ratio of 2.5 in sedated pigs 2 wk after surgery. Transmural bead columns are used in the present study and our 1-wk data from closed-chest sheep support these previous findings of transmural thickening gradients. Again, however, this approach does not avoid the requirement for surgery and the necessity to tunnel into the LV wall.

Ultimately, the best method for this type of study will likely involve magnetic resonance imaging (MRI). Indeed, Rademakers et al. (33) reported finding systolic subendocardial-to-subepicardial thickening ratios of 1.6 and Sinusas et al. (36) a thickening ratio of 1.5 from tagged MRI in dogs but these findings were derived using model assumptions about the behavior of the midwall from epicardial and endocardial measurements rather than from direct measurements, and such assumptions force a transmural gradient. Currently, the spatial resolution of MRI is not sufficient to make the required measurements directly. If the transmural thickness of the LV wall is ~10 mm, and one wants to resolve the thickening strain of one-third of the wall (3 mm) within 0.05, this requires a spatial resolution of 0.15 mm (0.05 × 3), a resolution available in the present bead column studies, but not currently with MRI. MRI resolution is, however, sufficient to measure total systolic wall thickening, and Bogaert and Rademakers (3) recently reported the results of a tagged MRI study of 87 normal humans showing regional wall thickening variations from 35% at the base to 42% at the apex, and from 33% in the posterior region, 36% in the lateral region, 39% in the septum, and 47% in the anterior region. This suggests that, in addition to variations due to species differences (dog, sheep, human), the results of the present study of the lateral region likely exhibit quantitative differences from the behavior of other regions in the same heart.

To summarize, the systolic wall thickening gradients we measure 1 wk after surgery are consistent with those reported in previous studies. Our 8-wk results do not contradict those from previous studies, because we could find no previous studies this late after surgery that did not involve some form of intervention. Thus we think that our experimental results are valid, at least in the lateral equatorial region of the left ventricle in anesthetized sheep.

We next explore some reasons why a transmural systolic wall thickening gradient present at 1 wk could disappear at 8 wk. Our first thought was that these hearts might be operating in a considerably different hemodynamic state from 1 to 8 wk after surgery, but the results shown in Table 1 do not support this view. Although group mean EDV increased by +8 ml (P = 0.04), EDP, ESV, ESP, SV, and EDV/EDP were unchanged between the two study periods. Although not achieving significance at the 0.05 level, group mean heart rate was slightly lower (~10 beats/min; P = 0.08) and the duration of systole was slightly longer (+26 ms; P = 0.06). If, however, a transmural gradient of systolic wall thickening is a fundamental property of heart wall dynamics, it does not seem likely that these small hemodynamic changes would eliminate it.

We next considered the possibility that perhaps some generalization swelling might have been present 1 wk after surgery that was resolved at 8 wk postsurgery. As shown by the data described in RESULTS and shown in Fig. 7, however, the tetrahedral volume computations for volumes contained within the bead columns at subepicardial, midwall, and subendocardial levels, as well as the total contained volume, do not support that contention. Subepicardial volume for the group showed no significant change from 1 to 8 wk, although the dominant systolic wall thickening increase occurred in the subepicardium over this interval. Subendocardial and total volume were also unchanged from 1 to 8 wk, and although midwall volume did increase slightly (P = 0.04), the increase was only 4 μl (~10%). Furthermore, this was an increase, not a decrease, in volume at 8 wk postoperatively, which is not in the direction suggesting a reduction of swelling from 1 to 8 wk. We thus think we are justified in ruling out swelling as an important factor in our findings.

Another possibility is that postsurgical adhesions might have influenced these results. However, the unchanged 3D EDTOT-ES laboratory coordinate displacements of the bead set origin (Fig. 1, O) and five of the six adjacent LV epicardial markers argues against a change in adhesion of the left ventricle to adjacent structures in the thorax in the interval from 1 to 8 wk after surgery, which adhesion might be postulated to affect the dynamics of the LV wall. Furthermore, we observed the same adhesion patterns in sheep at both 1 wk (another group of animals from our laboratory) and 8 wk after thoracotomy. This also suggests that adhesion is not the primary cause of the wall thickening gradient disappearance from 1 to 8 wk after surgery.
Another possibility considered was that the left ventricle might have changed shape between 1 and 8 wk. Both Bolli et al. (4) and Hexteborg et al. (15) stressed that a change in LV shape by itself can alter the transmural gradient of systolic thickening. Our unchanged sphericization index and ROC, however, suggest that a changing LV shape was not the basis for the differences observed in transmural thickening from 1 to 8 wk. There may, however, have been a small shape change in ED geometry within the bead region in the subepicardium and midwall from 1 to 8 wk. As shown in Table 4, although they were not statistically significant at the 0.05 level, ED radial dimensions did display a trend toward reduction from 1 to 8 wk, by 17 ± 19% (P = 0.06) in the subepicardium and by 12 ± 14% (P = 0.08) in the midwall. This thinning at ED from 1 to 8 wk could be linked to the small increase in EDV over this interval (Table 1), but one would need to invoke an unusual selective change in outer regional wall thickness in response to LV volume change, because the subendocardial region did not thin over this time interval. The importance of this possible trend is unknown, but this reduction might somehow enable a greater increase in radial systolic thickening in the outer half of the wall at 8 wk than was possible at 1 wk. Interestingly, the lack of geometric change in the bead region at ES (Table 5) suggests that during the 8 wk after implantation, the myocardium in this region remodels in a transmurally nonhomogeneous fashion such that ED geometry is altered without affecting the ES configuration, although the transmural gradient of both ED and ES remodeling E33 strain is preserved.

The calculated tetrahedral volume of the region bounded by the bead columns allowed us to place some limits on the constant-volume property of ventricular myocardium. Because 95% of the volume values can be expected to be within 2 SD of the mean for this lateral location in these hearts, the 95% confidence limits for the constancy of myocardial volume are ±8% (week 1) and ±11% (week 8) for the subepicardium, ±12% (week 1) and ±12% (week 8) for the midwall, ±13% (week 1) and ±14% (week 8) for the subendocardium, and ±6% (week 1) and ±7% (week 8) for the total transmural volume enclosed by the beads. These values are in agreement with the findings of previous studies (9, 21, 34, 37, 44).

Although the frame-to-frame volume variations that we observed (Fig. 7) are likely to reflect primarily our measurement noise at the frame rate, and because this noise becomes relatively more important on a percentage basis at smaller volumes computed from these bead sets nearer the endocardium, these data represent upper 95% confidence limits for total and transmural volume variations throughout the cardiac cycle. To the best of our knowledge, these are the most complete data reported concerning the constant-volume property of in vivo contracting myocardium to date and the only data concerning the transmural variations of myocardial volume throughout the cardiac cycle.

Another possibility was that the orientation of the first principal strain (E1), the eigenvector associated with systolic wall thickening might have changed between the 1- and 8-wk studies. Because radial wall thickening strain (E33) depends on both the magnitude and the direction of E1, if the E1 direction changed over time with respect to the radial (X3) axis, then E33 would not adequately characterize the maximum deformation of each transmural region. Table 3 shows that the angle of E1 with respect to the radial axis was small (mean angle, 18–30°) and did not change significantly from 1 to 8 wk. The magnitude of E1 increased significantly from 1 to 8 wk at both the 20 and 50% depths. Thus the E33 behavior reported in Table 2 does indeed reflect the maximum deformations of the regions studied, and a changing orientation of E1 is not a factor in the elimination of the transmural thickening gradient from 1 to 8 wk.

Edwards et al. (10) raised the possibility that selective outer wall trauma in studies such as those might lead to artifactually depressed outer wall function. We favor this view and suggest that a likely sequence is that the subepicardial and perhaps midwall thickening is reduced at 1 wk, and at 8 wk postoperatively, this damage is somehow reversed (Table 2 and Fig. 4). Indeed, the large variation in subendocardial-to-subepicardial systolic wall thickening ratio (from 1.0 to 11.0) reported in the previous studies discussed above may in part reflect the level of trauma inflicted. We should stress that we do not claim in the present study that our 1- or 8-wk data necessarily characterize normal dynamics. Scientifically, what is important is that they differ, as though we had somehow been able to vary the transmural gradient of systolic wall thickening in each heart as an independent variable without performing other interventions in these control experiments (although this was actually serendipitous). The glutaraldehyde fixation of the hearts in this study will allow us to perform quantitative histology for each heart and then to transform the cardiac strains reported here into fiber and sheet coordinates (6) that will be described in a future publication.

We next consider factors that might depress early postoperative subepicardial systolic thickening. Because approaches that do not require tunneling into the myocardium, such as single crystal epicardial Doppler studies (4, 10, 12), have the same transmural wall thickening gradients as those requiring tunneling (sonomicrometer crystals) (11, 16, 18, 25, 34), epicardial echocardiography (29), and bead columns (6, 23, 24, 26, 32, 43, 45), tunneling alone probably is not the culprit depressing subepicardial thickening. Furthermore, because transmural gradients are in evidence in both anesthetized (6, 10, 12, 23, 25, 26, 29, 32, 34, 43, 45) and conscious (4, 11, 16, 18, 24) animals and were present at 1 wk but not at 8 wk in our anesthetized animals, anesthesia also probably is not the primary cause of the thickening gradient. Inasmuch as similar gradients have been observed in the anterior (4, 6, 10, 16, 23, 24, 26, 29, 32, 34, 43, 45), lateral (11), and posterolateral (4, 10, 18, 25) regions of the left ventricle in previous studies, the findings from the lateral region in the present study may be representative. Finally, while our study was performed using sheep rather than dogs, which were used in most previous studies, species appear not to play a major role, because our results in sheep at 1 wk mimic those in previous studies showing a transmural thickening gradient. Furthermore, both Hexteborg et al. (14) and Hittinger et al. (16) reported results from chronically instrumented pigs and dogs that showed no significant transmural gradient of wall thickening, much as we found in our 8-wk sheep studies.

We provisionally conclude that the most likely candidate for reduced early subepicardial systolic wall thickening is some unknown process of selective outer wall trauma associated with the surgical procedures that depresses outer wall function for at least 1 wk after surgery and subsequently resolves over several weeks of postsurgical recovery. In addition to mechanical factors, such trauma could involve a number of biochem-
ical factors, such as altered transmural myocardial catecholamine stores (35), calcium handling (5), titin elasticity (13), or myosin light-chain kinase gradients (8).

The findings of the present study suggest some properties of mechanisms involved in systolic wall thickening, discussed in the subsections below.

A transmural radial strain gradient is not a fundamental requirement for systolic wall thickening. Our results show that virtually the same hemodynamics can be produced in the same hearts with and without a wall thickening gradient in the same region. (Tables 1 and 2 and Figs. 4 and 5). Some previous theoretical models addressing this subject (1, 11, 19, 25, 38) suggested the necessity for such a gradient, but these models used assumptions that are the mathematical equivalent of membranes maintaining constant-volume behavior in multiple transmural compartments. No such membranes have been observed.

Wall thickening at any transmural level can act independently of LVV. A clear example of wall thickening acting independently of LVV is shown in Fig. 6 (behavior was noted in all hearts studied), in which $E_{33}$ is shown to increase, remain unchanged, and decrease (sometimes all three in rapid sequence) during IVR, while LVV was not changing. Tight coupling between LVV and wall thickening has been a hallmark of some previous models (1, 11, 19, 25, 38), but these data show that this is not required.

Overall wall thickening does not require increased radial strain at all transmural levels. A clear example of wall thickening not requiring increased radial strain at all transmural levels is shown in Fig. 6 (behavior noted in all hearts studied), in which $E_{33}$ is shown to increase at one depth, remain constant at another depth, and decrease at yet another depth, all during the same interval. Note in Fig. 6B that for most of systole, the transmural thickening gradient was actually reversed, with subendocardial thickening less than subepicardial thickening. Two possible explanations for this behavior, which requires further study, include 1) altered excitation pattern, although no conduction abnormalities were observed during the experiments; and 2) alterations in transmural LV cellular calcium handling from 1 to 8 wk after surgery (5). A recent study of the anterior wall in the open chests of dogs, while revealing a large transmural gradient of $E_{33}$, reported a disproportionate reduction in subendocardial $E_{33}$ relative to subepicardial $E_{33}$ during IVR (2). Although coupling between transmural myocardial levels certainly exists, it clearly does not force the same dynamic thickening behavior, even qualitatively, at each depth or over time.

Systolic radial strain (wall thickening) in the lateral equatorial wall is almost always positive at ES, but may be positive, zero, or negative during the first half of systole. Figure 6B shows this clearly at the 80% depth, where $E_{33}$ is slightly negative during early systole, is zero briefly at mid-systole, and finally achieves a large positive value at ES. The increase in $E_{33}$ during IVR is a typical finding of the present study.

Wall thickening is not associated with major volume transfers between regions at different transmural levels. The results shown in Fig. 7 are typical, with each adjacent transmural compartment delineated and by the beads (subepicardial, mid-wall, and subendocardial as shown in Fig. 1) maintaining the same constant volume throughout the cardiac cycle. This suggests that major transmural compartmental fluid shifts do not occur during systole.

Summary. The five properties described above suggest that mechanistic theories of heart wall mechanics should take into account that, while maintaining a constant myocardial volume, transmural localized regions can change shape independent of LVV and in opposite directions. Rather than a transmural, tightly coupled, concentric series of uniform elastic compartments, heart wall mechanics seem more like a “transmural snake” whose “length” (i.e., wall thickness) measured from its “tail” (i.e., subepicardium) to its “head” (i.e., subendocardium), is determined by the dynamics of each transmural segment along its body, and such segments, while interconnected to their neighbors and each acting with constant volume, can deform almost independently, contracting, stretching, maintaining constant length, shearing, and changing angular relationships to one another at the same time. Although there are many possibilities, this behavior appears to be consistent with some models that recently have been advanced to describe dynamic cellular rearrangement throughout the cardiac cycle (2, 6, 20, 22, 23, 39, 41, 42).

Limitations. A major limitation of the present study is the requirement for surgery, opening the pericardium, and tunneling markers into the myocardium. Anesthesia likely depressed overall wall thickening in our experiments (previous studies, although not in this precise interpapillary region in sheep, described subepicardial wall thickening ranging from 0.04 to 0.33 and subendocardial thickening ranging from 0.18 to 0.83; Refs. 4, 6, 10–12, 16, 18, 23, 24, 26, 29, 32, 34, 43, and 44). Furthermore, it is possible that neither the 1-wk nor the 8-wk studies can be considered “normal” or suitable for extrapolation to dogs or humans. We have attempted to address these issues, but if a transmural systolic thickening gradient is a fundamental property of myocardial function, we would not expect it to disappear in seemingly normally pumping hearts 8 wk after surgery. We conclude that such a gradient of myocyte dynamics is not necessary to produce normal hemodynamics under closed-chest conditions, at least in the sheep heart.

The lateral equatorial region that we studied was picked intentionally to avoid the direct effects of underlying papillary muscle (Fig. 2). This could be a rather special region that is not directly influenced by one papillary muscle or the other (17) but is likely influenced by both. However, this is only one small, specialized region of the left ventricle (the bead set included only 0.15 ml of the LV myocardium), and thus considerable caution must be exercised before extrapolating these results to characterize the whole heart. Again, however, if a transmural thickening gradient were truly a fundamental characteristic of the myocardium, one would expect it to be observed everywhere in the left ventricle. Clearly, we have found an exception to a gradient rule, and only one exception is needed to call such a rule into question. We are currently extending this study, however, to look at the anterior region of the LV wall to determine whether the lateral wall behavior reported here is typical.

The approach used to average the data from each three-beat sequence was of sufficient concern that we analyzed all data using two completely different algorithms. The first algorithm is described in the Methods section, with the ED frame of each individual beat used as the reference configuration and the ES frame of that same beat used as the deformed configuration.
The conclusions of the present study cannot measure the dynamics of the most endocardial 10% of beads being at /H11011

ments, in conjunction with improved bead centroid defining software, in temporospatial resolution and noise reduction in

The bead-enclosed volumes indicate that the beads were not placed at equal transmural distances from the epicardium; the radial distance between beads tended to diminish from subepicardium to subendocardium. The displacement field strain analysis used in this study, however, does not require precisely equal transmural bead spacing; it requires only that the beads fully span the space. Our animated computer graphics reconstruction of bead positions showed that bead sets in all seven animals met this criterion.

The resolution of our current measurement system is on the order of 0.15 mm, and the measurements in this study, particularly in the radial direction, demand every bit of this resolution. We are currently developing a new charge-coupled device camera-based recording system that should allow improvements, in conjunction with improved bead centroid defining software, in temporospatial resolution and noise reduction in future studies.

We have discussed the issue of our deepest subendocardial beads being at ~90% actual wall depth. This means that we cannot measure the dynamics of the most endocardial 10% of the wall using this technique. This, however, does not change the conclusions of the present study.

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