Contribution of laminar myofiber architecture to load-dependent changes in mechanics of LV myocardium

YASUO TAKAYAMA,* KEVIN D. COSTA,* AND JAMES W. COVELL
Departments of Medicine and Bioengineering, University of California, San Diego, California 92093
Received 29 March 2001; accepted in final form 15 November 2001

Takayama, Yasuo, Kevin D. Costa, and James W. Covell. Contribution of laminar myofiber architecture to load-dependent changes in mechanics of LV myocardium. Am J Physiol Heart Circ Physiol 282: H1510–H1520, 2002; 10.1152/ajpheart.00261.2001.—The ventricular myocardium consists of a syncytium of myocytes organized into branching, transmurally oriented laminar sheets approximately four cells thick. When systolic deformation is expressed in an axis system determined by the anatomy of the laminar architecture, laminar sheets of myocytes shear and laterally extend in an approximately radial direction. These deformations account for ~90% of normal systolic wall thickening in the left ventricular free wall. In the present study, we investigated whether the changes in systolic and diastolic function of the sheets were sensitive to alterations in systolic and diastolic load. Our results indicate that there is substantial reorientation of the laminar architecture during systole and diastole. Moreover, this reorientation is both site and load dependent. Thus as end-diastolic pressure is increased and the left ventricular wall thins, sheets shorten and rotate away from the radial direction due to transverse shearing, opposite of what occurs in systole. Both mechanisms of thickening contribute substantially to normal left ventricular wall function. Whereas the relative contributions of shear and extension are comparable at the base, sheet shear is the predominant factor at the apex. The magnitude of shortening/extension and shear increases with preload and decreases with afterload. These findings underscore the essential contribution of the laminar myocardial architecture for normal ventricular function throughout the cardiac cycle.

myocardium; wall thickening; systole; diastole

THE VENTRICULAR MYOCARDIUM consists of a syncytium of myocytes organized into branching transmurally oriented laminar sheets approximately four cells thick (14). Recent evidence indicates that this laminar structure contributes importantly to systolic function. When systolic deformation is expressed in an axis system determined by the anatomy of the laminar architecture, laminar sheets of myocytes shear and laterally extend in an approximately radial direction. These deformations account for ~90% of normal systolic wall thickening in the left ventricular (LV) free wall. In a recent study (5) from this laboratory, we found regional variations in the relative contribution of sheet extension and shear to wall thickening. In the free wall of the ventricle, sheet extension accounted for the majority of wall thickening, whereas in the septum, sheet shear was the predominant factor. Systolic wall thickening is a commonly employed index of regional myocardial performance (8) in both clinical and experimental studies. However, wall thickening varies both transmurally and regionally (7). These variations were reported in clinical studies (3) using magnetic resonance imaging (MRI). Moreover, Villarreal et al. (36) have shown regional variations in the sensitivity of local indexes of function (e.g., circumferential shortening) to changes in loading conditions. Because both the architecture and the functional contribution of myocardial laminas vary regionally, we hypothesized that the regional variation of wall thickening was caused by differences in the local structure and function of the myocardial laminas.

Less is known about the function of the laminar architecture in diastole. Costa et al. (4) have examined finite strains in the wall of the canine LV during passive inflation of the ventricle and showed only small transverse shears, which would seem to indicate that there is little motion of the laminar sheets during diastole. In contrast, Spotnitz et al. (20) documented large changes in cleavage plane orientation during inflation of the passive rat LV. Moreover, diastolic deformation in the intact blood-perfused heart is probably different then in the passively inflated heart.

Extending our previous work detailing end-systolic function of myocardial laminas at a single systolic pressure (5, 15), the present study examined for the first time the three-dimensional (3-D) function of myocardial laminas during diastole. In addition, this study investigated whether the systolic and diastolic function of the sheets was sensitive to alterations in ventricular preload and afterload. In the present study, we investigated whether the changes in systolic and diastolic function of the sheets were sensitive to alterations in systolic and diastolic load. We hypothesized that 1) the functional role of the sheets is different in diastole and systole, 2) sheet function is load dependent both in

*Y. Takayama and K. D. Costa contributed equally to this work.
Address for reprint requests and other correspondence: J. W. Covell, Dept. of Medicine, School of Medicine, Univ. of California, San Diego, CA 92093 (E-mail: jcovell@ucsd.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
diastole and systole, and 3) the effects of load are regionally consistent at the apex and base of the LV free wall. The results of the studies indicate that there is substantial reorientation of the laminar architecture during systole and diastole. Moreover, this reorientation is both site and load dependent. Thus as end-diastolic pressure (EDP) is increased and the LV wall thickens, sheets shorten and rotate away from the radial direction due to transverse shearing, opposite of what occurs in systole. These mechanisms for changing ventricular wall thickness contribute substantially to normal LV wall function. Whereas the relative contributions of reorientation (interlaminar shear) and extension are comparable at the base, shear is the predominant factor at the apex. The magnitude of shortening/extension and shear increases with preload and decreases with afterload. These findings underscore the essential contribution of the laminar myocardial architecture for normal ventricular function throughout the cardiac cycle.

METHODS

The University of California at San Diego is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All experiments were conducted according to AAALAC guidelines for the use of animals in research and were approved by the Institutional Animal Care and Use Committee. A subset of data from the six animals included in this study has been presented previously (5). The present study describes previously unreported data on the effects of a series of diastolic and systolic load changes on the laminar architecture. The previous study reported findings under-...
sure in the LV was adjusted to 8–10 mmHg by infusion of warmed saline. The LV was perfusion fixed via the aortic root with buffered gluteraldehyde (2.5%) (44). All hearts were removed and stored in 10% buffered formalin (Fischer Scientific; Fairlawn, NJ).

Morphological studies. The myocardial cleavage plane and muscle fiber angles at each site were measured using the approach previously reported by LeGrice et al. (15). The specific procedures carried out on these hearts are described in detail in a recent publication (5). In brief, hearts were sectioned and tissue blocks containing the markers were removed from the basal and apical site in the anterior LV wall. Two 1-mm-thick transmural sections were cut from the block: one parallel to the longitudinal-radial surface (Fig. 2A) and one parallel to the circumferential-radial surface. These sections were cut into 50- to 100-μm-thick slices with the use of a vibrating microtome (Vibrotome 1000, Technical Products International) and the orientation of cleavage planes was determined using transmitted light with low magnification (×30) on a light microscope (Nikon Optiphot-2). The remaining transmural block of tissue was cut into 1-mm-thick slices in planes parallel to the epicardial-tangent plane (Fig. 2B). On the cut surfaces of each of the thick sections, the orientation of the muscle fibers was identified using reflected light at low power (×20). Images of the sections were acquired with the use of image processing software (NIH Image version 1.47) via a videocamera (model DXC-151, Sony) mounted on the microscope, and orientations of fibers and cleavage planes could be measured across each section and referenced to depth from the epicardium. Cleavage plane angles above the radial axis and fiber angles above the circumferential axis are recorded as positive and angles below are negative, consistent with the methods of Streeter and co-workers (34) and LeGrice and colleagues (15). These angles were then averaged in 1-mm steps across the wall. Sheet angle was defined as a rotation about the fiber axis relative to the radial axis (4).

Data Analysis

Calculation of deformation. The 3-D coordinates of the implanted beads were reconstructed from the biplane images at end diastole and end systole. End diastole was taken as the time of the peak QRS wave of the ECG. Pressure at the nadir of the dicrotic notch from the fluid-filled aortic root catheter was used to estimate the timing of ESP on the micromanometer tracing and then identify the timing of the end systolic cine frame. The analysis to obtain continuous, nonhomogeneous strain variations across the LV wall followed the least-squares finite-element method described by McCulloch et al. (21). In this method, the nodal parameters of a 3-D finite element with quadratic transmural interpolation of all three spatial coordinates were fitted by least squares to the coordinate of all the beads. A single finite element with six vertex nodes was fitted so that each of its three transmural edges approximated one of the bead columns in the undeformed reference state. The corresponding deformed bead coordinates were then used to fit an updated element configuration. To obtain the systolic strains in cardiac coordinates, the end-diastolic frame at each beat was used as the reference state, and the end-systolic configuration was used as deformed configuration. In the preload study, we elected to use the end-diastolic state at the lowest EDP (range of 3–4 mmHg) as the reference state, and end-diastolic configuration at low EDP, medium EDP, and high EDP during IVC occlusion was used as the deformed configuration. The Lagrangian Green’s strain tensor was then computed as a continuous function from the spatial gradients of the fitted finite element interpolating functions. Six independent finite strains were calculated in the cardiac coordinate system. These strains were expressed in a local coordinate system in which the first axis (X1) is circumferential, the second axis (X2) is longitudinal, and the third axis (X3) is radial (Figs. 1 and 2). The six finite strains included normal strains (E11, E22, and E33), which described stretch or shortening along the circumferential, longitudinal, or radial axis, respectively, and three shear strains (E12, E13, and E23), which described changes in angle between pairs of axes that were mutually perpendicular in the reference configuration.

To relate strains to a local 3-D structure of the ventricular wall, we used a previously described method to construct a local system of fiber-sheet coordinates (4) that defines the muscle fiber axis (Xf), the sheet axis (Xs), which lies within the sheet plane and is perpendicular to Xf, and the orthogonal Xo, axis, which is oriented normal to the sheet plane (Fig. 3). In brief, the two measured cleavage plane angles and the local muscle fiber angle were used to determine two separate transmural distributions of sheet angle. The final transmural distribution of sheet angle was determined by a quadratic fit (weighted to reflect the effect of fiber orientation on theoretical accuracy of cleavage angle measurements) to two transmural distributions (4). With values of fiber and sheet angle known at all depths, strains are converted from cardiac coordinates to the fiber-sheet coordinate system using a transformation matrix previously described (4). The resulting fiber-sheet strains are the following: 1) Ef, stretch (+) or shortening (−) along the fiber direction, 2) Es, stretch (+) or shortening (−) along the sheet axis, 3) En, stretch (+) or shortening (−) normal to the fiber sheet plane, 4) Ew, shear within the sheet plane, and 5) Ewm, shear that results

---

**Fig. 2.** Schematic drawing of two views of a transmural block of myocardium. **A:** a single sheet. See Fig. 1 for definitions of X1, X2, and X3. **B:** addition of muscle fibers to the sheet and position of epicardial markers.
Fiber Sheet Coordinates

Fig. 3. Schematic diagram of a transmural block of myocardium (as in Fig. 2B) with the fiber-sheet axis system labeled. Xf, fiber axis; Xn, sheet axis; Xs, sheet normal axis.

from sliding of adjacent sheets parallel to the fiber axis (Efn) or transverse to the fiber axis (Eun).

Strain data were determined at three relative depths through the total wall thickness; outer = 20% of the transmural depth from epicardium, midwall were at 50%, and inner were at 80%.

Contributions of fiber-sheet strain to wall thickening. We (5) have previously described the relationship between deformation expressed in the cardiac coordinate system and strains related to the laminar architecture. In general, all six of the sheet-strain components and the fiber and sheet angle may be involved in these relations. However, as the following equation reveals

\[ E_{33} = E_{nn} \cos^2 \beta + E_{nn} \sin^2 \beta + 2E_{nn} \sin \beta \cos \beta \] (1)

the radial wall-thickening strain (E33) depends only on the sheet angle \( \beta \) and the fiber-sheet components of strain in the \([X_n, X_s]\) plane perpendicular to the local fiber axis, namely \( E_{nn}, E_{sn}, \) and \( E_{ns} \). To assess the fiber-sheet strain determinants of systolic wall thickening, the contribution of each term on the right-hand side of Eq. 1 to \( E_{33} \) is presented (\( E_{ns}, \cos^2 \beta; E_{nn}, \sin^2 \beta; 2E_{nn}, \sin \beta \cos \beta \))

Reference Configuration

The choice of reference configuration for systolic strains expressed in cardiac coordinates traditionally has been the end diastole for that beat, and the reference configuration for diastolic cardiac strains has been zero or the lowest achievable filling pressure. We have elected to use this same strategy in this study. However, because the fiber and sheet angles were measured at only one filling pressure (8.8 ± 1.3 mmHg), we needed an approach to estimate the fiber and sheet angles at several different EDPs in each animal. We have previously demonstrated (5) that the 3-D laminar myocardial architecture of the LV wall at a given transmural depth may be mathematically described using two angle measures that define the local sheet structural axes relative to the local geometric axes of the LV. The fiber angle \( \alpha \) measures the orientation of the local muscle fiber axis relative to the circumferential (hoop) axis. The sheet angle \( \beta \) measures the local orientation of laminar myocyte bundles relative to the radial axis. Thus computing the changes in sheet architecture during LV wall deformation reduces to calculating the changes in \( \alpha \) and \( \beta \) associated with measurements of 3D finite strains in the myocardium. Details of this procedure, with an example calculation, are given in the APPENDIX.

Statistical Analysis

All values are reported as means ± SD unless otherwise noted. The transmural and longitudinal variations of each strain at various load conditions were analyzed using three-factor (pressure, site, and depth) repeated-measures ANOVA (SPSS for Windows version 6.1.4). When significant differences were detected by ANOVA, contrasts were performed to determine which individual differences were statistically significant. Statistical significance was accepted at the 95% confidence level (\( P < 0.05 \)).

RESULTS

Hemodynamics

We selected four contractions in each animal for the diastolic-loading studies. In all but two animals, these data were obtained during IVC occlusion. However, because good biplane images were not obtained during IVC occlusion in two dogs, data obtained during methoxamine infusion were used for these two animals. The contraction with the lowest value of EDP (average 3 ± 0 mmHg) was used as the reference configuration (lowest EDP). Three other contractions were selected at average EDPs of 8 ± 1 (low EDP), 13 ± 1 (medium EDP), and 18 ± 2 mmHg (high EDP).

Because both IVC occlusion and methoxamine changed ESP as well as EDP, the ESP tended to increase at high levels of EDP. In all six animals, the ESP of the reference beat averaged 104 ± 15 mmHg and the averages of ESP at each level of EDP were 105 ± 21 (low EDP), 129 ± 32 (medium EDP), and 153 ± 26 mmHg (high EDP). However, there was substantial variability in the magnitude of the increase in ESP between animals and only the ESP at high EDP was significantly greater than the ESP of the reference beat.

For the afterload study, a single contraction with an increased ESP (>35 mmHg compared with baseline) at matched EDP to the control contraction (EDP control = 12 ± 1, methoxamine = 12 ± 2) was selected during methoxamine infusion. The ESP increased from 119 ± 22 to 169 ± 23 mmHg. Heart rate decreased slightly but significantly during methoxamine infusion from 100 ± 11 beats/min at control to 94 ± 11 beats/min during methoxamine infusion (\( P = 0.0427 \)).

Anatomic Measurements

The centroids of the sets of three columns of beads at the basal site were located 23 ± 6% of the longitudinal distance from base to apex and at the apical site were...
located 80 ± 11% of the distance from base to apex. The average wall thickness was 12 ± 3 mm at the basal site and was 10 ± 2 mm at the apical site. All of the 12 bead sets for 6 dogs spanned at least 69% of the ventricular wall thickness, and 7 of 12 sites exceeded 90%.

The fiber and cleavage angle measurements and the resulting sheet angles at each site for these six dogs have been presented in detail in a previous publication (5). In brief, average of fiber and sheet angles in hearts fixed at 8.8 mmHg are shown in Table 1. Fiber angle distributions exhibit a nearly linear increase with depth beneath the epicardium. Sheet angles at the two sites are opposite in sign through most of the wall. Sheet angles at the two sites are opposite in sign through most of the wall.

**Calculated sheet angle.** Calculated sheet angle (β) changed significantly at the different EDPs. Figure 4 shows the calculated sheet angle at all four pressures and at three transmural sites. Note that there was a progressive change in angle at each site as EDP increased. At both sites, the magnitude of β increased as diastolic pressure increased, indicating reorientation of the sheets away from the radial direction as the wall thins. This effect was greater at the apex compared with the base. Much smaller changes in fiber angle (α) were found (typically <5°).

**Diastolic Strains**

End-diastolic strain expressed in cardiac coordinates (not shown) increased with increasing EDP at both sites. Thus overall both $E_{22}$ and $E_{12}$ significantly increased with EDP, whereas $E_{33}$ (Table 2) became more negative. $E_{11}$ increased but did not achieve statistical significance. At inner wall sites at the apex and base $E_{11}$ increased from 0.13 ± 0.14 to 0.41 ± 0.16 and 0.11 ± 0.20 to 0.29 ± 0.20, respectively, as EDP increased from low to high levels. Similarly, $E_{22}$ at the inner wall of the apex and base increased from 0.05 ± 0.08 to 0.17 ± 0.07 and 0.05 ± 0.03 to 0.15 ± 0.09, respectively.

Moreover, there was a significant difference in the magnitude of the strains at the two sites. The increase in $E_{11}$ with EDP at the apical site was significantly greater than at the basal site. $E_{12}$ failed to change at the apex (0.01 ± 0.05 low EDP to 0.01 ± 0.07 high EDP), whereas it became significantly more negative between base and apex.

**Table 1. Average fiber and sheet angle values at three transmural wall depths from base and apex measurement sites**

<table>
<thead>
<tr>
<th></th>
<th>Fiber Angle, °</th>
<th>Sheet Angle, °</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inner</td>
<td>Mid</td>
</tr>
<tr>
<td>Base</td>
<td>28.8 ± 18.2</td>
<td>−12.0 ± 11.7</td>
</tr>
<tr>
<td>Apex</td>
<td>62.3 ± 9.6</td>
<td>30.6 ± 7.7</td>
</tr>
</tbody>
</table>

Values are means ± SD for an average of angles in hearts fixed at 8.8 mmHg.

**Table 2. End-diastolic strains in fiber-sheet coordinates (inner wall)**

<table>
<thead>
<tr>
<th></th>
<th>EDP = 8 mmHg</th>
<th>EDP = 13 mmHg</th>
<th>EDP = 18 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{33}$</td>
<td>−0.04 ± 0.07+†‡</td>
<td>−0.12 ± 0.05+‡</td>
<td>−0.14 ± 0.05+‡</td>
</tr>
<tr>
<td>$E_{ff}$</td>
<td>0.06 ± 0.071*</td>
<td>0.14 ± 0.09*</td>
<td>0.18 ± 0.13*</td>
</tr>
<tr>
<td>$E_{sa}$</td>
<td>−0.04 ± 0.07</td>
<td>−0.06 ± 0.08</td>
<td>−0.07 ± 0.08</td>
</tr>
<tr>
<td>$E_{nn}$</td>
<td>0.1 ± 0.17*</td>
<td>0.14 ± 0.2*</td>
<td>0.19 ± 0.24*</td>
</tr>
<tr>
<td>$E_{sn}$</td>
<td>−0.02 ± 0.04</td>
<td>−0.06 ± 0.09</td>
<td>−0.07 ± 0.10</td>
</tr>
<tr>
<td>$E_{fm}$</td>
<td>0.03 ± 0.09+‡</td>
<td>0.06 ± 0.07+‡</td>
<td>0.08 ± 0.07+‡</td>
</tr>
<tr>
<td>$E_{mn}$</td>
<td>−0.01 ± 0.06+‡</td>
<td>−0.09 ± 0.07+‡</td>
<td>−0.14 ± 0.05+‡</td>
</tr>
<tr>
<td>Apex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{33}$</td>
<td>−0.7 ± 0.08</td>
<td>−0.15 ± 0.05</td>
<td>−0.21 ± 0.03</td>
</tr>
<tr>
<td>$E_{ff}$</td>
<td>0.06 ± 0.10</td>
<td>0.15 ± 0.1</td>
<td>0.21 ± 0.08</td>
</tr>
<tr>
<td>$E_{sa}$</td>
<td>−0.05 ± 0.029</td>
<td>−0.03 ± 0.11</td>
<td>−0.06 ± 0.16</td>
</tr>
<tr>
<td>$E_{nn}$</td>
<td>0.09 ± 0.11</td>
<td>0.14 ± 0.12</td>
<td>0.21 ± 0.18</td>
</tr>
<tr>
<td>$E_{sn}$</td>
<td>0.02 ± 0.02</td>
<td>0.02 ± 0.04</td>
<td>−0.00 ± 0.05</td>
</tr>
<tr>
<td>$E_{fm}$</td>
<td>0.05 ± 0.04</td>
<td>0.10 ± 0.03</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>$E_{mn}$</td>
<td>0.09 ± 0.1</td>
<td>0.2 ± 0.09</td>
<td>0.26 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SD. $E_{33}$, radial strain; $E_{ff}$, end-diastolic pressure; $E_{sa}$, fiber strain; $E_{nn}$, sheet strain; $E_{sn}$, sheet normal strain; $E_{fm}$, sheet-normal shear; $E_{mn}$, fiber sheet shear; $E_{mn}$, fiber normal shear. The reference configuration for the strains is EDP 3 mmHg. *Significant effect of depth on strain; †significant effect of pressure on strain; ‡significant difference between base and apex.

Fig. 4. Plot of the mean ± SD of change in sheet angle the basal site (A) and apical site (B). Inner, Mid, and Outer indicate the depth from the epicardial surface. EDP, end-diastolic pressure (mmH).
at the base (0.00 ± 0.04 low EDP to −0.06 ± 0.05 high EDP). The increase in $E_{23}$ with increased EDP also was greater at the apical site (−0.02 ± 0.05 low EDP to −0.010 ± 0.04) compared with −0.02 ± 0.04 low EDP to 0.0 ± 0.03 high EDP.

When end-diastolic strains were expressed in the fiber-sheet coordinate system, there was also significant deformation. The data are shown in Fig. 5 for the apical site at three depths and the high EDP and in Table 2 for the inner third of the LV wall at both sites.

Fiber strain ($E_{ff}$) increased significantly with increased EDP at both sites. At each EDP, $E_{ff}$ was slightly less at the outer wall than in the midwall and inner wall depths. Both interlaminar shear strains that reflect sliding of adjacent sheets ($E_{fs}$ and $E_{fn}$) showed a significant increase with pressure and depth and were greater at the apical site, and $E_{sn}$ changed sign from apex to base.

Contributions to wall thinning. Figure 6 describes the relative contribution of the components of laminar deformation (expressed as the three terms on the right-hand side of Eq. 1) to $E_{33}$. Although lateral shortening of sheets ($E_{ns} < 0$) and intra- and interlaminar shear both contributed significantly to wall thinning ($E_{33} < 0$) at the apical and basal sites, interlaminar shear was the predominant mechanism by which the wall thinned at the apex. Also, sheet thickening ($E_{nn} > 0$) has a small but consistent effect on wall thinning at the inner wall at the basal site, whereas this mechanism was negligible in the outer wall and at the apex.

Effects of Increased Systolic Pressure on Systolic Strains

Systolic deformation expressed in cardiac coordinates was significantly reduced by increases in ESP (not shown). Thus $E_{11}$, $E_{22}$, and $E_{33}$ were all significantly reduced at all transmural levels. There were no significant effects of increases in ESP on shear strains expressed in cardiac coordinates. Deformation expressed in sheet coordinates was also decreased significantly by increases in ESP. Thus there were significant reductions in $E_{ff}$, $E_{ns}$, $E_{fn}$, and $E_{sn}$ as shown in Fig. 7 for data at the inner wall of the ventricle. $E_{ff}$ decreased significantly at both sites during methoxamine at matched EDP. However, there were no significant differences among three depths and between the two sites. $E_{ns}$ also decreased with methoxamine and this effect was greater at the inner wall at both sites. $E_{sn}$ significantly increased (became less negative) at the apical site and decreased at the basal site. The reductions were all greater at the inner wall. The relative contribution of sheet motion to $E_{33}$ was not influenced by increased ESP, as shown in Fig. 8.
DISCUSSION

The ventricular myocardium consists of a syncytium of myocytes organized into branching transmurally oriented laminar sheets approximately four cells thick (14). Although this architecture has been recognized for over 50 years (6), until the recent development of techniques to determine 3-D finite deformation in the intact heart (3), it was not possible to assess the functional role of this unique laminar architecture. Recent evidence (5) based on these approaches indicates that this laminar structure may contribute importantly to systolic function. These studies have indicated that shearing deformation and lateral extension of the sheets accounts for almost all normal systolic wall thickening at several sites in the LV. The present study shows for the first time that the same mechanisms which give rise to wall thickening during systole also operate in reverse to account for wall thinning in diastole. Interestingly, the relative contributions of changes in sheet length and interlaminar shear to changes in wall thickness were relatively independent of load during both inflation and changes in systolic load despite large changes in deformation. This suggests a tightly regulated mechanism for changes in wall thickness in the normal beating heart.

Diastolic Strains

Resting tension in isolated cardiac muscle is borne by structures within the myocytes such as titin (39) and probably at higher loads by the extracellular matrix (18). In the whole heart, the relationship between filling pressure and volume is influenced by other factors including wall thickness, viscoelastic and other time-dependent factors (26) and coronary perfusion. Spotnitz et al. (33) were the first to point out that the laminar architecture of the ventricular wall changed as the heart was inflated. Their study showed that cleavage planes became more parallel to the epicardium as the heart was inflated. This rotation of the cleavage planes was thought to contribute to wall thinning as the heart dilated. The exact contribution of sheet deformation to changes in wall thickness ($E_{33}$) is given by Eq. 1 (5). This relationship shows clearly that sheet thickening and extension contribute to wall thickening as well as sheet rotation. Figure 6 indicates that both shortening of sheets and intralaminar shear importantly contribute to changes in wall thickness. The data indicated that the sheets thicken by as much as 20% in the subendocardium as the heart dilates ($E_{an}$ in Table 2). Because it seems safe to assume that myocyte diameters are decreasing as the heart is inflated, positive values of $E_{an}$ are consistent with rearrangement of myocytes within sheets (5). However, from our data, it is not possible to separate rearrangement of myocytes within the sheets from reorientation of the sheets themselves.

Omens et al. (24) examined 3-D deformation in the arrested nonperfused canine heart. They found that $E_{ff}$ was uniform across the wall at all degrees of inflation. However, the present study found that fiber strains on the epicardium were lower then those in the mid and inner aspects of the ventricular wall. The difference between the two studies likely is related to the preparations. The Omens et al. study (24) was done in the isolated arrested heart floating in warmed saline. The present study was done in the open-chest animal with the heart suspended in a pericardial cradle, where at low volumes the shape of the heart is likely to be different than in the in vitro preparation.

There are also important regional differences in the response of the laminar architecture to inflation. Sheet angles do not change much with inflation at the basal site (Fig. 4). However, changes at the apex are substantial. This is consistent with a greater contribution of $E_{sn}$ to wall thinning at the apex (Fig. 6). The anterior
papillary muscle and its insertion is interposed between these two sites and it seems likely that the stiff mitral annulus and the tethering effects of the chordae may influence the response of the basal site.

**Regional End-Systolic Strains and Effects of Afterload**

In the normal heart, the ventricular shape, myocyte architecture, ventricular activation sequence and the papillary muscle chordae tendineae system act together to produce substantial regional variations in LV function. Regional variations in shortening were first detected in humans by Kong et al. (12) using coronary angiography. These investigators, and Liedke et al. (17), by using angiographic techniques, showed greater shortening at the apex compared with the base of the LV. With dimension gauges implanted at the midwall in the direction of the midwall myofibers, shortening was found to be greatest in the apex (~20%) and less at the midwall and base of the ventricle (16). In the transplanted human heart, longitudinal shortening tended to be greater on the posterior wall and approximately equal to circumferential shortening at anterior locations (11). Similar results have also been obtained using finite deformation approaches (35). The presence of large deformations and substantial shearing makes it difficult to interpret the uniaxial data obtained from dimension gauges or other two-dimensional (2-D) approaches in terms of the local structure. The presence of small amounts of in plane and transverse shear produces substantial errors in the estimate of local fiber strain using uniaxial techniques (37). However, recent data using both 2-D and 3-D approaches and MRI have shown apex-base gradients in function in humans and some experimental animals (3, 13, 27). In the present study, $E_{11}$ in the inner wall at the apical site was significantly larger than $E_{11}$ at the basal site at baseline and in this same preparation $E_{33}$ was greater at the apex than the base. This was also true for $E_{ss}$ and $E_{sn}$. Fiber shortening ($E_{fr}$), however, was not different at the two sites.

The magnitude of $E_{11}$ and $E_{22}$ showed a consistent and substantial increase with depth. These transmural variations of strains have been observed in both human and experimental animal studies (3). However, in agreement with several other studies (23), $E_{rr}$ showed a relatively uniform distribution across the ventricular wall. Consistent with the difference in sheet angle $E_{sn}$ varied at the two sites and was always greater at the inner wall. The transmural differences in $E_{sn}$ were reported earlier in these animals (5) and seem likely to be due to transmural differences in the magnitude of systolic strain.

Increasing arterial pressure with methoxamine reduced strains expressed in both coordinate systems and tended to reduce inner wall strains to a greater extent thus reducing the transmural gradient. Matsuzaki et al. (19) also showed the increased afterload reduced nonuniformity of thickening between outer and inner layers. In contrast to earlier studies using uniaxial dimension gauges, we did not observe a proportionally greater reduction in deformation at the apex (16). We suspect that this is due to the difficulties with uniaxial measurements (38) at the midwall. Methoxamine infusion induced substantial and fairly uniform reduction in all strains expressed in sheet coordinates and the relative contribution of sheet strains to $E_{33}$ at each site remained uniform.

**Limitations**

The present study was designed to address the role of deformation of the laminar architecture in the regional response to changes in preload and afterload in the canine LV. The approach has several limitations. The major limitation is that we can measure the orientation of the sheets at only one configuration in each animal in the fixed heart and must use deformation data to estimate systolic and diastolic changes in local fiber and sheet orientation. To do this, we assume a structural model for the myocardium that is based on the average fitted fiber and sheet architecture at each site in the wall. Thus, although the equations transforming strains from cardiac to fiber and sheet coordinates are exact, errors in measurement of strains (the resolution of the radiographic system is ~0.02 mm) or transposing the anatomic reference system from microscopic measurements to the cardiac coordinate system in vivo will also contribute to the errors. Moreover, we have not taken into account the variance of both fiber and sheet orientation at each site. At the present time, it is not possible to determine fiber and sheet anatomy in the beating heart, although diffusion MRI shows considerable promise (29). Thus measurements must be done in different hearts fixed at different filling pressures, a task that has not been undertaken in larger hearts. In this preparation we did not use a bypass preparation that would have allowed better control of EDPs and ESPs. Thus there were changes in ESP as EDP was raised. Although these were not statistically significant, the changes in end-diastolic position of the sheet must be the result of both changes in ESP (and certainly end-systolic stress) as well as EDP.

In summary, the present study shows for the first time that the same mechanisms that give rise to wall thickening during systole also operate in reverse to account for wall thinning in diastole. Interestingly, the relative contributions of changes in sheet length and interlaminar shear to changes in wall thickness were relatively independent of load during both inflation and changes in systolic load, despite large changes in deformation. This suggests a tightly controlled mechanism for changes in wall thickness in the normal beating heart. Moreover, there were important regional variations in both sheet structure and function. For example, the contribution of intralaminar shear to changes in wall thickness differed at the apex and base, suggesting that the motion of laminae is more restricted at some sites. The concept that sheet motion contributes to normal diastolic and systolic ventricular function may have important implications for our un-
derstanding of cardiac pathophysiology. For example, a change in diastolic angle due to ventricular remodeling may influence the contribution of $E_{sn}$ to wall thickening. Moreover, disorders of the connective tissue matrix have been shown in various diseased hearts (1). These processes are likely to influence the ability of sheets to change orientation in both systole and diastole, and thus contribute to alterations in ventricular function.

APPENDIX

Calculating Changes in 3-D Tissue Architecture from Deformation Data

For this study, we required knowledge of the 3-D fiber-sheet architecture of the LV in several loading configurations different from the EDP at which the heart was fixed and histological measurements were actually made. Therefore, a method was devised in which measurements of 3-D strain from one loading configuration to another were used to determine corresponding changes in myocardial fiber and sheet orientation.

To illustrate the approach, we will use an example in which strains were measured during passive inflation of the ventricle at an EDP = 18 mmHg relative to a reference EDP = 3 mmHg. In this example, we will assume we know the fiber and sheet angles from anatomic measurements in the same heart at the inner apical site fixed at 3 mmHg filling pressure. These data are as follows: $\alpha = 71^\circ$, $\beta = -8.4^\circ$, $E_{11} = 0.560$, $E_{22} = 0.268$, $E_{12} = 0.033$, and $E_{33} = -0.213$.

We first consider the problem of calculating a change in muscle fiber orientation. Figure 9, top, depicts a section of apical anterior LV free wall cut parallel to the local epicardial-tangent plane in the reference configuration (EDP = 3 mmHg). The thin diagonal lines represent the local muscle fiber orientation. The $X_1$ and $X_2$ axes represent the local circumferential and longitudinal axes of the LV, and $X_f$ is aligned with the local muscle fiber axis. The local fiber angle ($\alpha = 71^\circ$) between $X_f$ and $X_1$ is indicated. Figure 9, bottom, depicts the same section of myocardium in the deformed configuration (EDP = 18 mmHg), indicating substantial biaxial stretch in the $\{x_1,x_2\}$ plane, with a small positive in-
plane torsional shear \( E_{12} \). With the use of continuum mechanics theory, the deformed fiber angle \( \alpha' \) is given by

\[
\cos \alpha' = \frac{2(E_{11} \cos \alpha + E_{12} \sin \alpha) + \cos \alpha}{\Lambda_1 \Lambda_1}
\]

where

\[
\Lambda_1 = \sqrt{2(E_{11} \cos^2 \alpha + E_{22} \sin^2 \alpha + 2E_{12} \sin \alpha \cos \alpha) + 1}
\]

Here, \( \Lambda_1 \) and \( \Lambda_1 \) are the stretch ratios measuring the deformed length of the vectors \( \mathbf{x}_1 \) and \( \mathbf{x}_1 \) respectively, relative to their undeformed lengths. In this example, \( \alpha' = 66^\circ \) represents a change in fiber orientation of \(-5^\circ\).

A similar approach was used to compute changes in the sheet angle \( \beta \) as illustrated in Fig. 10. Figure 10, top, depicts a transmural section of apical anterior LV free wall cut perpendicular to the local muscle fiber axis in the subendocardium (80% wall depth); at this depth, the fiber axis points into the page, and the thin diagonal lines represent the orientation of cleavage planes between adjacent laminar bundles of myocardium, with the endocardium on the left and the epicardium on the right. The radial, \( X_3 \), axis represents the local normal to the epicardial tangent plane in this reference configuration. The \( X_e \) axis is the in-plane, cross-fiber axis originally defined by Waldman et al. (38) to lie perpendicular to the local fiber axis and within a plane parallel to the epicardial tangent plane. \( X_c \) is the sheet axis, which is oriented perpendicular to the fiber axis and lies in the plane of the myocardial lamiae. The sheet angle \( (\beta = -8^\circ) \) between the \( X_e \) and \( X_3 \) axes is indicated. In addition, \( \gamma \) is the angle between the \( X_e \) axis and the \( X_c \) axis and is equal to \( 90^\circ - \beta \) in the undeformed reference state, in which \( X_e \) and \( X_3 \) are orthogonal.

Figure 10, bottom, illustrates the deformed configuration of this section of the LV wall at the increased EDP of 18 mmHg. As we show in this study, the myocardial lamiae (thin lines) have become less radially oriented as the heart wall thinned. However, due to large transverse shear strains, the \( x_e \) axis has also rotated away from its original orientation (by \(-28^\circ\)) and is no longer perpendicular to the heart wall surface. That is, the segment of myocardium that was originally radially oriented at the reference state is no longer “radial” at the elevated EDP. Consequently, the angle between the deformed \( x_e \) and \( x_3 \) axes has increased only minimally \((-12^\circ \) compared with \(-8^\circ \) in the reference configuration). Whereas this is theoretically correct, it is of limited practical value because, unlike the sheet axis, the radial axis is not an actual structural axis whose orientation can be uniquely identified and measured experimentally. Radial is experimentally defined as perpendicular to the LV surface at any given instant.

Therefore, of practical interest is the angle between \( x_e \) and the current local normal to the heart wall surface, represented by the dashed arrow in Fig. 10, bottom. This axis is perpendicular to the cross-fiber axis \( x_e \). Therefore, because it is readily identified anatomically, we use the \( x_e \) axis as a reference for calculating changes in sheet angle due to myocardial deformation. In particular, continuum mechanics theory was used to derive the following expression for the deformed sheet angle: \( \beta' = 90^\circ - \gamma' \), between \( x_e \) and the axis perpendicular to \( x_e \), in the current configuration

\[
E_{cc} = E_{11} \sin^2 \alpha + E_{22} \cos^2 \alpha - 2E_{12} \sin \alpha \cos \alpha
\]

are stretch ratios of the \( X_e \) and \( X_c \) vectors, and the cross-fiber-strain \( (E_{cc}) \) and cross-radial shear \( (E_{c3}) \) are given by

\[
\sin \beta' = \cos \gamma' = \frac{2(E_{cc} \sin \beta + E_{c3} \cos \beta) + \sin \beta}{\Lambda_1 \Lambda_1}
\]

where

\[
\Lambda_1 = \sqrt{2(E_{cc} \sin^2 \beta + E_{c3} \sin^2 \beta + 2E_{c3} \sin \beta \cos \beta) + 1}
\]

\[
\Lambda_3 = \sqrt{2E_{cc} + 1}
\]

Equation 3 is used to compute the sheet angle, which would be measured experimentally at various conditions of diastolic and systolic LV wall deformation, with \( E_{cc} \) and \( E_{c3} \) calculated using the undeformed fiber angle \( \alpha \) described above. In this example, \( E_{cc} = 0.509 \) and \( E_{c3} = 0.254 \), yielding \( \beta' = -40^\circ \), representing an angle change of \( 32^\circ \) from the reference configuration.

In general, if strains describing the deformation from state A to state B are given, and the values of \( \alpha \) and \( \beta \) in the reference state A are known, then computing the values of \( \alpha' \) and \( \beta' \) in state B is straightforward. Alternatively, if the values of \( \alpha' \) and \( \beta' \) in the deformed state are known, then the same equations may be used with estimated values of \( \alpha \) and \( \beta \) iteratively adjusted to yield the known values of \( \alpha' \) and \( \beta' \).

Equations 2 and 3 are exact. However, interpretation of the calculated angles \( \alpha' \) and \( \beta' \) as representing fiber and sheet angles that would be measured histologically requires two assumptions about the nature of the deformation of the LV wall: 1) circumferential segments of the myocardium remain circumferential and 2) planes parallel to the epicardium remain parallel to the epicardium. Because the LV maintains its thick-walled ellipsoidal geometry as it deforms, these assumptions seem reasonable and well within the \( 5^\circ - 10^\circ \) variance in the histological measurements.

We thank Dr. Jeff Holmes and Dr. Ian LeGrice for support and helpful discussions during the during the design and conduct of these studies. We appreciate the guidance of Dr. Larry Taber in deriving the equations for calculating angle changes due to finite deformations. We also thank Richard Pavelec for contributing managerial and surgical skills to the study.

This study was supported by National Heart, Lung, and Blood Institute Grant HL-32583.

Present address of Y. Takayama: Kansai Medical University/ Cardiovascular Center, 10-15 Fumizono-Cho, Higakuchiba, Osaka 570-0078, Japan.

Present address of K. D. Costa: Dept. of Biomedical Engineering, Columbia University, Mail Code 8904, 530 W. 120th St., New York, NY 10027.

REFERENCES


5. Costa KD, Takayama Y, McCulloch AD, and Covell JW. Laminar fiber architecture and three-dimensional systolic me-


