Effect of residual stress on transmural sarcomere length distributions in rat left ventricle

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Rodriguez, Edward K., Jeffrey H. Omens, Lewis K. Waldman, and Andrew D. McCulloch. Effect of residual stress on transmural sarcomere length distributions in rat left ventricle. Am. J. Physiol. 264 (Heart Circ. Physiol. 33): H1048–H1056, 1993.—It has been previously shown that the myocardium in the walls of the unloaded passive left ventricle (LV) is not stress free. To assess the functional significance of residual stress in the ventricular wall, we compared the transmural distributions of sarcomere length (SL) in specimens of rat LV myocardium fixed in the unloaded (residually stressed) and stress-free states. When a cross-sectional ring cut from the equatorial region of the freshly arrested rat hearts was cut radially to relieve residual stress, it sprang open into an arc with a mean opening angle of 45 ± 15° (SD) (n = 8). During immersion fixation in glutaraldehyde, the opening angle increased 9.3 ± 7.1° (SD) overall. SLs were measured at 16 equally spaced transmural locations from the free wall in the stress-free tissue sections and were compared with control measurements from adjacent cross-sectional rings in which residual stress had not been relieved. Average SL for the stress-free tissue (n = 11) was 1.84 ± 0.05 (SD) μm and for the unloaded tissue was 1.83 ± 0.06 (SD) μm. However, analysis of covariance on the pooled data showed that the transmural distributions were significantly different (P < 0.0001). Whereas SL was uniform across the wall in the stress-free state with a mean gradient of −0.014 ± 0.044 (SD) μm/total wall thickness, there was a significant decrease (P = 0.001) in SL from epicardium to endocardium in the intact unloaded tissue [slope = −0.114 ± 0.054 (SD) μm/total wall thickness]. This gradient may offset the opposite gradient in sarcomere extension during filling thus leading to a more uniform transmural distribution of SL at end diastole and hence more uniform development of systolic force.

residual strain; myocardium; zero-stress state; biomechanics

RESIDUAL STRESS is the stress that remains in a body in the absence of any external loads. In the biomechanics of tissues and organs, almost all model studies have been based on the assumption that there is no residual stress, and therefore that the unloaded state of the tissue is the zero-stress state. The presence of residual stress in an intact organ can be revealed by making cuts in the tissue to relieve it. If the shape of the unloaded tissue changes following the cuts, then residual stress was present. The change in shape from the cut, stress-free state to the intact, unloaded state may be described by the “residual strain.”

In the unloaded, isolated potassium-arrested rat heart, Omens and Fung (17) showed that the intact left ventricular (LV) myocardium is residually stressed. When they made a radial cut in an equatorial cross-sectional slice of the ventricular wall, the ring sprang open into an arc with a mean opening angle of 45 ± 10° (SD). A detailed study of the local tissue shape changes using small markers placed on the cross-sectional surface of the equatorial slice showed that the residual strain was negative (compressive) at the endocardium and positive (tensile) at the epicardium of the intact LV wall. This distribution of strain reflects the bending deformation required to restore the stress-free state into its intact unloaded configuration (the closed ring).

This finding is significant because it suggests a mechanism by which mechanical function of the LV wall may be optimized (5, 8). Stress analyses of LV filling that do not incorporate residual stresses have predicted a significant peak of circumferential stress at the endocardium compared with the epicardium under passive loading. Results by Demiray (1) showed that for a diastolic pressure of 20 mmHg, circumferential stress is 10 times higher at the endocardium than it is at the epicardium. Mirskey's models (15) predicted stresses 12 times greater at the endocardium than at the epicardium. The corresponding end-diastolic fiber extensions are significantly higher at subendocardial than subepicardial sites. Feit (2) reported that within the inner 29% of the ventricular wall, fibers could be so overextended (up to 50% more than epicardial fibers) that they would not contribute to the Frank-Starling mechanism. Humphrey and Yin (11) predicted end-diastolic circumferential strain to be about twice as large at the endocardium as it is in the epicardium. Tözeren (26) predicted from his models a diastolic fiber stretch ratio that was 20% higher at the endocardium than at the epicardium. However, measurements of sarcomere length (SL) at typical end-diastolic pressures show no such gradients in the dog (29) and distributions that have a subepicardial peak in the rat (7). These measured transmural distributions should be better suited to optimal systolic function because of the steep tension-SL relation in cardiac muscle (24). Hence, if residual stress can reduce the high transmural stress and strain gradients reported in the modeling literature, it may be an important physiological mechanism for optimizing systolic and diastolic function.

The way in which residual stress affects diastolic mechanics was shown by Guccione and co-workers (8). Using a thick-walled cylindrical model of the diastolic canine LV that incorporated residual stress in the analysis, they showed that transmural gradients of circumferential stress were significantly reduced when the assumed opening angle was increased from zero (no residual stress) to 90°. The stress-free configuration of their model was a cylindrical arc, which was closed before pressure loading, introducing circumferential residual stress that was compressive at the endocardium and tensile at the epicardium. This residual stress gradient offset the opposite gradients caused by filling (1, 15). The predicted end-diastolic SL also agreed more closely with experimental data than any other previous model.
They concluded that Fung's hypothesis (3, 5) of optimized function due to residual stress was supported by the model. However, this fundamental conclusion rests on two major assumptions: 1) that the gross changes in shape observed for the whole tissue reflect changes at the myofibril level so that the force-generating elements benefit from reduced diastolic stress and strain gradients and thus equalize the force of contraction across the wall; and 2) that the resting SL is constant across the wall in the zero-stress state. These two assumptions then imply that the distribution of SL in the intact, unloaded but residually stressed wall must be nonuniform, since a residual stress field, by definition, cannot be uniform (9). Residual stress then has the potential of affecting systolic force generation by influencing the transmural SL distribution that exists at end diastole.

The purpose of the present study was to confirm the model conclusions directly by measuring SL in the intact and stress-free states of the rat LV. Our hypothesis was that residual stress causes SL in the unloaded ventricular wall to be greater at the epicardium than at the endocardium. The true resting SL must be measured in the stress-free state. The SL distribution in the LV wall has not previously been measured independently of wall stress. Our results show that in the zero-stress state the transmural distribution of SL is uniform and significantly different from that in the unloaded, but residually stressed, state where SL decreases from the epicardium toward the endocardium. Therefore, residual stress directly affects transmural SL distribution.

METHODS

The isolated potassium-arrested rat heart preparation used by Omens and Fung (17) was extended in the present study to include fixation and histological preparation of the specimens for measurement of SLs. Twelve adult male (200–400 g) Sprague-Dawley rats were anesthetized with pentobarbital sodium (100 mg/kg) intraperitoneally. A tracheotomy was performed, and the animal was ventilated with 70% O2-30% N2 gas. After 5–10 min of ventilation, the chest was opened and the heart was arrested by clamping the ascending aorta and injecting 3–4 ml of cooled, heparinized (10 U/ml), hyperkalemic, Krebs-Henseleit solution directly into the LV through the apex. The perfusate contained (in mM) 68 NaCl, 30 KCl, 36 NaHCO3, 1.1 MgCl2, 6H2O, 1.6 Na2SO4, and 11 dextrose and was bubbled with 95% O2–5% CO2 gas. Also added to the perfusate were 30 mM of 2,3-butanedione monoxime to delay ischemic contracture. The arrested hearts were removed rapidly, and the ascending aorta was cannulated. The coronary circulation was perfused with chilled hyperkalemic solution for 3–5 min at ~50 mmHg of pressure.

Two adjacent equatorial cross-sectional slices, 2- to 3-mm thick, were cut with a razor blade from the arrested heart near the equator as shown in Fig. 1. Because the typical apex-to-base length for the hearts studied was ~14 mm, the slices removed represented ~40% of the total LV length. Typical outer diameters were ~11 mm. The slices were always studied simultaneously with adjacent sides facing upward for viewing. As observed previously (17), the shape of the ventricular cross-sections did not change noticeably after they were cut from the LV. One of the slices remained as an unloaded control while the other was later relieved of residual stress.

Several hundred stainless steel microspheres, 60–100 µm in diameter, were sprinkled on the upper side of one of the slices and pressed slightly into the tissue where they served as a grid of material markers for strain field measurements. The loose microspheres were rinsed off, and the two intact slices were submerged in a small dish of cool perfusate. The fluid served to support the slices in their natural unloaded shape and to minimize the effects of friction and gravity. A photograph was taken of the two slices in their intact state using an Olympus OM4-T 35-mm camera with a 90-mm macro lens (Tamron). The slice with the microspheres was then removed from the bath, and a radial cut was made through the LV free wall as indicated in Fig. 1. Within 15 s, the slice with the radial cut was replaced in the dish, and a second photograph was taken to record the configuration of the slice immediately after the cut. After the radial cut was made, the slice was considered to be stress free (17). Within 30 s of the second photo being taken, the perfusate was replaced with buffered glutaraldehyde (10 ml 50% glutaraldehyde, 1.49 g cacodylic acid sodium salt powder, 0.617 g NaCl, and 70 ml H2O), and a third photograph was taken to record the deformation arising from the introduction of the fixative. Additional photographs were taken at 1-min intervals for 7–10 min to record the shape changes of the cut slice during fixation.

Opening angle and strain calculation. Residual strain is the strain that is observed when residual stress is relieved. A simplified indication of the residual strain in the left ventricular wall was obtained from the photographs of the cut tissue by measuring the opening angle (17) as defined in Fig. 1. Briefly, the origin of the opening angle measurement was at the center of the LV cavity as determined from the photograph of the stress-free slice taken before the radial cut that relieved the residual stress. The center of the cavity was measured along a line joining the point where the radial cut intersects the epicardium to the point farthest away across the LV, on the RV endocardial surface of the septum. The rays that define the angle intersected the LV wall at the center of the cut edges. For 3 of 11 hearts studied, regional distributions of principal residual strain components were determined using the method of Omens and Fung (17) from the positions of the microspheres on the cut slice. The principal strain components were shown in that study to approximate the circumferential and radial strain components on the surface of the cut. The reference state for the residual strain was the stress-free configuration, since the residual strain of the tissue describes the local shape change from the zero-stress state to the unloaded state. Only three animals were analyzed to permit a qualitative rather than statistical
comparison of two-dimensional residual strain distributions with those reported earlier (17) and to visualize any correlation between the transmural variation of residual strain and the changes in SL. For a site around the circumference in the range of 5–30° from the radial cut, the coordinates of 25–30 microspheres from throughout the wall thickness were digitized from the photograph of the uncut state. The same beads were digitized in the stress-free state from the second photograph. From all the digitized markers, triangles of material points were selected with sides ranging from 0.3 to 0.8 mm in length. Triangles (15–20 triangles) were chosen to span the entire wall thickness at the chosen circumferential location. Using homogeneous strain analysis, two-dimensional strain components were computed for each triangle (17). Homogeneous strain analysis assumes that the deformation within a single triangle is constant. Strains were calculated with respect to a coordinate system where the circumferential direction was tangent to the epicardium and the radial direction was perpendicular to the circumferential direction and positive outward from the heart.

Histological preparation. After overnight or longer fixation, the slices were washed with distilled water and dehydrated in successive baths of 70, 95, and 100% ethyl alcohol for 1 h each. Specimens were cold infiltrated for up to 7 days and embedded with Polaron resin (EM, Chestnut Hill, MA). Earlier attempts using a different embedding medium (Historesin, Cambridge Instruments, Deerfield, IL) were found to produce incomplete infiltration and blocks that shredded during sectioning.

The embedded slices were mounted on specimen-holder blocks and sectioned with a Histostar microtome (LKB instruments) that had computer-controlled knife advance with a resolution of 0.5 μm and 3 degrees of freedom of the specimen holder for orienting the plane of cutting. Stress-free and unloaded slices were cut along planes tangent to the epicardium as shown in Fig. 2. Section thickness for all hearts was 5–7 μm. The original alignment before sectioning was determined so that the radial direction along which the sections were obtained was perpendicular to the local epicardial tangent plane. The specimens were oriented carefully to minimize any error in SL arising from the curvature of the wall. Sarcomeres from each section were later measured along the central regions of the cut where the fibers should have been most parallel to the plane of cutting. Misalignment of the tissue block was qualitatively and quantitatively judged by the following criteria: 1) uniformity of SL over the central regions of the section, 2) observation of greatest SL over the central region of the section and least SL in the peripheral regions affected by curvature (shredding at the edges can cause localized stretching of SL), and 3) overall cell shape that is elongated and not elliptical in the region of measurement. An analysis of SL distribution as discussed in Results and illustrated by Fig. 4 was done in selected sections to test the criteria used.

Fig. 2. Embedded slices were sectioned to obtain histological measurements of sarcomere length. Sections were cut along planes perpendicular to radial direction and collected from each heart at 16 equidistant transmural sites.

Sections were obtained throughout the wall thickness, and they were collected from each heart at 16 equidistant transmural sites. Collecting sections from locations at fixed proportions of the wall thickness allowed regional data to be pooled for different hearts. Equidistant collection of an equal number of sections from each heart permitted a more accurate interheart analysis. Relative to the region of the radial cut, sections were obtained at distances of 1–3 mm from the cut to either side on a random basis from heart to heart, but all at the same site for any one heart. The sections were stained with toluidine blue dye.

SL measurements. Sarcomeres were imaged under ×1,000 magnification with an oil immersion ×100 objective. The field of view was displayed on a monitor via a CCD video camera (Cohu) attached to the microscope (Olympus BH-2). The video image was digitized using a 512 × 512 pixel frame grabber (Data translation DT2651) board on the Q-bus of a VAXstation II/GPX computer. Custom software allowed image enhancement to be performed to improve the contrast of the striations, and the operator used a cursor to identify A bands of adjacent sarcomeres. Calibration was performed with a 0.01-mm reticle (Olympus). Groups of at least 20 adjacent sarcomeres were measured at a time, and 4 groups or more were obtained per section. Typically 150–200 SLs were averaged per section to obtain the mean SL. To avoid any bias, the observer measuring SL from the last six hearts was blinded for the state of stress (stress-free vs. unloaded) and transmural position of each section. Sarcomeres were measured from the central regions of the slide where lengths were less affected by curvature and other irregularities arising from the cutting of the equatorial slices.

Statistical analysis. The main problem addressed in the analysis of the data was the discrimination of the variation of SL due to the relief of residual stress from several other sources. These other sources of variability include the natural variability of SL within a section, the effects arising from the curvature of the wall and not corrected by block alignment, artifactual variations due to histology and sectioning, and the natural transmural variation within a given stress state. Some of these sources of variation had effects on SL of the same order of magnitude as those due to the relief of residual stress. Particularly near midwall the difference in SL between the stress-free state and the unloaded state was often less than the variation in SL between one transmural slice and the next. Data from 11 hearts were studied.

For each section corresponding to a particular transmural slice, average SL was calculated from all the measurements. We studied the differences in the transmural variations of SL between the stress-free state and the unloaded state by comparing absolute SL (in μm). Two-way analysis of covariance (ANCOVA) was used to examine the effect of the state of stress on SL as a linear function of wall depth, where depth was treated as a continuous variable (regressor). A repeated measures test was used, since the stress-free and unloaded data were measured for each transmural position. If depth was treated as a nominal variable rather than a continuous one, then each transmural site became an independent variable and the analysis was reduced to a one-way analysis of variance (ANOVA). Because ANCOVA cannot be used to study the statistical significance of the difference at one site (i.e., contrasts or single comparisons with 1 degree of freedom), ANOVA was used for this particular measure.

The null hypothesis was that the state of stress did not influence the transmural variation of SL with depth. The software employed for our statistical calculations was Superanova (Abacus Software), and the statistical results (P values obtained) from ANCOVA represent the statistical significance of the differences in the transmural SL distributions.
RESULTS

Opening angles, residual strains, and SLs were measured to study the effect of residual stress on the transmural distribution of SL in the rat LV. One heart out of 12 was excluded from the SL analysis owing to damage during sectioning with a defective knife. Opening angles measured from the photographs taken immediately after the cuts averaged 45 ± 15° (SD) for the eight hearts from which usable film was obtained. Immediately after introducing the fixative, there was an increase in opening angle averaging 8.1 ± 6.3° (SD). One minute after the addition of glutaraldehyde, the opening angle increased modestly [2.5 ± 2.2° (SD)] with minimal further change over time (Fig. 3). Because histology did not show any indication of contracture, the relatively large sudden increase in the opening angle of some of the samples on introduction of the fixative could have been due to localized areas of contracture or shrinkage away from the area of SL measurement. The small changes in opening angle during the remainder of the fixation process suggests that fixation was mostly uniform and rapid, producing relatively minor distortion of the tissue. An initial large opening angle (>70°) before fixation may be an indication of ischemic contracture occurring despite the action of the BDM in the perfusate or as the result of poor perfusion or unusual damage during the handling and cutting of the equatorial slices. Only two hearts included in the results had an opening angle after fixation that approached this range but were nevertheless included because histology did not show any signs of ischemia or contraction bands.

SLs from every histological section were measured at or near the central region where the effects of curvature and edge effects from cutting were smallest. The variation of SL over the surface of a typical slide is shown in Fig. 4. Notice that the most variation in length occurred near the cut edges (1.40–2.15 μm). The central region had a more uniform distribution of SLs, which for this case was ~1.90 μm. Not present in this example are the effects of curvature, which became more evident in histological sections from deeper layers where the curvature of the wall was considerably larger. In these sections, particularly near the edges, muscle fibers formed an angle with the plane of histological sectioning and appeared more elliptical. Sarcomere measurements taken at these sites appeared shorter.

For all hearts, the overall range of SLs for the unloaded state was 1.66–2.06 μm with transmural averages for the different hearts ranging between 1.80 ± 0.07 and 1.90 ± 0.08 (SD) μm. For the stress-free state the range was 1.71–1.98 μm with transmural averages ranging between 1.80 ± 0.04 and 1.87 ± 0.04 (SD) μm. The standard deviation of SLs for a given depth, calculated from the four or more groups measured per section, was ~0.05 μm. This gives an indication of the SL variability at a single transmural location.
Table 1. Statistical significance of site-to-site difference between unloaded and stress-free distributions

<table>
<thead>
<tr>
<th>Transmural Position</th>
<th>Significance (P)</th>
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<tbody>
<tr>
<td>1</td>
<td>0.005</td>
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<tr>
<td>2</td>
<td>0.043</td>
</tr>
<tr>
<td>3</td>
<td>0.126</td>
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<tr>
<td>4</td>
<td>0.508</td>
</tr>
<tr>
<td>5</td>
<td>0.868</td>
</tr>
<tr>
<td>6</td>
<td>0.901</td>
</tr>
<tr>
<td>7</td>
<td>0.590</td>
</tr>
<tr>
<td>8</td>
<td>0.456</td>
</tr>
<tr>
<td>9</td>
<td>0.148</td>
</tr>
<tr>
<td>10</td>
<td>1.000</td>
</tr>
<tr>
<td>11</td>
<td>0.679</td>
</tr>
<tr>
<td>12</td>
<td>0.039</td>
</tr>
<tr>
<td>13</td>
<td>0.320</td>
</tr>
<tr>
<td>14</td>
<td>0.057</td>
</tr>
<tr>
<td>15</td>
<td>0.039</td>
</tr>
<tr>
<td>16</td>
<td>0.057</td>
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</tbody>
</table>

Transmural positions start from epicardium (position 1) to endocardium (position 16).

The mean SLs as a function of normalized wall position for the two states of stress is shown in Fig. 5 as averaged for the 11 hearts analyzed. The unloaded SL distribution decreased from an interheart mean of 1.91 ± 0.08 (SD) μm at the epicardium to 1.78 ± 0.07 (SD) μm at the endocardium, while the stress-free distribution was more uniform across the wall with a transmural average of 1.84 ± 0.05 (SD) μm for the mean distribution for the 11 hearts analyzed. The two distributions are distinct in the first and last third of the wall thickness and overlap in the middle third. The error bars are ±SE. ANCOVA on the pooled data showed that the state of stress significantly affected transmural SL distributions (P < 0.0001).

The contrasts of the ANOVA were used to study the effects of the state of stress on SL at different transmural sites in the wall in the pooled data. It was expected that the differences in absolute SL between the stress-free state and the unloaded state would be most noticeable at the epicardial and endocardial regions and less so at the midwall regions. Table 1 shows P values for transmural comparisons. At the most epicardial layer P = 0.005 and at the most endocardial one P = 0.057, indicating that SL was significantly different between the control and stress-free state at these sites. However, this was not so in the middle layers where, for example, at the central site P = 0.456.

Slopes from linear regressions of SL as a function of transmural depth normalized by wall thickness were computed for the unloaded and the stress-free states in all hearts (Fig. 6 and Table 2). The mean slope was −0.114 ± 0.054 (SD) μm/total wall depth in the unloaded state and −0.014 ± 0.044 (SD) μm/total wall depth in the stress-free state. Mean wall thickness was 2.13 ± 0.20 (SD) mm. In 10 of 11 cases, the unloaded slope was more negative than the stress-free slope. In all cases but one, the regression coefficients (R) of the linear regressions were greater for the unloaded distributions than for the stress-free ones, which generally exhibited more variation. Individual fits were poor with R for the unloaded distributions ranging from 0.291 to 0.836 and those for the stress-free distributions ranging from 0.002 to 0.460. However, a paired t test showed the difference in slope to be significant (P = 0.001). Table 2 also shows the P values from ANCOVA performed separately for each heart. In 6 of 11 animals studied, ANCOVA showed a significant effect of the state of stress in the transmural SL distribution. Nevertheless, it should be noted that since the P value from an ANCOVA represents the statistical significance of the difference in the transmural SL distributions, the value for any one heart is sensitive to the noise present in the SL measurements. That is, for any one heart the P value is directly related to the goodness of the linear regression. This accounts for the considerable variability in the statistical significance of results from individual hearts. Therefore, ANCOVA applied on the pooled averaged data from the 11 hearts is a more reliable indication of the difference in SL distributions.

DISCUSSION

The purpose of the present study was to experimentally determine the effect that residual stress has on ventricular mechanical function by directly measuring SL in the intact and stress-free states of the rat LV. This was to determine the extent to which residual stress is present at the myofibril level and to understand how residual stress affects the transmural SL distribution of the ventricle.

Fig. 6. Slopes of linear fits of SL as a function of transmural position normalized by wall thickness. Each symbol corresponds to a different heart. In 10 of 11 cases, slopes were more negative and steeper in unloaded state of tissue than in stress-free state where SL distributions were in general more uniform but with greater variability. A paired t test showed that the 2 groups of slopes were significantly different (P = 0.001).

Table 2. Linear regressions of sarcomere length as a function of transmural depth normalized by wall thickness

<table>
<thead>
<tr>
<th>Heart No.</th>
<th>Slopes</th>
<th>P Values From ANCOVA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Unloaded</td>
<td>Stress-free</td>
</tr>
<tr>
<td>1</td>
<td>−0.113</td>
<td>−0.002</td>
</tr>
<tr>
<td>2</td>
<td>−0.098</td>
<td>−0.012</td>
</tr>
<tr>
<td>3</td>
<td>−0.160</td>
<td>−0.003</td>
</tr>
<tr>
<td>4</td>
<td>−0.089</td>
<td>0.042</td>
</tr>
<tr>
<td>5</td>
<td>−0.134</td>
<td>−0.053</td>
</tr>
<tr>
<td>6</td>
<td>−0.193</td>
<td>−0.068</td>
</tr>
<tr>
<td>7</td>
<td>−0.196</td>
<td>0.042</td>
</tr>
<tr>
<td>8</td>
<td>−0.056</td>
<td>0.040</td>
</tr>
<tr>
<td>9</td>
<td>−0.048</td>
<td>−0.031</td>
</tr>
<tr>
<td>10</td>
<td>−0.120</td>
<td>−0.020</td>
</tr>
<tr>
<td>11</td>
<td>−0.042</td>
<td>−0.087</td>
</tr>
<tr>
<td>Mean</td>
<td>−0.144</td>
<td>−0.014</td>
</tr>
<tr>
<td>±SD</td>
<td>0.054</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Slopes are in units of μm/total wall thickness. Mean wall thickness is 2.13 ± 0.20 (SD) mm. This measure does not include the papillary muscle and is taken after embedding. ANCOVA, analysis of covariance.
and thus the uniformity of systolic force generation. We found that the residual stress present in the LV wall affects the transmural distribution of SL. The difference between the SL distributions in the unloaded and the stress-free states (Fig. 5) was statistically significant. In all cases, the difference in SL between the two states was greatest at the innermost and outermost layers as expected from previous model analyses (8).

Although numerous sarcomeres were measured in each heart, statistical analysis from several hearts was required to observe a statistically significant difference in SL distributions in the two different states of stress owing to the large variability in the measurements. One of the sources of SL variability is ischemic contracture. Omens and Fung (17) have recognized the importance of delaying contracture in this type of preparation by perfusing the heart with a cardioplegic buffer. Contracture in our preparation was blocked by 2,3-butanediole monoxime, which is believed to block cross-linking between the actin and myosin filaments via saturation of calcium binding sites (28). The introduction of the fixative could have led to localized areas of contracture as evidenced by the 2–16° sudden increase in opening angle that occurred on contact of the specimen with glutaraldehyde. However, histology showed no contraction bands or other signs of contracture in the area of SL measurement. The small changes in opening angle (2.5 ± 2.2°) measured after the initial minute of the fixation process suggest that fairly rapid fixation was achieved with minimal changes in the stress-free shape of the area of interest.

The relative changes in SLs that occurred from the unloaded state to the stress-free state followed the same pattern of deformation reported by Omens and Fung (17), who measured residual strains. This can be seen in Fig. 7, where the stress-free and unloaded SL distributions are shown for a typical slice (heart 10) together with the greatest principal stretch, which closely approximates the circumferential stretch (17). Circumferential stretch ratios >1 near the epicardium indicate an increased circumferential segment length in the intact state compared with the stress-free state. Correspondingly, epicardial SL was greater in the unloaded state than in the stress-free state. Conversely near the endocardium, stretch ratios <1 indicate compression and correspond to shorter sarcomeres in the unloaded state than in the stress-free state. It is noticeable how circumferential stretch ratios are subject to less noise than the SL distributions. This is because each value is calculated from the area spanned by three markers. Therefore, each point is already an average over each triangular region. These distributions of residual strain and SL suggest that in the intact unloaded state of the tissue there is compressive circumferential residual stress in the inner layers and tensile stress in the outer layers.

The two-dimensional experiment used in the present study eliminates the effect of longitudinal residual stress by comparing an isolated circumferential cross-section before and after circumferential residual stress is relieved. A two-dimensional analysis of residual strain was necessary because of no practical method for a fully three-dimensional analysis of residual strain is yet available. Although transmural distributions of three-dimensional strain in the passively loaded dog could be obtained using biplane radiography to visualize implanted markers as performed in cardiac deformation studies (18, 27), these methods are not possible in the rat heart. Our unpublished observations also indicate that residual strain is difficult to measure uniquely in the larger animal. The two-dimensional study has some motivation in the equatorial

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Fig. 7. Transmural distribution of SL in unloaded and stress-free state of a typical heart (B) shown together with transmural distribution of principal stretch ratio (A) (approximates circumferential stretch (17)). Measurements from both distributions were taken in same region of LV wall. Variability of SL measurements is typical of that encountered in a single heart. A significant difference between stress-free and unloaded SL distributions is observed after averaging over more than one specimen. Stretch ratio is calculated from residual strain. Values larger than one in outer wall indicate circumferential stretching in this region when going from stress-free state to intact unloaded state. At endocardium, shortening occurs. Both SL and stretch ratio distributions are representative of an intact tissue that is under tension in the outer wall and under compression in the inner wall.
region where a cylindrical approximation to the wall geometry is best justified (8) but the present results only apply directly to the intact heart if longitudinal residual stress is negligible.

Changes in longitudinal length due to the cuts required to obtain the equatorial rings may affect SL distribution particularly at the epicardium and endocardium where fiber angle is steepest. However, the equatorial rings did not exhibit a significant off-plane deformation when relieved of residual stress. This suggests that in this area of the ventricle, circumferential residual stress was dominant and the effects of longitudinal residual stress were small. To study this in more detail, additional experiments were performed to examine the changes in epicardial circumferential and longitudinal length during the isolation of a single 5-mm thick equatorial ring. Epicardial strains were computed from the relative changes in positions of small suture markers placed at the epicardium. These calculations showed that the mean epicardial axial shortening from three animals was $6.5 \pm 3.5\%$ (SD), and the circumferential direction shortened by $1.8 \pm 3.6\%$ (SD) when the ring was cut from the intact ventricle. Reliable measurements of endocardial dimensions were not obtained, but some out-of-plane bulging of the subendocardium was observed, suggesting that some axial lengthening may have occurred at these layers. Judging from visual inspection, the circumferential variation of axial lengthening at the subendocardial layers was slightly nonuniform.

The shortening of the epicardium and the possible lengthening of the subendocardium when an equatorial section is obtained can be interpreted as longitudinal residual stress being relieved along the axial direction in a similar manner as occurs in the circumferential direction. The larger residual strains that occur along the circumferential direction are a result of the larger curvature of the ventricular wall about the long axis. Two experiments in which a longitudinal ring was obtained instead of a circumferential one were also performed. The opening that occurred when the basal portion of the ring was cut off and residual stress was relieved along the axial direction was examined. This was done by measuring the inner and outer cavity radii at the cut edges. In one case a significant opening was observed. In the other, the outer radius increased while the inner radius decreased due to changes in wall thickness or shearing. Although it is difficult to determine the transmural patterns of longitudinal deformation that occur when residual stress is relieved, some epicardial shortening has been observed while the subendocardium may lengthen slightly. This may have an effect on the intact transmural SL distribution. Specifically, it would make the unloaded, residually stressed, transmural SL distribution steeper than what was reported. This would make the importance of residual stress even more significant than the two-dimensional experiment suggests.

The finding that the changes in SL parallel the deformation observed with microsphere markers located on the tissue surface is evidence of the tight tethering between muscle fibers and the surrounding structural and load-bearing matrix. This is one of the assumptions made when interpreting the results from Guccione's (8) model, which suggested that residual stress may optimize cardiac performance by reducing peak stress gradients during filling. Other investigators (10, 12) have demonstrated a tight tethering between matrix and myofibrils by studying the deformation of microspheres lodged in the microcirculation of isolated muscle as it compares with changes in SL measured by laser diffraction. These studies, together with the present results, provide further evidence in favor of the assumption of homogeneity of deformation within a small region of myocardial tissue, which has been fundamental in many studies of cardiac mechanics in the literature (14, 18, 19, 27). This assumption is that if the markers are close enough, then the strain within the region spanned by the markers can be approximated to be constant.

The present results show a SL distribution for the intact slice with the longest sarcomeres located in the outer half of the wall, particularly toward the epicardium. The transmural distribution of SLs observed in the unloaded intact slices is similar to that of the arrested and isolated ventricle as suggested by measurements of SL by Grimm et al. (7) in rat LVIs that were perfusion fixed at various ventricular pressures. The hearts from Grimm's study that were fixed at zero transmural pressure showed greatest SLs occurring in the outer half of the wall. We propose that during filling, the nonuniform transmural SL distribution in the unloaded state becomes more uniform with the inner sarcomeres elongating more than the outer ones. In fact, Grimm's measurements of SL at increasing end-diastolic pressure show that the distributions become more uniform with higher volumes. Furthermore, results from Rodriguez et al. (19) show that during filling the inner sarcomeres stretch over a longer range than those near the epicardium (0.40 vs. 0.08 $\mu$m in the published example). This gradient of sarcomere extension during filling is due primarily to the incompressibility of the thick ventricular wall but is also affected by the interaction of fiber geometry with the changes in global shape such as a torsion (8) and changes in eccentricity that occur during filling (16). The importance of a uniform end-diastolic SL distribution can be appreciated when the steepness of the isometric tension-SL relationship is considered (24). If sarcomeres across the wall are not elongated to the same extent at end diastole, longer outer sarcomeres would develop significantly greater tension than deeper ones and thus lead to a gradient of transmural force generation. For example, data from ter Keurs et al. (24) show that peak isometric tension in isolated rat trabeculae at an extracellular Ca concentration of 2.5 $\text{mM}$ is $\sim 10\%$ higher at 2.0 $\mu\text{M}$ SL than at 1.9 $\mu\text{M}$. This reasoning is based on the classical interpretation of the Frank-Starling mechanism that systolic force is governed by end-diastolic SL. However, peak force seems to be independent of initial SL, which is inconsistent with this interpretation. Results from the work of ter Keurs et al. (24) in isolated muscle and from the work of Suga et al. (23) on the invariance of the end-systolic pressure volume relation in the intact ventricle indicate that peak force and ventricular pressure are independent of initial SL and loading conditions, respectively. Nevertheless, other
results suggest that there is a SL-history dependence of contraction. Effects such as shortening deactivation (25) and the force-velocity relation (13) do have an effect on peak force in the isolated muscle and on ventricular pressure in the intact heart (21). End-diastolic SL is therefore likely to determine the history of shortening. Furthermore, sarcomeres in the intact myocardium cannot shorten independently of other layers in the wall. They are constrained by their connective tissue interconnections and the incompressibility of the thick-walled myocardium. Work by Rodriguez et al. (19) on the reconstruction of SL and orientation in the beating canine heart shows that subepicardial, midwall, and subendocardial sarcomeres at the anterior free wall all shorten the same amount (in the order of 0.3 μm) during systole. Therefore, it is an oversimplification to interpret Starling’s law as meaning that systolic fiber stress is determined only by end-diastolic SL. However, owing to the coupling of myocytes in the wall of the intact heart and the SL-history dependence of cardiac muscle contraction, it is most likely that end-diastolic SL in the intact heart affects the history of SL during systole and hence the tension developed.

The direct measurement of the transmural SL distribution in the true stress-free state rather than in the unloaded state is useful for model studies of the mechanics of the diastolic and systolic LV wall. A basic requirement for all models of stress is the zero-stress reference state. In models of active contraction SL is the primary determinant of fiber tension. Almost all models assume that the unloaded state of the LV is the stress-free state and that SLs are initially constant. Even without including the mechanics of residual stress in the model, the present data on SL in the unloaded state will allow more accurate distributions of fiber stress to be predicted.

The degree to which the collagen matrix, the myofibrils, or other tissue structures are responsible for bearing residual stress is not known, but the present study shows that the effects of residual stress are present in the myofibril level, since relieving it causes changes in SL across the wall. Remodeling of the ventricular wall during growth or disease may change the distribution of residual stress and hence SL. Studies in blood vessels have shown that residual stress can change markedly during remodeling (5, 6). Sections of rat aorta at different stages of hypertrophy following aortic banding have been shown to have different opening angles when relieved of residual stress by radial cuts across the wall. These differences in opening angle are an indication of different distributions of residual stress in the tissue. In these specimens, evidence of morphometric remodeling was also observed (4, 6). Soon after abdominal aortic constriction with a surgically implanted band, wall thickness increased rapidly while internal radius decreased. After 3–5 days 50% of the total wall thickness observed was obtained, and after this time the increase was more gradual. Analytic studies (20, 22) have also shown that residual stress may arise from growth as the tissue elastically deforms to accommodate newly grown tissue without forming discontinuities in the growing body. The model by Rodriguez et al. (20) suggests that residual stress in the wall may change during growth and remodeling in certain types of hypertrophy. The elastic deformations that accommodate the newly grown tissue during growth and remodeling influence the transmural SL distributions in the heart and may give rise to altered distributions in the hypertrophied heart.

Although the relation between residual stress and tissue growth has been recognized, there have been no previous studies in the literature in which measurements of tissue morphology and structure have been related quantitatively to the residual stress present in the tissue. Regardless of the mechanism by which residual stress interacts with the sarcomeres, the main conclusion of the present study is that residual stress may not only optimize cardiac function by reducing end-diastolic stress gradients but may also affect the active performance of the heart through its effect on diastolic SL distributions in the intact ventricular wall. We have also provided the structural basis for the physical interpretation of modeling results that have shown how residual stress affects passive performance. Of future interest is the study of residual stress and SL during remodeling and hypertrophy. It has been suggested (4, 20, 22) that growth and remodeling are modulated by stress arising from loading and that they lead to altered amounts and distributions of residual stress. Data on distributions of residual stress, strain, and SL in the hypertrophied heart together with the present results for normal hearts may serve to elucidate how mechanical function of the ventricular myocardium is altered during hypertrophy and how stress contributes to myocardial growth and remodeling.

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REFERENCES


