Contribution of myocardium overlying the anterolateral papillary muscle to left ventricular deformation

Akinobu Itoh,1* Elizabeth H. Stephens,2* Daniel B. Ennis,3 Carl-Johan Carlhall,4 Wolfgang Bothe,1 Tom C. Nguyen,1 Julia C. Swanson,1 D. Craig Miller,1 and Neil B. Ingels Jr.1,5

1Department of Cardiothoracic Surgery, Stanford University School of Medicine, Stanford, California; 2Department of Bioengineering, Rice University, Houston, Texas; 3Department of Radiological Sciences, David Geffen School of Medicine, University of California, Los Angeles, California; 4Department of Clinical Physiology, Linköping University Hospital, Linköping, Sweden; and 5Department of Cardiovascular Physiology and Biophysics, Research Institute of the Palo Alto Medical Foundation, Palo Alto, California

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Both papillary muscle contraction and lengthening during diastole (35). Studies analyzing transmural myocardial strains overlying the papillary muscles have raised the question of whether the myocardium overlying the papillary muscles is unique relative to adjacent LV regions. Holmes et al. (22), in a study of canine hearts, found decreased deformation in regions overlying the anterolateral papillary muscle compared with more anteriorly located LV regions suggesting significant equatorial heterogeneity. These findings, however, were derived from comparisons between different hearts. We sought to extend these studies by examining three equatorial regions in the same heart during the same heartbeat.

The methods for the surgical placement of the transmural beadsets and data acquisition have been described in detail elsewhere (10, 11) and, therefore, will only be briefly summarized. Ten adult Dorsett hybrid sheep (52 ± 4 kg) were premedicated with ketamine (25 mg/kg intramuscularly) for venous and arterial catheter placement and monitoring. Anesthesia was induced and maintained with inhalational isoflurane (1.5–2.5%) and supplemental oxygen. Through a left thoracotomy, the pericardium was opened and the heart was confirmed to have no more than mild functional mitral regurgitation (MR) and have inspired papillary re-location techniques as part of functional MR treatment (23, 28, 29).

Although the importance of the papillary muscles has been well established, questions remain regarding their functional integration into the LV myocardium. The papillary muscles structurally link the MV to the LV myocardium within the valvular-ventricular complex. While the LV myocardium is known to be composed of sheets of complex but largely helically arranged fibers (20, 38), fibers within the papillary muscles themselves are highly aligned in the direction of tension (2, 22) and there is an abrupt change in myofiber angle from the ventricular compacta to the papillary muscles, particularly in the last 1–2 mm of the subendocardial LV wall (2, 22). Functionally, papillary muscle contraction appears to occur in concert with LV contraction, shortening during ejection and lengthening during diastole (35). Studies analyzing transmural myocardial strains overlying the papillary muscles have raised the question of whether the myocardium overlying the papillary muscles is unique relative to adjacent LV regions.
insufficiency in the aortic valve and MV by epicardial color Doppler echocardiography (Sonos 5500; Hewlett-Packard, Palo Alto, CA). Thirteen miniature tantalum radiopaque subepicardial markers were implanted to silhouette the LV myocardium; four markers spaced equally along the longitudinal meridians of three transverse planes (basal, equatorial, and apical; Fig. 1A) and one marker located at the LV apex. End-diastolic LV wall thickness was measured by epicardial echocardiography in three adjacent segments on the equatorial level midway between the atrioventricular groove and the LV apex (Fig. 1C): anterior wall (ANT): 14 ± 3 mm; LV wall over the anterolateral papillary muscle (PAP): 13 ± 2 mm; and lateral wall between the anterolateral and posteromedial papillary muscles (LAT): 13 ± 2 mm.

This wall thickness was then used to determine the implantation depth for three transmural beadsets (Fig. 1, B and C). A plexiglass template was used to guide insertion of the beads, perpendicular to the LV wall at each of the three locations (ANT, PAP, and LAT; Fig. 1C), in a manner similar to that previously described (10, 42). The deepest (subendocardial) beads were implanted at 90% of the echocardiographically determined end-diastolic wall thickness and two additional spherical 0.7-mm diameter beads were placed to span the myocardium. For each column, a fourth 1.7-mm diameter bead was sutured onto the overlying epicardium. To compare only the LV wall deformations at all locations, and not the deformation within the papillary muscle itself, the deepest beads of PAP were set to the same depth as ANT and LAT and did not penetrate into the papillary muscle. Upon study completion, the hearts were excised and thoroughly examined to confirm correct bead placement with MRI and histological examination.

In vivo marker data acquisition. Immediately after the operation, animals were transferred to the catheterization laboratory and studied in the right decubitus position with the chest open and anesthesia maintained. Two micromanometer-tipped pressure transducers (model MPC-500; Millar Instruments, Houston, TX) were calibrated and inserted into the LV chamber and ascending aorta via carotid artery catheters. Simultaneous biplane videofluoroscopic images of markers (60 Hz; Philips Medical Systems, Pleasanton, CA), ECG, LV pressure (LVP), and aortic pressure were recorded during three consecutive heartbeats in normal sinus rhythm with ventilation transiently arrested at end-expiration, as previously described (10). Data from the two-dimensional videofluoroscopic views were merged using three-dimensional (3-D) helical phantom image data and custom software (33), thus yielding the 3-D coordinates of each marker centroid every 16.7 ms. The accuracy of these 3-D reconstructions has previously been shown to be 0.1 ± 0.3 mm (16).

Quantitative analysis of myofiber angle. At the end of the study, the animals were euthanized by intravenous administration of KCl (80 meq) under 5% isoflurane. LVP was adjusted to match in vivo LV end-diastolic pressure with left atrial exsanguination. While this pressure was maintained, the hearts were fixed in situ with 300 ml of 5% buffered glutaraldehyde into a left coronary artery balloon catheter (Guidant; AguiTrac Peripheral Catheter, Santa Clara, CA). The hearts were then excised and stored in 10% formalin for 48 h. A LV long axis was defined by a skewer passing through the LV apex and midanterior mitral leaflet ("saddlehorn"). Each transmural beadset was excised surrounded by a rectangular cuboid, with a 10 × 10 mm square face, whose sides paralleled the local circumferential (Xc), longitudinal (Xl), and radial (Xr) lines (Fig. 1B). Each block was sliced into sequential 1-mm-thick sections parallel to the epicardial tangent plane (Xc-Xr) from epicardium to endocardium, providing a series of slices for measurement of myofiber angle (α). The myofiber angle, α, defined as the average angle between the local muscle fiber axis (Xf) and circumferential axis (Xc), with sign shown in Fig. 1B, was determined at 20, 50, and 80% depths from the epicardium using a MATLAB 2007b (The Mathworks, Inc, Natick, MA) program, as described previously (11).

Analysis of strains. The three transmural beadsets permitted simultaneous 3-D LV wall deformation measurements in the ANT, PAP, and LAT regions every 16.7 ms throughout multiple consecutive beats in each heart. The analysis of normal, principal, fiber, and cross-fiber strains has been described in detail previously (10, 11). Briefly, for each transmural beadset (ANT, PAP, and LAT) in each videographic frame, a local, right-handed orthogonal coordinate system (Fig. 1B) was defined with origin (O) at the center of the equilateral triangle of beads defining the epicardial tangent plane, the radial (Xr) axis was defined normal to the tangent plane and away from the LV cavity, the longitudinal (Xl) axis was defined at the intersection of the tangent plane and the plane containing Xr and a line (λ; Fig. 1A) through the origin (O) and the LV apex marker, and the circumferential (Xc) axis was defined mutually orthogonal to Xr and Xl.

Fig. 1. A: Locations of markers silhouetting the left ventricle (LV): 4 markers spaced equally along the longitudinal meridians of 3 transverse planes (basal, equatorial, and apical) and 1 marker located at the LV apex. Three transmural beadsets are shown placed in specific equatorial LV regions: anterior (ANT), LV overlying the anterolateral papillary muscle (PAP), and lateral (LAT), as determined by epicardial echocardiography. B: close-up of one of the 3 transmural beadsets. Markers are spaced evenly from endocardium to epicardium in a column oriented normal to the epicardial tangent plane. The deepest (subendocardial) beads were implanted at 90% of the echocardiographically determined end-diastolic wall thickness. For each column, a fourth 1.7-mm diameter bead was sutured onto the overlying epicardium. To compare only the LV wall deformations at all locations, and not the deformation within the papillary muscle itself, the deepest beads of PAP were set to the same depth as ANT and LAT and did not penetrate into the papillary muscle. Upon study completion, the hearts were excised and thoroughly examined to confirm correct bead placement with MRI and histological examination.

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Each cardiac cycle for each beat was visualized as a pressure-volume loop for precise timing definition of end diastole (ED) as the undeformed state (reference) and end systole (ES) as the deformed state as previously described (10).

For echocardiography, the displacement of the beads relative to ED was characterized by a continuous polynomial position field with quadratic dependence in $E_{xx}$ and linear dependence in $E_{xy}$ and $E_{xz}$ using least-squares fits (25). As described previously (10), the material gradient of the position field is the local deformation gradient tensor ($F$) and the Lagrangian strain ($E$) is then calculated as $E = (F^T F - I)/2$, where $I$ is the identity tensor. In terms of the coordinate system used in this study ($X_C$, $X_L$, and $X_R$), the three normal strains ($E_{CC}$, $E_{LL}$, and $E_{RR}$) measure local elongation or shortening along the circumferential ($E_{CC}$), radial ($E_{RR}$), and longitudinal ($E_{LL}$) axes. The three shear strains ($E_{CL}$, $E_{LR}$, and $E_{CR}$) represent angle changes between orthogonally oriented axes. The major principal strain ($E_1$) was calculated as the maximum eigenvalue of $E$. Strains were interpolated along the central axis of each transmural bead column at 1% increments of wall depth from the epicardium to the most subendocardial bead. The four beads are approximately located at depths of 0, 30, 60, and 90% of wall depth at ED, where 0% is at the epicardium and 100% is at the endocardium. The three depths chosen for detailed strain analysis were defined as 20% (subepicardial), 50% (midwall), and 80% (subendocardial) of the depth of the deepest bead measured from the epicardial surface as each time point, which approximately represents transmural depths of 18, 45, and 72% (10).

The myofiber angles ($\alpha$) measured at each depth were used to express normal myocardial strains at each depth in terms of myofiber ($E_{fiber}$) and cross-fiber ($E_{cross}$) strains, where the cross-fiber axis is defined as normal to both $E_{fiber}$ and $E_{RR}$ in the epicardial tangent plane. The strain tensor at a given depth was transformed from the local myocardial coordinate system ($X_R$, $X_L$, and $X_C$) into that relative to myofiber axis ($X_C$) and the perpendicular axis to $X_C$ ($X_R$) at a given depth in planes parallel to the epicardial tangent plane. Given that some myofiber lengthening may occur during isovolumic contraction (IVC) (7), the maximum positive $E_{fiber}$ during IVC ($E_{fiber, IVC}$), as well as $E_{fiber, IVC}$ relative to $E_{fiber, ES}$ ($E_{fiber, IVC-ES}$), were also analyzed for the different regions and layers, in addition to the fiber strain at ES relative to ED ($E_{fiber, ES}$).

**Statistical analysis.** Continuous data are reported as means ± SD. Comparisons between groups (LV location: ANT, LAT, and PAP; transmural depth: subepicardium, midwall, and subendocardium) were performed using two-way repeated-measures ANOVA with the Bonferroni post hoc test for multiple comparisons using SigmaStat (version 3.5; SPSS, Chicago, IL). Significance level was set at $P = 0.05$.

**RESULTS**

Group mean hemodynamic data are reported in Table 1. The normal strains $E_{LL}$ and $E_{RR}$ were significantly different between the ANT and LAT regions (Table 2), with LAT $E_{LL}$ and $E_{RR}$ at all transmural depths (except subepicardial $E_{RR}$) significantly less than those of ANT. No significant differences were found between ANT and LAT in $E_{CC}$. PAP normal strains, especially $E_{RR}$, were largely intermediate between those of ANT and LAT at each depth (ANT > PAP > LAT), with the exception of PAP subendocardial $E_{CC}$, which was less than ANT. A transmural gradient of strain (subendocardium > midwall > subepicardium) was evident in $E_{CC}$ for all regions, $E_{RR}$ for ANT and PAP, and a transmural gradient (subendocardium > subepicardium) was evident in LAT $E_{RR}$. $E_{LL}$ demonstrated a transmural gradient (subendocardium > subepicardium) in ANT and PAP.

Shear strains were small in magnitude and largely not significantly different between regions (Table 3). Transmural shear strain gradients were also largely not evident in the various regions.

**Principal strains in LAT were significantly smaller in magnitude than ANT for the major ($E_1$) and minor ($E_3$) principal strains at the subendocardial and midwall depths (Table 4). PAP major principal strains largely appeared intermediate between those of ANT and LAT (i.e., magnitude of ANT >

<table>
<thead>
<tr>
<th>Table 1. Hemodynamics</th>
<th>Measurements</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>54 ± 7</td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>90 ± 9</td>
<td></td>
</tr>
<tr>
<td>ESLVP, mmHg</td>
<td>83 ± 7</td>
<td></td>
</tr>
<tr>
<td>LVESV, ml</td>
<td>84 ± 12</td>
<td></td>
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<tr>
<td>LVEDV, ml</td>
<td>122 ± 15</td>
<td></td>
</tr>
<tr>
<td>$dp/dt_{max}$, mmHg/s</td>
<td>1.270 ± 80</td>
<td></td>
</tr>
</tbody>
</table>

Values are group means ± SD; $n = 10$. LV, left ventricle; ESLVP, end-systolic LV pressure; LVESV, LV end-systolic volume; LVEDV, LV end-diastolic volume; $dp/dt_{max}$, maximum change in pressure over time.

<table>
<thead>
<tr>
<th>Table 2. Systolic normal strains</th>
<th>Normal Strain</th>
<th>ANT</th>
<th>PAP</th>
<th>LAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{CC}$ Subepi</td>
<td>$-0.08 ± 0.03$</td>
<td>$-0.07 ± 0.03$</td>
<td>$-0.07 ± 0.02$</td>
<td></td>
</tr>
<tr>
<td>Midwall</td>
<td>$-0.14 ± 0.03$</td>
<td>$-0.11 ± 0.03$</td>
<td>$-0.13 ± 0.03$</td>
<td></td>
</tr>
<tr>
<td>Subendo</td>
<td>$-0.20 ± 0.04$</td>
<td>$-0.16 ± 0.04*$</td>
<td>$-0.19 ± 0.04$</td>
<td></td>
</tr>
<tr>
<td>$E_{LL}$ Subepi</td>
<td>$-0.07 ± 0.03$</td>
<td>$-0.06 ± 0.03$</td>
<td>$-0.02 ± 0.04*$</td>
<td></td>
</tr>
<tr>
<td>Midwall</td>
<td>$-0.09 ± 0.02$</td>
<td>$-0.09 ± 0.03$</td>
<td>$-0.02 ± 0.03*$</td>
<td></td>
</tr>
<tr>
<td>Subendo</td>
<td>$-0.12 ± 0.04$</td>
<td>$-0.10 ± 0.04$</td>
<td>$-0.03 ± 0.06*$</td>
<td></td>
</tr>
<tr>
<td>$E_{RR}$ Subepi</td>
<td>$0.17 ± 0.06$</td>
<td>$0.13 ± 0.05$</td>
<td>$0.10 ± 0.06$</td>
<td></td>
</tr>
<tr>
<td>Midwall</td>
<td>$0.34 ± 0.09$</td>
<td>$0.26 ± 0.11$</td>
<td>$0.17 ± 0.09*$</td>
<td></td>
</tr>
<tr>
<td>Subendo</td>
<td>$0.54 ± 0.19$</td>
<td>$0.42 ± 0.22*$</td>
<td>$0.25 ± 0.13*$</td>
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</tr>
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</table>

Values are group means ± SD; $n = 10$. ANT, anterior wall; PAP, LV over the papillary muscle; LAT, lateral wall on the equatorial level; $E_{CC}$, $E_{RR}$, and $E_{LL}$, measurements local elongation or shortening along the circumferential, radial, and longitudinal axes, respectively. Significant transmural gradients in strain magnitude (subendo > midwall > subepi, in italics) were observed in $E_{CC}$ (ANT, PAP, and LAT) and $E_{RR}$ (ANT and PAP). Significant transmural gradients (subendo > subepi, in italics) were observed in ANT and PAP in $E_{LL}$, and in LAT in $E_{RR}$. *$P < 0.05$ vs. ANT; †$P < 0.05$ vs. PAP by two-way repeated-measures ANOVA with the Bonferroni post hoc test for multiple comparisons.

<table>
<thead>
<tr>
<th>Table 3. Systolic shear strains</th>
<th>Shear Strain</th>
<th>ANT</th>
<th>PAP</th>
<th>LAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{CL}$ Subepi</td>
<td>0.01 ± 0.03</td>
<td>0.00 ± 0.03</td>
<td>0.00 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Midwall</td>
<td>0.01 ± 0.03</td>
<td>0.01 ± 0.02</td>
<td>$-0.01 ± 0.03$</td>
<td></td>
</tr>
<tr>
<td>Subendo</td>
<td>0.01 ± 0.02</td>
<td>0.02 ± 0.03</td>
<td>0.01 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>$E_{LR}$ Subepi</td>
<td>0.03 ± 0.03</td>
<td>0.03 ± 0.03</td>
<td>0.06 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Midwall</td>
<td>0.05 ± 0.05</td>
<td>0.05 ± 0.04</td>
<td>0.06 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Subendo</td>
<td>0.07 ± 0.08</td>
<td>0.07 ± 0.08</td>
<td>0.04 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>$E_{CR}$ Subepi</td>
<td>0.01 ± 0.04</td>
<td>0.02 ± 0.05</td>
<td>0.03 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Midwall</td>
<td>$-0.01 ± 0.04$</td>
<td>0.04 ± 0.04</td>
<td>0.01 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Subendo</td>
<td>$-0.04 ± 0.08$</td>
<td>0.05 ± 0.07*</td>
<td>0.01 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

Values are group means ± SD; $n = 10$. *$P < 0.05$ vs. ANT by two-way repeated measures ANOVA with the Bonferroni post hoc test for multiple comparisons. Significant transmural gradient (subepi vs. subendo, in italics) was observed in $E_{CR}$.
PAP > LAT). Transmural gradients (subendocardium > midwall > subepicardium) were evident in the major (E1) principal strains of ANT and PAP and a transmural gradient (subendocardium > subepicardium) in E1 was evident in LAT.

Myofiber angles in LAT were not significantly different from those of ANT at any transmural depth (Fig. 2) nor were the myofiber angles of PAP significantly different from ANT or LAT at the subepicardial or midwall depths. However, the PAP subendocardial myofiber angle was significantly greater (i.e., more longitudinal orientation, as opposed to circumferential) than ANT and LAT. All regions displayed a transmural gradient with myofiber angle increasing from the subepicardium to the subendocardium.

Myofiber strains at ES (E_fiber_ES) were not significantly different between those of ANT and LAT at any transmural depth (Table 5). PAP E_fiber_ES at the subepicardial and midwall depths was not significantly different than either ANT or LAT, but PAP E_fiber_ES at the subendocardial depth was significantly less than ANT. Cross-fiber strains (E_cross) in ANT were significantly greater than LAT at all transmural depths. PAP E_cross were also greater than LAT at all transmural depths. Transmural gradients (subendocardium > midwall > subepicardium) were evident for E_cross in all regions, and transmural gradients (subendocardium > subepicardium) were evident for E_fiber_ES in the ANT and LAT regions. The E_fiber_IVC was minimal for all regions and layers, and the E_fiber_IVC-ES demonstrated transmural gradients similar to E_fiber_ES. As evident for E_fiber_ES, ANT and LAT E_fiber_IVC-ES were not different at any depth, and PAP E_fiber_IVC-ES was only significantly smaller in magnitude compared with ANT and LAT in the subendothelial layer.

**DISCUSSION**

The two main findings of this study were as follows: 1) the myocardium overlying the papillary muscles displayed a transmural strain profile that is not unique but rather consistent with adjacent free wall regions, and 2) there was a gradient of transmural normal and principal strain profiles across the anterolateral equatorial left ventricle (ANT > PAP > LAT) despite similarities in myofiber angles and strains. When measured at the same transverse plane in the same animals during the same heartbeats, transmural strains in the anterior LV free wall (ANT) were greater than those of the LAT; strains in the myocardium overlying the PAP were largely intermediate between those of ANT and LAT, consistent with its location within this regional gradient.

**LV myocardium overlying anterolateral papillary is not unique.** The myocardium overlying the PAP displayed normal and major principal strains that were mostly intermediate between those of the ANT and LAT regions of the ventricle. Similarly, the shear strains in PAP were not markedly different from those of ANT or LAT nor were myofiber angles or strains. These results suggest that PAP is not functionally unique relative to adjacent regions of the ventricle.

The few instances in which PAP displayed unique strains all were found in the subendocardium and may possibly relate to...
loading of this myocardial region by coupling to the adjacent papillary muscle. E_{fiber}\_IVC was slightly, but significantly, different in PAP subendocardium relative to LAT and ANT, and E_{fiber}\_IVC of PAP subendocardium was slightly different than ANT. These differences may also relate, in part, to the difference in PAP subendocardium coupling to the anterior papillary muscle.

Others (2, 22) have noted an abrupt increase in myofiber angle within 1–2 mm of the junction between the myocardium and PAP. Given that the myofibers within the papillary muscle are highly aligned at an acute angle to the LV myocardium (2, 22), it is logical that the myofiber angle in the subendocardium bordering the papillary muscle would greatly increase, becoming more longitudinal to align with the fibers in the papillary muscle itself.

In light of these results, it appears that the myocardium overlying the PAP may not be severely depressed relative to other regions of the ventricle, as previously proposed (22, 31). Based on the results of the present study, the previously observed decreased deformation of the myocardium overlying the papillary muscle could, at least in part, be explained by the anterolateral gradient found here, where the anterior region displays strains of greater magnitude than regions located more laterally. Methodologic and technical differences between the study of Holmes et al. (22) and the present study should also be noted. The majority of animals in the study of Holmes et al. (22) had undergone MV replacement (33). Based on studies demonstrating that MV anuloplasty alters transmural strains (16), MV replacement likely affected their results. Compared with the normal and principal strains reported in the present study and similar studies (10–12), the anterior LV strains reported by Holmes et al. (22) were significantly less (~half) for both transmural depths measured (“inner wall” and “outer wall”). The decreased strains could have been due to overall decreased myocardial function in the studied hearts or altered regional strains secondary to MV replacement. Finally, Holmes et al. (22) made comparisons between the two regions from different animals during different heartbeats, rather than from the same heart during the same sampling instants, as reported here.

Anterolateral gradient of LV transmural strains despite similarities in myofibers. A gradient of LV transmural strains in which strains decreased moving from the anterior to lateral equatorial LV is supported by greater E_{RR} and E_{LL}, greater major and minor principal strains, and greater E_{cross} in ANT compared with LAT. Interestingly, myofiber angles and E_{fiber} were not significantly different between regions, which suggests that regional differences in normal and principal strains were not due to inherent differences in myofibers.

The finding of approximately two times greater wall thickening (E_{RR}) in ANT than LAT is consistent with the findings of Cheng et al. (12), although in that study the lateral and anterior regions were at different transverse planes (lateral equatorial vs. anterior basal). As in the present study, Cheng et al. (12) found no significant differences in myofiber angles or myofiber strains between the two locations despite differences in wall thickening. The results of these two studies, then, suggest that differences in coupling between these fundamental contractile units (myofibers) within the macrostructure of the myocardium likely account for the observed macroscopic differences in wall thickening. Possible mechanisms related to differences in coupling were discussed in detail by Cheng et al. (12). Regional differences in LV geometry may also relate to these differences in strain. According to and Rademakers (9), the anterior portion of the ventricle has a larger radius of curvature compared with the lateral LV free wall in the same transverse plane. This greater radius of curvature in the anterior LV wall would yield less pressure development for a given amount of wall thickening; therefore, greater wall thickening in ANT may be a compensatory mechanism to develop greater force to overcome its geometric shortcoming. Even with greater wall thickening in the anterior LV wall, however, Bogart et al. (9) found that the anterior LV region contributed less to the ejection fraction relative to the lateral LV region. In this study, ED thicknesses between the different regions were chosen to be comparable; therefore, differences in strain cannot be attributed to differences in thickness but rather must be due to fundamental differences in the mechanics of the different regions.

Transmural gradients evident throughout regions. The transmural gradients in E_{CC} and E_{RR}, major principal strain (E_1), fiber angles, and fiber strains evident in the three regions in this study confirm results in previous studies. Specifically, all three regions demonstrated significant transmural gradients in E_{CC} and E_{RR}, and ANT and PAP demonstrated gradients in E_{LL}. Similarly, all regions demonstrated transmural gradients in the major principal strain (E_1). Transmural gradients in myofiber angles were also noted in all regions and myofiber strains demonstrated transmural gradients in ANT and LAT.

Transmural gradients in normal strains, including radial strains (wall thickening) (9, 18, 32) and E_{CC} (9, 14, 22), have been demonstrated in a number of previous studies in multiple species using various methods. Given that the LV myocardium is a roughly constant volume system with a roughly cylindrical shape, mechanically there must be a transmural gradient in wall thickening, as well as in E_{CC} and E_{LL}, from purely physical considerations. It should be noted that while the midwall and epicardium demonstrated decreases in myocardial volume of 2–4% from ED to ES for all regions, the endocard-
The transmural gradient in myocardium demonstrated decreases in myocardial volume of 5–10% in all regions. Analysis of myocardial volume throughout the cardiac cycle by Ashikaga et al. (5) revealed similarly greater volume reduction of the subendocardial layer during ES relative to other layers that defies the postulated conservation of myocardial volume. The authors postulated that blood flow through vascular communications from the subendocardium into the ventricle, as well as trabeculations and cleavage spaces between myocardial sheets in the subendocardial region, resulted in greater volume reduction in the subendocardial layer (5). A similar, but lower magnitude transmural gradient in tissue volume changes were reported by Rodriguez et al. (36) using MRI methods.

The transmural gradient in myofiber strain has been previously demonstrated in the LV lateral equatorial region using the same transmural beadset technique (6, 11) and in humans using MRI (30), although others have not found evidence for such a transmural gradient (12, 14). Consistent with results from other studies (7), Efiber_IVC was minimal for all regions and layers and Efiber_IVC_ES demonstrated transmural gradients and regional heterogeneity similar to Efiber_ES.

The transmural gradient in myofiber angle has been well characterized and appears conserved across species (14, 39, 45). This gradient is fundamental to LV torsion (24), which in turn is key to LV force development, as well as minimization of transmural gradients of myofiber work and O2 consumption (3, 4, 24).

While shear strains are known to be important in LV torsion and thickening during systole as well as LV relaxation (6), shear strains were found to be small in magnitude in this study and largely not significantly different between regions.

Transmural strains of myocardium overlying papillary implicate robust cardiac structure. Based on what is known regarding papillary muscle mechanics, the finding of this study that the myocardial muscle overlying the PAP displays transmural strains consistent with those of adjacent regions is rather surprising. The papillary muscles undergo considerable motion and contraction throughout the cardiac cycle (27, 35), and given that the papillary muscles are mechanically contiguous with the overlying myocardium, one might expect the myocardium in that region to experience distinct forces relative to adjacent regions and therefore demonstrate unique transmural strain profiles. The lack of the distinction in transmural strains of the myocardium overlying the papillary muscles, however, can be seen as evidence for the robust nature of the cardiac structure. That is, the complex helical myofiber structure is built able to withstand these forces of the papillary muscles, and when these forces are removed, LV systolic function in that area that delicate balance of forces is altered and that region’s contractile properties do appear to change (13, 34, 40).

Implications. The findings of this study have a number of implications for our understanding of cardiac physiology and pathology. The finding that the myocardium overlying the papillary muscle is not unique despite the complex dynamics of the attached papillary muscle, points to a finely tuned, complex system. The heterogeneity of the left ventricle, as demonstrated by the anterolateral gradient in strains and transmural strain gradients, further points to the complexity of the mammalian ventricle and indicates that strategies such as engineered cardiac patches should optimally be designed to be regionally and depth specific. Lastly, the importance of coupling between the fundamental contractile unit of the myocyte we observed similarly points to LV structural complexity, specifically the importance of the configuration of myocytes within the macrostructure of the myocardium, e.g., their orientation, interconnections with one another, and interaction with the extracellular matrix. These aspects of myocyte configuration should be taken into account in clinical interventions such as engineered cardiac patches or the introduction of stem cells. Furthermore, the importance of this coupling points to the major role of the extracellular matrix in cardiac function (17, 43) and provides a mechanism for decreased cardiac function in disease states in which matrix components such as collagen are abnormal (41, 44). Potential interventions preventing or reversing such matrix changes could possibly improve LV pump performance in these patients, although this remains to be demonstrated.

Limitations. While the transmural beadset technique and analysis used in this study provides substantial insight into myocardial strains at different transmural depths in the beating heart, several limitations of this technique should be noted. First, it is likely that the markers themselves and the process of implantation may subtly affect overall cardiac function and local myofiber mechanics. Second, some of the results may be altered by anesthesia and be species specific, although certainly many characteristics of myocardial architecture and mechanics (i.e., gradient of myofiber angle across the LV wall) (14, 39, 45) appear to be conserved across species. One anatomical feature, however, that may not be conserved between human and animal hearts, is the attachment of papillary muscles to the ventricular myocardium. Axel found that human papillary muscles do not attach directly to the solid LV myocardial wall (8), unlike in animal hearts (15). Third, there are inherent uncertainties in myofiber angle measurements, variability in final depth of bead placement, and uncertainty in computational fitting to bead data and the videographic imaging modality itself. These uncertainties, however, are relatively small and would not account for the magnitude of regional differences found in this experiment. Fourth, this study included analysis at three equatorial locations; analysis of strains at more locations would be necessary to confirm a true anterolateral gradient in transmural myocardial strains as suggested by the results of this study. Finally, while previous studies have analyzed transmural strains in terms of laminar sheets utilizing β-angles, considerable controversy remains about the usefulness of such measurements and the measurements are subject to multiple sources of errors. Because of these limitations, laminar sheet analysis was not performed in this study.

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