Transmural Distribution of Three-Dimensional Systolic Strains in Stunned Myocardium

Reza Mazhari, PhD; Jeffrey H. Omens, PhD; Richard S. Pavelec, BS; James W. Covell, MD; Andrew D. McCulloch, PhD

Background—Regional function in stunned myocardium is usually thought to be more depressed in the endocardium than the epicardium. This has been attributed to the greater loss of blood flow at the endocardium during ischemia.

Methods and Results—We measured transmural distributions of 3D systolic strains relative to local myofiber axes in open-chest anesthetized dogs before 15 minutes of left anterior descending coronary artery occlusion and during 2 hours of reperfusion. During ischemia, regional myocardial blood flow was reduced 84% at the endocardium and 32% at the epicardium (P<0.005, n=7), but changes in end-systolic fiber length from baseline were transmurally uniform. Relative to baseline, radial segments in stunned tissue were significantly thinner at the endocardium than the epicardium at end systole (24±5% versus 16±3%; P<0.05, n=8), consistent with previous reports. Unlike radial and cross-fiber segments, however, the increase of end-systolic fiber lengths in stunned myocardium had no significant transmural gradient (23±8% epicardium versus 21±4% endocardium). We also observed significant 3D diastolic dysfunction in the ischemic-reperfused region transmurally.

Conclusions—Myocardial ischemia/reperfusion in the dog results in a significant transmural gradient of dysfunction between epicardial and endocardial layers in radial and cross-fiber segments, but not for fiber segments, despite a gradient in blood flow reduction during ischemia. Perhaps systolic fiber dysfunction rather than the degree of perfusion deficit during the preceding ischemic period may be the main determinant of myocardial dysfunction during reperfusion.

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Key Words: blood flow | diastole | mechanics | myocardial stunning | contractility

Myocardial stunning is mechanical dysfunction that persists after reperfusion despite the absence of irreversible damage and despite the return of normal or near-normal perfusion.1 Myocardial stunning results in alterations to systolic and diastolic regional function.2,3 This phenomenon can occur clinically during thrombolytic therapy, percutaneous transluminal coronary angioplasty, coronary artery bypass graft surgery, and exercise-induced ischemia.2,4 Studies in skinned and intact isolated muscle5 and heart6 preparations have demonstrated that contractile dysfunction after ischemia-reperfusion is caused by decreases in myofibril Ca2+ sensitivity and calcium-activated maximal force generation. It is also clear that this reduced sensitivity of myofilaments to calcium can be overcome by inotropic agents such as dobutamine.7

How the cellular mechanisms of stunning are related to losses of systolic function during reperfusion in the intact heart, however, is not fully understood. The severity of systolic dysfunction resulting from stunning can vary regionally in left ventricular (LV) myocardium. Bolli et al8 reported that ischemia-reperfusion in dogs resulted in greater systolic dysfunction in subendocardial layers than subepicardial muscle. Rynning et al9 came to the same conclusion on the basis of the observation that recovery of function was slower in longitudinally oriented segments than in circumferential segments. The latter study also illustrated that the systolic dysfunction in ischemia-reperfusion may depend on segment orientation as well as location.

It is well known that transmural cardiac strain distributions are 3D and heterogeneous, but no study of 3D regional mechanics has been conducted in myocardial stunning. The objective of this study was to measure 3D transmural strain with respect to local myofiber axes during coronary artery occlusion and reperfusion in the dog. At end systole, subendocardial segments were more severely affected than subepicardial segments in the radial and cross-fiber directions, consistent with previous reports, but in the muscle fiber direction, end-systolic segments in stunned myocardium were uniformly lengthened across the thickness of the wall. Even though the extent of ischemia during acute left anterior...
descending coronary artery (LAD) occlusion is most severe at the subendocardium, these results suggest that the effects of reperfusion on intrinsic myofiber shortening and tension development do not necessarily have a significant transmural gradient.

**Methods**

**Experimental Preparation**

The experimental preparation was similar to that described previously. Ten mongrel dogs (20 to 28 kg) were anesthetized with sodium pentobarbital (30 mg/kg IV) and ventilated. The anterior LV was exposed by thoracotomy, and the heart was suspended in a pericardial cradle. A snare and ultrasonic blood flow probe (T206, Transonic Systems Inc) were placed around the LAD distal to the first diagonal branch. A micromanometer-tipped catheter was introduced through the left carotid artery. A hydraulic occluder around the inferior vena cava allowed end-diastolic pressures (EDPs) to be lowered during occlusion and reperfusion to baseline values. Core body temperature was maintained at 37°C with circulating water heat columns (0.9-mm radiopaque markers (5 to 6 markers per column) were implanted in the LAD perfusion bed. By fluoroscopy (General Electric DXD-525II) with synchronized video cameras (COHU 4915), biplane views of the markers were acquired digitally (Data Translation DT3155). Video frame–synchronized signals were detected automatically, 3D coordinates of each biplane view were detected automatically, 3D coordinates of each bead were reconstructed by use of a perspective transformation derived from a 3D phantom, and 3D strains were calculated by a finite-element method.

During baseline, ischemia, and reperfusion, systolic strains were computed from the bead displacements between end diastole and end systole, the phases of which were determined from ECG and LV pressure recordings; end diastole represented the undeformed state, and end systole represented the deformed state. Because changes in these systolic strains during the experiment reflect changes in the end-diastolic configuration as well as the end-systolic state, end-diastolic and end-systolic acute “remodeling strains” were also computed by use of the changes in bead position from baseline to ischemia or from baseline to reperfusion at matching phases of the cardiac cycle. Remodeling strains describe 3D changes in regional segment geometry associated with acute alterations in material properties and loading conditions between baseline and ischemia or reperfusion. Therefore, for acute remodeling strains, end diastole (or end systole) at baseline was the undeformed state and end diastole (or end systole) during occlusion or subsequent reperfusion time points was the deformed state. All strains were resolved with respect to the measured local fiber axes to obtain segment length changes in fiber, cross-fiber (perpendicular to fiber direction, parallel to the wall), and radial orientations.

**Results**

**Exclusion**

Two dogs were excluded from analysis on the basis of the following a priori exclusion criteria (1 each): ventricular fibrillation occurring any time during the experiment, and subepicardial blood flow during coronary occlusion reduced to <50% of the nonischemic zone and to 25% of the nonischemic zone value in the subendocardial layer. ANOVA was used for statistical analysis, and a value of P<0.05 was considered to indicate statistical significance. All reported data are mean±SEM (n=8).

**Hemodynamics**

There were no significant changes in LVEDP, LV systolic pressure, heart rate, or maximum and minimum dP/dt during ischemia and reperfusion (Table 1). At 5 minutes of reperfu-

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**TABLE 1. Hemodynamic Measurements**

<table>
<thead>
<tr>
<th></th>
<th>Minutes of Reperfusion</th>
<th>Dobutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Ischemia</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>3.9±1.0</td>
<td>4.5±1.5</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>133.6±14.4</td>
<td>125.0±13.5</td>
</tr>
<tr>
<td>LAD flow, mL/min</td>
<td>17.6±7.7</td>
<td>0.0±0.0†</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>146±15</td>
<td>148±15</td>
</tr>
<tr>
<td>dP/dt(Δp, (mm Hg/s)×10³)</td>
<td>2.1±0.4</td>
<td>1.9±0.4</td>
</tr>
<tr>
<td>dP/dt(Δp, (mm Hg/s)×10³)</td>
<td>3.6±0.9</td>
<td>2.8±0.6</td>
</tr>
</tbody>
</table>

LVSP indicates LV systolic pressure; HR, heart rate. All values are mean±SD.

*P<0.05 ANOVA; †P<0.005 vs baseline; ‡P<0.005 vs 120 min reperfusion.
there was an ≈5-fold increase in LAD flow compared with baseline (P<0.005) due to reactive hyperemia. Compared with baseline and 120 minutes of reperfusion, dobutamine resulted in a significant increase in LV systolic pressure (P<0.005) and maximum dP/dt (P<0.005) and a significant decrease in LVEDP (P<0.005); dobutamine also significantly increased minimum dP/dt compared with baseline only (P<0.005). Complete LAD occlusion resulted in a nonuniform loss of blood flow transmurally (P<0.0005) (Table 2). Epicardial blood flow was ≈42% of flow in the remote nonischemic region, compared with 22% in the midwall and 15% in the endocardium.

Transmural Strain Distributions
Negative fiber and cross-fiber systolic strains at baseline, indicating shortening during ejection, were replaced during acute ischemia by positive strains (P<0.0001), which recovered partially after 2 hours of reperfusion (P<0.01 versus baseline and P=NS versus ischemia) and fully with dobutamine (P=NS versus baseline). As shown in Figures 1 and 2, A and B. Similarly, positive radial wall-thickening strains during systole were replaced by wall thinning during ischemia (P<0.0001) and reperfusion (P<0.001), as seen in Figures 1C and 2C. They also recovered fully with dobutamine (P=NS compared with baseline). At baseline, there were significant transmural gradients of cross-fiber and radial systolic strain (Figures 1 and 2, B and C), but fiber strains (Figure 2A) were transmurally uniform (P=NS). During reperfusion, there were significant transmural gradients (Figure 2) of end-systolic fiber (P<0.05) and cross-fiber (P<0.05) strain but not radial strain (P=NS).

These changes in systolic strain with ischemia and reperfusion also reflect acute end-diastolic remodeling; the end-diastolic remodeling strains in Figure 3 are referred to a matched EDP at baseline. End-diastolic radial thinning during ischemia and reperfusion relative to baseline was significantly greater (P<0.01) at the subendocardium than subepicardium. Dobutamine restored end-diastolic shape to baseline (P=NS compared with baseline).

As a measure of systolic shape change from baseline, independent of diastolic remodeling, end-systolic remodeling

![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** End-systolic strains of fiber (A), cross-fiber (B), and radial (C) segments during baseline, LAD occlusion, reperfusion, and dobutamine infusion. Strains were calculated with end diastole at each time point as undeformed and end systole as deformed state. Epi indicates epicardium; Endo, endocardium; Isch, ischemia; and Dobut, dobutamine. *P<0.05, Epi vs Endo.

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Transmural variation in end-systolic strains of fiber (A), cross-fiber (B), and radial (C) segments during baseline (*C*), LAD occlusion (*C*), and 120 minutes of reperfusion (*C*). Abbreviations as in Figure 1. *P<0.05, †P<0.001.
during ischemia and reperfusion (Figures 4 and 5) was significantly greater at the subendocardium than the subepicardium for cross-fiber ($P<0.005$) and radial ($P<0.005$) components, but not for fiber components. Hence, relative to baseline, fibers at end systole lengthened uniformly across the wall during ischemia and reperfusion. Dobutamine resulted in a significant change in end-systolic remodeling values compared with 120 minutes of reperfusion in all 3 components ($P<0.001$) (Figure 4), and values were not significantly different from zero ($P=NS$).

Discussion

The present measurements in anesthetized dogs are consistent with a previously report in which 15 minutes of coronary occlusion followed by reperfusion resulted in a greater loss of systolic wall thickening at the subendocardium than the subepicardium. They are also consistent with the observation in cats that longitudinal segments are more severely affected than circumferential segments during ischemia-reperfusion. But because these changes in regional systolic function also include the effects of nonuniform end-diastolic remodeling during ischemia and reperfusion, we also computed regional changes in end-systolic segment lengths relative to baseline. By this measure of systolic remodeling strain, gradients of cross-fiber and radial segment lengths remained, but systolic fiber lengths were increased uniformly across the wall in stunned myocardium. This suggests that the detrimental effects of ischemia-reperfusion on myofilament function may be similar throughout the wall thickness.

It has been proposed that blood flow reduction during the ischemic period is the primary determinant of postischemic dysfunction in vivo. In our experiments, however, we observed a nonuniform loss of blood flow transmurally, which would suggest that epicardial fiber dysfunction should be less severe than in endocardial layers, contrary to our observations. Conversely, multiple regression analysis by Przyklenk and Kloner showed that in anesthetized dogs, the degree of systolic dysfunction during the ischemic period and not the degree of perfusion deficit was the only significant correlate of systolic dysfunction during reperfusion. Our finding of a high correlation between the degree of systolic stretch during ischemia and reperfusion is consistent with a previous study by Przyklenk and Kloner. To clarify this relationship further, we performed multiple regression analyses on the effects of blood flow reduction and systolic stretch during ischemia on systolic stretch during 120 minutes of reperfusion. These analyses showed that systolic fiber stretch during 120 minutes of reperfusion depended significantly on the amount of systolic thinning during ischemia ($P<0.05$) and not on transmural perfusion ($P=0.25$). In the present study, end-systolic remodeling during ischemia and 120 minutes of reperfusion (Figure 5) both exhibited significant transmural gradients for radial and cross-fiber segments, and both exhibited no significant transmural gradient in the fiber direction. Thus, the degree of fiber segment dysfunction during ischemia may be the primary determinant of stunning injury during reperfusion. This may have significant clinical ramifications, because it implies that reduction of systolic ventricular loads during interventions such as balloon angioplasty may lead to less segment thinning in the ischemic/reperfused region during the intervention and ultimately may result in less severe dysfunction and faster recovery.

Studies in skinned and intact isolated muscle and heart preparations have demonstrated that contractile dysfunction after ischemia and reperfusion in vitro is associated with decreases in myofibril Ca$^{2+}$ sensitivity and calcium-activated maximal tension development. How these findings are related to in vivo findings, however, is not well understood. Although systolic fiber strain was uniformly distributed, cross-fiber and radial end-systolic remodeling strains varied transmurally (Figure 5). This was not necessarily unexpected, because myocardium is anisotropic: there is a stronger coupling between Ca$^{2+}$ sensitivity and fiber stress than transverse stresses. Hence, transverse strains could be affected by fiber stresses acting at a distance, whereas local fiber strains ought to be dominated by local fiber stresses. Moreover, the radial nonuniformity in systolic strain as shown here and by others is an expected consequence of the relative incompressibility (ie, conservation of volume) of myocardium.

The injury associated with stunning is believed to consist of an ischemic component and a reperfusion component that is proportional. For example, systolic dysfunction during reperfusion may depend on the balance of proton accumulation during ischemia and subsequent washout during early reperfusion. If ischemic injury is proportional to systolic dysfunction during ischemia and if reperfusion injury is proportional to blood flow, then net injury may be uniform; we observed uniform ischemic dysfunction in the fiber direction, and transmural blood flow ratios during reactive hyperemia are close to unity. It has also been proposed

![Figure 3](image-url)
that accumulation of some metabolites during ischemia may have a protective effect against ischemic injury, \(^{16}\) and it is conceivable that the greater imbalance of oxygen supply and demand may cause more protective species to accumulate at the endocardium than the epicardium.

An alternative explanation for these results may be that the level of perfusion during the experiments may have been supramaximal for engendering mechanical effects of stunning. Alternatively, because the lower severity of epicardial perfusion deficit could be a result of epicardial collateral flow, the uniform mechanical effect may reflect a time delay in development of collateral flow during early ischemia, resulting in early uniform perfusion deprivation, the effects of which are not offset by later epicardial collateral flow. Then again, many studies have demonstrated that collateral flow in the subepicardial layer is developed not earlier than 1 to 2 hours after occlusion, \(^{18}\) and the duration of ischemia in this study (15 minutes) falls short of that period.

Diastolic dysfunction is also seen in postischemic myocardium, \(^{3,19}\) but the underlying mechanisms are not well understood. We observed significant diastolic remodeling during ischemia and reperfusion in all 3 segment orientations, with the least transmural variation in the fiber component. A significant "diastolic creep" of circumferential and longitudinal segments has been described in intact hearts, \(^{20}\) whereas reperfusion in the isolated heart tends to increase diastolic stiffness. Although a significant loss of endomysial collagen fiber has been observed in the stunned myocardium, \(^{21}\) this probably occurs only after repeated cycles of ischemia and reperfusion. \(^{22}\) "Strain softening" \(^{23}\) as a result of systolic stiffening during the ischemic period may contribute to diastolic dysfunction in regionally stunned myocardium.

Disruption of diastolic intracellular calcium homeostasis in the stunned myocardium may also contribute to diastolic dysfunction. We saw a significant decrease in diastolic remodeling with dobutamine administration, suggesting that a mechanism associated with intracellular calcium handling may be involved.

The experimental methods used in this study for measuring regional wall mechanics have been used extensively in the past (see References 10, 11, and 13 for a selected few). The degree of damage caused by bead placement has been assessed previously \(^{24}\) and determined to be minimal. Observations were also made in this study in this regard while we assessed the degree of necrosis caused by reperfusion injury (TTC staining). Consistent with previous reports, \(^{24}\) we did not observe any significant damage caused by bead placement in the area of interest. Although this method is more invasive than some alternative methods (eg, MRI and 2D echocardiography), it provides a more complete description of transmural function than other modalities by providing a 3D description of the regional function (strains) from bead displacements. In addition, this method provides a higher spatial resolution (0.2 mm) and a superior accuracy at the epicardial and endocardial layers compared with other techniques (eg, MRI and transmyocardial Doppler). Although recent advances in MRI technology have enabled researchers to obtain 3D transmural strains, \(^{25}\) in the majority of the studies done with MRI, regional mechanics is reported only in the midmyocardial layer, because transmural resolution has been limited. \(^{26}\) Other noninvasive methods, such as transmyocardial Doppler, \(^{8,27}\) provide regional wall thickening...
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without having to insert any foreign object into the myocardial wall. This method, however, does not provide 3D deformation. Our results show that uniaxial measurements, especially radial and cross-fiber, can be misleading; but our observations of radial strains were consistent with previous reports of uniaxial studies. This suggests that the beads themselves did not impair radial systolic function in the area of interest.

Although the anesthetic used here may have cardiovascular effects, such as higher heart rates (5 to 10 bpm) seen here than in similar experiments, sodium pentobarbital has been among the most widely used in canine studies of ischemia and ischemia-reperfusion (see References 3, 4, 9, 20, and 28 for selected references). Relatively low diastolic pressures seen in the present study were consistent throughout the duration of the experimental protocol (with the exception of the dobutamine stress period). Higher diastolic pressures were avoided so as to minimize confounding effects on systolic contractile properties and regional blood flow independent of effects of reperfusion injury itself. Moreover, there is some evidence that some anesthetics may have confounding effects on the stunning process. Przyklenk and Kloner showed that heart rate itself did not affect degree of systolic dysfunction during the reperfusion phase in anesthetized open-chest dogs.

In summary, we found that coronary occlusion and reperfusion in the dog resulted in a significant transmural gradient of dysfunction between epicardial and endocardial layers in radial and cross-fiber segments, but not for fiber segments, despite a gradient in blood flow reduction during ischemia. A uniform degree of reperfusion injury and impairment of myofilament sensitivity in ischemic-reperfused myocardium might be sufficient to explain these observations. Perhaps systolic fiber dysfunction rather than degree of perfusion deficit during the preceding ischemic period may be the main determinant of myocardial dysfunction during reperfusion.

Acknowledgments

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References

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