Flow–function relations during graded coronary occlusions in the dog: effects of transmural location and segment orientation

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Abstract

Objective: The sensitive relationship between regional myocardial perfusion and local systolic deformation during acute myocardial ischemia is not independent of the transmural location or segment orientation. The aim of this study was to determine the effects of fiber orientation and transmural location on the relationships between regional myocardial flow and three-dimensional systolic wall strain during graded coronary artery occlusions. Methods: Transmural distributions of three-dimensional strain by biplane radiography of implanted radiopaque markers and myocardial blood flows using fluorescent microspheres were measured in the ischemic region during graded left anterior descending (LAD) coronary artery occlusions in 12 anesthetized dogs. Results: Occlusion of the coronary artery did not significantly alter mean heart rate or end-systolic pressure. As flow decreased during graded occlusions, ischemia significantly changed systolic circumferential, longitudinal, radial, fiber and cross-fiber strains (p < 0.004). There was a significant effect of transmural position on circumferential, cross-fiber and radial strains, but not on fiber or longitudinal strains. Ischemia significantly altered all normal strains: circumferential, longitudinal, fiber, cross-fiber and radial. There was a strong interaction effect between transmural location and blood flow for circumferential, cross-fiber and radial strains, but not fiber or longitudinal strains. Conclusion: During non-transmural ischemia, there is evidence of strong transmural tethering in the cross-fiber direction, whereas the fiber-strain flow relation is independent of transmural position. Thus, whether the relationship between local myocardial bloodflow and systolic strain during acute ischemia is dependent on transmural location, depends on segment orientation. © 1998 Elsevier Science B.V.

Keywords: Heart; Blood flow; Fluorescent microspheres; Systolic strain; Regional function; Dog

1. Introduction

There is sensitive relationship between regional myocardial perfusion and local systolic segment deformation during acute myocardial ischemia [1–3]. However, the flow–function relation is not independent of the transmural location or orientation of the myocardial segment being used to measure local function. For example, Vatner [1] found that a 70% reduction in subendocardial flow caused a 50% loss of subendocardial fiber shortening, whereas Prinzen and colleagues [4] reported that reducing subepicardial perfusion by only 32% decreased systolic fiber strain on the epicardium by 60%. On the other hand, Weintraub and co-workers [5] reported that longitudinal shortening was completely eliminated at the subepicardiac while local perfusion was reduced by less than 50%.

A similar disparity has been reported in the relationship between regional myocardial flow and systolic wall thickening. Significantly impaired transmural and subepicardial systolic wall thickening have been observed during partial coronary artery occlusions in the dog despite no significant reduction of subepicardial perfusion [3,6]. Consequently, these investigators found that epicardial segment function correlates more strongly with endocardial flow and endocardial function than with epicardial flow. This has lead to the hypothesis that epicardial motion is ‘tethered’ or ‘coupled’ to endocardial deformation [3–5].
Contrary to the coupling hypothesis, Torry and colleagues [7] reported that the degree of local segment dysfunction closely paralleled the pattern of regional perfusion through the thickness of the ventricular wall. Thus, they concluded that perfusion, rather than transmural interaction, largely determined subepicardial function in nontransmural ischemia.

Bertha and Folts [8] also dismissed the tethering hypothesis in their assessment of the relationship between subendocardial and subepicardial fiber shortening during gradual coronary flow reductions. They observed that at the lowest flow levels, active fiber shortening was still maintained in the subepicardium even though simultaneous severe bulging was observed in the subendocardium. During partial coronary artery occlusion with normal subepicardial blood flow, Gallagher and co-workers [9] found that circumferential subepicardial and subendocardial shortening were eliminated whereas subepicardial shortening in the direction of the myofibers remained normal. This result pointed to the importance of accounting for segment orientation in relation to myofiber architecture when assessing regional flow–function relations.

Using biplane radiography of an array of implanted myocardial markers, it is possible to measure the transmural distribution of the systolic strain tensor, and the alterations associated with acute regional ischemia [10]. This provides a complete three-dimensional measure of local myocardial segment function, which can also be related to the local myofiber structure [11]. Therefore, we used this approach to identify the effects of fiber orientation and transmural location on three-dimensional flow–function relations during graded coronary artery occlusions in anesthetized dogs. The analysis showed that whether the relationship between local myocardial bloodflow and systolic strain during acute ischemia is dependent on transmural location depends on segment orientation. In particular, there is evidence of strong tethering in the cross-fiber direction, whereas the fiber-strain flow relation was independent of transmural position.

2. Methods

Transmural distributions of three-dimensional strain and myocardial blood flows were measured in the ischemic region during graded left anterior descending (LAD) coronary artery occlusions in anesthetized dogs. The University of California, San Diego (UCSD) is accredited by the American Association for Accreditation of Laboratory Animal Care. All animal protocols were approved by the UCSD Animal Subjects Committee and conducted in accordance with the Institute of Laboratory Resources’ Guide for the Care and Use of Laboratory Animals published by the National Research Council (Washington DC, 1996).

Twelve random-sourced dogs weighing from 24 to 33 kg were anesthetized with pentobarbital (30 mg/kg) and maintained at a surgical plane with supplemented doses. The animals were intubated and ventilated with room air using a Harvard respiratory pump. Care was taken to ensure full lung expansion throughout the experiment. Blood gases were monitored intermittently, and blood pH was maintained between 7.36 and 7.45. Hemodynamic conditions were observed and kept stable throughout each experiment. End-diastolic pressure (EDP) was raised and lowered by intravenous dextran infusion (70% in 0.9% NaCl) and blood withdrawal. The class I_ antiarrhythmic agent procainamide was given via intermittent intravenous administration over several minutes (200–400 mg total) before the first coronary ligation. There were no lasting changes in heart rate, peak systolic pressure or QRS interval after the procainamide infusion. Intravenous lidocaine (2 mg/kg) was also administered as needed to control arrhythmias.

The heart was exposed through a median sternotomy and left lateral thoracotomy at the fifth intercostal space and supported by a pericardial cradle. A section of the LAD coronary artery distal to the first or second diagonal branch was dissected free for placement of a mechanical occluder. A micromanometer (Konigsberg Instruments Inc., Pasadena, CA) was inserted into the left ventricle (LV) through the left atrial appendage and was calibrated with a fluid-filled catheter introduced from the femoral artery into the left ventricle. Once the LV pressures were matched, the fluid-filled catheter was withdrawn into the aortic root to monitor aortic pressure. Limb leads were placed for an electrocardiogram.

To measure transmural distributions of systolic deformation in the ischemic region, 3 columns (~10 mm separation) of 4–6 radiopaque beads (1.0–1.2 mm diameter) were implanted in the LV anterior free wall in the area perfused by the LAD (Fig. 1). Five additional beads (2-mm diameter) were sutured to the epicardial surface at the apex, base, and above each column of beads. The anterior–posterior and lateral axes of the X-ray machine were adjusted so that the radiopaque markers were all visible in both imaging planes, and synchronized fluoroscopic video images were recorded on two VHS videotape recorders (Panasonic AG6300) with dubbed time codes. Biplane video and hemodynamics were recorded at baseline and during successive graded occlusions of the LAD coronary artery using the mechanical occluder, with end-diastolic pressures maintained relatively constant. Finally, bead positions in a geometric phantom were recorded and used to reconstruct three-dimensional coordinates from the two-dimensional images [12].

Fluorescent microspheres 15 μm in diameter (Molecular Probes Inc., Eugene, OR) were used to measure myocardial perfusion immediately after each video-recording period.

Microspheres labeled with 7 different fluorochromes were available with the following emission wavelengths: blue, 420 nm; blue-green, 457 nm; green, 488 nm;
blocks from the ischemic zone were cut from tissue adjacent to the occlusion site of the left anterior descending coronary artery. The heart was then excised; tissue which contained all of the radiopaque beads was carefully cut out. Tissue containing the bead array was excised and fixed by immersion in 10% formalin phosphate buffer for at least 24 h. A transmural block of tissue adjacent to the beads was used to define the cardiac coordinate system shown in the inset.

The three-dimensional coordinates of the beads were measured digitally from biplane images at end-diastole and end-systole, determined from the time of the peak R-wave, and 40 ms before peak negative dP/dt [16], respectively. The epicardial reference beads were used to transform the coordinates for these linear curves ranging from 29 to 966 spheres/ml/1U and -196 to 74 spheres/ml, respectively, with all r² values greater than or equal to 0.985. Samples from tissue were diluted so that the measurements would fall on the linear portion of the calibration curves.

Blood flows were calculated using the equation $Q_m = (C_m \times Q_r)/C_r$ where $Q_r$ is the withdrawal rate of the reference sample (ml/min), $C_m$ is the myocardial microsphere concentration, and $C_r$ was the reference blood sample microsphere concentration.

The techniques for measuring fiber angles have been described previously [15]. A transmural block of tissue containing the bead array was excised and fixed by immersion in 10% formalin phosphate buffer for at least 24 h. A sample of the fixed tissue cut adjacent to the beads was dehydrated through graded ethanol treatments and embedded in paraffin. Sections 10 μm thick were cut parallel to the epicardium at 9–10 transmural locations, stained with picrosirius red, and viewed under low-power (×20) light microscopy. The fiber angle was then measured with respect to the circumferential axis by digital image analysis as a function of depth through the ventricular wall.

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three-dimensional bead coordinates into a right-handed orthogonal system of ‘cardiac’ coordinates [17] defined by local circumferential, longitudinal, and radial axes (Fig. 1).

A finite element method was used to compute transmural distributions of three-dimensional strain at end-systole (with respect to an undeformed reference state at end-diastole) [18,19]. At 10% increments of relative wall depth, the six components of the strain tensor were computed in cardiac coordinates: the three normal strains, representing circumferential, longitudinal, and radial segment length change, with positive strain indicating segment lengthening and negative strain corresponding to shortening. In addition shear strains are found, which reflect angle changes during deformation between originally orthogonal pairs of coordinate axes. The torsional, axial and azimuthal shear strains represent shearing in the circumferential–longitudinal, circumferential–radial and longitudinal–radial reference coordinate planes, respectively.

For the description of flow–function relations, strains are reported at three depths: 20% (subepicardial), 50% (midwall), and 80% (subendocardial) of wall depth. Since the local myofiber angles were measured and fitted by a linear function of wall depth, the strain tensor was also rotated into a ‘fiber’ coordinate system giving local fiber and cross-fiber in-plane strains, leaving the radial strain component unchanged.

### 2.1. Statistical analysis

Differences in pressures and heart rate between the baseline and ischemic conditions were compared using a paired *t*-test. Blood flow comparisons between baseline and ischemic states were performed with a non-parametric Wilcoxon signed rank test while transmural distributions of blood flow were analyzed using a non-parametric Friedman test. Variations in strain as a function of wall depth were analyzed using two-way repeated-measures analysis of variance (ANOVA) with transmural location as the within factor and occlusion state as the between factor. Strains were analyzed using two-factor ANOVA with blood flow group and transmural location as nominal factors. Statistical significance was accepted at the 95% confidence level (*p* < 0.05).

### 3. Results

#### 3.1. Exclusions

Data from one dog were excluded from analysis because of a large error in the three-dimensional reconstruction of the bead coordinates, possibly due to movement of the X-ray tubes or image intensifiers. Of the 57 runs recorded in the other 11 dogs, five were excluded for the following reasons: two because withdrawal of the reference blood sample was started late, two because arrhythmias occurred during the data acquisition, and one because the video recordings were not synchronized. Endocardial strains interpolated at a transmural depth of 80% of wall thickness were excluded from 4 dogs in which the average relative wall depth of the three deepest beads was less than 80%.

#### 3.2. Hemodynamics

Average left ventricular weight was 129 ± 27 g (mean ± SD). Hemodynamic data are summarized in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Total occlusion</th>
<th><em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>105.4 ± 18.6</td>
<td>109.6 ± 17.0</td>
<td>0.0642</td>
</tr>
<tr>
<td>ESP (mm Hg)</td>
<td>120.9 ± 20.1</td>
<td>116.4 ± 22.1</td>
<td>0.5861</td>
</tr>
<tr>
<td>EDP (mm Hg)</td>
<td>9.1 ± 2.2</td>
<td>12.6 ± 5.2</td>
<td>0.0110</td>
</tr>
</tbody>
</table>

Statistical significance was taken as *p* ≤ 0.05. ESP, end-systolic pressure; EDP, end-diastolic pressure.
Occlusion of the coronary artery did not significantly alter mean heart rate or end-systolic pressure. A small increase in mean end-diastolic pressure (3.5 mmHg) occurred between baseline and total coronary artery occlusion.

Since the distribution of measured regional blood flows was not Gaussian, non-parametric statistics were used. In the ischemic region, median transmural myocardial blood flow was 1.01 ml/min/g and it was reduced to 0.13 ml/min/g at total occlusion. At all three transmural depths in the ischemic region, there was a significant decrease in blood flow during total coronary occlusion (p < 0.03). In the non-ischemic region, median transmural myocardial flow was 1.29 ml/min/g at baseline and 1.38 ml/min/g at total occlusion, and did not change significantly during total LAD occlusion at any wall depth.

3.3. Transmural strain distributions

Negative circumferential and longitudinal strains, indicating in-plane shortening, and positive radial wall thickening all increased in magnitude from epicardium to endocardium during baseline, as seen in the representative measurements from one animal (Fig. 2). As flow decreased during graded occlusions, shortening gradually gave way to lengthening (positive strains) and systolic wall thickening was replaced by thinning (negative strain).

The transmural gradients of strain tended to decrease with progressive ischemia so that the loss of circumferential, longitudinal and radial strain was largest at the subendocardium and smallest at the epicardium as seen for the mean results for baseline and total occlusion runs (Fig. 3). Mean longitudinal–radial (azimuthal) transverse shears were small and positive at baseline but changed sign during total occlusion. By two-way analysis of variance comparing baseline and total occlusion runs only, ischemia significantly changed all three normal strains (p < 0.002), and the longitudinal–radial transverse shear strain (p = 0.0096), but not the other two shear strain components.

There was a significant effect of transmural position on circumferential strain (p = 0.0001), radial strain (p = 0.0001).
and both transverse shears ($p = 0.012$ and $p = 0.040$). From the interaction effects, ischemia significantly altered the transmural variations of circumferential, longitudinal, and radial strains ($p = 0.0001$ for all) but not the shears.

End-systolic strains were also computed with respect to fiber and cross-fiber coordinates (Fig. 4). Mean fiber strains were negative at baseline and positive during total occlusion tending to increase slightly in magnitude across the ventricular wall. By two-way analysis of variance comparing baseline and total occlusion runs only, ischemia significantly changed fiber and crossfiber strains ($p = 0.004$ and $p = 0.0001$, respectively). There was a significant effect of transmural position on crossfiber strain ($p = 0.001$) but not fiber strain ($p = 0.7$). Negative strains in the cross-fiber direction at baseline had a significant transmural variation increasing markedly toward the endocardium ($p = 0.0001$). After LAD occlusion, systolic fiber and cross-fiber strains changed significantly and on average were positive (lengthening) and transmurally uniform. From the interaction effects, ischemia significantly altered the transmural variations of fiber and crossfiber strains ($p = 0.0001$ for both). Radial strains in the fiber coordinate system are the same as those in cardiac coordinates.

### 3.4. Transmural myocardial flow

The effect of transmural depth on blood flow in the ischemic region was not statistically significant during either baseline or total occlusion. Fig. 5 shows endocardial to epicardial blood flow ratios according to the overall transmural blood flow grouped as follows: $0.0–0.1$, $0.1–0.2$, $0.2–0.5$, $0.5–0.8$, $0.8–1.2$, and $1.2–2.0$ ml/min/g. At high transmural blood flows, the average ratio of regional endocardial to epicardial flow in the ischemic region was 0.004, and both transverse shears ($p = 0.012$ and $p = 0.040$). From the interaction effects, ischemia significantly altered the transmural variations of circumferential, longitudinal, and radial strains ($p = 0.0001$ for all) but not the shears.

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greater than unity. However, as transmural flow decreased, the ratio tended to decrease such that at the lowest transmural blood flows, flow to the endocardium was about 70% of that of the epicardium. For comparison, at baseline endocardial/epicardial flow ratios were 1.22 ± 0.16 (mean ± 1SE) compared with 0.86 ± 0.18 (mean ± 1SE) at total occlusion (p = NS).

In the nonischemic region, endocardial/epicardial flow ratios were 1.29 ± 0.22 (mean ± 1SE) at baseline and 1.18 ± 0.16 (mean ± 1SE) at total occlusion, and were not significantly different from each other. There was no significant effect of transmural depth on non-ischemic zone perfusion during baseline or total LAD artery occlusion.

For the six flow groups in Fig. 5, the corresponding median transmural blood flows relative to the median baseline flows were 6%, 11%, 32%, 61%, 92% and 147% (from the 0.0–0.1 to 1.2–2.0 groups, respectively). The relative flows for each of the 3 transmural layers were similar to these transmural values. The baseline flows were not normally distributed, so the mean relative flows were lower than the median values but the trends between groups were the same.

3.5. Flow–function relations

Fig. 6 shows the relationship between average transmural strain components and local myocardial flow. The strains were grouped according to tissue blood flows using the same intervals as used in Fig. 5. Average circumferential strains had positive or small negative values in the low flow groups. As local blood flow increased, circumferential strains tended to become more negative throughout the wall. Average longitudinal strains were all positive in the low blood flow groups and tended to become small or negative at high blood flows. Average radial strains were negative during the low flow group and tended to become positive at high blood flows.

The results of a two-way analysis of variance for both the normal and shear strains in cardiac coordinates are summarized in Table 2. Table 2. Results from the two-way analysis of variance of the normal and shear strain data in cardiac coordinates, with transmural location and blood flow group as the nominal factors

<table>
<thead>
<tr>
<th>Strain orientation</th>
<th>Transmural location</th>
<th>Blood flow</th>
<th>Location * Blood flow interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean square</td>
<td>p-value</td>
<td>mean square</td>
</tr>
<tr>
<td>Circumferential</td>
<td>0.0574</td>
<td>0.0010</td>
<td>0.0426</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>0.0001</td>
<td>0.9810</td>
<td>0.0142</td>
</tr>
<tr>
<td>Radial</td>
<td>0.0638</td>
<td>0.0246</td>
<td>0.1066</td>
</tr>
<tr>
<td>Circumferential–longitudinal</td>
<td>0.0008</td>
<td>0.5653</td>
<td>0.0022</td>
</tr>
<tr>
<td>Circumferential–radial</td>
<td>0.0133</td>
<td>0.0001</td>
<td>0.0014</td>
</tr>
<tr>
<td>Longitudinal–radial</td>
<td>0.0014</td>
<td>0.4333</td>
<td>0.0078</td>
</tr>
</tbody>
</table>

Mean square values indicate the strength of the given effect on the strain, while p-values indicate the significance of the effect.

The analysis indicated a significant effect of blood flow on all of the normal strain components as well as the longitudinal–radial transverse shear component. There was also a significant effect of transmural location on the circumferential and radial strains and on the circumferential–radial transverse shear. In the circumferential direction, there was a significant interaction effect between transmural location and blood flow, indicating a dependence of the circumferential strain–blood flow relation on wall depth. Conversely, there was no significant interaction between the longitudinal strain–flow relation and location.

Fig. 7 plots the average strain components referred to fiber coordinates grouped according to local blood flow. Fiber strains were positive in the low-flow groups and became negative at high blood flows. Similarly, average midwall and endocardial strains in the cross-fiber direction
tended to become more negative as the blood flow was increased; however, epicardial cross-fiber strains did not appear to follow this same trend.

Two-way analysis of variance of the fiber strain components and blood flow groups are shown in Table 3. There was a highly significant effect of local blood flow on both fiber and cross-fiber strains. Transmural location had a significant effect on cross-fiber strains but not on fiber strains. In the cross-fiber direction, the ANOVA indicated a significant interaction effect between transmural location and blood flow; such an effect was not present in the fiber direction. Thus, the relationship between blood flow and cross-fiber strain appears to be dependent upon wall depth whereas the relationship between blood flow and fiber strain appears to be unique and independent of transmural location. Results in the radial direction are the same as those previously discussed in cardiac coordinates.

There were no significant differences between the normal cardiac and fiber/cross-fiber strains in the highest flow group (1.2–2.0 ml/min/g) compared with those measured during baseline conditions.

4. Discussion

In this study, regional ischemic systolic function in the left ventricular wall was assessed from the transmural distributions of three-dimensional finite strains during graded left anterior descending coronary artery occlusions. We examined the relationship between regional myocardial blood flow and regional systolic function, and found a dependence of this relationship on transmural location, segment orientation, and fiber architecture.

4.1. Effects of ischemia on 3D mechanics

Reductions in blood flow had a significant effect on three-dimensional systolic segment function. Circumferential, longitudinal, radial, fiber, and cross-fiber strains were all significantly altered as a result of the graded LAD occlusions. However, of the shear strains, only the longitudinal–radial transverse shear was significantly affected by ischemia. These results are in general agreement with a previous study by Villarreal and colleagues [10] in which three-dimensional finite strains were computed in much the same manner as our study. Villarreal and co-workers also observed that the transmural gradient of the circumferential strains at baseline tended to be lost in ischemia. In the present study, we noted a similar trend not only for the circumferential strains, but also for the radial and cross-fiber strains. The transmural distributions of the longitudinal, fiber, and shear strains appeared more uniform than the other components, and remained so after total occlusion.

4.2. Flow–function relationship depends on transmural location and orientation

As reported by others [3,5,6], the flow–function relationship was not, in general, independent of transmural position. This finding is consistent with the hypothesis that mechanical interaction results in a tethering between the endocardium and epicardium [5,20].

However, our study also indicated for the first time that the degree of mechanical interaction was highly dependent on segment orientation, i.e. the orientation in which function is measured. Specifically, for segments parallel to the plane of the wall, the interaction was highest and highly significant for cross-fiber and circumferential shortening, but lower and not statistically significant in the fiber and longitudinal directions. Although the radial strain did not show a statistically significant interaction, it did show the greatest strength of the effect of the interaction on strain, suggesting that the flow–function interaction for this strain component is dependent on position. This evidence supports the conclusion that transmural interactions are minimized for myocardial strain in the fiber direction and emphasizes the importance of accounting for segment orientation with respect to fiber anatomy as suggested by Gallagher and colleagues [9]. Thus, we conclude that there appears to be a unique relationship between local myocardial blood flow and function, independent of transmural location, in the fiber direction; however, such a unique relationship does not exist in cross-fiber directions, due to an effect of transmural tethering.

4.3. Regional wall thickening

In their review of systolic wall thickening, Hexeberg and colleagues [20] conclude that thickening of a discrete myocardial layer is not independent of the surrounding
layers and that it is not appropriate to relate a change in local thickening to a change in local blood flow.

We found that in the radial direction, the dependence of the flow–function relationship on transmural position was, in fact, the strongest of all the directions as indicated by the mean square value of the ANOVA (Tables 2 and 3). For example, as shown in Fig. 6, positive radial strains (wall thickening) became zero or less at the epicardium when flow was reduced to the 0.2–0.3 ml/min/g range. However, midwall radial strains did not give way to thinning until the local blood flow fell to the 0.1–0.2 ml/min/g range, and endocardial wall thickening did not begin until the myocardial blood flow fell below 0.1 ml/min/g. These data suggest that radial function is tethered in some fashion between layers of myocardium, hence this local function is not uniquely determined by local perfusion.

Edwards and co-workers [6] also found evidence of tethering as measured in the radial direction when they noted that subepicardial thickening correlated most closely with subendocardial blood flow. Although we found a large transmural interaction in the radial direction (mean-squared value), the smaller marker separation in the radial direction (compared with the column separation) resulted in larger variations in the radial strains, and thus the interaction that we observed did not achieve statistical significance.

Torry and colleagues [7] found that, under conditions of non-transmural ischemia, the increasing degree of radial dysfunction from subepicardium to subendocardium paralleled the increased loss of blood flow through the wall. Thus, they concluded that perfusion, rather than tethering, was the primary determinant of subepicardial function under conditions of non-transmural ischemia.

In our study, during the high levels of mean transmural blood flow, the local epicardial to endocardial flow ratio was close to 1, indicating relatively uniform transmural flow during the baseline conditions. However, during the baseline conditions, we observed a significant transmural gradient of radial strain, with maximum wall thickening occurring at the endocardium as expected [11]. The graded LAD occlusions produced a non-uniform transmural pattern of perfusion in which the average epicardial to endocardial flow ratio increased as mean transmural flow was reduced. Yet, at total LAD occlusion, we observed that the transmural gradient in radial strain had markedly diminished. This observation supports the notion that perfusion may not be as important a determinant as mechanical tethering in contributing to radial dysfunction.

4.4. Limitations

Several limitations of the present study should be noted. The main determinant of the strain measurement is the resolution of the radiographic images used to compute the three-dimensional coordinates of the implanted beads, and hence the accuracy in locating the actual bead coordinates. The error in finding the centroid of the beads was estimated to be 0.2–0.3 mm by Waldman and colleagues [11]. Using the finite element fitting technique, this corresponds to a practical resolution of about ±0.02 in the strains [21], although due to the bead spacing, radial components may have greater resolution. Local injury due to the implantation of the bead set, differences in the location of the bead set, and differences in heart sizes are also possible sources of variation for strains among the animals.

Other limitations to this study result from the resolution and accuracy of the blood flow measurements. The fluorescent microsphere technique itself correlates well with results for regional blood flow using radioactive microspheres [22,23]. Even though our measurements of transmural flow are close to several previously reported values, the endocardial/epicardial flow ratio did not decrease as much as previously reported. For example, Edwards et al. [6] report a baseline endocardial/epicardial flow ratio of 0.82, reduced to 0.43 after partial LAD stenosis. Torry and colleagues [7] give a baseline ratio of 0.81 and then 0.58 during partial coronary occlusion. In the present study, our mean ratios were 1.22 and 1.29 in the ischemic and non-ischemic zones at baseline, and 0.86 and 1.18 in these same zones during maximum occlusion. Thus, the baseline ratios in this study may be somewhat higher than normal and the decrease with occlusion may not be as great as expected.

There are local variations in myocardial perfusion especially during ischemia. Blood flows were found in close proximity to the location of the mechanical measurements (within 2 cm), but blood flow could be different at the center of the transmural radiopaque marker set. The transmural location of the blood flow measurement may not exactly match the transmural locations of the strain measures. Loss of microspheres during tissue digestion, filtering of the samples, or sample handling is one probable source of error. Although care was taken, the addition of unequal volumes of solvent to each sample would have also affected the total microsphere count. Inconsistencies in the measurements of the fluorescence of each sample could have resulted in additional errors. There is also ‘spillover’ from each color band to adjacent color bands, which will decrease the accuracy of the fluorescent microsphere method. Glenny et al. [13] report fairly large spillovers from blue-green, green and yellow-green into the adjacent color bands (up to 18% of the correct color band emission). We calculated the spillover from these three colors on our fluorometer to compare with the Glenny values. We found that our maximum value was about one-half that given by Glenny: the spillovers from blue-green, green and yellow-green to the two adjacent colors were: 2.7 and 6.8; 7.1 and 0.2; 0.1 and 0.1 respectively. Overall, the spillovers for all seven colors are comparable to the corresponding values given by Glenny. But nevertheless, there is some spillover which we do not correct.
for, and acknowledge this as a limitation of the microsphere technique. These limitations of the fluorescent microsphere technique may have contributed to an increase in the variation observed in the blood flow data, and precluded the determination of more accurate distributions of strain–flow relationships.

5. Conclusion

In summary, using three-dimensional strain distributions as an index of myocardial systolic function, we found that the uniqueness of the local blood flow–function relationship is largely dependent on segment orientation. The relationship appeared to be unique and independent of transmural location in the fiber direction but not in the cross-fiber or radial directions. The apparent dependence of the flow–function relationship on wall depth in the cross-fiber and radial directions supports the conclusion that a mechanical tethering occurs between the transmural layers of the myocardium, and this tethering most strongly affects cross-fiber, rather than fiber function. This is also consistent with the hypothesis that perfusion directly governs myocardial fiber tension development which in turn influences fiber shortening.

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