Subendocardial and Subepicardial Wall Thickening During Ischemia in Exercising Dogs

David C. Homans, MD, Eugene Sublett, BS, Paul Lindstrom, Tracy Nesbitt, and Robert J. Bache, MD

To determine whether ischemia in the exercising dog is associated with preservation of subepicardial thickening relative to subendocardial thickening, 10 dogs were chronically instrumented with circumflex artery flow probes, hydraulic occluders, and pairs of ultrasonic microcrystals for determination of wall thickness in the circumflex artery distribution. One pair of crystals spanned the entire ventricular wall (transmural), and the other spanned the outer half of the ventricular wall. Inner wall thickness was computed as the difference between transmural wall thickness and outer wall thickness. Dogs performed control treadmill exercise and exercise with a coronary stenosis that reduced circumflex artery flow to resting control levels. Percent systolic thickening at rest for the transmural, inner, and outer regions was 21.3 ± 11.8%, 35.5 ± 20.3%, and 10.3 ± 5.0% (mean ± SD), respectively. During exercise without stenosis, systolic thickening increased to 143 ± 37% of control for outer wall crystals and 137 ± 26% of control for the inner portion of the wall. During exercise, the addition of a coronary stenosis caused a reduction in thickening to 17.7 ± 28.5% of control for the outer wall and 40.1 ± 32.3% of control for the inner portion of the wall; these were not significantly different. In contrast, normalized inner wall blood flow during exercise with circumflex artery stenosis (25.0 ± 16.0%) was significantly less than for the outer portion of the wall (48.5 ± 20.9%). Further, there was a close relation between changes in inner wall thickening and inner wall blood flow (r = 0.84), whereas there was only a very weak relation between changes in outer wall blood flow and function (r = 0.62; p = 0.04). During ischemia in the exercising dog, outer wall thickening is depressed out of proportion to reductions in outer wall blood flow and is not preserved relative to inner wall thickening. (Circulation 1988;78:1267–1276)

In the normal heart, there is a transmural variation in myocardial blood flow with a perfusion gradient favoring the subendocardium.1 Similar transmural variations in systolic thickening of the left ventricular wall have been noted, with greatest thickening occurring in the inner wall at rest.2–4 Exercise normally results in an increase in blood flow with maintenance of the perfusion gradient favoring the subendocardium.4 This is associated with parallel increases in both inner and outer wall systolic thickening.5 When blood flow is limited by a coronary artery stenosis during exercise, however, coronary flow fails to increase appropriately, and subendocardial blood flow is reduced relative to subepicardial flow.6 Under these circumstances, transmural wall thickening generally decreases.7,8 The transmural pattern of regional dysfunction under these circumstances has not been previously investigated.

The purpose of this investigation was to test the hypothesis that the greater severity of subendocardial hypoperfusion occurring during exercise in the presence of a coronary stenosis is accompanied by relatively greater reductions in inner wall thickening relative to outer wall thickening. To test this hypothesis, the transmural pattern of perfusion and systolic wall thickening were studied in chronically instrumented mongrel dogs during treadmill exercise in the presence and absence of coronary artery stenosis.

Materials and Methods

Studies were performed on 14 mongrel dogs (weight, 21.2 ± 1.4 kg) previously trained to run on a motor-driven treadmill. The animals received pre-
operative antibiotic prophylaxis with gentamicin 4 mg/kg i.v. and subsequently were anesthetized with pentobarbital sodium (25–30 mg/kg i.v.) and ventilated with a Harvard respirator (South Natick, Massachusetts). A left thoracotomy was performed in the fourth intercostal space. A polyvinyl chloride catheter (3.0 mm o.d.) filled with heparin saline solution (200 units/ml) was inserted into the root of the aorta through the internal thoracic artery for pressure measurement and blood withdrawal. The pericardium was then incised, and the heart was supported in a pericardial sling. The proximal 1.5 cm of the circumflex coronary artery was dissected free, and an electromagnetic flow probe and hydraulic occluder were fitted around it. A chronically implantable micromanometer (Model P-5, Konigsberg Instruments, Pasadena, California) and a fluid-filled polyvinyl chloride catheter were inserted into the left ventricle through the apical dimple. Three pairs of ultrasonic microcrystals were then inserted for measurement of left ventricular wall thickening. Epicardial crystals were 3 mm in diameter, and endocardial crystals were 1.5 mm in diameter. Two pairs were placed in the distribution of the circumflex coronary artery, and one pair was placed within the distribution of the anterior descending coronary artery. One of the circumflex pairs of crystals was placed across the entire thickness of the left ventricular wall; the endocardial crystal was advanced through a stab wound in the epicardium obliquely until it was positioned as near as possible to the endocardium without penetrating into the left ventricular cavity. The epicardial member of this pair was positioned on the epicardial surface in that location which minimized the intercrystal distance and was sutured in place. For the second pair of circumflex crystals, the inner crystal was advanced obliquely to a depth that approximated the midwall (3–6 mm below the epicardial surface). The epicardial member of this pair was positioned as noted above and sutured in place. An additional pair of transmural wall thickness crystals was inserted within the region of the anterior descending artery. A fluid-filled polyvinyl chloride catheter was then inserted into the left atrial cavity through the left atrial appendage and secured with a pursestring suture. The pericardium was loosely closed, and all catheters and the electronic cables were tunneled dorsally to the base of the neck where they were exteriorized. The thoracotomy was then repaired, and the animals were allowed to recover from surgery. After operation, the dogs were treated with gentamicin (2 mg/kg q.t.d. for 3 days) for antibiotic prophylaxis and with pentazocin (25 mg/kg t.i.d. orally for 3 days) for analgesia.

All experiments were performed 7–10 days postoperatively when the animals were in good physical condition. Catheters were protected with a nylon vest that the dogs had been trained to wear (Alice King Chatham, Los Angeles, California). Measurements of aortic and left ventricular pressures were obtained with fluid-filled catheters connected to Statham P23Db pressure transducers (Cleveland, Ohio) fastened to the nylon vest at midchest level. Left ventricular pressure and dP/dt were determined from the implanted micromanometer that was calibrated to the fluid-filled left ventricular pressure catheter. Circumflex coronary artery flow was measured with a Statham SP2202 electromagnetic flowmeter. Ultrasonic microcrystal measurements of wall thickness were obtained by activating the implanted piezoelectric crystals with a Triton Ultrasonic Dimension System (Model 120, Triton, San Diego, California), which was modified to not interfere with the electromagnetic flowmeter function. All data were recorded on an eight-channel direct-writing oscillograph (Model 8800, Hewlett-Packard, Palo Alto, California). Myocardial blood flow was measured with serial injections of microspheres, 15 μm in diameter, labeled with gamma-emitting nuclides (125I, 51Cr, 85Sr, 90Nb, 111Sn, 46Sc, and 51Co). Before injection, the microspheres were agitated for at least 15 minutes in an ultrasonic bath. During each intervention, approximately $3 \times 10^6$ microspheres were injected into the left atrial catheter during a 15-second interval, and the atrial catheter was flushed with 10 ml isotonic saline. Beginning 5 seconds before each microsphere injection and continuing for 90 seconds, a reference sample of blood was withdrawn from the aortic catheter at a constant flow rate of approximately 15 ml/min. To simplify these already complex experiments and to shorten the total duration of exercise required, only three different radioisotopes were injected during each experiment.

Before each study, dogs underwent warm-up exercise for approximately 5 minutes, and during this time, the exercise level required to achieve heart rates of 200–240 beats/min was determined (mean treadmill speed and grade were 6.4 km/hr and 15%, respectively). After warm-up exercise, 30 minutes was allowed for the dog to return to preexercise control levels of heart rate, aortic pressure, and left ventricular dP/dt. Hemodynamic and ultrasonic dimension data were then recorded, and microspheres were injected into the left atrium for determination of resting regional myocardial blood flow. Treadmill exercise was then begun, and the grade and speed were rapidly advanced to the predetermined level required to raise the heart rate to 200–240 beats/min. When heart rate, aortic, and left ventricular systolic pressures, dP/dt, and wall thickening had remained stable for 1 minute, hemodynamic and ultrasonic dimension data were recorded during exercise in the absence of circumflex stenosis. The hydraulic occluder was then inflated with a mechanical inflation device to reduce circumflex coronary artery flow to the resting level as determined by the electromagnetic flowmeter tracing. Occasional adjustments of the occluder were required to maintain flow at the desired level. The dogs maintained exercise at this stable level for 10
minutes. Five minutes into the exercise period, a second injection of radioactive microspheres was made into the left atrium for determination of myocardial blood flow during exercise in the presence of a stenosis. The dog continued exercising for an additional 5 minutes, and the treadmill was then stopped. Immediately upon cessation of treadmill exercise, the hydraulic occluder was abruptly deflated, permitting unimpeded reactive hyperemia throughout the postexercise period. Five minutes after exercise, the dogs were removed from the treadmill and placed in a sling adjacent to the treadmill to maintain upright posture, and they were allowed to rest quietly in a darkened laboratory for the remainder of the study. At 10 minutes after exercise, a third injection of radioactive microspheres was performed for determination of regional myocardial blood flow. Hemodynamic and ultrasonic dimension data were recorded continuously throughout exercise and for 1 hour after exercise.

After the study had been completed, the dogs were killed with a lethal dose of pentobarbital sodium, and the heart and both kidneys were excised. The circumflex coronary artery was cannulated at the site of the hydraulic occluder, and 10 ml Evans blue dye was injected to identify the area of the left ventricle supplied by the circumflex coronary artery distal to the site of stenosis. The heart was then fixed in 10% buffered formalin. After fixation, the atria, right ventricle, and aorta were removed, and the left ventricle was weighed. The pairs of ultrasonic microcrystals were then inspected to ensure proper placement; crystal alignment of the transmural pair was considered acceptable when the center of the inner crystal was less than 3 mm from a perpendicular line passing through the center of the epicardial crystal. Outer wall crystal pairs were considered acceptable when the center of the midwall crystal was less than 2 mm from a perpendicular line originating at the center of the epicardial crystal. The inner crystals of the transmural wall thickness were always positioned in the inner third of the myocardium. Transmural and epicardial crystal pairs were always placed so that the centers of each epicardial crystal were separated by no more than 1.5 cm. Circumflex crystals were located at least 1 cm within the perfusion boundary of the circumflex coronary artery, identified by the blue-stained myocardium, and anterior descending pairs were at least 1 cm outside the perfusion boundary of the circumflex coronary artery.

Pairs of ultrasonic microcrystals with interposed myocardium were then excised, and the myocardium was divided into four layers from epicardium to endocardium. Myocardial samples were weighed on an analytical balance and placed in vials for determination of radioactivity (sample weight, 1.59 ± 0.44 g). Myocardial and blood reference specimens were counted in a gamma counting system (Model 5912, Packard Instruments, Downers Grove, Illinois) with a multichannel analyzer at window settings selected according to peak energies of each radionuclide. The activity recorded in each energy window was corrected for background, and overlapping accounts contributed by accompanying isotopes according to the method of Domenech et al.9

Blood flow to each myocardial specimen was computed as $Q_m = Q_r \times C_m/C_r$, where $Q_m$ is the myocardial blood flow (ml/min), $Q_r$ is the reference blood flow rate (ml/min), $C_m$ is the counts per minute of the myocardial specimen, and $C_r$ is the counts per minute of the reference blood specimen. Blood flow was divided by the sample weight and expressed as milliliters per minute per gram of myocardium. The ratio of subendocardial flow to subepicardial flow was obtained by dividing flow to the innermost layer by the corresponding flow to the epicardial layer. Inner wall flow was computed as the mean value of the inner two layers and outer wall flow was computed as the mean value of the outer two layers.

For ultrasonic microcrystal determination of wall thickening, the end-diastolic wall thickness was measured at the initiation of the upstroke of the left ventricular pressure tracing recorded by the Koningsberg micromanometer, and the end-systolic wall thickness was measured 20 msec before peak negative dP/dt on the differentiated left ventricular pressure tracing.10 The values for 6–10 successive beats were averaged. Estimated inner wall thickness was computed as the instantaneous difference between transmural thickness and outer wall thickness by electronically subtracting the analog signals (Figure 1). Percent systolic wall thickening was defined as end-diastolic wall thickness minus end-systolic wall thickness divided by end-diastolic wall thickness. Circumflex flow measurements were obtained with a Gould-Statham SP-2202 electromagnetic flowmeter; the volume of antegrade circumflex coronary artery flow was determined by electrical integration of the electromagnetic flowmeter tracing.

Hemodynamic, myocardial blood flow, and ultrasonic dimension data were compared by analysis of variance for repeated measures. When an overall difference was found, individual comparisons were made with the Student’s paired t test with Bonferroni’s correction for multiple simultaneous comparisons. A p value of less than 0.05 was considered significant. Unless otherwise specified, values are reported as mean ± SD. Linear regression was performed with the least-squares technique.

**Results**

In four dogs, crystal alignment was not considered acceptable; data are presented from the remaining 10 dogs. Myocardial sample weights and the numbers of microspheres per sample (for microspheres injected during exercise with stenosis) are depicted in Table 1.

**Hemodynamics**

The hemodynamic data are summarized in Table 2. The resting heart rate was 130 ± 23 beats/min.
Heart rate increased during exercise without coronary stenosis and increased slightly more during exercise in the presence of a coronary stenosis. By 10 minutes after exercise, the heart rate remained slightly, but significantly, greater than baseline. Similar trends were noted in aortic systolic pressures; however, the magnitude of exercise-induced change was less than for heart rate. There was a tendency for a lower systolic pressure to occur during exercise in the presence of a stenosis; however, this was not statistically significant. There was a slight but statistically significant increase in left ventricular end-diastolic pressure during exercise in the absence of a coronary stenosis, and this increased substantially more during exercise in the presence of coronary stenosis. There were also significant increases in left ventricular $dP/dt$ during exercise when compared with resting control values. During exercise in the absence of a coronary stenosis, circumflex coronary artery flow nearly doubled compared with resting control; during exercise in the presence of a coronary stenosis, flow was reduced to values slightly below resting control; however, this was not statistically significant. Thus, a severe coronary artery stenosis was used during exercise. Aortic pressure, left ventricular end-diastolic pressure, left ventricular $dP/dt$, and coronary artery flow had returned to control values by 10 minutes after exercise.

**Regional Myocardial Blood Flow**

Mean transmural flow, ratios of subendocardial to subepicardial flow, and flow to each of four transmural layers are depicted in Table 3 and Figure 2. At rest, transmural blood flows to circumflex segments containing transmural crystal pairs and outer wall pairs did not differ and were comparable to anterior descending perfused crystal pairs. During exercise in the presence of a coronary stenosis, there was a marked increase in anterior descending perfused transmural myocardial flow to $2.84 \pm 0.94$

**TABLE 1. Sample Weights and Microspheres by Layer**

<table>
<thead>
<tr>
<th>Layer</th>
<th>Sample weight (g)</th>
<th>Microspheres per sample (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer 1</td>
<td>$1.94 \pm 0.64$</td>
<td>$1,165 \pm 1,025$</td>
</tr>
<tr>
<td>Layer 2</td>
<td>$1.50 \pm 0.31$</td>
<td>$933 \pm 899$</td>
</tr>
<tr>
<td>Layer 3</td>
<td>$1.37 \pm 0.27$</td>
<td>$668 \pm 746$</td>
</tr>
<tr>
<td>Layer 4</td>
<td>$1.57 \pm 0.57$</td>
<td>$646 \pm 766$</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Sample weights and number of microspheres per sample by layer (1, subepicardium; 4, subendocardium). Number of microspheres trapped per sample was computed for microspheres injected during exercise with stenosis.
ml/min/g. In contrast, there was a reduction of mean transmural flow to circumflex myocardium (0.97 ± 0.58 for transmural crystal pairs and 0.96 ± 0.42 ml/min/g for segments containing outer wall crystal pairs). The presence of a coronary stenosis resulted in a profound transmural redistribution of myocardial blood flow during exercise. Myocardial blood flow to the innermost layer of the anterior descending perfused myocardium rose to 3.31 ± 0.89 ml/min/g during exercise in the presence of a coronary stenosis. Myocardial blood flow to the innermost layer during exercise with stenosis was 0.71 ± 0.58 ml/min/g for circumflex segments containing transmural crystal pairs and 0.46 ± 0.35 ml/min/g for segments containing outer wall thickness crystal pairs (p = 0.17). The ratio of subendocardial blood flow to subepicardial blood flow at rest was 1.63 ± 0.54 for transmural crystal pairs and 1.62 ± 0.49 for subepicardial crystal pairs. During exercise with stenosis, it fell to 0.61 ± 0.45 for transmural pairs and 0.38 ± 0.21 for epicardial crystal pairs (p = 0.12).

To provide an index of the relative reduction in flow to the outer and inner halves of the ventricular wall in circumflex perfused regions, values for circumflex outer (mean of layers 1 and 2) and inner (mean of layers 3 and 4) wall flows were divided by values of blood flow to the corresponding portions of the anterior wall of the left ventricle. These data are illustrated in Figure 3. During exercise with stenosis, outer wall flow to circumflex segments instrumented with transmural crystal pairs was reduced to 46.8 ± 19.9% of flow to corresponding layers of anterior descending perfused myocardium; outer wall flow to segments instrumented with outer wall crystal pairs was reduced to 48.5 ± 20.9% of flow to the anterior descending myocardium. Flow reductions to the inner wall were significantly more severe; inner wall flow to circumflex segments containing transmural crystal pairs was reduced to 25 ± 16.0% of anterior descending myocardium, whereas inner wall flow to segments containing subepicardial crystal pairs was reduced to 19.1 ± 12.2% of anterior descending perfused myocardium (p < 0.01 inner wall vs. outer wall comparisons for both transmural and outer wall crystal pairs). Thus, under resting conditions, the transmural distribution of myocardial blood flow slightly favored the subendocardium in both anterior descending and circumflex perfused regions. During exercise in the presence of a coronary stenosis, this pattern of flow distribution persisted in anterior descending perfused myocardium with increases in flow to all transmural layers of the ventricular wall. There was significant maldistribution of myocardial blood flow in the circumflex region during exercise in the presence of the coro-

### Table 2. Hemodynamics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Without stenosis</th>
<th>With stenosis</th>
<th>10 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>130 ± 23</td>
<td>211 ± 20.6*</td>
<td>220 ± 30.3*</td>
<td>155 ± 18.5</td>
</tr>
<tr>
<td>Aortic systolic pressure (mm Hg)</td>
<td>134 ± 17</td>
<td>159 ± 19</td>
<td>149 ± 17</td>
<td>130 ± 13</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>5.5 ± 3.7</td>
<td>9.9 ± 5.9</td>
<td>20.5 ± 10.1†</td>
<td>5.0 ± 4.6</td>
</tr>
<tr>
<td>dP/dt + (x 10³)</td>
<td>2.42 ± 0.37</td>
<td>4.56 ± 0.95*</td>
<td>3.82 ± 0.98</td>
<td>2.30 ± 0.37</td>
</tr>
<tr>
<td>dP/dt − (x 10³)</td>
<td>2.19 ± 0.47</td>
<td>3.12 ± 0.49*</td>
<td>2.63 ± 0.82</td>
<td>2.25 ± 0.27</td>
</tr>
<tr>
<td>Cx flow (ml/min)</td>
<td>49.4 ± 14.7</td>
<td>93.9 ± 34.5*</td>
<td>37.2 ± 18.4</td>
<td>47.0 ± 13.0</td>
</tr>
</tbody>
</table>

*tp<0.05 vs. control; †p<0.05 vs. exercise without stenosis.

### Table 3. Myocardial Blood Flow

<table>
<thead>
<tr>
<th>Location</th>
<th>Resting</th>
<th>Exercise</th>
<th>After exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cx transmural</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layer 1</td>
<td>1.03 ± 0.46</td>
<td>1.26 ± 0.79</td>
<td>0.92 ± 0.40</td>
</tr>
<tr>
<td>Layer 2</td>
<td>1.32 ± 0.41</td>
<td>1.12 ± 0.69</td>
<td>1.22 ± 0.34</td>
</tr>
<tr>
<td>Layer 3</td>
<td>1.46 ± 0.34</td>
<td>0.80 ± 0.53</td>
<td>1.28 ± 0.28</td>
</tr>
<tr>
<td>Layer 4</td>
<td>1.51 ± 0.40</td>
<td>0.71 ± 0.58</td>
<td>1.48 ± 0.37</td>
</tr>
<tr>
<td>Mean</td>
<td>1.33 ± 0.35</td>
<td>0.97 ± 0.54</td>
<td>1.23 ± 0.28</td>
</tr>
<tr>
<td>I:O</td>
<td>1.63 ± 0.54</td>
<td>0.61 ± 0.45</td>
<td>0.79 ± 0.74</td>
</tr>
<tr>
<td>Cx epicardial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layer 1</td>
<td>1.03 ± 0.37</td>
<td>1.43 ± 0.99</td>
<td>0.98 ± 0.37</td>
</tr>
<tr>
<td>Layer 2</td>
<td>1.41 ± 0.36</td>
<td>0.99 ± 0.61</td>
<td>1.53 ± 0.45</td>
</tr>
<tr>
<td>Layer 3</td>
<td>1.56 ± 0.33</td>
<td>0.74 ± 0.42</td>
<td>1.43 ± 0.35</td>
</tr>
<tr>
<td>Layer 4</td>
<td>1.54 ± 0.41</td>
<td>0.46 ± 0.35</td>
<td>1.23 ± 0.34</td>
</tr>
<tr>
<td>Mean</td>
<td>1.26 ± 0.35</td>
<td>0.96 ± 0.42</td>
<td>1.21 ± 0.33</td>
</tr>
<tr>
<td>I:O</td>
<td>1.62 ± 0.49</td>
<td>0.38 ± 0.21</td>
<td>1.32 ± 0.32</td>
</tr>
<tr>
<td>LAD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layer 1</td>
<td>1.06 ± 0.37</td>
<td>2.14 ± 0.84</td>
<td>1.11 ± 0.50</td>
</tr>
<tr>
<td>Layer 2</td>
<td>1.53 ± 0.45</td>
<td>2.90 ± 1.11</td>
<td>1.67 ± 0.77</td>
</tr>
<tr>
<td>Layer 3</td>
<td>1.49 ± 0.35</td>
<td>2.97 ± 1.06</td>
<td>1.75 ± 0.80</td>
</tr>
<tr>
<td>Layer 4</td>
<td>1.53 ± 0.26</td>
<td>3.31 ± 0.89</td>
<td>1.81 ± 0.61</td>
</tr>
<tr>
<td>Mean</td>
<td>1.40 ± 0.33</td>
<td>2.84 ± 0.94</td>
<td>1.59 ± 0.64</td>
</tr>
<tr>
<td>I:O</td>
<td>1.54 ± 0.43</td>
<td>1.74 ± 0.77</td>
<td>1.73 ± 0.53</td>
</tr>
</tbody>
</table>

All values are mean ± SD.

Cx transmural, flow to circumflex transmural crystal pairs; Cx epicardial, flow to circumflex myocardium containing subepicardial crystal pairs (in layers 1 and 2); LAD, outermost layer; layer 3, innermost layer; I:O, ratio of subendocardial to subepicardial blood flow.

There are no statistically significant differences between circumflex and epicardial layers for any value.
nary stenosis, however, with hypoperfusion being most severe in the subendocardial layers. By 10 minutes after exercise, both the values of mean transmural flow, which is the flow to each layer of the myocardial wall, and the transmural distribution of regional myocardial blood flow were no longer different than during resting control conditions.

Regional Myocardial Function

The mean end-diastolic intercrystal distance for transmural circumflex crystal pairs was 8.9 ± 1.4 mm. Mean end-diastolic crystal separation for outer wall thickening pairs was 4.4 ± 1.6 mm (range, 3.2–6.4 mm). Percent thickening of transmural crystal pairs, outer wall thickness pairs, and estimated inner wall values is summarized in Table 4. Resting systolic thickening of transmural circumflex crystal pairs was not different from LAD thickening. Percent systolic thickening of the outer wall crystal pairs was significantly less (10.3 ± 5.0%, p<0.05) than transmural circumflex pairs (21.2 ± 11.8%). Estimated inner wall thickening was significantly greater than either transmural or outer wall thickening (35.5 ± 20.3%, p<0.05 vs. transmural thickening). Thus, a transmural variation in regional function at rest was observed with greater degrees of systolic wall thickening in the inner portion of the left ventricular wall. During exercise in the absence of a stenosis, there were similar relative increases in transmural wall thickening, outer wall thickening, and computed inner wall thickening. During exercise in the presence of a coronary stenosis, there were significant reductions in transmural thickening to 8.3 ± 9.0%, outer wall thickening to 2.2 ± 3.4%, and inner wall thickening to 16.9 ± 10.0% (p<0.05 for all values compared with resting control). By 10 minutes after exercise, there has been partial recovery of systolic wall thickening for all three groups.

Figure 4 summarizes wall thickening (normalized to the resting control value) for transmural crystal pairs, outer wall pairs, and computed inner wall values. During exercise in the absence of a stenosis, there were comparable increases in the systolic wall thickening observed at each coronary location.

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

**Figure 2.** Plots of myocardial blood flow (mean±SEM) to anterior descending and circumflex crystal pairs during exercise (n=10). Circumflex myocardial blood flows were all significantly lower than corresponding anterior descending flows; however, there were no differences between transmural and epicardial circumflex flows. TM, mean of transmural blood flow.

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Bar graph of circumflex myocardial blood flow (mean±SEM) as a percentage of myocardial blood flow to the corresponding layers of the control (anterior descending) crystal pairs. Inner, mean of layers 3 and 4; outer, mean of layers 1 and 2. LAD, left anterior descending coronary artery. *Significant difference between inner and outer layers (p<0.05, n=10).

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Bar graph of systolic thickening (mean±SEM) as a percentage of resting control value for transmural crystal pairs, outer wall pairs, and estimated inner wall values. There were no significant differences between transmural, subepicardial, and subendocardial values (n=10).

### Table 4. Percent Systolic Wall Thickening

<table>
<thead>
<tr>
<th>Location</th>
<th>Rest Without stenosis</th>
<th>With stenosis</th>
<th>10 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD</td>
<td>26.3 ± 12.3</td>
<td>34.9 ± 21.4</td>
<td>37.8 ± 20.1</td>
</tr>
<tr>
<td>Transmural</td>
<td>21.3 ± 11.8</td>
<td>31.0 ± 15.2</td>
<td>8.3 ± 8.9</td>
</tr>
<tr>
<td>Outer</td>
<td>10.3 ± 5.0</td>
<td>14.9 ± 5.3</td>
<td>2.2 ± 3.4</td>
</tr>
<tr>
<td>Inner</td>
<td>35.5 ± 20.3*</td>
<td>53.6 ± 31.0*</td>
<td>16.8 ± 16.8</td>
</tr>
</tbody>
</table>

10 min, 10 minutes after exercise; LAD, left anterior descending coronary artery; transmural, transmural circumflex crystals; outer, outer wall circumflex thickness; inner, inner wall circumflex crystals.

*p<0.05 vs. outer crystals.
thickening of all three groups. During exercise in the presence of a coronary stenosis, transmural systolic wall thickening was reduced to 33.2±26.8% of the control value, outer wall thickening was reduced to 17.7±28.5% of the resting control value, and computed inner wall thickening was reduced to 40.12±32.8% of the control value (p = NS inner vs. outer wall thickening). Thus, a marked reduction in systolic wall thickening relative to the control value was present in all layers. Circumflex myocardial blood flow was not measured during exercise without stenosis. Therefore, to obtain a measure of the extent of hypoperfusion caused by the stenosis, circumflex flow during exercise with stenosis was normalized by dividing it by the flow to the control (LAD) segment during exercise with stenosis. Figure 5 is a plot of the normalized systolic thickening during exercise in the presence of a coronary stenosis (relative to exercise without stenosis) versus normalized transmural blood flow during exercise-induced ischemia for transmural crystal pairs. Systolic thickening during exercise with stenosis is "normalized" to systolic thickening of the same segments in the absence of stenosis. Regional myocardial blood flow (mean transmural blood flow) during exercise-induced ischemia is normalized to anterior descending crystal pairs during exercise with stenosis (n=10). Line depicts the regression equation for the range of observations.

FIGURE 5. Regression plot of normalized thickening versus normalized blood flow during exercise-induced ischemia for transmural crystal pairs. Systolic thickening during exercise with stenosis is "normalized" to systolic thickening of the same segments in the absence of stenosis. Regional myocardial blood flow (mean transmural blood flow) during exercise-induced ischemia is normalized to anterior descending crystal pairs during exercise with stenosis (n=10). Line depicts the regression equation for the range of observations.

FIGURE 6. Regression plot of normalized subendocardial systolic wall thickening during exercise-induced ischemia versus normalized inner wall myocardial blood flow. Blood flow is mean of layers 3 and 4 (n= 10). Line depicts the regression equation for the range of observations.

FIGURE 7. Regression plot of normalized outer wall thickening during exercise-induced ischemia versus normalized outer wall blood flow (mean of layers 1 and 2, n=10). Line depicts the regression equation for the range of data.

FIGURE 7. Regression plot of normalized outer wall thickening during exercise-induced ischemia versus normalized outer wall blood flow (mean of layers 1 and 2, n=10). Line depicts the regression equation for the range of data.

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Discussion

The purpose of this study was to test the hypothesis that the preferential hypoperfusion of the subendocardium that occurs during exercise in the presence of a coronary stenosis results in more severe systolic dysfunction of subendocardial layers. Subendocardial systolic wall thickening was not selectively reduced relative to transmural or outer wall thickening. Furthermore, although reductions in transmural and subendocardial systolic thickening were directly correlated with the reductions in blood flow observed in these regions, only a very poor relation was found for the outer layers of the ventricular wall.

In the normal heart, transmural variations in myocardial blood flow exist that favor the subendocardium both at rest and during exercise. There is a transmural gradient in extravascular compressive forces that are maximal in the subendocardial regions and that would oppose blood flow to the inner layers of the ventricular wall. Subendocardial flow is maintained by an associated transmural gradient in
coronary vascular resistance; the lower subendocardial resistance results in a distribution of perfusion that favors the subendocardium. This gradient of perfusion is maintained during exercise and is associated with preservation of subendocardial wall thickening that remains greater than subepicardial wall thickening during exercise in the absence of a coronary stenosis.

In agreement with previous studies, exercise in the presence of a coronary artery stenosis resulted in moderate-to-severe hypoperfusion of subendocardial layers with only mild-to-moderate reductions in subepicardial flow in this study. The mechanisms responsible for this preferential hypoperfusion of subendocardial layers include an increase in the fraction of total flow that occurs during systole, intense vasodilation of coronary resistance vessels with loss of the transmural gradient of coronary vascular resistance that normally favors flow to the subendocardium, and a marked reduction in distal coronary artery perfusion pressure. Although changes in subendocardial wall thickening were found to correlate closely with these reductions in subendocardial blood flow in the current study, the poor correlation between changes in subepicardial wall thickening and subepicardial blood flow suggests that hypoperfusion is not the major determinant of subepicardial wall thickening under conditions of ischemia during exercise. Potential mechanisms that might account for reductions in subepicardial wall thickening out of proportion to the degree of hypoperfusion might include greater sensitivity of the outer wall to ischemia (due to differing myocardial oxygen consumption or greater regional wall stress) or physical constraints inhibiting fiber shortening in subepicardial layers during ischemia (tethering). Previous studies have demonstrated differences in the mechanical properties, blood flow, and metabolism of inner and outer layers that might have significant effects on the systolic function and vulnerability to ischemia of these regions.

After coronary artery occlusion, myocardial infarction occurs earliest and is most extensive in the subendocardial region, even when hypoperfusion is evenly distributed across the ventricular wall. Further, regional myocardial oxygen consumption has been reported to be higher and oxygen tension lower in subendocardial layers than subepicardial layers, suggesting that the subendocardium is most vulnerable to ischemia and that subendocardial function might be selectively reduced during coronary flow reductions. Gallagher et al. however, noted reductions of up to 75% in transmural wall thickening in the presence of hypoperfusion that only affected the subendocardial layers. This raised the possibility that subepicardial dysfunction was occurring in the absence of reductions in subepicardial flow.

Mechanical differences exist between subepicardial and subendocardial layers that have relevance to the measurement of systolic function in these layers. Streeter et al. demonstrated that fibers are oriented circumferentially in the midportion of the ventricular wall, whereas subepicardial fibers are more longitudinally oriented. The possibility of transmural variations in wall stress has also been raised; however, not all models predict transmural variations in wall stress. Systolic performance, as estimated by segment length changes or wall thickness changes, appears to be greater in inner layers than outer layers of the ventricular wall. This greater wall thickening of inner wall layers may be accounted for by conservation of mass throughout the cardiac cycle, however, and does not necessarily suggest enhanced contractility of inner wall layers.

The impact of differences in fiber orientation between inner and outer layers on the measurement of regional function has been explored in detail by several investigators. Although segment shortening has generally been measured with crystals placed in the midwall and oriented circumferentially, Gallagher et al. measured subepicardial segment shortening in a longitudinal orientation parallel to the direction of subepicardial fibers. They noted that posterior wall subepicardial segment shortening measured in the circumferential orientation fell substantially with little or no change in subepicardial myocardial blood flow during coronary artery stenosis, whereas epicardial segment shortening parallel to subepicardial fibers was much better preserved. Weintraub et al. measured segment shortening parallel to subepicardial fiber orientation in the anterior wall and still found that the changes in epicardial segment shortening correlated poorly with changes in subepicardial blood flow during coronary stenosis. Gallagher et al. used transmural wall thickening as a means to integrate systolic function across the entire ventricular wall and compared this with changes in segment shortening parallel to epicardial fibers in both the anterior and posterior areas of the left ventricle. They found that subepicardial fiber shortening fell more rapidly in the anterior wall of the ventricle than in the posterior wall, suggesting significant regional heterogeneity in the response of epicardial fibers to transmural variations in myocardial perfusion.

One explanation for these disproportionate reductions in subepicardial systolic performance has been the concept of "tethering." It is possible that noncontracting subendocardial layers would physically constrain overlying subepicardial fibers from shortening appropriately. This might explain a parallel fall in subepicardial and subendocardial systolic performance when only subendocardial blood flow is reduced. This would not, however, explain the finding of Hattori et al. that subepicardial fiber shortening in the posterior wall actually decreased more rapidly than subendocardial fiber shortening during progressive coronary artery stenosis. Furthermore, Heikkila et al. found that epicardial
freezing injury had little effect on transmural wall thickening despite severely reduced subepicardial shortening (as assessed by motion of metal markers placed within the ventricular wall). This latter finding suggests that an equivalent level of tethering of subendocardial fibers to subepicardial fibers may not occur. It is possible that wall stress to subepicardial layers may increase during regional myocardial ischemia, perhaps as a result of reduced wall thickness, increased radius of curvature, or failure of subendocardial fibers to bear a proportionate fraction of the systolic load. This may combine with tethering to restrict subepicardial fiber shortening.

To circumvent the potentially confounding influences of crystal alignment and fiber orientation that may occur when segment shortening is used to evaluate systolic function, Gallagher et al.\(^3\) used adjacent pairs of ultrasonic microcrystals to measure wall thickness across the entire ventricular wall and the outer half of the ventricular wall. Transmural and inner wall thickening, but not outer wall thickening, were found to correlate highly with reductions of myocardial blood flow during coronary artery stenosis at rest with this model. Using a single epicardial Doppler crystal to estimate transmural, inner, and outer wall thickening, Bolli et al.\(^28\) suggested a disparity between endocardial and epicardial function during recovery from 15-minute occlusions, with subendocardial wall thickening recovering at a somewhat faster rate than subepicardial wall thickening. Again, these findings would not have been predicted if tethering of subepicardial fibers to subendocardial fibers were the only mechanism underlying reductions in systolic thickening of the outer layers because both layers would be expected to undergo parallel changes in function. Regional systolic performance of the outer layers of the ventricular wall, therefore, appears to be a complex process in which not only reductions in myocardial blood flow but also regional variations in geometry or wall stress, tethering influences, and possible transmural variations in wall stress may contribute to the observed reductions in fiber shortening. The present study extends these findings to exercise in the presence of a coronary artery stenosis. During acute coronary artery occlusion, prominent pH and osmotic changes can occur, and the potential exists for the local accumulation of metabolites that could diffuse from subendocardium to subepicardium and alter subepicardial performance. During exercise in the presence of a coronary stenosis, residual perfusion attenuates local accumulation of metabolites and pH or osmotic changes. Thus, the disproportionate impairment of subepicardial performance in this study appears to be due to mechanical forces.

The possibility must be considered that the disparity between systolic performance and myocardial blood flow during ischemia might be an artifact of the methodology used in these studies. The relative proportion of wall thickening in the inner and outer layers has been found to be similar in studies with several different methodologies to measure inner and outer wall thickening (ultrasonic microcrystals, epicardial Doppler technique, and M-mode echocardiography with an intramyocardial echo target\(^7,4,32,35\)). Furthermore, the parallel increases in systolic performance of the outer and inner wall layers during exercise in the absence of coronary stenosis in this study and in the previous study of Gallagher et al.\(^3\) suggest that selective damage to the outer wall layers as a result of crystal implantation would be unlikely. Therefore, it is doubtful that methodological artifacts are responsible for the findings of severely reduced subepicardial systolic performance during exercise-induced ischemia.

In conclusion, there is a striking dissociation between reductions in myocardial blood flow and reductions in systolic thickening of the outer layers of the left ventricular wall during exercise in the presence of a coronary stenosis. The net effect of this is to cause a more prominent reduction in regional systolic performance than might be anticipated on the basis of the relatively modest perfusion abnormalities alone.

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