Experimental Studies

Coronary Blood Flow Autoregulation and Flow Heterogeneity in the Stunned Heart

Carla B. SHNIER, M.D., Brian A. CASON, M.D., Anne F. HORTON, M.S., and Robert F. HICKEY, M.D.

SUMMARY

We used an anesthetized swine model of regionally "stunned" myocardium to determine the effect of stunning on coronary autoregulation and blood flow heterogeneity. In 18 domestic swine, stunning was accomplished by reducing blood flow to the left anterior descending coronary artery (LAD) by approximately 75% of baseline for 15 min and restoring it to normal for 1 hour. We quantified coronary autoregulation using both the slope of coronary pressure-flow curves and an autoregulation index. We quantified blood flow heterogeneity using radioactive microspheres to determine the variability in flow (dispersion index) among forty 200 mg segments of myocardium from the center of the stunned, LAD-perfused left ventricle. Before and after stunning, we measured autoregulation, myocardial blood flow and flow heterogeneity, as well as hemodynamic indices of myocardial oxygen demand. Fifteen min of ischemia and 1 hour of reperfusion produced both a 46% reduction in mechanical function, and a 7% drop in systemic arterial pressure, but no change in heart rate or rate pressure product. Myocardial oxygen consumption was 15% reduced and myocardial blood flow 16% reduced in the stunned myocardium when measured at one hour of reperfusion. Fifteen min after reperfusion, the slope of the coronary pressure flow plots and the coronary venous oxygenation were increased whereas the autoregulation index decreased. These findings all indicate reduced autoregulation during early reperfusion. However, after one hour of reperfusion, the slope of the coronary pressure-flow relation (0.41 ± 0.19 vs. 0.48 ± 0.26 ml·min⁻¹·mmHg⁻¹) and the autoregulation index (0.43 ± 0.16 vs. 0.30 ± 0.32) were unchanged from control measurements (p > 0.05). Blood flow heterogeneity remained normal in the stunned myocardium. These findings challenge the hypothesis that the mechanical dysfunction of the stunned myocardium is due to suboptimal perfusion resulting from poor coronary autoregulation or maldistribution of blood flow. (Jpn Heart J 35: 645–660, 1994)

Key words: Reperfused myocardium Coronary blood flow control Swine

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MYOCARDIAL stunning is a reversible contractile dysfunction which persists after reperfusion of the ischemic myocardium, despite the absence of necrosis.\textsuperscript{1) The etiology of the “stunned myocardium” is unknown, although several hypotheses exist. One hypothesis is that in the stunned myocardium, local control of blood flow is impaired, leading to suboptimal perfusion or persistent ischemia. This hypothesis is supported by the finding of increased microscopic heterogeneity of venous oxygen saturations in the stunned myocardium, with an excess of veins having low saturations.\textsuperscript{2) The blood flow regulation hypothesis is also supported by work showing an increased resting resistance,\textsuperscript{3)} and an increased propensity to vasoconstriction\textsuperscript{4-6) in coronary arteries after they have been subject to ischemia and reperfusion. If confirmed, this hypothesis could explain the improvement in contractile function seen when coronary flow to the stunned myocardium is artificially increased.\textsuperscript{7)}

Despite the data suggesting that blood flow control may be abnormal in the stunned myocardium, few studies have described the effects of reversible ischemia on coronary autoregulation or on the distribution of blood flow. An important form of coronary flow control, coronary autoregulation is the intrinsic ability of the heart to maintain near constant blood flow following changes in perfusion pressure. Impaired autoregulation could lead to suboptimal perfusion and myocardial dysfunction. To test the hypothesis that coronary autoregulation is impaired in the stunned myocardium, we measured autoregulation before stunning, and after both 15 min and 60 min of reperfusion in a swine model of ischemia/reperfusion. We also studied blood flow heterogeneity in the stunned myocardium because maldistribution of blood, even with normal overall flow, could cause patchy myocardial ischemia and contractile dysfunction. To determine if stunning increases the macroscopic heterogeneity of blood flow, we compared the blood flow distribution in 200 mg segments of myocardium before and 60 min after ischemia.

**Materials and Methods**

**Anesthesia:** This experimental protocol was approved by our Animal Welfare Committee and followed the guidelines for animal use by the American Physiological Society. Twenty eight domestic swine weighing 40–50 kg were premedicated with ketamine hydrochloride (1.0 g i.m.) and then anesthetized by mask with isoflurane (1.0–2.5%) in oxygen. Under general anesthesia, a tracheostomy was performed and ventilation was controlled to maintain normal pH and PaCO\textsubscript{2}. To minimize ventricular arrhythmias, lidocaine hydrochloride was given as a bolus (3 mg•kg\textsuperscript{-1} iv.) followed by a constant intravenous infusion of 2 mg•min\textsuperscript{-1}. After completion of the surgical preparation, isoflurane was discon-
continued and anesthesia was maintained with sodium pentobarbital ($15 \, \text{mg} \cdot \text{kg}^{-1}$ loading dose followed by a $40 \, \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ infusion) and high-dose fentanyl ($50 \, \text{mcg} \cdot \text{kg}^{-1}$ bolus followed by a $0.5 \, \text{mcg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ infusion). Central body temperature was maintained at $36.5-38.5^\circ\text{C}$ by warmed intravenous fluids and surface warming. Arterial blood gases were measured using a Radiometer (Copenhagen, Denmark) ABL-II blood gas analyzer. Hemoglobin and oxyhemoglobin saturation were measured using a Radiometer hemoximeter OSM3 with internal correction made for swine hemoglobin absorption characteristics.

**Surgical preparation and instrumentation:** The heart was exposed through a median sternotomy and suspended in a pericardial cradle. A calibrated micromanometer (Millar Instruments, Houston) was inserted through the left atrium into the left ventricle for measurement of the left ventricular pressure and its first derivative with respect to time ($dP/dt$). Epicardial pacing wires were sutured to the right atrium and electrical pacing was begun at a rate 20% higher than the intrinsic heart rate. Pacing then continued at the same rate throughout the experiment. Both carotid arteries were cannulated with 14-gauge catheters to supply blood to an extracorporeal circuit, and to provide periodic arterial blood samples and continuous systemic pressure monitoring. All transduced signals were recorded on a Grass Instruments polygraph (Quincy, MA). Both internal jugular veins were cannulated to provide intravenous access for drug and saline infusions. Finally, the anterior interventricular coronary vein was cannulated with a 20-gauge catheter to allow intermittent sampling of the blood draining the myocardial risk zone.

**LAD cannulation and perfusion:** Immediately before cannulation of the left anterior descending artery, the pig was anticoagulated with a 10,000 U intravenous bolus of heparin followed by a 5000 U$\cdot$h$^{-1}$ continuous infusion. The left anterior descending artery was dissected free of surrounding tissue for a 2 cm length near its origin at the base of the heart. The coronary artery was then cannulated with a 3 mm O.D. plastic cannula and perfused with oxygenated blood pumped from the carotid artery (Masterflex digital roller pump, Cole-Parmer, Chicago, IL). Before perfusing the heart, blood was passed through a 40 micron filter (Pall Biomedical, Glen Cove, NY) to trap air and particulate debris. Systolic shortening was measured to ensure adequacy of coronary blood flow. If systolic shortening did not return to the pre-cannulation level within 3 min, cannulation was deemed unsuccessful and the pig was excluded from the study. The LAD pressure was measured at the tip of the perfusion cannula through a 25-gauge catheter inside the cannula. Flow was measured by an in-line electromagnetic flowmeter (Micron Medical, Los Angeles, CA), calibrated by timed blood collection in a graduated cylinder. Except during intentional ischemia, LAD flow was determined by setting the pump output so that mean coronary
artery pressure matched mean systemic pressure.

**Sonomicrometry:** Myocardial contractile function was quantified in both the left anterior descending and the circumflex perfusion zones using segmental systolic shortening. Systolic shortening was calculated as:

\[
\text{Systolic Shortening} \% = \frac{\text{end-diastolic length} - \text{end-systolic length}}{\text{end-diastolic length}}
\]

Diastolic and systolic segment lengths were measured using 2 mm lensed ultrasonic crystals (Dimension 3, La Jolla, CA), which were embedded in the subendocardial muscle through a small epicardial incision. The crystals were positioned approximately 11 mm apart, facing each other and parallel to the short axis of the heart. Crystal position and movement was displayed on an oscilloscope and the physiologic recorder. Crystal position was confirmed at autopsy. Systolic shortening readings were averaged over 5 heart beats. End-diastole was defined as the onset of positive left ventricular dP/dt; end-systole was defined as the time of peak negative dP/dt.

**Coronary pressure-flow plots and autoregulation measurements:** To determine the coronary pressure-flow relationship, 4–6 paired values of coronary blood flow and pressure were obtained over a pressure range of 60–130 mmHg. The initial values in all relationships were the intracoronary pressure, which equaled systemic pressure and the corresponding flow. Subsequent pressure-flow coordinates were established by manually increasing the pump output by 2–4 ml/min-1 and recording the resulting pressure after 30 s of pressure-flow stability. Heart rate and systemic blood pressure were kept constant for all points in each pressure-flow relationship.

The degree of coronary autoregulation was assayed in two ways. First, the slope of the pressure-flow relationship was established by least-squares linear regression. Second, the degree of autoregulation was quantified using the autoregulation index of Norris (ArI). This index is a ratio of the measured change in conductance for a given change in perfusion pressure to the change in conductance (F/P) which would have occurred if autoregulation were perfect and flow did not change. It is calculated as follows:

\[
\text{ArI} = \left[ \frac{F}{P} - \frac{F_i}{P_i} \right] \left[ \frac{F}{P} - \frac{F_i}{P_i} \right]^{-1}
\]

where Fi is the initial flow at starting pressure, Pi, and F is the coronary flow at the new steady-state increased pressure, P. An ArI of 1.0 indicates perfect coronary autoregulation, where measured flow is constant despite changes in perfusion pressure. By contrast an ArI of 0 indicates no autoregulation, since a given change in coronary pressure causes no change in vascular conductance and re-
results in a proportional change in flow. ArI was calculated for each autoregulation plot using intracoronary pressures between 80–120 mmHg. The linear equation for each pressure-flow regression was solved for flow at coronary pressures of 80 (Pi) and 120 mmHg (P), and these resulting flow values were used in the ArI formula as Fi and F, respectively. This method of calculating ArI was chosen in the belief that the regression line determined by multiple points provided the most accurate information for calculation. The fit of coronary pressure-flow values was highly correlated (mean r² control = 0.96 ± 0.04; mean r² stunned = 0.96 ± 0.03).

**Myocardial blood flow:** Regional myocardial blood flow was measured during the control period, in the middle of the ischemic period and after 60 min of reperfusion using radioactive microspheres 15 microns in diameter. Approximately 1 million microspheres, labeled with one of 54Mn, 153Gd, or 65Zn radionuclides, were agitated for 4–5 min in dextran solution and released into the extracorporeal circuit proximal to the roller pump perfusing the LAD artery. Each 0.5 cc injection of microsphere-dextran suspension was flushed slowly with 5 cc of autologous blood to prevent changes in coronary artery pressure and agitated mechanically in the circuit to ensure an even distribution of the spheres in blood. Simultaneously, a reference blood sample was withdrawn over three min from the distal extracorporeal perfusion circuit at a rate of 3 ml min⁻¹. After the experiment, the stunned myocardium perfused by the LAD artery was delineated by a dye infusion technique: blood stained with Evans blue dye was infused into the cannulated LAD artery at normal aortic pressures, while the remainder of the heart was perfused at the same pressures with unstained blood from the aortic root. The blue area, representing myocardium perfused by the LAD artery, was excised and weighed.

**Blood flow dispersion using radioactive microspheres:** A 2.5 × 2.0 cm area of stunned myocardium around the LAD crystal site was divided into 20 transmural segments which were then subdivided into endocardial and epicardial layers. These 40 myocardial samples were weighed (mean ± SD = 194 ± 21 mg) and assayed for radioactivity using a Packard (Meriden, CT) gamma counter. After adjusting counts for background radiation and isotope overlap, the blood-flow to each myocardial segment was calculated using the equation:

\[
\text{regional, myocardial blood flow} = \frac{\text{Counts}_i \times \text{Flow}_{\text{ref}}}{\text{Counts}_{\text{ref}}}
\]

where Countsᵢ = counts in the myocardial sample, Countsᵢᵠ = counts in the reference blood sample, and Flowᵢᵠ = reference sample flow. Reference flow was calculated by dividing the net weight of the reference sample by the specific gravity of blood, 1.05. Heterogeneity of blood flow within the LAD zone was quantified
using the dispersion index which is defined as:

\[
\text{dispersion} = \frac{SD_{bf} \times 100}{X_{bf}}
\]

where \(SD_{bf}\) = the standard deviation of the blood flow measurements within the zone of interest and \(X_{bf}\) = mean of those blood flow measurements.

**Experimental protocol:** After LAD cannulation and 30 min stabilization, the coronary pressure-flow relationship was measured as described above. Hemodynamic indices of myocardial oxygen demand, such as heart rate, systemic blood pressure and filling pressures were measured at baseline. Blood flow within the LAD zone was determined by microsphere injection. Arterial and coronary venous hemoglobin concentrations, oxygen saturations and blood gases were measured to calculate myocardial oxygen consumption (MVO₂). MVO₂ was calculated for the LAD-perfused zone using the Fick Principle, as the product of the LAD blood flow (flowmeter) and the difference between the coronary arterial and coronary venous oxygen contents.

After these baseline measurements were completed, the myocardium was stunned by making the LAD zone severely ischemic for 15 min. This was accomplished by lowering the pump flow by approximately 75% of baseline to the point where systolic shortening in the LAD zone was near zero but still present (i.e., no net bulging). Radioactive microspheres were injected at the midpoint of the ischemic period. After 15 min of ischemia, reperfusion was carried out in a graded fashion to prevent malignant arrhythmias. Flow was first returned to the baseline level and held there for 5 min and then it was increased to match LAD and systemic mean pressures. Coronary pressure-flow relations, hemodynamic indices of myocardial oxygen demand, systolic shortening and MVO₂ were measured after 60 min of reperfusion. In addition, after 60 min of reperfusion, the blood flow to the LAD-zone was measured with radioactive microspheres. Coronary pressure flow relationships were also measured at 15 min of reperfusion in the last 9 animals studied.

**Data analysis:** ArIs, slopes of the pressure-flow relations, hemodynamic variables, oxygen consumption, systolic shortening and LAD blood flow for all the pigs at baseline, at 15 and 60 min of reperfusion were expressed as mean ± SD and were compared using repeated measures analysis of variance and paired comparisons were made with the Newman-Keuls test. Probability values of <0.05 were considered significant. ArIs and slopes were also compared using the non-parametric Wilcoxon signed rank test because the normality of data distributions was in question.
RESULTS

Quality of the preparation: Twenty-eight pigs were studied initially. Eight were excluded from analysis because of unsuccessful cannulation; 2 others were excluded because they failed to show autoregulation of coronary blood flow at baseline. Results from the remaining 18 pigs are presented. We noted that the acid-base status, ventilation, oxygenation (with an FIO₂ of 1.0) and hemoglobin concentration were normal and stayed constant throughout the experiment. (pH = 7.42 ± 0.06, PO₂ = 462.2 ± 59.0 mmHg, PCO₂ = 40.3 ± 8.3 mmHg, Hb = 10.9 ± 0.9g/100 ml⁻¹).

Hemodynamics (Table I): Heart rate was controlled by atrial pacing at a rate approximately 20% higher than the intrinsic rate and did not vary throughout the experiment. Likewise, neither the rate pressure product (14,700 ± 2200 mmHg·beats·min⁻¹) nor LVEDP (5 ± 3 mmHg) changed from control values in the stunned myocardium. However, the mean systemic arterial pressure decreased by 7% of baseline in the stunned myocardium after 60 min of reperfusion. This decrease is reflected in an equal drop in coronary pressure because, by experimental design, coronary pressure was matched to systemic arterial pressure except during ischemia.

Regional myocardial contractile function (Table II): The regional myocardial contractile function, expressed as an absolute percentage of systolic shortening was 20.9 ± 4.0% in the LAD-perfused zone at baseline and 1.2 ± 1.6% during ischemia. Function improved rapidly at the onset of reperfusion and then remained fairly stable at approximately half of baseline function (11.3 ± 4.3% absolute) after 60 min of reperfusion. By contrast, the systolic shortening in the circumflex (control) area increased when the adjacent myocardium was ischemic, and, although it tended to normalize during reperfusion, it remained greater than baseline after 60 min of reperfusion.

<table>
<thead>
<tr>
<th>Table I. Hemodynamics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 18)</td>
</tr>
<tr>
<td>Heart rate (beats·min⁻¹)</td>
</tr>
<tr>
<td>Mean systemic arterial pressure (mmHg)</td>
</tr>
<tr>
<td>Mean coronary artery pressure (mmHg)</td>
</tr>
<tr>
<td>RPP × 10⁻⁹ (mmHg·beats·min⁻¹)</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
</tr>
</tbody>
</table>

Values are mean ± SD. RPP = rate pressure product, (heart rate × systemic systolic blood pressure); *p < 0.05 vs control by paired t test. The decrease in coronary artery pressure in the stunned myocardium followed the decrease in systemic arterial pressure because coronary artery pressure was set to equal systemic pressure by experimental design.
Table II. Systolic Shortening and Coronary Blood Flow

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 18)</th>
<th>Ischemia (n = 18)</th>
<th>Stunned (15' reperf. n = 9)</th>
<th>Stunned (60' reperf. n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%SS Cx</td>
<td>12.8±3.6</td>
<td>17.0±3.3*</td>
<td>15.1±3.0</td>
<td>14.5±3.6*</td>
</tr>
<tr>
<td>%SS LAD</td>
<td>20.9±4.0</td>
<td>1.2±1.6*</td>
<td>10.1±3.5*</td>
<td>11.3±4.3*</td>
</tr>
<tr>
<td>LAD blood flow (ml/100 g^-1/min^-1)</td>
<td>77.2±25.5</td>
<td>26.3±28.1*</td>
<td>117.6±73.8*</td>
<td>70.3±23.3</td>
</tr>
</tbody>
</table>

Values are mean ± SD. %SS Cx = percentage systolic shortening in the myocardium perfused by the circumflex artery; %SS LAD = percentage systolic shortening in the myocardium perfused by the left anterior descending artery; *p < 0.05 vs. control by ANOVA and Newman-Keuls test.

Table III. Autoregulation

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 18)</th>
<th>Stunned (15' reperf. n = 9)</th>
<th>Stunned (60' reperf. n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoregulation Index</td>
<td>0.43±0.16</td>
<td>0.10±0.33*</td>
<td>0.30±0.32</td>
</tr>
<tr>
<td>Slope (ml/100 g^-1/min^-1/mmHg^-1)</td>
<td>0.41±0.19</td>
<td>0.85±0.51*</td>
<td>0.48±0.26</td>
</tr>
<tr>
<td>MVO₂ (ml/100 g^-1/min^-1)</td>
<td>8.43±2.35</td>
<td>6.79±1.72</td>
<td>7.07±1.94</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Slope refers to the slope of the pressure-flow regression line. LAD blood flow was measured by calibrated electromagnetic flowmeter; MVO₂ = myocardial oxygen consumption; *p < 0.05 vs. control and vs. 60' reperfusion by ANOVA and Neuman-Keuls test.

Figure 1. Coronary pressure-flow relationships before and after myocardial ‘stunning’ in a typical experiment. Autoregulation is impaired after 15 minutes of reperfusion. By 60 minutes reperfusion, the coronary pressure-flow relationship is similar to the control state.

Autoregulation (Table III): In measurements made 15 min after reperfusion, autoregulation was impaired in the stunned myocardium. In these measurements the ArI decreased from 0.43 ± 0.16 to 0.10 ± 0.33 and the slope of the pressure-flow plot increased from 0.41 ± 0.19 to 0.85 ± 0.51 ml/100 gm/min^-1/mmHg^-1.
Figure 2. Regional coronary venous oxyhemoglobin saturation

Table IV. Myocardial Blood Flow and Dispersion

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia</th>
<th>Stunned (60' reperfusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmural flow (mLg⁻¹min⁻¹)</td>
<td>0.93±0.38</td>
<td>0.21±0.10</td>
<td>0.79±0.34*</td>
</tr>
<tr>
<td>Epicardial flow (mLg⁻¹min⁻¹)</td>
<td>0.97±0.39</td>
<td>0.32±0.16</td>
<td>0.85±0.37*</td>
</tr>
<tr>
<td>Endocardial flow (mLg⁻¹min⁻¹)</td>
<td>0.89±0.38</td>
<td>0.11±0.07</td>
<td>0.72±0.31*</td>
</tr>
<tr>
<td>Endo/Epi flow Ratio</td>
<td>0.92±0.19</td>
<td>0.35±0.19</td>
<td>0.87±0.15</td>
</tr>
<tr>
<td>Total dispersion</td>
<td>21.5±2.2</td>
<td>79.8±64.2</td>
<td>22.0±65.5</td>
</tr>
<tr>
<td>Epicardial dispersion</td>
<td>19.3±8.4</td>
<td>41.7±59.2</td>
<td>16.8±7.1</td>
</tr>
<tr>
<td>Endocardial dispersion</td>
<td>17.7±8.0</td>
<td>62.5±64.0</td>
<td>20.6±7.8</td>
</tr>
</tbody>
</table>

All values in this table are the means ± SD of 18 pigs. Statistical comparisons were made between control and stunned period only. Numerical values for the ischemic period are shown to illustrate the degree of ischemia. *p < 0.05 vs. control by paired t test and by Wilcoxon signed rank test.

However, this impairment was temporary. After 60 min of reperfusion, neither the ArI nor the slope of the pressure-flow plots was different from control. Our control CBF appeared to have good autoregulation based on similar values of ArI and slope of pressure-flow plot obtained on awake dogs.9)

An example of coronary pressure-flow plots from a typical experiment (Figure 1) shows that the pressure-flow relationship at 60 min of reperfusion was similar to the control relationship. By contrast, the pressure-flow relationship at 15 min reperfusion showed impaired autoregulation, compared to the control state.

Coronary venous oxygen saturation (Figure 2): Regional coronary venous O₂ saturation was 30.6 ± 6.8% during the control, which is a level previously shown to be associated with good autoregulation.10) During the hyperemia which
followed ischemia, coronary venous saturation rose to $57.3 \pm 21.3\%$ then decreased gradually toward the control level ($p = 0.07$) as autoregulation was re-established during the 1 hour of reperfusion.

**Blood flow to the LAD-perfused left ventricle (Table IV):** The transmural blood flow to the LAD-perfused left ventricle, as measured with radiolabeled microspheres, was $0.93 \pm 0.38 \text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ during the control period. During ischemia, transmural flow dropped to $0.21 \pm 0.10 \text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. Predictably, ischemia was more severe in the endocardium than in the epicardium, which lowered the endocardial to epicardial flow ratio from $0.92 \pm 0.19$ to $0.35 \pm 0.19$ ($p < 0.05$). After 60 min of reperfusion, transmural and endocardial blood flows were slightly lower than control values ($p < 0.005$), but there was no significant change in blood flow dispersion compared to the control myocardium.

**DISCUSSION**

The major finding of this study is that coronary pressure-flow autoregulation is disrupted temporarily after 15 min of reperfusion, and that all the indices of autoregulation recover after one hour of reperfusion. Specifically, despite a persistent 46% reduction in contractile function, neither the autoregulation index (ArI) nor the slope of the pressure-flow relation changed from baseline. In addition, we found that myocardial stunning is not accompanied by increases in blood flow heterogeneity when blood flow is measured in 40 contiguous 200 mg segments of myocardium. Further, our study clearly indicates a significant reduction in function at a time that autoregulation and blood flow heterogeneity has returned to baseline. Thus our findings do not support the hypothesis that persistent disturbances of coronary blood flow contribute to the prolonged dysfunction of the stunned myocardium.

**Autoregulation:** In the control period, all pigs demonstrated autoregulation by both measures used in this study: the ArI and slope of the coronary pressure-flow relation. The slope of the pressure-flow relation in our anesthetized pigs was similar to that reported in awake chronically-instrumented dogs.$^{11}$ At 60 min of reperfusion, 15 of 18 pigs had positive ArIs indicating that, for the most part, some degree of autoregulation was present in the stunned myocardium. In a small minority of individuals (3/18), ArI was negative after 60 min of reperfusion, indicating some impairment of autoregulation in these individuals which did not reach statistical significance for the entire group.

However, blood flow control was indeed affected, albeit temporarily, by 15 min of ischemia. Fifteen min after reperfusion the coronary pressure slope had increased from $0.41 \pm 0.18$ to $0.85 \pm 0.51$ ($p < 0.02$). Similarly, ArI decreased from $0.38 \pm 0.18$ to $0.10 \pm 0.33$ ($p < 0.05$). This finding of a diminished
Autoregulation is supported by changes seen in coronary venous oxygenation. Fifteen min after the start of reperfusion, coronary venous oxygen saturation was very high (57%), reflecting high venous PO$_2$ (47 ± 16 mmHg), loss of autoregulation and blood flow in excess of myocardial demand. This finding of disrupted autoregulation in the presence of a high coronary venous oxygen saturation supports previous work showing that coronary autoregulation is correlated with physiologically low PO$_2$ in the coronary vein. Coronary venous oxygen saturation decreased during reperfusion as the coronary arteries regained their normal tone, so that saturation was not different from baseline after 60 min. After 60 min of reperfusion, the ArI, slope of the pressure-flow relationship, venous oxygen saturation and venous PO$_2$ were not statistically different from baseline. However, all four of these parameters showed a slight trend toward less autoregulation than before stunning. This observation supports the notion that ischemia disrupts autoregulation transiently in this model. Unlike contractile function, which is still severely depressed after 1 hour of reperfusion, autoregulation recovers almost completely in this model of the stunned myocardium.

The motivation for this study came from previous studies in isolated and in situ coronary arteries which showed that ischemia and reperfusion increased coronary reactivity to vasoconstrictor agents, decreased responsiveness to vasodilator stimuli, and/or destroyed coronary blood flow autoregulation. Based on these previous studies, we expected autoregulation to be abnormal in the stunned myocardium. The discrepancies between our results and those cited above merit some explanation.

Bolli et al showed that after sublethal ischemia and reperfusion in dogs, the resting blood flow and the maximal vasodilation of the post-ischemic artery were reduced, leading to the conclusion that reversible ischemia caused both myocardial and microvascular “stunning.” Pressure-flow autoregulation was not tested. These results differ from ours for several reasons. First, our models of stunned myocardium were different. Bolli subjected dog hearts to 90% regional flow reduction by total coronary occlusion, followed by abrupt reperfusion; we subjected pig hearts to a 75% regional flow reduction and two-stage reperfusion, such that reflow was restricted to the control level for 5 min before hyperemic reperfusion was permitted. The milder insult in the pig model is reflected in less contractile dysfunction. At 1 hour of reperfusion, the stunned zone in our study was moderately hypokinetic, while after 4 hours of reperfusion, the stunned myocardium in Bolli’s dog study was genuinely dyskinetic in 25 to 75% of the cases. The greater flow deprivation and more traumatic reperfusion may account for the microvascular “stunning” in this dog model.

Second, we measured the effect of ischemia and reperfusion on the coro-
nary pressure-flow autoregulation; Bolli et al and other investigators$^{5,14}$ tested its
effect on vasodilatory reserve. Normal autoregulation implies that the coronary
vessels can maintain constant blood flow over physiologic (70–130 mmHg) pres-
sure changes and match blood flow to myocardial demand. By contrast, normal
vasodilatory reserve implies that all coronary vessels can maximally vasodilate. A
reduction in vasodilatory reserve may indicate an anatomic disruption of the
coronary bed or a reduction of the response to a maximal vasodilatory stimulus.
Thus, while both measures are indicators of coronary function, vasodilatory re-
serve and autoregulatory ability are different entities and can not be expected to
respond identically to temporary ischemia.

There is some support in the literature for our findings. A recent study by
Ito et al$^{15}$ failed to show a change in coronary autoregulation after 15 min of
myocardial ischemia and 30 min of reperfusion in dogs. However, given the
number of animals tested and the variability of their data, statistical power analy-
sis indicates that the probability of detecting a true difference of 50% or more in
ArI was <0.6.$^{16}$ By contrast, our study illustrated the resiliency of the
autoregulatory system in twice the number of experimental subjects, which were
more uniformly and more severely stunned than the subjects of Ito et al.$^{15}$ We
can conclude, with 90% certainty, that the autoregulation index is unchanged by
46.5% or more in the stunned myocardium. Another difference between the
studies is that Ito et al$^{15}$ measured flow over a greater pressure range (20–160
mmHg) and at the lowest coronary pressures the myocardium developed
ischemic dyskinesis.

Other studies which attest to the resiliency of the coronary vasculature after
ischemia include one in swine showing that 20 min of coronary artery occlusion
and 45 min of reperfusion did not impair maximal coronary conductance.$^{17}$
More recently, Laxson et al$^{14}$ found that stunning dog hearts by repeated coro-
nary artery occlusions did not impair the normal relationship between coronary
blood flow and myocardial oxygen utilization, and did not change the transmural
distribution of flow.

**Blood flow heterogeneity:** We measured the blood flow distribution in small
segments (201 ± 4.1 mg mean weight) of myocardium to investigate the possibil-
ity that small regions of insufficient flow contribute to the mechanical dysfunction
of the stunned myocardium. We found that the blood flow heterogeneity in-
creased during ischemia, but that it returned to normal, both transmurally and in
the endocardium, after 60 min of reperfusion. The blood flow heterogeneity did
not change in the epicardium where ischemia was the least severe. Our baseline
dispersion index agrees closely with that documented in other preparations.$^{18,19}$
Our findings also support those of Austin et al$^{19}$ who showed that heterogeneity
of blood flow increased with pharmacologically-mediated or physiologically-in-
duced vasodilatation. In our study, ischemia caused vasodilation and an increase in heterogeneity, but within 60 min of reperfusion, vascular tone was regained and the dispersion index normalized. The normalization of the endo/epi flow ratios and the return of autoregulation corroborate the finding of normal blood flow distribution in the stunned myocardium and suggest that the persistent decrease in systolic shortening is not due to inadequate blood supply to small areas of the myocardium.

Injection of microspheres has, by itself, the potential to affect autoregulation and blood flow heterogeneity. We injected approximately $7 \times 10^5$ microspheres for each of the three measurements of regional CBF. We sought to inject a sufficient number of spheres per tissue piece to permit accurate calculation of regional blood flow but not to induce embolization that would change baseline CBF or autoregulation. The number of microspheres per 200 mg tissue piece varied from 910 to 4100, which was approximately one-half of the number injected by Baer et al. These investigators found no changes in coronary-pressure flow relationships or regional myocardial blood flow from this number of spheres. Moreover, our indices of autoregulation and heterogeneity returned to control values, indicating that neither stunning nor microsphere injections affected these variables.

**MVO$_2$:** We found that MVO$_2$ in the post-ischemic myocardium was 85% of control. This was higher than expected, given the 46% reduction in systolic shortening and the 7% reduction in systemic blood pressure because, at least in the normal heart, myocardial oxygen consumption parallels mechanical work. One explanation of why the MVO$_2$ is so minimally depressed is that regional dyskinesis in the non-ischemic myocardium is very costly in terms of oxygen consumption. The oxygen consumption of the bulging segment is 70% that of the normally contracting myocardium and three-fold higher than myocardium that is fully arrested. The early systolic dyskinesis in the stunned myocardium could account for high MVO$_2$ in the face of minimal net contraction.

Previous reports have described oxygen consumption in the stunned myocardium as reduced, increased, or unchanged. The study by Smith et al. reported findings similar to ours in that stunned myocardium did less work, had lower blood flow and lower oxygen consumption than control myocardium. Interestingly, during intracoronary infusion of isoproterenol, the stunned hearts in Smith et al's study increased systolic function, blood flow and oxygen consumption to near normal without any evidence of ischemia.

The well-documented response of the stunned myocardium to exogenous catecholamines provides a possible explanation for the apparent unpredictability of myocardial oxygen consumption in the post-ischemic state. The method of stunning, the type and depth of anesthesia, and the elapsed time after the initia-
tion of reperfusion may influence catecholamine levels, and hence, the systolic function and MVO₂ after a given ischemic insult. For example, both of the above-cited studies documenting unchanged\(^{14}\) or increased\(^{2}\) MVO₂ in the stunned myocardium used repeated coronary occlusions to stun the heart, while the studies in which stunning lowered the MVO₂ used single coronary occlusions. Laxson’s study,\(^{14}\) which found no decrease in MVO₂ despite marked reduction in systolic function, was done in conscious dogs which likely had greater systolic shortening and higher MVO₂ than they would have had if under anesthesia. The results of Stahl’s study,\(^{2}\) which showed MVO₂ to be higher in the stunned myocardium, are difficult to interpret given that the hemoglobin concentration of blood perfusing the myocardium was only measured at the end of the experiment. If hemoglobin concentrations changed over the course of the experiment, their control MVO₂ calculation would be in error.

The accuracy of our regional MVO₂ determination depends upon the validity of anterior interventricular venous (AIV) blood sampling as an indicator of the oxygen extraction of myocardium supplied by the LAD artery. It was previously shown that in anesthetized swine whose LAD coronary artery was cannulated and perfused, more than 90% of blood in the AIV is supplied by the LAD artery during normal flow.\(^{24}\) Thus, AIV sampling is a good indicator of the metabolic status of the LAD-perfused area in the absence of ischemia.

**Limitations and advantages of our model:** Though myocardial stunning has most often been studied *in situ* in the dog heart, we were able to produce a similar model of stunning in the swine. Swine, unlike dogs, have minimal innate coronary collateral circulation\(^{25}\) and so flow to the test myocardium during ischemia can be more precisely controlled during regional perfusion. To produce stunning, we lowered pump flow to 25% of baseline, which mimics average collateral flow after complete coronary occlusion in the common dog model of stunning. The advantage in using pigs is that a given decrement in coronary blood flow will result in a more predictable degree of ischemia,\(^{11}\) and therefore a more uniform model of stunning in which to study coronary autoregulation. The non-uniformity of stunned myocardium in dogs subjected to 15 min of total coronary occlusion has been addressed by Bolli et al.\(^{26}\) They found that the large variability in severity and duration of post-ischemic myocardial dysfunction was determined primarily by the variability in collateral blood flow to the test myocardium during reversible ischemia.

Potential disadvantages of our experimental preparation are that anesthesia, coronary cannulation and extracorporeal circulation may affect autoregulation and the distribution of coronary blood flow. Caution is therefore required in the extrapolation of our findings to the intact or anesthetized animal. However, it was reassuring to find that autoregulation was present at baseline and that flow
heterogeneity was comparable to that measured in both conscious animals and in non-cannulated hearts of anesthetized animals.\textsuperscript{18,19}

In summary, we found that myocardial stunning in this model does not disrupt coronary autoregulation or macroscopic blood flow heterogeneity. Despite a marked (46.5\%) reduction in systolic shortening, two indices of coronary autoregulation, the ArI and the slope of the coronary pressure-flow relation, were unchanged from baseline after 1 hour of reperfusion. Blood flow heterogeneity, as measured by the dispersion of blood flow in 200 mg segments of myocardium, was also normal in the stunned heart. These findings indicate that neither disrupted global autoregulation nor blood flow heterogeneity contribute to contractile dysfunction after one hour of reperfusion.

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