Ineffective Perfusion-Contraction Matching in Conscious, Chronically Instrumented Pigs With an Extended Period of Coronary Stenosis

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Abstract—Several models purported to represent hibernating myocardium involve a coronary stenosis (CS) to reduce blood flow (BF) and function without eliciting necrosis in anesthetized pigs. The goal of the present study was to determine whether sustained moderate reduction in coronary BF in conscious pigs induced hibernating myocardium, ie, perfusion-contraction matching with no necrosis. These experiments were conducted in conscious pigs chronically instrumented with a coronary artery BF probe and hydraulic occluder, left ventricular (LV) pressure gauge, and wall thickening (WT) crystals in the potentially ischemic and nonischemic zones. The hydraulic occluder was inflated to induce a stable 41±4% reduction in BF for 24 hours. Ischemic zone systolic WT fell initially with CS and then continued to decline during the period of CS even though blood flow did not change further, suggesting the induction of myocardial stunning. At 2 days after release of CS, WT was still depressed by 48±15%. Assessment of necrosis by histology or triphenyltetrazolium chloride showed 40±5% multifocal patchy necrosis interspersed with normal tissue involving the inner one third to one half of the ventricular wall. Regional myocardial BF (radioactive microsphere technique) was assessed by dividing the entire LV into an average of 48±59 pieces and examining the spatial distribution of BF within the area at risk (AAR). BF in the samples in the area of patchy necrosis was reduced (−66±4% from a baseline of 1.55±0.27 mL min−1 g−1), whereas BF was maintained in samples in the AAR without necrosis (−2±7% from a baseline of 1.25±0.22 mL min−1 g−1). These findings indicate that when hypoperfusion induced by CS in conscious pigs is sustained, the result is necrosis rather than hibernating myocardium. The remainder of the AAR, which lacked necrosis, might have been mistaken for hibernating myocardium had only histology been evaluated and BF not been measured and found to be at normal levels. (Circ Res. 1998;82:1199-1205.)

Key Words: hibernating myocardium ■ coronary blood flow ■ coronary artery disease ■ myocardial ischemia ■ myocardial stunning

The concept of myocardial hibernation is based primarily on clinical observations,1–4 but an appropriate animal model has yet to be established. Numerous models have been established for what is currently referred to as acute or short-term hibernation,5–9 but few long-term models exist that established for what is currently referred to as acute or short-term hibernation,5–9 but few long-term models exist that affect the myocardium. One unique feature of the present study was the continuous monitoring and documentation of flow/function mismatch in hibernating myocardium are not shared by all.10,11

The first goal of the present investigation was to determine whether a moderate reduction in CBF similar to that used in studies of acute hibernation,5,6 when sustained and monitored for 24 hours, induced hibernating myocardium in conscious pigs. There are 2 major limitations to previous studies: (1) experiments were conducted in anesthetized animals for <3 hours’ duration, or (2) the stenosis was prolonged to 24 hours or even 1 week, but blood flow was not measured continuously. The goal of the present study was to avoid these 2 limitations. The pig was selected because the coronary anatomy of this species resembles that found in humans. Furthermore, the lack of preformed collateral vessels allows more precise regulation of the stenosis and concurrent flow reduction. The conscious pig was studied to avoid complicating factors such as anesthesia and recent surgery, which could affect the myocardium. One unique feature of the present study was the continuous monitoring and documentation of...
reduced CBF. To do this, CBF was reduced by \( \approx 40\% \) and monitored continuously for 24 hours in conscious, chronically instrumented pigs. As noted above, most previous studies assumed continuously reduced CBF over a 24-hour to several-week period without actual verification by direct measurement. A second goal was to assess the spatial distribution of myocardial blood flow in the heart, which was done by sectioning the LV into an average of 488 ± 59 samples for measurement of blood flow. Most previous studies on spatial distribution of myocardial blood flow have examined the nonischemic heart. A third goal was to determine histologically whether necrosis was observed within the AAR, examined 2 days after reperfusion. After it was observed that the 24-hour CS protocol resulted in patchy areas of necrosis, 2 additional pigs were studied with 5-hour CS and 2 days of reperfusion to determine whether necrosis resulted from the shorter period of myocardial ischemia.

**Materials and Methods**

Ten domestic swine, weighing 22.1 ± 0.7 kg, were sedated with telazol 5 mg/kg IM and atropine 0.05 mg/kg IM. General anesthesia was induced with sodium thiopental 10 to 20 mg/kg IV and maintained with halothane (0.5 to 1.5 vol%) after tracheal intubation. Under sterile surgical technique, a left thoracotomy was performed at the fifth intercostal space. Tygon catheters (Norton Plastics) were implanted in the descending aorta and in the left atrium for measurement of pressures and radioactive microsphere injection. A solid-state miniature pressure gauge was implanted in the LV cavity to obtain LV pressure and LV dP/dt. The left anterior descending coronary artery was isolated, and a CBF probe (Transonic Systems Inc) and a hydraulic occluder made of Tygon tubing were implanted in 7 of the pigs, whereas in 1 pig, the instrumentation was implanted on the left circumflex coronary artery. In all pigs, 2 pairs of ultrasonic crystals were implanted transmurally across the LV free wall in the anterior and posterior regions for measurement of regional wall thickness. The subendocardial crystal was introduced obliquely so that the myocardium between the 2 crystals would not be impaired by injury or fibrosis. The epicardial and endocardial crystals were properly aligned during surgical implantation by positioning of the crystals to obtain a received signal on the oscilloscope with the greatest amplitude and shortest transit time. The crystals distal to the occluder were implanted in the central ischemic zone as defined by a test coronary artery occlusion at the time of operation. The correct placement of the crystals was also confirmed at necropsy. The wires and catheters were externalized between the scapulae, the incision was closed in layers, and the chest was evacuated. Each pig was treated with 1 g cephalxin IV before surgery and with cephalxin 15 mg/kg twice a day PO for 14 days after surgery. Animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, revised 1996).

Hemodynamics were recorded on a magnetic tape recorder (Honeywell) and on multiple-channel ink-writing oscillographs (Gould-Brush). Aortic and left atrial pressures were measured with a strain-gauge manometer (Statham Instruments) connected to the respective fluid-filled catheters. The solid-state LV pressure gauge was cross-calibrated against measurements of systolic aortic and left atrial pressures. LV dP/dt was calculated with an operational amplifier connected as a differentiator, which has a frequency response of 700 Hz. Mean arterial pressure was determined with a resistance-capacitance filter having a 2-second time constant. Regional myocardial function was measured with an ultrasonic transit-time dimension gauge. This instrument measures the transit time of acoustic signals traveling at a sonic velocity of 1.58 \( \times 10^4 \) mm/s between the intramyocardial crystal pairs. The drift of this instrument, although minimal, was effectively compensated for by repeated calibrations. A cardiofretometer triggered by the LV pressure pulse provided instantaneous and continuous records of heart rate.

All pigs were introduced to a sling for training 1 to 2 hours daily over a 1- to 2-week period before surgery, and training was resumed after 1 week of postoperative recovery. The experiments in conscious pigs were initiated 10 to 14 days after surgery. Seven pigs were used for the 24-hour CS and 2-day reperfusion protocol. Two pigs were used for a 5-hour CS, 2-day reperfusion protocol. An additional pig was used as a sham, ie, it underwent 24-hour recording in the sling without stenosis. Intravenous maintenance fluids (lactated Ringers solution with 20 mEq/L KCl) were administered at 15 mL \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \) during the period of moderate coronary flow reduction, and the animals were fed periodically. The position of each pig within the sling was continuously changed. At times, all of the legs were placed in the sling, and the pigs would rest as they would in a pen or cage. Valium was administered at 0.5 to 1.0 mg/kg for tranquilization before initiation of the experimental protocol and additionally as required, ie, if the pigs became transiently agitated. Periods of agitation were \( \approx 2 \) minutes in duration and were rapidly treated with Valium. The total amount of time that agitation occurred over 24 hours was on average \( \approx 14 \) minutes per animal, whereas in the pigs with 5 hours of CS, this rarely occurred. Global and regional baseline hemodynamic data were recorded, and a CS was induced by introduction of saline into the hydraulic occluder to reduce CBF by \( \approx 40\% \). The degree of CBF reduction was then continuously monitored and sustained for the entire 24-hour or 5-hour period. Because CBF tended to rise over the stenosis period, continuous adjustment of the hydraulic occluder was required to accurately maintain the CBF reduction.

Premature contractions, as noted from the hemodynamic recordings, developed during the CS period in 5 of the 7 animals with 24-hour CS. In 3 of the 7 pigs, arrhythmias began at 7 to 10 hours
After completion of perfusion, the LV was cut into 7 to 8 slices, and pressure was maintained at 120 to 140 mm Hg for both cannulas.

Regional CBF was measured by the radioactive microsphere technique. Six million microspheres (15 ± 1 μm) labeled with 95Nb, 82Sr, 141Ce, 46Sc, 113Sn, 51Cr, 114In, or 103Ru were suspended in 0.01% Tween 80 solution. A total of 5 to 8 LV transmural samples from ischemic and nonischemic regions from each heart were embedded in paraffin, sectioned at 5-μm thickness, and stained with hematoxylin and eosin. These histological sections were subjectively evaluated for the presence of histopathological lesions. The TTC-negative infarct regions were evaluated morphometrically with a digitizer from the individual slice photographs. The extent of the patchy necrosis was evaluated using the histological sections for percentage area necrosis of the remaining AAR. The total histological necrosis was estimated from the area of the digitized grossly TTC-negative area and the estimated percentage of its AAR with patchy necrosis. There was no evidence of platelet or fibrin plugs in the microvasculature.

For measurement of regional myocardial blood flow, the slices were trimmed of excess epicardial fat and fibrous tissue, the apical tissue near the Konigsberg insertion site was discarded, and the remaining tissue was cut further into 488 ± 59 pieces for the entire heart, with each piece weighing an average of 0.163 ± 0.001 g. Each piece was numbered and mapped by position and presence or absence of infarct (TTC technique). Microscopic examination verified infarct of the TTC-negative myocardial samples. The average number and weight of the tissue samples within the AAR were 178 ± 27 samples per heart and 0.148 ± 0.001 g per sample, respectively. The total weight of tissue for each animal averaged 26.4 ± 3.2 g for the ischemic zone distal to the occluder and 52.9 ± 6.7 g for the nonischemic zone as determined by dual perfusion. The samples were counted in a gamma counter (Searle Analytical) with appropriate background correction.

### Table 1. Hemodynamics and Regional Myocardial Function at Baseline and 1, 12, and 24 Hours of CS and 2 Hours and 2 Days of Reperfusion (n = 7)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 h</th>
<th>12 h*</th>
<th>24 h*</th>
<th>Ave CS</th>
<th>2 h R*</th>
<th>2 d R*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF, mL/min</td>
<td>38 ± 3</td>
<td>23 ± 3</td>
<td>22 ± 3</td>
<td>23 ± 2</td>
<td>23 ± 3†</td>
<td>35 ± 5</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>LV systolic pressure, mm Hg</td>
<td>131 ± 2</td>
<td>129 ± 3</td>
<td>125 ± 6</td>
<td>123 ± 6</td>
<td>127 ± 6</td>
<td>122 ± 6</td>
<td>125 ± 5</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>11 ± 1</td>
<td>17 ± 2</td>
<td>18 ± 2</td>
<td>19 ± 2</td>
<td>18 ± 2†</td>
<td>17 ± 3</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>LV dp/dt, mm Hg/s</td>
<td>3052 ± 166</td>
<td>2606 ± 87</td>
<td>2598 ± 147</td>
<td>2512 ± 152</td>
<td>2610 ± 148†</td>
<td>2519 ± 177</td>
<td>3024 ± 242</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>113 ± 3</td>
<td>114 ± 3</td>
<td>109 ± 5</td>
<td>110 ± 5</td>
<td>112 ± 4</td>
<td>109 ± 4</td>
<td>109 ± 6</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>129 ± 6</td>
<td>135 ± 6</td>
<td>135 ± 6</td>
<td>126 ± 7</td>
<td>132 ± 6</td>
<td>135 ± 8</td>
<td>127 ± 7</td>
</tr>
<tr>
<td>Ischemic wall thickening, mm</td>
<td>2.7 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.6 ± 0.2†</td>
<td>0.7 ± 0.2</td>
<td>1.4 ± 0.4</td>
</tr>
</tbody>
</table>

Ave CS indicates average of all hourly measurements during the entire period of CS; R, reperfusion.

*P<0.05 different from baseline by ANOVA and Student t test.

### Table 2. Regional Myocardial Blood Flow (mL·min⁻¹·g⁻¹) at Baseline and 1, 12, and 24 Hours of CS (n = 7)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 h</th>
<th>12 h*</th>
<th>24 h*</th>
<th>Ave CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic zone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocardium</td>
<td>1.52 ± 0.24</td>
<td>0.50 ± 0.16</td>
<td>0.44 ± 0.13</td>
<td>0.52 ± 0.15</td>
<td>0.49 ± 0.14†</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>1.48 ± 0.26</td>
<td>0.86 ± 0.19</td>
<td>0.84 ± 0.17</td>
<td>0.90 ± 0.17</td>
<td>0.86 ± 0.17†</td>
</tr>
<tr>
<td>Epicardium</td>
<td>1.17 ± 0.21</td>
<td>1.13 ± 0.18</td>
<td>1.15 ± 0.15</td>
<td>1.23 ± 0.15</td>
<td>1.16 ± 0.14</td>
</tr>
<tr>
<td>Transmural</td>
<td>1.39 ± 0.24</td>
<td>0.84 ± 0.17</td>
<td>0.82 ± 0.13</td>
<td>0.89 ± 0.14</td>
<td>0.85 ± 0.14†</td>
</tr>
<tr>
<td>Normal zone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocardium</td>
<td>1.58 ± 0.23</td>
<td>1.86 ± 0.19</td>
<td>1.78 ± 0.26</td>
<td>1.91 ± 0.23</td>
<td>1.84 ± 0.21</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>1.48 ± 0.22</td>
<td>1.83 ± 0.18</td>
<td>1.75 ± 0.24</td>
<td>1.92 ± 0.22</td>
<td>1.82 ± 0.19</td>
</tr>
<tr>
<td>Epicardium</td>
<td>1.24 ± 0.18</td>
<td>1.54 ± 0.13</td>
<td>1.49 ± 0.19</td>
<td>1.62 ± 0.16</td>
<td>1.54 ± 0.15</td>
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<tr>
<td>Transmural</td>
<td>1.43 ± 0.21</td>
<td>1.74 ± 0.17</td>
<td>1.67 ± 0.23</td>
<td>1.82 ± 0.20</td>
<td>1.74 ± 0.18</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.

*P<0.05 different from baseline by ANOVA and Student t test.

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Absolute values for tissue blood flows (mL·min⁻¹·g⁻¹) were obtained and expressed as mL·min⁻¹·g⁻¹. Data for blood flow are reported for the ischemic and nonischemic zones. Spatial distribution was created by grouping tissue flow samples each 0.1 mL·min⁻¹·g⁻¹ and counting the frequency of occurrence in each group.

Data/Statistics
All data were stored on a PC computer and reported as mean±SEM. Comparisons between the baseline and average stenosis groups were made with Student’s t test for grouped data. Hemodynamics and regional myocardial blood flows during the 24-hour CS and reperfusion were analyzed with repeated-measures ANOVA. If the ANOVA indicated statistical significance, the average value during CS was compared with baseline by Student’s t test, with P<0.05 taken as the level for significance.

Results
Hemodynamics During the 24-Hour CS
Changes in CBF, nonischemic zone wall thickening, and IZWT during the 24-hour CS and the 2-day reperfusion period are shown in Figure 1. Hemodynamic and regional function measurements at baseline and at 1, 12, and 24 hours and the average values over the 24-hour period of CS, along with recovery values at 2 hours and 2 days after coronary artery reperfusion, are listed in Table 1. Although CBF was reduced by an average of 41±4% during the CS, IZWT had decreased by an average of 63±7% from baseline initially, continued to decrease, and was reduced by 78±8% from baseline at the end of the 24-hour CS. After 2 days of reperfusion, CBF and IZWT were still depressed by 24±8% and 48±15%, respectively. During the first several hours of CS, while CBF was maintained, IZWT decreased progressively. The difference in slopes of these 2 measurements was significant (Figure 1), suggesting that stunning occurred during the stenosis, causing a further decline in IZWT. LV end-diastolic pressure increased significantly (P<0.05) during the 24-hour CS, from 11±1 mm Hg to an average of 18±2 mm Hg. LV dP/dt decreased significantly during the stenosis period, from a baseline of 3052±166 mm Hg/s to an average of 2610±148 mm Hg/s and had recovered to baseline values (3024±242 mm Hg/s) after 2 days of reperfusion after the stenosis. No significant changes were observed during the stenosis period for either mean arterial pressure or heart rate from baseline values of 113±3 mm Hg and 129±6 bpm, respectively.

Blood Flow Distribution Within the AAR and Nonischemic Area
Absolute values for tissue blood flows (mL·min⁻¹·g⁻¹) for the ischemic and nonischemic zones are listed in Table 2. Myocardial blood flows assessed during the CS (1, 12, and 24 hours) were averaged and are represented in Figure 2A. During the CS, transmural tissue blood flows, assessed by the microsphere technique, decreased by an average of 39±10%, compared with 41±4% by the flowmeter method. Analysis of subendocardial and midmyocardial blood flows from the AAR showed a clear redistribution of blood flow during the CS. Subendocardial and midmyocardial blood flows decreased from baseline by 68±9% and 42±11%, respectively, during the CS. In contrast, subepicardial blood flow remained unchanged from baseline flow. The redistribution of blood flow during the CS resulted in a significant, P<0.01, decrease in the endocardial/epicardial flow ratio from 1.30±0.09 to 0.42±0.11.

Infarct Distribution Within the AAR
The AAR, as assessed by dual perfusion of the heart with saline (AAR) and Monastral blue (non-AAR), averaged 36±2% of the LV and septum. After slicing and TTC staining, the areas of solid and patchy infarct (histopathologically identified) were calculated as a percentage of the AAR. Each heart demonstrated either subendocardial infarct in the AAR surrounded by areas of patchy necrosis or just areas of patchy necrosis involving the endocardial and midmyocardial layers (Figure 3).

Histologically, the ischemic tissue examined 2 days after the reintroduction of blood flow revealed multifocal subendocardial lesions of healing necrosis with some myocardial fibers with coagulation necrosis. The healing ischemic lesions were characterized by macrophage and other mononuclear cell infiltrations. The patchy areas of healing necrosis ranged from ~50 μm to 1 mm in diameter. These lesions, found in the endocardial and midmyocardial third of the AAR, were observed in all pigs with 24-hour CS and 2 days of reperfu-
sion and in the 2 pigs studied with 5-hour CS and 2 days of reperfusion. There was no histological evidence of platelet or fibrin plugs in the vasculature. In the 1 pig that was monitored for 24 hours without CS being induced, no infarct was observed.

The distribution of infarcted tissue by layer (Figure 2B) showed a progressive decrease from the subendocardium to the subepicardium. Subendocardial and midmyocardial tissue averaged 90±4% and 55±7% infarcted samples, respectively. In contrast, only 2±1% of the tissue samples from the subepicardial layer were infarcted.

Spatial Flow Distribution
Spatial distribution of subepicardial flow during CS demonstrated no significant change from baseline (Figure 4). Spatial distribution of subendocardial and midmyocardial flow during CS demonstrated a significant shift to the left, with a considerable number of tissue samples with blood flow <20% of baseline, even though CBF was decreased by only 41±4%. The bimodal distribution of samples in the subendocardium and midmyocardium during CS is consistent with the concept that the low flows, ie, <0.5 mL·min⁻¹·g⁻¹, were most likely those that demonstrated necrosis. In fact, blood flow in the samples in the area of patchy necrosis was reduced (−66±4% from a baseline of 1.55±0.27 mL·min⁻¹·g⁻¹), whereas blood flow was maintained in samples in the AAR without necrosis (−2±7% from a baseline of 1.25±0.22 mL·min⁻¹·g⁻¹).

Discussion
The initial goal of the present study was to determine whether or not a sustained, moderate reduction in CBF induced hibernating myocardium in conscious pigs. After 24 hours of sustained, 41±4% CBF reduction (59±4% of baseline) and 2 days of full reperfusion, patchy infarct was present in an average of 40±5% of the AAR. This finding is clearly inconsistent with previous models of acute, subacute, or chronic hibernation in pigs, which have shown little or no infarct after sustained CS.5– 8,10–14,20 These previous studies can be grouped into those in which blood flow was documented to be severely reduced,5– 8 those in which blood flow was either minimally reduced or not reduced at all,12–14,20 and those in which blood flow was not measured.10,11 The latter studies, in which blood flow was not measured, will not be discussed further. Those studies in which blood flow was measured and found not to be reduced substantially12,13 are consistent with the results of the present investigation, ie, we also could not demonstrate areas of necrosis in which tissue flow was not reduced after sustained CS. The discrepancy arises between those studies purporting to show a fixed reduction in blood flow and no evidence of necrosis5– 8 and our study demonstrating a fixed reduction in blood flow and substantial necrosis. The studies with no evidence of necrosis can be subdivided into those in which blood flow was documented to be severely reduced,5– 8 those in which blood flow was either minimally reduced or not reduced at all,12–14,20 and those in which blood flow was not measured.10,11 The latter studies, in which blood flow was not measured, will not be discussed further. Those studies in which blood flow was measured and found not to be reduced substantially12,13 are consistent with the results of the present investigation, ie, we also could not demonstrate areas of necrosis in which tissue flow was not reduced after sustained CS. The discrepancy arises between those studies purporting to show a fixed reduction in blood flow and no evidence of necrosis5– 8 and our study demonstrating a fixed reduction in blood flow and substantial necrosis. The studies with no evidence of necrosis can be subdivided into those in which blood flow was measured continuously and that were relatively short-term, ie, several hours,5,6 and those in which blood flow was measured intermittently.7,8 In the latter group, it is possible to surmise that blood flow was not reduced constantly over the 24-hour period. Interestingly, in the study by Chen et al,5 when blood

Figure 4. Spatial distribution of subendocardial (Endo) and midmyocardial (Mid) flows in the AAR during CS demonstrated a shift to the left, with a significant number of tissue samples with blood flow less than the average transmural CBF reduction of 40%. The bimodal distribution of samples suggests that samples with flows <0.5 mL·min⁻¹·g⁻¹ demonstrated necrosis. Spatial distribution of subepicardial (Epi) flow in the AAR during CS demonstrated no significant change from baseline (n=7).

Figure 3. Histological analysis using hematoxylin and eosin demonstrated patchy areas of subendocardial necrosis. Arrows denote these areas. Left, low power (bar=500 μm), and right, high power (bar=50 μm), illustrating healing necrosis with remnants of myofibers with coagulation necrosis (arrows).
flow was measured continuously in 3 pigs, infarct was observed in 2. Indeed, in our experience, blood flow tends to rise in the model we used over the 24-hour period, and adjustment to the occluder was required to maintain a 40% reduction in blood flow. In preliminary experiments, when this was not corrected, blood flow returned toward baseline, but ischemic zone wall function remained depressed because of concurrent myocardial stunning. This led to a situation of depressed function without depressed blood flow and a false interpretation, ie, that blood flow continued to be reduced. Thus, studies of CS in which regional function rather than blood flow is regulated are flawed by the fact that blood flow can return to baseline without significant improvement in regional function because of concurrent myocardial stunning. Needless to say, infarction would not be observed in those studies, and in the absence of repeated blood flow measurements during the period of stenosis, the conclusion that myocardial hibernation had occurred would have been incorrect.

The differences between our results and those in which blood flow was measured continuously and found to be reduced are more difficult to resolve. However, the majority of these studies were conducted in open-chest, anesthetized swine with relatively short (<2-hour) periods of blood flow reduction. It is conceivable that the markedly diminished energy requirements of the heart in that state may have yielded short-term protection; eg, LV dP/dt was 1275±200 mm Hg/s in the study by Schulz et al, compared with 2610±148 mm Hg/s in our study and compared with 1320±150 mm Hg in dogs with heart failure, which is mechanistically akin to myocardial protection during cardiothoracic surgery. It was exactly for this reason that we opted to study conscious pigs. The present results might be reconciled with these previous studies if perfusion-contraction matching can be maintained for relatively short periods of time, eg, <3 hours, but when the stenosis is maintained longer in the pig, necrosis develops. Interestingly, the 2 pigs studied with 5-hour CS and 2 days of reperfusion also demonstrated areas of patchy necrosis in the subendocardial third of the AAR.

Although the experimental design of the present study did not have the limitation of using an anesthetized preparation, it did have limitations. In the conscious state, any change in activity of the animal could result in further imbalance between myocardial oxygen supply and demand. Indeed, we did observe occasional episodes of agitation that required treatment with small doses of Valium in the animals with 24-hour stenosis. However, the total time of agitation, ie, <15 minutes over 24 hours, was not long enough to be responsible for the myocardial necrosis that developed. In a previous study from our laboratory with gradual stenosis over a 3-week period induced by ameroid constriction, necrosis was not observed. The major difference between that study and the present investigation is that blood flow was not reduced in the subendocardium in the previous study and was reduced by 68±9% in this present protocol. It is also important to keep in mind, however, that in patients with chronic coronary artery disease and CS, minute-to-minute variations in activity and arousal occur as these individuals undergo normal daily activity and stress far in excess of the stress incurred by the pigs in this study resting comfortably in the sling. Furthermore, in the pigs with 5-hour CS, periods of agitation rarely occurred.

The presence of infarcted tissue in the present study was located predominantly in the subendocardial rather than midmyocardial layers, with little in the subepicardium. This correlated well with blood flow, which was reduced more in the subendocardium than midmyocardium and minimally in the subepicardium. The results from the subendocardium and subepicardium were clear. In the subendocardium, blood flow was reduced by 68±9%, and infarction developed, whereas there was no reduction in blood flow in the subepicardium, and no infarction was observed. The results for the midmyocardium were mixed, because blood flow was reduced by 42±11% and infarction was observed in only 55±7% of the samples. However, the spatial distribution of flow analysis demonstrated that samples with severe flow reduction (<66±4% from a baseline of 1.55±0.27 mL·min⁻¹·g⁻¹) resulted in infarction, and samples with mild or moderate flow reduction were spared.

The difference in baseline blood flow for the 2 populations of samples could be explained in part by the predominantly subendocardial location of the infarcted samples but could also be attributed to the predilection of myocardial tissues with high baseline blood flows to undergo necrosis after myocardial ischemia. In most studies, myocardial blood flow during CS is presented as 1 value, not only in terms of continuous versus intermittent measurement but also with regard to blood flow averages. As pointed out by Austin et al, Bassingthwaighte et al, and others, blood flow is distributed spatially (Figure 4). At any given time in the baseline state, 5% of the normal myocardium exhibits >40% reduction in blood flow, which was the average effect of the CS in the present study. This occurs in humans as well as experimental animals. Does this mean that 5% of the myocardium is hibernating even in the absence of coronary artery disease? Not necessarily; more likely, it means that there is a spatial distribution of oxygen demand. Conversely, those samples in the midmyocardium demonstrating 40% reduction in blood flow and no necrosis may not have been hibernating but actually exhibiting the normal spatial distribution of blood flow characteristic of any given fraction of normal myocardium.

Most previous studies have examined spatial distribution of myocardial blood flow under baseline conditions. Little is known regarding spatial distribution of blood flow in the presence of CS. The present investigation is the first to demonstrate a clear spatial distribution of blood flow in the myocardium distal to a coronary artery stenosis. Interestingly, there was no shift in the subepicardial distribution but rather a severe shift to the left in the subendocardium. The absence of a subepicardial shift in a porcine model of the ischemic heart could not have been predicted. This demonstrates that collateral channels are not required for the ischemia-induced redistribution of myocardial blood flow that is observed with CS.

Interestingly, wall thickening in the ischemic zone declined over the initial 6 hours of CS despite no change in blood flow. This is supported by the observation that the slopes of the CBF
and wall thickness measurements during the initial period of CS were significantly different (Figure 1). Because of this and the fact that wall thickening in the ischemic zone improved gradually over the subsequent 2 days after full reperfusion (Figure 1), in addition to infarct, an element of myocardial stunning was present in this model. These data are consistent with the emerging concept that stunned myocardium is an essential component of hibernating myocardium.

In summary, sustained CS in conscious pigs with documented sustained moderate 40% reduction in myocardial blood flow results in substantial subendocardial and midmyocardial infarction rather than sustained perfusion-contraction matching and hibernating myocardium. The remainder of the AAR, which was spared necrosis, might have been mistaken for hibernating myocardium had blood flow not been measured and found to be at normal levels.

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