Ischemic cardiomyopathy in pigs with two-vessel occlusion and viable, chronically dysfunctional myocardium

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Fallavollita, James A. and John M. Canty, Jr. Ischemic cardiomyopathy in pigs with two-vessel occlusion and viable, chronically dysfunctional myocardium. Am J Physiol Heart Circ Physiol 282: H1370–H1379, 2002; 10.1152/ajpheart.00138.2001.—A chronic left anterior descending coronary artery (LAD) stenosis leads to the development of hibernating myocardium with severe regional hypokinesis but normal global ventricular function after 3 mo. We hypothesized that two-vessel occlusion would accelerate the progression to hibernating myocardium and lead to global left ventricular (LV) dysfunction and heart failure. Pigs were instrumented with a fixed 1.5-mm constrictor on the proximal LAD and circumflex arteries. After 2 mo, there were no overt signs of right-heart failure and triphenyl tetrazolium chloride infarction was trivial (1.4 ± 0.1% of the LV). Compared with shams, regional function [myocardial systolic excursion (ΔWT); 2.1 ± 0.3 vs. 4.6 ± 0.4 mm, P < 0.05] and resting perfusion (0.90 ± 0.13 vs. 1.32 ± 0.09 ml·min^{-1}·g^{-1}, P < 0.05) were reduced, consistent with hibernating myocardium. Pulmonary systolic (45.9 ± 3.3 vs. 36.5 ± 2.2 mmHg, P < 0.05) and wedge pressures (19.1 ± 1.6 vs. 11.2 ± 0.9 mmHg, P < 0.05) were increased with global ventricular dysfunction (ejection fraction 43 ± 2 vs. 50 ± 2%, P < 0.05). Early LV remodeling was present with increased cavity size and mass. Reductions in sarcoplasmic reticulum Ca^{2+}-AT-Pase and phospholamban were confined to the dysfunctional LAD region with no change in collateral circulation. This combined stenoses of the LAD and circumflex arteries accelerate the development of hibernating myocardium and result in compensated heart failure.

hibernating myocardium; stunned myocardium; pigs; collaterals; heart failure; ischemic cardiomyopathy

ISCHEMIC CARDIOMYOPATHY is an increasingly prevalent cause of death and disability from cardiovascular disease (20). Unfortunately, major deficiencies exist in our understanding of how chronic coronary artery disease progresses to symptomatic left ventricular (LV) dysfunction due to the lack of experimental animal models where the progression of physiological, cellular, and molecular responses can be evaluated longitudinally. Although some patients develop heart failure due to muscle loss and replacement fibrosis in association with large or multiple myocardial infarts (1, 6, 29), pathological studies support the view that many patients have global LV dysfunction, disproportionately severe in relationship to the amount of replacement fibrosis identified at postmortem exam (3, 31). Two of the factors that could contribute to this dissociation include a chronic depression in myocyte contractile function due to alterations in sarcoplasmic reticulum (SR) function and LV remodeling with diffuse interstitial connective tissue proliferation and myocyte cell dropout due to apoptosis (28). An additional explanation for the dissociation between the severity of LV dysfunction and fixed structural changes could be reversible contractile dysfunction arising from repetitive episodes of ischemia in regions supplied by severe coronary stenoses with chronically reduced coronary flow reserve (20). Although viable dysfunctional myocardium due to chronic stunning or chronic hibernation has been demonstrated in a subset of patients with reversibly depressed LV function, its importance as a cause of ischemic cardiomyopathy in the absence of infarction has not been clearly established.

In a previous study, we demonstrated that pigs with a chronic left anterior descending coronary artery (LAD) stenosis develop a critical impairment in coronary flow reserve that results in hibernating myocardium but preserved global LV systolic function (18). This was accompanied by cellular features typical of those found in advanced heart failure, including a progressive regional downregulation in SR gene expression (17) and substantial regional myocyte loss due to increased apoptosis. These regional findings suggested that reversible ischemia from chronic reductions in coronary flow reserve may represent an important early and reversible event in the progression of chronic LV dysfunction (22). Importantly, there was minimal fibrosis, no infarction, and no evidence of global LV dysfunction or heart failure in pigs with hibernating myocardium from a single LAD occlusion.

We hypothesized that global LV dysfunction similar to that encountered in patients with chronic ischemic cardiomyopathy could be produced if the extent of
myocardium at risk of reversible ischemia was increased. To test this, we produced chronic stenoses on the proximal LAD as well as the proximal circumflex artery of pigs. Echocardiographic assessment of global and regional function was combined with physiological studies to assess systemic and pulmonary hemodynamics to evaluate global LV performance under resting conditions. Our results demonstrate that this instrumentation results in a moderate global impairment in systolic LV function with markedly elevated filling pressures similar to those encountered in compensated congestive heart failure. Moreover, there was viable dysfunctional myocardium with acceleration in the development of phenotypic features typical of hibernating myocardium.

**METHODS**

All procedures and protocols conformed to institutional guidelines for the care and use of animals in research. The initial anesthetic preparation has been previously described (16, 18) but modified in that proximal stenoses were placed on two of the three coronary arteries (15). Briefly, juvenile pigs were premedicated with a Telazol (tiletamine 50 mg/ml and zolazepam 50 mg/ml)/ketamine (100 mg/ml) mixture (0.037 ml/kg im) and given prophylactic antibiotics (cephalothin 50 mg/kg iv and gentamicin 5 mg/kg im). A thoracotomy was performed under isoflurane anesthesia (1–3%). In 40 pigs, the proximal LAD and left circumflex coronary (LC) arteries were dissected free and instrumented with 1.5-mm (ID) Delran constrictors (18). The right coronary artery (RCA) that supplies the posterior descending artery was not manipulated. After the incision was closed and the pneumothorax was evacuated, antibiotics were repeated. Pain was controlled with an analgesic (butorphanol 0.025 mg/kg im) and an intercostal nerve block (2% Marcaine). Sudden death occurred in 25 animals an average of 42 ± 4 days after instrumentation. An additional four animals died after sedation for the terminal physiological study, and one febrile animal was terminated 1 wk after initial instrumentation. One animal had an LC stenosis that was <50%. This animal was excluded from further analysis because functionally significant stenoses were not present in both coronary distributions. Eight animals served as sham-instrumented controls, in which the LAD and LC arteries were dissected free but not instrumented with a stenosis.

Four of the nine dysfunctional animals and all of the sham-instrumented controls had a baseline echocardiogram 1 wk after instrumentation to assess regional and global LV function. After an overnight fast, pigs were sedated with a Telazol/xyazine (100 mg/ml) mixture (0.022 ml/kg im). Transthoracic echocardiography was performed with a 2.25-MHz phased-array transducer (Ultramark 9, ATL) from a right parasternal approach with standard M-mode measurements obtained from a midventricular short-axis view. This permitted simultaneous recording of the anteroseptal (LAD perfusion territory) and posterior walls (normally perfused RCA territory). End diastole was defined as the onset of the QRS complex and end systole was the point of minimum chamber diameter. Myocardial systolic excursion (ΔWT) was defined as end-systolic thickness minus end-diastolic thickness. Percent fractional shortening was defined as [(LV end-diastolic dimension) – (LV end-systolic dimension)]/(LV end-diastolic dimension)·100.

**Experimental protocol.** Approximately 2 mo after instrumentation, pigs underwent a terminal physiological study in the closed-chest anesthetized state. After an overnight fast, animals underwent a repeat echocardiogram with Telazol/xyazine sedation. Subsequently, animals were intubated, ventilated with oxygen and anesthetized with isoflurane (1–2%) supplemented with Telazol/xyazine (0.011 ml/kg im as needed). An 8-Fr sheath was placed into the left carotid artery through which a 5-Fr Millar micromanometer was placed into the LV apex for pressure measurement. The side port was used for arterial pressure. An 8-Fr sheath was placed in the right carotid artery and an 8-Fr pigtail catheter was inserted into the LV apex for microsphere flow measurement and ventriculography. A sampling catheter was inserted into the femoral artery for arterial reference sampling during microsphere flow injections. Right heart catheterization was performed through an 8-Fr introducer placed in the jugular vein using a 7-Fr balloon-tipped thermodilution catheter that was advanced into the pulmonary artery and connected to a Gould P23 dB transducer. Animals were heparinized (100 units/kg iv) and hemodynamics were allowed to equilibrate for ~30 min before beginning the protocol.

Regional perfusion was quantified with colored microspheres that were analyzed as we have previously described (18). We injected 3 × 10^6 microspheres into the left ventricle after starting a 90-s reference arterial withdrawal sample (6 ml/min). After the resting flow measurement, anteropapillary wall motion (centerline score) and ejection fraction were assessed with hand injections of radiographic contrast in the lateral projection as previously described (16, 17). A second microsphere measurement of perfusion was performed during adenosine vasodilation (0.9 mg·kg^-1·min^-1) with phenylephrine infused to maintain arterial pressure (10.0 ± 1.3 μg·kg^-1·min^-1). Finally, stenosis severity was determined by coronary angiography (18).

Colored microspheres were processed as previously described (18). Samples were cut into three layers of approximately equal thickness to assess the transmural distribution of perfusion. Myocardial samples were taken from central regions of the stenotic LAD and LC regions and compared with the normally perfused RCA region. Postmortem, histology, and Western analysis of SR proteins. Hearts were weighed and sectioned into concentric rings. Thin rings were immersed in triphenyl tetrazolium chloride (TTC) to quantify infarction by digital planimetry. Samples were also obtained for quantification of connective tissue and regional SR protein levels. Histological samples were immersed in Z-fix (Anatech), sectioned, and stained with Masson’s trichrome, and connective tissue was quantified by point counting (18). Protein was isolated from flash-frozen subendocardial samples, electrophoresed, and separated on SDS polyacrylamide gels, and transferred to nitrocellulose or polyvinylidene difluoride membranes as previously described (17). Linearity of density and protein loading for the candidate SR proteins was demonstrated over a range of 20–60 μg of total protein for the SR Ca^{2+}·ATPase, between 25 and 100 μg of total protein for phospholamban, and between 40 and 140 μg total protein for calsequestrin, using commercially available antibodies previously shown to cross react with the swine (17).

**Data analysis.** Hemodynamics were digitized with an analog-to-digital converter at a sampling rate of 500 Hz and analyzed on line with the Notocord Heme software system. LV pressure was differentiated numerically to obtain the first derivative of LV pressure (dP/dt). We quantified regional wall motion from hand tracings of the lateral ventriculograms using the centerline method as previously described in detail (16). Data are presented as means ± SE. Measurements from the LAD and LC regions were compared with the
normally perfused RCA region of the same heart using paired t-tests and the Bonferroni correction for multiple comparisons. Differences in hemodynamics and flow between rest and vasodilation were compared using paired t-tests. Experimental groups were compared with shams using unpaired t-tests. Sham-instrumented animals had smooth luminally normal arteries.

RESULTS

The nine animals instrumented with stenoses were studied 72 ± 3 days after instrumentation when they weighed 49.0 ± 5.0 kg (dysfunctional group). The eight sham-instrumented animals (sham group) weighed 47.1 ± 2.9 kg at 74 ± 1 days after instrumentation (no differences between groups). All animals were in good health at the time of terminal study with no overt clinical signs of congestive heart failure. There were no differences in hematocrit or arterial blood gases between the groups (average values: hematocrit 32 ± 1%, pH 7.42 ± 0.01, PCO2 45 ± 1 Torr, and PO2 437 ± 12 Torr).

A representative coronary angiogram from a dysfunctional animal is shown in Fig. 1. Significant proximal stenoses were present in both the LAD and LC arteries with an average diameter reduction of 95 ± 2 and 87 ± 3%, respectively. Complete occlusion with collateral-dependent myocardium was present in 4 LAD and 2 LC vessels. Left ventriculography demonstrated severe anteroapical hypokinesis with moderately reduced global function (average ejection fraction 43 ± 2%, P < 0.02 vs. shams, Table 1).

Gross and microscopic pathology. The LV mass-to-body weight ratio was higher in dysfunctional animals but of borderline significance (2.89 ± 0.16 vs. 2.65 ± 0.08 g/kg, P = 0.08). There was TTC evidence of small, healed infarction in five of the dysfunctional animals. The extent of TTC infarction averaged 1.4 ± 0.7% of LV mass. On histological evaluation, point counting of connective tissue averaged 3.6 ± 0.2% in the normally perfused RCA region, 16.5 ± 9.3% in the stenotically LAD region (P < 0.05 vs. RCA), and 8.9 ± 1.7% in the stenotic LC region (P = 0.06 vs. RCA). There were no regional differences in connective tissue staining in sham animals (LAD 3.3 ± 0.4% vs. RCA 4.6 ± 0.9%; P, not significant). Because of the marked variability in connective tissue among individual dysfunctional animals, the difference between dysfunctional and sham groups did not reach statistical significance.

Systemic and pulmonary hemodynamics. Hemodynamic parameters are summarized in Tables 1 and 2 and Fig. 2. Under resting conditions, systemic hemodynamics in dysfunctional animals were similar to sham controls. In contrast, resting LV end-diastolic pressure (LVEDP) was increased in dysfunctional animals (26 ± 3 vs. 17 ± 1 mmHg, P < 0.01), and LV dP/dt was reduced compared with sham controls (1,587 ± 125 vs. 1,894 ± 119 mmHg/s, P = 0.10). Right heart catheterization (Table 1) demonstrated mild pulmonary systolic hypertension (46 ± 3 vs. 37 ± 2 mmHg, P < 0.05) and a moderate increase in pulmonary wedge pressure to 19 ± 2 mmHg compared with 11 ± 1 mmHg in sham controls (P < 0.01). Although LV filling pressures were elevated in dysfunctional animals, there was no alteration in resting cardiac output. Collectively, these hemodynamic findings demonstrate moderate impairment of LV systolic function at rest despite a chronic elevation in LV filling pressure in animals with two-vessel coronary artery stenoses.

Echocardiographic measurements of function. Trans-thoracic echocardiography performed at the time of terminal study confirmed impaired global and regional LV function in animals instrumented with chronic stenoses (Table 3, Figs. 3 and 4). Initial echocardiograms performed 7 ± 1 days after instrumentation (1 wk) demonstrated no differences in regional or global function between dysfunctional and sham-instrumented animals (Table 3, Fig. 3). At ~10 wk after instrumentation, ΔWT had increased commensurately with end-diastolic wall thickness in sham-instrumented animals and in the normally perfused posterior region of the

Table 1. Pulmonary hemodynamics, cardiac output, and ventriculography

<table>
<thead>
<tr>
<th></th>
<th>Pulmonary Systolic, mmHg</th>
<th>Pulmonary Mean, mmHg</th>
<th>Pulmonary Wedge, mmHg</th>
<th>Cardiac Output, l/min</th>
<th>Cardiac Index, ml·min⁻¹·kg⁻¹</th>
<th>Anteroapical Wall Motion, centerline score</th>
<th>Ejection Fraction Ventriculography, %</th>
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<tr>
<td>Dysfunctional</td>
<td>46 ± 2*</td>
<td>31 ± 4</td>
<td>19 ± 2*</td>
<td>5.7 ± 0.9</td>
<td>108 ± 10</td>
<td>−2.1 ± 0.3*</td>
<td>43 ± 2*</td>
</tr>
<tr>
<td>Sham</td>
<td>37 ± 2</td>
<td>25 ± 2</td>
<td>11 ± 1</td>
<td>5.0 ± 0.4</td>
<td>106 ± 4</td>
<td>−1.4 ± 0.1</td>
<td>50 ± 2</td>
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</tbody>
</table>

Values are means ± SE. *P < 0.05 dysfunctional vs. sham.
dysfunctional group (Table 3). However, anteroseptal ΔWT in dysfunctional animals failed to increase as the animals grew and regional function was significantly reduced compared with sham controls (systolic excursion 2.1 ± 0.3 vs. 4.6 ± 0.4 mm, P < 0.01; wall thickening 26 ± 4 vs. 65 ± 7%, P < 0.05). Function of the normally perfused posterior wall was similar in each group.

To assess whether global LV remodeling occurred in dysfunctional animals, ventricular volumes were estimated from the minor axis LV dimensions (Fig. 4). The validity of these estimates is supported by the fact that the estimated stroke volume by echocardiography compared favorably with that from thermodilution measurements averaged 46 ± 3% in dysfunctional animals vs. 62 ± 4% in sham controls (P < 0.05). End-systolic volume index was significantly higher in dysfunctional than sham animals (1.31 ± 0.18 vs. 0.85 ± 0.05 ml/kg, P < 0.05). The increase in end-diastolic volume index was of borderline significance (2.38 ± 0.25 vs. 1.90 ± 0.15 ml/kg, P = 0.13).

Regional myocardial perfusion. Systemic hemodynamics corresponding to the microsphere flow measurements at rest and during adenosine vasodilation are shown in Table 2. There were no differences in heart rate, aortic pressure or double product between dysfunctional and sham animals either under resting conditions or during vasodilation. There were no regional differences in flow in sham-instrumented animals. Full-thickness perfusion in sham animals averaged 1.32 ± 0.09 ml·min⁻¹·g⁻¹ and was higher in the subendocardium with an endo/epi flow ratio of 1.22 ± 0.03. During adenosine, there was more than a sixfold increase in flow in sham animals to 8.66 ± 1.20 ml·min⁻¹·g⁻¹ (P < 0.01 vs. rest) with a slight reduction in the endo-to-epi ratio to 0.88 ± 0.04, (P < 0.01 vs. rest).

The transmural distribution of perfusion at rest and during adenosine in dysfunctional animals is illustrated in Fig. 5 and values of flow reserve are summarized in Table 4. Under resting conditions, full-thickness perfusion in the normally perfused RCA region averaged 1.15 ± 0.09 ml·min⁻¹·g⁻¹ with an endo-to-epi ratio of 1.31 ± 0.06. Full-thickness LAD perfusion was reduced to 0.90 ± 0.13 ml·min⁻¹·g⁻¹ (P < 0.05 vs. RCA regions and sham controls) with an endo-to-epi ratio of 1.04 ± 0.15 (P, not significant). In the LC region, full-thickness flow averaged 1.12 ± 0.11 ml·min⁻¹·g⁻¹ and the endo-to-epi ratio averaged 1.08 ± 0.08 (both P, not significant vs. RCA and sham controls). As shown in Fig. 5, resting perfusion to the inner two-thirds of the LAD region was significantly reduced compared with the normally perfused remote region (LAD subendocardium 0.92 ± 0.14 vs. 1.27 ± 0.10 ml·min⁻¹·g⁻¹ in the RCA, P < 0.05).

During adenosine vasodilation, there were more marked differences in perfusion among the three regions (Fig. 5). Full-thickness flow during adenosine averaged 5.99 ± 0.63 ml·min⁻¹·g⁻¹ in the RCA region, 2.78 ± 0.57 ml·min⁻¹·g⁻¹ in the LC region (P < 0.01 vs. RCA), and 1.44 ± 0.11 ml·min⁻¹·g⁻¹ in the LAD region (P < 0.01 vs. RCA). Flow in the subendocardium of the LAD region was critically impaired, tending to fall below resting values during adenosine vasodilation (0.64 ± 0.10 ml·min⁻¹·g⁻¹, P = 0.08 vs. rest). This was consistent with a transmural steal and accompanied by a pronounced reduction in the endo-to-epi ratio to 0.29 ± 0.06 (P < 0.01 vs. rest). Vasodilated subendo-
cardiac flow in the LC region was also significantly reduced. Although flow increased during adenosine, the difference did not reach statistical significance (2.26 ± 0.60 ml·min⁻¹·g⁻¹, P = 0.08 vs. rest; endo-to-epi ratio 0.65 ± 0.10, P < 0.01 vs. rest). Thus these data indicate there were physiologically significant reductions in vasodilated perfusion in two of the three major coronary artery distributions. Resting perfusion in the dysfunctional LAD region was depressed compared with shams and remote regions and was consistent with the development of hibernating myocardium.

**Regional reductions in SR proteins.** Subendocardial samples from the LAD and normally perfused region were assayed for candidate proteins to determine whether there were regional alterations as we have previously found in pigs with hibernating myocardium from a single LAD stenosis (17). Protein levels for the SR Ca²⁺-ATPase (LAD 6.5 ± 1.3 vs. 9.3 ± 1.4 densitometric units in normal hearts, P < 0.05) and phospholamban (LAD 7.7 ± 2.1 vs. 12.1 ± 2.4 densitometric units in normal hearts, P = 0.05) were reduced by ∼30–35% in the dysfunctional LAD region compared with normally perfused remote RCA regions. There were no regional differences in calsequestrin nor were there regional differences in sham-instrumented control animals.

**DISCUSSION**

There are two important new findings from this investigation. First, pigs instrumented with two proximal coronary artery stenoses develop regional dysfunction accompanied by a moderate reduction in LV performance. This was characterized by reductions in LV ejection fraction in the setting of pronounced increases in LV filling pressures at rest. Although this did not produce reductions in resting cardiac output nor clinical signs characteristic of advanced right heart failure, it was similar to the hemodynamic profile of patients with moderate (NYHA Class II and III) compensated congestive heart failure in the setting of chronic coronary artery disease. Our results also clearly show that hibernating myocardium can be responsible for chronic global LV dysfunction in the absence of infarction when the extent of myocardium at risk of reversible ischemia involves at least two of the three major coronary artery distributions. Development of hibernating myocardium was accelerated compared with the 3–4 mo time frame required in pigs.
tractile abnormalities and/or structural changes associated with ischemic cardiomyopathy remains unclear (5).

Our results demonstrate that viable dysfunctional myocardium can cause global LV dysfunction characterized by markedly elevated LV filling pressures with only trivial amounts of myocardial fibrosis by TTC staining. Furthermore, like humans, there was LV dilatation with a borderline increase in LV mass-to-body weight ratio. Because cardiac output at rest was normal and there were no clinical signs of heart failure, our findings in pigs most likely represent a compensated state of heart failure where resting pulmonary capillary wedge pressures are elevated to maintain cardiac output within the normal range. In support of this, clinical studies evaluating resting hemodynamics in patients with compensated NYHA Class II and III heart failure have found pulmonary wedge pressure to be increased to values approaching 20 mmHg with near-normal resting cardiac output (25). Whether pigs with viable dysfunctional myocardium in our study would ultimately progress to more advanced heart failure with severe right-sided pressure elevation, edema, and reduced cardiac output will require studies performed at later time points.

Relationship to previous models of heart failure from ischemic heart disease. Although there are several experimental models to study heart failure, most work in large animals has focused on pacing-induced heart failure or the decompensated state of hypertrophy arising from hypertension or chronic aortic outflow tract obstruction. Large animal models of heart failure due to ischemic heart disease have been limited. These have been primarily focused on heart failure in association with large myocardial infaracts or myocardial microembolization (30). Because the extent of acute infarction needed to produce heart failure is associated with an extremely poor short-term survival in large animals, the majority of studies have focused on the rat because it can survive myocardial infarcts approaching 60% of LV mass. The one exception to this has been the circumflex occlusion model in growing swine developed by Zhang et al. (37). This model leads to biventricular hypertrophy with variable degrees of heart failure and LV remodeling. Nevertheless, LVEDPs were only increased to 16 mmHg and were lower than what we found in the present study.

Chronic coronary artery stenosis models leading to LV dysfunction have previously been evaluated in rats and dogs. Capasso et al. (12) showed that a fixed

instrumented with a single LAD stenosis, where global LV function and filling pressures were normal (17, 18). Thus our results support the hypothesis that chronic ischemic heart disease can lead to heart failure with viable, chronically dysfunctional myocardium.

Ischemic cardiomyopathy and viable dysfunctional myocardium. Ischemic cardiomyopathy is the most common cause of congestive heart failure and accounts for nearly 70% of the patients enrolled in recent large clinical trials (20). Patients with cardiomyopathy due to coronary artery disease have a survival rate poorer than those with idiopathic dilated cardiomyopathy (2, 20, 29). Postmortem studies demonstrate extensive arteriosclerosis with total occlusion of at least one of the three major epicardial arteries, LV dilatation, and an increase in LV mass (1, 31). History of prior myocar-dial infarction may be decades remote from the event. Because cardiac output at rest was normal and there were no clinical signs of heart failure, our findings in pigs most likely represent a compensated state of heart failure where resting pulmonary capillary wedge pressures are elevated to maintain cardiac output within the normal range. In support of this, clinical studies evaluating resting hemodynamics in patients with compensated NYHA Class II and III heart failure have found pulmonary wedge pressure to be increased to values approaching 20 mmHg with near-normal resting cardiac output (25). Whether pigs with viable dysfunctional myocardium in our study would ultimately progress to more advanced heart failure with severe right-sided pressure elevation, edema, and reduced cardiac output will require studies performed at later time points.

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Table 4. Regional stenosis severity, flow, and flow reserve

<table>
<thead>
<tr>
<th>Stenosis, %</th>
<th>Resting Flow, ml·min⁻¹·g⁻¹</th>
<th>Adenosine Flow, ml·min⁻¹·g⁻¹</th>
<th>Flow Reserve, Adenosine/Resting Flow</th>
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<tbody>
<tr>
<td></td>
<td>Endo</td>
<td>Full thickness</td>
<td>Endo</td>
</tr>
<tr>
<td>LAD</td>
<td>95 ± 2</td>
<td>0.92 ± 0.14†</td>
<td>0.90 ± 0.13†</td>
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<tr>
<td>LC</td>
<td>87 ± 3</td>
<td>1.14 ± 0.12</td>
<td>1.12 ± 0.11</td>
</tr>
<tr>
<td>RCA</td>
<td>1.27 ± 0.10†</td>
<td>1.15 ± 0.09</td>
<td>5.79 ± 0.71</td>
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</table>

Values are means ± SE, n = 9 hearts. Endo, subendocardium; LAD, left anterior descending artery; LC, left circumflex artery; RCA, right coronary artery. *P < 0.05 vs. Endo; †P < 0.05 vs. RCA.
diameter stenosis on the left main coronary artery of rats moderately impaired coronary flow reserve and led to global LV dysfunction within several days. These changes persisted for at least several weeks and, like the present study, were accompanied by minimal increases in connective tissue. Unlike the present findings, however, there was histological evidence of necrosis and considerably more variability in late hemodynamic parameters. Some animals developed only an increase in LVEDP, others had an increased LVEDP and reduced systolic pressure, and still others developed clinical evidence of right-sided heart failure. This heterogeneity most likely reflected variability among animals with regard to the stenosis severity and its physiological effects on subendocardial flow during vasodilation. Firoozan et al. (19) produced globally reduced LV function in dogs instrumented with up to four amloid occluders. Although there was evidence of progressive LV dilatation in this model, there were no sham controls for comparison. Anatomic and physiological stenosis severities were not assessed. In addition, the role of pathological changes, such as infarction and increased connective tissue staining, were not systematically quantified. Finally, although clinical signs of right heart failure were reported in this study, cardiac output and left and right ventricular filling pressures were not measured. Thus there are many unanswered questions regarding this model, including the degree of hemodynamic impairment and the relationship between regional dysfunction and reductions in coronary flow during vasodilation.

Our results extend the findings of these previous studies in several important ways. They demonstrate that the two-vessel swine model results in a reproducible level of LV dysfunction in a large animal model of chronic coronary artery disease. In addition, although there was no clinical evidence of right heart failure, there were chronic elevations in LV filling pressure at rest. This contrasts with our previous findings in pigs with a single LAD stenosis where LV end-diastolic pressure was similar in animals with hibernating myocardium and sham controls (17). Although the elevated filling pressures maintained a normal cardiac output at rest despite globally reduced systolic function, it is likely that exercise-induced increases in cardiac output were limited. This could conceivably arise from the development of acute demand-induced ischemia distal to the critical coronary stenoses as well as due to the inability to increase cardiac output because the extent of viable dysfunctional myocardium was so extensive.

Finally, the regional nature of this model allows the normally perfused RCA distribution supplying the posterior myocardium to be used as an internal reference for perfusion measurements and to assess alterations in protein. In this regard, we did not find significant differences in SR protein expression in the normally perfused RCA regions of dysfunctional versus sham animals. Thus there was no evidence for global remodeling of the normal myocardium with regard to SR function or perfusion. Nevertheless, we cannot exclude the possibility that such changes might occur in such regions at later time points.

Comparison with the one-vessel model of hibernating myocardium in pigs. Results of the present study extend previous work from our laboratory as well as others, in which pigs instrumented with a stenosis on the proximal LAD developed viable, chronically dysfunctional myocardium (17, 18, 26, 27). We have previously shown there was a progression from chronically stunned to hibernating myocardium as stenosis severity increases (9, 16). Transition to hibernating myocardium occurred after 3–4 mo (average 107 days) and was accompanied by regional reductions in oxygen consumption (27) and flow (17, 18). Although the LAD supplies ~40% of the pig left ventricle (35), LV ejection fraction in animals with hibernating myocardium and one-vessel occlusion was the same as sham-instrumented controls (17). In the present study, both the LAD and LC arteries were instrumented, thereby increasing the total LV mass at risk. Based on previous measurements in pigs, we would estimate the risk area subjected to reversible ischemia to exceed 60% of the left ventricle (35).

We have previously characterized the temporal progression of regional dysfunction in relationship to the physiological significance of a coronary stenosis in pigs instrumented with a single LAD stenosis. After 2 mo (63 ± 2 days), animals with one-vessel occlusion had dysfunctional myocardium with normal resting perfusion, consistent with chronic stunning (16). Although subendocardial flow during adenosine vasodilation could only increase to 1.51 ml·min⁻¹·g⁻¹, it was considerably higher than the value of 0.64 ml·min⁻¹·g⁻¹ in the present study. In addition, LAD subendocardial flow during vasodilation in the present study frequently fell below the resting value, whereas subepicardial flow increased, consistent with a transmural steal. Although variability in stenosis severity among experimental groups could contribute to this difference, a more likely explanation is that the temporal acceleration in the development of hibernating myocardium in this model reflected a more severe impairment in maximal LAD flow during vasodilation. This most likely arose from an attenuation of source collateral flow from the stenotic circumflex artery. The role of the physiological stenosis severity in the progression to stunned to hibernating myocardium is also supported by recent studies from our laboratory where the development of hibernating myocardium could be accelerated to occur in 1–2 wk when a critical limitation in LAD flow during vasodilation was acutely placed and maintained on the LAD in chronically instrumented pigs (34). These findings lend further support to the hypothesis that the key physiological factor determining the transition from chronically stunned to hibernating myocardium is the physiological significance of the coronary stenosis and a severely limited ability to increase subendocardial flow during vasodilation.

Although the reductions in function are almost certainly reversible, the degree of infarction and increases in connective tissue in the present study were slightly

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H1376  GLOBAL LV FAILURE WITH VIABLE DYSFUNCTIONAL MYOCARDIUM

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greater (1.4% of the LV mass by TTC) than our experience with one-vessel occlusion. Part of this is related to the fact that we included all instrumented animals in this analysis. In our one-vessel model studies, we chose to exclude the rare animals in which >1% of the LV was infarcted (14, 16). Inclusion of all animals in the present study was felt to be more analogous to the situation in patients with coronary artery disease and ischemic cardiomyopathy. Nevertheless, the extent of infarction was trivial, indicating that this is essentially a model of heart failure from viable dysfunctional myocardium arising from chronic episodes of reversible ischemia. In support of reversible dysfunction, our finding of 16.5% connective tissue in the LAD distribution is within the range (11–24%) reported in patients with hibernating myocardium that improved function after revascularization (13, 23, 32). The results of the present investigation would not be substantively different even if animals with >1% infarction were excluded.

**Perfusion-contraction matching in viable chronically dysfunctional myocardium.** Controversy exists regarding the mechanisms responsible for viable dysfunctional myocardium and whether resting flow is actually normal or depressed (8–11, 21, 36). Some investigators have suggested that regional dysfunction is disproportionately depressed relative to the modest reductions in flow in regions of hibernating myocardium (8). Others have suggested heterogeneity of mechanisms when myocardial flow and function are analyzed on a segmental basis, with chronic stunning and hibernating myocardium being present in the same heart (19, 33). Our results with direct microsphere measurements indicate that flow is chronically reduced in hibernating LAD myocardium compared with normal remote regions as well as sham controls. Data to assess whether flow and function are matched or mismatched in clinical studies are complicated by the wide variations in regional perfusion among patients (7, 11, 21) and the paucity of information regarding the relationship between flow and radial wall motion versus more sensitive indices of regional function such as wall thickening.

Our previous studies provided limited insight into flow-function matching and this is the first study using this model that assessed regional wall thickening. Accordingly, we evaluated the relationship between relative reductions in flow and function at rest in Fig. 6. Flow (both subendocardial and full thickness) and systolic excursion from the hibernating LAD region are shown relative to the normally perfused region of the same animal. Although our analysis is limited by the paucity of functional data from chronically dysfunctional myocardium, the data support the notion that there is some degree of flow-function matching in chronic hibernating myocardium because resting flow in chronic stunning would be normal. This finding is consistent with the matched reductions in flow and wall thickening in viable dysfunctional myocardium found among some, but not all, regions of ameroid-instrumented dogs (19) and preliminary studies from our laboratory in pigs with hibernating myocardium from a single LAD stenosis (24). It is interesting to note that the majority of the individual data points show a greater reduction in regional function than the reduction in relative flow. Although this may suggest that flow and function are not ideally matched in hibernating myocardium, this finding is also consistent with a component of stunning superimposed on hibernating myocardium in this model (4). Further studies in this and other chronic models will be required to clarify the precise relationship between flow and function in hibernating myocardium.

**Methodological limitations.** Although we have previously demonstrated that pigs with a single LAD stenosis have contractile reserve to β-adrenergic stimulation, we did not assess this in pigs with two stenoses because of a high rate of sudden death during spontaneous excitement in this model. We have no reason to believe that this would be absent because there was minimal evidence of irreversible injury and a similar degree of increased connective tissue, but confirming this will require additional studies. Delineation of the LC region was done by subjectively evaluating the coronary anatomy in conjunction with the distribution of flow during vasodilation. Because flow was analyzed with colored microspheres and we needed to obtain samples for protein isolation and histology, we were unable to identify the perfusion territory of individual vessels with vital dyes. Although we were careful to restrict our analyses to core regions and avoided potential border areas between perfusion territories, it is possible that the intermediate reductions in vasodilated flow in the LC region could, to some extent, be a result of an admixture of normal and severely stenotic tissue. Because of this and an inability to assess re-
regional wall thickening in the lateral region by M-mode echocardiography or ventriculography, we have confined our primary flow and function analyses to the LAD and normally perfused RCA regions. Furthermore, because we could not analyze wall thickening and flow in small myocardial regions, we cannot determine whether heterogeneity in mechanisms exists with chronically stunned and hibernating myocardium coexisting in the same perfusion territory. Additional studies will be required to evaluate flow-function relations on a three-dimensional basis in smaller myocardial segments.

Clinical implications. Our study shows that viable, chronically dysfunctional myocardium can lead to reductions in global function and elevations in LV filling pressure that are characteristic of compensated heart failure. Thus global LV dysfunction from repetitive episodes of ischemia can develop in the absence of myocardial infarction. Because large dysfunctional regions after myocardial infarction can stimulate global LV remodeling, it is possible that ischemic cardiomyopathy could develop from remodeling in response to viable dysynergic regions. Because apoptosis has been demonstrated in hibernating myocardium, myocyte loss may be accentuated as the extent of dysfunctional myocardium increases. Whether extending the time frame over which the observations are conducted or increasing the area at risk of reversible ischemia leads to remote zone remodeling and/or progresses to more advanced phases of heart failure will require further study.

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