Hibernating Myocardium Retains Metabolic and Contractile Reserve Despite Regional Reductions in Flow, Function, and Oxygen Consumption at Rest

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Abstract—Hibernating myocardium, characterized by reductions in flow and function at rest, has limited contractile reserve in response to increases in external workload. We hypothesized that this attenuation of function reflects an adaptive downregulation that prevents the development of metabolic evidence of ischemia during stress. To test this hypothesis, pigs were chronically instrumented with a proximal left anterior descending artery stenosis for 3 months, resulting in severe anteroseptal hypokinesis with reduced resting perfusion (0.78±0.05 versus 0.94±0.07 mL · min⁻¹ · g⁻¹ in remote, P<0.01; and 0.99±0.08 in controls, P<0.05). Open-chest studies confirmed resting dysfunction compared with normal controls (segment shortening 9.2±2.2% versus 23.5±1.1%, P<0.05). Resting myocardial oxygen consumption was reduced (63±3 versus 77±6 μL · g⁻¹ · min⁻¹ in controls, P<0.05), yet lactate consumption was normal. Although subendocardial perfusion failed to increase during graded, intravenous epinephrine infusion (n=8), peak segment shortening (to 17.3±3.1%, P<0.05) and oxygen consumption (to 90±6 μL · g⁻¹ · min⁻¹, P<0.01) increased from the depressed resting levels. There was no lactate production in hibernating myocardium, and lactate uptake increased during stress (0.7±0.1 to 1.2±0.1 μmol · g⁻¹ · min⁻¹, P<0.05). The absence of metabolic evidence of ischemia was also confirmed during atrial pacing to a rate of 120 bpm (n=8). Thus, despite reductions in function and oxygen consumption at rest, hibernating myocardium retains the ability to increase metabolism without the development of acute ischemia. This supports the hypothesis that the downregulation of oxygen consumption and function in hibernating myocardium is an adaptive response that prevents a supply-demand imbalance during submaximal increases in cardiac workload when coronary flow reserve is limited. (Circ Res. 2003;92:48-55.)

Key Words: hibernating myocardium ■ oxygen consumption ■ lactate ■ contractile reserve ■ β-adrenergic stimulation

Hibernating myocardium is characterized by chronic reductions in flow and function that occur in the absence of infarction or subjective evidence of acute ischemia.1 Although flow is reduced at rest, the myocardium retains the ability to at least transiently respond to increases in external workload. While considerable attention has focused on assessing contractile reserve in hibernating myocardium, the metabolic responses at rest and during increases in the external determinants of myocardial oxygen consumption are largely unknown. In normal hearts, acute transient ischemia causes dysfunction that is accompanied by a rapid fall in high-energy phosphate levels and a switch to anaerobic glycolysis as evidenced by regional lactate production. When moderate ischemia is maintained for hours, however, reductions in regional function match the reduced levels of oxygen delivery. This prevents irreversible injury by reestablishing a balance between supply and demand, a phenomenon termed “short-term hibernation.”2 Although this reverses lactate release and regenerates high-energy phosphate levels, the duration over which the heart is able to adapt without developing subendocardial necrosis is less than 24 hours.3–5 This is at least partly due to the fact that, in the presence of a coronary stenosis, transient increases in the external determinants of myocardial oxygen consumption lead to an immediate deterioration in the tenuous balance between supply and demand.6,7 Thus, tissue high-energy phosphate levels decrease and the heart again releases lactate during superimposed stress.

Chronic hibernating myocardium also retains the ability to transiently increase function in response to β-adrenergic stimulation, despite a critical limitation in coronary flow reserve, but the metabolic consequences of this are unknown. Although functional changes after graded increases in demand are blunted because of the inability of subendocardial flow to increase, regional wall motion does not deteriorate.8 These findings could be due to intrinsic cellular adaptations induced in hibernating myocardium that downregulate regional energy utilization to prevent the development of

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metabolic ischemia. Thus, a downregulation of resting flow and function could allow the myocardium to respond to modest increases in the external determinants of workload without the immediate development of metabolic evidence of ischemia. This could conceivably minimize periods of repetitive acute supply-demand imbalance that lead to acute myocardial stunning.

We therefore tested the hypothesis that hibernating myocardium entails an adaptive downregulation in regional metabolism that, in contrast to short-term hibernation, allows the heart to increase oxygen consumption in response to stress without immediately leading to metabolic evidence of ischemia. We used an established porcine model of chronic hibernating myocardium and evaluated submaximal metabolic responses with atrial pacing and β-adrenergic stimulation with epinephrine. While function and metabolic responses were blunted, the results demonstrate that hibernating myocardium can increase function and oxygen consumption without developing lactate release during steady-state submaximal stress. This supports the view that hibernating myocardium is an adaptive state retaining the ability to elicit metabolic reserve without the immediate development of anaerobic glycolysis.

Materials and Methods

Experimental procedures conformed to institutional guidelines for the care and use of animals in research. Detailed methodology is contained in the online data supplement, available at http://www.circressaha.org. Hibernating myocardium was produced in pigs by chronic instrumentation of the left anterior descending artery (LAD) with a 1.5-mm (ID) Delrin stenosis, as previously described.9,10

Studies were conducted in 16 chronically instrumented animals and in 15 normal controls under isoflurane (1% to 3%) anesthesia supplemented with Telazol (tiletamine/zolazepam)/xylazine (Fort Dodge Animal Health, Fort Dodge, Iowa; IM every 30 to 60 minutes).

Abbreviated Experimental Protocol

Hemodynamic parameters, microsphere flow, and myocardial function (contrast ventriculography) were assessed at rest in the closed-chest state. After median sternotomy, the anterior interventricular vein was catheterized to selectively sample venous blood from the LAD region. Piezoelectric crystals were placed in the anterior wall to quantify segment shortening. Pacing was performed with a bipolar lead on the right atrial appendage.

In the first protocol, 8 animals with hibernating myocardium and 7 normal controls had hemodynamic parameters, microsphere flow, and myocardial function assessed at rest and during ~15-minute stages of atrial pacing in 30-bpm increments starting at 90 bpm. Attempts to pace at heart rates ≥150 bpm resulted in systemic hypotension and a fall in the determinants of oxygen consumption. Because the change in oxygen consumption over this limited heart rate range was small, a second protocol assessed flow, function, and metabolism during graded β-adrenergic stimulation with epinephrine. In pigs with hibernating myocardium (n = 8), epinephrine was infused intravenously in 15-minute stages to incrementally increase heart rate by ~15 bpm to a maximum rate of ~130 bpm. In normal control animals (n = 8), hemodynamics, flow, and function were assessed at rest and at epinephrine infusions titrated to match heart rates to stages 2 and 4 of the protocol for animals with hibernating myocardium (~105 and ~130 bpm, respectively). When hemodynamics returned to baseline in normal controls, we assessed these parameters during pacing at 90 and 120 bpm. Coronary flow reserve was assessed in all animals during adenosine infusion (0.9 mg · kg⁻¹ · min⁻¹) with phenylephrine confounded (11 ± 1 μg · kg⁻¹ · min⁻¹) to maintain mean arterial pressure.9,10

Regional Ventricular Function

Contrast ventriculography and coronary angiography were performed and quantified as previously described.9,10 Anteroapical wall motion was semiquantitatively scored (3–normal, 2–mild hypokinesis, 1–severe hypokinesis, and 0–akinesis). Segment shortening in the LAD perfusion territory was quantified with subendocardially placed piezoelectric crystals.11 Segment shortening (percentage) was calculated as (end-diastolic length–end-systolic length)/(end-diastolic length) × 100.

Oxygen and Lactate Consumption

Blood gases, total oxygen content (hemoximeter), and whole-blood lactate levels (enzymatic digestion) were quantified by an automated blood gas analyzer (Radiometer Medical A/S). Oxygen and lactate consumption were calculated as the respective differences between arterial and anterior interventricular vein contents multiplied by full-thickness perfusion measured by the microsphere technique.

Microsphere Perfusion and Myocardial Sampling

Regional flow was measured with 15-μm fluorescent microspheres (Triton Inc), as previously described.8 After the protocols, hearts were arrested with KCl and excised for sampling. A midventricular ring was divided into 12 wedges, each of which was subdivided into subendocardial, midmyocardial, and subepicardial thirds. Samples were weighed and processed for flow quantification as detailed in the online data supplement. Four concentric rings were immersed in triphenyltetrazolium chloride (TTC), and those with evidence of infarction were scanned. Infarction was quantified by digital planimetry and expressed as a percentage of left ventricular (LV) mass.12 Necrosis was not directly quantified in the myocardial ring used for microsphere flow measurements.

Data Analysis

Data are reported as the mean±SEM. Hemodynamic data were continuously digitized and averaged over 30 seconds (Heme, Notocord). Comparisons between the LAD and the normally perfused remote region (Remote) were compared using paired t tests. Differences between hibernating and normal controls (Normal) during pacing or epinephrine were compared using a two-way ANOVA. Dunnett’s test was used for post hoc comparisons. A value of P≤0.05 was considered significant.

Results

At the time of study, there were no differences in weight between the instrumented (86±7 kg) and normal groups (79±4 kg, P = NS). Blood gas and hemoglobin values for instrumented animals (pH, 7.40±0.01; PCO₂, 47±1 torr; PO₂, 484±9 torr; and Hb, 10.8±0.3 g/dL) and normal controls (pH, 7.40±0.01; PCO₂, 44±1 torr; PO₂, 424±17 torr; and Hb, 11.5±0.3 g/dL) were normal. Seven of the 16 animals with hibernating myocardium had evidence of necrosis by TTC staining. Necrosis in animals with hibernating myocardium averaged 0.5±0.2% of LV mass.

Flow, Function, and Metabolism in Hibernating Myocardium at Rest (n = 16)

Systemic hemodynamics and the results of ventriculography in the closed-chest resting state are presented in Table I. Heart rate and systolic pressure were similar in the two groups of animals, but LV end-diastolic pressure (LVEDP) was higher in animals with hibernating myocardium. In instrumented animals, the average LAD stenosis was 93±2% with complete occlusion and collateral-dependent myocardium in 9 of 16. There was regional and global dysfunction, with severe anteroapical hypokinesis.
and reductions of ejection fraction and peak positive and peak negative dP/dt (Table 1).

Regional dysfunction in chronically instrumented animals was associated with reduced resting perfusion, consistent with hibernating myocardium (Figure 1). Compared with the remote region, resting flow was significantly reduced in each transmural layer with the greatest reduction in the subendocardium (24%). LAD subendocardial and full-thickness flow were also significantly reduced compared with normal controls. Hibernating myocardium exhibited a severe reduction in regional flow reserve, with full-thickness flow during adenosine markedly reduced compared with normal controls (1.73±0.22 versus 5.54±0.34 mL · min⁻¹ · g⁻¹, P<0.001, Figure 2). Subendocardial flow during adenosine was critically impaired with no increase over resting levels (0.91±0.17 versus 0.74±0.05 mL · min⁻¹ · g⁻¹, P=NS).

Resting hemodynamics, function, and metabolism under open-chest conditions are summarized in Table 2. Like the closed-chest state, there were no differences in heart rate or systolic pressure between groups, but LVEDP was higher in animals with hibernating myocardium. Hibernating myocardial dysfunction and metabolic responses to submaximal pacing stimulation (n=8) were also characterized by a 60% reduction in subendocardial segment shortening (9.2±2.2% versus 23.5±1.1%) and significant reductions in peak positive and peak negative dP/dt. Full-thickness perfusion in the hibernating LAD region was reduced compared with normal controls (0.66±0.04 versus 1.03±0.08 mL · min⁻¹ · g⁻¹, P<0.01).

Oxygen consumption was reduced ∼20% in hibernating myocardium (63±3 versus 77±6 μL · g⁻¹ · min⁻¹, P<0.05), but lactate uptake was present in every animal with no difference between groups (0.7±0.1 versus 0.7±0.1 μmol · g⁻¹ · min⁻¹). There were no differences in any resting parameter between animals with and without evidence of necrosis.

Physiological and Metabolic Responses to Submaximal Pacing Stimulation (n=8)

Flow, function, and metabolic responses of hibernating myocardium were initially evaluated with atrial pacing from 90 to 120 bpm (Figure 3). At each pacing rate, peak positive dP/dt, LAD segment shortening, oxygen consumption, and full-thickness flow in hibernating myocardium were reduced compared with normal controls. Flow was also reduced in the hibernating subendocardium at both 90 (0.58±0.07 versus 1.30±0.10 mL · min⁻¹ · g⁻¹, P<0.01) and 120 bpm (0.84±0.06 versus 1.37±0.09 mL · min⁻¹ · g⁻¹, P<0.01). LAD segment shortening did not increase in hibernating or normal myocardium,11 but there was a small reduction in end-diastolic length in both groups (Hibernating, 19.6±1.0 versus 19.4±1.0 mm, P<0.05; Normal, 24.0±1.6 versus 23.2±1.5 mm, P<0.01). Both groups demonstrated a trend toward an increase in oxygen consumption, with no metabolic evidence of ischemia in any animal. Lactate uptake persisted and there was no change in coronary venous pH (Hibernating, 7.35±0.01 to 7.34±0.01; Normal, 7.34±0.01 to 7.34±0.01).

### Table 1. Closed-Chest Hemodynamics and Ventriculography at Rest

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate</th>
<th>Systolic Pressure</th>
<th>LVEDP</th>
<th>Left Ventricular dP/dt, min Hg/s</th>
<th>Ejection Fraction</th>
<th>Wall Motion Score</th>
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<td>mm Hg</td>
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<tr>
<td>Hibernating</td>
<td>16</td>
<td>79±3</td>
<td>129±3</td>
<td>24.1±1.2</td>
<td>1268±57*</td>
<td>0.44±0.02*</td>
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<tr>
<td>Normal</td>
<td>15</td>
<td>79±2</td>
<td>133±3</td>
<td>17.9±1.5</td>
<td>1525±81</td>
<td>0.60±0.03*</td>
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*P<0.05 vs Normal.

Figure 1. Resting flow in animals with hibernating myocardium and normal controls. In normal controls (n=15, right), there were no regional differences in the transmural distribution of resting perfusion. In contrast, among instrumented animals (n=16, left), there were reductions in resting flow in each transmural layer of the LAD distribution (gray circles) compared with the normally perfused remote myocardium (black squares), consistent with hibernating myocardium. Subendocardial and full-thickness flow in hibernating myocardium were also reduced compared with normal controls. Endo indicates subendocardium; Mid, midmyocardium; Epi, subepicardium; and FT, full thickness.

Figure 2. Rest and vasodilated flow in hibernating and normal myocardium. In normal controls (n=15, right), adenosine vasodilation (black squares) resulted in ∼6-fold increase in LAD flow over resting values (gray circles). However, in hibernating myocardium (n=16, left), vasodilated flow reserve was severely reduced with only a 2.5-fold increase in full-thickness flow (FT). Significant reductions in vasodilated flow were present in each transmural layer, with flow in the subendocardium (Endo) unable to increase over resting levels. Mid indicates midmyocardium; Epi, subepicardium.
Attempts to pace at higher rates resulted in a reduction in the determinants of oxygen consumption in both groups of animals. Nevertheless, even at pacing rates of 150 bpm, there was no lactate production in any animal (data not shown).

Flow, Function, and Metabolism at Graded Levels of Epinephrine Infusion (n=8)

Because the range of metabolic stress produced by atrial pacing was limited, additional studies were performed during β-adrenergic stimulation with epinephrine. Hemodynamics, flow, function, and metabolism during graded epinephrine infusion are summarized in Figure 4. Arterial and venous PO₂ and oxygen contents were similar between groups at rest and during epinephrine stimulation (see online data supplement). Heart rate responses were identical in the two groups, and there were no differences in epinephrine dose between groups at any stage (see online data supplement). During epinephrine, systolic blood pressure in the hibernating group was slightly greater than normal controls. At the maximum epinephrine infusion (E₄), LVEDP was higher in animals with hibernating myocardium (14.3±2.3 versus 8.2±1.5 mm Hg in controls, P<0.05). In normal controls, β-adrenergic stimulation produced progressive increases in function, full-thickness perfusion, oxygen consumption, and lactate uptake. Responses in hibernating myocardium were markedly attenuated, with blunted increases in function, flow, and metabolism. At each level of epinephrine stimulation, segment shortening, LAD perfusion, and oxygen consumption were reduced in hibernating myocardium compared with normal controls. However, there was no metabolic evidence of

<table>
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<th>TABLE 2. Open-Chest Hemodynamics, Function, and Metabolic Parameters at Rest</th>
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<tr>
<td>Heart Rate, bpm</td>
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<td>Hibernating</td>
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<td>Normal</td>
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*P<0.05 vs Normal.

Figure 4. Hemodynamics, function, flow, and metabolism during graded epinephrine infusion. A, Hemodynamics. In animals with hibernating myocardium (n=8, gray circles), epinephrine increased heart rate by 10 to 15 bpm in each of four stages (E₁ through E₄). Normal controls (n=8, black squares) received two doses of epinephrine corresponding to E₂ and E₄. Both groups demonstrated progressive increases in +dP/dt, although at any infusion rate values were reduced in animals with hibernating myocardium. B, Function and flow. In contrast to the responses of normal myocardium to graded epinephrine infusion, increases in flow and function were blunted in hibernating myocardium. At comparable levels of stimulation, segment shortening, subendocardial, and full-thickness flow in hibernating myocardium were reduced compared with normal controls. C, Metabolic parameters. In hibernating myocardium during epinephrine stimulation, increases in O₂ consumption and lactate uptake were blunted compared with normal myocardium. However, there was no evidence of acute myocardial ischemia as lactate uptake persisted and venous pH was similar in both groups.
ischemia during stress, as regional function never deteriorated, lactate uptake progressively increased, and coronary venous pH was similar to normal controls (peak dose, Hibernating, 7.27±0.01; Normal, 7.28±0.01, P=NS). The functional and metabolic responses to epinephrine were similar in animals with and without evidence of necrosis.

**Flow, Function, and Metabolism at the Peak Functional Response**

Segment shortening responses to β-adrenergic stimulation were variable among individual animals, but no animal demonstrated a biphasic response (improvement in function at low levels but functional deterioration at higher epinephrine doses). To determine whether averaging of function may have obscured potential differences from rest, a subset analysis was performed to evaluate the data on the basis of the peak functional response for each animal (Figure 5). When the maximum improvement in function was evaluated compared with baseline, the increase in regional function in hibernating myocardium was significant (10.8±2.4% to 17.3±3.1%, P<0.05). At peak LAD function, there were no differences in heart rate (Hibernating, 115±7 versus Normal, 120±6 bpm; P=NS) or epinephrine dose (Hibernating, 0.16±0.06 versus Normal, 0.16±0.02 μg·kg⁻¹·min⁻¹; P=NS) between groups. At the peak level of function, hibernating myocardium demonstrated an almost 50% increase in full-thickness perfusion (0.95±0.07 versus 0.65±0.07 mL·min⁻¹·g⁻¹, P<0.01) and a commensurate increase in oxygen consumption (90±6 versus 63±6 μL·g⁻¹·min⁻¹, P<0.01). There was also a significant increase in lactate uptake in hibernating myocardium (0.7±0.1 versus 1.2±0.1 μmol·g⁻¹·min⁻¹, P<0.05), with no regional lactate production in any animal. The increase in subendocardial flow was not statistically significant (0.62±0.06 to 0.84±0.10 mL·min⁻¹·g⁻¹, P=0.11). Thus, hibernating myocardium retained metabolic reserve, demonstrating an ability to increase oxygen consumption from a depressed resting level in response to submaximal β-adrenergic stimulation.

**Relation Among Myocardial Lactate Uptake, Oxygen Delivery, and Oxygen Consumption**

Figure 6 summarizes the relationship between myocardial lactate uptake, oxygen delivery, and oxygen consumption in animals with hibernating myocardium versus normal controls. Although hibernating myocardium was associated with depressed levels of oxygen consumption at all workloads, the relationship between oxygen delivery and myocardial oxygen consumption was similar to that of normal myocardium. The blunted increase in oxygen consumption was not related to impaired oxygen delivery during submaximal metabolic stimulation since lactate uptake increased in response to stress.

**Discussion**

There are several important new findings from our study. First, hibernating myocardium was accompanied by a re-
regional downregulation in oxygen consumption at rest that was commensurate with reductions in resting flow and subendocardial function assessed by segment shortening. These changes were not a reflection of acute ischemia, as myocardial lactate extraction and coronary venous pH were normal. Although the functional response of hibernating myocardium after increases in the external determinants of myocardial demand was blunted, hibernating myocardium retained the ability to increase oxygen consumption in response to stress. Importantly, oxygen consumption increased in the absence of metabolic ischemia since lactate uptake increased and subendocardial function did not deteriorate. This contrasts with the critical match between flow, function, and oxygen consumption in short-term hibernation where there is an immediate deterioration of metabolism with lactate release developing after transient β-adrenergic stimulation.6 Collectively, these findings support the hypothesis that intrinsic adaptations characteristic of chronic hibernating myocardium serve to downregulate regional energy utilization so as to prevent an imbalance between supply and demand during submaximal increases in the external determinants of myocardial oxygen consumption.

Comparison to Metabolic Responses of Short-Term Hibernation

Previous studies have demonstrated that the myocardium is able to adapt to moderate levels of ischemia by acutely downregulating function in the face of limited coronary blood flow.13 This acute adaptive response, termed “short-term hibernation,” is able to preserve myocardial viability over a time frame considerably longer than that after a total occlusion in the absence of collateral perfusion. Previous studies have demonstrated that this adaptive response is characterized by an acute downregulation in metabolism. For example, Fedele et al14 showed that acute moderate ischemia leads to stable reductions in flow, function, and oxygen consumption for up to 3 hours. While ischemia was initially accompanied by marked lactate release, this returned to net lactate uptake within 3 hours, with a return of coronary venous pH toward normal. Although metabolic evidence of ischemia diminished, the magnitude of lactate uptake remained significantly lower than baseline. Pantely et al15 subsequently demonstrated that creatine phosphate and ATP levels fall rapidly during the early phase of moderate ischemia, but creatine phosphate was regenerated and ATP levels stabilized despite a persistent reduction in flow. These observations support the notion that there was an acute adaptive downregulation of myocardial energy utilization that could restore a balance between a limited supply and demand over a longer time frame than possible after total coronary occlusions.

Short-term hibernation is a favorable adaptive response that preserves myocardial viability at the expense of contractile function. Nevertheless, it is unable to allow the heart to respond metabolically to stress and ultimately, cannot prevent the development of irreversible tissue injury. Schulz and colleagues showed that β-adrenergic stimulation in the setting of short-term hibernation could transiently elicit contractile reserve at the expense of metabolic evidence of ischemia reflected by recurrent lactate release and further deterioration of high-energy phosphate levels.6 When this was continued for a period of hours, myocardial infarction developed leading to the notion that short-term hibernation was an extremely tenuous adaptation.7 While tissue viability could be maintained for at least several hours in the setting of moderate ischemia, subendocardial necrosis was consistently observed when moderate flow reductions were maintained for ≥24 hours.3–5

Although the resting reductions in flow, function, and oxygen consumption in chronic hibernating myocardium are similar to these acute responses, lactate metabolism and the response to submaximal metabolic stress are markedly different. During steady-state increases in the external determinants of demand, myocardial oxygen consumption increased from depressed levels yet remained lower than normal responses. In contrast to lactate release characteristic of short-term hibernation, lactate uptake increased during β-adrenergic stimulation and pacing in chronic hibernating myocardium. This was in large part related to the ability of flow to increase from the depressed resting levels. Thus, the adaptive downregulation characteristic of chronic hibernating myocardium allows the heart to increase oxygen consumption without developing an immediate supply-demand imbalance or deterioration of aerobic metabolism.

Metabolism in Viable Chronically Dysfunctional Myocardium in Humans

To our knowledge, the present study, using selective venous catheterization, provides the first direct confirmation that oxygen consumption is reduced in hibernating myocardium. Our findings are consistent with those of Mills et al,16 who demonstrated that resting oxygen consumption was decreased in a similar model of chronic coronary stenosis. The relative reductions in oxygen consumption are also consistent with estimates of oxygen consumption in patients with hibernating myocardium. For example, Vanoverschelde et al17 used positron emission tomography (PET) to study patients with chronic coronary occlusion, no history of infarction, and viability confirmed by preserved uptake of the glucose analogue 18F-2-deoxyglucose (FDG). Estimates of oxygen consumption were derived from the myocardial clearance of 18F-acetate. Collateral-dependent myocardium with abnormal regional wall motion had an ≈20% reduction in resting blood flow compared with remote normal myocardium (0.77±0.06 versus 0.95±0.07 mL·min⁻¹·g⁻¹, P<0.001). Among dysfunctional segments, oxidative metabolism was reduced by 28% (0.049±0.005 versus 0.068±0.007 per minute, P<0.001). Similar quantitative results have also been reported in viable segments after myocardial infarction.18,19

Interestingly, oxygen consumption has also been shown to be reduced in dysfunctional segments with normal resting flow or chronically stunned myocardium,20 but in collateral-dependent myocardium with normal wall motion, resting perfusion and oxygen consumption were normal.17 The similarity of oxygen consumption in pigs with hibernating myocardium further supports its utility as a valid model of hibernating myocardium in humans. Furthermore, the fact that oxygen consumption was reduced compared with normal controls excludes the possibility that relative reductions in the
estimates of oxygen consumption by PET reflect increases in oxygen utilization and hyperkinesis in normally perfused remote regions.\textsuperscript{17}

The observation that inotropic stimulation can increase regional oxygen consumption in pigs with hibernating myocardium is also consistent with the limited data available from patients with chronic coronary disease. For example, dobutamine stimulation increased regional function and oxygen consumption in dysfunctional segments of patients with severe ischemic cardiomyopathy.\textsuperscript{20} Even among segments with no contractile reserve (62\% of which were considered viable), dobutamine resulted in a regional increase in flow and oxygen consumption. Similar findings were reported in viable segments of patients with anterior infarction.\textsuperscript{19} Despite reduced oxygen consumption at rest, dobutamine stimulation only increased oxygen consumption in regions that showed improvement in function after revascularization. In fact, oxygen consumption actually decreased in nonviable segments.\textsuperscript{19}

**Relation Between Perfusion and Oxygen Consumption at Rest and During Stress**

An important correlate of our observations is that reduced flow at rest is dissociated from metabolic evidence of ischemia in hibernating myocardium. Although differences in perfusion between normal and hibernating regions increase in response to submaximal stress,\textsuperscript{8} metabolism does not deteriorate as during acute ischemia in normal myocardium. Evaluation of the relationship between lactate uptake, oxygen delivery, and oxygen consumption (Figure 6) supports the notion that hibernating myocardium operates over a lower range of the oxygen supply-demand relationship. Although flow and oxygen consumption are reduced at rest, increases in metabolism are met by appropriate increases in oxygen delivery and lactate uptake. Other laboratories have demonstrated attenuation of metabolism that is not limited by oxygen delivery in pacing-induced heart failure,\textsuperscript{21} left ventricular remodeling,\textsuperscript{22} and decompensated hypertrophy.\textsuperscript{23} Hibernating myocardium appears to have a similar regional cellular phenotype as these global states, characterized by impaired energy utilization, cellular hypertrophy, and reductions in the sarcoplasmic reticulum calcium uptake proteins.\textsuperscript{10,24} The similarity of the cellular, molecular, and metabolic responses raises the possibility that they reflect a common myocardial response to stress. Further studies will be required to elucidate the specific bioenergetic mechanisms responsible for these changes in hibernating myocardium.

**Methodological Limitations**

In the present study, it was not possible to directly measure subendocardial oxygen consumption. Therefore, we cannot address the potential influences of transmural differences in myocardial metabolism or admixtures of normal and hibernating myocardium in the LAD perfusion territory. While unlikely, the measured increase in oxygen consumption could have resulted solely from increases in subepicardium or from an admixture of blood from hibernating and normal regions. Although the possibility of subendocardial ischemia during inotropic stimulation cannot be excluded, it is unlikely given the fact that subendocardial function\textsuperscript{25,26} and coronary venous lactate release averaged across the myocardial wall\textsuperscript{26} are sensitive indices of subendocardial hyperfusion. The absence of any reduction in myocardial lactate uptake and the lack of functional deterioration strongly support our hypothesis that the subendocardium was not metabolically ischemic. However, the ischemic threshold of chronic hibernating myocardium was not assessed in this investigation, and regional lactate production almost certainly would occur at higher levels of stress or perhaps with different stimuli. Differences in the metabolic response to graded versus transient \(\beta\)-adrenergic stimulation could also account for the differences in contractile reserve in pigs with hibernating myocardium and will require further study.\textsuperscript{8} Finally, our results do not address the mechanism(s) of functional and metabolic downregulation in hibernating myocardium, which may include alterations in \(\beta\)-adrenergic receptor density,\textsuperscript{7,27} affinity, and signal transduction.\textsuperscript{28}

**Clinical Implications**

Our results are consistent with the hypothesis that chronic hibernating myocardium represents an adaptive state where function and metabolism are downregulated to limit the development of metabolic ischemia in response to submaximal increases in the external determinants of myocardial workload. Thus, while blood flow is reduced at rest and the difference in perfusion between normal and hibernating myocardium increases during stress, it is not truly an ischemic state. An adaptive uncoupling between perfusion, metabolism, and function may be responsible for the lack of progressive functional and pathological deterioration of hibernating myocardium.\textsuperscript{29}

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Hibernating Myocardium Retains Metabolic and Contractile Reserve Despite Regional Reductions in Flow, Function and Oxygen Consumption at Rest

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Short title – Metabolic Reserve in Hibernating Myocardium

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ONLINE SUPPLEMENTAL METHODS AND RESULTS

Studies were conducted in 16 chronically instrumented animals after 121±7 days, and in 15 normal controls. Anesthesia was induced with Telazol (tiletamine/zolazepam)/xylazine and, after endotracheal intubation, maintained with isoflurane (1-3%). Telazol/xylazine was repeated every 30-60 minutes. A catheter was placed in the left atrium for pressure monitoring and microsphere injection. LV pressure was measured with a catheter-tipped micromanometer (Millar Instruments). Arterial pressure and microsphere reference samples were taken from a femoral artery. Pharmacological agents and fluids (0.9% NaCl) were infused through a jugular vein. Pigs were heparinized (100 units/kg) and hemodynamics equilibrated for ~30 minutes before the protocol.

Experimental Protocol

In the first protocol, physiologic parameters were assessed in 8 animals with hibernating myocardium and 7 normal controls during graded atrial pacing. Right atrial pacing was initiated at 90 bpm and increased in 30 bpm increments. After allowing ~10 minutes for equilibration, hemodynamic parameters, microsphere flow and regional function were assessed. All measurements and arterial and selective LAD venous blood were obtained within ~5 minutes. Attempts to pace at heart rates ≥150 bpm resulted in systemic hypotension and a fall in the determinants of oxygen consumption in both groups of animals.

β-adrenergic stimulation with epinephrine was performed on a second group of 8 pigs with hibernating myocardium. A graded infusion of epinephrine was titrated to increase heart rate by ~15 bpm. During each stage, parameters were allowed to equilibrate for ~10 minutes. Over the next 5 minutes, a microsphere measurement was performed, arterial and selective LAD venous blood was obtained, and hemodynamics and regional function were recorded. In animals with hibernating myocardium, four stages were performed to a maximum rate of ~130 bpm. One animal received only three epinephrine doses but the data at peak dose was used for both Stages 3 and 4. In 8 normal control animals, epinephrine was titrated to achieve heart rates equivalent to stages 2 and 4 of the protocol for
animals with hibernating myocardium (~105 and ~130 bpm, respectively). The epinephrine dose for each stage and the corresponding arterial and venous pO\textsubscript{2} and oxygen content values are shown in Table 1. When hemodynamics returned to baseline, these parameters were also assessed during atrial pacing at 90 and 120 bpm.

**Myocardial Function and Stenosis Severity**

Global and regional left ventricular function was assessed in the closed chest state using contrast ventriculography. Ejection fraction was determined by the area-length method and anteroapical wall motion was semi-quantitatively scored (3-normal, 2-mild hypokinesis, 1-severe hypokinesis, 0-akinesis)\textsuperscript{1,2}. Coronary angiography was performed by selective catheterization of the left main coronary artery and LAD stenosis severity (% diameter) determined by caliper measurements as previously described\textsuperscript{1,2}.

Segment shortening in the LAD perfusion territory was quantified with subendocardially-placed piezoelectric crystals\textsuperscript{3} distal to the second diagonal branch and aligned to the subendocardial fiber plane. End-diastole and end-systole were defined from micromanometer pressure tracings as the onset of +dP/dt and 20msec prior to peak –dP/dt, respectively. Segment shortening (%) was calculated as (end-diastolic length – end-systolic length)/(end-diastolic length) • 100.

**Blood Gas Methods**

Paired arterial and coronary venous blood samples were analyzed with an ABL System 605 with OSM 3 Hemoximeter (Radiometer Medical A/S). Standard blood gas values were quantified with calibrated pH, O\textsubscript{2} and CO\textsubscript{2} electrodes. Total hemoglobin was quantified by spectrophotometry, corrected for the pig. Total oxygen content (Table 1) was determined by the product of hemoglobin concentration • fraction of saturated hemoglobin • 1.39 (ml O\textsubscript{2} per gram hemoglobin). Whole blood lactate levels were quantified by lactate oxidase metabolism. Regional oxygen and lactate consumption were calculated as the respective differences between arterial and anterior interventricular vein contents multiplied by full-thickness perfusion measured with fluorescent microspheres.
**Myocardial Sampling and Infarct Quantification**

At the conclusion of the protocol, the heart was excised and the left ventricle sectioned into multiple concentric rings. A ring from the apical third of the left ventricle including the segment shortening crystals was used for quantification of microsphere flow. The rings immediately apical and basal to the flow ring, a basal ring, and the apex were immersed in 1% triphenyltetrazolium chloride in phosphate buffer warmed to 37°C. After incubation, the rings were transferred to buffered formalin (Z-fix), and digitally scanned the following day to improve contrast between normal and infarcted myocardium. Necrosis was quantified by digital planimetry. Approximate mass was quantified using the trapezoid rule and expressed as % of left ventricular mass. Necrosis was present in 7 of the 16 animals with hibernating myocardium, involving 0.5-2.4% of the left ventricle.

**Microsphere Perfusion**

Regional myocardial flow was measured with 15µm fluorescent microspheres (Triton Inc.). Approximately 2 million fluorescence-labeled microspheres were injected into the left atrium ~10 minutes into each stage. An arterial reference sample was started immediately before injection and continued for 90 seconds. Myocardial samples from a mid-ventricular ring were digested in 4M KOH with 1% Tween 80 and filtered. Dyes were eluted with a measured volume of di-(ethylene glycol) ethyl ether acetate (CAS# 112-15-2) and analyzed with a luminescence spectrometer (LS-50, Perkin Elmer). Excitation/emission wavelength pairs were set to minimize spectral interactions among an 8 fluorescent dye set. Samples corresponding to the LAD and the normally perfused remote region were identified by assessing the circumferential distribution of flow during adenosine vasodilation. Reported values are the weighted-mean of all samples within each region.
Table 1. Arterial and Venous pO$_2$, O$_2$ Content and Epinephrine Dose.

<table>
<thead>
<tr>
<th></th>
<th>Hibernating (n=8)</th>
<th>Normal (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arterial</td>
<td>Venous</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>pO$_2$</td>
<td>O$_2$ Content</td>
</tr>
<tr>
<td>Dose (µg/kg/min)</td>
<td>(torr)</td>
<td>(ml/dl)</td>
</tr>
<tr>
<td>Rest</td>
<td>435±47</td>
<td>16.6±0.6</td>
</tr>
<tr>
<td>Epi #1</td>
<td>0.03±0.01</td>
<td>453±40</td>
</tr>
<tr>
<td>Epi #2</td>
<td>0.06±0.01</td>
<td>448±48</td>
</tr>
<tr>
<td>Epi #3</td>
<td>0.12±0.02</td>
<td>436±51</td>
</tr>
<tr>
<td>Epi #4</td>
<td>0.21±0.05</td>
<td>397±53</td>
</tr>
</tbody>
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