Myocardial Metabolism During Increased Work States in the Porcine Left Ventricle In Vivo

Barry M. Massie, Gregory G. Schwartz, Jorge Garcia, Judith A. Wisneski, Michael W. Weiner, Twyman Owens

It is not known whether myocardial energy requirements can be increased to the degree that they exceed myocardial O₂ availability in the absence of abnormalities of coronary blood flow or coronary reserve. To determine whether this form of "demand ischemia" occurs, 10 swine were subjected to pressure overload induced by aortic constriction, inotropic and chronotropic stimulation by dobutamine, and the combination of these interventions. In an additional 9 animals, intravenous adenosine was administered during the combination of constriction and dobutamine to determine whether further increases in coronary flow could be achieved and if they would attenuate the metabolic changes. Left ventricular anterior wall transmural blood flow was measured by radioactive microspheres. Energy phosphates were assessed by 31P magnetic resonance spectroscopy using the Fourier series window technique to increase the proportion of signal derived from the subendocardium. Myocardial lactate release was quantified independent of net lactate uptake using an isotropic tracer technique. The three interventions produced 39% to 195% increases in myocardial O₂ uptake from control measurements. The phosphocreatine to ATP ratio (PCr/ATP), uncorrected for partial saturation, fell significantly, from 1.39±0.10 at control conditions to 1.25±0.10 with dobutamine alone and 1.15±0.08 with dobutamine plus constriction (P<.05 for both). Myocardial lactate release rose from 0.21±0.03 μmol·g⁻¹·min⁻¹ at control conditions to 0.45±0.05 and 0.59±0.10 μmol·g⁻¹·min⁻¹, respectively (P<.05 for both), with these two interventions. Although transmurally averaged left ventricular blood flow rose from 0.97±0.09 mL·g⁻¹·min⁻¹ at control conditions to 3.25±0.47 mL·g⁻¹·min⁻¹ (P<.001) and subendocardial blood flow increased from 1.02±0.09 to 2.92±0.45 mL·g⁻¹·min⁻¹ (P<.001) at the highest of the three increased work states, the subendocardial to subepicardial flow ratio declined progressively from 1.13±0.08 to 0.87±0.04 (P<.05). With a further increase in aortic constriction, myocardial O₂ uptake and subepicardial blood flow rose, whereas subendocardial blood flow did not change, and there was a further decline in PCr/ATP and a rise in lactate release. Although adenosine increased the average myocardial blood flow during high work state from 3.79±0.91 to 6.29±1.08 mL·g⁻¹·min⁻¹ (P<.001), the further rise in subendocardial flow from 3.08±0.62 to 3.78±0.68 mL·g⁻¹·min⁻¹ was not significant, nor were the accompanying changes in PCr/ATP or lactate metabolism. The progressive decline in the subendocardial to subepicardial ratio and PCr/ATP with a concomitant increase in myocardial lactate release is consistent with the occurrence of demand ischemia in the porcine left ventricle during high work states, and the lack of a significant increase in subendocardial blood flow or attenuation of the metabolic changes suggests limitation of subendocardial coronary reserve under these conditions. (Circ Res. 1994;74:64-73.)

Key Words • myocardial metabolism • magnetic resonance spectroscopy • dobutamine • phosphocreatine • ATP • lactate • myocardial ischemia

Myocardial ischemia occurs when the rate of ATP synthesis by oxidative phosphorylation cannot be sustained at the rate of ATP hydrolysis because of insufficient delivery of O₂. As a result, phosphocreatine (PCr) declines, ADP rises, and anaerobic glycolysis increases. Such metabolic changes may result either from a primary reduction in myocardial blood flow and O₂ delivery (supply ischemia) or from increased myocardial energy requirements when coronary stenosis prevents a commensurate rise in myocardial blood flow (mixed supply-demand ischemia). However, it is not known whether myocardial energy requirements can be increased to the degree that they exceed myocardial O₂ availability in the absence of coronary stenosis or other abnormalities of the coronary circulation—a condition that could be termed pure demand ischemia. As with supply ischemia and mixed supply-demand ischemia, pure demand ischemia should also be associated with reductions in high-energy phosphates, increased myocardial lactate release, and relative hypoperfusion of the subendocardium. However, in pure demand ischemia, these metabolic alterations would be expected to occur despite substantial increases in the absolute transmural and subendocardial blood flow.

The goal of the present study was to determine whether pure demand ischemia occurs in the porcine left ventricle in response to a combination of left ventricular pressure overload and inotropic stimulation and whether such changes can be attenuated by pharmacologic coronary vasodilation during adenosine infusion. Myocardial energy phosphates were assessed by 31P magnetic resonance spectroscopy (13P-MRS), glycolytic metabolism by isotope dilution measurements of myocardial lactate release, and transmural blood flow by radioactive microspheres.

Materials and Methods

Experimental Preparation

Female adolescent Yorkshire-Landrace swine, weighing 33 to 45 kg, were studied in accordance with the Guidelines of the American Physiological Society for Animal Use. The prepara-
After these control measurements at the spontaneous heart rate, additional recordings of blood pressures and segment shortening were made at atrially paced rates of 100 to 150 beats per minute in increments of 10 beats per minute to examine segment shortening at heart rates comparable to those subsequently obtained during catecholamine infusion.

Each measurement was then repeated under three conditions: (1) constriction of the proximal ascending aorta, which was maintained to produce a systolic pressure gradient of 50 mm Hg between the left ventricle and aortic arch, (2) dobutamine infusion at 15 \( \mu \)g \cdot \text{kg}^{-1} \cdot \text{min}^{-1}, \) and (3) the combination of aortic constriction plus dobutamine infusion. Each condition was maintained for approximately 40 minutes (10 minutes to achieve steady-state hemodynamic changes plus 20 minutes for \( ^{31} \text{P-MRS} \) plus 10 minutes for blood flow and biochemical measurements). In five animals, the constriction was released, dobutamine was discontinued, and a set of measurements was obtained after a 1-hour recovery period. In the remaining five animals, the aortic constriction was then increased until the distal aortic systolic pressure declined to 80 mm Hg, and an additional set of measurements was obtained.

Protocol 2. An additional nine animals were studied using a protocol in which coronary flow and metabolic changes with pharmacologically induced vasodilation were examined in the basal state and during the combination of dobutamine infusion and aortic constriction, as described above. After baseline measurements, adenosine was administered intravenously at a dosage titrated to maintain mean aortic pressure proximal to the site of aortic constriction at 60 mm Hg (0.73 ± 0.18 \( \mu \)g \cdot \text{kg}^{-1} \cdot \text{min}^{-1}). Measurements were repeated, adenosine was discontinued, and a 60-minute recovery period was allowed; then dobutamine and aortic constriction were begun. After measurements at this high-work state condition, adenosine was again infused in the same dosage, and a final set of measurements was performed.

Measurements

\( ^{31} \text{P-MRS} \). Measurements were performed using a Philips Gyroscan research system (1.0-m bore) operating at 2 T (Philips Medical Systems, Best, The Netherlands), using previously described techniques. The magnet was shimmed on cardiac water protons to a line width of less than 35 Hz. The pulse length was chosen to provide maximal weighting of the signal from the subendocardium relative to the subepicardium, based on computer modeling of the surface coil radiofrequency field and an estimated wall thickness of 11 mm. A repetition rate of 3 seconds was used, and 200 transients were collected for each spectrum. In addition, further subendocardial weighting was obtained by using the Fourier series window (FSW) localization technique previously described from our laboratory, which enhances the contribution of signal from the inner half of the left ventricular wall.

The summed free induction decays were processed by an exponential multiplication of 15 Hz, a convolution difference of 200 Hz, and phasing with zero and first-order phase correction. The peak areas of PCR and the \( \alpha \)- and \( \beta \)-phosphates of ATP were determined by triangulation using Philips Medical Systems software, and the ratio of the PCR and ATP peak area (PCr/ATP) was determined by dividing the PCr resonance area by the mean of the \( \alpha \)- and \( \beta \)-ATP areas. The ratio of PCr to inorganic phosphate (P) was estimated from the ratio of their peak heights, rather than areas, because overlap of signal from 2,3-diphosphoglycerate of the chamber blood with that of P, resonance precluded determination of the P linewidth. The effect of partial saturation was examined in three animals by determining PCr/ATP in spectra obtained with 3- and 15-second repetition times. Fully relaxed PCr/ATP was found to be 1.4 times greater than the partially saturated ratio, both in one-pulse and FSW acquisitions. The saturation factor was not different under basal versus high work states. However, because saturation factors were not measured in...
every pig, the data are presented without correction for partial saturation.

**Blood flow analysis.** Myocardial blood flow was measured by the radioactive microspheres technique.9 Two to three million 15-μm-diameter radioactive microspheres ([35S]Cr, [54]Mn, [52]Sr, [54]Sn, [56]Ga, and [58]In) were injected into the left atrium for 30 seconds, and a reference blood sample was simultaneously withdrawn from a carotid artery with a calibrated pump at a rate of 11 ml/min for 4 minutes. After formalin fixation, approximately 3-g sections of myocardium were cut from the region immediately below the center of the surface coil and from a second region in the posterolateral left ventricle. Each section was then divided into subendocardial, midwall, and subepicardial layers of equal thickness for determination of the transmural distribution of blood flow. Mean transmural blood flow was calculated by averaging the measurements in the three layers.

**Analyses of O2 Content and Metabolic Substrates.** The O2 content of the arterial and anterior interventricular vein blood samples was determined from the sum of hemoglobin-bound and -dissolved O2 contents, measured by an oximeter calibrated for pig blood (Radiometer OSM3, Copenhagen, Denmark) and a blood gas analyzer (Radiometer ABL-30), respectively. Anterior wall myocardial O2 uptake (MV02 in micromoles per gram per minute) was determined by multiplying the transmurally averaged anterior wall blood flow times the arterial-venous O2 difference.

The methods for measuring glucose, lactate, and free fatty acid concentrations have been described previously.6,10-12 Blood samples for lactate and glucose were mixed immediately with cold 7% perchloric acid and centrifuged. The protein-free filtrate was removed and stored at -4°C for future analysis. Lactate concentration was determined by an enzymatic spectrophotometric method,13 and glucose concentration was measured by the hexokinase/glucose-6-phosphate dehydrogenase-coupled enzymatic method.14 Blood for free fatty acid analysis was placed in iced heparinized glass tubes and centrifuged at 4°C, and the plasma was stored at -4°C. Free fatty acid concentrations were determined using gas chromatography by a modification of the method of Ko and Royer.15 The uptake of these substrates was calculated as the product of the arterial-venous concentration difference times the transmurally averaged anterior wall blood flow.

**Isotopic determination of myocardial lactate release.** Some lactate is released by the myocardium even under nonischemic aerobic conditions, in which there is net uptake.6,10,11 The isotope dilution method enables lactate release to be quantified independent of net lactate uptake. As previously described,6,10,11 a primed continuous infusion of L-[1-14C]lactate (specific activity, 55 mCi/mmol) was administered intravenously to maintain a stable arterial specific activity of [14C]lactate over the study period. A 20-minute stabilization period is needed to allow [14C]lactate to plateau in the arterial circulation and coronary veins. From the simultaneously drawn arterial and coronary venous blood samples, the specific activity of lactate is measured by determining 14C in a scintillation counter from samples in which lactate has been separated by ion-exchange chromatography and measured chemically. A lactate isotopic extraction ratio is defined as follows:

**Arterial Lactate (dpm/mL) - Venous Lactate (dpm/mL)**

The isotopic extraction uptake is then determined by multiplying the arterial chemical lactate concentration by the isotopic extraction ratio and is expressed as micromoles per milliliter. The difference between the myocardial lactate uptake determined from the isotope technique and the arterial-coronary sinus chemical concentration difference is the amount of lactate released in micromoles per milliliter. This value times the transmurally averaged blood flow provides lactate release in micromoles per gram per minute.

**Hemodynamics and sonomicrometry.** Heart rate, blood pressure, and segment length were monitored continuously throughout the experiment and recorded at 100 mm/s on a multichannel recorder (Gould Instruments, Cupertino, Calif) before, during, and after 3P-MRS under each experimental condition to confirm hemodynamic stability. Left ventricular pressures were recorded immediately before and after each spectrum, since it was necessary to disconnect the catheter during spectroscopy to avoid introducing excessive radiofrequency noise. End-diastolic segment length was measured at the initial rise in the positive dP/dt signal, and end-systolic measurements were determined 20 milliseconds before the peak negative dP/dt. Fractional systolic segment shortening was determined as [(end-diastolic length) - (systolic length)]/end-diastolic length and then normalized and expressed as a fraction of shortening fraction recorded at approximately the same heart rate during atrial pacing under control conditions. Measurements made on 10 consecutive beats encompassing at least one full respiratory cycle were averaged.

**Data Analysis**

The significance of differences in the hemodynamic, blood flow, and metabolic measurements between the four experimental conditions (control, aortic constriction, dobutamine, and combined constriction and dobutamine) was determined by repeated-measures ANOVA. If a significant change (P<.05) was identified, Duncan’s multiple-comparison procedure was used to evaluate the significance of differences between individual conditions. A threshold of P<.05 was used to determine the significance of these differences. All data are reported as mean±SEM. All weights are given as wet weights.

**Results**

**Hemodynamic and Left Ventricular Function Measurements**

Hemodynamic and segment-length measurements are presented in Table 1. Aortic constriction caused increases in left ventricular systolic pressure and end-systolic segment length and a decrease in segment shortening. Dobutamine increased heart rate, left ventricular systolic pressure, and segment shortening while decreasing end-diastolic and end-systolic segment lengths. Combined aortic constriction and dobutamine caused essentially additive effects, producing no significant change in segment shortening from control after correction for differences in heart rate. Left ventricular end-diastolic pressure increased significantly only at the highest work rate, state, rising from 11±2 mm Hg at control conditions to 21±3 mm Hg (P<.05).

The product of heart rate times left ventricular systolic pressure rose from the control value by a mean of 40% with constriction, 123% with dobutamine, and 220% with the combination. MVO2 increased by means of 38%, 125%, and 194%, respectively (Table 2).

**Myocardial Blood Flow and O2 Measurements**

Table 2 provides the data on changes in myocardial blood flow and MVO2 in both the anterior and posterior walls. Mean blood flow and flow in each layer rose incrementally with the three interventions, but more so in the subepicardium than in the subendocardium. As is illustrated in Fig 2, despite the 182% increase in subendocardial blood flow at the highest-work state condition, the subendocardial to subepicardial flow ratio declined progressively, from 1.13±0.08 at control
TABLE 1. Hemodynamic and Left Ventricular Function Measurements

<table>
<thead>
<tr>
<th></th>
<th>I: Control</th>
<th>II: Constriction</th>
<th>III: Dobutamine</th>
<th>IV: Constriction + Dobutamine</th>
<th>Additional Significant Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>86±3</td>
<td>107±14</td>
<td>160±8*</td>
<td>167±11*</td>
<td>II vs III, II vs IV</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>123±8</td>
<td>96±6*</td>
<td>138±6</td>
<td>102±7*</td>
<td>II vs III, III vs IV</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>92±6</td>
<td>71±4*</td>
<td>79±4*</td>
<td>65±5*</td>
<td>II vs IV</td>
</tr>
<tr>
<td>LV end-diastolic P, mm Hg</td>
<td>124±9</td>
<td>149±7</td>
<td>166±12*</td>
<td>220±11*</td>
<td>II vs IV, III vs IV</td>
</tr>
<tr>
<td>LV systolic P, mm Hg</td>
<td>11±2</td>
<td>15±2</td>
<td>8±2</td>
<td>21±3*</td>
<td>III vs IV</td>
</tr>
<tr>
<td>End-diastolic length, mm</td>
<td>10.6±0.7</td>
<td>10.8±0.6*</td>
<td>10.0±0.6</td>
<td>10.2±0.6*</td>
<td>II vs III, II vs IV</td>
</tr>
<tr>
<td>End-systolic length, mm</td>
<td>9.1±0.6</td>
<td>10.2±0.8*</td>
<td>8.2±0.6*</td>
<td>8.5±0.5</td>
<td>II vs III, III vs IV</td>
</tr>
<tr>
<td>Shortening fraction</td>
<td>0.15±0.01</td>
<td>0.09±0.02*</td>
<td>0.18±0.01</td>
<td>0.15±0.02</td>
<td>II vs III, III vs IV</td>
</tr>
<tr>
<td>Normalized shortening†</td>
<td>1.0</td>
<td>0.62±0.04*</td>
<td>1.38±0.05*</td>
<td>1.15±0.06</td>
<td>II vs III, III vs IV</td>
</tr>
</tbody>
</table>

*P<.05 vs control.
†Normalized to control measurement at similar heart rate.

Conditions to 0.87±0.05 at the highest work state (P<.05). Similar findings were observed in the posterior wall, where the subendocardial to subepicardial flow ratio declined from 1.04±0.06 to 0.78±0.04 (P<.05).

**^{31}P-MRS Measurements**

Fig 3 shows representative spectra demonstrating the decline in PCR/ATP at the high work state. Table 3 includes the data obtained by **^{31}P-MRS. PCR/ATP declined significantly from 1.46±0.10 at control conditions to 1.35±0.07 at the highest work state with one-pulse acquisitions and from 1.39±0.10 to 1.15±0.08 using the FSW technique. In addition, PCR/P, also fell significantly, although this measurement must be interpreted with caution because of the partial overlap of the P, and 2,3-diphosphoglycerate resonances.

**Myocardial Substrate Utilization**

Table 4 contains the chemical measurements of the arterial and coronary venous substrate concentrations. The arterial concentrations of glucose and lactate did not change significantly during the course of the experiment. However, arterial and venous free fatty acid concentrations increased with dobutamine infusion and aortic constriction plus dobutamine. The coronary venous chemical lactate concentration rose significantly with the combination of constriction plus dobutamine compared with all other conditions. The only significant change in the arterial-venous substrate differences was a narrowing of the transmyocardial chemical lactate difference at the two highest work states. This resulted in a progressive decline in the extraction of chemical lactate, from 45±3% at control conditions to 24±4% at the highest work state. Free fatty acids accounted for the major increase in exogenous substrate uptake, with the work state increasing to approximately eight times the control value at the highest work state (0.240 versus 0.031 μmol g⁻¹ min⁻¹, P<.05). The 62% and 58% increases in glucose and chemical lactate uptake, respectively, did not achieve statistical significance.

Isotopically measured anterior wall lactate release rose progressively with increasing myocardial work. The twofold and threefold increases in lactate release observed with dobutamine and constriction plus dobutamine, respectively, were both significant. Fig 4 juxtaposes the two metabolic markers of ischemia used in the present study. PCR/ATP decreases progressively while myocardial lactate release increases progressively, with the differences reaching statistical significance at the highest work states.

**Recovery and Response to Further Increase in Work State**

In the five animals that were allowed to recover, most measurements, including blood pressure, segment shortening, PCR/ATP, and arterial and venous lactate contents, had returned to control values after a 1-hour period. Heart rate remained above baseline (103±8 versus 88±5 beats per minute, P<.05), and transmurally averaged blood flow tended to be higher (1.17±0.19 versus 0.99±0.08 mL g⁻¹ min⁻¹, P<.10), but the subendocardial to subepicardial blood flow ratio had returned to baseline. These findings indicate that the preparation was stable.

In the remaining five animals, the aortic constriction was increased to a second more severe level during dobutamine infusion. The data from this intervention are shown in Fig 5. MVO₂ rose from 14.1±3.4 μmol g⁻¹ min⁻¹ with constriction plus dobutamine to 17.5±3.9 μmol g⁻¹ min⁻¹ (P<.05) with more severe constriction plus dobutamine. Although mean transmural blood flow rose slightly from 3.6±0.8 to 3.8±0.5 mL g⁻¹ min⁻¹ (P=NS), subendocardial blood flow actually declined slightly from 3.4±0.7 to 3.1±0.5 mL g⁻¹ min⁻¹ (P=NS), as did the subendocardial to subepicardial ratio, from 0.91±0.02 to 0.80±0.10 mL g⁻¹ min⁻¹ (P<.05). These changes were accompanied by a further decline in PCR/ATP (from 1.19±0.08 to 1.03±0.05, P<.05) and a further rise in lactate release (from 0.547±0.091 to 0.875±0.182 μmol g⁻¹ min⁻¹, P=.08).

**Effect of Adenosine Infusion**

Table 5 gives the major findings during the adenosine protocol. Under basal conditions, adenosine caused a marked increase in myocardial blood flow in all layers. During the high work state, blood flow to the subepicardium and midwall rose significantly. However, subendocardial flow rose far less, indicating that coro-
nary reserve was limited in at least part of this layer. Adenosine infusion had no significant effect on either PCr/ATP or lactate release at high work state.

**Discussion**

**Metabolic Changes With Increasing Work State**

Myocardial ischemia may be defined as a decrease or an inadequate increase in myocardial blood flow causing contractile dysfunction and/or an alteration in myocardial metabolism. In most instances, this occurs as a result of a primary reduction of coronary flow, a condition that may be termed supply ischemia. Both in patients with subcritical coronary stenoses and in experimental models with partial coronary stenosis, the metabolic abnormalities of ischemia may also occur without a decrease in flow, or even with a limited increase, under conditions of enhanced O\textsubscript{2} requirements.\textsuperscript{16-23} This combination of impaired blood flow or flow reserve with increased demand represents mixed supply-de-

**TABLE 2. Myocardial Blood Flow and Oxygen Consumption**

<table>
<thead>
<tr>
<th></th>
<th>I: Control</th>
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<th>IV: Constriction + Dobutamine</th>
<th>Additional Significant Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ant BF, mL·g\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>0.97±0.09</td>
<td>1.36±0.13</td>
<td>2.17±0.23*</td>
<td>3.12±0.45*</td>
<td>II vs III, II vs IV, III vs IV</td>
</tr>
<tr>
<td>Ant Endo BF, mL·g\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>1.03±0.10</td>
<td>1.38±0.13</td>
<td>2.13±0.22*</td>
<td>2.90±0.43*</td>
<td>II vs II, II vs IV</td>
</tr>
<tr>
<td>Ant Endo/Epi BF</td>
<td>1.13±0.08</td>
<td>1.05±0.05</td>
<td>0.95±0.04*</td>
<td>0.87±0.04*</td>
<td>II vs IV</td>
</tr>
<tr>
<td>Post BF, mL·g\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>0.97±0.09</td>
<td>1.42±0.13*</td>
<td>2.22±0.28*</td>
<td>3.37±0.52*</td>
<td>II vs IV, III vs IV</td>
</tr>
<tr>
<td>Post Endo BF, mL·g\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>1.01±0.09</td>
<td>1.37±0.14*</td>
<td>2.07±0.27</td>
<td>2.94±0.48*</td>
<td>II vs IV, III vs IV</td>
</tr>
<tr>
<td>Post Endo/Epi BF</td>
<td>1.04±0.06</td>
<td>0.95±0.04*</td>
<td>0.89±0.03*</td>
<td>0.78±0.05*</td>
<td>II vs IV, III vs IV</td>
</tr>
<tr>
<td>Art O\textsubscript{2} content, pmol/mL</td>
<td>6.7±0.6</td>
<td>6.6±0.9</td>
<td>7.1±0.7</td>
<td>6.9±0.9</td>
<td></td>
</tr>
<tr>
<td>Ven O\textsubscript{2} content, pmol/mL</td>
<td>1.4±0.4</td>
<td>1.3±0.5</td>
<td>1.7±0.5</td>
<td>1.3±0.5</td>
<td></td>
</tr>
<tr>
<td>A-V O\textsubscript{2} difference, pmol/mL</td>
<td>5.3±0.2</td>
<td>5.3±0.3</td>
<td>5.4±0.25</td>
<td>5.5±0.3</td>
<td></td>
</tr>
<tr>
<td>MV\textsubscript{O\textsubscript{2}}, pmol·g\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>5.1±0.5</td>
<td>7.0±0.6</td>
<td>11.5±1.1*</td>
<td>14.9±1.2*</td>
<td>II vs III, II vs IV, III vs IV</td>
</tr>
</tbody>
</table>

Ant indicates anterior wall; BF, blood flow; Endo, subendocardial; Epi, subepicardial; Post, posterior wall; Art, arterial; Ven, coronary venous; A-V, arterial-coronary venous; and MV\textsubscript{O\textsubscript{2}}, myocardial O\textsubscript{2} uptake (anterior wall).

*P < .05 vs control.

**Discussion**

To date, neither clinical nor experimental evidence of myocardial ischemia has been demonstrated when myocardial energy demand is increased in the absence of coronary artery stenosis or other abnormalities of the coronary circulation, such as those produced by small vessel disease or left ventricular hypertrophy.

The findings of the present study suggest that the phenomenon of pure demand ischemia may occur during marked increases in left ventricular work state. With the combination of dobutamine plus aortic constriction, there was a significant decline in PCr/ATP, a rise in coronary venous lactate, a decrease in the extraction of lactate, and increased myocardial lactate release. These metabolic changes suggest inadequate oxidative phosphorylation and increased anaerobic glycolysis and are characteristic of myocardial ischemia. Although the changes in PCr/ATP and lactate release achieved sta-
Table 3. Measurements Assessed by 31P Magnetic Resonance Spectroscopy

<table>
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<tbody>
<tr>
<td>PCr/ATP</td>
<td>1.46±0.10</td>
<td>1.54±0.08</td>
<td>1.39±0.08</td>
<td>1.35±0.07*</td>
<td>II vs IV</td>
</tr>
<tr>
<td>PCr/ATP (FSW)</td>
<td>1.39±0.10</td>
<td>1.33±0.08</td>
<td>1.25±0.10*</td>
<td>1.15±0.08*</td>
<td>II vs IV</td>
</tr>
<tr>
<td>PCr/Pi (FSW)</td>
<td>3.37±0.27</td>
<td>2.91±0.21</td>
<td>3.04±0.23</td>
<td>2.69±0.15*</td>
<td></td>
</tr>
</tbody>
</table>

PCr indicates phosphocreatine; FSW, measured using Fourier series window localization technique; and Pi, inorganic phosphate. Values are mean±SEM. Data are not corrected for partial saturation. *P<.05 vs control.

Although demand ischemia may have produced the metabolic changes observed in the present study, both the decline in PCr/ATP and rise in lactate release are quantitatively small compared with those usually observed during supply ischemia due to coronary stenosis.2,3,6 Our results do not permit distinction of mild ischemia affecting the entire subendocardium from a spotty heterogeneous subendocardial ischemia. Marked heterogeneity of regional myocardial blood flow has been described in the canine left ventricle,24 and the metabolic findings of the present study could reflect similar heterogeneity in the imbalances between myocardial O2 supply and demand under these conditions of high work state.25,26

The 31P-MRS techniques used in the present study cannot provide spectra localized to small areas of ischemia, so it is likely that the decreases in PCr/ATP reflect the admixture of signal from both ischemic and nonischemic regions. For this reason, the isotope dilution measurements of myocardial lactate release are

Table 4. Arterial and Coronary Vein Substrate Concentrations and Myocardial Substrate Uptake

<table>
<thead>
<tr>
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<th>Additional Significant Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial FFAs, µmol/mL</td>
<td>0.24±0.06</td>
<td>0.23±0.04</td>
<td>0.52±0.14*</td>
<td>0.40±0.09</td>
<td>II vs III</td>
</tr>
<tr>
<td>CV FFAs, µmol/mL</td>
<td>0.21±0.04</td>
<td>0.17±0.02</td>
<td>0.41±0.12*</td>
<td>0.31±0.07</td>
<td>II vs III, II vs IV</td>
</tr>
<tr>
<td>A-CV FFAs, µmol/mL</td>
<td>0.03±0.01</td>
<td>0.07±0.02</td>
<td>0.10±0.05</td>
<td>0.08±0.05</td>
<td></td>
</tr>
<tr>
<td>FFA uptake, µmol·g⁻¹·min⁻¹</td>
<td>0.03±0.01</td>
<td>0.09±0.03</td>
<td>0.20±0.07*</td>
<td>0.24±0.06*</td>
<td>II vs IV</td>
</tr>
<tr>
<td>Arterial glucose, µmol/mL</td>
<td>6.77±0.85</td>
<td>7.21±1.01</td>
<td>6.48±0.74</td>
<td>6.55±0.66</td>
<td></td>
</tr>
<tr>
<td>CV glucose, µmol/mL</td>
<td>6.22±0.87</td>
<td>6.90±0.98</td>
<td>6.22±0.75</td>
<td>6.25±0.70</td>
<td></td>
</tr>
<tr>
<td>A-CV glucose, µmol/mL</td>
<td>0.45±0.10</td>
<td>0.31±0.09</td>
<td>0.27±0.09</td>
<td>0.25±0.08</td>
<td></td>
</tr>
<tr>
<td>Glucose uptake, µmol·g⁻¹·min⁻¹</td>
<td>0.43±0.11</td>
<td>0.40±0.11</td>
<td>0.57±0.19</td>
<td>0.70±0.28</td>
<td></td>
</tr>
<tr>
<td>Arterial lactate uptake, µmol/mL</td>
<td>1.21±0.13</td>
<td>1.18±0.09</td>
<td>1.06±0.07</td>
<td>1.12±0.09</td>
<td>II vs IV, III vs IV</td>
</tr>
<tr>
<td>CV lactate, µmol/mL</td>
<td>0.65±0.06</td>
<td>0.72±0.06</td>
<td>0.73±0.06</td>
<td>0.84±0.06*</td>
<td>II vs IV, III vs IV</td>
</tr>
<tr>
<td>A-CV lactate, µmol/mL</td>
<td>0.55±0.08</td>
<td>0.46±0.05</td>
<td>0.34±0.06*</td>
<td>0.29±0.06*</td>
<td>II vs IV</td>
</tr>
<tr>
<td>Lactate uptake, µmol·g⁻¹·min⁻¹</td>
<td>0.55±0.10</td>
<td>0.63±0.10</td>
<td>0.71±0.13</td>
<td>0.87±0.18</td>
<td></td>
</tr>
<tr>
<td>Lactate extraction, %</td>
<td>45±3</td>
<td>39±3</td>
<td>31±4*</td>
<td>24±4*</td>
<td>II vs III, II vs IV, III vs IV</td>
</tr>
<tr>
<td>Lactate release, µmol·g⁻¹·min⁻¹</td>
<td>0.21±0.03</td>
<td>0.29±0.07</td>
<td>0.45±0.05*</td>
<td>0.59±0.10*</td>
<td>II vs IV</td>
</tr>
</tbody>
</table>

FFA indicates free fatty acid; CV, coronary vein; and A-CV, arterial minus coronary vein. Values are mean±SEM. *P<.05 vs control.
particularly important. Since lactate release from any myocardial region will affect this measurement, despite the admixture of blood from regions extracting lactate, it is possible to detect the contribution of very localized areas of anaerobic glycolysis while, as in the present study, the myocardium as a whole exhibits net lactate consumption.

Alternative Interpretations

Although the findings of the present study are consistent with the concept of demand ischemia, alternative interpretations must be considered as well. In particular, other plausible explanations exist for the observed metabolic and blood flow changes.

The lower value of PCr/ATP at high work states could reflect a physiological rise in ATP hydrolysis products, reflecting positive feedback for the control of oxidative phosphorylation, rather than ischemia. Although a decline in PCr/ATP with an increasing work state has been observed in the left ventricle of the rat, cat, and neonatal sheep, a similar decline has not been observed previously in the left ventricle of older large mammals. Ligeti et al have speculated that these interspecies differences may represent adaptations to differing cardiovascular demands. The pig is less likely than the dog to be adapted for either endurance or for an extreme range of MVO₂ and therefore may resemble the rat and cat in its metabolic response to high work states. The disparity between the results of the present study and those of Balaban and coworkers also may be due to the much higher MVO₂ levels achieved in the current experiments. For example, the rate-pressure product and MVO₂ in the present study were 47% higher and 52% higher, respectively, than the levels achieved in these earlier canine studies. Another potential explanation for the present results may be the differences in the coronary circulation between the pig and the dog, which could predispose the pig to ischemia. Finally, in most previous studies, spectra were obtained without any procedure for increasing the contribution of signal from the subendocardium, where changes in PCr/ATP would be more likely. Nonetheless, in the one canine study that obtained subendocardial spectra, no change in high-energy phosphates was observed with increasing work state.

Data obtained in perfused hearts have demonstrated that PCr/ATP and [ADP] are substrate dependent. At constant MVO₂, when glucose was provided as the sole substrate, PCr/ATP was lower than when palmitate or octanoate was provided. This raises the possibility that a shift in substrate utilization in vivo could cause a similar decline in PCr/ATP. However, this is unlikely to be the mechanism for the lower PCr/ATP value at high work states in the present study, since free fatty acids rather than glucose accounted for most of the increase in substrate uptake from the control value.

The utilization of lactate by the heart is affected substantially by the substrate milieu, and dobutamine significantly increased arterial free fatty acids. A rise in free fatty acids is associated with an increase in myocardial free fatty acid uptake and a shift away from lactate oxidation, but such a shift is not normally associated with an increase in lactate release measured by isotopic techniques and thus cannot account for the present results.

Lactate release, reflecting anaerobic glycolysis, is a very sensitive indicator of myocardial ischemia. A small amount of lactate release is observed under normoxic basal conditions in both animal models and in young healthy human subjects. There are two potential explanations for this finding. First, lactate release may result from anatomic or temporal microheterogeneity of blood flow, with small regions of oxygen...
TABLE 5. Major Findings During Adenosine Protocol

<table>
<thead>
<tr>
<th>Hemodynamics</th>
<th>Control</th>
<th>Adenosine + Dobutamine</th>
<th>AoCon + Dobutamine</th>
<th>Significant Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>114±5</td>
<td>119±10</td>
<td>192±6*</td>
<td>175±10*</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>95±6</td>
<td>68±3*</td>
<td>105±7</td>
<td>90±8</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>115±6</td>
<td>95±8</td>
<td>240±11*</td>
<td>222±11*</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>8±1</td>
<td>7±1</td>
<td>15±4</td>
<td>11±3</td>
</tr>
</tbody>
</table>

**Anterior wall blood flow**

| Subepicardium, mL·g⁻¹·min⁻¹ | 0.88±0.12 | 6.08±0.94* | 4.63±1.22* | 8.85±1.52* | All          |
| Midwall, mL·g⁻¹·min⁻¹       | 1.07±0.16  | 4.79±0.65* | 3.65±0.89* | 6.25±1.16* | III vs IV    |
| Subendocardium, mL·g⁻¹·min⁻¹| 1.16±0.15  | 3.15±0.51* | 3.08±0.62* | 3.78±0.68* | None         |
| Endo/Epi                  | 1.32±0.05  | 0.53±0.06* | 0.74±0.06* | 0.46±0.05* | II vs III, III vs IV |

**Metabolism**

| MVO₂, μmol·g⁻¹·min⁻¹ | 5.8±0.7 | 5.3±1.0 | 14.3±1.0 | 12.8±2.0 | II vs III and IV |
| PCR/ATP                | 1.44±0.02 | 1.41±0.04 | 1.31±0.04 | 1.34±0.04 | None |
| Lactate release, μmol·g⁻¹·min⁻¹ | 0.18±0.02 | 0.38±0.06 | 0.46±0.03* | 0.60±0.12* | None |

AoCon indicates aortic constriction; HR, heart rate; bpm, beats per minute; MAP, mean arterial pressure; LVSP, left ventricular (LV) systolic pressure; LVEDP, LV end-diastolic pressure; Endo/Epi, subendocardial to subepicardial blood flow ratio; MVO₂, anterior wall O₂ uptake; and PCR, phosphocreatine. Values are mean±SEM.

*P<.05 vs control.

supply-demand imbalance even under basal conditions. Alternatively, a small amount of lactate release may accompany normal aerobic glycolytic metabolism, as has been demonstrated when glucose and insulin are administered. Thus, the increased lactate release at high work states might be a concomitant of an increased aerobic glycolytic rate. This latter explanation is less likely to be responsible for the present findings during high work states, because during aortic constriction plus dobutamine, lactate release rose by 181% while glucose uptake increased by only 63%; however, the rates of glucose oxidation and glycolgenolysis were not determined.

The decline of the subendocardial to subepicardial blood flow ratio at the highest work state is not a priori evidence of subendocardial ischemia. This ratio may fall at high heart rates or with vasodilator or catecholamine infusion, especially when there is a decline in coronary perfusion pressure. However, previous studies that demonstrated a decline in the ratio of subendocardial to subepicardial blood flow did not provide metabolic evidence to corroborate or exclude ischemia under these conditions. The failure of subendocardial blood flow to rise significantly when MVO₂ was incremented with additional aortic constriction or during adenosine contrasts with the further increase in subepicardial flow and thus strongly suggests that subendocardial reserve under these conditions was close to exhaustion.

It is also noteworthy that left ventricular systolic function, as characterized by segment shortening, was not impaired at the high work state, as would have been expected with supply ischemia. However, mixed supply-demand ischemia is characterized more by diastolic than systolic dysfunction, and the same result might be anticipated for pure demand ischemia. The rise in left ventricular end-diastolic pressure without an increase in end-diastolic segment length would be consistent with impairment of diastolic function. However, given the marked changes in heart rate, afterload, and administration of dobutamine in this protocol and the use of segment shortening as the only measurement of function, too much weight should not be placed on these observations.

Therefore, although the major findings of the present study—the decrease in PCR/ATP, the rise in lactate release, and the decline in the subendocardial to subepicardial blood flow ratio—are not individually conclusive evidence of demand ischemia; taken together, ischemia due to an imbalance between myocardial O₂ demand and supply provides the most coherent unifying explanation for the results.

**Limitations**

Although both the single pulse and the FSW ¹³P-MRS spectra weighted the subendocardial signal, both include substantial signal from the subepicardium. Since it is assumed that subendocardial ischemia is more severe, these measurements may have underestimated the metabolic changes. Therefore, the finding of decreased PCR/ATP, even with suboptimally localized spectroscopy, implies an even greater reduction in the subendocardium.

Measurements of the coronary arterial-venous difference of substrates must also be interpreted with caution. Chemical lactate extraction varies with the concentration of alternative substrates and may decline in the absence of ischemia. Conversely, chemical measurements of coronary arterial venous lactate may obscure lactate produced and released by the myocardium. However, the isotope dilution technique used in the present study overcomes this limitation.
As has been discussed previously, exhaustion of coronary reserve within the subendocardial layer is the most likely explanation for the metabolic changes at the high work states. However, it is possible that this limitation of coronary reserve is dependent on the experimental model. Although systolic and mean arterial pressure rose with both dobutamine and aortic constriction, the diastolic pressure in the proximal aorta stayed the same or, during dobutamine and adenosine infusion, declined. At the same time, in some experiments the left ventricular end-diastolic pressure rose. Thus, a declining coronary perfusion gradient may have contributed to exhaustion of subendocardial coronary reserve. Finally, these experiments were not conducted in conscious animals, and anesthesia is also known to reduce coronary reserve. Thus, although subendocardial blood flow increased by nearly threefold during the highest work state, to values similar to those observed during severe exercise in conscious pigs, the results may not pertain to conscious animals.

Implications

The concept of demand ischemia has usually been used to describe situations in which coronary blood flow is limited by a subcritical stenosis and ischemia is induced by increasing MVo2. There are few previous data in either anesthetized or awake animals indicating that ischemia occurs with increases in work state in the absence of abnormalities of coronary flow or coronary reserve. The present results are consistent with such an interpretation, and it is noteworthy that the changes occurred at increments of MVo2 that are achieved during strenuous exertion. The most likely explanation for this phenomenon is exhaustion of coronary flow reserve, presumably involving the myocardium in a heterogeneous manner. Alternative mechanisms include O2 demand that exceeds the capacity for O2 diffusion or the Vmax of one or more steps in the oxidative metabolic pathways.

Acknowledgments

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