Regeneration of Myocardial Phosphocreatine in Pigs Despite Continued Moderate Ischemia


The effects of 1 hour of mild and moderate reductions in coronary blood flow on myocardial high-energy phosphate levels were evaluated. Thirty anesthetized pigs were instrumented with left anterior descending arterial and venous catheters, crystals for instantaneous wall thickness, and a fluid-filled occluder. Measurement of myocardial blood flow was performed with microspheres, and a series of myocardial biopsies also was performed. In 10 pigs, overall coronary blood flow was lowered by 22%, with a fall in subendocardial-to-subepicardial flow ratio from 1.11 to 0.54 and in wall thickening from 33% to 15%. Subendocardial flow fell 48%. Coronary blood flow and thickening were constant during 1 hour of ischemia. Phosphocreatine (μmol/g wet wt) in the subendocardial third of the ischemic zone fell from 7.6 to 3.8 at 5 minutes of ischemia (p<0.005 versus control) and returned to normal (7.9) at 60 minutes (p=NS), despite ongoing ischemia. Subendocardial ATP (μmol/g wet wt) fell slowly from 4.3 and leveled off at 2.1 at 60 minutes of ischemia (p<0.001 versus control). Similar regeneration of phosphocreatine was found in seven additional pigs, with a 43% transmural reduction in coronary blood flow and a 66% reduction in subendocardial flow. No significant changes in ATP and phosphocreatine were noted in two different control groups (n=13 pigs). The regeneration of phosphocreatine despite ongoing ischemia and low ATP levels was not related to changes in myocardial oxygen demand or consumption, or in regional function during the period of ischemia. This may reflect 1) a successful downregulation of the energy needs of the ischemic myocardium to maintain cell viability, or 2) a metabolic abnormality in the ability of the cells to produce ATP primarily or by use of phosphocreatine. (Circulation Research 1990;67:1481-1493)

When a coronary artery is occluded, myocardial function in the region of the heart served by the vessel rapidly declines.1 This is associated with a prompt fall in phosphocreatine (PCr) and a slower decline in ATP.2,3 Function and the levels of these two high-energy phosphates remain low during vessel occlusion. If flow is restored before the onset of permanent tissue damage, full recovery of function and ATP levels occurs but requires a considerable period of time (days to weeks).3-5

A clinically common but less studied situation is a prolonged period of a mild to moderate reduction of flow to a region of the heart.6,7 This often occurs in the setting of a chronic flow limitation due to coronary artery disease and is associated with reduced function in the hypoperfused region. When adequate flow is restored, function improves. This has been referred to as “hibernating” or “idling” myocardium.6,7 If myocardial cells can indeed survive prolonged reductions in flow, the hypoperfused myocardium must be able to adjust successfully to low-flow states and restore energy balance to maintain cell viability. How this is accomplished has not been established. The effects of a prolonged reduction, but not cessation, of flow on the regulation of metabolism and function to a region of the left ventricle are not fully understood. Our main objectives in this study were to measure the changes that occurred in the metabolism and function of a region of the left ventricle subjected to 1 hour of a mild or moderate constant reduction in flow and to establish the relation between changes in high-energy phosphate levels and regional function during constant ischemia. To accomplish this, we measured transmural function along with flow and myocardial high-energy phosphate levels in three layers in a region of the left ventricular (LV) free wall during a constant reduc-
tion in flow for 60 minutes. Regional myocardial oxygen consumption (MVO₂) was unchanging during ischemia, and two levels of coronary flow reduction were studied in two different groups of animals: mild (20–25% reduction) and moderate (40–50% reduction). In addition, we performed two sets of control studies to evaluate the experimental preparation.

**Materials and Methods**

**Preparation**

Pigs weighing 30–65 kg were studied. The surgical preparation was similar to that previously reported and is summarized below. Premedication was with xylazine (2 mg/kg i.m.) and ketamine (10 mg/kg i.m.). The animals were anesthetized throughout the experiment with α-chloralose (100 mg/kg i.v. initially, then 50 mg/kg i.v. every 2 hours). They were ventilated with oxygen-enriched air by a piston respirator to maintain pH at 7.35–7.45, Paco₂ at 35–45 mm Hg, and PaO₂ at 100–150 mm Hg. Rectal temperature was maintained at 98–100°F using a cooling/heating pad.

Aortic and inferior vena caval catheters were inserted from vessels in the groin. The heart was exposed through a median sternotomy and suspended in a pericardial cradle. Catheters were inserted into the LV apex (LV pressure and LV dP/dt) and left atrium (microsphere injections). The proximal left anterior descending (LAD) coronary artery had three items placed in or around it. An electromagnetic flow probe (2.0 or 2.5 mm) was placed proximally followed by an inflatable fluid-filled occluder around the vessel. A small Silastic catheter (0.6 mm o.d., 0.3 mm i.d.) was inserted into the mid-LAD by the Herd-Barger technique for recording phasic and mean pressure distal to the occluder. The frequency response of the system has been reported. Another Silastic catheter was inserted into the anterior cardiac vein as it coursed parallel to the LAD. It was placed well below the site of the LAD occluder so that venous blood returning only from the distal LAD vascular bed would be sampled. Simultaneous arterial and LAD coronary vein blood samples were drawn, and oxygen content was measured. MVO₂ for the region served by the LAD beyond the occluder was the product of transmural LAD flow by microspheres and the arterial–coronary vein oxygen difference.

A pair of ultrasonic crystals (5 mHz) was placed in the region served by the LAD vascular bed. One crystal was sewn to the epicardial surface, and the second was inserted obliquely into the subendocardium. The crystals were used to measure diastolic and systolic wall thickness with the timing of end systole and end diastole as reported by others. Systolic wall thickening was the difference between systolic and diastolic thickness as a percent of diastolic thickness.

Pressures were measured with Statham P23Gb transducers (Gould Instruments, Cleveland). Flow from the electromagnetic flow probe was measured with a Statham 2202 flowmeter. Pressures, electro-

magnetic flow, ultrasonic crystals, and electrocardiographic signals were recorded on an eight-channel Mark 200 Brush pen recorder (Gould). Oxygen content of arterial and coronary venous samples was determined in duplicate with an IL 382 hemoximeter (Instrumentation Laboratory, Lexington, Mass.).

During the protocols, myocardial flow was measured with radionuclide spheres of 11×0.3-μm diameter labeled with 58Cr, 59Nb, 103Ru, and 147Ce. The spheres were injected into the left atrium over 30 seconds. A reference sample was withdrawn from the descending aorta over 2 minutes at a rate of 10 ml/min starting 5 seconds before the injection of the spheres. The spheres were suspended in 0.9% saline with 0.01% Tween 80 and were vigorously agitated before use. The number of spheres injected was calculated so that a 0.5-g sample of myocardium contained 800 spheres.

Transmural myocardial biopsies (3-mm diameter) were performed using a high-speed drill. For later identification of the epicardium, a dilute solution of Evans blue dye was painted on the surface of the region to be biopsied. Tissue samples were injected into liquid nitrogen within 1 second. An assistant immediately inserted a plug into the hole, and hemostasis was secured by a 4-0 silk suture using a superficial horizontal mattress stitch around the plug. The biopsy and insertion of the plug were accomplished within 1–2 seconds. Premature ventricular contractions occurred as a result of the biopsy but did not last more than 3–5 seconds. Hemodynamic variables returned to baseline within 30 seconds after the biopsy was obtained. Analysis of the biopsy samples is described below.

At the end of the experiments, the heart was removed and each coronary artery was cannulated. To determine the myocardium perfused by the LAD, a blue dye was injected into the LAD, and a red dye was injected into the right carotid artery (RCA) and circumflex coronary artery (Cx) at the same rate and pressure. The heart then was placed in formalin for 3–5 days. Myocardium from the LAD and non-LAD (Cx and RCA) regions was divided into three layers: a subendocardial, a midmyocardial, and a subepicardial layer. Approximately 5 g tissue from each layer was counted for 5 minutes using a Nuclear Data ND 600/660 multichannel analyzer (Nuclear Data Inc., Schaumburg, Ill.). Tissue adjacent to biopsy sites was excluded. Coronary flow was calculated for each layer and expressed in milliliters per minute per gram tissue.

The blue-stained epicardial surface of the frozen biopsy specimens was easily identified in all samples. This enabled division of the specimens into subendocardial, mid-wall, and subepicardial thirds of approximately equal weight while immersed in liquid nitrogen. Tissue extraction and biochemical analysis of each sample were performed using the methods of Lowry and Passonneau. ATP content was measured by spectrofluorometry with glucose-6-phosphate dehydrogenase and hexokinase in an NADP to
NADPH–linked reaction and expressed in micromoles per gram wet tissue weight (μmol/g wet wt). PCr content was determined with ADP and creatine kinase, by measuring ATP formed. ADP content was determined by spectrofluorometry with lactate dehydrogenase and pyruvate kinase in an NAD to NADH–linked reaction. AMP content was determined using myokinase (adenylate kinase) and ATP, and then measuring ADP formed. The coefficient of variation for the chemical analyses is ±2.4% for ATP and ±3.4% for PCr, based on 50 analyses of pooled samples.

Protocols for Ischemia Groups

These experiments were initiated 45 minutes after the preparation was completed and after an appropriate dose of anesthesia was given. The following variables were monitored during the entire protocol: heart rate, electrocardiogram, wall thickening, LAD flow (phasic and mean), distal LAD pressure (phasic and mean), and LV pressure and LV dp/dt. Initial (control) measurements also included 1) blood samples for oxygen content from an artery and the coronary vein, drawn just before an injection of microspheres into the left atrium to measure regional flow, and 2) a biopsy taken from the Cx region. This sequence was reversed in one half of the animals. When the hemodynamic variables returned to baseline and had been stable for 5 minutes, a 60-minute period of ischemia was initiated. Flow was decreased by inflating the occluder to reduce mean flow–probe flow by either 20–25% of control (mild ischemia group) or 40–50% of control (moderate ischemia group). During the experiment, the flow measured by flow probe was used to judge the level and constancy of flow to the ischemic region during the 60 minutes of ischemia. However, transmural flow by the microsphere method was the absolute arbiter that flow to the central ischemic region was reduced to the desired level and was maintained constant during the 60 minutes of ischemia. If the three transmural flow determinations by microspheres during the 60 minutes of ischemia varied by more than 15%, the results from that animal were not used in the data analysis. At 5–15 minutes, 25–35 minutes, and 50–60 minutes of ischemia, blood samples for oxygen content were drawn, microspheres were given, and a biopsy was taken from the ischemic LAD region. At 60 minutes of ischemia, the occluder was released and flow was unimpeded for the next 30 minutes. Hemodynamic variables were recorded during this time.

After the 30-minute period of reperfusion, the animals were killed, and the hearts were removed. A different colored dye was injected into each coronary artery at the same rate and pressure to identify the region perfused by each vessel. This enabled us to be certain that the first biopsy was taken from the Cx (nonischemic) region and that the three biopsies taken during ischemia and the crystals were all located well within the LAD (ischemic) region (at least 1 cm from the border zone). The alignment, depth, and orientation of the crystals also were confirmed.

Nonischemic Group Protocol

Three pigs underwent the same surgical preparation and protocol described above except that flow was never reduced. These animals were included to establish that MVO₂, function, and metabolite levels were constant during the course of the protocol when flow was not decreased and thus ischemia was not induced.

Preinstrumentation and Postinstrumentation Control Group

To assess the effects of instrumentation of the LAD, 10 additional pigs were studied. Preinstrumentation transmural myocardial biopsies were obtained from the distal LAD and Cx vascular beds. The proximal LAD artery then was dissected and instrumented with the electromagnetic flow probe, the fluid-filled occluder, and the Silastic rubber catheter to measure distal LAD pressure, as described previously. Forty-five minutes after instrumentation, aortic and distal LAD pressure and flow again were recorded. Transmural biopsies were repeated in the LAD and Cx vascular beds.

Postinstrumentation biopsy sites were chosen proximal to and at least 10 mm away from the preinstrumentation sites. This was done to minimize the influences of the first biopsy on the blood flow to the site of the second biopsy in both vascular beds. The time between preinstrumentation and postinstrumentation biopsies was 2–2½ hours, and the time between the LAD and Cx biopsies was approximately 5 minutes.

The specimens later were divided into the layers described for the samples in the other experiments and were assayed in the same way.

Statistical Analyses

Results are given as mean±SD. Estimates were made for any missing values, and corrections were made for bias due to this.¹⁷ Variables were tested by one-way analysis of variance with repeated measures. Range testing was done with Tukey’s test using the corrected statistics.¹⁷

Results

A total of 35 pigs was studied. Five were excluded: two because microsphere flows during ischemia varied beyond established limits, one because of equipment failure, and two because the animals developed ventricular fibrillation after a biopsy during the early phase of ischemia. Thus, adequate data were collected in 30 pigs: 17 in the ischemia groups (by design, 10 mild, seven moderate), three in the nonischemic group, and 10 in the preinstrumentation and postinstrumentation control group.

Mild Ischemia Group

Hemodynamic variables. Table 1 presents hemodynamic and oxygen data for the mild ischemia group.
By design, LAD mean flow was reduced during ischemia. This decrease averaged 22% for the group. Subendocardial flow was reduced by 48% and mid-wall flow by 28%, whereas subepicardial flow did not change significantly. The reduced flow led to a significant increase in oxygen extraction, whereas MVO₂ did not change significantly. Percent wall thickening decreased by 55% from the control value (p<0.001). Of great importance is that all the values remained constant during the 60 minutes of ischemia.

Compared with control values, LV systolic pressure decreased slightly, heart rate increased, and double product increased moderately during the period of ischemia. These changes were statistically significant, but relatively small.

**Metabolites.** The transmural content of ATP was 4.40±0.31 μmol/g wet wt during the control period. This decreased to 3.37±0.54 at 5 minutes and remained depressed to 2.91±1.04 at 30 minutes and 3.05±0.83 at 60 minutes of ischemia (p<0.001 versus control). Figure 1 shows the content of ATP and PCr by layer across the LV free wall. To provide an estimate of variability, means and standard deviations are listed in Table 2. During ischemia, ATP content fell in each layer. A graded response was noted, with the largest decrease in ATP content in the subendocardium (p<0.001 versus control) followed by the mid-wall (p<0.005 versus control). The ATP content in the outer layer was not significantly changed. Although the trend of ATP contents was downward during the 60 minutes of ischemia, no statistically significant changes occurred between the 5-minute and 60-minute values.

Changes in PCr content during ischemia were markedly different than noted for ATP. Transmural PCr content during the control period was 8.13±0.89 μmol/g wet wt. At 5 minutes of ischemia, it decreased to 5.47±1.63 but then rose to 7.03±1.55 at 30 minutes and 8.43±1.53 at 60 minutes. Only the value at 5 minutes is significantly different from control (p<0.001). Figure 1 and Table 2 show the PCr contents by layer. The subendocardial layer showed the greatest decrease at 5 minutes of ischemia (p<0.005), but by 60 minutes of ischemia the PCr content in each layer had returned to the control value. Regeneration of PCr occurred despite persistent depression of ATP content and the constant reduction in flow. It was independent of the severity of the flow reduction because PCr regeneration occurred in each of the three layers.

ADP and AMP were appropriately low during the control period. The only statistically significant change in ADP or AMP during ischemia was an increase in subendocardial ADP at 5 minutes of ischemia.

**Moderate Ischemia Group**

Seven animals were studied during a more severe reduction in transmural flow to determine if PCr regeneration still would occur. Complete data were collected at control and at 5 and 30 minutes of ischemia. Two animals developed ventricular fibrillation just after the 30-minute biopsy was obtained. Data at 60 minutes are from the five remaining animals.

**Hemodynamic variables.** Table 3 presents hemodynamic and oxygen data for the moderate ischemia
content of ATP and PCr in each of the three layers, and Table 2 provides an estimate of variability. Once again, a transmural gradient of ATP was present during ischemia, with lowest levels in the subendocardium. The pattern is very similar to the mild ischemia group except that ATP content is more severely reduced in each region, reflecting the greater severity of flow reduction. No significant changes occurred in the ATP values during the 60 minutes of ischemia.

Despite the more substantial reduction in flow, PCr content still increased during the 60 minutes of flow reduction after reaching a low value at 5 minutes of ischemia. The transmural value during control was 7.93±1.01 μmol/g wet wt. At 5 minutes of ischemia, it fell to 3.79±1.53 (p<0.001 versus control) but at 30 minutes had increased to 6.63±2.13 and reached a value above control at 8.22±1.23 at 60 minutes of ischemia. Figure 2 and Table 2 give the PCr content by layer. The values are significantly reduced from control at 5 minutes for all three layers and at 30 minutes for the subendocardial region. By 60 minutes, the PCr content had returned to baseline in all regions. The ability of the myocardium to regenerate PCr was not affected by the severity of ischemia over the range we tested (up to a 66% flow reduction).

The control ADP and AMP contents in the moderate ischemia group were higher than those in the mild ischemia and nonischemic groups (Table 2). These animals entered the study in a physiological state similar to the other groups. The difference may just reflect variability related to the small number of animals in each group. As in the mild ischemia group, ADP and AMP rose slightly at 5 minutes and then returned to baseline during continued ischemia.

Nonischemic Group

The hemodynamic (Table 4) and metabolic data (Table 2; Figure 3) from the three animals in this group are given. These variables were stable over the 60 minutes that the experiments were performed. Thus, changes noted in the metabolite levels of the ischemic group were not due to deterioration of the preparation over time or effects of the biopsies. These results attest to the stability of the preparation and our ability to minimize the effects of the multiple biopsies.

Preinstrumentation and Postinstrumentation Group

Hemodynamic variables. Heart rate increased from 74±17 beats/min before the preinstrumentation biopsies to 97±25 just before the postinstrumentation biopsies (p<0.05). Aortic systolic pressure decreased from 136±17 to 118±27 mm Hg (p<0.05). The rate–pressure product did not change significantly.

Metabolites. Transmural results are shown in Table 5. Preinstrumentation and postinstrumentation ATP and PCr levels were similar in theCx (noninstrumented) vascular bed. After instrumentation, ATP levels in the LAD distribution decreased by 7% (p<0.05). Postinstrumentation LAD PCr concentration did not change significantly.
TABLE 2. Metabolites

<table>
<thead>
<tr>
<th></th>
<th>Mild ischemia group</th>
<th></th>
<th></th>
<th></th>
<th>Moderate ischemia group</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>5</td>
<td>30</td>
<td>60</td>
<td>Control</td>
<td>5</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>ATP (µmol/g wet wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subendo</td>
<td>4.25±0.46</td>
<td></td>
<td></td>
<td></td>
<td>4.24±0.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td>4.60±0.32</td>
<td></td>
<td></td>
<td></td>
<td>4.51±0.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subepi</td>
<td>4.33±0.34</td>
<td></td>
<td></td>
<td></td>
<td>4.35±0.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP (µmol/g wet wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subendo</td>
<td>0.02±0.03</td>
<td></td>
<td></td>
<td></td>
<td>0.06±0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td>0.03±0.03</td>
<td></td>
<td></td>
<td></td>
<td>0.05±0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subepi</td>
<td>0.03±0.03</td>
<td></td>
<td></td>
<td></td>
<td>0.06±0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD. Subendo, subendocardial layer; mid, midmyocardial layer; subepi, subepicardial layer.

$\ast$p<0.001 vs. control.
$\ast\ast$p<0.005 vs. control.
$\ast\ast\ast$p<0.01 vs. control.
$\ast\ast\ast\ast$p<0.025 vs. control.
$\ast\ast\ast\ast\ast$p<0.05 vs. control.

The ATP and PCr contents in the three layers of the LV wall before and after instrumentation are shown in Figures 4 and 5. When the values in the different layers were assessed after instrumentation, ATP in the LAD region showed a significant decrease only in the middle layer (Figure 4A). The LAD PCr contents did not change significantly (Figure 5A). No changes occurred in the ATP and PCr contents in the Cx region after instrumentation of the LAD (Figures 4B and 5B). Thus, minimal changes in ATP and PCr contents occurred as a result of the instrumentation of the LAD. These results also attest to the stability of the preparation and our ability to minimize the effects of multiple biopsies. In addition, the results establish the stability of the high-energy phosphate contents in the preparation.

**Discussion**

These experiments show the following. 1) Graded reductions in flow across the LV wall from epicardium to endocardium are associated with graded reductions in ATP and PCr contents at 5 minutes of ischemia. Thus, metabolic vulnerability of the myocardium exists during ischemia and is most pronounced in the subendocardial region. 2) ATP contents fall significantly during ischemia with the extent of the decrease related to the severity of ischemia. ATP remains steady between 5 and 60 minutes of constant ischemia. 3) After reaching a low level at 5 minutes of ischemia, PCr content, in contrast, increases significantly by 30 minutes of constant ischemia and reaches control levels by 60 minutes of ischemia. Regeneration of PCr occurs in all layers of the myocardium, even in regions with up to a 66% reduction in flow. 4) The regeneration of PCr was not due to changes in variables that might lead to an amelioration of ischemia. During the 60 minutes that transmural flow was reduced, the regional flows, the rate-pressure product, MVO₂, and wall thickening all remained constant.

**High-Energy Phosphate Levels During Reduced Flow**

Previous studies of high-energy phosphate contents during mild to moderate ischemia are few, and the results have not been uniform. Prinzen et al.¹⁸ reported ATP and PCr contents during the initial 5 minutes of a flow reduction in dogs. In the subendocardial region, a 68% decrease in flow was associated with a 46% fall in PCr at 1 minute, with a further decline at 5 minutes. However, ATP content did not change significantly. Their results for PCr agree with our data at 5 minutes of ischemia, whereas in contrast, we noted a significant decrease in ATP at 5 minutes of a comparable degree of ischemia.

The stability in ATP at a reduced level during 60 minutes of constant low flow that we noted agrees with the results of Neill et al.¹⁹ They showed stability of ATP at a reduced level between 30 minutes and 5 hours at mild and moderate decreases of flow in dogs. PCr was not measured in that study.

Liedtke et al.²⁰ measured transmural ATP and PCr levels at 60 minutes after a 50% reduction in transmural flow in an in situ working pig heart. They noted no decrease in PCr level, as we did at 60 minutes.
TABLE 2. Metabolites (Continued)

<table>
<thead>
<tr>
<th></th>
<th>Nonischemic group</th>
<th>Ischemia (min)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (min)</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.65±0.40</td>
<td>3.60±0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.39±0.14</td>
<td>4.25±0.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.59±0.08</td>
<td>4.63±0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.61±0.40</td>
<td>9.33±1.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.75±0.41</td>
<td>9.54±1.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.16±0.49</td>
<td>9.29±0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03±0.03</td>
<td>0.02±0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03±0.03</td>
<td>0.02±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03±0.03</td>
<td>0.02±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.36±0.22</td>
<td>0.30±0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.36±0.26</td>
<td>0.32±0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35±0.23</td>
<td>0.33±0.20</td>
</tr>
</tbody>
</table>

However, they would have missed the early fall and subsequent return to normal of PCr levels. They also reported a reduction in PCr level after 60 minutes of a 60% reduction in transmural flow. This contrasts with our results, which showed that PCr was always back to the control value at 60 minutes irrespective of the severity of ischemia, within the limits of flow reduction we tested. We anticipate that more severe reductions of flow in our model eventually would blunt or prevent the regeneration of PCr.

Our study is unique because it defines the effects of reduced flow on metabolite levels in the same animal at three different times during a 60-minute period in three layers of the LV wall. This enabled us to identify the early reduction and subsequent regeneration of PCr despite ongoing ischemia.

PCr regeneration with low levels of ATP has been reported during reperfusion of myocardium previously made ischemic by occlusion of a coronary artery (stunned myocardium).21–24 PCr content actually exceeds initial values by 15 minutes after reperfusion and then gradually returns to baseline levels over the next several hours. ATP content, in contrast, requires several days to increase to baseline values. The mechanism of this PCr “overshoot,” despite low ATP levels in the reperfused stunned myocardium, has not been definitely elucidated, nor is it certain whether the regeneration of PCr during hypoperfusion that we noted occurs by a similar mechanism.

Relation Between Function and High-Energy Phosphate Levels

Reduced contractile function has been postulated as a mechanism of reducing oxygen demand of ischemic myocardium, enabling restoration of metabolic balance.7 The rebound recovery of PCr despite low ATP in the stunned myocardium may be due to the decrease in mechanical function of the heart that occurs after reperfusion,24 although others have not found a good correlation between the PCr overshoot and reduced function.3,25 Fedele et al25 have reported that coronary venous pH and PCO2 improved over time in a pig model with constant, reduced coronary flow. This occurred despite a constant MVO2. The

TABLE 3. Hemodynamics and Oxygen Data for Moderate Ischemia Group (n=7)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia (min)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV Pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>118±14</td>
<td>113±15</td>
<td>110±13</td>
</tr>
<tr>
<td>Diastolic</td>
<td>7±4</td>
<td>13±4</td>
<td>14±5†</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>88±23</td>
<td>89±20</td>
<td>93±24</td>
</tr>
<tr>
<td>Rate–pressure product (×102)</td>
<td>104±29</td>
<td>101±29</td>
<td>102±28</td>
</tr>
<tr>
<td>LAD flow (ml/min/g) (microspheres)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmural</td>
<td>1.05±0.29</td>
<td>0.56±0.16‡</td>
<td>0.61±0.16‡</td>
</tr>
<tr>
<td>Subendocardial</td>
<td>1.10±0.26</td>
<td>0.32±0.08‡</td>
<td>0.37±0.11‡</td>
</tr>
<tr>
<td>Midmyocardial</td>
<td>1.11±0.37</td>
<td>0.47±0.17‡</td>
<td>0.58±0.20‡</td>
</tr>
<tr>
<td>Subepicardial</td>
<td>0.96±0.25</td>
<td>0.84±0.24</td>
<td>0.85±0.26</td>
</tr>
<tr>
<td>Mean LAD pressure (mm Hg)</td>
<td>99±14</td>
<td>41±8*</td>
<td>43±7*</td>
</tr>
<tr>
<td>A–CV O2 difference (vol %)</td>
<td>9.0±1.6</td>
<td>9.6±1.5</td>
<td>9.5±1.6</td>
</tr>
<tr>
<td>O2 extraction (%)</td>
<td>80±6</td>
<td>89±2‡</td>
<td>88±2*</td>
</tr>
<tr>
<td>MVO2 (ml/min/100 g)</td>
<td>9.28±2.00</td>
<td>5.21±0.91‡</td>
<td>5.62±1.03‡</td>
</tr>
<tr>
<td>Wall thickening (%)</td>
<td>31±9</td>
<td>8±9*</td>
<td>10±10*</td>
</tr>
</tbody>
</table>

Values are mean±SD. LV, left ventricular; LAD, left anterior descending coronary artery; A–CV, arterial–coronary vein; MVO2, myocardial oxygen consumption.

*p<0.005 vs. control.
†p<0.05 vs. control.
‡p<0.001 vs. control.
§p<0.01 vs. control.
MODERATE ISCHEMIA GROUP

Circulation Research Vol 67, No 6, December 1990

FIGURE 2. Phosphocreatine (PCr) (top panel) and ATP (bottom panel) contents across the left ventricular wall in the moderate ischemia group. PCr regeneration still occurs in each layer despite a more severe reduction in flow than in the mild ischemia group (Figure 1). See Table 2 for numerical levels and significance values. C, control.

NON-ISCHEMIC GROUP

FIGURE 3. Phosphocreatine (PCr) (top panel) and ATP (bottom panel) contents across the left ventricular wall in the nonischemic group. Metabolite contents remained stable during the experiment when ischemia was not induced. Biopsies were performed at the identical times as in the ischemic groups. See Table 2 for numerical levels.

TABLE 4. Hemodynamics and Oxygen Data for Nonischemic Group (n=3)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>LV Pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>107±2</td>
<td>111±5</td>
</tr>
<tr>
<td>Diastolic</td>
<td>9±2</td>
<td>10±4</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>104±11</td>
<td>104±16</td>
</tr>
<tr>
<td>Rate-pressure product (×10²)</td>
<td>112±14</td>
<td>116±22</td>
</tr>
<tr>
<td>LAD flow (ml/min/g) (microspheres)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmural</td>
<td>1.03±0.7</td>
<td>1.34±0.7</td>
</tr>
<tr>
<td>Subendocardial</td>
<td>1.14±0.21</td>
<td>1.36±0.65</td>
</tr>
<tr>
<td>Midmyocardial</td>
<td>1.11±0.15</td>
<td>1.36±0.74</td>
</tr>
<tr>
<td>Subepicardial</td>
<td>1.05±0.15</td>
<td>1.24±0.75</td>
</tr>
<tr>
<td>Mean LAD pressure (mm Hg)</td>
<td>93±7</td>
<td>94±5</td>
</tr>
<tr>
<td>A–CV O₂ difference (vol %)</td>
<td>9.3±1.4</td>
<td>8.9±1.3</td>
</tr>
<tr>
<td>MVO₂ (ml/min/100 g)</td>
<td>10.1±1.6</td>
<td>11.8±6.2</td>
</tr>
<tr>
<td>Wall thickening (%)</td>
<td>33±4</td>
<td>37±2</td>
</tr>
</tbody>
</table>

Values are mean±SD. LV, left ventricular; LAD, left anterior descending coronary artery; A–CV, arterial–coronary vein; MVO₂, myocardial oxygen consumption.
The authors found that in three additional pigs, a progressive decline in contractile function occurred during the initial 40 minutes of reduced flow. They suggested that a progressive decline in function during hypoperfusion resulted in a final myocardial oxygen demand more closely matched to myocardial oxygen supply and a reduction in the severity of metabolic derangement.

We did not find a progressive decline in function. Rather, we observed that function tended to remain stable at a depressed level during the 60 minutes of a constant reduction in flow. Others also have shown that contractile function is stable during a constant prolonged flow reduction. Reduced contractile function still may serve as a mechanism to decrease oxygen demand and restore metabolic balance. If so, our results indicate that this adjustment must occur at or near the onset of ischemia rather than progressively during the ischemia. However, the metabolic cost of shortening appears small, and it may be unlikely that much of an energy savings can be achieved by reducing shortening until regional dyskinesis occurs.

**Mechanisms of Phosphocreatine Regeneration**

The classical view of the role of PCr in myocardial metabolism is that it serves as a reservoir of high-energy phosphate that maintains cellular ATP levels high over a wide range of energy demand. Our results are at odds with this view. The fundamental question is how this regeneration of PCr comes about.

The simplest explanation for the increase in PCr is derived from examination of the equilibrium expression for the creatine kinase (CK) reaction:

$$K_{eq} = \frac{[\text{ATP}][\text{Cr}]}{[\text{ADP}][\text{PCr}][\text{H}^+]$$

If the cardiac cell is considered to be a single compartment and if a steady state has been achieved, then this equilibrium may be evaluated by examination of its component parts.

**TABLE 5. Transmural High-Energy Phosphate Concentrations for Preinstrumentation and Postinstrumentation**

<table>
<thead>
<tr>
<th>Group (n=10)</th>
<th>ATP (μmol/g wet wt)</th>
<th>Phosphocreatine (μmol/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td></td>
<td>4.46±0.42</td>
<td>4.13±0.51*</td>
</tr>
<tr>
<td></td>
<td>7.93±0.60</td>
<td>8.70±0.79</td>
</tr>
<tr>
<td>LAD</td>
<td>4.43±0.29</td>
<td>4.52±0.21</td>
</tr>
<tr>
<td>Cx</td>
<td>8.36±0.66</td>
<td>8.35±0.53</td>
</tr>
</tbody>
</table>

Values are mean±SD. LAD, left anterior descending coronary artery; Cx, circumflex coronary artery. *p<0.05 vs. Pre value.

**Figure 4.** ATP contents preinstrumentation and postinstrumentation in the left anterior descending (LAD) (panel A) and circumflex (CX) (panel B) regions. LAD ATP content after instrumentation of the LAD decreased significantly in the middle (MID) layer (p=0.025) and approached significance in the subendocardial (SUB ENDO) layer (p=0.06). ATP contents in the CX region were unchanged after instrumentation of the LAD region. SUB EPI, subepicardial layer.
this model, the steady-state changes observed in ATP and PCr during ischemia must be offset by changes in the other components. It has been shown that during ischemia, pH falls and free ADP rises. Only a small increase in free ADP content is necessary to stimulate oxidative phosphorylation. These changes would necessitate a rise in creatine if equilibrium is to be maintained. During ischemia, whole heart creatine does not change appreciably. However, studies using isolated perfused hearts or isolated rat atria suggest that nearly half of intracellular creatine is bound and hence not immediately available to participate in the CK reaction. It is possible that during graded ischemia, creatine is liberated from a bond to a free pool, and as such provides a driving force for the observed rise in PCr content.

A second explanation involves compartmentation of PCr generation and degradation. This model predicts a central transport function for PCr (the "PCr shuttle"). Specifically, ATP is generated within mitochondria, and its high-energy phosphate is transferred to creatine by CK bound to the mitochondrial inner membrane (miCK). The PCr thus formed diffuses readily through the cytosol to the myofibril, where a second CK isoenzyme catalyzes the formation of ATP for contraction and ion transport. This model has been strengthened by two observations. First, the predominant CK isoforms are localized to the mitochondrial inner membrane and M-line of the sarcomere, respectively. Second, localization of miCK to the inner mitochondrial membrane near the adenine nucleotide antiporter makes the formation of PCr at the mitochondria kinetically favorable.

In the compartmentation model, it is possible that during mild ischemia, the CK reaction is selectively impeded at the myofibril relative to the rate of mitochondrial ATP and PCr generation (both of which also may be reduced compared with basal levels). This could occur through loss of intracellular Mg or cytoplasmic CK. If this were the case, PCr would accumulate while ATP remained low, because the mitochondrial pool of ATP is about half of total cellular ATP. Limitation of high-energy phosphate transfer at the myofibril could explain the reduced contractile function seen during graded ischemia. In addition, because mitochondrial CK would be available to provide ATP for a variety of other necessary cellular processes, this mechanism potentially could preserve myocardial integrity in the face of mild ischemia.

ATP could have remained low despite the regeneration of PCr because of a loss of intracellular ADP, which can be rapidly broken down to AMP and then adenosine. This apolar compound can easily leave the cell, depleting the adenine nucleotide pool. However, we saw neither an increase nor a decrease in total ADP or AMP contents during the 60 minutes of low flow.

Studies can be cited to support each of the potential mechanisms cited above. Our data do not necessarily favor one mechanism over the others to
explain the PCr regeneration we observed during constant ischemia.

**Study Limitations**

Regional myocardial oxygen determinations are based on oxygen contents measured from a coronary vein that drains the LAD region. Previous work in dogs has established that this site has little or no admixture of venous blood from adjacent vascular beds during reduced flow. The lack of significant collateral flow in pigs compared with dogs should further enhance the accuracy of measuring oxygen consumption in the ischemic LAD vascular bed.

The fall in blood pressure with the prolonged reduction in flow and multiple biopsies was offset by an increase in heart rate so that the double product was relatively unchanged. An assumption made in stating that the double product is unchanged is that a decrease in one component and a comparable increase in the other component are equivalent determinants of myocardial oxygen needs. The lack of a significant change in the measured MVO₂ during ischemia supports this assumption.

The three “sham” experiments without ischemia demonstrated that the concentrations of ATP and PCr were relatively stable during the course of the experiment, as assessed by multiple biopsies, when no ischemia was induced. Furthermore, no changes in LV wall thickening or other indexes of cardiac performance were noted. Thus, the changes seen during mild and moderate ischemia are unrelated to time, spontaneous variability, or the multiple biopsies performed.

The studies of myocardial high-energy phosphate contents before and after instrumentation similarly demonstrate changes that are much smaller than those seen in our protocols. Our control findings are similar to those previously reported in pigs. Others also have found a small gradient of high-energy phosphate levels across the LV wall. Differences in metabolite contents in different regions of the left ventricle have not been found by others, or have been quite small, as we found in the preinstrumentation and postinstrumentation LAD and Cx territories.

Differences in high-energy phosphate contents have been reported between systole and diastole. These differences are of a small magnitude and are much less than the changes we observed.

ADP content measured by chemical analysis after tissue extraction represents total ADP content (free and bound). Greater than 90% of ADP is felt to be bound to actin and myosin. The small amount of free intracellular ADP content may be an important regulator of oxidative phosphorylation. It is likely that our methods would not have detected subtle, but important, changes in free ADP levels that could influence energy production during ischemia.

**Study Implications**

The essential need for control data in studies of interventions designed to ameliorate ischemia is emphasized by our results. Without it, any intervention applied during ischemia could have been interpreted as beneficial, judging by the increase in PCr. The spontaneous increase in PCr adds uncertainty in defining what constitutes an improvement or beneficial effect of interventions during ongoing ischemia that is short of total cessation of coronary flow. The definition of a steady state during ischemia with stable reductions in coronary flow becomes problematic.

It has been reported that a close relation exists between a decrease in the PCr/ATP ratio and reductions in myocardial blood flow during brief periods of ischemia. Our results indicate limitations in using this ratio. The PCr/ATP ratio in our study increased dramatically over time despite a constant level of reduced flow. The dynamic nature of the ratio during ischemia requires caution in interpretation of this ratio as an estimate of the flow to ischemic myocardium.

This study demonstrates that present understanding of myocardial energy metabolism is incomplete. The widely held view of PCr as an energy sink for the contractile apparatus clearly is oversimplified.

**Conclusions**

Myocardial PCr content is dynamic during a constant level of prolonged mild to moderate ischemia. PCr decreases during the initial minutes of ischemia but is then regenerated until it returns to control levels despite reductions in ATP to less than 50% of control. The PCr regeneration was not related to decreases in myocardial oxygen demand or consumption, or changes in regional function during the 60 minutes of ischemia. The mechanism(s) and significance of the increase in PCr is uncertain. It may reflect a “downregulation” of the energy needs of the ischemic myocardium so that ATP levels, although reduced, are adequate to maintain cell viability and also are adequate to allow repletion of PCr content. It may, alternatively, reflect a metabolic abnormality in the ability of the cells to produce ATP primarily or by use of PCr.

**References**


22. Vary TC: Relationship between adenosine nucleotide metabolism and irreversible ischemic tissue damage in isolated perfused rat heart. *Circ Res* 1979;45:218–225


37. Turner DC, Wallimann T, Eppenberger HM: A protein that binds specifically to the M-line of skeletal muscle is identified as the muscle form of creatine kinase. *Proc Natl Acad Sci USA* 1973;70:702–705


40. Greenfield RA, Swain JL: Disruption of myofibrillar energy use: Dual mechanisms that may contribute to post-ischemic dysfunction in stunned myocardium. *Circ Res* 1987;60:283–289


51. Brooks WM, Haseler LJ, Clarke K, Willis RJ: Relation between the phosphocreatine to ATP ratio determined by \(^{31}\)P nuclear magnetic resonance spectroscopy and left ventricular function in underperfused guinea pig heart. J Mol Cell Cardiol 1986;18:149–155

KEY WORDS • adenosine triphosphate • coronary blood flow • myocardial function • myocardial metabolism • myocardial oxygen consumption
Regeneration of myocardial phosphocreatine in pigs despite continued moderate ischemia.

Circ Res. 1990;67:1481-1493
doi: 10.1161/01.RES.67.6.1481

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/67/6/1481

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/