Response of High-Energy Phosphates and Lactate Release During Prolonged Regional Ischemia In Vivo

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**Background.** The functional impairment of persistently ischemic, or “hibernating,” myocardium may serve to maintain myocardial cell viability through a reduction of energy requirements. Although previous studies have, in a variety of experimental models, independently shown variable responses in lactate metabolism and intracellular phosphates during prolonged ischemia, the responses of these metabolites under identical flow conditions have not been adequately described.

**Methods and Results.** To examine the responses of high-energy phosphates and lactate metabolism to prolonged ischemia induced by partial coronary artery stenosis, 12 open-chest pigs were studied using $^{31}$P nuclear magnetic resonance spectroscopy. Concurrent measurements of blood flow, segment shortening, high-energy phosphates, and lactate release (in nine animals) were made during 2 hours of regional ischemia. Subendocardial blood flow and segment shortening were persistently depressed during ischemia, with parallel reductions in ATP, phosphocreatine (PCr), and the ratio of phosphocreatine to inorganic phosphate (PCr/Pi). Pi was persistently elevated during the ischemic period. In contrast, lactate release increased significantly from 0.23±0.04 to 1.34±0.28 μmol/ml after 15 minutes of ischemia ($p<0.05$) but then decreased to 0.73±0.17 μmol/ml at 2 hours ($p<0.05$ versus 15 minutes, $p=NS$ versus control). Similarly, pH increased significantly from a nadir of 6.82±0.07 at 30 minutes of ischemia to 6.98±0.05 at 2 hours.

**Conclusions.** Changes in high-energy phosphates parallel changes in blood flow and function during prolonged ischemia, whereas there is a partial amelioration in lactate production and acidosis. These data support the concept that reduction of myocardial energy requirements during prolonged flow reduction results in signs of reduced ischemia. (*Circulation* 1992;85:342–349)

"Hibernating myocardium," or persistent contractile dysfunction of viable myocardium due to chronically reduced coronary blood flow,1–3 is usually seen in patients with severe coronary artery stenosis. Although the mechanism of this functional impairment is unknown, it has been established that the metabolic...
The ability of $^{31}$P nuclear magnetic resonance (NMR) to repeatedly and nondestructively measure high-energy phosphates allows investigation of the metabolic response to prolonged ischemia. Accordingly, simultaneous measurements of myocardial blood flow, segment shortening, phosphates, intracellular pH, and lactate release were performed during 2 hours of prolonged regional ischemia in the open-chest pig to determine the responses of high-energy phosphates and lactate metabolism. Specifically, the present study tested the hypothesis that although there are persistent changes in phosphate compounds during reduced blood flow, lactate production and acidosis initially increase but then fall during prolonged ischemia. Proof of this hypothesis would support the concept that reduction of myocardial function during ischemia reduces the metabolic consequences of ischemia and permits cell viability.

Methods

Yorkshire-Landrace pigs weighing approximately 35 kg were used for these experiments. The animal preparation has been previously described in detail. Briefly, the animal was pretreated with ketamine hydrochloride (10 mg/kg i.m.) and anesthetized with $\alpha$-chloralose (80 mg/kg i.v. bolus, followed by 20 mg/kg i.v. every hour or as required) and halothane (0.25%). A tracheotomy was performed, and the animal was intubated and ventilated to maintain arterial blood gases in the normal physiological range (Po$_2$ > 100 mm Hg, pH > 7.30). After cannulation of both carotid arteries for hemodynamic monitoring and blood sampling, a median sternotomy was performed, and the heart was suspended in a pericardial cradle.

A section of the proximal left anterior descending coronary artery (LAD) was isolated, and an adjustable hydraulic occluder (In-Vivo Metric, Healdsburg, Calif.) was placed around the section. A Doppler flow probe (TMI, Iowa City, Iowa) was placed around the coronary artery, either immediately proximal to the occluder or in a segment at least 1 cm distal to the occluder. A catheter was placed in the left atrium for microsphere administration, and wires were attached for atrial pacing. In nine animals, a 20-gauge plastic catheter was inserted into the interventricular vein so that its distal end was distal to the coronary occluder. Piezoelectric crystals for segment shortening were inserted into the subendocardium approximately 1 cm apart in a region supplied by the coronary artery beyond the occluder. The coronary artery was transiently occluded to identify the ischemic zone by epicardial discoloration and to ensure that the crystals were positioned in the ischemic zone. A 2.5-cm-diameter two-turn surface coil weighing 1 g and mounted on a flexible plastic strap was then lightly sutured to the epicardium over the center of the ischemic zone, and the pig was transferred to a plastic cradle with a heated water blanket. This coil and its attachment technique have previously been shown to have no effect on segment shortening.

A pigtail catheter was temporarily placed into the left ventricle through the carotid sheath, and left ventricular dP/dt was determined at a heart rate of 100 beats per minute using a short length of arterial pressure tubing. This dP/dt measurement was used to time the systolic period for segment shortening following the method of Gallagher et al. Baseline measurements of segment shortening and coronary blood flow were then obtained using a Triton Technology sonomicrometer model 120 and flowmeter model 100 (Triton Technology, San Diego, Calif.), respectively. All hemodynamic and functional measurements were recorded at 100 mm/sec using a Gould physiological recorder (Gould Inc., Cupertino, Calif.).

Experimental Protocol

Baseline measurements of hemodynamics, segment shortening, and coronary blood flow were obtained. Absolute myocardial blood flow was determined using left atrial injection of 15-µm radioactive microspheres ($^{51}$Cr, $^{54}$Mn, $^{57}$Co, $^{85}$Sr, $^{89}$Nb, $^{113}$Sn, or $^{153}$Gd) and timed withdrawal from a carotid artery. An intravenous infusion of l-[1-14C]lactate (New England Nuclear, Boston, Mass.) was started with a priming dose of 20 µCi and a continuous infusion of 25 µCi/hr. Two spectra were obtained under control conditions to establish the baseline measurement of high-energy phosphates. Simultaneous arterial and anterior interventricular vein blood samples were obtained for chemical analysis and specific activity of lactate. The coronary artery occluder was then slowly inflated using a microsyringe (VWR Scientific, San Francisco, Calif.) to achieve a noticeable change in myocardial segment shortening and an approximate 20% reduction in resting coronary artery blood flow as measured by the Doppler flow probe. Coronary flow was observed throughout the protocol, and the occluder was manually adjusted to maintain this degree of coronary flow reduction.

Spectra were obtained at 15, 30, 60, 90, and 120 minutes of stenosis with concurrent measurements of myocardial blood flow (by radioactive microspheres), hemodynamics, segment shortening, and, in nine animals, lactate release. After the last measurement at 120 minutes of ischemia, the coronary artery occluder was deflated to allow reperfusion. In each instance, reperfusion was verified by reactive hyperemia of coronary blood flow. The animal was then killed with intravenous sodium pentobarbital, the location of the coil was marked with a suture, the heart was excised, and the subendocardial position of the crystals was verified by inspection.

Spectroscopy

Studies were performed using a 1.0-m bore Philips Gyrosan Research system operating at 2 T (Philips Medical Systems, Shelton, Conn.). The animal was placed into the magnet so that the surface coil was in the longitudinal isocenter. Heart rate was maintained throughout the experiment at 100 beats per minute using a pulse generator (model 5880A, Medtronic,
Inc., Minneapolis, Minn.). Because radio frequency interference can be generated by wires entering the magnet bore, a custom switching circuit was required to allow simultaneous acquisition of NMR spectra and functional measurements (segment shortening and coronary blood flow). This circuit used a trigger pulse from the magnet pulse programmer to temporarily isolate the crystal and Doppler circuits during spectroscopy pulsing and acquisition (typically, 150 msec). Thus, although spectra were being acquired with a pulse repetition rate of 3 seconds, functional data were acquired for all except 150 msec of that interval.

After tuning the surface coil, shimming was manually adjusted on protons using a Gordon-Timms arrangement, yielding line widths under 35 Hz. A hexamethyl phosphorous triamide standard was placed in the center of the surface coil, and the 90° flip angle for this pulse length was determined. Spectroscopy was then performed using single radio frequency pulses of 180°, a pulse length chosen from pilot experiments, and computer modeling of the surface coil radio frequency field to achieve heavier weighting of the signal from the subendocardium relative to the subepicardium. Spectroscopy was electrocardiographically gated to the onset of every fifth diastole, and 100 free induction decays were summed for each measurement, resulting in a total acquisition time of 5 minutes for each spectrum.

Spectral Analysis

Spectra were analyzed using NMR1 software (NMRI, Syracuse, N.Y.) by an observer blinded to the experimental protocol. A convolution difference of 200 Hz was used, followed by an exponential multiplication of 15 Hz and phasing with a zero and first-order phase correction. Peak areas were fitted using Lorentzian line shapes for the phosphomonoesters, inorganic phosphate (Pi), phosphodiester, Pcr, and the γ, α, and β resonances of ATP. The chemical shift of the Pi resonance was determined from the maximum of the actual spectrum rather than the fitted spectrum. For each spectrum, the quantities calculated as a fraction of control values were Pi, Pcr, and ATP; the ratio of Pcr/Pi; and the chemical shift of Pi in parts per million (ppm). Calculation of the ratio of Pcr/Pi was performed to minimize the variations in data acquisition between different animal preparations and because this ratio may have independent physiological significance as a reflection of the phosphorylation potential. The chemical shift of Pi was measured to calculate the change in pH during ischemia using the following equation

$$\text{pH} = \text{pK} - \log((\delta_0 - \delta_0')/(\delta_0 - \delta_0))$$

where δ0 is chemical shift (ppm), δa is 3.10 ppm, δb is 5.75 ppm, and pK is 6.60.14 Under control conditions, assignment of the Pi resonance was difficult due to the large overlapping resonance from 2,3-diphosphoglycerate (2,3-DPG) of the chamber blood. Thus, under control conditions, pH was not reliable. With ischemia, the Pi peak was easily resolved from the 2,3-DPG resonance, allowing accurate determination of pH.

Chemical Analysis for Lactate Release

Methods for analysis of the blood samples for lactate release have been described in detail previously. Briefly, the weighed blood samples were mixed immediately with a measured volume of cold 7% perchloric acid and centrifuged. The protein-free supernatant was removed and stored at −4°C for analysis. For the 14C analysis, lactate was separated from the other substrates by ion exchange chromatography, and [14C]lactate content was determined by scintillation counting. The chemical lactate content was measured by an enzymatic spectrophotometric method. The amount of lactate released (μmol/ml blood) was calculated as:

$$[14C] \text{lactate uptake} = ([A] - [V])$$

where [14C]lactate uptake was determined from the isotopic lactate extraction ratio across the myocardial bed, [A] is arterial blood lactate concentration, and [V] is interventricular vein lactate concentration.

Blood Flow Analysis

After formalin fixation, approximately 2-g sections of myocardium were cut from the region below the center of the surface coil and from two regions of myocardium distant from the ischemic region. Each section was then divided into subendocardial and subepicardial halves. After blotting dry and weighing, segments were counted for 10 minutes in a gamma counter. These counts were corrected for the known radioactivities and decay rates of the isotopes as well as for the weight of each sample and the counts in the reference arterial withdrawal. These data were analyzed separately for subendocardial and subepicardial segments in both the ischemic and nonischemic segments.

Function Analysis

Using the timing calibrations performed at the beginning of the experiment, the end-diastolic dimensions were determined at the beginning of left ventricular contraction at the initial rise of the (+)-dP/dt signal. The end-systolic dimensions were determined at 20 msec before the peak (−)-dP/dt.12 Fractional systolic segment shortening was defined as [(end-diastolic length) − (end-systolic length)]/end-diastolic length.

Data Analysis

Results for the spectral and functional analysis were related to time of ischemia and reperfusion. Statistical analysis was performed using a commercial statistics program (STATVIEW 512+, Brain Power, Inc., Calabasas, Calif.) operating on a Macintosh SE microcomputer (Apple Computer, Inc., Cupertino, Calif.). Changes in each variable over time were determined
using analysis of variance for repeated measures and the Fisher protected least-significant difference procedure. A value of \( p < 0.05 \) was considered significant. All data are expressed as mean±1 standard error.

**Results**

**Effects of Ischemia on Myocardial Blood Flow and Function**

Of 12 animals completing the 2-hour protocol, lactate measurements were made in nine animals. There were no group differences in any measured variable between the nine animals with lactate measurements and the total group of 12 animals. Therefore, results are reported for the nine animals with simultaneous measurements of all variables.

Mean arterial blood pressure was unchanged during the protocol and averaged 92±10 mm Hg. Subendocardial blood flow in the myocardium under the coil decreased from 0.96±0.16 to 0.30±0.06 ml/min/g at 15 minutes \( (p<0.05) \) (Figure 1 and Table 1) and remained depressed throughout the ischemic period, although a slight but not significant increase occurred over time. Subepicardial blood flow in the same region was reduced by 33% from control at 15 minutes \( (p=NS) \). The ratio of subendocardial to subepicardial blood flow \( (\text{endo/epi}) \) in the distribution of the LAD remained stable and significantly reduced throughout the ischemic period (Table 1). There were no significant changes in subendocardial blood flow in the nonischemic region of the heart, although subepicardial blood flow increased by 60 minutes of ischemia without any change in the endo/epi ratio.

The response of segment shortening was similar to that of myocardial blood flow, with segment shortening falling from 0.19±0.02 under control conditions to 0.06±0.02 after 30 minutes of ischemia (Figure 1). Thus, in this model, regional ischemia was established and maintained at a fairly constant level over 2 hours, with persistent reductions in both subendocardial blood flow and segment shortening.

**Effects of Ischemia on Myocardial Metabolism**

The metabolic responses of the myocardium are given in Figures 2–6. Figure 2 shows spectra acquired during control conditions and after 15 and 120 minutes of ischemia. These spectra demonstrate the three resonances of ATP \( (\beta, \alpha, \gamma) \), the resonance from PCr, and the resonance from Pi. Under control conditions, unambiguous assignment of the Pi chemical shift is difficult due to the overlapping 2,3-DPG resonance. During ischemia (15 and 120 minutes), PCr was reduced with a concomitant increase in Pi and a downfield shift of Pi due to intracellular acidosis. These abnormalities persisted during the 2 hours of ischemia.

Figure 3 shows that the reduction in ATP was progressive but not rapid. ATP fell to 0.87±0.03 of control at 15 minutes but was only significantly reduced \( (0.70±0.05 \text{ of control}) \) after 30 minutes of ischemia. PCr concentrations fell significantly within the first 15 minutes of ischemia to 0.60 of control and remained significantly depressed to the end of the ischemic period (Figure 4). Pi markedly increased with ischemia to more than twice its control value and remained elevated throughout the ischemic period (Figure 5). These changes resulted in a similar persistent reduction in the ratio of PCr to Pi.

In contrast to the prolonged elevation of Pi during ischemia, lactate release increased initially after 15 minutes of ischemia \( (0.23±0.04 \text{ to } 1.34±0.28 \mumol/ml, p<0.05) \). As shown in Figure 5, lactate release at 2 hours of ischemia \( (0.73±0.17 \mumol/ml) \) then de-

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**TABLE 1. Myocardial Blood Flow (ml/min/g±SEM) and Ratio of Subendocardial (Endo) to Subepicardial (Epi) Flow (Endo/Epi) in Regions Supplied by Left Anterior Descending Coronary Artery (Ischemic) and Left Circumflex (Normal) at Each Time Point Before and During Prolonged Stenosis**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Ischemic Endo</th>
<th>Ischemic Epi</th>
<th>Ischemic Endo/epi</th>
<th>Normal Endo</th>
<th>Normal Epi</th>
<th>Normal Endo/epi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.96±0.16</td>
<td>1.07±0.16</td>
<td>0.89±0.06</td>
<td>1.14±0.18</td>
<td>1.10±0.14</td>
<td>1.04±0.06</td>
</tr>
<tr>
<td>15</td>
<td>0.30±0.06</td>
<td>0.72±0.16</td>
<td>0.42±0.04</td>
<td>1.13±0.25</td>
<td>1.18±0.30</td>
<td>0.96±0.05</td>
</tr>
<tr>
<td>30</td>
<td>0.34±0.06</td>
<td>0.82±0.11</td>
<td>0.41±0.05</td>
<td>1.41±0.15</td>
<td>1.51±0.16</td>
<td>0.93±0.05</td>
</tr>
<tr>
<td>60</td>
<td>0.41±0.07</td>
<td>0.91±0.11</td>
<td>0.45±0.09</td>
<td>1.61±0.20</td>
<td>1.69±0.23</td>
<td>0.95±0.05</td>
</tr>
<tr>
<td>90</td>
<td>0.42±0.11</td>
<td>1.02±0.23</td>
<td>0.41±0.13</td>
<td>1.70±0.27</td>
<td>1.68±0.23</td>
<td>1.01±0.08</td>
</tr>
<tr>
<td>120</td>
<td>0.35±0.10</td>
<td>0.97±0.23</td>
<td>0.36±0.12</td>
<td>1.60±0.21</td>
<td>1.72±0.20</td>
<td>0.93±0.05</td>
</tr>
</tbody>
</table>

\(^{*}p<0.05 \text{ vs. control blood flow.}\)
increased to a value significantly different from the 15-minute value but not significantly different from control. Concurrent with these changes in lactate metabolism, intracellular pH was 6.87±0.06 after 15 minutes of ischemia and increased significantly to 6.97±0.05 at 120 minutes (Figure 6).

**Discussion**

The results of these experiments show that 2 hours of prolonged myocardial ischemia caused parallel changes in PCr, Pi, and PCr/Pi as well as contractile function. In contrast, although lactate release was initially elevated, it returned toward its control value during the 2 hours of ischemia. These data support the concept that reduction of myocardial function during ischemia reduces the metabolic consequences of ischemia and promotes cell viability.

**Metabolic Changes During Prolonged Ischemia**

*High-energy phosphates.* The metabolic changes during prolonged ischemia seen in the present study may be compared with findings by other investigators. ATP concentrations were measured by Neill and Ingwall after 30 minutes and 5 hours of ischemia in the dog. The response of [ATP] was dependent on the degree of blood flow reduction, with modest but stable reductions in [ATP] seen when tissue blood flows were more than 0.3 ml/min/g. When flow was below this threshold, [ATP] fell progressively over 5 hours. In the present study, [ATP] also fell slowly and remained stable over 2 hours with a mean subendocardial blood flow of 0.3–0.4 ml/min/g. These data indicate that at these levels of flow reduction, ATP production and consumption are evenly matched,

![Figure 2](image2.png)

**Figure 2.** Spectra acquired under control conditions and after 15 and 120 minutes of ischemia. Spectra demonstrate resonances of inorganic phosphate (Pi) (contaminated with some 2,3-diphosphoglycerate of chamber blood), phosphocreatine (PCr), and the three phosphates of ATP. (See text for details.)

![Figure 3](image3.png)

**Figure 3.** Plot of ATP as a function of time during prolonged ischemia. ATP became significantly reduced after 30 minutes of ischemia and decreased progressively to 57% of control.

![Figure 4](image4.png)

**Figure 4.** Plots of phosphocreatine (PCr) and ratio of phosphocreatine to inorganic phosphate (PCr/Pi) (both as a fraction of control) as a function of time during 2 hours of prolonged stenosis. Both PCr and PCr/Pi were significantly reduced at all time points during the ischemic period. *p<0.05.
isolated neonatal pig hearts. PCr concentrations did not change between control conditions and after 2 hours of low-flow ischemia, although a possible fall of PCr during this period could not be excluded. ATP, however, fell by 24% during the ischemic period.

In contrast to these other findings of PCr recovery during prolonged ischemia, high-energy phosphate concentrations remained depressed during the ischemic period in the present study. For the group of 12 animals, there was no significant increase in PCr during the ischemic period. Furthermore, the slight but not significant increase in PCr during ischemia paralleled the small changes in subendocardial blood flow. Although PCr recovery at 60 minutes (of at least 50% of the original reduction) was seen in two animals, the majority of animals did not exhibit recovery of PCr. The absence of significant change in phosphate concentrations during ischemia, as reflected in the stable concentrations of PCr, ATP, or Pi, suggests that the degree of PCr use (and/or ATP consumption) was greater in the present experiment than in the study of Pantely et al.

The most probable explanation for the differences between these studies involves the severity of ischemia achieved in the present study and the methodology used for measurement of PCr. In the present study, subendocardial blood flow was reduced by 70% compared with a 48% reduction in the study by Pantely et al. Thus, it is possible that PCr recovery could occur under conditions of mild compared with more significant ischemia, signifying a threshold for metabolic recovery. However, the animals exhibiting PCr recovery in the present study did not have lesser degrees of blood flow reduction than the other animals, which argues against this mechanism. It is also possible that any PCr regeneration in the subendocardium was in part masked by the contribution of the subepicardial tissue to the spectra.

Lactate and pH. In contrast to the paucity of data regarding phosphate compounds during prolonged ischemia, the response of lactate metabolism has been studied under a number of conditions. Apstein et al noted an increased early lactate production in globally ischemic isolated rat and rabbit hearts that decreased with prolonged ischemia. Fedele et al demonstrated a similar response in pigs with a reduction of regional subendocardial blood flow to 0.73 ml/min/g for 3 hours. Concurrent measurements of regional coronary venous pH (sampled from the anterior interventricular vein), PCO₂, and lactate consumption showed that ischemia initially produced abnormalities of these measures that substantially resolved during the 3 hours of ischemia. Specifically, anterior interventricular vein pH fell from 7.35 to 7.28 at 5 minutes but recovered to 7.35 at 3 hours. Lactate consumption changed to production early in ischemia but returned to a balance between consumption and production after 2 and 3 hours. Eberli et al also found an initial increase in lactate production after 15 minutes of underperfusion of isolated rabbit hearts that was abolished after 1 hour.
Finally, Arai et al.\textsuperscript{19} noted declining subendocardial lactate content during 1 hour of mild ischemia in the in situ pig after an initial increase. Together, results of these studies suggest that during mild prolonged ischemia, the rate of glycolysis initially increases but subsequently declines.

In contrast to these studies, Downing and Chen\textsuperscript{17} observed a stable level of lactate production in isolated neonatal pig hearts subjected to 2 hours of low-flow ischemia but no significant reduction in glycogen concentration measured enzymatically. These findings in neonatal pig hearts may be explained by a less severe degree of myocardial ischemia than that maintained in the previous studies, preservation of myocardial glycogen concentrations to provide substrate for continued glycolysis, or a difference between neonatal and adult hearts in their response to ischemia.

In the present study, as observed in most previous measurements, lactate release increased during acute ischemia but recovered in part with prolonged ischemia. Differences between these and previous data, such as the lack of complete return to control levels of lactate metabolism, can be attributed to the degree of ischemia, which was more severe in the present study, as well as the more precise methodology of isotopically measuring lactate release compared with chemical lactate production. This decline in lactate release may be caused by such factors as myocardial glycogen depletion,\textsuperscript{20,21} inhibition of glycolysis by acidosis,\textsuperscript{22} loss of glycolytic cofactors,\textsuperscript{23} or persistently reduced energy demand due to reduced contractility. In the present study, it is not possible to differentiate among these potential mechanisms.

The implications of these data regarding hibernating myocardium are that the metabolic response of the myocardium to prolonged ischemia appears to involve a degree of metabolic recovery that allows cell viability despite reduced blood flow. In the present study, recovery of initially elevated lactate production occurred during prolonged ischemia. Because lactate production is an indicator of myocardial ischemia,\textsuperscript{13} these changes in lactate production imply that the degree of ischemia of the myocardium was reduced despite persistent changes in blood flow, function, and phosphate concentrations. These data are consistent with the hypothesis that reduction of myocardial function during ischemia reduces ATP requirements sufficiently to permit cell viability. These results can also be compared with those found in a model of externally reduced contractility reported by this laboratory. Using the same porcine experimental model, intracoronary lidocaine was found to significantly reduce regional contractile function and ameliorate the metabolic consequences of regional flow reduction.\textsuperscript{24} Similarly, reduced regional contractility during prolonged ischemia, mediated by an endogenous inhibitor such as Pi,\textsuperscript{25} is probably responsible for the improvement in lactate metabolism seen in the present study.

**Limitations**

An important consideration in interpreting the results of the present study is the applicability of a 2-hour period of coronary artery stenosis to the issue of hibernation, a process that is thought to be chronic involving much longer time periods (days to months). It is not clear whether further prolonged periods of significant regional ischemia would result in different findings, a question that must be answered using chronic animal models.

The technical limitations of the present study primarily concern the inaccuracies in determining phosphate concentrations using \textsuperscript{31}P NMR. To weight the spectral acquisition to the subendocardium, a radio frequency pulse was used that allowed contamination of the myocardial signal by chamber blood. This contamination resulted in a phosphomonoester resonance from the 2,3-DPG of chamber blood overlaying the resonance from myocardial Pi. Thus, under control but not ischemic conditions, assignment of the Pi resonance chemical shift was difficult and unreliable. Although a consistent method was used to identify this resonance, the intensity of Pi was probably overestimated under control conditions, and the ratio of PCR to Pi was underestimated. Despite these difficulties, the calculated pH of 7.17 under control conditions agrees with other data indicating a normal pH of approximately 7.1.\textsuperscript{26}

In addition to the errors in measuring the phosphate metabolites, there are inherent errors in sampling venous blood from the anterior interventricular vein. Specifically, there is potential for dilution of lactate measurements during ischemia by contamination of venous drainage from the nonischemic circumflex artery bed. To minimize these effects, the venous catheter was placed just distal to the site of the arterial occluder. Furthermore, previous studies have shown that under similar low-flow conditions, flow from the circumflex bed was 3.5% of LAD flow in dogs\textsuperscript{27} and generally less than 5% of LAD flow in pigs.\textsuperscript{28} Thus, although it is not possible to quantitate the degree of contamination by nonischemic venous drainage, it is unlikely that the degree of contamination was significant. Also, because swine do not have significant development of collaterals during ischemia,\textsuperscript{29} contamination by nonischemic tissue would not explain the changing lactate release over the 2-hour measurement window.

Furthermore, as in previous studies using this technique,\textsuperscript{14,30} lactate release data in the present study are expressed as micromoles per milliliter of blood sampled. Although the rate of lactate release (\textmu mol/min) may be calculated by multiplying by myocardial blood flow, this calculation requires assumptions about the spatial homogeneity of blood flow over the entire region draining into the anterior interventricular vein as well as transmural flow differences within the region. Thus, the value of myocardial blood flow in the tissue sampled for lactate release and its stability over time are relative un-
knowns. Given these uncertainties, actual lactate release was chosen as the most reliable measure of changes in lactate production.

Conclusions

The present study of prolonged regional ischemia has demonstrated that despite persistently reduced blood flow and contractile dysfunction as well as abnormalities in phosphate concentrations, there was a reduction in initially elevated lactate production during 2 hours of ischemia. This change in lactate production provides metabolic evidence for diminished ischemia over time and supports the concept that reduced contractile function during prolonged ischemia lowers ATP requirements and permits cell viability.

References


KEY WORDS • high-energy phosphates • ischemia • nuclear magnetic resonance
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