Velocity of the Creatine Kinase Reaction Decreases in Postischemic Myocardium: A 31P-NMR Magnetization Transfer Study of the Isolated Ferret Heart

Stefan Neubauer, Baron L. Hamman, Stanton B. Perry, John A. Bittl, and Joanne S. Ingwall

Recovery of postischemic function may be limited by energy synthesis by mitochondria, energy transfer via the creatine kinase reaction, or energy utilization at myofibrils. To identify the limiting step, we defined the relations among oxygen consumption, creatine kinase reaction velocity and cardiac performance in myocardium reperfused following mild, moderate, and severe ischemia. Isolated isovolumic ferret hearts were perfused with Krebs-Henseleit buffer at 37° C. After 30 minutes of control, hearts were made ischemic for 20, 40, or 60 minutes and reperfused for 40 minutes. During preischemia, cardiac performance (estimated as the rate-pressure product), was 14.8 \times 10^{3} \text{ mm Hg/min}, oxygen consumption was 16.7 \mu \text{ mol/min/g dry weight}, and creatine kinase reaction velocity measured by 31P-nuclear magnetic resonance saturation transfer was 12.7 mM/sec. For hearts reperfused after 20, 40, or 60 minutes of ischemia, rate-pressure product was 11.5, 6.5, and 1.1 \times 10^{3} \text{ mm Hg/min}; oxygen consumption was 13.5, 14.2, and 6.9 \mu \text{ mol/min/g dry weight}; and creatine kinase reaction velocity was 9.6, 5.0, and 2.0 mM/sec, respectively. Thus, with increasing severity of insult, creatine kinase reaction velocity decreased monotonically with performance (r=0.99). Changes in creatine kinase reaction velocity were predicted from the creatine kinase rate equation (r=0.99; predicted vs. measured velocity) and can therefore be explained by changes in substrate concentration. Oxygen consumption did not correlate with performance or creatine kinase velocity, consistent with abnormalities in mitochondrial energy production. In all cases, creatine kinase reaction velocity was an order of magnitude faster than the maximal rate of ATP synthesis estimated by oxygen consumption. We conclude that, in postischemic myocardium, creatine kinase reaction velocity decreases in proportion to performance, but high-energy phosphate transfer does not limit availability of high-energy phosphate for contraction. (Circulation Research 1988;63:1-15)

In normoxic heart, the rate of oxidative phosphorylation, the velocity of the creatine kinase reaction, and cardiac performance are closely coupled. In the isolated, buffer-perfused heart, the velocity of the creatine kinase reaction (creatine phosphate\rightarrow[^\gamma\text{P}]\text{ATP}) measured by 31P-nuclear magnetic resonance (NMR) magnetization transfer and ATP synthesis estimated by oxygen consumption follow increases or decreases of the rate-pressure product. In the heart of the living rat, Bittl et al observed similar coupling between creatine kinase reaction velocity and cardiac performance. The biochemical basis for the coupling between work load and creatine kinase reaction velocity may be related to four factors: substrate concentration, total enzyme activity, intracellular isoenzyme localization and pH. Under normoxic conditions with constant creatine kinase activity and isoenzyme distribution, work load–dependent changes in cytosolic substrate concentration should account for changes in creatine kinase reaction velocity. The creatine kinase rate equation, which relates reaction velocity to substrate concentration, accurately...
predicts the reaction velocity measured empirically by 3P-NMR saturation transfer for both in vivo and ex vivo hearts over a wide range of cardiac performance, suggesting that reaction velocity is regulated by substrate concentration. Changes in substrate concentration also account for changes in the reaction velocity in the neonatal rabbit heart with threefold differences in creatine pools. However, during myocardial injury caused by mild ischemia or mild hypoxia, the rate equation did not predict measured reaction velocity. Thus, changes in substrate concentration were not sufficient to explain changes in reaction velocity in injured myocardium.

Another determinant of creatine kinase reaction velocity is total enzyme activity. Bittl et al demonstrated that under basal metabolic conditions, the 3P-NMR measurements of creatine kinase reaction velocity in brain, heart, and skeletal muscle were linearly related to Vmax for creatine kinase in each tissue.

The importance of isoenzyme distribution and localization on creatine kinase reaction velocity has also been investigated. Perry et al studied newborn rabbit hearts, which lack the mitochondrial isoenzyme of creatine kinase (mito-CK) and 18-day-old rabbits, which have nearly normal levels of this isoenzyme. They showed that for a given level of performance, creatine kinase reaction velocity correlated with the amount of mito-CK accumulation. Bittl et al., using isolated adult rabbit heart, showed that mito-CK activity decreased as the duration of ischemia increased. During reperfusion, they observed a linear inverse relation between the extent of mito-CK depletion and the ability of the heart to recover mechanical function. Taken together, these results suggest a role of the mito-CK isoenzyme for the recovery of postischemic function.

Recovery of postischemic function may be limited by energy production (oxidative phosphorylation), energy utilization (actomyosin ATPase), or energy transfer (via creatine kinase). The data of Bittl et al, consistent with the hypothesis that energy production and transfer are compromised and limit mechanical function in the postischemic heart. Other studies have suggested that mitochondrial function, and thus ATP synthesis, is compromised. Furthermore, it has been suggested that ATP utilization at the myofibrils is impaired as well.

The present study was undertaken to define myocardial substrate concentration, creatine kinase activity, isoenzyme distribution, and pH on cardiac performance, creatine kinase reaction velocity, and oxygen consumption in the postischemic heart injured mildly or severely by 20–60 minutes of total ischemia. We report that during reperfusion, creatine kinase reaction velocity matches changes in cardiac performance, that changes in cytosolic substrate concentrations explain changes in creatine kinase reaction velocity, and that oxidative phosphorylation is impaired. Based on the comparison of the rate of high-energy phosphate transfer via creatine kinase with the maximal rate of ATP synthesis by oxidative phosphorylation, we conclude that high-energy phosphate transfer does not limit delivery of high-energy phosphate for contraction.

Materials and Methods

Isolated, Buffer-Perfused Ferret Heart

Male, castrated ferrets, weighing 1,500–2,000 g, were anesthetized with chloroform. A transverse laparotomy and a left and a right anterolateral thoracotomy were performed, and the heart was rapidly excised and immersed in ice-cold buffer. The aorta was dissected free, and mounted onto a cannula attached to a perfusion apparatus, as previously described for rat heart. Retrograde perfusion of the heart was begun in the Langendorff mode at a constant temperature of 37°C and a constant coronary perfusion pressure of 100 mm Hg. This perfusion pressure was chosen because it yielded an optimal creatine phosphate/ATP ratio, indicative of maximum oxygen delivery. In preliminary experiments, this ratio was highest and was unchanged over a pressure range of 80 to 120 mm Hg, pressures which changed myocardial performance by 38%. In addition, the ratio of wet-to-dry weight of hearts perfused for 10 minutes was not different from the ratio for hearts perfused for 130 minutes (data not shown), indicating that the amount of edema formation with time was minimal. Before entering the heart, perfusate was passed through a membrane filter with a pore size of 5 μm (Gelman Sciences, Ann Arbor, Michigan). A small vent made of polyethylene tubing was pierced through the apex of the left ventricle to allow drainage of flow from Thebesian veins. For control perfusion, phosphate-free Krebs-Henseleit buffer was used containing (mM/l) NaCl 118, KCl 4.7, CaCl2 1.75, MgSO4 1.2, EDTA tetrasodium 0.5, NaH2CO3 25.0, and glucose 11.0. Equilibrating the buffer with 95% O2-5% CO2 yielded a pH of 7.4. Coronary flow was measured by collecting coronary sinus effluent in a calibrated cylinder. Hearts perfused in this manner could be maintained in a mechanical and metabolic steady state for at least 130 minutes.

Cardiac Performance Measurements

A water-filled latex balloon was inserted into the left ventricle through an incision in the left atrial appendage, via the mitral valve, and secured by a ligature. The balloon was connected to a Statham P23Db pressure transducer (Gould Instruments, Cleveland, Ohio) via a small-bore polyethylene tube for continuous measurement of left ventricular pressure and heart rate on a Hewlett-Packard 7754B recorder (Palo Alto, California). Performance was estimated as the product of heart rate and developed pressure (mm Hg/min). Starling curves were performed by increasing the volume of the intraventricular balloon by 0.05 ml increments to raise the end-diastolic pressure and to allow for peak left ventricular developed pressure.
Protocol

FIGURE 1. Scheme of the experimental protocol for hearts subjected to 20, 40, or 60 minutes of ischemia and 40 minutes of reperfusion.

NMR Measurements

The perfused hearts were placed into a 30-mm NMR sample tube and inserted into a custom probe that was seated in the bore of a superconducting wide-bore (89 mm) 8.4 Tesla magnet (Oxford Instruments, Bedford, Massachusetts). A Nicolet 1280 computer (Nicolet Instrument, Madison, Wisconsin) was used to generate 31P-NMR spectra from a Nicolet NT-360 spectrometer operating in the pulsed Fourier transform mode at 145.75 MHz. Before inserting hearts into the probe, an 18-channel Oxford Instrumentation Shim Supply was used to homogenize the magnetic field. A sample of phosphoric acid (500 mM), occupying a volume and position similar to that of the beating heart, was inserted into the probe, and the signal intensity was maximized by minimizing the line width of phosphorus (typically about 40 Hz).

Spectra were accumulated over 2-minute periods, averaging data from 44 free induction decays that were obtained using a pulse time of 60 μsec, a pulse angle of 60° and an interpulse delay of 2.60 seconds. For magnetization transfer experiments each broadband pulse was preceded by a low-power, narrow-band pulse at the resonance frequency of [γ-P] ATP for 0, 0.3, 0.6, 1.2, 2.4 or 4.8 seconds. For each of the six saturation transfer spectra, 32 scans were accumulated by repetitively cycling through the six different times of presaturation. Thus, the degree of saturation and the delay between the high-power pulse was the same for each spectrum. In addition, any metabolic deterioration occurring during the saturation transfer measurement was equally distributed among the spectra. A complete saturation transfer measurement was acquired in 16 minutes. Separate studies showed that the narrow-band pulse directly attenuated the creatine phosphate magnetization by less than 5% when the carrier frequency was placed 359 Hz downfield from the resonance of creatine phosphate. Stability of the preparation was assessed by comparing the spectra without selective saturation obtained before and after each magnetization transfer experiment.

Magnetization transfer measurements of the forward creatine kinase reaction, creatine phosphate→[γ-P]ATP, were analyzed according to the two-site chemical exchange model of Forsen and Hoffman. Briefly, the longitudinal relaxation times \( T_1 \) for creatine phosphate and the rate constants for the creatine kinase reaction were obtained from the parameters for the single-exponential function relating magnetization area and saturation time with nonlinear regression analysis:

\[
f(x) = (M_0 - M_\infty) \times \exp(-x/T_1) + M_\infty
\]

The slope of this function yields the value for \( T_1 \). \( T_1 \) is the longitudinal relaxation time in the presence of saturation but in the absence of exchange for creatine phosphate; \( T_1 \) (calculated for each experiment except for experiments following 60 minutes of ischemia when summed spectra were used) was calculated from the relation \( T_1/\tau_i = M_0/M_\infty \), where \( M_0 \) is the magnetization in the absence of saturation and \( M_\infty \) is the magnetization of creatine phosphate after infinite saturation at [γ-P]ATP. The unidirectional rate constant for the forward reaction, \( k \), was calculated from the equation \( k = 1/T_1 - 1/T_2 \). Multi-

| Table 1. Physiological and Metabolic Stability of Isolated, Buffer-Perfused Ferret Heart |
|------------------------------------|-------------------------------|-------------------|
|                                   | 0 min                         | 130 min           |
| RPP (10³ mm Hg/min)               | 17.1 ± 1.1                    | 16.0 ± 0.8        |
| PLVDP (mm Hg)                     | 135 ± 5                       | 132 ± 4           |
| CF (ml/min)                       | 33 ± 2                        | 34 ± 2            |
| MVO₂ (µmol/min x g dry wt)        | 15.2 ± 2.1                    | 14.7 ± 1.8        |
| ATP (% of control)                | 100                           | 102 ± 2           |
| CrP (% of control)                | 100                           | 84 ± 2*           |
| Pi (% of control)                 | 100                           | 104 ± 4           |
| total P (% of control)            | 100                           | 102 ± 3           |
| CK flux (mM/s)                    | 11.8 ± 0.7                    | 11.7 ± 1.5        |

RPP, rate-pressure product; PLVDP, peak left ventricular developed pressure; CF, coronary flow; MVO₂, oxygen consumption; CrP, creatine phosphate; Pi, inorganic phosphate; total P, total NMR-observable phosphate.

*p<0.05 vs. 0 minutes (paired t test).
plying the rate constant by substrate concentration yielded reaction velocity.

The resonance areas corresponding to ATP, creatine phosphate, inorganic phosphate, monophosphate esters, and nicotinamide adenine dinucleotide, which are proportional to the number of phosphorus atoms in the respective compound, were measured using the Nicolet INTEGRATION program. Saturation factors were determined by comparing spectra obtained for pulse angle of 60° and interpulse delay of 2.6 seconds to spectra obtained using a pulse angle of 90° and an interpulse delay of 12 seconds (fully relaxed spectra): 1.24 (creatine phosphate), 1.00 (γ-, α- and β-P resonance of ATP). In each heart, the area of the [β-P] ATP resonance of the first spectrum obtained under control conditions was arbitrarily set to 100% and used as the reference value for all resonances in the set of 31P-NMR spectra obtained for the protocol. By comparing the [β-P]ATP resonance area of pre-ischemic hearts to the resonance area of a standard ATP solution, the ATP concentration of a normal ferret heart was determined to be 4.35 μmol/g wet wt. Assuming that 50% of the wet weight of buffer-perfused hearts represents intracellular water, the intracellular ATP concentration was estimated to be 8.7 mM. Intracellular pH (pHi) was measured by comparing the chemical shift between inorganic phosphate and creatine phosphate with values obtained from a standard curve.15

**Experimental Protocol**

To demonstrate hemodynamic and metabolic stability of the preparation, some hearts (n = 6) were perfused with oxygenated buffer for 130 minutes (the time required for the longest protocol used in this study).

In other hearts, the protocol shown in Figure 1 was used. After a 30-minute control period, total normothermic ischemia was induced for 20, 40, or 60 minutes (n = 5 per group). Hearts were then reperfused for 40 minutes. After completion of the protocol, hearts were frozen for subsequent enzyme analysis. During ischemia, a temperature of 37°C was maintained by means of a variable temperature unit attached to the NMR probe. Throughout the protocol, cardiac performance was continuously recorded. Starling curves were obtained at the beginning and the end of the control period as well as at 17 and at 40 minutes of reperfusion. During ischemia, end-diastolic pressure increased by more than 5 mm Hg only for hearts made ischemic for 60 minutes (after 46 ± 2 minutes); during early reperfusion, end-diastolic pressure increased in all groups. By partially deflating the left ventricular balloon, the end-diastolic pressure was maintained at approximately 10 mm Hg at all times during ischemia and reperfusion.

Saturation transfer measurements were carried out during the control period (7–23 minutes) and during reperfusion (22–38 minutes). During control perfusion, two spectra without selective saturation were obtained, one before and one after the saturation transfer measurement. During ischemia, as well as during the first 16 minutes of reperfusion, spectra were continuously recorded. At the end of the protocol a final spectrum was recorded.

Some hearts (n = 4) developed irreversible ventricular fibrillation when reperfused after 60 minutes of ischemia and were excluded from the study.

**Oxygen Consumption**

Because of the necessity to use short, metallic lines of tubing impermeable to oxygen for oxygen-consumption measurements, these measurements cannot be made simultaneously with NMR measurements. Accordingly, additional hearts were subjected to protocols identical to the ones described above for NMR studies (n = 3 for control perfusion; n = 4 per group for hearts subjected to 20, 40, or 60 minutes of ischemia and subsequent reperfusion). Oxygen tension was measured in the perfusion medium at the level of the aortic cannula and in the coronary effluent in the right ventricle with a Clark-
TABLE 2. Heart Rate, Left Ventricular Developed Pressure, and
Peak Left Ventricular Developed Pressure During Control and at
the End of Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>20 minutes ischemia</th>
<th>40 minutes ischemia</th>
<th>60 minutes ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (beats/min)</td>
<td>156±4</td>
<td>155±12</td>
<td>151±7</td>
<td>156±5</td>
</tr>
<tr>
<td>LVDP (mm Hg)</td>
<td>102±4</td>
<td>76±6</td>
<td>53±6</td>
<td>9±2</td>
</tr>
<tr>
<td>PLVDP (mm Hg)</td>
<td>127±4</td>
<td>92±7</td>
<td>75±8</td>
<td>17±2</td>
</tr>
<tr>
<td>(%PLVDP)</td>
<td>100</td>
<td>72</td>
<td>58</td>
<td>13</td>
</tr>
</tbody>
</table>

LVDP, left ventricular developed pressure; PLVDP, peak left ventricular developed pressure.

Values are mean ± SEM.

Oxygen consumption was calculated according to the following formula: 

\[(\text{perfusion PO}_2 \text{ difference across the heart}) \times (\text{solubility of O}_2/\text{mm Hg}) \times (\text{coronary flow})/(\text{g dry wt})].

Biochemical Measurements

From each heart, 5-10 mg of tissue were homogenized in 0.1 M K$_2$HPO$_4$ buffer, pH 7.4, with 1 mM EGTA, 1 mM β-mercaptoethanol, and 0.1% Triton at 4°C (final tissue concentration 5 mg/ml). Aliquots were taken for measurement of total creatine kinase activity, creatine kinase isoenzyme distribution, total lactate dehydrogenase activity, and total citrate synthase activity as previously described. Protein was measured by the method of Lowry et al and total creatine by the method of Kammermeier.

Statistical Analysis

To test for significant differences within one group of hearts, the paired t test was used. Enzyme contents or substrate concentrations of experimental groups were compared using the Neumann-Keuls multiple range test if significant differences among groups were indicated by one-way analysis of variance. The relations among rate-pressure product, oxygen consumption, ATP, creatine kinase reaction velocity, and mito-CK activity were tested with linear regression analysis. Calculations were aided by the Statistics and Data Management Package (Bolt, Beranek and Newman; Cambridge, Massachusetts). All data are presented as mean ± SEM.

Results

Stability of Preparation

The isolated adult ferret heart, perfused with Krebs-Henseleit buffer at constant pressure, is a new model for the study of cardiac metabolism and physiology. To demonstrate physiological and metabolic stability of this preparation over a time equivalent to the duration of the longest protocol used in this study, hearts were perfused for 130 minutes. Results shown in Table 1 demonstrate that the rate-pressure product and peak left ventricular developed pressure; coronary flow; oxygen consumption; ATP, inorganic phosphate and total NMR-observable phosphate contents; and creatine kinase reaction velocity all showed less than a 10% decline over 130 minutes of control perfusion. Creatine phosphate content declined to 84% of control during this time. Thus, the isolated, buffer-perfused ferret heart exhibits physiological and metabolic stability, and it does so for a longer time than, for example, the isolated rat heart, in which instability occurs after 90 minutes of buffer perfusion.

Cardiac Performance During Control and Reperfusion

Figure 2 shows values for the rate-pressure product during control and reperfusion periods of hearts subjected to ischemia for 20, 40, or 60 minutes. During the control period, cardiac performance was stable and was not different among the experimental groups. With the onset of ischemia, active pressure development ceased within approximately 2.5 minutes. After 40 minutes of reperfusion, the rate-pressure product recovered to 72±5, 50±5, and 9±1% of control for hearts made ischemic for 20, 40, and 60 minutes, respectively. Following 20 and

![Figure 3. Coronary flow (CF) of the three experimental groups during control and reperfusion.](http://circres.ahajournals.org/content/65/3/852/F3.large.jpg)
40 minutes of ischemia, cardiac performance improved monotonically during early reperfusion for 20–30 minutes when a new steady state was reached; after 60 minutes of ischemia, function reached a new plateau by 5 minutes of reperfusion. Data presented in Table 2 show that the depression of postischemic function was solely due to a reduction of left ventricular developed pressure. Heart rate was the same during control and early (data not shown) and late reperfusion for all groups. Changes in postischemic depression of the peak left ventricular developed pressure closely followed changes observed for the rate-pressure product. Coronary flow (Figure 3) sharply increased during the first 2 minutes of reperfusion. This "reactive hyperemia" was greatest after 40 minutes of ischemia, and was blunted following 60 minutes of ischemia. By 15 minutes of reperfusion, coronary flow returned to preischemic values for all groups.

Oxygen Consumption

Myocardial oxygen consumption (Figure 4) was constant during the preischemic period. After 20 minutes of reperfusion a new steady state was reached in all hearts. Oxygen consumption was 81%, 87%, and 41% of control for hearts recovering from 20, 40, and 60 minutes of ischemia. Thus, oxygen consumption recovered to a much greater extent than cardiac performance following 40 and 60 minutes of ischemia. Consistent with abnormalities in mitochondrial ATP production, there was a poor relation between oxygen consumption and performance during postischemia (y = 0.64x + 7.43; r = 0.83, p = 0.37).

Biochemical Analysis

Table 3 summarizes the results of enzymatic analysis of hearts frozen after 130 minutes of control perfusion or after reperfusion following 20, 40, or 60 minutes of ischemia. Total creatine kinase activity was not different among control hearts and hearts reperfused after 60 minutes of ischemia, about 25% of the total creatine kinase activity was lost. Creatine kinase isoenzyme distribution was not affected by ischemia: All hearts contained ~90% of the MM and 10% of the mitochondrial isoenzyme; the BB and MB isoenzymes were not detectable. Lactate dehydrogenase activity was 4.0 IU/mg protein in a normal ferret heart and was decreased (by approximately 25%) only after 60 minutes of ischemia. In contrast, activity of the mitochondrial protein citrate synthase was not significantly reduced.

<table>
<thead>
<tr>
<th>Total CK activity</th>
<th>%MM</th>
<th>% mito-CK</th>
<th>mito-CK activity</th>
<th>LDH</th>
<th>Citrate synthase</th>
<th>mito-CK/CS</th>
<th>Total creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>(IU/mg protein)</td>
<td></td>
<td></td>
<td>(IU/mg protein)</td>
<td>(IU/mg protein)</td>
<td>(IU/mg protein)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11.0±0.6</td>
<td>90±2</td>
<td>10±2</td>
<td>1.14±0.23</td>
<td>4.0±0.2</td>
<td>1.12±0.05</td>
<td>0.99±0.16</td>
</tr>
<tr>
<td>20 minutes ischemia</td>
<td>10.2±0.6</td>
<td>89±3</td>
<td>11±3</td>
<td>1.15±0.28</td>
<td>3.6±0.3</td>
<td>1.06±0.06</td>
<td>1.11±0.29</td>
</tr>
<tr>
<td>40 minutes ischemia</td>
<td>11.2±0.6</td>
<td>90±2</td>
<td>10±2</td>
<td>1.09±0.28</td>
<td>3.9±0.1</td>
<td>1.09±0.06</td>
<td>0.86±0.30</td>
</tr>
<tr>
<td>60 minutes ischemia</td>
<td>8.2±0.4*</td>
<td>91±2</td>
<td>9±2</td>
<td>0.74±0.18</td>
<td>2.9±0.2*</td>
<td>0.86±0.30</td>
<td>0.74±0.18</td>
</tr>
</tbody>
</table>

CK, creatine kinase; % MM, % mito, percentage of total creatine kinase activity present as the MM or mitochondrial creatine kinase isoenzyme form; mito-CK/CS, mitochondrial creatine kinase activity divided by citrate synthase activity; LDH, lactate dehydrogenase.

*p<0.05 60 minutes vs. control, 20, or 40 minutes (Neumann-Keuls multiple range test).
by ischemia and reperfusion. The ratio of mito-CK and citrate synthase activities was unchanged in reperfused hearts. The total creatine pool was reduced only after 60 minutes of ischemia (by 40%). Thus, cytosolic, but not mitochondrial, proteins and metabolites changed only after 60 minutes of ischemia.

Earlier studies of isolated mitochondria suggested that mito-CK activity is lost during reperfusion in proportion to the duration of ischemia. Our results using whole heart homogenates show that neither total creatine kinase activity nor isoenzyme distribution is altered by 20 or 40 minutes of ischemia followed by reperfusion. After 60 minutes of ischemia, total creatine kinase activity, and hence mito-CK activity, decreased by only 25%. To test whether the mito-CK isoenzyme dissociated from the outer aspect of the inner mitochondrial membrane but retained its activity in the cytosol, additional hearts were subjected either to control perfusion (n = 3) or to 60 minutes of ischemia followed by 40 minutes of reflow (n = 3). Hearts were then homogenized in buffer containing sucrose (0.075 M), mannitol (0.225 M) and EGTA (0.002 M) titrated with Trisma base to pH 7.5. The suspension was centrifuged at 5,000 rpm for 10 minutes at 4°C. For all hearts, the cytoplasmic fraction contained 90% of the activity of the cytosolic enzyme lactate dehydrogenase, and 80% of total creatine kinase activity, while the pellet contained 75% of the activity of the mitochondrial protein citrate synthase. For both control and postischemic hearts, all the mito-CK activity was found in the mitochondria-containing pellet, while no activity was recovered in the cytoplasmic fraction: 0% for control and 3 ± 3% for postischemic hearts. Thus, in the ferret heart, mitochondria retained their mito-CK activity even after 60 minutes of ischemia.

**NMR Measurements**

**Metabolite concentrations.** Figure 5 depicts a typical 31P-NMR spectrum from a ferret heart. In such a heart, the creatine phosphate/ATP ratio is 1.93 ± 0.08 (after correcting for partial saturation), which is higher than ratios reported for glucose-perfused hearts of any other species. A small resonance can be seen for inorganic phosphate, and there is, in contrast to other reports, an additional small peak tentatively assigned as glycerophosphoryl choline.

In Figure 6, time-dependent changes in myocardial ATP content are shown during control perfusion, during 20, 40, or 60 minutes of ischemia and during reperfusion. ATP was stable during the preischemic period, and declined almost linearly during ischemia. During early reperfusion, ATP content tended to increase, but by 40 minutes of reperfusion, recovery of ATP was not significant for any group of hearts (paired t test). Postischemic ATP was depleted in proportion to the duration of ischemia: 64%, 33%, and 13% of control values for hearts subjected to 20, 40, and 60 minutes of ischemia, respectively.

Figure 7 shows that creatine phosphate content fell below the threshold of detectability (approximately 0.6 mM) within 7 minutes after the onset of ischemia. Upon reperfusion, recovery of creatine phosphate occurred within 4 minutes in all hearts. Following 20 and 40 minutes of ischemia, creatine phosphate showed an overshoot that persisted throughout the reperfusion period. Following 60 minutes of ischemia, creatine phosphate content returned to control levels during early reperfusion; the small decrease in creatine phosphate levels with late reperfusion (13%) was not significant.

**Figure 5. Control spectrum from a ferret heart.** This spectrum was obtained 2 minutes by signal-averaging 44 scans with a pulse angle of 60° and an interpulse delay of 2.6 seconds. MPE, monophosphate esters; Pi, inorganic phosphate; GPC, glycerophosphoryl choline; CrP, creatine phosphate; γ-, α-, and β-phosphorus atoms of ATP.
FIGURE 6. Changes in ATP content (% of control) of the three experimental groups during control perfusion, ischemia, and reperfusion. In all groups, ischemia was induced at 30 minutes. Reperfusion began at 50, 70, and 90 minutes for hearts following 20 minutes (○), 40 minutes (□), and 60 minutes (△) of ischemia, respectively.

Inorganic phosphate concentration was low (1.8 mM) during preischemia (Figure 8). During ischemia, the inorganic phosphate accumulation showed a biphasic pattern: a rapid increase during the first 3 minutes coinciding with the disappearance of most of the creatine phosphate resonance, and a slower rise thereafter parallel to the reduction of the ATP resonance areas. By 60 minutes of ischemia, the inorganic phosphate resonance had increased 18-fold, corresponding to a cytosolic concentration of 32 mM. During reperfusion, inorganic phosphate returned to control levels following 20 and 40 minutes of ischemia, but remained elevated (approximately sixfold) after 60 minutes of ischemia, corresponding to a concentration of 10 mM.

The total NMR-observable phosphate pool (during control 604 ± 16% of the [β-P]ATP resonance) was not significantly changed at the end of ischemia but declined (p<0.01, paired t test) upon reperfusion following 40 and 60 minutes of ischemia (to 476 ± 22% and 441 ± 19%, respectively).

FIGURE 7. Changes in creatine phosphate (CrP) content of the three experimental groups during control perfusion, ischemia, and reperfusion. See Figure 6 for explanation of symbols.

Intracellular pH (Figure 9) was 7.16 ± 0.01 during control perfusion and declined monotonically during ischemia. At 20 minutes of ischemia pHi was 6.47 ± 0.06; in some of the hearts subjected to 40 minutes of ischemia and in all of the hearts subjected to 60 minutes of ischemia, pHi fell below 6.00 (lower threshold for pH-determination from a standard curve) by the end of ischemia. With reperfusion, pHi recovered to normal values within 4 minutes in all hearts. Following 60 minutes of ischemia, pHi declined slightly, but not significantly (paired t test), during late reperfusion. pHi was not different among hearts reperfused after 20, 40, or 60 minutes of ischemia (multiple range test).

Table 4 shows cytosolic concentrations of the reactants of the creatine kinase reaction during preischemia and postischemia. Free cytosolic ADP concentration was calculated assuming that creatine kinase is in equilibrium and using $K_{obs}$ from Lawson and Veech.25 Calculated [ADP] in a normal ferret heart was 93 μM. In reperfused hearts, [ADP] decreased in proportion to the severity of insult,
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Figure 8. Changes in inorganic phosphate (Pi) content of the three experimental groups during control perfusion, ischemia and reperfusion. For interpretation of the time scale, see Figure 6.

Fallings to values of 4 μM in hearts recovering from 60 minutes of ischemia.

Measurement of creatine kinase reaction velocity by saturation transfer NMR. Figure 10 depicts stacked plots of 31P-NMR spectra obtained from saturation transfer experiments for preischemic and postischemic hearts. The spectra show that the extent of saturation transfer from the creatine phosphate to the [γ-P]ATP resonance is reduced in proportion to the duration of ischemia.

Data from all saturation transfer experiments are shown in Table 4. The T1 of creatine phosphate in preischemic hearts was 2.65±0.15 seconds and was not significantly changed after 20 or 40 minutes of ischemia and reperfusion. For hearts reperfused after 60 minutes (which exhibited very little creatine kinase velocity), T1 was estimated by summing spectra from the five experiments (to obtain a sufficiently high signal to noise ratio) and was found to be 2.15 seconds. Thus, T1 of creatine phosphate was essentially unchanged in all reperfused hearts.

Both the rate and extent of magnetization transfer from creatine phosphate to [γ]ATP decreased in postischemic hearts. For preischemic hearts and hearts reperfused after 20, 40, or 60 minutes of ischemia, the pseudo-first order unidirectional rate constant decreased from 0.70 to 0.43, 0.20, and 0.17/sec, respectively. Creatine kinase reaction velocity fell from 12.2 to 9.7, 5.0, and 2.4 mM/sec, respectively. Thus, both rate constant and reaction velocity were depressed in proportion to the duration of ischemia. Results presented in Table 4 and Figure 11 show that changes in creatine kinase reaction velocity in postischemic hearts could be predicted by the creatine kinase rate equation using literature values for Km and K for substrates (p = 0.015). Absolute reaction velocity values calculated from the rate equation, however, were offset by a factor of ~2 for all hearts (y = 2.3x - 1.7; where y is calculated velocity and x is measured velocity).

Relations Among Performance, Metabolite Concentrations, Enzyme Activities, and Creatine Kinase Reaction Velocity

For preischemic and postischemic hearts, cardiac performance and creatine kinase reaction velocity showed close linear correlation (Figure 12, upper...
TABLE 4. Substrate concentrations and kinetic parameters for the forward creatine kinase reaction

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>20 minutes ischemia</th>
<th>40 minutes ischemia</th>
<th>60 minutes ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (mM)</td>
<td>8.7</td>
<td>5.6</td>
<td>2.9</td>
<td>1.1</td>
</tr>
<tr>
<td>CrP (mM)</td>
<td>17.4</td>
<td>22.6</td>
<td>25.2</td>
<td>14.4</td>
</tr>
<tr>
<td>ADP (mM)</td>
<td>0.093</td>
<td>0.040</td>
<td>0.015</td>
<td>0.004</td>
</tr>
<tr>
<td>Free creatine (mM)</td>
<td>21.3</td>
<td>18.8</td>
<td>15.4</td>
<td>7.0</td>
</tr>
<tr>
<td>$V_{\text{max}}$ (mM/s)</td>
<td>80</td>
<td>74</td>
<td>82</td>
<td>59</td>
</tr>
<tr>
<td>$T_1$ [CrP] (s)</td>
<td>2.65 ± 0.15</td>
<td>2.44 ± 0.20</td>
<td>2.81 ± 0.34</td>
<td>2.15 (summed spectra)</td>
</tr>
<tr>
<td>k (sec$^{-1}$)</td>
<td>0.70 ± 0.03</td>
<td>0.43 ± 0.04</td>
<td>0.20 ± 0.04</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>$V_{\text{measured}}$ (mM/sec)</td>
<td>12.2</td>
<td>9.7</td>
<td>5.0</td>
<td>2.4</td>
</tr>
<tr>
<td>$V_{\text{predicted}}$ (mM/sec)</td>
<td>27.0</td>
<td>18.1</td>
<td>11.2</td>
<td>2.6</td>
</tr>
</tbody>
</table>

CrP, creatine phosphate; $V_{\text{max}}$, maximal creatine kinase reaction velocity calculated from total creatine kinase activity at 37°C; k, pseudo-first order rate constant of the forward direction of the creatine kinase reaction; $V_{\text{measured}}$, creatine kinase reaction velocity measured by saturation transfer; $V_{\text{predicted}}$, creatine kinase reaction velocity calculated from the creatine kinase rate equation assuming $K_m$ and $K_i$ values (mM) according to Bittl et al.$^1$ $K_m$: 0.07 (ADP), 2.0 (CrP), 0.93 (ATP), 17.3 (free creatine); $K_i$: 0.09 (ADP), 1.13 (CrP), 0.75 (ATP), 22.4 (free creatine).

Discussion

Isolated Ferret Heart as a Model of Ischemic Injury

We chose to use the isolated ferret heart for these studies primarily because of the requirements to maximize the signal-to-noise ratio and to minimize the time required to perform the magnetization transfer experiment. This is especially crucial during postischemia, when instability of the preparation may occur over time. The adult ferret heart (approximately 9 g) fits well in a 30-mm probe, placing a maximum amount of cardiac tissue in the NMR-sensitive volume. For the ferret heart, a saturation transfer measurement required only 16 minutes, while in the rat heart (1.5 g in a 20 mm probe), approximately 35 minutes were required.$^2$

Hearts used for this study met the following criteria for adequate perfusion: The hearts showed stability for all physiological and metabolic (aside from a small loss of creatine phosphate) parameters for 130 minutes of control perfusion. We optimized the creatine phosphate/ATP ratio by varying perfusion pressure and found it optimal and unchanged for a range from 80 to 120 mm Hg. Therefore, we chose a perfusion pressure of 100 mm Hg. The creatine phosphate/ATP ratio of 1.93 ± 0.08 for 8-g ferret heart is lower than the ratios reported by Morris et al.$^{24}$ or by Kusuoka et al.$^{26}$ for constant

![Figure 10](http://circres.ahajournals.org/)

FIGURE 10. Stacked plots of NMR spectra from saturation transfer experiments of hearts subjected to control perfusion and to 20, 40, or 60 minutes of ischemia followed by 40 minutes of reperfusion. The magnetization transfer measurement was made during reperfusion (22–38 minutes). The duration (in seconds) of presaturation of the [γ-P] ATP is denoted to the right of each spectrum. For assignment of peaks, see Figure 5.
flow preparations but similar to that found by Kusuoka et al. for constant pressure preparations. Lower work loads caused by lower perfusion pressures, lower heart rates, and lower temperatures used by these investigators should increase this ratio. In our study of the isolated adult ferret heart working at physiological temperature and spontaneous rate, the creatine phosphate/ATP ratio remained high, while the inorganic phosphate/ATP ratio was low and did not increase over time. The ratio of inorganic phosphate to ATP content is a useful indicator of the extent of creatine phosphate breakdown. In our preparation, the ratio was 0.26, much lower than that reported by Morris et al. Thus, we conclude that the perfusion conditions we used were adequate to support stable physiological and metabolic function.

In this study, myocardial injury was produced by 20, 40, or 60 minutes of global, normothermic ischemia followed by reperfusion. Twenty minutes of ischemia followed by 40 minutes of reperfusion produced mild, reversible injury, or stunning. Extensive recovery of function (72%) occurred within 40 minutes of reperfusion. Creatine phosphate, inorganic phosphate, and pH were all close to preischemic control levels, while ATP was 64% of preischemic values. No alteration of cardiac enzyme activities was detectable, and there was no gross dissociation of performance and oxygen consumption. On the other hand, 60 minutes of ischemia followed by reperfusion produced severe, irreversible damage. Cardiac performance barely recovered (9%), ATP was depleted to undetectable levels, and inorganic phosphate remained elevated sixfold. Cytosolic enzyme activities, namely creatine kinase and lactate dehydrogenase as well as the total creatine pool, were depleted to a similar extent (approximately 25%). Although cardiac performance was almost completely depressed, oxygen consumption was still 41% of control, indicating oxygen wasting due to abnormalities in mitochondrial energy production. Forty minutes of ischemia and reperfusion may represent a state of transition from reversible to irreversible injury. Performance recovered approximately 50%; oxygen consumption was 87% of control levels; creatine phosphate, inorganic phosphate, and pH were all normal; and ATP content was approximately 33% of control. Little or no loss of cytosolic enzymes occurred. These results suggest that phenomena associated with irreversible injury, such as enzyme depletion, occurred between 40 and 60 minutes of ischemia, while mitochondrial dysfunction assessed by comparing the coupling of oxygen consumption and cardiac performance occurred between 20 and 40 minutes of ischemia. Thus, we examined myocardium that was stunned (20 minutes of ischemia), irreversibly injured (60 minutes of ischemia), and in a transitional state from reversible to irreversible injury (40 minutes of ischemia).

Creatine Kinase Reaction Velocity

A major advantage of the preparation used for our studies is that all physiological and metabolic parameters were stable during reperfusion, even following 60 minutes of ischemia. A necessary condition for the magnetization transfer measurement is metabolic stability: ATP and creatine phosphate contents were not significantly different before and after the 16-minute period required for magnetization transfer measurement. Moreover, to minimize even small changes that might occur during the magnetization transfer experiment, we obtained saturation transfer spectra by recycling through the six presaturation times, thereby equally distributing any instability among spectra. Thus, we eliminated deterioration of the preparation as a possible source of error. Postischemic functional parameters changed only slightly. Also, no cardiac arrhythmias occurred in postischemic hearts. Therefore, the conditions were fulfilled for accurate measurement of creatine kinase kinetics.

Under normoxic conditions, both in the isolated, buffer-perfused rat heart and in the rat heart in situ, creatine kinase reaction velocity closely matched cardiac performance and oxygen consumption. This was interpreted as evidence for coupling of energy transfer, production, and utilization. Creatine kinase reaction velocity could be predicted from the creatine kinase rate equation, indicating that changes in cytosolic substrate concentrations regulate the reaction velocity. Both in the mildly
ischemic isolated rat heart and in the hypoxic rat heart in vivo, decreases in cardiac performance were matched by similar decreases of creatine kinase reaction velocity. It was suggested that the basis for cardiac failure in these settings was the decreased rate of high-energy phosphate turnover. In hearts injured by mild hypoxia or ischemia, the rate equation did not predict creatine kinase reaction velocity, indicating that factors other than cytosolic substrate concentrations may modulate creatine kinase reaction velocity. In the postischemic heart, we report here that creatine kinase reaction velocity matches cardiac performance for each degree of myocardial injury, consistent with the concept that the rate of turnover of high-energy phosphate limits cardiac performance in injured hearts. Changes of creatine kinase reaction velocity could be predicted from the rate equation in postischemic hearts, suggesting that upon recovery from injury, unlike during injury, changes in cytosolic substrate concentrations again regulate creatine kinase reaction velocity. Since the cytosolic concentrations of ADP and creatine are near or below their $K_m$ values for the reaction, both ADP and creatine are limiting. The rate equation overestimated reaction velocity by a factor of two. This may be due to use of $K_m$ or $K_i$ values for rat, bovine, and dog creatine kinase, instead of ferret; no values are available for ferret creatine kinase.

Changes in total enzyme activity (proportional to $V_{max}$) occurred only after 60 minutes of ischemia and thus play a minor role in regulating reaction velocity in postischemic myocardium. Creatine kinase isoenzyme distribution did not change even after 60 minutes of ischemia, and thus cannot account for changes in reaction velocity. $pHi$ was the same in preischemic and postischemic myocardium. Thus, of the four factors that can alter creatine kinase reaction velocity in vivo—substrate concentration, total enzyme activity, isoenzyme localization, and $pHi$—substrate concentration is the major factor determining reaction velocity in the postischemic myocardium.

Prior to this investigation, there has been only one study reporting values for creatine kinase reaction velocity during postischemia. Nunnally and Hollis studied isolated rabbit hearts reperfused after 35 minutes of ischemia. They did not report postischemic performance or metabolite levels. From their spectra and pressure recordings, we estimate that performance and ATP content were approximately 50% of control. In agreement with our results, they found that $T_1$ of creatine phosphate did not change in reperfused hearts. In contrast, however, the reverse reaction velocity ($k_{rev}$) 

$$\gamma-P - ATP \rightarrow creatine phosphate$$

was increased approximately threefold during postischemia, while forward reaction velocity ($k_{for}$) (creatine phosphate $\rightarrow \gamma-P$ATP) was unchanged. We found for ferret hearts reperfused after 40 minutes of ischemia, where performance and ATP levels were also depressed approximately 50%, that $k_{for}$ decreased from 0.70 to 0.20 sec$^{-1}$. One possible explanation for this discrepancy could be that in the study of Nunnally and Hollis, where stability of hearts during reperfusion was not reported, creatine phosphate or ATP levels were unstable during postischemia. This would lead to an overestimation of $k_{for}$ and $k_{rev}$.

**Mechanism for Depressed Postischemic Function**

The mechanisms responsible for the depression of postischemic function have not been identified. Proposed mechanisms include impaired energy production, utilization, or transfer; abnormalities of electromechanical coupling related to $Ca^{2+}$ metabolism; activation of phospholipases; and free radical formation. Another mechanism, changes in coronary flow or perfusion pressure due to
occlusion of small vessels by postischemic edema formation or intravascular coagulation (no-reflow phenomenon), can be excluded in our experiments since both coronary perfusion pressure (100 mm Hg) and coronary flow were identical in control and postischemic hearts. Similarly, in our studies, neither elevated inorganic phosphate levels nor intracellular acidosis can be responsible for depressed function in hearts reperfused after 20 and 40 minutes of ischemia because inorganic phosphate and pH were normal in these hearts. Increased inorganic phosphate levels and slightly decreased pH may, however, contribute to depressed function of hearts reperfused after 60 minutes of ischemia.

In this report, we measure each of the possible rate limiting steps for ATP turnover: Maximal ATP synthesis via oxidative phosphorylation was estimated from oxygen-consumption measurements, ATP utilization at the myofibrils was assessed from the rate-pressure product, and high-energy phosphate transfer via creatine kinase was measured using 31P-NMR magnetization transfer. Each of these three components have been proposed as the mechanism limiting postischemic performance.

Mitochondria isolated from myocardium injured by ischemia and reperfusion have been shown to be severely damaged.31 Such mitochondria accumulated large quantities of Ca2+, and oxidative phosphorylation (state 3 respiration and respiratory control ratio) was severely impaired. Furthermore, the efficiency of oxidative phosphorylation was compromised, as the rate of oxygen consumption became uncoupled from the rate of ATP synthesis, resulting in a decrease of the P/O ratio.11,12 In our study, at least for hearts injured by 40 or 60 minutes of ischemia, mitochondrial function was also impaired. We found that, compared with the recovery of performance, oxygen consumption of these hearts was disproportionately high. The ratio of ATP synthesis via oxidative phosphorylation estimated from oxygen consumption measurements (assuming a P/O ratio of 3, ATP synthesis rate = oxygen consumption [µmol/min x g dry weight] x 6) to cardiac performance was 6.8 for preischemic hearts and 7.0, 13.0, and 37.6 (µmol ATP/mm Hg x g dry weight) for hearts recovering from 20, 40, or 60 minutes of ischemia, respectively. These data indicate that mitochondrial ATP synthesis is abnormal, the P/O ratio is lower than 3, and oxygen is wasted. This discordance holds true, even when these results are corrected for basal oxygen consumption (3.0 µmol/g dry wt/min).32 This discrepancy between oxygen consumption and cardiac performance during reperfusion has been reported by others.33 Thus, reduced ATP synthesis rates could limit recovery of postischemic function.

Postischemic function may also be limited by ATP utilization by the myofibril. Greenfield and Swain13 report that in the intact dog, activity of the myofibrillar creatine kinase isoenzyme, MM creatine kinase (CKmm), decreased by 30% after 15 minutes of ischemia followed by reperfusion. They also found that postischemic ADP levels decreased from control values of 216 µM to 92 µM. Since the apparent Km of ADP for CKmm was 105 µM, they conclude that ADP limits myofibrillar creatine kinase reaction velocity in postischemic myocardium. Postischemic dysfunction was therefore attributed to limitation of energy utilization at myofibrils caused by decreased CKmm activity and by restricted substrate (ADP) availability. In our study, total tissue CKmm activity was not reduced until after 60 minutes of ischemia, where it declined by approximately 25%. Thus, in our study, reduced myofibrillar CKmm activity could limit postischemic ATP utilization only if it is postulated that CKmm dissociates from the myofibrils, but retains its activity in the cytoplasm. We did not measure this. The calculated ADP concentrations of postischemic ferret hearts in this study (93, 40, 15, and 4 µM for control hearts and hearts reperfused after 20, 40, and 60 minutes of ischemia, respectively) were all below the apparent Km of CKmm (105 µM) obtained for dog myofibrillar creatine kinase. Km of ADP for ferret myofibrillar CKmm is not known; however, if the kinetic properties of ferret myofibrillar creatine kinase are the same as for dog, it is possible that energy utilization by the myofibril is limited by substrate (ADP) availability.

Finally, energy transfer via creatine kinase could limit recovery of postischemic performance. The close correlation between cardiac performance and creatine kinase reaction velocity observed in this study is consistent with this hypothesis. Furthermore, Bittl et al11 have demonstrated that in the isolated rabbit heart reperfused after 10–60 minutes of ischemia mitochondrial creatine kinase activity is lost in proportion to the duration of ischemia and that recovery of postischemic function correlated well with mito-CK activity. Perry et al4 showed that in rabbit hearts at different developmental stages mito-CK activity correlated with creatine kinase reaction velocity. Taken together, these data suggest that mito-CK plays a role for energy transfer, and that energy transfer plays an important role for the recovery of postischemic function. Although we found a close correlation between energy transfer and performance in the postischemic myocardium, we observed no decrease of tissue mito-CK activity or the mito CK/citrate synthase ratio after 20 or 40 minutes of ischemia and only a 25% decrease (mito-CK) or no decrease (mito-CK/citrate synthase) after 60 minutes of ischemia. Thus, we could not test whether decreased mito-CK activity contributes to decreased creatine kinase activity and cardiac performance in the ferret heart.

By comparing the rate of ATP synthesis estimated from oxygen consumption measurements and the rate of high-energy phosphate transfer via the creatine kinase reaction, we can assess whether energy transfer becomes limiting in postischemic hearts. Assuming a P/O ratio of 3, an upper limit for
ATP synthesis can be calculated from oxygen consumption. The ratio of creatine kinase reaction velocity to maximum ATP synthesis rate was 18.2, 18.0, 8.8, and 8.6 for control hearts and hearts reperfused after 20, 40, and 60 minutes of ischemia, respectively. These values for postischemic hearts are most likely underestimated because of futile oxygen consumption. Thus, in all preischemic and postischemic hearts, creatine kinase reaction velocity is an order of magnitude greater than maximum ATP synthesis. We conclude that high-energy phosphate transfer via creatine kinase cannot be limiting for the recovery of function in the mildly or severely injured postischemic myocardium.

In summary, using $^3$P-NMR magnetization transfer to measure creatine kinase reaction velocity, oxygen consumption to estimate maximum ATP synthesis via oxidative phosphorylation, and the rate-pressure product to estimate ATP utilization, we have defined the relationships among synthesis, transfer and utilization of ATP in the postischemic ferret heart. We found that high-energy phosphate synthesis, transfer and utilization are all depressed. High-energy phosphate transfer via creatine kinase decreased in proportion to decreased cardiac performance. The decreases in creatine kinase velocity were explained by changes in cytosolic ADP and creatine levels. The low cytosolic creatine kinase velocity were explained by changes in cytosolic ADP and creatine levels. Thus, we conclude that cytosolic high-energy phosphate transfer via creatine kinase is not limiting. Instead, we postulate that decreased ATP synthesis limits postischemic cardiac performance.

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KEY WORDS • postischemia • cardiac performance • creatine kinase reaction velocity • oxygen consumption
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