ATP Synthesis During Low-Flow Ischemia
Influence of Increased Glycolytic Substrate

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Background—Our goals were to (1) simulate the degree of low-flow ischemia and mixed anaerobic and aerobic metabolism of an acutely infarcting region; (2) define changes in anaerobic glycolysis, oxidative phosphorylation, and the creatine kinase (CK) reaction velocity; and (3) determine whether and how increased glycolytic substrate alters the energetic profile, function, and recovery of the ischemic myocardium in the isolated blood-perfused rat heart.

Methods and Results—Hearts had 60 minutes of low-flow ischemia (10% of baseline coronary flow) and 30 minutes of reperfusion with either control or high glucose and insulin (G+I) as substrate. In controls, during ischemia, rate-pressure product and oxygen consumption decreased by 84%. CK velocity decreased by 64%; ATP and phosphocreatine (PCr) concentrations decreased by 51% and 63%, respectively; inorganic phosphate (Pi) concentration increased by 300%; and free [ADP] did not increase. During ischemia, relative to controls, the G+I group had similar CK velocity, oxygen consumption, and tissue acidosis but increased glycolysis, higher [ATP] and [PCr], and lower [Pi] and therefore had a greater free energy yield from ATP hydrolysis. Ischemic systolic and diastolic function and postischemic recovery were better.

Conclusions—During low-flow ischemia simulating an acute myocardial infarction region, oxidative phosphorylation accounted for 90% of ATP synthesis. The CK velocity fell by 66%, and CK did not completely use available PCr to slow ATP depletion. G+I, by increasing glycolysis, slowed ATP depletion, maintained lower [Pi], and maintained a higher free energy from ATP hydrolysis. This improved energetic profile resulted in better systolic and diastolic function during ischemia and reperfusion. These results support the clinical use of G+I in acute MI. (Circulation. 2000;101:2090-2096.)

Key Words: creatine kinase • glucose • insulin • ischemia • metabolism

During normoxia, ATP delivery to the myofilaments and membrane ion pumps is ensured by ATP synthesis from glycolysis and oxidative phosphorylation. The creatine kinase (CK) reaction contributes to the ability of cardiac muscle to increase and sustain high levels of work, as shown during the ATP supply-demand mismatch of hypoxia1 and high cardiac work loads2 when phosphoryl transfer from phosphocreatine (PCr) to ADP slows the rate of ATP depletion. Moreover, maintaining high ATP and low ADP and inorganic phosphate (Pi) levels maximizes the free energy available from ATP hydrolysis.

The relative contributions of oxidative versus glycolytic ATP synthesis rates to total ATP synthesis; the time course and extent of changes in [ATP], [ADP], [Pi], pH, and the free energy of ATP hydrolysis; and concomitant systolic and diastolic dysfunction that occurs during the low-flow ischemia of an acute myocardial infarction (MI) region are not known. Previous work in animals has suggested that oxidative metabolism remains the major ATP source in the ischemic region immediately after coronary ligation.3 Despite its importance in supporting cardiac function under stress, changes in CK reaction velocity are also undefined during low-flow ischemia. Thus, our first goal was to define the alterations that occur in anaerobic glycolysis, oxidative phosphorylation, and CK velocity simultaneously with changes in systolic and diastolic function during ischemia and reperfusion. These results support the clinical use of G+I in acute MI. (Circulation. 2000;101:2090-2096.)

Key Words: creatine kinase • glucose • insulin • ischemia • metabolism

During low-flow ischemia, the activity of aerobic and anaerobic pathways are determined in part by the amount of residual coronary flow and available carbon substrates. Increasing glycolytic substrate with high glucose and insulin (G+I) has been reported to be beneficial in low-flow ischemia and patients with acute MI,5-9 but the bioenergetic consequences of G+I in this setting are unknown. G+I increases glycolysis,5 but it may also affect oxidative phosphorylation and CK velocity by increasing pyruvate delivery to the Krebs cycle, by worsening tissue acidosis, and by
changing ADP and/or P, concentrations. Therefore, our second goal was to define the effects of increased glycolytic substrate on each of these possibilities.

To achieve these goals, 31P NMR spectroscopy and magnetization transfer (MT) measurements were performed in isolated blood-perfused hearts subjected to a clinically relevant degree of low-flow ischemia (10% of baseline coronary flow) and provided with either normal levels of insulin and the major physiological substrates (glucose, free fatty acid, and lactate) (the control group) or to the control levels of free fatty acid and lactate but with increased levels of glucose and insulin (the G+I group).

Methods

Heart Preparation

Isovolumic LV and coronary perfusion pressures were recorded in RBC-perfused rat hearts (balloon in LV) isolated from fasted male Wistar rats (300 to 350 g). For experiments using NMR spectroscopy, the heart was placed in a 20-mm glass NMR tube in an NMR spectrometer and perfused with phosphate-free Krebs-Henseleit buffer containing bovine RBCs at a hematocrit of 40% as described. Animal care was in accord with the protocol of the Boston University School of Medicine and Harvard Medical School Institutional Animal Care and Use Committees.

Experimental Protocols

After 60 minutes of baseline normoxic perfusion at a constant coronary flow rate (coronary perfusion pressure 80 mm Hg), hearts underwent 60 minutes of low-flow ischemia (constant coronary flow at 10% of baseline), were reperfused for 30 minutes at the baseline coronary flow rate, and then were freeze-clamped. In control hearts (n=12), the initial concentrations of 5.5 mmol/L glucose and 15 \( \mu \text{mol/L} \) insulin were maintained throughout the protocol. In the G+I group (n=12), from 5 minutes before the onset of low-flow ischemia until the end of reperfusion, high glucose and insulin were infused into the aortic cannula; final concentrations were 19.5 mmol/L glucose and 250 \( \mu \text{mol/L} \) insulin. Half of the experiments (n=6 per group) were performed without NMR spectroscopy to permit the collection of venous effluent for measurement of MV\( \dot{O}_2 \) and lactate.

31P NMR Measurements

Standard 1-pulse 31P NMR spectra (Figure 1) obtained at 161.94 MHz as previously described were acquired over 2 minutes by signal-averaging 52 scans of 60\( \mu \text{s} \) (27 \( \mu \text{s} \)) read pulses separated by an interscan delay of 2.3 seconds immediately before and after each MT experiment and each change in coronary flow. Four MT experiments (Figure 2) were carried out: 1 before ischemia, 1 after 10 minutes and again after 38 minutes of ischemia (early and late ischemia, respectively), and 1 after 10 minutes of reperfusion.

Metabolite Contents and Conversion to Concentrations

Integrated signal intensities in 31P NMR spectra corresponding to the ATP, phosphocreatine (PCr), and P, contents of the heart were measured by NMR1 curve-fitting routine (New Methods Research Inc). Signal intensities of the \([\beta-P]ATP \) resonance peaks during baseline perfusion were indistinguishable for the glucose and insulin and the control groups and were assigned a concentration value of 10.8 mmol/L, independently determined by high-pressure liquid chromatography. Other metabolite concentrations were determined from the ratio of their signal intensities (corrected for differential relaxation) relative to that of \([\beta-P]ATP \) during the stabilization period multiplied by 10.8 mmol/L. Intracellular pH was determined by comparing the chemical shift difference between the \( P_i \) and PCr resonances with values obtained from a external standard curve.

[ADP] was calculated by 2 methods. The first makes the assumption that the CK reaction was at or near equilibrium throughout the protocol: \( K_{eq}=\frac{[ATP][free \ creatine]}{[ADP][PCr]}[H^+], \) where \( K_{eq} \) was set to 1.66 \( \times \) 10^3 at pH 7.1 for baseline and reperfusion periods, and 1.47 \( \times \) 10^3 at pH 6.2 for ischemia. The second method assumes that the CK reaction is at equilibrium only during the baseline period. The pseudo–first-order rate constant of the CK reaction, \( k_{tok} \), measured by MT is the product of the second-order rate constant, \( k' \), and free [ADP]. We calculated the second-order rate constant at baseline (by dividing \( k_{tok} \) by [ADP]) and assumed it remained constant. [ADP] for ischemia and reperfusion was estimated by dividing \( k_{tok} \) (measured by MT) by \( k' \), referred to as [ADP]'. This second method of [ADP]' calculation recognizes that \( k' \) may vary during the experimental protocol. Total tissue creatine measured in a subset of hearts was not different between groups and was unaffected by ischemia and reperfusion (22 to 23 mmol/L).
The free-energy release from ATP hydrolysis was calculated from the equation 
\[ \Delta G = \Delta G' + RT \ln \left( \frac{\text{[ATP]} \times \text{[ADP]} \times \text{[Pi]}}{\text{[ATP]}_{\text{in}} \times \text{[Pi]}_{\text{in}} \times \text{[ADP]}_{\text{in}}} \right) \]
where \( \Delta G' \) (30.5 kJ/mmol) is the value of ATP hydrolysis under standard conditions of molarity, temperature, pH, and \([\text{Mg}^{2+}]\). \( R \) is the gas constant (8.314 J mol\(^{-1}\) K\(^{-1}\)), and \( T \) is temperature in kelvin. Input values for ATP and P, were obtained from \(^{31}\)P NMR measurements, [ADP], and [ATP].

Lactate concentration was measured in coronary arterial and venous sample supernatants after digestion with perchloric acid. Lactate production rates (\( \mu \text{mol g dry wt}^{-1} \cdot \text{min}^{-1} \)) were calculated from the coronary flow rate and heart weight. Total lactate production was determined from the area under the curve of lactate production rate versus time during ischemia and the first 90 seconds of reperfusion. Tissue lactate and glycogen were measured in the hearts frozen at the end of reperfusion. MVO\(_2\) was calculated from the arteriovenous O\(_2\) content differences derived from O\(_2\) saturation curves for the Krebs-RBC perfusate over the experimental range of pH and Po\(_2\). Lactate production and MVO\(_2\) were reported as (mmol/L/s) by using measured wet/dry heart weight ratios and a value of 0.5 mL intracellular water per gram wet weight. Dry/wet weight is constant during low-flow ischemia and decreases by only 6% after reperfusion.

**CK Reaction Velocity**

The CK reaction is
\[ k_{\text{rev}} \frac{[\text{ADP}][\text{PCr}]}{[\text{ATP}][\text{Creatine}]} \]

The MT measurements of the forward CK reaction (PCr\( \leftrightarrow \gamma\text{-P}[\text{ATP}]) were analyzed according to the 2-site chemical exchange model yielding the pseudo-first-order rate constant, \( k_{\text{in}} \).\(^{11}\) Multiplying \( k_{\text{in}} \) by [PCr] yields the measured reaction velocity, \( V_{\text{meas}} = k_{\text{in}}[\text{PCr}] \). CK velocity can also be calculated from the rate equation\(^{11}\):
\[ V_{\text{rev}} = V_{\text{meas}}[\text{ADP}][\text{PCr}]/(1 + D K_{\text{D}}) \]
Measured input values are 55 (mmol/L)/s for \( V_{\text{meas}} \) (total CK activity under saturating conditions at 37°C), the cytosolic concentrations of PCr and ADP for each heart and kinetic constants from Reference 17.

**Statistical Analysis**

Data acquired sequentially in individual hearts were tested by a 2-way ANOVA for repeated measures. When 2-way ANOVA gave a significant (\( P<0.05 \)) difference either among the sequential measures or between the 2 groups, the data were further analyzed:

1. 1-factor ANOVA and 2-tailed Student’s \( t \) tests were used to test for statistical significance among the sequential experimental periods (stabilization, early ischemia, late ischemia, and reperfusion).
2. Either a 2-tailed unpaired Student’s \( t \) test or a Mann-Whitney rank test comparing control and G+I-treated hearts was used to test for statistical significance between the 2 groups. All calculations were aided by Statview 512+ (Brain Power Inc). All data are presented as mean±SEM.

**Results**

**Hemodynamics**

During ischemia, systolic function (and also developed pressure and rate-pressure product) decreased to \( \approx \)20% of baseline (Table 1 and Figure 3). G+I slightly increased systolic function during late ischemia and substantially increased it during early reperfusion (Figure 3). G+I exerted a highly protective effect on diastolic function, markedly blunting the increase in diastolic pressure during both ischemia and reperfusion. G+I also decreased coronary resistance (coronary perfusion pressure at constant coronary flow) during ischemia and reperfusion.

**Metabolite Concentrations**

Control [ATP] and [PCr] decreased early and progressively during ischemia and did not recover during reperfusion (Figures 1 and 4 and Table 2). In contrast, for the G+I group, [ATP] did not decrease during early ischemia, [ATP] and [PCr] were higher than in controls at late ischemia, and neither increased during reperfusion. [ADP] calculated by 2 methods (see Methods) did not increase in either group during ischemia but tended to be lower in the G+I group. P, increased 3-fold by end ischemia in controls but did not change with G+I. There were no differences in resonance areas for monophosphate esters between the 2 groups during ischemia or reperfusion. Intracellular pH decreased to \( \approx \)6.2 in both groups during ischemia.

\( \Delta G_{\gamma} \) was similar and high in the control and G+I groups for preischemia and early ischemia. During late ischemia, \( \Delta G_{\gamma} \) (calculated with either set of [ADP] values) decreased by \( \approx \)2 to 4 kJ/mol for the control group but stayed high in the G+I group.

**Oxidative Phosphorylation**

O\(_2\) consumption (MVO\(_2\)), used as the index of oxidative phosphorylation, was comparable in the 2 groups, declining to \( \approx \)18% of baseline during ischemia and recovering to 72% to 95%. Thus, increasing the supply of glucose did not increase MVO\(_2\).
Glycolysis, assessed from ischemic lactate production ($\mu$mol $\cdot$ g dry wt $^{-1}$ $\cdot$ min $^{-1}$), was increased in the G+I hearts. In controls, lactate production peaked at 20 minutes (1.66±0.62) and thereafter gradually declined to 1.02±0.38 at end ischemia. In contrast, in G+I hearts, lactate production progressively increased to 40 minutes (2.93±0.38) and remained high (2.44±0.3) ($P<0.01$ versus controls at end ischemia). Total ischemic lactate production was 166±32 μmol/g dry wt for controls and 273±22 μmol/g dry wt for G+I hearts ($P<0.05$).

**Table 1. Cardiac Performance**

<table>
<thead>
<tr>
<th></th>
<th>Pre-Ischemia</th>
<th>End Ischemia</th>
<th>Reperfusion</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>G+I</td>
<td>Control</td>
</tr>
<tr>
<td>CF, mL $\cdot$ min $^{-1}$ $\cdot$ g wet wt $^{-1}$</td>
<td>2.42±0.12</td>
<td>2.65±0.17</td>
<td>0.24±0.01</td>
</tr>
<tr>
<td>CPP, mm Hg</td>
<td>80±3</td>
<td>78±2</td>
<td>20±2</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>268±18</td>
<td>243±12</td>
<td>225±15</td>
</tr>
<tr>
<td>LVDP, mm Hg</td>
<td>117±5</td>
<td>129±7</td>
<td>23±3</td>
</tr>
<tr>
<td>LV EDP, mm Hg</td>
<td>9±0.5</td>
<td>9±0.4</td>
<td>43±2.1</td>
</tr>
<tr>
<td>RPP, $10^3$ mm Hg $\cdot$ bpm</td>
<td>30.0±1.2</td>
<td>30.7±1.6</td>
<td>4.4±0.5</td>
</tr>
<tr>
<td>MV$\dot{O}_2$, μmol $\cdot$ min $^{-1}$ $\cdot$ g wet wt $^{-1}$</td>
<td>5.8±0.5</td>
<td>6.6±0.7</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>ATP synth, (mmol/L)/s</td>
<td>1.0±0.1</td>
<td>1.1±0.1</td>
<td>0.18±0.02</td>
</tr>
</tbody>
</table>

CF indicates coronary flow; CPP, coronary perfusion pressure; HR, heart rate; LVDP, LV developed pressure; LV EDP, LV end-diastolic pressure; RPP, rate-pressure product of HR $\times$ LVDP; and ATP synth, ATP synthesis rate from oxidative phosphorylation (based on MV$\dot{O}_2$, assuming a P:O ratio of 2.5.23 All data are reported as mean±SEM, n=12 per group, except for MV$\dot{O}_2$, which is n=6 per group.

*P<0.05 control vs G+I.

**Figure 3.** Hemodynamic measurements in control (○) and G+I hearts (●). Data are shown during preischemic perfusion (0 to 60 minutes), low-flow ischemia (60 to 120 minutes), and reperfusion (120 to 150 minutes). n=12 per group. *P<0.05 vs equivalent control values.

**Figure 4.** ATP, PCr, and P$_i$ concentrations and intracellular pH for control (○; n=6) and G+I (●; n=6) hearts determined from 1-pulse $^{31}$P NMR spectra taken during protocol as in Figure 3. High G+I slows decrease in high-energy phosphate concentrations and protects against a rise in P$_i$ during low-flow ischemia. Intracellular pH falls similarly for both groups. *P<0.05 vs preischemia; +P<0.05 vs control.
TABLE 2. Substrate Concentration and Flux Through the Forward CK Reaction

<table>
<thead>
<tr>
<th></th>
<th>[ATP]</th>
<th>[PO4]</th>
<th>[Pi]</th>
<th>[ADP]$_m$</th>
<th>[ADP]$_f$</th>
<th>$k_{for}$</th>
<th>$V_{for}$</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>mmol/L</td>
<td>mmol/L</td>
<td>mmol/L</td>
<td>μmol/L</td>
<td>μmol/L</td>
<td>1/s</td>
<td>(mmol/L/s)</td>
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<tr>
<td>Preischemia</td>
<td></td>
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<tr>
<td>Control</td>
<td>10.8</td>
<td>18.5±1.2</td>
<td>2.8±0.3</td>
<td>29±5.5</td>
<td>29±5.5</td>
<td>0.58±0.04</td>
<td>10.2±1.2</td>
</tr>
<tr>
<td>G+I</td>
<td>10.8</td>
<td>18.8±1.3</td>
<td>3.1±0.4</td>
<td>30.7±7.6</td>
<td>30.7±7.6</td>
<td>0.55±0.04</td>
<td>10.0±1.2</td>
</tr>
<tr>
<td>Early ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.1±0.5*</td>
<td>8.9±1.0*</td>
<td>5.4±0.5*</td>
<td>13.6±1*</td>
<td>33.8±9.3</td>
<td>0.61±0.1</td>
<td>5.2±0.4* (51)</td>
</tr>
<tr>
<td>G+I</td>
<td>9.5±1.4</td>
<td>11.9±1.8*</td>
<td>3.7±0.6</td>
<td>17.9±5</td>
<td>33.9±11.2</td>
<td>0.50±0.07</td>
<td>7.0±1.7 (69)</td>
</tr>
<tr>
<td>G+I vs control††</td>
<td>$P=0.054$</td>
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<tr>
<td>Late ischemia</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>5.3±0.3*†</td>
<td>6.9±0.6*†</td>
<td>8.5±0.6†</td>
<td>11.1±0.8†</td>
<td>27.4±6.8</td>
<td>0.53±0.04</td>
<td>3.7±0.4* (36)</td>
</tr>
<tr>
<td>G+I</td>
<td>8.1±1.4††</td>
<td>10.9±1.7*</td>
<td>3.8±0.6</td>
<td>7.7±1.5*</td>
<td>20.4±4.5</td>
<td>0.42±0.06</td>
<td>5.0±1.4* (49)</td>
</tr>
<tr>
<td>G+I vs control††</td>
<td>$P=0.04$</td>
<td>$P=0.052$</td>
<td>$P=0.001$</td>
<td></td>
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<tr>
<td>Reperefusion</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>4.7±0.6*†</td>
<td>9.7±1.4*</td>
<td>5.2±0.6§</td>
<td>48.9±5.8*§</td>
<td>18.6±3.3</td>
<td>0.42±0.05*</td>
<td>4.3±0.9* (42)</td>
</tr>
<tr>
<td>G+I</td>
<td>6.0±0.8*‡§</td>
<td>14.4±0.9*</td>
<td>4.2±0.5</td>
<td>29.5±2.9§</td>
<td>14.6±4.5</td>
<td>0.37±0.03</td>
<td>4.2±0.9* (41)</td>
</tr>
<tr>
<td>G+I vs control††</td>
<td>$P=0.021$</td>
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</table>

$k_{for}$ indicates pseudo-first-order constant for the forward CK reaction (measured using magnetization transfer); $V_{for}$, $k_{for}×[PCr]_t$=velocity of the forward CK reaction (measured using magnetization transfer); $V_{calc}$, velocity of the forward CK reaction calculated using the rate equation; and $ΔG_{AI}$, free energy release of ATP hydrolysis. $V_{calc}$ and $ΔG_{AI}$ are calculated with [ADP]$_f$. Values in parentheses are % of baseline.

*P<0.05 vs stabilization; †P<0.05 late ischemia vs. early ischemia; ‡P<0.05 reperfusion vs. early ischemia; §P<0.05 reperfusion vs. late ischemia.

[[ATP], [PO4], and [Pi]] correspond to average values during the magnetization transfer experiments, whereas data shown in Figure 4 correspond to concentrations calculated from control spectra before and after each magnetization transfer experiment.

†Calculated by use of the equilibrium input values for the CK reaction, as discussed in Methods.

‡Calculated by use of the second-order rate constant determined for ischemic conditions from the equation: $k' = k_{for}/[ADP]_f$: $k' = 0.25±0.06$ for control and $0.24±0.05$ for G+I.

*Given as absolute values.

††There are no significant intergroup differences except where noted.

At end reperfusion, tissue lactate (10.6±1.2 versus 11.6±1.9 μmol/g dry wt, $P=NS$) and tissue glycogen (39.9±10.6 versus 23.9±7.3 μmol glucose equivalent/g dry wt, $P=NS$) were similar for the control and G+I groups.

**Creatine Kinase**

During preischemia, the measured pseudo-first-order rate constant of the CK reaction ($k_{for}$) and CK velocity ($V_{calc}$) were similar for the 2 groups (Figure 2 and Table 2). During ischemia, measured CK velocity decreased similarly in both groups, to 36% to 49% of baseline, and remained depressed (41% to 42%) during reperfusion. Predicted CK velocity ($V_{calc}$) was calculated by use of input values for both [ADP]$_m$ and [ADP]$_f$. The rate equation predicted that during preischemia, $V_{calc}$ would be equal for the control and G+I groups, and during ischemia, $V_{calc}$ would decrease similarly in both groups; both were observed. $V_{calc}$ values were also depressed during ischemia but somewhat less than $V_{calc}$. Thus, both measured and calculated estimates of CK velocity during ischemia showed a moderate inhibition and no influence of the G+I substrate. During reperfusion, the rate equation predicted higher velocities than those measured by MT for both groups.

**Washout of CK Activity**

Coronary venous effluent was collected and assayed for CK activity, and the cumulative CK leakage was calculated. G+I did not affect CK leakage during ischemia ($≈2.6$ IU/g wet wt) or reperfusion (32 IU/g wet wt). Thus, leakage was only $≈0.2\%$ of total tissue CK (1100 IU/g) during ischemia and only 3% during reperfusion.

**Discussion**

**Model of Ischemia**

In this study, red blood cell (RBC)–perfused hearts with a normal hematocrit were hypoperfused at a coronary flow rate that approximated myocardial perfusion levels that have been measured in acute MI in patients. Under these conditions, the coronary flow level and normal hemoglobin content provided enough oxygen delivery that oxidative phosphorylation was still the major source of ATP production and accounted for $≈90\%$ of ATP synthesis despite the ischemic state.

To simulate acute MI perfusion conditions, we imposed an ischemic coronary flow rate of 10% of normal. This value is representative of perfusion levels immediately after a coronary occlusion in humans and dogs. However, an acute MI region undoubtedly contains a gradation of perfusion levels. In regions of relatively more severe ischemia, both glycolysis and oxidative phosphorylation would be relatively more inhibited; with less severe ischemia, oxidative phosphorylation would probably contribute a relatively greater fraction of ATP synthesis.

Many characteristics of this model differ from either total (zero-flow) ischemia or high-flow hypoxia. With normal substrate availability, and like both total ischemia and hyp-
region. Recently, Ponticos and colleagues\textsuperscript{19} presented evidence that CK can be inhibited by as much as 60\% by phosphorylation by AMP kinase under conditions in which PCr/creatine and ATP/ADP levels fall. We suggest that inhibition of CK by this mechanism is likely to occur under the conditions of our study.

### ATP Synthesis With Normal Substrates
At baseline, ATP synthesis rate from oxidative phosphorylation (estimated from MV\textsubscript{O} \textsubscript{2}) and from glycolysis\textsuperscript{20} were \textasciitilde 1.0 and \textasciitilde 0.01 mmol/mL of cell water/s, respectively. During low-flow ischemia, these rates were \textasciitilde 0.2 and \textasciitilde 0.02 (mmol/L)/s, respectively (assuming 1 net mole of glycolytic ATP produced per mole of lactate produced). Comparing low-flow ischemia to baseline, the ATP synthesis rate from oxidative phosphorylation decreased to 20\%, while glycolytic ATP production doubled. The ratio of glycolytic/oxidative ATP synthesis increased 10-fold, from 0.01/1.0 (1\%) to baseline at 0.02/0.2 (10\%). Nonetheless, 90\% of ATP synthesis in this simulated acute MI milieu was via oxidative phosphorylation.

### ATP Synthesis With Increased G+I
A major result of the present study is that G+I increased [ATP] during low-flow ischemia primarily by increased glycolysis and not by increasing oxidative phosphorylation assessed by O\textsubscript{2} utilization. G+I did not stimulate oxidative phosphorylation by increasing [ADP] or [P\textsubscript{i}], because neither was increased by G+I. The increased [ATP] in the G+I group may be partly due to a slight increase in the P:O ratio due to a shift in substrate oxidation from free fatty acid to pyruvate. Finally, our results show that the increase in [ATP] in the G+I group was not the result of increased PCr utilization or of any mechanisms secondary to increased acidosis.

What was the fate of the ATP that resulted from G+I-mediated increase of glycolytic synthesis during ischemia? The amount of glycolytic ATP synthesis during the entire 60-minute ischemic period (estimated from lactate production) was 61 and 104 mmol glycolytic ATP/L cell water in the control and G+I groups, respectively (or \textasciitilde 30 and 52 mmol per heart, respectively). Therefore, the differential rate of ischemic glycolytic ATP synthesis attributable to the G+I was 0.37 mmol \cdot heart \textsuperscript{-1} \cdot min \textsuperscript{-1}, or \textasciitilde 0.7 mmol/L cell water/min. This would have increased [ATP] markedly above the controls, by \textasciitilde 42 mmol/L, at end ischemia if no increase in ATP utilization had occurred in the G+I group. The observation that there was only a 6.8 mmol/L differential in high-energy phosphate concentration between groups at end ischemia suggests that the major portion of the “extra” glycolytic ATP resulting from the G+I group was not the result of increased PCr accumulation or of any mechanisms secondary to increased acidosis.

### Inhibition of CK
Our results for both measured and calculated CK velocity show that CK reaction velocity is lower during low-flow ischemia. This is also demonstrated by the failure of CK to use more of the available PCr to slow the rate of ATP depletion. This result was unexpected and suggests that, in contrast to minimal perturbation of CK velocity observed under conditions of mild underperfusion,\textsuperscript{18} CK is substantially inhibited in the metabolic milieu of an acute infarct

| $V_{\text{calc}}$ (mmol/L)/s | $V_{\text{calc}}$# (mmol/L)/s | $\Delta G_{-p}^*$ & $\Delta G_{-p}^{**}$ |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| 5.8$\pm$0.9                | 6.1$\pm$1.2                | 60.9$\pm$0.5               | 60.9$\pm$0.5               |
| 2.5$\pm$0.1 (43)           | 4.4$\pm$1.0 (76)           | 40.5$\pm$0.3               | 50.7$\pm$1.0               |
| 3.4$\pm$0.5 (55)           | 4.7$\pm$1.3 (77)           | 61.7$\pm$1.0               | 60.1$\pm$1.1               |
| 2.4$\pm$0.4$\times$ (41)   | 4.3$\pm$0.9 (74)           | 58.6$\pm$0.5$\times$       | 50.7$\pm$0.5$\times$       |
| 1.7$\pm$0.8$\times$ (28)   | 3.3$\pm$0.6$\times$ (55)   | 63.2$\pm$1.3               | 60.8$\pm$1.4               |
| 11.3$\pm$0.9$\times$ (195) | 4.3$\pm$0.8 (74)           | 55.9$\pm$0.9$\times$       | 50.7$\pm$0.9$\times$       |
| 8.1$\pm$0.9$\times$ (133)  | 4.3$\pm$1.1 (70)           | 58.5$\pm$0.9$\times$       | 50.7$\pm$1.4               |
| $P=0.039$                   |                            |                             |                             |
trol. Second, intracellular pH also depends on ATP hydrolysis (a proton-generating reaction), which was less with G+I.

**Higher \( \Delta G \) with G+I**

Another major finding is that G+I markedly increased the free energy release from ATP hydrolysis, \( \Delta G_{\text{p}} \). Free energy release from ATP hydrolysis is not constant but rather depends on the concentrations of ATP, ADP, and P. With G+I, [ATP] was higher, [ADP] was relatively unchanged, and [P] was lower than for controls. We calculated \( \Delta G_{\text{p}} \) and \( \Delta G'_{\text{p}} \) using values of [ADP] and [ADP]'. Regardless of which estimate of [ADP] was used, at late ischemia, greater free energy (4.0 to 4.4 kJ/mol) was available for all cellular ATPase reactions for the G+I group.

**Study Limitations**

Our protocol was designed to simulate, as closely as possible, the myocardial metabolic milieu present in an acutely infarcted region in humans. However, because the G+I was started 5 minutes before the onset of ischemia, our protocol does not mimic the timing of drug administration of an acute MI. Because global ischemia was imposed, the entire LV of the isolated heart served as a model of the acute MI region. Despite the imposition of global coronary flow reduction, heterogeneous ischemia most likely exists in this model, as it does in acute clinical MI. Our NMR spectra report average tissue values for the entire heart and do not report any intracellular or regional variations and/or gradients; such average values may belie the extent of metabolite and free energy changes in different regions. Because it is impossible to measure free [ADP] directly, we calculated its value by 2 independent methods. Although there were some differences in estimates for [ADP] according to the method of calculation, both methods resulted in the same overall conclusions regarding the bioenergetic benefits of the G+I substrate.

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**References**

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