Hypoperfusion-induced contractile failure does not require changes in cardiac energetics

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1Cardiac Muscle Research Laboratory, Boston University School of Medicine, Boston, Massachusetts 02118; 2Kardiologie, Universitätsklinik, Inselspital, 3010 Bern, Switzerland; and 3NMR Laboratory for Physiological Chemistry, Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

Saupe, Kurt W., Franz R. Eberli, Joanne S. Ingwall, and Carl S. Apstein. Hypoperfusion-induced contractile failure does not require changes in cardiac energetics. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1715–H1723, 1999.— Decreasing coronary perfusion causes an immediate decrease in contractile function via unknown mechanisms. It has long been suspected that this contractile dysfunction is caused by ischemia-induced changes in cardiac energetics. Our goal was to determine whether changes in cardiac energetics necessarily precede the contractile dysfunction as one would expect if a causal relationship exists. In 14 isolated rat hearts, we gradually decreased coronary perfusion using a coronary perfusate with a normal hematocrit and normal concentrations of the major metabolic substrates. Using 31P NMR spectroscopy to measure ATP, phosphocreatine (PCr), P1, and ADP concentrations ([ATP], [PCr], [P1], [ADP]), pH, and amount of free energy released from ATP hydrolysis (ΔG0,ATP), we found that none of these variables changed significantly until several minutes after systolic pressure had significantly decreased. Even when developed pressure had decreased by over one-third, only very slight changes in [P1], pH, and ΔG0,ATP had occurred, with no significant changes in [ATP], [PCr], or [ADP]. Additionally, the rate of high-energy phosphate transfer between ATP and PCr did not decrease enough during hypoperfusion to explain the contractile dysfunction. We conclude that nonenergetic factors are the dominant cause of the initial decrease in systolic function when myocardial perfusion is decreased.

hibernation; ischemia; perfusion-contraction matching; metabolism

A DECREASE in myocardial perfusion causes an immediate decrease in left ventricular pressure generation. This decreased contractile work in response to hypoperfusion has complex clinical ramifications because it has the detrimental effect of decreasing left ventricular function but the beneficial effect of protecting the hypoperfused myocardium by decreasing its oxygen requirement (22, 30). The mechanisms that cause and subsequently maintain decreased systolic function when myocardial perfusion is decreased are poorly understood. One long-standing hypothesis is that coronary hypoperfusion causes a change in the energetic state of the heart, which subsequently inhibits systolic pressure generation (8, 16, 24). Specifically, it has been suggested that decreased systolic function is caused by depletion of high-energy phosphates [ATP and phosphocreatine (PCr)] and/or accumulation of metabolic by-products such as H+, ADP, and P1 (8, 13, 14, 24, 25, 29, 31). Evidence that supports this hypothesis comes largely from studies in which a severe degree of myocardial hypoperfusion was induced in a few seconds in buffer/crystalloid-perfused isolated hearts. These studies have arrived at a variety of conclusions, including that the decreased systolic function during hypoperfusion is caused by 1) a decrease in phosphorylation potential ([ATP]/[ADP] × [P1]), where brackets indicate concentration) (6, 2) an increase in [P1] (13, 3) an increase in both [P1] and [H+]) (8, and 4) something other than cardiac energetics (23).

A rapid decrease in oxygen delivery to the heart causes obligatory changes in cardiac energetics that are a function of both the rapidity and severity of the decrease in oxygen delivery. Arai et al. (1) demonstrated that a rapid decrease in coronary perfusion causes a much larger change in cardiac energetics than does gradually lowering flow to the same level. Therefore, studies in which myocardial perfusion is decreased to a low level in a few seconds, particularly those that use a buffer/crystalloid perfusate that has a low oxygen content, maximize both the speed and magnitude of the resulting energetic derangement and may observe temporal relationships between changes in cardiac function and energetics during the early minutes of myocardial hypoperfusion that are unrelated to causality.

The goal of our study was to determine during myocardial hypoperfusion whether changes in cardiac energetics necessarily precede the contractile dysfunction as one would expect if they cause it. Our approach was to decrease myocardial perfusion and oxygen delivery gradually to minimize energetic derangement. This was accomplished not only by decreasing myocardial perfusion slowly but also (in contrast to most prior studies in isolated hearts) using a coronary perfusate with a normal hematocrit, oxygen content, and normal concentrations of the major metabolic substrates of glucose, lactate, and long-chain fatty acids. 31P NMR spectroscopy was used to measure [ATP], [PCr], [P1], [ADP], and intracellular pH as coronary perfusion was decreased in these isolated rat hearts.

Recently, other variables related to the energy status of the heart have been suggested as the cause of hypoperfusion-induced contractile failure. For example, it has been suggested that rapid “shuttling” of high-energy phosphates is necessary to maintain normal contractile function and that a decreased rate may

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be the cause of systolic dysfunction during myocardial hypoperfusion (4, 19, 31). Likewise, it has been suggested that the decreased systolic function during hypoperfusion might be caused by a decrease in the amount of free energy released from ATP hydrolysis ($\Delta G_{\text{ATP}}$), which is a function of [ATP], [ADP], and [Pi] (17, 27). Therefore, in addition to measuring [ATP], [PCr], [Pi], and [ADP], and intracellular pH, we also calculated $\Delta G_{\text{ATP}}$ and measured creatine kinase flux rate (CK$_{\text{flux}}$) using the magnetization transfer technique.

**MATERIAL AND METHODS**

Experimental preparation. The isolated isovolumic rat heart preparation, perfused with a red blood cell-containing perfusate at a 40% hematocrit, was used. This methodology has previously been described in detail elsewhere (7). Male Sprague-Dawley rats (n = 14) weighing 400–450 g were deeply anesthetized with 80 mg/kg ip pentobarbital sodium. Hearts were rapidly excised and cannulated by the aorta on a constant-flow perfusion apparatus. Flow was adjusted so that coronary perfusion pressure (CPP) was ~95 mmHg. After equilibration, flow was held constant and CPP was determined by coronary vasomotor tone. A small portion of the left atrium was removed so that a polyethylene (PE-160) drain could be inserted and advanced through the apex of the left ventricle to allow drainage of the Thebesian veins. To measure isovolumic left ventricular pressure, a fluid-filled latex balloon attached to a Statham P23 Db pressure transducer (Gould, Oxnard, CA) was inserted through the mitral valve into the left ventricle. The balloon was filled until a left ventricular end-diastolic pressure (EDP) of ~10 mmHg was achieved, and the balloon volume was then held constant. To pace the heart, salt bridge pacing wires consisting of PE-160 were inserted through the apical catheter. The heart was paced at a cycle length of 5.8 Hz (350 beats/min). Hearts were then inserted in a 20-mm-diameter glass NMR tube that was inserted into an Oxford Instrument 9.4-tesla magnet connected to a General Electric GN400 spectrometer. CPP was monitored via a sidearm of the aortic cannula connected to a Gould-Statham P23 Db pressure transducer. The perfusate level in the NMR tube was maintained by aspiration just above the left atrium by continuous suction through a polyethylene tube. CPP and isovolumic left ventricular pressure were continuously measured using a commercially available data acquisition system (MacLab). All data were sampled at 200 Hz and stored on a hard disk for analysis.

Perfusion solution. The perfusion solution consisted of packed bovine red blood cells resuspended in a phosphate-free modified Krebs-Henseleit solution at a hematocrit of 40%. The packed red cells were processed as previously described (7). The red cell suspension was essentially white blood cell and platelet free. Packed red blood cells were stored in nominally calcium-free buffer at 4°C and washed daily before use. The modified phosphate-free Krebs-Henseleit buffer contained (in mM): 118 NaCl, 4.7 KCl, 2.0 CaCl$_2$, 1.2 MgSO$_4$, 25.5 NaHCO$_3$, 5.5 glucose, 1.0 lactate, 0.5 NaEDTA, 15 μM insulin, and 0.4 μM of acetylcholine. Blood was equilibrated with 20% O$_2$-3% CO$_2$-balance N$_2$ to achieve a PO$_2$ of ~140 mmHg and a pH of 7.4.

To make the red cell perfusate feasible for $^{31}$P NMR spectroscopy, KH$_2$PO$_4$ was not included in the Krebs-Henseleit buffer. This was done to avoid contamination of phosphorus spectra with signal from outside of the heart. Furthermore, to reduce the 2,3-diphosphoglycerate signal from the red blood cells in the NMR-sensitive volume, a solution of mannitol (0.2 M) was superfused around the heart at twice the coronary flow rate to provide rapid removal of venous effluent. These measures effectively reduced the contamination of the $^{31}$P NMR spectra by noncardiac phosphate and also markedly reduced the 2,3-diphosphoglycerate signal, thus permitting $^{31}$P NMR study of the red blood cell-perfused heart.

$^{31}$P NMR spectroscopy. Myocardial energetics were studied using $^{31}$P NMR spectroscopy. Briefly, spectra were collected with the resonance frequency for $^{31}$P of 161.94 MHz in a GE-400, 9.4-tesla spectrometer using a pulse width of 27 μs to give a 60° flip angle. Using an interpulse delay of 2.14 s, we collected 104 scans in each 4-min period. Individual free induction decays were zero filled and weighted with a 20-Hz line-broadening decaying exponential before Fourier transformation.

Magnetization transfer was used to measure the pseudo-first-order rate constant ($k_{\text{for}}$) for the forward CK reaction (PCr + MgADP + H$^+$ → MgATP + creatine). Magnetization transfer was performed by applying a low-power radiofrequency pulse centered at the γ-phosphate of ATP for either 0.0 s (M$_0$) or 4.8 s (M$'$). The $k_{\text{for}}$ for this reaction was calculated as $k_{\text{for}}$ = (M$_0$ − M$'$)/(1 × M$'$), with T1 set to 3.5 s (10). Flux through the CK reaction (rate of ATP synthesis from PCr) was calculated as $k_{\text{for}}$ × [PCr]. We measured $k_{\text{for}}$ at baseline and after 30 min of hypoperfusion. The two-point (M$_0$, M$'$) method for measuring $k_{\text{for}}$ was used because it allowed us to complete a measurement of $k_{\text{for}}$ in 15 min.

Protocol. Hearts (n = 14) were initially perfused at a CPP of 90–100 mmHg for a 28-min baseline period. After this baseline period, CPP was decreased at a rate of ~30% per minute for 3 min by decreasing coronary flow. Coronary flow was decreased by a total of 69–70% in each heart. This level of hypoperfusion was chosen because pilot data indicated that it caused a decrease in developed pressure (systolic – diastolic pressure) of ~50%. Physiological data were collected at 2-min intervals throughout the protocol except during the early minutes of hypoperfusion, when they were recorded at 1-min intervals. Cardiac energetics were assessed using two types of $^{31}$P NMR spectroscopy. Concentrations of ATP, PCr, P$_i$, and H$^+$ were determined with one-pulse spectra, and CK$_{\text{flux}}$ was measured using magnetization transfer. One magnetization transfer measurement was obtained during the baseline period, and the second was obtained after 30 min of myocardial hypoperfusion. A pair of one-pulse spectra was collected just before and after each measurement of CK$_{\text{flux}}$. One-pulse spectra were sequentially collected for determination of cardiac energetics at the onset of myocardial hypoperfusion. For the one-pulse spectra, the baseline spectra are plotted at time 0 and all other spectra are plotted at the midpoint of their 4-min collection time (2, 6, and 10 min after the start of hypoperfusion). After 30 min of hypoperfusion, when cardiac energetics and contractile function were relatively stable, CK$_{\text{flux}}$ was again measured.

Data analysis and statistics. The area under the P$_i$, PCr, and γ-phosphate of ATP peaks of each $^{31}$P spectrum was measured using commercially available software (NMR1). From fully relaxed spectra (interpulse delay of 10 s), it was determined that the area under the P$_i$ and PCr peaks needed
to be corrected for partial saturation by being multiplied by 1.15 and 1.2, respectively. Area units were converted to intracellular concentrations by assuming that the ATP concentration in each heart during the control period was 10.8 mM (2). Setting the area under the γ-phosphate peak of ATP during the control period of each heart equal to 10.8 mM provided a conversion factor used to convert the PCr and P, area units into concentrations. Intracellular pH was measured by comparing the chemical shift between P, and PCr resonances to a standard curve.

ADP was calculated from the CK equilibrium equation using Keq = [ATP][free creatine]/[ADP][PCr][H+], where Keq was set equal to 1.66 × 109 at pH 7 (26, 34). A total creatine concentration of 33 mM was used in all calculations. ΔGATP was calculated from the equation ΔGATP = ΔG° - RTln[ATP]/[ADP][P,], where ΔG° (-30.5 kJ/mol) is the value of ATP hydrolysis under standard conditions of molarity, temperature, pH, and [Mg2+]; R is the gas constant (8.314 J·mol⁻¹·K⁻¹); and T is temperature in Kelvin (20).

Values for physiological data and energetics are expressed as means ± SE. Data were analyzed statistically using ANOVA with repeated measures and a Fisher post hoc test. Differences were considered statistically significant at P < 0.05.

RESULTS

During the baseline period there were no significant changes in any measure of cardiac energetics or contractile function, as shown in Table 1, where values at the start and end of the baseline magnetization transfer were not different. After the 28-min baseline period, CPP was gradually lowered from 95 ± 2 mmHg at baseline to 63 ± 2 mmHg after 1 min (34% decrease) to 43 ± 2 mmHg after 2 min (27% decrease) to 34 ± 1 mmHg after 3 min (26% decrease) (Fig. 1). Coronary flow was 2.43 ± 0.25 ml/min at baseline and 0.72 ± 0.07 ml/min after 10 min of hypoperfusion. The lowering of CPP caused systolic pressure to decrease from its baseline value of 80 ± 3 mmHg to 72 ± 3 mmHg after 1 min, 67 ± 2 mmHg after 2 min, and 59 ± 3 mmHg after 3 min (each significantly less than baseline). During this time, heart rate was maintained constant at 350 beats/min with epicardial pacing and EDP did not change from its baseline value of 8 ± 1 mmHg.

Table 1. Effect of hypoperfusion on creatine kinase reaction

<table>
<thead>
<tr>
<th>Baseline Magnetization Transfer</th>
<th>Magnitization Transfer at 30 min of Hypoperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start</td>
<td>End</td>
</tr>
<tr>
<td>ATP, mM</td>
<td>10.9±0.1</td>
</tr>
<tr>
<td>PCr, mM</td>
<td>24.6±0.8</td>
</tr>
<tr>
<td>P, mM</td>
<td>5.3±0.6</td>
</tr>
<tr>
<td>Coronary perfusion pressure, mmHg</td>
<td>96.4±2.4</td>
</tr>
<tr>
<td>Developed pressure, mmHg</td>
<td>77.4</td>
</tr>
<tr>
<td>kₚ₀, s⁻¹</td>
<td>0.40±0.02</td>
</tr>
<tr>
<td>CKₚ₀, mM/s</td>
<td>9.8±0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 rat hearts. PCr, phosphocreatine; developed pressure, systolic pressure – diastolic pressure; kₚ₀, pseudo first-order rate constant; CKₚ₀, creatine kinase flux rate. *Significantly different from baseline (P < 0.05).

Fig. 1. Effect of gradually lowering coronary perfusion pressure (starting at time 0) on systolic pressure. Coronary perfusion was lowered gradually to minimize changes in cardiac energetics that occur during myocardial hypoperfusion. Systolic pressure was significantly decreased relative to baseline after 1 min of hypoperfusion. *Significantly different from baseline.

Figure 2 is a representative example of contractile function and 31P spectra measured at baseline and after 10 min of hypoperfusion. In this example, the heart responded to hypoperfusion by decreasing left ventricular systolic pressure from 96 mmHg at baseline to 52 mmHg. This reduction in systolic pressure was accompanied by only small changes in cardiac energetics, because [ATP] was 10.7 mM at both time points, [PCr] decreased from 24.5 to 21.8 mM, [P,] increased from 7.0 to 9.5 mM, and pH decreased from 7.23 to 7.16. This example highlights the fact that large hypoperfusion-induced decreases in systolic pressure occur in the presence of very small changes in cardiac energetics.

Effect of hypoperfusion on cardiac energetics. The effect of gradually lowering CPP on cardiac energetics is shown in Figs. 3 and 4. During the first 2 min of hypoperfusion, when systolic pressure fell significantly, there were no changes in [ATP], [PCr], [P,], [ADP], ΔGATP, or pH.

After 6 min of hypoperfusion, [ATP], [PCr], [ADP], and ΔGATP had not changed significantly from baseline (Figs. 3 and 4). In contrast, [P,] increased from 6.1±0.8 to 8.6±0.4 mM, and pH decreased from 7.15±0.01 to 7.09±0.02 (each P < 0.05). After 10 min of hypoperfusion, [ATP] and [ADP] were still not significantly different from baseline but [PCr], [P,], pH, and ΔGATP were each significantly different from their baseline values. The total amount of NMR-observable phosphate ([ATP] × 3 + [PCr] + [P,]) did not significantly change during myocardial hypoperfusion (61.5±0.8 mM at baseline, 62.2±1.2 mM after 2 min, 64.7±1.7 mM after 6 min, and 63.2±1.7 mM after 10 min of hypoperfusion).

To further examine the relationship between [P,] and systolic function during hypoperfusion, we plotted these
two variables at each time point (baseline, 2, 6, and 10 min after the start of hypoperfusion) for each of the 14 hearts (Fig. 5). There was not a significant correlation between systolic pressure and [Pi]. When data from all hearts were averaged at each of the four time points, however, there was a strong linear correlation between systolic pressure and [Pi] (Fig. 5, inset). This suggests that the length of time of hypoperfusion is a key determinant of both the fall in systolic pressure and increase in [Pi]. If [Pi] was an important determinant of systolic function during the early minutes of hypoperfusion, hearts with the largest increases in [Pi] would be expected to have the largest decreases in systolic pressure. This was not the case, because no correlation existed between the change in [Pi] and the change in systolic pressure during the first 2 min of hypoperfusion (Fig. 6).

**DISCUSSION**

The mechanisms that link myocardial perfusion level with contractile state are of great clinical importance but are poorly understood. Several studies have demonstrated that when myocardial perfusion is rapidly decreased, changes in cardiac energetics can occur before the decrease in systolic function (6, 13). This temporal relationship has led investigators to suggest that the early changes in cardiac energetics may be the

**Fig. 2.** Left ventricular pressure tracings (top) and 31P NMR spectra (bottom) at baseline and after 10 min of myocardial hypoperfusion. In this example, systolic pressure decreased from 96 to 52 mmHg after 10 min of hypoperfusion with only slight changes in cardiac energetics, because phosphocreatine concentration ([PCr]) decreased 2.7 mM, [Pi] increased 2.5 mM, and [ATP] did not change. This example demonstrates that large hypoperfusion-induced changes in systolic function can occur with only very minor changes in cardiac energetics. PME, phosphate monoesters.

**Fig. 3.** Systolic pressure and 3 measures of cardiac energetics ([ATP], [PCr], and [Pi]) during 10 min of gradually induced myocardial hypoperfusion. Systolic pressure fell significantly after 1 min, but there were no significant changes in [ATP] or [PCr] during the first 6 min. [Pi] was significantly increased from baseline at 6 and 10 min. *Significantly different from baseline.

**CKflux.** The rate of high-energy phosphate transfer between ATP and PCr (CKflux) is determined by measuring the rate of disappearance of the PCr resonance area when the γ-ATP resonance is selectively saturated, as shown in Fig. 7. The difference in the PCr resonance areas between M₀ and M₆ is proportional to CKflux. In this example, we see that during myocardial hypoperfusion [PCr] is somewhat decreased but that the decrease in PCr resonance area during M₆ is very similar at baseline and during hypoperfusion. In this example, systolic pressure decreased from 96 mmHg at baseline to 50 mmHg during M₆, while CKflux decreased from 8.8 to 7.0 mM/s. On average, there was a 27% decrease in CKflux during myocardial hypoperfusion (P < 0.05), an amount that would not be expected to cause any significant change in systolic function (11, 32) (Table 1).
cause of the decreased systolic function. To test this, we determined whether the temporal relationships are obligatory as one would expect them to be if they are causal. We found that when myocardial perfusion was decreased gradually, significant changes in cardiac energetics, including \( \Delta G_{\text{ATP}} \), occurred only after systolic pressure had decreased. The rate of high-energy phosphate transfer between ATP and PCr (CK flux) decreased only modestly during hypoperfusion, making it unlikely to have caused the hypoperfusion-induced systolic dysfunction. We conclude that nonenergetic factors are the dominant cause of the initial decrease in systolic function when myocardial perfusion is decreased slowly.

Relationship between systolic function and cardiac energetics during myocardial hypoperfusion. One approach to studying the mechanism by which myocardial hypoperfusion causes decreased systolic function has been to measure cardiac energetics using \(^{31}\)P NMR spectroscopy in isolated, buffer-perfused hearts as coronary perfusion is rapidly decreased, the goal being to determine what variable(s) change fast enough when perfusion is decreased to potentially explain the rapid decrease in systolic function. Although these studies use similar methodologies, they come to a variety of conclusions. Clarke et al. (6) concluded that phosphorylation potential ([ATP]/[ADP] \( \times \) [Pi]) controls contractile function during myocardial hypoperfusion. Elliott et al. (8) concluded that changes in [Pi] and [H\(^{+}\)] are the major determinants of contractile failure secondary to myocardial hypoperfusion. He et al. (13) measured cardiac energetics with unprecedented time resolution.

![Fig. 4.](image1)

**Fig. 4.** Three other measures of cardiac energetics plotted with systolic pressure (*) during myocardial hypoperfusion. [ADP] (□) did not change during protocol, and pH (●) and amount of free energy released from ATP hydrolysis (ΔG_{\text{ATP}}; □) did not show significant changes from baseline until 6 and 10 min into hypoperfusion. *Significantly different from baseline.

![Fig. 5.](image2)

**Fig. 5.** Relationship between systolic pressure and [Pi] at each time point (baseline, 2, 6, and 10 min into hypoperfusion) in all 14 hearts. There was no significant correlation between systolic pressure and [Pi]. When data points were grouped by time (inset), significant linear correlation (r\(^2\) = 0.99) existed, suggesting that time of hypoperfusion is key determinant of decrease in systolic pressure.

![Fig. 6.](image3)

**Fig. 6.** Scatter plot of changes in systolic pressure and [Pi] during first 2 min of hypoperfusion for each of 14 hearts. If [Pi] was important determinant of systolic function during early minutes of hypoperfusion, hearts with largest increases in [Pi] would have been expected to have largest decreases in systolic pressure. This was not the case, because no significant correlation existed between these 2 variables.
during repeated 33-s bouts of rapidly induced hypoperfusion in buffer-perfused rat hearts. They found that \([\Pi]\) increased significantly during the first second of hypoperfusion, before a change in systolic function, and concluded that \([\Pi]\) may play an important role in inhibiting contractile function during myocardial hypoperfusion. Our results were similar to those of He et al. (13) in many important respects, considering the differences in methodology such as the degree of hypoperfusion and its speed of induction (very rapid induction of zero-flow ischemia compared with gradual induction of low-flow ischemia) and the composition of the perfusate (buffer compared with reconstituted blood). Most notably, data at our 10-min time point were very similar to their 10-s time point because in both cases developed pressure was decreased by 40–45\%, \([\Pi]\) was increased \(-3\) mM, \([\text{PCr}]\) was decreased \(2–4\) mM, and \([\text{ATP}]\) was unchanged from baseline (13). Our different methodologies did, however, cause one critical difference in results. In the study of He et al. (13), the hypoperfusion-induced increase in \([\Pi]\) preceded the decrease in systolic function, whereas a significant increase in \([\Pi]\) did not occur until several minutes after the decrease in systolic function when our methodology, designed to minimize ischemia-induced energetic changes, was employed.

The fact that changes in cardiac energetics can occur before decreased systolic pressure is of interest, but from a mechanistic point of view the more important question is, must they? In our study, all of the variables related to cardiac energetics that we measured changed only after systolic pressure had significantly decreased. We interpret this to mean that temporal correlations between changes in cardiac function and energetics that may occur in some protocols do not describe an obligatory, i.e., causal, relationship. Our findings are similar to those of Koretsune et al. (23), who demonstrated in four buffer-perfused ferret hearts that the start of contractile failure secondary to zero-flow ischemia occurred 15 s before significant changes in any measure of cardiac energetics, including Pi. Koretsune et al. (23) proceeded to demonstrate that loss of coronary turgor, not cardiac energetics, was the main cause of the systolic dysfunction during the first minutes of rapidly induced ischemia. He et al. (13) also noted that CPP decreased more rapidly than systolic pressure and suggested that the loss of coronary vasculature turgor may play some role in the rapid decrease in systolic pressure when coronary flow is decreased. More recent work from this group, however, demonstrates that, during a rapid decrease in coronary flow, the timing of the decrease in CPP correlates poorly with the timing of the decrease in contractile function (12).

One likely reason for the differences in findings among investigators who have measured cardiac energetics while rapidly decreasing myocardial perfusion is that the timing and size of the resulting changes in cardiac energetics are strongly influenced by the speed at which coronary perfusion is decreased. Contractile function and ATP consumption do not downregulate as fast as coronary flow and oxygen supply can be experimentally decreased. To minimize any decrease in the concentration of ATP, two metabolic pathways are activated. First, ATP is synthesized from PCr and ADP...
via the CK reaction (PCr + ADP → ATP + creatine). This reaction is closely coupled to ATP hydrolysis so that the net reaction of the two coupled reactions is PCr → creatine + P. Second, ATP synthesis via anaerobic glycolysis is increased, which causes production of lactic acid and a fall in myocardial pH. Therefore, the first observable changes in energetics when coronary flow is decreased are decreased [PCr], a reciprocal increase in [P], and a fall in pH (13). The faster coronary flow is decreased, the greater is the imbalance between ATP supply and demand and the resultant changes in [PCr], [P], and pH, as demonstrated by Arai et al. (1), who found that rapidly decreasing coronary flow causes larger changes in cardiac energetics compared with decreasing flow to the same level gradually.

To avoid issues of timing, several studies have examined the subacute steady-state relationship between cardiac energetics and systolic function during graded myocardial hypoperfusion (9, 33). These studies have reported that progressive decreases in myocardial perfusion cause both progressive decreases in systolic function and progressive changes in cardiac energetics. Schaefer et al. (33) found that, during graded myocardial hypoperfusion, the magnitude of the decrease in systolic function closely correlated with the magnitude of the decrease in the [PCr]-to-[P] ratio, increases in [P], and decreases in pH. They suggest that [P] and [H+] may play important roles in inhibiting systolic function during ischemia. Subsequent work from this group showed a strong linear correlation between increased [P] and decreased developed pressure during graded hypoperfusion (9).

Our data (where myocardial perfusion was decreased gradually) are very similar to these steady-state data of Figueredo et al. (9) in several important respects. First, at 2 min after myocardial hypoperfusion was initiated, and at their mildest level of hypoperfusion, contractile function is significantly decreased, whereas [P] is not different from baseline. We interpret this to indicate that contractile failure secondary to myocardial hypoperfusion can occur without a significant increase in [P]. Second, in their study, and in our study when data were grouped by time points (Fig. 5, inset), there is a strong linear correlation between [P] and systolic pressure. The slope of this relationship in both studies is such that an ~50% decrease in systolic pressure occurs coincident with an ~4 mM increase in [P]. Although a causal relationship might be inferred from this correlation, at least in our study, a plot of [P] and systolic pressure at each time point in each heart demonstrates that there is no relationship between these two variables (Fig. 5). Additionally, there was no correlation between the change in [P] during the first 2 min of hypoperfusion and the change in systolic pressure (Fig. 6). We therefore conclude that the correlation seen in Fig. 5, inset, is due not to a causal relationship but instead to the fact that [P] and systolic pressure are both functions of the degree of hypoperfusion and therefore correlate with each other.

Although the increase in [P] from 6.1 ± 0.8 mM at baseline to 7.2 ± 0.5 mM after 2 min of hypoperfusion was not statistically significant by repeated-measures ANOVA, the question remains as to whether it is biologically significant. In preparations where [P] can be independently manipulated, it is clear that elevating [P] can inhibit systolic tension development but that the degree of any inhibition is a function of the background concentrations of many molecules, including ATP and PCr (28). In the experiments that most closely model our metabolic conditions, Mekhfi and Ventura-Clapier (28) demonstrated in skinned ventricular muscle fibers that increasing [P] from 0 to 12 mM caused no inhibition of tension when the increase in [P] was accompanied by a reciprocal decrease in PCr and a constant [ATP]. In light of these data, it is unlikely that even the statistically significant 3.0 mM increase in [P] that we observed after 10 min of hypoperfusion would contribute to the concomitant 30-mmHg decrease in systolic pressure given that [ATP] was constant and there was a reciprocal decrease in [PCr] of 2.4 mM.

Effect of coronary hypoperfusion on ΔG_ATP and CKflux. In recent reviews, Heusch (15) and Heusch and Schulz (17) raised the possibility that ischemia-induced down-regulation of contractile function may be caused not by decreases in [ATP] or [PCr], or by accumulation of ADP, Pi, or H+, but by changes in other measures of cardiac energetics such as ΔG_ATP or an inadequate rate of ATP synthesis from PCr (CKflux).

To address this, we calculated ΔG_ATP, which is not constant but is determined in vivo by the concentrations of ATP, ADP, and Pi (20). In our study, the calculated values for ΔG_ATP did not significantly decrease until 6 min after hypoperfusion had begun, at least 4 min after systolic pressure had significantly decreased. From this we conclude that the change in ΔG_ATP is unlikely to have caused the systolic dysfunction. We cannot rule out the possibility that small changes in ΔG_ATP can cause a decrease in systolic pressure, but this is very unlikely because Balschi et al. (3) demonstrated that, to impair baseline cardiac function, ΔG_ATP must be lowered well below 54 kJ/mol, and in our study ΔG_ATP did not fall below 57 kJ/mol. Similarly, it has been estimated that the ΔG_ATP required for the major ATPases in cardiac muscle (myosin-ATPase, Ca2+-ATPase, Na+-K+-ATPase) are all <57 kJ/mol (20).

Another major aspect of cardiac energetics that has been suggested to link perfusion level with contractile state is the rate at which ATP is synthesized from PCr, also called CKflux. In the healthy, well-oxygenated heart, the rate of ATP synthesis from PCr is 5–10 times that of ATP synthesis from oxidative phosphorylation and glycolysis combined, indicating that each molecule of ATP synthesized via these pathways is chemically “shuttled” between ATP and PCr many times before it is hydrolyzed to ADP and Pi. The idea that decreased “shuttling rate” of high-energy phosphates would impair systolic function is conceptually similar to the idea that depressed CKflux during heart failure contributes to the contractile dysfunction (18). Likewise, Rauch et al. (31) conclude that disturbance of PCr shuttle might be of importance in early contractile failure. To directly
test this possibility, we measured CK$_{\text{flux}}$ at baseline and after 30 min of myocardial hypoperfusion, a time when cardiac function and energetics were relatively stable. Hypoperfusion caused CK$_{\text{flux}}$ to decrease from 9.2 to 7.2 mM/s, a 27% decrease. This modest decrease in CK$_{\text{flux}}$ would not be expected to cause a significant inhibition of systolic function because acute inhibition of CK by >90% caused no change in left ventricular developed pressure (11). Similarly, a chronic decrease in CK activity to <5% of normal in mice did not cause impaired systolic function in isolated hearts (32). As Bittl and Ingwall (5) showed, CK$_{\text{flux}}$ varies with cardiac workload such that when workload is low, CK$_{\text{flux}}$ is decreased. Therefore, it is likely that the decrease in CK$_{\text{flux}}$ we observe during hypoperfusion is caused by the decreased myocardial oxygen consumption and cardiac work, as opposed to the decrease in CK$_{\text{flux}}$ causing the decrease in cardiac work.

Limitations. Several limitations of our study merit mention. First, because we were unable to completely prevent cardiac energetics from changing during hypoperfusion, we cannot definitively conclude that changes in cardiac energetics do not contribute to the systolic dysfunction. Instead, we demonstrated that significant decreases in systolic pressure can occur before significant changes in cardiac energetics, and we interpret this to mean that changes in cardiac energetics are not the dominant cause of the systolic dysfunction. We have no direct evidence of the mechanism responsible for the almost immediate decrease in systolic pressure during hypoperfusion, only evidence against a large role for changes in cardiac energetics.

A second limitation is that we did not measure cardiac energetics with the 0.5-s time resolution of He et al. (13). Therefore, we cannot be certain that some variable did not exhibit a large but temporary change before our first measurement of cardiac energetics 2 min after the start of myocardial hypoperfusion. Such a pattern is extremely unlikely because He et al. show that all energetic variables show only steady, not biphasic, changes during the first 30 s of myocardial hypoperfusion. Similarly, Clarke et al. (6) demonstrated with a 10-s time resolution that pH, Pi, and high-energy phosphates all change in a simple, steady direction during the early minutes of myocardial hypoperfusion and not in a complex biphasic way.

Finally, our technique for measuring CK$_{\text{flux}}$ requires the heart to be in a near-energetic steady state, a condition not met during the early minutes of myocardial hypoperfusion. To minimize the duration of steady state needed, we used a simplified technique for measuring CK$_{\text{flux}}$ that only required 15 min. Even so, relatively steady-state conditions were not met until 30 min into myocardial hypoperfusion. For this reason, we cannot say how CK$_{\text{flux}}$ changed before that time, and our data only demonstrate that CK$_{\text{flux}}$ contributes little if any to the sustained decrease in contractile function during myocardial hypoperfusion. It seems reasonable to suspect that large changes in CK$_{\text{flux}}$ do not occur during the early seconds of myocardial hypoperfusion because we know of no reports that describe rapid changes under any circumstances.

In summary, we report that, when CPP is gradually lowered, systolic function decreases before changes in [ATP], [PCr], [ADP], [P$_i$], [H$^+$], or $\Delta G_{\text{ATP}}$. Even after 6 min of hypoperfusion, when developed pressure had decreased by over one-third, only very slight changes in [P$_i$] and pH had occurred, with no significant changes in [ATP], [PCr], [ADP], or $\Delta G_{\text{ATP}}$. The decrease in high-energy phosphate transfer rate during hypoperfusion, CK$_{\text{flux}}$, was not of sufficient magnitude to explain the decrease in systolic function observed after 30 min of hypoperfusion. We interpret these data to mean that the hypoperfusion-induced decrease in systolic pressure is not causally linked to changes in any of these measures of cardiac energetics. Although there are undoubtedly other possible energetic explanations for the decrease in systolic function secondary to hypoperfusion not studied in the present paper, it seems likely that nonenergetics mechanisms are of primary importance (13, 21, 23, 35). Particularly intriguing are recent studies demonstrating that the endocardial and coronary vascular epithelial cells produce substances that modulate contractile function in a manner that is dependent on coronary flow, shear stress, and the partial pressure of oxygen (35). The role of these substances in causing the rapid downregulation of contractile function during hypoperfusion remains to be determined in the intact heart.

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REFERENCES


