Energy Metabolism and Contractile Function After 15 Beats of Moderate Myocardial Ischemia

Andrew E. Arai, George A. Pantely, William J. Thoma, Cheryl G. Anselone, and J. David Bristow

Difficulties in studying myocardial metabolism with adequate time resolution have led to contradictory conclusions regarding the mechanisms causing contractile abnormalities during the early stages of ischemia. In acutely instrumented swine, we investigated whether abnormalities in subendocardial ATP, phosphocreatine, or lactate content develop rapidly enough during the first few heart beats after onset of partial myocardial ischemia to contribute to contractile failure. Within the first 15 beats of a 40–50% reduction in left anterior descending coronary artery blood flow, regional myocardial function was significantly reduced but continuing to deteriorate. Rapidly frozen transmural left ventricular biopsies obtained on the 15th heart beat (±1.5 beats) after the onset of ischemia revealed significant decrements in subendocardial phosphocreatine and ATP levels to 77% (p <0.05) and 84% (p <0.005) of control values, respectively, but minimal change in lactate content. Metabolic effects as assessed by transmural averages took longer to become detectable; thus, there was a tendency to underestimate the importance of subendocardial metabolic effects on myocardial function. When left ventricular preload was assessed during this early time period, left ventricular end-diastolic wall thickness only decreased by 3%, and left ventricular end-diastolic pressure did not change significantly despite a large fall in coronary perfusion pressure. Thus, in an in vivo pig model with techniques optimized to detect subendocardial metabolic changes within the period of very early moderate myocardial ischemia, abnormalities in high energy phosphate compounds occurred rapidly enough to contribute to developing myocardial dysfunction, whereas preload-mediated mechanisms related to vascular distending pressure could not explain the functional deterioration under these conditions. (Circulation Research 1992;70:1137–1145)

KEY WORDS • phosphocreatine • ATP • lactate • ischemia • myocardial metabolism

Myocardial function deteriorates rapidly after the sudden onset of ischemia or hypoxia. Allen and Orchard1 have reviewed many of the potential mechanisms leading to this decline in myocardial function. The factors most likely to cause contractile failure can be divided into three major categories: 1) metabolic factors, which encompass alterations in cellular energetics and accumulation of degradation products such as inorganic phosphate (P_i) or hydrogen ions; 2) activation factors, including shortening of the action potential duration, which would reduce systolic calcium release; and 3) mechanical factors, such as perfusion pressure, which might decrease systolic function as mediated through changing myocardial distension (preload) or conceivably by other vascular–myocardial coupling mechanisms. All of these variables could lead to abnormal myocardial function during the early phase of ischemia, but the relative importance of each mechanism in vivo is less well understood. Several investigators have tried to assess this latter question by examining whether or not a given parameter changes rapidly enough during ischemia to cause contractile failure.3–4 This approach assumes that any metabolic factor that changes at a rate slower than the change in myocardial function is unlikely to have a substantial causal role.

Three elegant studies using nuclear magnetic resonance spectroscopic techniques optimized to detect changes in myocardial energetics and related metabolites during the first minute of ischemia have come to different conclusions. Schwartz et al3 have concluded that a close relation exists between segment shortening and phosphorous metabolites during the first 24 seconds of ischemia. Clarke et al3 have concluded that only the phosphorylation potential changes at a rate faster than the loss of myocardial function. Koretsune et al5 have found that metabolic changes occur too slowly to account for the functional decline and have provided direct evidence that mechanical factors related to perfusion pressure may be important.

The current study was designed to test the hypothesis that phosphocreatine (PCr) and ATP content in the subendocardium do not change rapidly enough during the first few seconds of moderate ischemia in vivo to account for the onset of regional myocardial dysfunc-

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tion. Our pilot studies indicated that measurable changes in regional myocardial function occur within the first few heart beats of ischemia. We used rapid-freezing biopsy techniques that could be timed to a given heart beat after the onset of ischemia. Since some reported nuclear magnetic resonance measurements suggested that metabolite contents change too slowly to account for a change in myocardial function, it seemed likely that we would not detect any significant change in ATP or PCr by the 15th heart beat of ischemia (~10 seconds). To minimize the chances of a type II statistical error, these experiments were designed to have high statistical power to detect a change in these particular metabolites on this time scale. Our experimental design provides the strictest challenge to dissociate myocardial dysfunction from metabolic changes in that the degree of blood flow reduction was less severe and of shorter duration than it was in any prior study of early ischemia.

Materials and Methods

Domestic swine of either sex that weighed between 42 and 44 kg were studied in an acute, open-chest surgical preparation allowing continuous hemodynamic evaluation and intermittent metabolic determinations. All animals were fasted overnight. After premedication with xylazine (2 mg/kg i.m.) and ketamine (10 mg/kg i.m.), anesthesia was induced with α-chloralose (100 mg/kg i.v.) and was maintained with supplemental doses of α-chloralose and morphine sulfate as needed. Acid-base status was kept within normal limits (pH, 7.35–7.45; PaCO₂, 35–45 mm Hg; and PaO₂, 100–150 mm Hg) by titration of mechanical ventilation and administration of intravenous bicarbonate. Temperature was regulated with a cooling/heat pad as needed. After the surgical preparation was completed, normal acid-base status was present for at least 30 minutes before initiating an experimental protocol.

The surgical preparation used in these protocols was similar to that previously reported. A short section of the proximal left anterior descending coronary artery (LAD) was dissected free to allow placement of an electromagnetic flow probe and a hydraulic occluder around the artery with no arterial branches between these devices. Catheters were placed in the distal LAD to monitor LAD perfusion pressure, in the interventricular vein parallel to the LAD for obtaining blood samples, in the left atrium for microsphere injection, in the aorta for reference blood samples, and in the inferior vena cava for fluids and supplemental anesthesia. A catheter-tipped manometer was introduced into the left ventricular (LV) apex to monitor LV pressure and LV dp/dt. Finally, ischemic zone function was monitored by continuously measuring systolic wall thickness between an ultrasonic crystal inserted into the subendocardium and one sutured to the epicardial surface. With this preparation, blood flow through the LAD could be reduced to a preselected fraction of control and maintained at that level throughout the remainder of the experiment.

Analytic Methods

Metabolic determinations consisted of 3-mm transmural drill biopsies of the LV free wall. Biopsies obtained with this system were frozen in liquid nitrogen within 1–2 seconds. Samples were stored at −70°C until the time of processing. Transmural biopsies weighed 137.7±40.5 mg (200A electronic analytical balance, Precisa, Zurich, Switzerland). These biopsies were later divided into subendocardial, middle, and subepicardial sections based on which end of the biopsy retained the blue stain painted on the epicardial surface before biopsy. The subendocardial, middle, and subepicardial divisions averaged 33.2±0.3%, 33.3±5.5%, and 33.4±0.7%, respectively (as a percent of the whole biopsy). The samples were extracted and processed for lactate content, ATP content, and PCr content using spectrophotometric enzyme-linked analysis.

In addition, arteriovenous oxygen differences and arteriovenous lactate differences across the ischemic zone of the LV were obtained. Blood samples were collected anaerobically in cold syringes containing heparin fluoride to inhibit glycolysis. Venous samples obtained in this manner have less than 2% contamination from circumflex collaterals and thus have very high specificity for the ischemic zone in swine. Oxygen content was measured on an IL 382 hemoximeter (Instrumentation Laboratory, Lexington, Mass.). Plasma for lactate content was deproteinized with perchloric acid and neutralized with potassium hydroxide and imidazole buffer. Plasma samples were then frozen until the time of enzymatic analysis.

The heart was removed at the end of the experiment. The right and circumflex coronary arteries were catheterized close to their origins, and the LAD was catheterized at the level of the occluder. Colored dyes were simultaneously injected at a constant rate into each coronary artery to distinguish the ischemic myocardium from the nonischemic zone. This verified that the ultrasonic crystals and biopsies during ischemia were all within the ischemic zone. Control biopsies were usually obtained from the circumflex distribution to minimize damage to the ischemic zone.

Myocardial blood flow was measured with 11.4±0.1-μm radiolabeled microspheres (147sCe, 117sNb, 99sRu, or 99sIr) using standard techniques. The heart was fixed in formalin to facilitate analysis of the transmural distribution of blood flow. The LV was divided into ischemic and nonischemic zones after discarding the border zone and areas immediately surrounding biopsies. The zones of interest were further subdivided into subendocardial, middle, and subepicardial sections. Five 1 gram aliquots of tissue from each section were counted on a Micra Multichannel Analyzer with a germanium detector.

Experimental Protocol

The actual experiment began with a complete set of control data. These data included resting hemodynamics (Figure 1), arteriovenous blood samples across the LAD perfusion zone, the first transmural coronary biopsy, and injection of the first set of microspheres. After a few minutes, blood flow through the LAD was acutely reduced approximately 40–50% and was maintained at that level throughout the remainder of the experiment. The second biopsy was obtained as close as possible to the 15th heart beat after ischemia was initiated. Finally, between approximately 5 and 10 minutes of ischemia, a third set of data including hemodynamics, microsphere
FIGURE 1. Recordings showing hemodynamics and regional wall thickening in swine during the first 16 beats of partial ischemia. WT, ischemic zone left ventricular (LV) wall thickness (scale increases from top to bottom; small vertical lines indicate timing of end diastole and end systole). ECG, electrocardiogram; CAP, coronary artery pressure in the left anterior descending coronary artery distal to the occluder; p, phasic waveform; CAQ, electromagnetic left anterior descending coronary artery blood flow; m, electrical mean waveform; LVP, LV pressure via catheter-tipped manometer; dP/dt, first derivative of LVP. Note that end-diastolic WT changes little with respect to the reference line, whereas end-systolic WT decreases by the 15th beat of ischemia. Vertical dotted lines indicate the onset of ischemia and time of biopsy.

blood flow, arteriovenous blood samples, and the third myocardial biopsy was obtained.

Pilot studies were performed on several pigs to determine an optimal degree of blood flow reduction and timing of biopsy during early ischemia. Instrumentation and methods for these animals were similar to the main protocol.

Statistical Analysis

All results are reported as mean±SD in text and figures. These experiments were designed to maximize sensitivity to detect changes in parameters between control and 15 beats of ischemia, since we were most interested in learning whether changes in metabolite contents occur rapidly enough to contribute to an early decrease in contractile function. The two-tailed paired-sample t test was used to compare control data with the data obtained at 15 beats of ischemia. Finally, the 5–10-minute data were collected to give a frame of reference for comparison with our other reported experiments. Statistical comparisons between control and the 5–10-minute biopsy data were not performed because this group behaved similarly to our previously reported series and the additional comparisons would have weakened the statistical power to detect changes at 15 beats of ischemia.

Calculation of the statistical power (1−β) was based on the number of animals (n), the degrees of freedom (v=n−1), the standard deviation of the difference from control to biopsy, the change in metabolite content observed, and the t statistic. Prospectively, this helped to plan appropriate sample sizes. After completion of the protocol, the power to detect a 20% change in ATP content and PCr content was calculated to be 0.997 and 0.84, respectively. Thus, these experiments resulted in a high power to detect small changes in ATP and PCr during very early ischemia.

Results

Results are reported for eight pigs in the primary protocol. There are occasional references to four similarly instrumented pigs observed during pilot studies. The hemodynamic response to a sudden partial reduction in LAD blood flow is illustrated in Figure 1. For this animal, the control state was characterized by a heart rate of 74 beats per minute, an LV systolic pressure of 138 mm Hg, and an LV end-diastolic pressure of 10 mm Hg. Systolic LAD perfusion pressure distal to the occluder was within 6 mm Hg of LV systolic
pressure, and the dicrotic notch demonstrated the fidelity of this catheter system. The events after an abrupt partial reduction in LAD blood flow (first dotted line) demonstrated the rapidity with which contractile abnormalities developed after the change in LAD blood flow and LAD perfusion pressure. Mean LAD blood flow was reduced by 50% but then remained stable until the first biopsy (second dotted line), which triggered the premature ventricular complex at the far right. LAD perfusion pressure distal to the occluder fell rapidly in parallel with LAD blood flow. Contractile function gradually decreased after the onset of partial ischemia as indicated by the reduced systolic LV wall thickening. The timing of end systole is critical during moderate ischemia because postsystolic thickening does not contribute to external cardiac work. When standard criteria for timing based on the electrocardiogram and LV dP/dt were used, systolic wall thickening fell from 38% at control to 22% by the 16th beat. The biopsy occurred on the 16th heart beat and was followed by a brief period of instability.

Radioactive microspheres injected into the left atrium helped to validate the accuracy of the electromagnetic flow probe readings of LAD blood flow as well as to assess the transmural distribution of blood flow. All electromagnetic flow probes used in these experiments have been calibrated with known flow rates of whole blood ex vivo. In addition, brief occlusions verified the zero-flow baseline before and after the experiment. Figure 2 shows the comparison of LAD blood flow measurements by the two techniques. The two y axes were scaled to allow a visual comparison of the relative blood flow reduction during ischemia. Because of the time necessary for microsphere blood flow determination, we relied on the electromagnetic flow probe as the only measure of LAD blood flow at 15 beats of ischemia. To summarize, LAD blood flow was reduced to approximately 50% of control levels by 15 beats of ischemia and remained at this level until the end of the experiment. The reduction in blood flow as determined by the flow probe at 15 beats of ischemia or by microspheres at 10 minutes of ischemia was statistically significant (p<0.005 for both). Mean LAD perfusion pressure distal to the occluder fell from 99±16 to 44±8 mm Hg by 15 beats of ischemia (p<0.001) and did not change further.

Figure 3 summarizes results for the eight animals studied during a 50% reduction in LAD blood flow. The dotted lines indicate the response of each individual animal until the time of the first biopsy during ischemia (open circles). Average normalized wall thickening (±SD) for the group is shown for every third beat (dark circles with error bars). The time axis was calibrated as the number of heart beats from the onset of ischemia. Since the average heart rate was 90 beats per minute, 15 beats is equivalent to 10 seconds of ischemia. The final heart beat refers to the regional function just before the 10-minute biopsy point.

Of note in Figure 3, regional function began to deteriorate within the first few beats of ischemia. By the 15th beat of ischemia, regional function was significantly different from control data (p<0.001). During these initial 15 beats of ischemia, about half of the reduction in wall thickening that eventually developed by 10 minutes (final) was already present. A new plateau usually occurred within 30 to 60 seconds and remained stable throughout the experiment. The precision of the timing of biopsy was borne out by the fact that all but one animal was biopsied within one heart beat of the target desired.

Other measures of myocardial function also changed significantly within the first 15 beats of ischemia. LV systolic pressure fell from 124±17 to 114±12 mm Hg by 15 beats of ischemia (p<0.05) and stabilized at 111±12 mm Hg by the end of the experiment. LV dP/dt fell from 1,611±297 to 1,358±212 mm Hg/sec by the 15th beat (p<0.01) and did not change further by the end of the experiment (1,324±221 mm Hg/sec). Heart rate re-
Several metabolic observations were made during early partial myocardial ischemia. Crosshatched bars indicate subendocardium, solid bars indicate the middle zone, and open bars indicate subepicardium. Subendocardial PCr and middle zone PCr fall significantly within the first 15 beats of ischemia (*p<0.05 vs. control data).

Mammalian hearts were allowed to recover for 15 minutes after each partial ischemia, and the content in subepicardial and subendocardial areas was measured. ATP content decreased further (0.73 μmol/g wet wt) during the first 15 beats of ischemia.

Issues concerning the sensitivity of various techniques in terms of their ability to detect early changes in ATP or PCr content become evident here. In these experiments, transmural changes in PCr content and ATP content were not statistically significant by the time of the first biopsy. For example, transmural ATP content fell by only 8%. Thus, studies that only measure subepicardial metabolite content or transmural metabolite levels may be too insensitive to detect metabolic changes on this rapid a time scale.

If changes in ATP, PCr, or Pi contribute significantly to developing contractile abnormalities, one would expect a correlation between these parameters and regional wall thickening. Figure 6 shows the relation between regional wall thickening and subendocardial PCr content. Table 1 presents the correlation statistics of regional wall thickening as the dependent variable versus subendocardial PCr or ATP content. The last two lines of the table present the correlation statistics for all pairs of data except the 10-minute biopsy of one animal. The wall thickening versus PCr regression analysis was strongly influenced by this single point, which is indicated by the accompanying asterisk in Figure 6. In this animal, subendocardial PCr content was normal at control, decreased to 61% of control at 15 beats of
ischemia, but was back to normal by 10 minutes of ischemia despite low ATP and less regional thickening.

Myocardial lactate accumulation was slower than the changes in myocardial function and slower than the changes in other metabolites (Figure 7). Although six of eight animals demonstrated a small increase in subendocardial lactate content \( (p<0.01) \), the net change was too small to have clear significance from a standpoint of metabolite accumulation or pH change. However, this is suggestive that glycolytic flux may actually increase very rapidly after a change in myocardial perfusion. Arteriovenous sampling with this model required at least 30–60 seconds, so it was impossible to perform this measurement rapidly enough to describe the very earliest changes in arteriovenous lactate production. The control state showed a net lactate consumption \((80 \mu\text{mol/min per 100 g})\). By 10 minutes, the ischemic zone was producing lactate at a rate of 96 \( \mu\text{mol/min per 100 g} \) \( (p<0.001 \text{ versus control state}) \).

**Discussion**

The moderate reduction in coronary blood flow and the short duration of ischemia in these experiments pose a severe challenge to the hypothesis that contractile dysfunction develops before the onset of metabolic abnormalities. The results delineate the rapidity with which abnormalities in contractile function develop after partial reductions in regional myocardial blood flow. Of five measures of myocardial function analyzed, all except LV end-diastolic pressure were significantly changed from control to the 15th heart beat of ischemia. However, the 15th heart beat still represents a dynamic time period in the process of developing contractile dysfunction since regional wall thickening, LV systolic pressure, and LV end-diastolic pressure continued to change after this time. Thus, the biopsy on the 15th heart beat captured the metabolic abnormalities present during a period of deteriorating myocardial function. In brief, significant changes in PCr and ATP content had already developed in the subendocardium by this time, but lactate accumulation took longer to develop. Finally, nearly half of the fall in PCr and ATP content seen between control and 10 minutes occurred within the first 15 beats. These data suggest that changes in high energy phosphate compounds or their metabolites (such as Pi) occur rapidly enough during early partial ischemia to contribute to the contractile abnormality that develops. Analysis of the current work helps reconcile some of the diversity of conclusions reached by other studies of early ischemia.\(^2,4\)

Before comparing this work with the results of similar studies, several characteristics of this system should be considered. These experiments were performed in situ. Despite anesthesia, these hearts operated within physiological ranges of heart rate, blood pressures, preload, and coronary blood flow. The degree of ischemia only moderately changed systemic hemodynamics. Consequently, the ischemic segment was stressed by normal systolic pressures. Under these conditions, the ischemic segment continued to perform external work, as evidenced by some residual systolic wall thickening. Finally, the biopsy technique assessed metabolites with a rapid time resolution \((±1.5 \text{ heart beats})\) and across three layers of the LV wall.

**Comparison With Prior Studies**

Several studies have examined the metabolic and functional events during relatively early ischemia. For a variety of reasons, most metabolic observations during early ischemia have been performed between 1 and 15 minutes after complete reductions in blood flow. Much of this work has been summarized in a review by Allen and Orchard.\(^1\)

Myocardial biopsy techniques have been used to address the metabolic and functional consequences during the first 60 seconds of ischemia or anoxia. In a protocol similar to ours using a 50% reduction in LAD blood flow in dogs, Covell et al\(^13\) noted a 40% decrease in PCr but no change in ATP an average of 47 seconds into ischemia. Others noted 10–20% changes in ATP content during the first 60 seconds of ischemia, but these changes were not statistically significant.\(^14,15\) Making metabolic measurements at several time periods during the first 30 seconds of anoxia in isolated perfused rat hearts, Hears\(^16\) found changes in ATP and PCr content at 10 seconds similar to those we reported at 15 beats of ischemia. Hears concluded that these meta-

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**TABLE 1. Relations Between Regional Wall Thickening and Subendocardial Metabolite Content in Swine**

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>( r )</th>
<th>( r^2 )</th>
<th>Regression equation</th>
<th>SEE</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subendo PCr</td>
<td>0.64</td>
<td>0.41</td>
<td>( y=0.59x+0.12 )</td>
<td>0.24</td>
<td>(&lt;0.05)</td>
</tr>
<tr>
<td>Subendo ATP</td>
<td>0.58</td>
<td>0.34</td>
<td>( y=1.02x-0.25 )</td>
<td>0.25</td>
<td>(&lt;0.025)</td>
</tr>
<tr>
<td>Subendo PCr*</td>
<td>0.84</td>
<td>0.71</td>
<td>( y=0.75x+0.06 )</td>
<td>0.16</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Subendo ATP*</td>
<td>0.60</td>
<td>0.36</td>
<td>( y=0.99x-0.19 )</td>
<td>0.24</td>
<td>(&lt;0.025)</td>
</tr>
</tbody>
</table>

\( r \), Correlation coefficient; SEE, standard error of the \( y \) estimate; subendo, subendocardial; PCr, phosphocreatine.

*Regression analysis was repeated except for the 10-minute biopsy data from one animal (see text).
bolic changes actually preceded development of myocardial dysfunction. Overall, it is reasonably well accepted that PCr begins to fall in the first minute of ischemia. Although many studies have shown a trend toward 10–20% changes in ATP levels, our study appears to be the first to have the statistical power to demonstrate the change in subendocardial ATP content (or conversely exclude a change had it not been present) at such an early stage of ischemia.

Because of limitations inherent to biopsy and chemical extraction techniques, several groups have used nuclear magnetic resonance spectroscopy to assess metabolic events during the first minute of myocardial ischemia. Unfortunately, three groups have come to different conclusions concerning whether changes in high energy phosphate compounds or their metabolites occur rapidly enough to account for the early onset of ischemic myocardial dysfunction.4–6 Our results may help reconcile some of the differences between these studies. Schaefer et al17 and Schwartz et al18 have provided the strongest evidence for a causal relation between high energy phosphate metabolites and ischemic myocardial function in vivo. Several minutes into steady-state partial ischemia, there was found to be a strong correlation between regional myocardial function and the subendocardial PCr/Pi ratio.17 In a protocol averaging the effects of 10 cycles of ischemia and reperfusion, subendocardial PCr, P, or PCr/Pi ratio and segment shortening maintained a close relation during the first 24 seconds of coronary occlusions.2 Although their measurements were not statistically different from control measurements until near the end of 24 seconds of ischemia, trends in their data for ATP content and PCr content were nearly superimposable on our data at approximately 10 seconds of ischemia. Thus, our results agree closely with those of Schwartz et al18 during early ischemia in similar experimental models using distinctly different methods to assess metabolism.

In contrast, Clarke et al19 have concluded that only the cytosolic phosphorylation potential changes rapidly enough to account for declining rate–pressure product in globally ischemic guinea pig hearts. Three aspects of their techniques should be emphasized in comparison with the current study: 1) Because of the relatively low signal-to-noise ratio characteristic of nuclear magnetic resonance spectroscopy, they averaged the effects of 20 cycles of occlusion and reperfusion in each heart to attain sufficient sensitivity to describe the metabolic consequences of the first 60 seconds of ischemia. This protocol resulted in progressive loss of ATP and PCr content that was due to repeated occlusions; thus, changes of magnitude similar to those observed in the first few seconds of the current study were obscured. 2) Spatial resolution limited to transmural metabolite determinations decreased sensitivity for detecting subendocardial effects and overestimated the time constants for the rate of metabolic changes (see “Appendix”). 3) They reported the phosphorylation potential based on calculated values of [ADP]. Since determining the phosphorylation potential involves dividing the relatively large [ATP] by the small [ADP], errors in calculating [ADP] became magnified in the process. In summary, three aspects of the study by Clarke et al19 limit the strength of their conclusions.

Characteristics of crystalloid-perfused hearts may explain why Koretsune et al4 have reached conclusions nearly opposite those of the current study. They did not detect any change in PCr or P during the first 30 seconds of ischemia as developed pressure fell to 50% of control values in isolated perfused ferret hearts. Furthermore, they found that global LV function deteriorated less rapidly after microembolization-induced ischemia. They concluded that metabolic factors did not occur rapidly enough to contribute to ischemic dysfunction but that perfusion pressure was a likely regulating force. However, the isolated perfused heart may behave quite differently from other systems described; it has been demonstrated that even much longer durations of decreased perfusion pressure do not cause the same metabolic changes expected during ischemia.18 During regional ischemia, as shown in the current study, the normal segment develops systolic dysfunction against which the ischemic segment must at least maintain wall stress (and thus high energy requirements). In contrast, decreasing developed systolic pressure during global LV ischemia may act to reduce myocardial oxygen requirements. This could slow the rate at which ATP and PCr are consumed.

Additionally, the conclusion that perfusion pressure modulates myocardial function does not appear to apply to the heart in situ. One might speculate that the high coronary perfusate flow rates (10–20 times normal coronary blood flow rates) may invoke preload-mediated mechanisms not usually seen in vivo. Finally, the finding that microembolization caused slower development of contractile abnormalities may be explained by the uniform transmural distribution of ischemia (and thus less severe subendocardial ischemia). The importance of subendocardial vulnerability to ischemia30,21 and subendocardial segment shortening22 have previously been discussed. So although Koretsune et al4 make arguments concerning the regulation of systolic function by perfusion pressure in the isolated heart, it is difficult to extrapolate those conclusions to the intact system.

Mechanism of Contractile Dysfunction During Ischemia

The change in ATP content23 and decrease in free energy of ATP hydrolysis24 are likely too small to account for alterations in myocardial function. We presume that the more likely mechanism linking function to ATP and PCr content involves the accumulation of P, in skinned ventricular trabeculae, P, reduced maximum Ca2+-activated force of contraction and shifted the relation between force and intracellular Ca2+ toward higher Ca2+ concentrations.25 Thus, net hydrolysis of ATP and PCr, which would be expected to release P, could indirectly depress contractile function at levels of ATP and PCr that otherwise seem adequate to maintain normal cellular processes. Because of the nonlinearity of the relation between P, and developed tension, contractile function has been shown to be more sensitive to the first few millimolar accumulations of P, than subsequent equimolar changes.25 This would have a tendency to amplify contractile consequences of P, released from net degradation of PCr and ATP during early ischemia.
Changes in cytosolic pH are very unlikely to cause contractile failure by 15 beats of ischemia. Since lactate has not started to accumulate to any significant degree, pH changes will largely be determined by breakdown of ATP and PCR. The magnitude of changes in these metabolites by 15 beats of ischemia is expected to cause a slight cytosolic alkalosis rather than an acidosis. Similarly, abnormal myocardial calcium metabolism is unlikely to contribute to early myocardial dysfunction.

**Limitations**

The major limitations of the current study are inherent to myocardial biopsy and chemical extraction. This requires careful correlation with the results observed by other methodologies such as nuclear magnetic resonance spectroscopy. In many respects, these techniques provide complementary information. In particular, our extraction technique has not allowed us to measure P, levels. So from our data, we can only conclude that the observed decrease in PCr and ATP would be expected to release P, and potentially indirectly affect regional function.

The relation between phosphorus metabolites and regional myocardial function should not be oversimplified. The correlation between subendocardial PCr and regional wall thickening (Figure 6) does not necessarily indicate a causal mechanism. This relation appears modulated during reperfusion and during late ischemia. Additionally, Path et al noted a steep relation between PCr and regional function during low flow states but poor correlation at higher coronary blood flows. A dissociation between high energy phosphate compounds and myocardial function has also been described in the posts ischemic myocardium. Inotropic agents have been shown to improve regional function of “stunned myocardium” without depleting high energy phosphate compounds. Conversely, pharmacologically repletion the ATP stores of posts ischemic myocardium does not improve function.

**Conclusions**

Although the current study cannot conclude to what degree various changes in metabolites cause the contractile dysfunction of myocardial ischemia, it does contribute several important observations. Changes in subendocardial PCR and ATP occur very rapidly during early ischemia and on a time scale compatible with deteriorating regional function. Changes in lactate content are slower and support earlier reports that pH is unlikely to account for the early changes in function. This work corroborates some of the data obtained by NMR spectroscopy. Our data are compatible with the theory that accumulation of cytosolic P, contributes significantly to early contractile failure. In addition, two measures of LV preload do not change during the first 15 beats of ischemia. This indicates that a preload alteration related to perfusion pressure is not an important regulator of function under these conditions. Finally, techniques that do not focus on the subendocardium or only provide transmural metabolite determinations have a tendency to underestimate the severity of metabolite changes and underestimate the rate at which these changes occur.

**Appendix**

Basing time constant measurements such as those reported by Clarke et al on transmural metabolite determinations will overestimate the half-life if significant transmural gradients develop. A simple calculation based on a two-compartment model for PCR loss during ischemia can illustrate this point. Assume for simplicity that the subendocardial and subepicardial PCR compartments both start at equal concentrations and are of equal size, that the subendocardial half-life of PCR hydrolysis is 20 seconds, and that the subepicardial PCR content does not change significantly over time. Simple arithmetic reveals a half-life of 54 seconds for the hydrolysis of PCR based on transmural measurements. This is almost three times the subendocardial half-life used to set up this example. All experiments based on transmural metabolite determinations will tend to underestimate the magnitude of change of a given subendocardial metabolite and overestimate the half-life of disappearance.

**References**


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