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Doppler Strain Imaging Closely Reflects Myocardial Energetic Status in Acute Progressive Ischemia and Indicates Energetic Recovery After Reperfusion

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Abstract

Background—Capitalizing on mechanoenergetic coupling, we investigated whether strain echocardiography can noninvasively estimate the ratio of adenosine triphosphate (ATP) to adenosine diphosphate (ADP), a marker of energetic status during acute myocardial ischemia and reperfusion.

Methods—Twenty-eight pigs were divided into 7 groups (1 baseline, 4 ischemic, and 2 reperfusion). Ischemia was induced by left anterior descending coronary artery occlusion. Longitudinal systolic lengthening (SL) and postsystolic shortening (PSS) strain were measured by echocardiography. The ATP/ADP ratio was obtained from myocardial biopsies in the ischemic and control regions.

Results—SL and PSS strain and the ATP/ADP ratio progressively decreased ($P < .05$) with increased duration (12, 40, 120, and 200 minutes) of ischemia. A mathematical formula ($\text{ATP/ADP} = -0.97 + 0.25 \times \text{PSS strain} + 0.20 \times \text{SL strain}$) estimated best the ATP/ADP ratio ($r = 0.94$, $P < .05$). Reperfusion after 12 but not after 120 minutes of ischemia significantly improved the ATP/ADP ratio and decreased SL and PSS strain.

Conclusions—Strain echocardiography closely reflected changes and enabled the noninvasive estimation of the ATP/ADP ratio. A higher ATP/ADP ratio is associated with functional improvement after reperfusion.

Keywords

Strain echocardiography; Ischemia; Energy phosphates

The impairment of energetic metabolism plays a major role in the pathophysiology of ischemia and heart failure, 2 leading contributors to mortality and morbidity in Western civilization.¹ In acute ischemia, the progressive alteration of energetic metabolism is linked to functional impairment, and severe energy deprivation is associated with irreversible injury.^{1,2} The analysis of myocardial strain by echocardiography has evolved as a valuable

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tool for the noninvasive quantitation of regional functional impairment.^{3–6} On the basis of the close relation of myocardial mechanical function to the underlying energy metabolism⁷ (ie, mechanoenergetic coupling¹), we hypothesized that the computational analysis of echocardiographic strain parameters would enable quantitative estimates of energetic impairment in myocardial ischemia. Such estimates could provide noninvasive insights into the severity of energetic alterations and allow the prognosis of myocardial viability. To test this hypothesis, we characterized the relationship between changes in strain parameters and changes in the ratio of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) by a mathematical formula for different time points of acute progressive ischemia induced in experimental pigs. The ATP/ADP ratio was used because it is a sensitive indicator of the mismatch between myocardial ATP production and consumption.^{1,8–10} To initially assess the prognostic utility of the echo-cardiographically estimated ATP/ADP ratio, we associated the ratio with the level of recovery of mechanical function in myocardium reperfused after 12 minutes (stunning) and 120 minutes (necrosis) of ischemia.

METHODS

Animal Preparation

The study was approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. Anesthesia was induced with an intramuscular injection of ketamine HCl (20 mg/kg) and xylazine (2 mg/kg) and maintained with an intravenous infusion of ketamine HCl (2 mg/kg/L), fentanyl (0.02 mg/kg/L), and etomidate (0.08 mg/kg/L). Each animal was intubated and mechanically ventilated. Following sternotomy, the heart was exposed on a pericardial cradle, 7Fr pressure catheters (Millar Instruments, Inc., Houston, TX) were inserted into the left ventricle and the ascending aorta via common carotid artery access under ultrasound guidance, and the animals were fully anticoagulated with heparin. Pressure and electrocardiographic monitoring continued throughout the study.

Control and Testing Regions

The apical anterior wall served as the testing region in the left anterior descending coronary artery (LAD) territory, whereas midposterior myocardium represented the control region. Delineation of the testing region following LAD occlusion and identification of reperfusion were performed using an intravenous bolus (1 mL) of ultrasonic contrast (Definity; Bristol-Myers Squibb, New York, NY).

Strain Echocardiography

An ultrasound system (Vivid 7; GE Healthcare, Milwaukee, WI) equipped with a 3.5-MHz transducer was used to obtain narrow-sector epicardial long-axis tissue velocity projections with high temporal resolution (≥ 220 frames/s) for offline analysis (EchoPAC; GE Healthcare). Strain-rate measurements were obtained from tissue velocity data, and strains were integrated from strain rates over the period of an electrocardiographic RR interval.⁴ Measurements of 3 consecutive cardiac cycles were averaged to reduce the influence of noise. Longitudinal systolic lengthening (SL) strain, end-systolic (ES) strain, and peak shortening (PkS) strain were expressed as percentage deformation with respect to the preceding end-diastolic state. Post-systolic shortening (PSS) strain was expressed as the difference between ES and PkS strain magnitudes. The lengthening/shortening (L/S) ratio was calculated as $SL/(SL - PkS)$.⁵ Myocardial systolic compliance was calculated as SL strain divided by systolic left ventricular pressure rise (%/mm Hg). Pressure rise was measured as the difference between peak systolic left ventricular pressure (LVPS) and end-diastolic left ventricular pressure (LVPD).⁵ Wall motion score (WMS) was assessed in both the control and testing regions on a scale ranging from 1 to 5 as normokinesis, hypokinesis, akinesis, dyskinesis, and aneurysm.¹¹

High-Energy Phosphate Analysis From Myocardial Tissue Samples

Myocardial samples (100–200 mg) from the testing and control regions were rapidly dissected and immediately immersed into liquid nitrogen to avoid the loss of high-energy nucleotides. The frozen samples were ground, homogenized in 10 volumes of 0.6 M HClO₄/1 mM ethylenediaminetetraacetic acid and centrifuged at 4°C. Supernatant was neutralized with 2 M K₂HCO₃, and the precipitate was removed by another centrifugation at 4°C. Nucleotide concentrations were determined by high-performance liquid chromatography (HPLC Series 1100; Hewlett-Packard Corporation, Waldbronn, Germany) using MonoQ HR 5/5 column (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, United Kingdom) and triethylamine bicarbonate (pH 8.8) as an elution buffer.¹² ATP and ADP values (nmol/mg protein), their ratio, and the sum of ATP, ADP, and adenosine monophosphate were calculated.

Hemodynamic Data Analysis

LVPS and LVPD, the peak positive and negative time derivatives of pressure (+dP/dt and -dP/dt, respectively), and heart rate were calculated from ventricular pressure tracings.

Viability Analysis by Tissue Staining

The heart was dissected into transverse slices approximately 1 cm thick that were incubated in 2% triphenyltetrazolium chloride (TTC) solution at 37°C for 5 minutes. Viable myocardium became brick red, whereas necrotic myocardium remained unstained. Infarction trans-murality was quantified as the maximal radial percentage of necrosis; a radial extent of necrosis >90% was considered transmural infarction. The spatial extent of infarction was measured as a percentage of planimetered necrosis within the entire myocardial slice area.

Study Protocol

Twenty-eight animals were randomly assigned into 7 subgroups of 4 animals each. One subgroup was used for baseline measurements. In 4 subgroups, the middle LAD was occluded for 12, 40, 120, and 200 minutes, respectively, without reperfusion. In another 2 subgroups, the LAD was occluded for 12 and 120 minutes, respectively, followed by 100-minute reperfusion. At the end of each time period, hemodynamic measurements and echocardiographic scans were performed, myocardial tissue samples were promptly obtained, the animal was euthanized, and the heart was excised and dissected to slices for TTC staining.

Statistical Analysis

Data are presented as mean ± SD. SAS/STAT (SAS Institute, Inc., Cary, NC) GLM procedures followed by Scheffé pairwise corrections were used to assess the differences of echocardiographic, hemodynamic, and energetic parameters at different time points of experiments. The SAS/STAT REG procedure or linear regression analysis using the least squares method was performed to show the relation between strain parameters and the ATP/ADP ratio as well as hemodynamic parameters. Multivariate linear regression analysis with backward elimination¹³ was used to explore all strain parameters measured at different time points of acute progressive ischemia and define a formula for estimating the ATP/ADP ratio from strain values. The intraobserver and interobserver variability of strain measurements were assessed in 10 randomly picked animals in the apical anterior midposterior segments and are presented as the difference between measurements expressed as a percentage of the mean. We also calculated retrospectively the power of the study using nQuery Advisor software (Statistical Solutions, Farmer's Cross, Ireland). The study had 80% power at $P = .005$ for 7 subgroups to detect a change of 1.2 in the ATP/ADP ratio (using SD = 0.3). For

strain values, we were able to detect a difference of 1.9% in SL strain ($SD = 0.5\%$), 4.6% in ES strain ($SD = 1.2\%$), 5.8% in PkS strain ($SD = 1.5\%$), 3.9% in PSS strain ($SD = 1.0\%$), and 0.39 in the L/S ratio ($SD = 0.10$). P values $< .05$ were considered statistically significant.

RESULTS

Of a total of 34 animals, 28 were used in the study; 6 animals were excluded (4 for sustained arrhythmia during ischemia or reperfusion, 1 for pericarditis, and 1 for perioperative bleeding).

Left Ventricular Hemodynamic Data

LVPs had a decreasing trend during progressive ischemia and tended toward an increase in both reperfusion groups (Table 1). Changes in $+dP/dt$ were not statistically significant. The magnitude of $-dP/dt$ decreased during ischemia compared with baseline and tended to be higher following reperfusion compared with ischemia. LVPD did not significantly change, and heart rate showed a trend toward higher values with longer duration of ischemia.

Course of Strains and Systolic Compliance During Progressive Ischemia

Both SL and PSS strain developed in the testing region at 12 minutes of ischemia and decreased thereafter (Figure 1). This decrease was nearly exponential (Figure 2). With the development of SL strain at 12 minutes of ischemia, the mean negative value of ES strain at baseline changed to a positive value, with a decreasing trend toward 200 minutes of ischemia, whereas absolute values of PkS strain decreased monotonically to their minimal magnitudes at 200 minutes of ischemia (Figure 1, Table 2). The L/S ratio significantly increased from baseline to 12 minutes of ischemia and remained stable with longer duration of ischemia. Compared with 12 minutes of ischemia, systolic compliance showed a trend toward decrease at 40 minutes of ischemia and was significantly lower at 120 and 200 minutes of ischemia (Table 2). WMS at baseline was 1.2 and tended to decrease from 3.5 at 12 minutes of ischemia to 3.5, 3.0, and 2.8 at 40, 120, and 200 minutes of ischemia, respectively. In the control region, strain parameters (Table 2) remained without significant changes and WMS remained equal to 1.0 at baseline and all testing stages.

Change in Strain Patterns and Systolic Compliance After Reperfusion

After 12 minutes of ischemia and 100 minutes of reperfusion, SL and PSS strain decreased significantly compared with 12 minutes of ischemia without reperfusion; the polarity of ES strain returned to mean negative values, although never reaching the baseline magnitude; PkS strain improved compared with all ischemic intervals; the L/S ratio trended to a lower mean value; and systolic compliance decreased compared with 12 minutes of ischemia without reperfusion (Table 2). After 120 minutes of ischemia and 100 minutes of reperfusion, strain parameters as well as systolic compliance remained without significant changes compared with 120 or 200 minutes of ischemia without reperfusion. Intraobserver and interobserver variability were, respectively, 14.5% and 15.4% for SL strain, 12.4% and 16.1% for ES strain, 13.2% and 15.3% for PkS strain, 12.7% and 14.3% for PSS strain, and 11.0% and 17.2% for the L/S ratio.

Course of High-Energy Phosphate Levels During Progressive Ischemia

In the testing region, ATP levels declined exponentially with increasing duration of ischemia (Table 3), whereas ADP levels (not shown) increased insignificantly at 12 minutes of ischemia and thereafter progressively decreased to their minimal values at 200 minutes. As a result of these ATP and ADP changes, the ATP/ADP ratio as well as the sum of adenosine phosphates decreased monotonically from baseline to the 200-minute point of ischemia. In

the control region, no significant changes in ATP and ADP levels, the ATP/ADP ratio, or the sum of adenosine phosphates were observed.

Change of High-Energy Phosphate Levels After Reperfusion

Following 12 minutes of ischemia and 100 minutes of reperfusion, ATP levels were reduced by approximately 30% from their baseline levels (ie, were similar to ATP levels at 12 minutes of ischemia without reperfusion). We observed a marked decrease in ADP, which kept the ATP/ADP ratio value comparable with that at baseline and, therefore, significantly higher than during the other ischemic periods. Following 120 minutes of ischemia and 100 minutes of reperfusion, ATP and ADP levels and the ATP/ADP ratio did not change significantly compared with 120 minutes of ischemia without reperfusion.

Regression Analysis of Strain Parameters Versus Hemodynamic Data During Ischemia

Peak SL strain was not correlated with $+dP/dt$ ($r = -0.19$, $P = .48$) or maximum left ventricular pressure ($r = -0.27$, $P = .304$). There was no correlation between ES strain ($r = -0.09$, $P = 0.745$), PkS strain ($r = 0.39$, $P = .131$), or PSS strain ($r = -0.27$, $P = 0.311$) and $-dP/dt$. LVPD was not correlated with PkS strain ($r = 0.26$, $P = 0.324$) or PSS strain ($r = -0.21$, $P = 0.434$). There was no correlation between the L/S ratio and any of the left ventricular pressure parameters.

Viability Evaluation

No necrosis was detectable at baseline and after 12 minutes of ischemia with and without reperfusion. After 40 minutes of ischemia, small subendocardial necrosis (<10%) was identified in 1 animal. Transmural necrosis was detected in all animals that underwent 120 minutes of ischemia with and without reperfusion and 200 minutes of ischemia with no reperfusion. The spatial extent of infarction was $29 \pm 8\%$, $32 \pm 6\%$, and $33 \pm 5\%$, respectively.

Regression Analysis of High-Energy Phosphates and Strain Parameters

During ischemia, the ATP/ADP ratio was correlated closely with SL strain ($r = 0.73$, $P < .001$) and PSS strain ($r = 0.86$, $P < 0.0001$) (Figure 2), as well as with PkS strain ($r = -0.70$, $P < .003$), the L/S ratio ($r = -0.52$, $P < .05$), and systolic compliance ($r = 0.67$, $P < .005$). Levels of ATP were separately correlated with SL strain, PSS strain, PkS strain, and the L/S ratio ($r = 0.76$, $P < .001$; $r = 0.85$, $P < .0001$, $r = -0.72$, $P < .002$; $r = -0.52$, $P < .05$, respectively). There was no correlation between ES strain and the ATP/ADP ratio ($r = 0.37$, $P = .15$) or ATP levels ($r = 0.35$, $P = .18$). Levels of ADP were also correlated with SL strain ($r = 0.76$, $P < .001$), PSS strain ($r = 0.82$, $P < .0001$), PkS strain ($r = -0.67$, $P < .005$), and systolic compliance ($r = 0.62$, $P < .05$, respectively). ADP was not correlated with ES strain ($r = 0.44$, $P = .87$) or the L/S ratio ($r = -0.48$, $P = .06$). WMS was not correlated with the ATP/ADP ratio in ischemic myocardium ($r = 0.41$, $P = .117$).

Mathematical Formula for Estimating the ATP/ADP Ratio

Through multivariate linear regression analysis of all permutations of strain parameters described in this study and using backward statistical elimination, we arrived at the following formula that estimated best ($r = 0.94$, $P < .05$) the ATP/ADP ratio during the course of persisting acute ischemia: $ATP/ADP = -0.97 + 0.25 \times PSS \text{ strain} + 0.20 \times SL \text{ strain}$.

DISCUSSION

The main finding of this preclinical study is that changes in SL and PSS strain in acute progressive ischemia are correlated with markers of myocardial energetic status such as ATP levels and the ATP/ADP ratio. We have shown that higher values of SL and PSS strain in the early stages of acute ischemia are associated with a relatively preserved pool of adenine phosphates that maintain viability, and such myocardium therefore functionally improves after reperfusion. Lower magnitudes of SL and PSS strain that develop with the prolongation of acute ischemia are associated with depleted adenine phosphates, the development of myocardial necrosis, and no functional improvement after reperfusion. On the basis of these relationships, the present study describes a noninvasive computational strain echocardiographic approach for estimating the ATP/ADP ratio, a marker of myocardial energetic status.

Relationship of Strain Values to Myocardial Energetic Status in Acute Ischemia

Coronary occlusion is followed by the rapid development of contractile failure,^{14,15} and the lack of an active component of regional myocardial motion was reported to occur at 3 to 5 minutes of severe ischemia.¹⁶ Consistent with these reports, the myocardium responded to acute coronary occlusion in our study by peak magnitudes of SL and PSS strain at 12 minutes of ischemia. Although these early functional alterations were accompanied by decreases in the ATP/ADP ratio of 37% and ATP level of 28% from their baseline values, myocardial viability remained preserved. These findings correspond closely to previously published measurements¹ and are consistent with an inhibition of myosin adenosine triphosphatase function, which accompanies decreases in the free energy of ATP hydrolysis by 25% at approximately 12 minutes of severe ischemia and in the ability of myocytes to survive an approximate 25% decrease in ATP level.¹⁷ The noncontracting myocardium was stretched during systole by the rising ventricular pressure, which resulted in SL strain. Then, during late systole and early diastole, when ventricular pressure declines, the myocardium passively recoiled and produced PSS strain.^{5,16} As ischemia continued, strain parameters, including SL and PSS strain, progressively decreased in magnitude, which was observed in this study and is consistent with previous studies.^{5,18–20} This decrease was closely correlated with a decline in the ATP/ADP ratio and accompanied by nontransmural necrosis (1 animal) at 40 minutes and transmural necrosis at 120 and 200 minutes of ischemia. We speculate that the observed decrease in strain parameters was related to increasing myocardial stiffness^{15,21,22} and decreased systolic compliance⁵ during ischemia. The noncontracting, progressively less compliant myocardium increasingly resists the stretching forces generated by systolic ventricular pressure. As a consequence, SL strain decreases in its magnitude, and the passive recoil following aortic valve closure also becomes limited. The latter manifested as a progressive decrease in PSS strain magnitude. Several variables, including edema, cellular and hemorrhagic infiltrates, the extent of myocardial necrosis,^{19,20} changes in calcium homeostasis, and ischemic contracture and muscle rigor,^{23,24} can contribute to increasing stiffness and decreased compliance of an acutely ischemic and reperfused myocardium.^{14,15} The development of ischemic contracture and the formation of rigor bonds have been associated with decreases in both ATP levels and the ATP/ADP ratio^{1,8,15,23,24} and might explain the close correlations of SL strain, PSS strain, and systolic compliance to the ATP/ADP ratios found in this study.

Relationship of Strain Values to High-Energy Phosphate Levels and the ATP/ADP Ratio After Reperfusion of Stunned and Necrotic Myocardium

Following the reperfusion of stunned myocardium (ie, after 12 minutes of ischemia), SL strain, PSS strain, and the L/S ratio decreased and suggested partially active myocardial motion.⁵ This myocardial mechanical improvement was accompanied by normalization of

the mean ATP/ADP ratio, which was driven more by a decrease in the ADP level than by an increase in the ATP level, an observation consistent with work done by others.¹ It has also been previously described that a stunned muscle maintains ATP consumption in spite of the lower force development and that its calcium responsiveness decreases.²⁵ The changes in strain patterns could thus be explained on the basis of force generation, which (although low) would decrease the magnitude of SL strain and contribute to active contraction (expressed as negative values of ES strain and a decrease of the PSS strain component) during the ejection phase. After the reperfusion of a necrotic myocardium, there were (as expected) no changes in high-energy phosphate levels and the ATP/ADP ratio value, and only an insignificant decrease in strain magnitudes was observed.

Estimation of the ATP/ADP Ratio From Strain Curves

On the basis of the described relationship between high-energy phosphate levels and strain magnitudes, a mathematical formula for estimating the energetic status was derived that reflects a linear relation of mathematically combined SL and PSS strain magnitudes to the ATP/ADP ratio as contractile failure develops following 12 minutes of acute ischemia. The time point at which reperfusion takes place during the progressive decrease in high-energy phosphate levels determines whether and how fast functional recovery would occur.¹ This was demonstrated in our study by testing 2 different time points of reperfusion, and the changes in myocardial energy-related SL and PSS strain magnitudes before reperfusion (Table 2) suggest a role of combined SL and PSS strain analyses in estimating postreperfusion recovery. SL and PSS strain can be present at low magnitudes in nonischemic myocardium.^{26–30} The presence of SL and PSS strain at baseline and the progression of SL and PSS strain magnitudes during the initial 12 minutes of ischemia to their peak values would lead to an underestimation of the ATP/ADP ratio. However, using ES strain as an independent parameter would differentiate nonischemic (a highly negative ES strain value) from acutely ischemic (a positive or nearly zero ES strain value) myocardium (Table 2). In addition, it is unlikely that strain echocardiography would be done during the initial 12 minutes of acute ischemia.

Left Ventricular Blood Pressure and Decline in Values of Strain Parameters

Considering the load dependency of strain parameters,^{16,31} the decrease in SL and PSS strain could have been affected by the progressive decline of LVPS. However, there was no correlation between the strain magnitudes and left ventricular pressure parameters. Still, the possibility that SL and PSS strain were influenced by loading conditions exists, although in our experimental setting, left ventricular pressure was not the primary factor leading to the decline in SL and PSS strain magnitudes.

Interpretation of Wall Motion Scoring With Regard to Alterations in Myocardial Strain and Energetics in Acute Ischemia

In obtaining the WMS of myocardial motion abnormalities, a change from dyskinesis (ie, SL) to akinesis in acute ischemia could be interpreted as “improvement.” However, our study highlights that decreases in the magnitudes of SL and PSS strain may occur in persisting ischemia and are associated with significant worsening of the energy metabolic status, which can eventually lead to transmural necrosis. Therefore, the assessment of regional functional abnormalities in acute ischemia requires careful clinical interpretation. In addition, on the basis of our evaluation, WMS was not correlated with changes in energetics in ischemic myocardium. These findings support the use of strain for the analysis of left ventricular motion and their interpretation on the basis of the estimated energetic status.

Potential Diagnostic Applications

Currently, noninvasive techniques such as positron emission tomography and magnetic resonance spectroscopy are used to assess viability by quantifying regional myocardial metabolism and energetic.³² However, these imaging methods still have rather limited availability, and broadly available echocardiography could complement these methods in assessing the viability of acutely ischemic myocardium at bedside. Recently, the combination of the L/S ratio and systolic myocardial compliance measured by strain echocardiography has been used to differentiate between viable and necrotic myocardium in acute ischemia.⁵ These parameters are further elaborated in the present study. However, clinical validation studies and head-to-head comparisons with other modalities will be required to test the clinical applicability and diagnostic power of this information.

Limitations

General anesthesia and an open-chest experimental model provide different hemodynamic conditions compared with closed-chest echocardiographic scans in conscious patients. This difference can affect the relation between the strain magnitudes and the ATP/ADP ratio, and clinical tests will be required to overcome this limitation.

The mathematical formula presented does not provide an estimation of the ATP/ADP ratio during varying degrees of coronary stenosis, in multiple-vessel disease, and in the presence of preformed collaterals and does not address the influence of reperfusion at different time points of ischemia.

Although we evaluated the extent of necrosis by the commonly employed method of TTC staining, we acknowledge that there are limitations with this technique.³³ We were unable to determine the influence of microscopic necrosis on the relationship between myocardial energetic status and deformation parameters in the early stages of acute ischemia (40 minutes), because subendocardial necrosis was indicated by TTC in only 1 animal, and the presence of microscopic necrosis was conceivable. However, to evaluate the association of necrosis and energetic or strain parameters was not the primary aim of our study and has been discussed elsewhere.^{17,20,34–36}

ATP levels and the ATP/ADP ratio were the primary parameters in this study. We plan to explore other relevant energetic parameters, such as phosphorylation potential and the free energy of ATP hydrolysis.

CONCLUSION

Quantitative measurements of local myocardial function with Doppler strain imaging closely reflect serial changes in high-energy phosphate metabolism occurring in acute myocardial ischemia. The present study introduces a conceptual approach to the noninvasive estimation of myocardial energetic status during acute persisting ischemia using echocardiography. However, the clinical diagnostic utility of the method remains to be determined.

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Figure 1.

An example of data obtained from an animal during acute progressive ischemia. (*Top*) Left ventricular pressure (LVP) with pressure derivative (dP/dt) tracings. (*Middle*) Longitudinal strain tracings obtained from a testing region at different stages of acute progressive ischemia. At baseline, no systolic lengthening (SL) or postsystolic shorting (PSS) strain was present; peak shortening (PkS) strain coincided with end-systolic (ES) strain. At 12 minutes of acute ischemia, SL and ES strain values were highest and progressively decreased thereafter. PkS strain continued in progressive decline as well. PSS strain, calculated as the difference between ES and PkS strain, was maximal at 12 minutes and then decreased to its minimal value at 200 minutes of ischemia. (*Bottom*) Electrocardiogram (ECG) tracings at respective time intervals.

Figure 2.

(A), Course of systolic lengthening (SL) and postsystolic shortening (PSS) strain during acute persistent ischemia. Both SL and PSS strain increased rapidly from baseline to the 12-minute time point of ischemia. Then they monotonically decreased toward their minimal values at 200 minutes of ischemia. (B), Regression analyses comparing the decreasing values of SL and PSS strain with energetic status characterized by the ratio of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). *Triangles* indicate baseline values.

Table 1

Hemodynamic measurements

	Baseline	No reperfusion				100-min reperfusion	
		12-min ischemia	40-min ischemia	120-min ischemia	200-min ischemia	12-min ischemia	120-min ischemia
L VPS	90.6 ± 9.8	86.8 ± 16.3	85.1 ± 6.8	73.6 ± 15.3	78.2 ± 14.0	92.1 ± 10.0	88.8 ± 6.7
+ dP/dt	1,256 ± 175	1,267 ± 489	1,103 ± 391	1,868 ± 292	1,198 ± 397	1,782 ± 688	1,805 ± 589
-dP/dt	-2,447 ± 840	-1,747 ± 836	-1,602 ± 335	-977 ± 424*	-1,107 ± 274	-1,869 ± 462	-1,791 ± 340
L VPD	10.3 ± 2.1	10.9 ± 2.9	10.5 ± 1.4	10.7 ± 6.4	10.9 ± 2.8	10.5 ± 3.0	9.8 ± 1.1
HR	79.8 ± 11.2	89.6 ± 11.5	86.4 ± 18.7	119.8 ± 17.1	93.0 ± 12.6	95.8 ± 21.4	95.0 ± 13.0

+ dP/dt, Peak positive rate of pressure change; -dP/dt, peak negative rate of pressure change; HR, heart rate; LVPS, peak systolic left ventricular pressure; LVPD, peak end-diastolic left ventricular pressure.

* $P < .05$ vs baseline.

Table 2

Strain echocardiographic data

Parameter	Baseline	No reperfusion				100-min reperfusion	
		12 min ischemia	40 min ischemia	120 min ischemia	200 min ischemia	12 min ischemia	120 min ischemia
Testing region							
SL strain (%)	0.1 ± 0.2	6.0 ± 1.2*	4.3 ± 0.6*†	3.3 ± 0.5*†	3.1 ± 0.3*†	3.9 ± 0.2*†	2.5 ± 0.4*†‡
ES strain (%)	-9.7 ± 1.5	2.9 ± 1.2*	3.4 ± 1.3*	1.6 ± 0.4*	1.3 ± 1.4*	-2.4 ± 1.2*†‡§¶	1.3 ± 0.3*//
PKS strain (%)	-9.7 ± 1.5	-5.8 ± 1.0*	-3.5 ± 2.8*	-2.5 ± 1.5*	-2.2 ± 1.1*	-7.7 ± 1.6*‡§¶	-1.4 ± 0.9*//
PSS strain (%)	0.1 ± 0.2	9.1 ± 1.0*	6.9 ± 1.6*	4.1 ± 1.6*†	3.5 ± 1.3*†‡	5.3 ± 0.6*†	1.3 ± 0.3*†‡//
L/S	0.01 ± 0.03	0.51 ± 0.05*	0.6 ± 0.18*	0.6 ± 0.15*	0.6 ± 0.08*	0.34 ± 0.04	0.67 ± 0.19*
SC (%/mm Hg)	N/A	0.08 ± 0.02	0.06 ± 0.01	0.05 ± 0.01†	0.05 ± 0.01†	0.03 ± 0.01†	0.05 ± 0.01†
Control region							
SL strain (%)	0.1 ± 0.1	0.1 ± 0.2	0.3 ± 0.5	0.2 ± 0.4	0.4 ± 0.8	0.1 ± 0.3	0.2 ± 0.4
ES strain (%)	-14.6 ± 0.7	-15.1 ± 2.2	-15.1 ± 3.7	-14.8 ± 1.3	-12.8 ± 2.0	-14.7 ± 2.0	-13.8 ± 2.5
PKS strain (%)	-15.3 ± 0.7	-15.2 ± 2.2	-15.4 ± 3.3	-14.2 ± 0.8	-13.3 ± 2.0	-14.8 ± 2.1	-13.9 ± 2.3
PSS strain (%)	0.8 ± 0.6	0.1 ± 0.2	0.3 ± 0.6	0.3 ± 0.4	0.6 ± 0.7	0.1 ± 0.2	0.1 ± 0.2
L/S	0.02 ± 0.03	0.01 ± 0.01	0.02 ± 0.03	0.01 ± 0.03	0.03 ± 0.5	0.01 ± 0.02	0.02 ± 0.03

ES, End-systolic; L/S, lengthening/shortening ratio; PKS, peak shortening; PSS, postsystolic shortening; SC, systolic compliance; SL, systolic lengthening.

* $P < .05$ vs baseline.

† $P < .05$ vs 12-minute ischemia.

‡ $P < .05$ vs 40-minute ischemia.

§ $P < .05$ vs 120-minute ischemia.

// $P < .05$ vs 12-minute ischemia and 100-minute reperfusion.

¶ $P < .05$ vs 200-minute ischemia.

Table 3

High-energy phosphate levels

	Baseline	No reperfusion				100-min reperfusion	
		12 min ischemia	40 min ischemia	120 min ischemia	200 min ischemia	12 min ischemia	120 min ischemia
ATP (nmol/mg protein)							
Testing wall	28.0 ± 2.7	20.2 ± 1.8*	8.0 ± 3.3*†	2.2 ± 2.0*†‡	1.6 ± 0.6*†‡§	19.8 ± 1.8*‡§¶	2.4 ± 1.9*†//
Control wall	28.5 ± 3.2	28.5 ± 2.5	26.1 ± 7.0	24.4 ± 6.3	25.5 ± 7.2	27.2 ± 3.2	29.2 ± 6.7
ATP/ADP							
Testing wall	4.1 ± 0.3	2.6 ± 0.4*	1.5 ± 0.6*†	0.6 ± 0.3*†‡	0.6 ± 0.1*†‡	4.3 ± 0.1†‡§¶	0.8 ± 0.4*†//
Control wall	3.4 ± 0.6	3.4 ± 0.7	3.3 ± 1.2	3.0 ± 0.9	3.1 ± 1.1	3.0 ± 0.6	3.2 ± 0.4
ATP + ADP + AMP (nmol/mg protein)							
Testing wall	35.5 ± 2.9	28.9 ± 1.3	12.5 ± 4.2*†	9.8 ± 3.0*†‡	5.0 ± 1.0*†‡	24.7 ± 2.1*‡§¶	5.8 ± 2.8*†//
Control wall	38.1 ± 3.7	37.9 ± 1.5	35.0 ± 6.7	33.6 ± 5.7	34.6 ± 6.4	37.1 ± 2.6	37.7 ± 8.5

ADP, Adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate.

* $P < .05$ vs baseline.

† $P < .05$ vs 12-minute ischemia.

‡ $P < .05$ vs 40-minute ischemia.

§ $P < .05$ vs 120-minute ischemia.

// $P < .05$ vs 12-minute ischemia and 100-minute reperfusion.

¶ $P < .05$ vs 200-minute ischemia.