Myocardial creatine kinase kinetics in hearts with postinfarction left ventricular remodeling

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Murakami, Yo, Jianting Zhang, Marcel H. J. Eijgelsloven, Wei Chen, Wenda C. Carlyle, Yi Zhang, Guangrong Gong, and Robert J. Bache. Myocardial creatine kinase kinetics in hearts with postinfarction left ventricular remodeling. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H892–H900, 1999.—This study examined whether alterations in myocardial creatine kinase (CK) kinetics and high-energy phosphate (HEP) levels occur in postinfarction left ventricular remodeling (LVR). Myocardial HEP and CK kinetics were examined in 19 pigs 6 wk after myocardial infarction was produced by left circumflex coronary artery ligation, and the results were compared with those from 9 normal pigs. Blood flow (microspheres), oxygen consumption (MVO₂), HEP levels ([³¹P magnetic resonance spectroscopy (MRS)], and CK kinetics ([³¹P MRS]) were measured in myocardium remote from the infarct under basal conditions and during dobutamine infusion (20 µg·kg⁻¹·min⁻¹·iv). Six of the pigs with LVR had overt congestive heart failure (CHF) at the time of study. Under basal conditions, creatine phosphate (CrP)-to-ATP ratios were lower in all transmural layers of hearts with CHF and in the subendocardium of LVR hearts than in normal hearts (P < 0.05). Myocardial ATP (biopsy) was significantly decreased in hearts with CHF. The CK forward rate constant was lower (P < 0.05) in the CHF group (0.21 ± 0.03 s⁻¹) than in LVR (0.38 ± 0.04 s⁻¹) or normal groups (0.41 ± 0.03 s⁻¹); CK forward flux rates in CHF, LVR, and normal groups were 6.4 ± 2.3, 14.3 ± 2.1, and 20.3 ± 2.4 µmol·g⁻¹·s⁻¹, respectively (P < 0.05, CHF vs. LVR and LVR vs. normal). Dobutamine caused doubling of the rate-pressure product in the LVR and normal groups, whereas CHF hearts failed to respond to dobutamine. CK flux rates did not change during dobutamine in any group. The ratios of CK flux to ATP synthesis (from MVO₂) under baseline conditions were 10.9 ± 1.2, 8.03 ± 0.9, and 3.86 ± 0.5 for normal, LVR, and CHF hearts, respectively (P < 0.05); during dobutamine, this ratio decreased to 3.73 ± 0.5, 2.58 ± 0.4, and 2.78 ± 0.5, respectively (P = not significant among groups). These data demonstrate that CK flux rates are decreased in hearts with postinfarction LVR, but this change does not limit the response to dobutamine. In hearts with end-stage CHF, the changes in HEP and CK flux are more marked. These changes could contribute to the decreased responsiveness of these hearts to dobutamine.

heart failure; high-energy phosphates; 31-phosphorus nuclear magnetic resonance spectroscopy; coronary occlusion

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conformed with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health [DHSS Publication No. (NIH) 85–23, Revised 1985].

Infarct production by coronary ligation. Young Yorkshire swine (45 days; ~10 kg) were anesthetized with pentobarbital sodium (30 mg/kg iv) and then intubated and ventilated with a respirator and supplemental oxygen. Arterial blood gases were maintained within the physiological range with adjustments of the respiratory settings and oxygen flow. A left thoracotomy was performed, and 0.5 cm of the proximal left circumflex coronary artery (LCX) was dissected free and completely occluded with a ligature. After ligation, the animals were observed in the open-chest state for 60 min. When ventricular fibrillation occurred, electrical defibrillation was performed immediately. This procedure was usually successful. The chest was then closed; if the heart was dilated, the pericardium was left open. The animals were given standard postoperative care including analgesia until they ate normally and were active. LCX occlusion was performed in 24 pigs. Five of these pigs died suddenly during the first week after LCX ligation surgery; studies were performed in the remaining 19 pigs with LCX occlusion (Fig. 1).

 Surgical preparation. Twenty-four animals with LVR and nine size-matched normal animals were anesthetized with α-chloralose (100 mg/kg followed by 20 mg·kg$^{-1}$·h$^{-1}$ iv) and then intubated and ventilated with a respirator and supplemental oxygen. Arterial blood gases were maintained within the physiological range with adjustments of the respiratory settings and oxygen flow. A heparin-filled polyvinyl chloride catheter (3.0-mm OD) was introduced into the right femoral artery and advanced into the ascending aorta. A sternotomy was performed, and the heart was suspended in a pericardial cradle. A second heparin-filled catheter was introduced into the left ventricle through the apical dimple and secured with a purse-string suture. A similar catheter was inserted into the left atrium through the atrial appendage. A microcatheter (0.3-mm ID) was inserted into the anterior interventricular vein for coronary venous blood gas measurement. A 25-mm-diameter NMR surface coil was sutured onto the LV anterior wall, with care taken to avoid the infarct region. The pericardial cradle was then released and the heart allowed to assume its normal position in the chest. The surface coil leads were connected to a balanced-tuned external circuit. The animals were then placed in a Lucite cradle and positioned within the magnet.

A $^{31}$P NMR spectroscopic study was performed 6 wk after LCX ligation surgery. If CHF developed, as indicated by the development of cyanosis, ascites, slow growth rate, and decreased activity, the final $^{31}$P NMR spectroscopy study was performed immediately.

Spatially localized $^{31}$P NMR spectroscopic technique. Measurements were performed in a 40-cm bore 4.7-T magnet interfaced with a SISCO console (Spectroscopy Imaging Systems, Fremont, CA). The LV pressure signal was used to gate NMR data acquisition to the cardiac cycle, whereas respiratory gating was achieved by triggering the ventilator to the cardiac cycle between data acquisitions (31, 32, 46, 47). Spectra were recorded during late diastole with a pulse repetition time of 6–7 s. This repetition time allowed full relaxation for ATP and P$\text{i}$ resonances and ~90% relaxation for the CrP resonance (31, 32). CrP resonance intensities were corrected for this minor saturation. The correction factor was determined for each heart from two spectra recorded consecutively without transmural differentiation, one with a 15-s repetition time to allow full relaxation and the other with the 6- to 7-s repetition time used in all the other measurements.

Radio frequency transmission and signal detection were performed with a 25-mm-diameter surface coil. A capillary containing 15 µl of 3 M phosphonoacetic acid was placed at the coil center to serve as a reference. The proton signal from water, detected with the surface coil, was used to homogenize the magnetic field and to adjust the position of the animal in the magnet so that the coil was at or near the magnet and gradient isocenters. This was accomplished with the use of a spin-echo experiment and a readout gradient. The information gathered in this step was also utilized to determine the spatial coordinates for spectroscopic localization (31, 32). Chemical shifts were measured relative to CrP, which was assigned a chemical shift of ~2.55 parts per million (ppm) relative to 85% phosphoric acid at 0 ppm. Spatial localization across the LV wall was performed with the rotating-frame experiment using adiabatic plane-rotation pulses for phase modulation (RAPP)-imaging-selected in vivo spectroscopy (ISIS)/Fourier series window (FSW) method (RAPP-ISIS/FSW) (10). Detailed data with regard to voxel profiles, voxel volume, and the accuracy of the spatial localization have been published elsewhere (10, 31, 32). Briefly, signal origin was restricted by using the static magnetic field magnitude ($B_0$) gradient and adiabatic inversion pulses to a 17 × 17-mm column coaxial with the surface coil and perpendicular to the LV wall. Within this column, the signal was further localized by using the radio frequency magnetic field magnitude generated by the surface coil ($B_1$) gradient to five voxels centered about 45°, 60°, 90°, 120°, and 135° spin-rotation increments (10, 31, 32). FSW localization utilized a nine-term Fourier series expansion. The Fourier coefficients, the number of free induction decays acquired for each term in the Fourier

 ![Fig. 1. Apex view of a remodeled left ventricle 44 days after left circumflex coronary artery ligation. The damaged postlateral wall changed to a thin scar. The posterior papillary muscle was spared. Both ventricles were hypertrophied. The animal had a 23-kg body weight and did not show signs of end-stage congestive heart failure (CHF).](http://ajpheart.physiology.org/Downloadedfromhttp://ajpheart.physiology.org/)
expansion, and the multiplication factors employed to construct the voxels have been reported previously (10, 31, 32). The voxel locations relative to the coil were set by using the $B_1$ magnitude at the coil center, which was experimentally determined in each case by measuring the 90° pulse length for the phosphonoacetic acid reference located in the coil center. A total of 96 scans accumulated over 10 min were used to construct each set of spatially localized spectra calculated on the basis of the two-site chemical exchange model (6, 39) such that $k_5 = \Delta M/M)/(1/T1^* - 1/T1)$, where $k_5$ and $T1^*$ represent the estimated pseudo first-order rate constant and the intrinsic longitudinal relaxation time of CrP, respectively. $\Delta M = M_0 - M_{\text{infinite}}$, where $M_0$ and $M_{\text{infinite}}$ represent the magnetization at saturation zero and infinite times, respectively. $T1^*$ is a time constant that fits the integral of CrP magnetization decay as the time of saturation of ATPγ increased from 0 to infinite. The CK forward flux rate was calculated as the product of $k_5$ and myocardial CrP concentration.

Myocardial blood flow. Myocardial blood flow was measured with radioactive microspheres that were 15 µm in diameter and labeled with $^{141}$Ce, $^{51}$Cr, $^{95}$Nb, $^{85}$Sr, or $^{46}$Sc (NEN, Boston, MA) as previously described (46). For each measurement, $3 \times 10^8$ microspheres were administered into the left atrial catheter and flushed with 5 ml of normal saline. A reference sample of arterial blood was drawn from the aortic catheter at a rate of 15 ml/min, starting 5 s before microsphere injection and continuing for 120 s. Radioactivity in the myocardial and blood reference specimens was determined by using a gamma spectrometer (model 5912; Packard Instruments, Downers Grove, IL) and was corrected for contaminant activity from the associated isotopes and for background activity. Myocardial oxygen flow ($Q_m$) was calculated from the withdrawal rate of the reference blood specimen ($Q_r$), the radioactivity of the reference specimen ($C_m$), and myocardial radioactivity ($C_m$) using the equation $Q_m = Q_r(C_m/C_m)$. Blood flow was expressed as milliliters per minute per gram of myocardium.

Myocardial oxygen consumption and ATP production. For studies in which myocardial oxygen consumption ($MV_O2$) was determined, blood specimens were withdrawn anaerobically into iced syringes from the aortic and coronary venous catheters (3 ml each). $P_{AO2}$, $P_{CO2}$, and pH were measured with a blood gas analyzer (model 1304; Instrumentation Laboratory, Lexington, MA) calibrated with known gas mixtures. Hemoglobin content ($Hb$) was determined by the cyanmethemoglobin method. Coronary venous and aortic oxyhemoglobin saturation values were calculated from the blood $P_{AO2}$, pH, and temperature using the oxygen dissociation curve. Blood oxygen content was calculated as $Hb 	imes 1.34 \times SO_2 + (0.0031 \times P_{AO2})$. $MV_O2$ was computed as the product of myocardial blood flow measured with microspheres and the difference in oxygen content between aortic and coronary venous blood. The rate of ATP production was calculated from the $MV_O2$ values (the rate of oxidative phosphorylation using P:O = 3 and wet wt/dry wt = 4.5) by assuming that mitochondrial uncoupling was not present during any portion of the protocol. After the study was completed, a drill biopsy of myocardium beneath the surface coil was obtained and quickly frozen in liquid nitrogen for subsequent analysis of ATP and total Cr content using an HPLC technique (34). The heart was then fixed in 10% buffered Formalin. The atria, right ventricle, aorta, and large epicardial vessels were dissected from the left ventricle. The left ventricle was sectioned into four transverse rings of equal thickness (−2.0 cm) parallel to the mitral valve ring. The region of myocardium directly beneath the surface coil was removed and sectioned into three transmural layers from epicardium to endocardium, weighed, and placed into vials for counting of the radioactivity.

Experimental protocol. Aortic and LV pressures were measured with fluid-filled pressure transducers (Statham) positioned at midchest level and recorded on an eight-channel direct-writing recorder (Coulbourn Instruments, Lehigh Valley, PA). LV pressure was recorded at normal and high gain separation. LV pressure was recorded at normal and high gain separation. LV pressure was recorded at normal and high gain separation. LV pressure was recorded at normal and high gain separation. After the study was completed, a drill biopsy of myocardium beneath the surface coil was obtained and quickly frozen in liquid nitrogen for subsequent analysis of ATP and total Cr content using an HPLC technique (34). The heart was then fixed in 10% buffered Formalin. The atria, right ventricle, aorta, and large epicardial vessels were dissected from the left ventricle. The left ventricle was sectioned into four transverse rings of equal thickness (−2.0 cm) parallel to the mitral valve ring. The region of myocardium directly beneath the surface coil was removed and sectioned into three transmural layers from epicardium to endocardium, weighed, and placed into vials for counting of the radioactivity.

Data analysis. Hemodynamic data were measured from the charts recorded. The number of integral values for CrP, ATP, and P:O during each experimental condition were expressed as the ratios CrP/ATP and ATP/ATP. $31P$ NMR spectra from the first, third, and fifth voxels were taken to represent subepicardium, midmyocardium, and subendocardium, respectively.

One-way analysis of variance with replications was used to compare data during different experimental conditions within the same group. When a significant effect was found, individual comparisons were made using Scheffé's method. The
LV end-diastolic pressure was higher (each the baseline LV systolic pressure (LVSP) was lower and in response to dobutamine. However, in pigs with CHF, from those in normals either under basal conditions or hemodynamic variables were significantly different in Table 2. In pigs with compensated LVR, none of the significantly increased blood flow in each layer of the myocardium was not significantly different among the different groups under basal conditions. In normal hearts and in hearts with compensated LVR, dobutamine significantly increased blood flow in each layer of the LV wall. In hearts with CHF, myocardial blood flow did not change in response to dobutamine. The ratio of endocardial to epicardial blood flow was not significantly different under basal conditions among the three groups and did not change significantly during dobutamine infusion (Table 3).

Table 1. Anatomic data

<table>
<thead>
<tr>
<th></th>
<th>LV Wt, g</th>
<th>Body Wt, kg</th>
<th>LV Wt/Body Wt, g</th>
<th>Scar Wt, g</th>
<th>Scar Wt/LV Wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>63.2±7.4</td>
<td>23.4±3.1</td>
<td>2.7±0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVR</td>
<td>84.5±8.6*</td>
<td>25.6±4.4</td>
<td>3.3±0.4*</td>
<td>7.1±1.4</td>
<td>0.08±0.02</td>
</tr>
<tr>
<td>CHF</td>
<td>82.7±9.8*</td>
<td>19.9±2.6</td>
<td>4.0±0.5†</td>
<td>10.8±1.6</td>
<td>0.13±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. LVR, left ventricular remodeling; CHF, congestive heart failure. *P < 0.05, †P < 0.01 vs. normal.

unpaired t-test was used for the comparison of intergroup data. A value of P < 0.05 was required for significance. All values are expressed as means ± SE.

RESULTS

Of the 19 animals with LCX ligation, 6 animals developed cyanosis and/or ascites before the end of the 6-wk observation period. These six animals formed the CHF group. The remaining 13 pigs with LCX ligation formed the LVR group.

Anatomic data. All hearts had a transmural infarct with myocardium in the region perfused by the LCX replaced by scar. The anatomic data are summarized in Table 1. In 13 animals with compensated LVR, the LV weight-to-body weight ratio was increased by 22% compared with that in 9 size-matched normals (P < 0.05). In hearts with CHF, this ratio was increased by 48% (P < 0.01).

Hemodynamic data. Hemodynamic data are shown in Table 2. In pigs with compensated LVR, none of the hemodynamic variables were significantly different from those in normals either under basal conditions or in response to dobutamine. However, in pigs with CHF, the baseline LV systolic pressure (LVSP) was lower and LV end-diastolic pressure was higher (each P < 0.05). In response to dobutamine, heart rate and LVSP increased to similar levels in the normal and LVR groups (Table 2). However, in hearts with CHF, neither LVSP nor the rate-pressure product (LVSP × heart rate) increased significantly in response to dobutamine.

Myocardial blood flow and oxygen consumption. Myocardial blood flow and oxygen consumption data are summarized in Table 3. Blood flow per gram of myocardium was not significantly different among the different groups under basal conditions. In normal hearts and in hearts with compensated LVR, dobutamine significantly increased blood flow in each layer of the LV wall. In hearts with CHF, myocardial blood flow did not change in response to dobutamine. The ratio of endocardial to epicardial blood flow was not significantly different under basal conditions among the three groups and did not change significantly during dobutamine infusion (Table 3).

MV2 was not significantly different among groups under basal conditions (Table 3). In response to dobutamine stimulation, MV2 approximately doubled in both normal and LVR hearts. Oxygen consumption tended to increase in CHF hearts during dobutamine, but this did not achieve statistical significance.

31P NMR spectroscopic and myocardial biopsy measurements. In hearts with LVR, the myocardial CrP level was decreased compared with that in normal hearts (Table 4). One heart with CHF had substantial wall thinning so that only the three outer voxels cover the myocardium while the two inner voxels included mostly LV chamber blood, as evidenced by prominent 2,3-diphospho-D-glycerate resonances and little HEP. In this heart, the basal ΔM/M (to be discussed in CK kinetics) was <10% of normal. This animal died of ventricular fibrillation (VF) during the T1 experiment. Another animal with CHF and a similar degree of LV dilatation (which died of VF shortly after the dobutamine infusion was begun) also had severe decreases of CrP/ATP and the CK flux rate (data not shown). The data from these two animals were not pooled in the CHF group because the animals did not complete the protocol; LV dilatation was so severe in these two animals (compared with that of the other animals with CHF that did complete the protocol) that the two inner voxels covered LV chamber blood only.

Under basal conditions, myocardial CrP/ATP was decreased significantly in the subendocardial layer of the LVR hearts. In hearts with CHF, CrP/ATP was decreased significantly in all transmural layers. Pi levels were too low to be detected under basal conditions in each group. In response to dobutamine infusion, CrP/ATP decreased in both normal and LVR hearts. In response to dobutamine stimulation, there was a tendency toward an increase of Pi levels across the LV wall, but this was not significant. In hearts with CHF, myocardial HEP levels did not change significantly in response to dobutamine, and an increase of Pi level was seen in only one of the six pigs studied.

Table 2. Hemodynamic data

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate, beats/min</th>
<th>Mean Aortic Pressure, mmHg</th>
<th>LV Systolic Pressure, mmHg</th>
<th>LV End-Diastolic Pressure, mmHg</th>
<th>Rate-Pressure Product, mmHg·beats·min⁻¹·10⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>128±14</td>
<td>71±8</td>
<td>96±12</td>
<td>6±1</td>
<td>12.8±2.1</td>
</tr>
<tr>
<td>LVR</td>
<td>139±15</td>
<td>74±8</td>
<td>98±11</td>
<td>9±1</td>
<td>13.6±4.4</td>
</tr>
<tr>
<td>CHF</td>
<td>141±19</td>
<td>63±9</td>
<td>72±16*</td>
<td>16±4†</td>
<td>10.1±3.0</td>
</tr>
<tr>
<td>Dobutamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>178±31</td>
<td>75±10</td>
<td>143±12</td>
<td>5±1</td>
<td>25.5±4.7</td>
</tr>
<tr>
<td>LVR</td>
<td>189±23</td>
<td>82±9</td>
<td>133±11</td>
<td>9±1</td>
<td>25.1±5.1</td>
</tr>
<tr>
<td>CHF</td>
<td>152±24</td>
<td>55±9†</td>
<td>74±15†</td>
<td>16±3†</td>
<td>11.2±2.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, †P < 0.01 vs. normal.
Myocardial ATP levels (from biopsy specimens) were lower in LVR hearts and were significantly decreased in hearts with CHF (Table 4). Myocardial total Cr tended to be lower in LVR hearts, but this did not achieve statistical significance.

CK kinetics. Typical magnetization transfer spectra from a normal heart and a heart with CHF are shown in Fig. 2, whereas CK kinetic data are summarized in Table 4. The ratio \( \Delta M/M \) (which is linearly related to the CK flux rate) was normal in LVR hearts but was significantly decreased in hearts with CHF. \( \Delta M/M \) did not change significantly during dobutamine infusion in any group of animals. T1 was not significantly different from normal in either LVR or CHF hearts. The forward rate constant \( (k_f) \) was not different from normal in LVR hearts but was significantly decreased in hearts with CHF. \( \Delta M/M \) in the two animals with severe LV failure that died before completion of the protocol were 0.14 and 0.17 (data not included in Table 4). The calculated forward flux rate of CK was decreased by 30% in hearts with CHF. During infusion of dobutamine, flux through the CK reaction decreased significantly in normal and LVR hearts but not in hearts with CHF (Table 4).

ATP synthesis/utilization rates, calculated by using the oxygen consumption data (Table 3) and a ratio \( \text{P:O} = 3 \), were not significantly different under basal conditions (Table 4) among the three groups and increased similarly in normal and LVR hearts during dobutamine stimulation. To examine the relationship between the rates of ATP production and CK flux, the ratio of CK flux to ATP utilization (CrP/ATP) from the three groups under baseline conditions and during dobutamine stimulation are plotted in Fig. 3. During basal conditions in normal hearts, the CK flux rate was more than an order of magnitude faster than the ATP synthetic rate. As the workload increased, the ratio decreased in proportion to the increased ATP synthesis rate during dobutamine infusion. In LVR hearts the CK forward rate constant was not different from normal, but the significantly decreased CrP in these hearts resulted in a 30% decrease in calculated CK flux. The increased oxygen consumption during dobutamine infusion caused a decrease in the ratio of ATP synthesis to CrP flux to a value of ~1:3. In hearts with CHF, CK flux during basal conditions was approximately four times the calculated ATP synthesis rate. During dobutamine infusion there was a trend toward a decrease in this ratio, but this did not achieve statistical significance.

**DISCUSSION**

The main findings of the present study are 1) postinfarction LVR is accompanied by a decrease in myocardial CK flux; 2) in compensated remodeled hearts, this abnormality does not limit LV function in response to catecholamine stimulation; and 3) in hearts with CHF, the alterations are more severe and might contribute to the inability of these hearts to respond to inotropic stimulation.

Characteristics of animal model. Because of the paucity of innate coronary collateral vasculature in...
swine, coronary artery occlusion results in a full-thickness myocardial infarct that is subsequently replaced by scar. Using this experimental model, we have previously observed that LV dilatation and dysfunction occur in all of the animals within 6–8 wk after coronary artery ligation (48). In a previous study of this experimental model, animals that developed overt heart failure had LV dilatation with an approximate doubling of systolic wall stress and a decrease of ejection fraction to 27 ± 6.1% compared with 56 ± 5.6% in normal animals. Animals without overt heart failure had a lesser degree of LV dilatation with a 50% increase in systolic wall stress and a decrease of ejection fraction to 36 ± 2% (48). In the present study, approximately one-third of the animals developed heart failure with ascites, peripheral cyanosis, and decreased activity, generally with dyspnea at rest, before the planned 6-wk termination of the study. Animals with CHF also had right ventricular dilatation, suggesting that elevated filling pressures of the failing left ventricle had caused pulmonary hypertension. In a previous study, we found that the severity of LV systolic dysfunction was related to the size of the initial infarct (48). Similarly, in the present study, the scar weight-to-LV weight ratio was larger in hearts with CHF than in hearts in the LVR group (Table 1). In the current study in animals with heart failure, heart rate and LV systolic pressure failed to respond to dobutamine, in agreement with previous studies demonstrating blunted responses to catecholamines in the failing heart (5, 9, 17). In addition, myocardial blood flow did not increase significantly during dobutamine stimulation (Table 3). Failure of coronary flow to increase during dobutamine was likely the result of metabolic regulation of myocardial blood flow, because we previously observed in this model (7) that coronary vasodilator reserve in both LVR or CHF hearts was not significantly impaired when tested with a maximum vasodilating dose of adenosine.

Myocardial HEP levels. Myocardial ATP levels have been reported to be decreased in some (20, 46, 48) but not all (14, 27) animal models of LV dysfunction. In those reports in which ATP was found to be decreased, the changes were relatively small (~20% below the normal level). In the present study, myocardial ATP content tended to be lower in animals with heart failure than in animals with compensated remodeling. This is in agreement with the report of Shen et al. (36) demonstrating that, in dogs in which heart failure was induced by rapid ventricular pacing, myocardial CrP and ATP levels progressively decreased as heart failure evolved. The mechanisms that determine the set point
of the normal myocardial ATP level are not known, and it is unclear whether the decreased ATP content in failing hearts contributes to LV dysfunction. In stunned myocardium, although ATP is decreased as much as 40–50%, inotropic stimulation can restore normal function, indicating that a decreased level of myocardial ATP by itself is not sufficient to cause LV dysfunction (24, 42).

Consistent with previous reports, hearts with LVR are characterized by significant decreases of myocardial CrP and CrP/ATP (12–14, 26, 27, 44–48). The decrease of CrP/ATP indicates an increase of myocardial free ADP and suggests an alteration in oxidative phosphorylation regulation. In animals with chronic pressure overload, the decrease in myocardial CrP/ATP is proportional to the severity of LVH (14, 44–48). Furthermore, Neubauer et al. (26) have reported that myocardial CrP/ATP is a good predictor of mortality in patients with congestive cardiomyopathy. Chemical energy generated in the mitochondria is transported to the contractile apparatus and consumed by actomyosin ATPases in the cross bridges. An imbalance of the energy delivery-demand relationship could occur at several sites along the chemical energy production-utilization cycle. In remodeled ventricles LV dilatation causes an increase of systolic wall stress that would be expected to increase energy demand. Furthermore, increased intercapillary diffusion distances could act to limit oxygen and carbon substrate delivery. LVH/CHF hearts have a decreased capacity to utilize free fatty acids, whereas increased glucose utilization could decrease the myocardial energy state (19, 21, 44).

In response to dobutamine stimulation, both normal and LVR hearts showed slight but significant decreases of CrP/ATP and increases of ΔPi/CrP. Similar findings were observed in a previous study by Massie et al. (22, 23). Although these changes might be the result of demand ischemia, it is unlikely that ischemia would occur when the workload of heart was only doubled and coronary reserve was not exhausted (23, 48). To assess the possibility of demand ischemia, a model of the myocyte oxygenation level. It is more likely that the changes in myocardial CrP/ATP and ΔPi/CrP are the result of alterations of the regulation of oxidative phosphorylation. Myocardial CK flux, contractile reserve, and ATP utilization rate. In the present study, the CK flux rate was reduced in hearts with LVR, and this change was most severe in failing hearts. In a separate study, using the same animal model, we found significant decreases of mitochondrial CK expression at both the transcriptional and translational levels (11). A decrease in mitochondrial CK might require higher cytosolic ADP levels to support a given rate of ATP synthesis and might limit the maximal rate of ATP synthesis. A significantly increased myocardial free ADP has been found in this model in a previous study (48).

As a consequence of the combined decrease in the myocardial CrP level and (in the failing hearts) the CK forward rate constant, the myocardial CK flux rate was decreased by 30% in LVR hearts and by 68% in hearts with CHF. These data are in agreement with data from previous studies in rodent hearts with myocardial hypertrophy or failure (3, 12–14). The CK system facilitates myocardial energy metabolism as an HEP transport shuttle and buffer (33, 43). Cr and CrP diffuse more readily than ATP and ADP in the myocyte, giving rise to the CK/CrP shuttle hypothesis (1–4). Because in normal myocardium the ratio of phosphor exchange between CrP and ATP is an order of magnitude higher than the ATP utilization rate, the shuttle hypothesis does not require an increase in the CK flux rate to accommodate physiological increases in myocardial workload. Indeed, in the present study, the CK flux rate decreased when the workload of the hearts was increased. In the present study, the ratio of the CK flux rate to the rate of oxidative phosphorylation was calculated to examine whether the rate of ATP utilization would approach the rate of flux through the CK reaction during catecholamine stimulation (Fig. 3). Interestingly, during high work states in both normal and LVR hearts, this ratio decreased to a level similar to that of the CHF hearts during baseline conditions (Fig. 3). It is possible that this ratio of CK flux rate to the rate of oxidative phosphorylation reflects a minimum value to maintain optimal cross-bridge function. Optimal cross-bridge function has been demonstrated to require the presence of the CK system (16, 33). In a study of dogs with CHF induced by rapid pacing, Traverse et al. (38) found that the increase of MVo2 during treadmill exercise was substantially less than normal. In previous studies, decreased contractile reserve was observed in hearts in which CK activity was suppressed by sulfhydryl inhibition (37), guanidino substrate replacement (16), and CK-M subunit gene knockout (40). These data support the concept that a decrease of the CK flux rate might contribute to decreased contractile reserve in the failing heart.

In conclusion, the present study demonstrated that postinfarct LVR was associated with alterations in the myocardial CK kinetics. These alterations did not restrict LV function or contractile reserve in hearts with compensated LVR. In hearts with end-stage CHF, these changes were more severe, raising the possibility that alterations in the CK system might contribute to the decreased ability of these hearts to respond to inotropic stimulation.

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of mice deficient in muscle creatine kinase lack burst activity.


