High-Energy Phosphate Metabolism and Creatine Kinase in Failing Hearts
A New Porcine Model

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Background—This study aimed to create a pig model of heart failure secondary to severe aortic stenosis and to examine the relationship between the alterations in myocardial high-energy phosphate (HEP) metabolism and protein expression of creatine kinase (CK) isoforms.

Methods and Results—Sixteen pigs with left ventricular hypertrophy (LVH) secondary to ascending aortic banding and 10 normal pigs (N) were studied. Myocardial protein levels of CK isoforms (Western blot), HEP levels, and CK kinetics (31P MR spectroscopy) were measured under basal conditions. Nine of the 16 animals with LVH developed congestive heart failure (CHF), as evidenced by ascites (100 to 2000 mL). LV weight/body weight ratio (g/kg) was 2.18 ± 0.15 in N hearts, 3.04 ± 0.14 in hearts with LVH (P < 0.01), and 4.23 ± 0.36 in hearts with CHF (P < 0.01 versus LVH). Right ventricle weight/body weight ratio and LV end-diastolic pressure were significantly higher in hearts with CHF (each P < 0.01 versus N or LVH). Myocardial phosphocreatine/ATP ratios and the CK forward flux rates were decreased in LVH hearts, most severely in hearts with CHF. CK-M/β-actin ratios were 2.21 ± 12 (N), 1.69 ± 0.15 (LVH), and 1.39 ± 0.27 (CHF, P < 0.05 versus N). CK-mitochondria (CK-Mt)/β-actin ratios were 1.40 ± 0.09 (N), 1.24 ± 0.09 (LVH), and 1.02 ± 0.08 (CHF, P < 0.05 versus N or LVH). The severity of the reduction of CK flux rate was linearly related to the severity of the decrease of CK-Mt/β-actin (r = 0.68, P < 0.01).

Conclusions—In this new model of heart failure/hypertrophy, the abnormal myocardial HEP metabolism is related to the decreased CK-Mt protein level, which in turn is related to the severity of the hypertrophy. (Circulation. 2001;103:1570-1576.)

Key Words: heart failure ■ hypertrophy ■ creatine kinase ■ phosphates ■ spectroscopy

Heart failure is associated with abnormal myocardial energy metabolism.1,2 The mechanisms of this abnormality and its contribution to the evolution from compensated left ventricular hypertrophy (LVH) to congestive heart failure (CHF) are not known.2,3 One difficulty in studying this important medical problem is the lack of a large-animal model that has significant clinical relevance and clearly exhibits the transition from compensated LVH to CHF.

In the heart, the contractile utilized ATP is synthesized mainly in the mitochondria through oxidative phosphorylation and transported to the contractile apparatus, where it is consumed by myosin ATPase to generate force.3,4 The creatine kinase (CK) system is postulated to play an important role in myocardial energy metabolism by maintaining high ADP levels at the site in mitochondria at which ATP is generated and low levels at the site of ATP utilization.3,4 These considerations are embodied in the CK shuttle hypothesis, which postulates that the CK system acts to facilitate ATP production, transportation, and utilization. A fetal shift of the myocardial CK isoforms with a decreased mitochondrial CK (CK-Mt) was found in hearts with LVH or CHF of different species.2,4,5 In normal hearts, the fraction of CK-Mt in small animals is ~28%; this fraction is only 10% in large-animal or human hearts.2,5,6 Because of the low CK-Mt fraction, in hearts with cardiac hypertrophy the reduction of CK-Mt might be the “weak link” of the energy shuttle and consequently contribute to the transition from compensated LVH to heart failure. In dogs with LVH secondary to pressure overload produced by banding of the ascending aorta, we found that myocardial high-energy phosphate (HEP) levels and the phosphocreatine (PCr)/ATP ratio were significantly decreased.7 These abnormalities were proportional to the degree of hypertrophy but were not the result of persistent abnormalities of myocardial perfusion.7 Whether these abnormalities in HEP metabolism are related to the decreased CK-Mt is not known. The present study was designed to

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develop a new porcine model of CHF secondary to severe pressure overload and to examine the relationship between the abnormal steady-state myocardial HEP metabolism and changes in protein levels of the CK isofrom.

**Methods**

Studies were performed in accordance with the “Position of the American Heart Association on Research Animal Use,” adopted November 11, 1984, and protocols were approved by the Animal Care Committee of the University of Minnesota.

**Production of LVH**

Sixteen Yorkshire pigs at ~45 days of age were anesthetized with sodium pentobarbital (25 to 30 mg/kg IV), intubated, and ventilated with a respirator. A right thoracotomy was performed in the third intercostal space, and the ascending aorta, ~1.5 cm above the aortic valve, was mobilized and encircled with a polyethylene band 2.5 mm in width. While LV and distal aortic pressures were measured simultaneously, the band was tightened until a 60 to 70 mm Hg peak systolic pressure gradient was achieved across the narrowing. The chest was then closed, the pneumothorax was evacuated, and the animals were allowed to recover. LVH occurred progressively as the area of aortic constriction remained fixed in the face of normal body growth. Two months after banding, animals were returned to the laboratory for study.

**Experimental Preparation**

Ten normal pigs and 16 pigs with LVH were premedicated with sodium pentobarbital (25 to 30 mg/kg IV), followed by an infusion of 4 mg · kg⁻¹ · h⁻¹. A smaller dose of pentobarbital (~20 mg/kg IV) was used for animals with CHF to prevent loss of animals from general anesthesia. Animals were intubated and ventilated with a respirator with supplemental oxygen; arterial blood gases and pH were maintained within the physiological range. A polyvinyl chloride catheter, 3.0-mm OD, filled with heparin-saline was introduced into the right femoral artery and advanced into the ascending aorta. A left thoracotomy was performed in the fifth intercostal space, and the heart was suspended in a pericardial cradle. A heparin-saline-filled catheter was introduced into the LV through the apical diple and secured with a purse-string suture. A similar catheter was placed into the left atrium through the atrial appendage. An NMR surface coil was sutured to the anterior LV wall overlying the region perfused by the LVH, and 4 CHF ventricles for subsequent analysis of ATP content. The heart was then fixed in 10% buffered formalin for subsequent determination of Cr total content and molecular analysis.

**Myocardial Blood Flow**

Myocardial blood flow was measured with microspheres, 15 μm in diameter, labeled with ¹⁴⁴Ce, ⁵²Cr, ⁸⁵Sr, or ⁶⁵Sc (NEN Corp) as previously described.⁷

**NMR Technique**

The NMR technique has been described in detail.⁷–¹⁰ Briefly, measurements were performed in a 40-cm-bore 4.7-T magnet interfaced with a Spectroscopy Imaging Systems Corp console. 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.⁷–¹⁰

**Calculation of Myocardial Free ADP Levels**

The myocardial free ADP levels were calculated from the CK equilibrium expression¹¹ with an equilibrium constant of 1.66×10⁹⁰ and cytosolic pH=7.1: [ADP]=[(ATP)(Cr)₀]/[(PCr)(H⁺)(Kₜ)]. PCr and ATP values were obtained from the spectra calibrated by the biopsy-measured ATP levels. Free creatine was calculated by subtracting the PCR values from the biopsy-obtained measurement of total creatine (Cr_total).

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**CK Kinetics Measured With ³¹P MR Spectroscopy Saturation Transfer Technique**

The applied ³¹P MR spectroscopy magnetization saturation transfer technique was described previously.¹²,¹³ Briefly, a Gaussian pulse train was used to saturate ATP-γ resonance. This Gaussian pulse train was applied repetitively to ensure the complete saturation of ATP-γ resonance.

The CK kinetics was calculated with the magnetization transfer method as previously described.¹²,¹³ The forward rate constant of CK (kf) and intrinsic relaxation time for PCr (T₁) were calculated on the basis of the 2-site chemical exchange model¹²: kf=(ΔM/M)/T^*₁ and 1/T^₁=1/T^*₁−kₜ, where kₜ and T¹ represent the estimation of pseudo–first-order rate constant and the intrinsic relaxation time of PCr, respectively; ΔM=M₀−M_infinite where M₀ and M_infinite represent the magnetization at saturation zero and infinite times, respectively; and T^*₁ is a time constant that fits the integral of PCr magnetization decay as the time of saturation at ATP-γ increased from 0 to infinite. The CK forward flux rate (Flux_k) was calculated as the products of kₜ and myocardial PCr concentration (Flux_k=kₜ(PCr)).

**Hemodynamic Measurements**

Aortic and LV pressures were monitored with pressure transducers positioned at mid chest level. LV pressure was recorded at normal and high gain for measurement of end-diastolic pressure.

**Experimental Protocol**

Aortic and LV pressures were measured with Spectramed pressure transducers positioned at mid chest level and recorded on an 8-channel direct writing recorder (Coulbourne Instrument Co). LV pressure was recorded at normal and high gain for measurement of end-diastolic pressure. Midway through the 10-minute MR spectroscopy acquisition period, a microusb injection was performed for determination of myocardial blood flow.

**Tissue Preparation**

At the end of the study, a drill biopsy was taken from normal, LVH, and CHF ventricles for subsequent analysis of ATP content with a high-performance liquid chromatography technique.¹⁴ The animal was then killed, the heart was excised, and a full-thickness myocardial specimen ~3 g in weight was taken and frozen (~70°C) for subsequent determination of Cr_total content and molecular analysis.¹⁴ The heart was then fixed in 10% buffered formalin for myocardial blood flow measurement as described.⁶,⁷

**Myocardial CK Activity and Isozyme Measurements**

Specimens from 6 hearts of each group were studied to examine myocardial protein levels of CK isoforms by Western blot as previously described.¹⁵

**Western Blot Analysis**

A parallel Western blot analysis was performed. The protein extracts were run on 12% SDS-PAGE gels for 3 hours at 180 V with the Protein Electrophoresis apparatus (BioRad). Commercially prepared molecular weight standards and purified proteins (CK-MM, CK-MB, CK-BB, and CK-Mt, all obtained from Aalto) were run as controls. The protein subunits were transferred for 1 hour at 100 V in transfer buffer (25 mmol/L Tris, 192 mmol/L glycine, 20% methanol). Monoclonal mouse antibodies specific to the CK-M and CK-B subunits (OEM Concepts Inc) and polyclonal rabbit antibodies specific to CK-Mt (from Dr Strauss’ laboratory, Washington University, St Louis, Mo) were sequentially directed against their respective protein subunits bound to the membrane. Densitometry of the film allowed for a relative quantification of the CK protein subunits.

**Total CK and CK Activity**

Total CK activity was measured with a creatine kinase diagnostic kit (CK-10) (Sigma Diagnostics).¹⁶
Data Analysis
Data were analyzed with 1-way ANOVA with replications. A value of $P<0.05$ was required for significance. When the ANOVA yielded a significant result, individual comparisons were made by the method of Scheffé. Data are reported as mean±SEM.

Results

Animal Model
The signs of cyanosis and ascites (peritoneal fluid >50 mL) were used to separate LVH hearts with and without CHF. Nine of the 16 pigs with aortic banding developed CHF, as evidenced by ascites (100 to 2000 mL). Two of these 9 pigs that also had cyanosis died during the instrumentation surgery. The other 7 pigs formed the CHF group. The remaining 7 pigs with aortic banding formed the LVH group. The anatomic data are summarized in Table 1. The ratio of LV weight to body weight (LVW/BW; g/kg) increased by 39% in hearts with CHF ($P<0.01$ versus LVH or normal). The ratio of RV weight to body weight (RVW/BW; g/kg) increased by 112% ($P<0.01$ versus LVH or normal) only in hearts with CHF.

Hemodynamic Data
Hemodynamic data are shown in Table 2. LV systolic pressure was significantly higher in hearts with LVH. LV end-diastolic pressure was significantly increased only in CHF hearts ($P<0.01$ versus LVH or normal, Table 2).

Biopsy and CK Kinetics Data
Myocardial ATP and Cr$_{total}$ content decreased by 29% and 26%, respectively, in hearts with CHF ($P<0.05$, Table 5). In hearts with compensated LVH, neither ATP nor Cr$_{total}$ level changed significantly (Table 5). The calculated myocardial free ADP was significantly increased in hearts with LVH or CHF (each $P<0.05$ versus normal). Myocardial PCr was significantly decreased in hearts with LVH, most severely in hearts with CHF (Table 4).

Alterations of CK Isoform Expression in the LVH and CHF Hearts
The CK isoform protein levels normalized to $\beta$-actin from all hearts are summarized in Figure 1. Myocardial $\beta$-actin contents were not significantly different among the 3 groups. Each isoform protein expression level was normalized to the $\beta$-actin. Therefore, only the ratios of CK isoform to $\beta$-actin from different groups are compared in this figure. In human and large-animal models, the fractions of the isoforms to the total CK were $\approx 90\%$, $\approx 3\%$, and $\approx 10\%$ for CK-M, CK-B,

### Table 1. Anatomic Data

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BW, kg</th>
<th>LVW, g</th>
<th>RW, g</th>
<th>LVW/BW</th>
<th>RW/BW,</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>38.8±2.6</td>
<td>86.4±10.9</td>
<td>32.6±1.5</td>
<td>2.18±0.15</td>
<td>0.86±0.05</td>
</tr>
<tr>
<td>LVH</td>
<td>7</td>
<td>46.5±4.2</td>
<td>142.3±14.9*</td>
<td>47.7±5.9†</td>
<td>3.04±0.14†</td>
<td>1.02±0.03</td>
</tr>
<tr>
<td>CHF</td>
<td>7</td>
<td>33.3±6.7</td>
<td>142.6±31.5*</td>
<td>53.9±8.5*</td>
<td>4.23±0.36†</td>
<td>1.82±0.25</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

* $P<0.05$ vs normal; † $P<0.01$ vs normal; ‡ $P<0.05$ vs LVH; § $P<0.01$ vs LVH.

### Table 2. Hemodynamic Data

<table>
<thead>
<tr>
<th>Heart Rate, bpm</th>
<th>Mean Aortic Pressure, mm Hg</th>
<th>LV Systolic Pressure, mm Hg</th>
<th>LV End-Diastolic Pressure, mm Hg</th>
<th>Rate-Pressure Product, 1000×(mm Hg×bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>122±6</td>
<td>85±3</td>
<td>116±6</td>
<td>6±1</td>
</tr>
<tr>
<td>LVH</td>
<td>123±2</td>
<td>90±6</td>
<td>142±9*</td>
<td>7±1</td>
</tr>
<tr>
<td>CHF</td>
<td>145±7</td>
<td>79±4</td>
<td>133±8</td>
<td>20±2†‡</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

* $P<0.05$ vs normal; † $P<0.01$ vs normal; ‡ $P<0.01$ vs LVH.

Myocardial PCr/ATP was significantly decreased in every layer across the LV wall of the LVH hearts. The reduction of PCr/ATP was most severe in hearts with CHF (Table 4).
TABLE 4. Myocardial PCr/ATP Ratio

<table>
<thead>
<tr>
<th></th>
<th>EPI</th>
<th>MID</th>
<th>ENDO</th>
<th>Mean</th>
<th>ENDO/EPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.06±0.10</td>
<td>1.16±0.12</td>
<td>1.13±0.13</td>
<td>1.12±0.11</td>
<td>1.08±0.09</td>
</tr>
<tr>
<td>LVH</td>
<td>1.11±0.12</td>
<td>1.12±0.13</td>
<td>1.15±0.10</td>
<td>1.13±0.12</td>
<td>1.05±0.02</td>
</tr>
<tr>
<td>CHF</td>
<td>1.29±0.11</td>
<td>1.40±0.15</td>
<td>1.32±0.06</td>
<td>1.34±0.10</td>
<td>1.04±0.06</td>
</tr>
</tbody>
</table>

EPI indicates epicardium; MID, midmyocardium; and ENDO, endocardium. Values are mL · min⁻¹ · g⁻¹, mean±SEM.

Discussion

The present study was designed to develop a new porcine model of CHF secondary to pressure overload and to examine the relationship between alterations in myocardial HEP metabolism and changes in myocardial protein levels of CK isoforms. The main findings are that (1) in response to this level of aortic stenosis, 56% of the animals developed severe LVH and CHF, as evidenced by ascites; the remaining animals developed compensated LVH; and (2) in LVH hearts, the severity of the altered HEP metabolism is related to the decreased protein level of CK-Mt, which is most severe in hearts with CHF.

LVH and CHF Secondary to Pressure Overload

In the present study, signs of ascites and cyanosis were used to separate hypertrophied hearts with and without CHF. All the CHF pigs had ascites; 2 of them also had cyanosis. The RVW/BW and LV end-diastolic pressure were significantly higher only in hearts with CHF (Tables 1 and 2). Taken together, these data demonstrate unequivocally that >50% of the animals developed heart failure in response to this level of aortic stenosis.

Using a porcine model, Massie et al found that an aortic stenosis that produced a 25 mm Hg pressure gradient across the narrowing resulted in an increase of LV mass by ~38% in 6 months. No signs of heart failure were observed in these animals. Using this animal model, they found that myocardial HEP metabolism was not significantly different from that of normal hearts either at basal or high cardiac work states. In dogs at 8 weeks of age, an aortic banding that produced a 25 mm Hg pressure gradient across the narrowing resulted in an increase of LV mass by ~100% in 12 months. Using this dog model of compensated LVH, we found that myocardial HEP metabolism was abnormal, which was linearly related with the severity of myocardial hypertrophy but not related to the persistent myocardial ischemia. In the present study, we applied a more severe aortic stenosis that produced an ~70 mm Hg pressure gradient across the narrowing.

Reduction of ATP Content

Recently, there has been an increasing volume of evidence that myocardial ATP concentration is decreased in the failing heart. The underlying mechanism(s) of this abnormality is not defined. In the present study, myocardial ATP level decreased by 29% in hearts with CHF (Table 5). The cause of the reduction of the ATP level in CHF hearts is unknown. In dogs with rapid pacing–induced heart failure, Shen et al found that the myocardial total adenine nucleotide (TAN) pool and ATP content decreased with a time course identical to that of LV dysfunction, being progressively more severe during continuous rapid pacing. The loss of the TAN pool itself could result in the reduction of ATP, because the resynthesis of adenine nucleotide is a slow and energy-costly process through de novo synthesis, in which inosine monophosphate is produced from ribose-5-phosphate, which utilizes 6 HEP bonds.

Loss of Myocardial Cr_total Content

The myocardial Cr_total concentration is reduced in failing hearts (Table 5). Recently, Neubauer et al found that in hearts with CHF, the decreased myocardial Cr_total level may be caused by a significant reduction of myocardial creatine.
transporter. As a result of the decrease of the myocardial ATP/ADP ratio, myocardial free energy release per unit ATP hydrolysis (ΔG) is significantly reduced.23 A reduced ΔG was previously shown to be related to the decreased LV contractile performance.23 Shen et al19 reported that in dogs, during the progressive increase of the severity of LV dysfunction in response to rapid pacing, the reduction of myocardial Crtotal occurred earlier and faster than the decrease of myocardial ATP/TAN levels. It was therefore hypothesized that the loss of the Cr total in the failing heart may serve as a marker for the onset of LVH.24 In the present study, myocardial ATP and Crtotal were significantly lower only in hearts with CHF (Table 5). It is interesting to note that the Crtotal of the 2 pigs that had severe heart failure and died on the surgery table was only 31 and 47 μmol/g dry wt, respectively. This is only ∼50% of the mean value of the CHF hearts and ∼33% of the normal values (Table 5). This >60% decrease of Crtotal would make the calculated free ADP level normal, assuming that reduction of ATP was not >30% (Table 5). These data support the concept that loss of the Cr total in the failing heart may serve as a compensatory mechanism in response to the reduction of the TAN pool19 and consequently preserve myocardial free energy release per unit ATP hydrolysis.19,23

Myocardial PCr/ATP Ratio, CK Energetics, and Isoform Expression

Hearts with LVH are characterized by significant decreases of myocardial PCr and PCr/ATP ratio (Table 4, References 18 to 20). In hearts with postinfarction LV remodeling, we found that the decreased PCr/ATP ratio in myocardium remote from LV scar was independent of the myocardial ischemia.20 The decrease of PCr/ATP ratio indicates an alteration of oxidative phosphorylation regulation and decrease of ΔG.23,24 In patients with dilated cardiomyopathy, the PCr/ATP ratios also provided prognostic information and predicted survival better than LV ejection fraction or New York Heart Association class.25 All these data indicate that the reduction of PCr/ATP is related to the severity of LVH and LV dysfunction. Providing direct evidence to demonstrate a causal factor, however, is a different matter. Data from the present study indicate that the abnormal myocardial PCr/ATP ratio is related to the reduced protein level of CK-Mt. Because CK-Mt is located at the end point of the cascade of ATP production, the reduction of this protein may limit the ATP production rate, which could result in a lower PCr/ATP ratio.

The coupling of CK-Mt and the adenine nucleotide translocator (ANT) is important to maintain a normal mitochondrial oxidative phosphorylation regulation. The reduction of CK-Mt could result in an alteration of mitochondrial oxidative phosphorylation regulation, which could be manifested by a decreased PCr/ATP ratio. The uncoupling of CK-Mt with ANT could also result in a decreased ATP production rate and consequently a decreased steady-state PCr/ATP ratio.

Alterations in Myocardial Bioenergetics and LV Dysfunction

How this reduction of myocardial HEP levels (Table 5) contributes to the contractile performance of the failing hearts is not known. It is not likely, however, that this ∼30% decrease of myocardial ATP concentration would cause the dysfunction of failing hearts. The fact that myocardial ATP concentration is many times greater than the Km values of either the ATPase at the contractile apparatus or sarcoplasmic reticulum calcium ATPase (SERCA);2,26 makes it unlikely that this reduction would directly depress the contractile performance of the LV.

A fatal shift of the CK enzyme system has been reported in several models of LVH/CHF hearts (for review see Reference 2). The CK system plays an important role in myocardial energy metabolism as an energy transport shuttle or energy buffer or by its function in enzyme-enzyme coupling with myosin ATPase.2–4 Creatine diffuses more readily than ADP in the myocyte, giving rise to the "CK/CP shuttle" hypothesis. In previous studies, a decreased contractile reserve was observed in hearts with a suppressed CK system induced by sulfhydryl inhibition,23 by guanidino substrate replacement,27 or by CK-M and CK-Mt gene knockout.28 Taken together, these observations suggest that a severely altered myocardial CK system could contribute to LV dysfunction of the CHF hearts.

### TABLE 5. CK Kinetics and Myocardial Biopsy Data

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>LVH</th>
<th>CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ATP], μmol/g dry wt</td>
<td>22.4±2.5</td>
<td>19.7±1.2</td>
<td>15.8±1.75*</td>
</tr>
<tr>
<td>[Cr]tot, μmol/g dry wt</td>
<td>120±7.9</td>
<td>115±14.9</td>
<td>89±18.3*</td>
</tr>
<tr>
<td>[ADP]*, nmol/g dry wt</td>
<td>273±27</td>
<td>372±32*</td>
<td>352±25*</td>
</tr>
<tr>
<td>[PCr]*, μmol/g dry wt</td>
<td>47.6±2.7</td>
<td>33.3±1.8†</td>
<td>22.8±1.2†‡</td>
</tr>
<tr>
<td>ΔM/M</td>
<td>0.45±0.10</td>
<td>0.49±0.05</td>
<td>0.37±0.04</td>
</tr>
<tr>
<td>T1, s</td>
<td>2.02±0.32</td>
<td>2.52±0.45</td>
<td>2.00±0.38</td>
</tr>
<tr>
<td>kᵣ, s⁻¹</td>
<td>0.49±0.07</td>
<td>0.41±0.03</td>
<td>0.31±0.03*</td>
</tr>
<tr>
<td>CK forward flux, μmol · g dry wt⁻¹ · s⁻¹</td>
<td>20.5±4.3</td>
<td>13.4±1.1*</td>
<td>8.8±1.3‡</td>
</tr>
<tr>
<td>CK total activity, IU/mg</td>
<td>11.8±1.6</td>
<td>10.5±1.2</td>
<td>6.1±0.9†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P<0.05 vs normal; †P<0.01 vs normal; ‡P<0.05 vs LVH; §P<0.01 vs LVH.
CK-Mt and ANT are parts of the mitochondrial permeable transition pore (mtPTP). A reduction of CK-Mt can induce the accumulation of reactive oxygen species, which opens the mtPTP and triggers myocardial apoptosis.\(^{29}\) Perhaps it is the decreased CK activity and energy state (as indicated by a decrease of ATP/ADP ratio, Table 5) and increase of reactive oxygen species that contribute to the opening of mtPTP and initiate apoptosis, which then contributes to the LV dysfunction. Whether the alteration of CK-Mt contributes to apoptosis warrants future studies.

**Conclusions**

Our findings indicate that in this new model of pressure-overload LVH and failure, the abnormal myocardial HEP metabolism is related to the reduced protein level of mitochondrial CK.
Figure 3. Scatterplots showing decrease of myocardial PCr/ATP ratios (A) and CK flux rate (B) in relation to decrease of CK-Mt/β-actin ratios in hearts with LVH. Both decrease of PCr/ATP ratio and CK flux rate are related to protein level of CK-Mt in hypertrophied hearts.

Acknowledgments

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