Myocardial ATP hydrolysis rates in vivo: a porcine model of pressure overload-induced hypertrophy

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Myocardial ATP hydrolysis rates in vivo: a porcine model of pressure overload-induced hypertrophy. Am J Physiol Heart Circ Physiol 309: H450–H458, 2015. First published May 29, 2015; doi:10.1152/ajpheart.00072.2015.—Left ventricular (LV) hypertrophy (LVH) and congestive heart failure are accompanied by changes in myocardial ATP metabolism. However, the rate of ATP hydrolysis cannot be measured in the in vivo heart with the conventional techniques. Here, we used a double-saturation phosphorous-31 magnetic resonance spectroscopy-magnetization saturation transfer protocol to monitor ATP hydrolysis rate in swine hearts as the hearts became hypertrophic in response to aortic banding (AOB). Animals that underwent AOB (n = 22) were compared with animals that underwent sham surgery (n = 8). AOB induced severe LVH (cardiac MRI). LV function (ejection fraction and systolic thickening fraction) declined significantly, accompanied by deferent levels of pericardial effusion, and wall stress increased in aorta banded animals at week 1 after AOB, suggesting acute heart failure, which recovered by week 8 when concentric LVH restored LV wall stresses. Severe LV dysfunction was accompanied by corresponding declines in myocardial bioenergetics (phosphocreatine-to-ATP ratio) and in the rate of ATP production via creatine kinase (at week 1). The first time, the same linear relationships of the rate increase of the constants of the ATP hydrolysis rate (kATP→a) vs. the LV rate-pressure product increase during catecholamine stimulation were observed in vivo in both normal and LVH hearts. Collectively, these observations demonstrate that the double-saturation, phosphorous-31 magnetic resonance spectroscopy-magnetization saturation transfer protocol can accurately monitor myocardial ATP hydrolysis rate in the hearts of living animals. The severe reduction of LV chamber function during the acute phase of AOB is accompanied by the decrease of myocardial bioenergetic efficiency, which recovers as the compensated LVH restores the LV wall stresses.

Left ventricle hypertrophy; adenosine triphosphate; heart failure; MR spectroscopy

NEW & NOTEWORTHY

This is the first report to monitor the in vivo ATP hydrolysis rate in hypertrophied hearts induced by pressure overload. The study identified a transient left ventricular failure in the acute phase following pressure overloading, which was then compensated as left ventricular hypertrophied to normalize wall stress.

LEFT VENTRICULAR (LV) HYPERTROPHY (LVH) and congestive heart failure are accompanied by myocardial bioenergetic abnormalities, including a decline in the rate of ATP flux through the creatine kinase (CK) system (8, 14, 17, 22, 23). The CK system buffers cellular ATP levels by shuttling high-energy phosphate between phosphocreatine (PCr) and ATP (5), and the forward (PCr→ATP) rate of CK-mediated ATP flux can be measured via phosphorus-31 magnetic resonance spectroscopy magnetization-saturation transfer (31P-MRS-MST). However, conventional MRS-MST techniques are unable to determine the rate of myocardial ATP hydrolysis in vivo, because the method requires the quantification of myocardial free inorganic phosphate (Pi) levels, which are low, and because the signal for Pi magnetization overlaps with the signal for 2,3-diphosphoglycerate (2,3-DPG) from the erythrocytes in the LV cavity blood (4, 6). For the experiments described in this report, we measured the ATP hydrolysis rate in the hearts of living animals by using a double-saturation 31P-MRS-MST method that bypasses the need to measure Pi levels directly. The accuracy of this method has been rigorously examined for assessments in skeletal muscle and in the heart (21), while the studies presented here were performed with pigs that had undergone aortic banding surgery to induce concentric LVH, as well as in sham-operated normal pigs. Our results demonstrate that both LV function and myocardial bioenergetics decline severely during the acute phase of aortic stenosis secondary to aortic banding (AOB), but recover remarkably during the compensatory phase, as the LV becomes hypertrophic and LV wall stress returns to normal.

METHODS

The experimental protocol was approved by the University of Minnesota Research Animal Resources Committee. All experimental and animal maintenance procedures were performed in accordance with the Animal Use Guidelines of the University of Minnesota and were consistent with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23). Porcine model of pressure-overload cardiac hypertrophy. Experiments were performed with 2-mo-old (~15 kg) female Yorkshire swine (Manthei hog farm, Elk River, MN). Pressure-overload LVH was surgically induced in 22 animals (i.e., the AOB group), as described previously (16, 24), and control assessments were performed in 8 animals that underwent all surgical procedures, except the banding step (i.e., the Normal group). Briefly, animals were anesthetized with inhaled isoflurane (2% vol/vol), and a left thoracotomy was performed to expose the aorta; then, for animals in the AOB group, a plastic band was placed across the aortic arch distal to the second branch and tightened until the peak systolic pressure gradient across the narrowed region reached 40 mmHg. The chest was closed in layers, and the animal was allowed to recover. Postoperative analgesia was provided for 24 h after surgery via intramuscular injections of buprenorphine (0.03 mg/kg) and ketoprofen (12 mg/kg) and for 3 days after surgery with a fentanyl patch. Four animals in the AOB group died of acute LV failure during the 7-day period immediately following AOB surgery, before data collection at the week 1 time point could be completed.

MRI assessments of cardiac function. Assessments were performed on a 1.5-Tesla clinical scanner (Siemens Sonata, Siemens Medical Systems, Islen, NJ) with a phased-array four-channel surface coil and...
ECG gating (20). Cardiac MRI was performed 1 day before the terminal open chest NMR studies were carried out. Animals were anesthetized with 2% inhaled isoflurane and positioned in a supine position within the scanner. Cardiac function (ejection fraction and thickening fraction) was evaluated and quantified via short-axis cine images and QMASS software (Medis Medical Imaging Systems, Leiden, The Netherlands). Aortic narrowing was evaluated via a cine sequence with imaging planes positioned perpendicular to the aorta, and the severity of aortic narrowing (aortic stenosis) was quantified as the percentage difference in cross-sectional area between the narrowed region and a region proximal to the narrowed region. LV systolic wall stress was calculated from the anatomic and hemodynamic measurements according to the Laplace model (9):

\[
LV \text{ wall stress} = \frac{LV \text{ systolic pressure} \times LV \text{ chamber radius}}{(2 \times LV \text{ wall thickness})}
\]

Myocardial blood flow measurement. Myocardial blood flow was assessed via first-pass perfusion MRI and quantified with automated software (CIMRA; CSON Medical, Minneapolis, MN), as previously described (7, 20). Upon completion of the final set of in vivo magnetic resonance (MR) experiments, the results from first-pass perfusion MRI were corroborated via the injection of fluorescently labeled microspheres, as previously described (21).

Double-saturation \(\text{\(^{31}\text{P}-\text{MRS-MST}\)}\) measurements of myocardial ATP utilization. \(\text{\(^{31}\text{P}-\text{MRS}\)}\) measurements were performed as described previously (21). Briefly, animals were anesthetized with 2% isoflurane and ventilated with supplemental oxygen on a respirator. Polyvinyl chloride catheters (3-mm outer diameter) were inserted into the jugular vein, ascending aorta (through the left external carotid artery), and LV (through the apical dimple) for drug administration and hemodynamic monitoring; the LV catheter was inserted after the heart had been exposed via a sternotomy and suspended in a pericardial cradle. Ventilation rate, volume, and inspired oxygen content were adjusted to maintain physiological values for arterial \(\text{PO}_2\), \(\text{PCO}_2\), and \(\text{pH}\), and aortic and LV pressures were continuously monitored throughout the study. \(\text{\(^{31}\text{P}-\text{MRS}\)}\) measurements were performed on a 65-cm bore, 9.4-Tesla magnet and Vnmrj console (Varian, CA) via a double-tuned (\(^1\text{H}\) and \(^31\text{P}\)) surface coil (28-mm diameter) that was sutured to the epicardium of the anterior LV wall.

A detailed description of the theoretical and mathematical basis for our \(\text{\(^{31}\text{P}-\text{MRS-MST}\)}\) protocol has been published previously (21). Briefly, myocardial ATP turnover can be modeled as a chemical exchange network consisting of three components: \(\text{PCr}\), ATP, and \(\text{Pi}\): \(\text{PCr} \leftrightarrow \text{ATP} \leftrightarrow \text{Pi}\). The rate of \(\text{PCr} \rightarrow \text{ATP}\) reaction can be readily measured by conventional \(\text{\(^{31}\text{P}-\text{MRS-MST}\)}\) protocols, whereas the rate of \(\text{ATP} \rightarrow \text{Pi}\) reaction can be indirectly measured by subtracting the \(\text{PCr} \rightarrow \text{ATP}\) flux from the total ATP turnover flux. The complete protocol consists of the following: 1) measuring the intrinsic longitudinal relaxation time (\(T1\)) constants of spin for \(\text{PCr}\) via a progressive saturation experiment [repetition time (TR) = 6 s, number of repetitions (NEX) = 12]; 2) measuring intrinsic \(T1\) constants of spin for \(\text{ATP} \rightarrow \text{Pi}\) via an inversion recovery experiment with both \(\text{PCr}\) and \(\text{Pi}\), saturated (TR = 4 s, NEX = 24); and 3) measuring \(\text{PCr}\) magnetization saturation and \(\text{ATP} \rightarrow \text{Pi}\) magnetization saturation while selectively saturating \(\text{ATP} \rightarrow \text{Pi}\) or both \(\text{PCr}\) and \(\text{Pi}\) (TR = 6 s, NEX = 16). Selective saturation was achieved by using a B1-insensitive train to obliterate signal pulse sequence to ensure frequency-selective elimination of magnetization in the presence of B1-inhomogeneity from the surface coil (2, 19). Spin magnetization was excited with frequency-selective hyperbolic secant pulses of varying power levels and subsequently eliminated with dephasing gradients. Double saturation was achieved by creating a composite pulse from two hyperbolic secant pulses with different excitation frequencies. A larger bandwidth was used for the excitation frequency to compensate for any change in the chemical shift of \(\text{Pi}\), that could result from variations in pH.

Validation of the \(\text{\(^{31}\text{P}-\text{MRS-MST}\)}\) protocol. Our double-saturation \(\text{\(^{31}\text{P}-\text{MRS-MST}\)}\) approach determines the rate of the ATP → Pi reaction indirectly, by measuring the total rate of ATP utilization (\(k_{\text{ATP,total}}\) where \(k\) is the rate constant), which incorporates both the ATP → PCr and ATP → Pi reactions, and then subtracting the rate of the ATP → PCr reaction, which is determined via conventional methods (i.e., \(k_{\text{ATP} \rightarrow \text{PCr}} = k_{\text{ATP,total}} - k_{\text{ATP} \rightarrow \text{Pi}}\)). The accuracy of our approach was verified in skeletal muscle, where, \(P\) levels can be measured directly and, consequently, conventional methods can be used to determine \(k_{\text{ATP} \rightarrow \text{Pi}}\) and \(k_{\text{ATP} \rightarrow \text{PCr}}\) independently. Validation was performed by 1) measuring \(k_{\text{ATP,total}}\) with our new method; 2) determining \(k_{\text{ATP} \rightarrow \text{PCr}}\) and \(k_{\text{ATP} \rightarrow \text{Pi}}\), with conventional methods; and then 3) comparing \(k_{\text{ATP,total}}\) to the sum of \(k_{\text{ATP} \rightarrow \text{Pi}}\) and \(k_{\text{ATP} \rightarrow \text{PCr}}\). The values obtained for \(k_{\text{ATP,total}}\) (1.09 ± 0.5 s⁻¹; via our new method) and for the sum of \(k_{\text{ATP} \rightarrow \text{Pi}}\) and \(k_{\text{ATP} \rightarrow \text{PCr}}\) (1.10 ± 0.14 s⁻¹; via conventional methods) were in good agreement, which confirms that our double-saturation \(\text{\(^{31}\text{P}-\text{MRS-MST}\)}\) approach can accurately determine the rate of ATP hydrolysis in living muscle tissue. A more detailed description of the validation procedure has been published previously (21).

Induction of high cardiac work state. After all of the baseline MR data were obtained, a high cardiac work state was induced via intravenous infusion of dobutamine and dopamine (each 10 \(\mu\)g kg⁻¹ min⁻¹), as described previously (10, 11, 22). MR data for the high-cardiac work state were collected 5 min after dobutamine/dopamine infusion was initiated, to ensure that systemic hemodynamics had stabilized.

Histological assessments. On completion of the in vivo studies, tissue samples were obtained from the myocardium below the surface coil and immediately frozen in liquid nitrogen or processed for histology. Myocardial ATP levels were measured by using a fluorometric ATP Assay Kit (Abcam), as directed by the manufacturer’s instructions. Paraffin- and OCT-embedded sections of heart tissue were stained with hematoxylin and eosin for assessments of cardiomyocyte size, or with fluorescent anti-CD31 and anti-smooth muscle actin (SMA) antibodies for assessments of vascular and arteriolar density. Cardiomyocyte size was quantified by measuring the cells’ high-power fields per section, 20 sections per heart.

Statistics and data analysis. Data are presented as means ± SD and were analyzed for significance with Sigmastat version 3.5 (San Jose, CA); a \(P\) value of <0.05 was considered significant. Comparisons between two different groups were analyzed via the \(t\)-test, comparisons within the same group were analyzed via the paired \(t\)-test, and comparisons among more than two groups were analyzed for significance with one-way ANOVA. When ANOVA indicated significance, post hoc analyses were performed via the \(t\)-test with Bonferroni correction. The relationships between LVH and cardiac function, wall stress, or myocardial bioenergetics and between the rate-pressure product (RPP) and the myocardial ATP hydrolysis rate were evaluated via linear regression and analysis of covariance.

RESULTS

AOB induced severe stenosis and myocardial hypertrophy. One week after the banding procedure, aortic cross sections in animals of the AOB group were reduced by 66% at end diastole; by week 8, the constriction increased to 75% at end diastole (Fig. 1, A–C), and LV walls were 50% thicker (\(P < 0.05\)) in AOB animals than in Normal animals (Fig. 1D, Table 1). Furthermore, the ratio of LV weight to body weight (Fig. 1E) was 24% greater at week 1 (\(P < 0.05\)), and 42% greater at week 8 (\(P < 0.05\)), in AOB animals than in Normal animals, and significant, although less dramatic, increases in the ratio of right ventricular weight to body weight were also observed in AOB animals

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at week 8. Thus the AOB procedure led to the development of severe stenosis and cardiac hypertrophy.

AOB led to significant, but transient, declines in LV chamber function. All four animals from the AOB group that died within the first week displayed evidence of ascites and pericardial effusion. Three out of seven animals from the AOB group whose week 1 MRI was successfully completed showed evidence of significant pericardial effusion. The Normal group was not subject to terminal MRS study at week 1, but only at week 8, whereas the AOB group was separated into two subgroups and subject to the terminal MR spectroscopic study at week 1 and week 8, respectively. The animal numbers for each group (Fig. 2) are as follows: Normal group, n = 8; AOB week 1, n = 7; AOB week 8, n = 11. At week 1 after AOB surgery, hemodynamic measurements, including LV systolic pressure (LVSP) and the RPP, were similar in AOB and Normal animals under both the baseline cardiac workload and after a high cardiac workload was induced via catecholamine infusion (Table 2). However, measurements of LV ejection (Fig. 2A) and thickening (Fig. 2B) fractions were significantly lower in the AOB group than in Normal animals, and MR images of hearts from the AOB group displayed evidence of ascites and pericardial effusion (Fig. 2C). At week 8 after surgery, LVSP and RPP had increased significantly in animals from the AOB group and were significantly greater than in Normal animals under both workload conditions (Table 2).

Table 1. Cardiac MRI measurements

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>AOB, week 8</th>
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<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>LV end-diastolic volume, ml</td>
<td>58.9 ± 1.6</td>
<td>90.4 ± 8.4*</td>
</tr>
<tr>
<td>LV end-systolic volume, ml</td>
<td>33.3 ± 1.4</td>
<td>40.2 ± 6.1*</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>25.6 ± 1.7</td>
<td>50.2 ± 8.3*</td>
</tr>
<tr>
<td>End-diastolic wall thickness, mm</td>
<td>7.8 ± 0.6</td>
<td>11.9 ± 0.6*</td>
</tr>
<tr>
<td>LV weight, g</td>
<td>133 ± 10</td>
<td>213 ± 17*</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>49.4 ± 4.5</td>
<td>56.0 ± 5.6*</td>
</tr>
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Values are means ± SD; n, no. of animals. AOB, aortic banding; LV, left ventricular. *P < 0.05 vs. Normal.
Cardiac functional parameters (LV ejection fraction and thickening fraction) had also improved and did not differ significantly from measurements in the Normal group, and MR images of AOB hearts showed no evidence of LV failure (data not shown). Measurements of systolic (Fig. 2D) and diastolic (Fig. 2E) wall stress also differed significantly between groups at week 1, but not at week 8. Collectively, these observations suggest that the hearts of AOB animals were in the acute phase of LV failure at week 1 and were unable to respond to the developing stenosis by increasing the force of contraction; the impaired response may also have been exacerbated by the agent used for general anesthesia during the open-chest procedure. By week 8, cardiac function had been restored by the hypertrophic response to pressure overload, and the hearts had entered the compensated phase of concentric LVH.

**Pressure-overload induced LVH was associated with significant changes in myocardial bioenergetics.** Myocardial ATP metabolism can be modeled as a chemical exchange network with three components, PCr$\leftrightarrow$ATP$\leftrightarrow$Pi. Thus we determined whether the AOB procedure and consequent myocardial hypertrophy were associated with changes in myocardial ATP metabolism by using our double-saturation $^{31}$P-MRS-MST technique (21) (Fig. 3) to measure the following: 1) the ratio of the PCr and ATP concentrations (PCr/ATP); 2) the rate constants for the CK-mediated conversion of PCr to ATP ($k_{\text{PCr\to ATP}}$) and for ATP hydrolysis ($k_{\text{ATP\to Pi}}$); and 3) the

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**Table 2. Hemodynamic measurements**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>AOB, week 1</th>
<th>AOB, week 8</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>HWL</td>
<td>Baseline</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>91 ± 6</td>
<td>169 ± 17</td>
<td>102 ± 11</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>86 ± 12</td>
<td>148 ± 27</td>
<td>87 ± 4</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>4 ± 2</td>
<td>5 ± 2</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>RPP × 10$^3$, mmHg × beats/min</td>
<td>6.9 ± 2.9</td>
<td>22.2 ± 9.3</td>
<td>8.8 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. HWL, high-cardiac workload; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; RPP, rate-pressure product (heart rate × LVSP). *P < 0.05 vs. Normal. †P < 0.05 vs. AOB, week 1.
corresponding rates of ATP flux \{\text{Flux}_{\text{PCr}\rightarrow\text{ATP}} = k_{\text{PCr}\rightarrow\text{ATP}} \times (\text{PCr}/\text{ATP}) \times [\text{ATP}]; \text{Flux}_{\text{ATP}\rightarrow\text{P}} = k_{\text{ATP}\rightarrow\text{P}} \times [\text{ATP}]\} in the hearts of animals from the AOB and Normal groups.

PCr/ATP were significantly reduced in AOB hearts under both baseline and high cardiac workloads at week 1 (Fig. 4A), when the hearts were in the acute phase of LV failure; baseline measurements improved significantly in the AOB group at week 8, during the compensated phase, but remained significantly lower than those in the Normal group. The rate constant \(k_{\text{PCr}\rightarrow\text{ATP}}\) (Fig. 4B) and flux \(\text{Flux}_{\text{PCr}\rightarrow\text{ATP}}\) in the hearts of animals from the AOB and Normal groups. AOB was not associated with changes in \(k_{\text{ATP}\rightarrow\text{P}}\) during the acute phase (Fig. 4C). At high cardiac workload, the higher RPPs were associated with higher \(k_{\text{ATP}\rightarrow\text{P}}\) in the AOB week 8 group hearts (Fig. 4D). At baseline, \(\text{Flux}_{\text{PCr}\rightarrow\text{ATP}}\) per gram of myocardium was significantly lower in AOB week 1 hearts (Fig. 4D); whereas \(\text{Flux}_{\text{ATP}\rightarrow\text{P}}\) did not differ significantly between groups at baseline (Fig. 4D).

The high cardiac work state induced by catecholamine stimulation was accompanied by increases in both RPP (Table 2) and \(k_{\text{ATP}\rightarrow\text{P}}\) (Fig. 4C). One animal from the AOB-week 1 group failed to complete the terminal MRS protocol and thus was removed from the plots in Fig. 5, C and D. Two animals from AOB-week 8 group failed to complete the hemodynamic measurements and thus were removed from plot in Fig. 5C. The linear relationship between the rate of ATP hydrolysis \(k_{\text{ATP}\rightarrow\text{P}}\) and the RPP is evident in all groups (Fig. 5A). The slopes of this relationship are not significantly different among the groups (\(P > 0.05\)). All three groups showed significant correlation between RPP and rate constants (Normal, \(P = 0.0005\); AOB-week 8, \(P < 0.0001\); AOB-week 1, \(P = 0.0044\)). This is the first report that rate of ATP hydrolysis is linearly related to the increase of cardiac work states in the in vivo heart based on the experimental data.

Furthermore, linear regression relationships are also observed between LVH vs. ejection fraction (Fig. 5B), diastolic wall stress (Fig. 5C), and myocardial PCr/ATP (Fig. 5D) in AOB animals from week 1 to week 8. These correlations demonstrated that, during the first 8 wk following pressure overload induced by AOB, transient LV failure (Fig. 2C) was compensated as LV hypertrophied to restore the LV wall stress, the chamber function, and the myocardial energetics (Fig. 5, B–D).
Pressure overload-induced LVH was associated with increases in cardiomyocyte size and declines in vascular density. To determine whether the concentric LVH is accompanied by the increases in the size of individual cardiomyocytes, histological analyses were performed in sections of heart tissue from AOB animals that were killed at week 8 and compared with equivalent assessments in body weight-matched animals from the Normal group (Fig. 6A). Cardiomyocytes were significantly larger (Fig. 6B), while capillary density (i.e., the number of vessels per millimeter squared that expressed the endothelial marker CD31) (Fig. 6C) and arteriole density (i.e., the number of vessels per millimeter squared that coexpressed CD31 and SMA) (Fig. 6D) were significantly lower, in sections from AOB animals than in sections from the Normal group. However, the declines in vessel densities did not appear to occur through degeneration of the vascular network, because the number of vessels per cardiomyocyte, as well as the rate of myocardial blood flow under both baseline and high cardiac workloads (Table 3), were similar in both groups.

DISCUSSION

LVH is known to be accompanied by a decline in bioenergetic efficiency, as indexed by the PCr/ATP, and the severity of the decline is linearly related to the extent of hypertrophy, but whether the bioenergetic deficiencies include a change in the myocardial rate of ATP hydrolysis is unknown. For the experiments described in this report, we used a double-saturation 31P-MRS-MST protocol to determine, for the first time, that the rate constant of ATP hydrolysis rate increases linearly related to cardiac workload increases (Fig. 5A), and the maximal rate constant of ATP hydrolysis rate achieved is significantly lower in failing heart (Figs. 4C and 2C, AOB-week 1).

Severe pressure overload was induced by constricting the aorta with a plastic band. The hearts experienced an acute phase of LV failure, as indicated by evidence of cyanosis, ascites, and pericardial effusion, significant declines in cardiac functional parameters (LV ejection and thickening fractions), and significant increases in LV volume and wall stress. The acute phase of heart failure was also associated with significant declines in myocardial PCr/ATP and in the rate of CK-mediated ATP production. The rate constant of myocardial ATP hydrolysis did not change significantly. The slope of the relationship between the ATP hydrolysis rate constant and RPP did not change significantly. The slope of the relationship between the ATP hydrolysis rate constant and RPP was the same as the regression slope observed in the hearts of Normal animals. ATP hydrolysis rates change during the development of compensated LVH. The physiological changes that occur in response to myocardial pressure overload are well understood. The initial increase in wall stress leads to acute LV dilatation and functional decline, and these abnormalities are remedied during the compensatory phase, as the heart adapts to the increased pressure by becoming hypertrophic. This pattern (i.e., acute decline and subsequent recovery of LV function) can be observed in the animals studied here: wall stresses were significantly higher, and ejection fractions significantly lower in AOB animals than in Normal animals at week 1 after AOB.
but not at week 8. The rate constant \( k_{\text{ATP} \rightarrow \text{Pi}} \) under high cardiac workload was significantly lower in hearts of AOB at week 1. Our measurements also confirm that \( k_{\text{ATP} \rightarrow \text{Pi}} \) increases under high cardiac workload and is linearly related to RPP, while measurements of \( k_{\text{PCr} \rightarrow \text{ATP}} \) under baseline and high cardiac workloads were similar. These observations are expected, because the increased demand for energy during the high-cardiac work state necessitates an increase in ATP hydrolysis, while the corresponding increase in CK-mediated ATP production is undetectable, because the flux of the PCr \( \rightarrow \text{ATP} \) reaction is much greater than the flux of ATP hydrolysis \( (23) \); however, this is the first time that these expectations have been verified experimentally by measuring \( k_{\text{ATP} \rightarrow \text{Pi}} \) in the hearts of living animals. Our results also indicated that the slope of the relationship between \( k_{\text{ATP} \rightarrow \text{Pi}} \) and RPP was similar between normal hearts and hearts with compensated concentric LVH (Fig. 5A), which suggests that the link between energy consumption and the mechanical efficiency of myocardial contraction (e.g., force generation and/or the cross-bridge holding economy) is undisrupted in LVH hearts.

In vivo measurements of myocardial bioenergetics via double-saturation \( ^{31} \text{P-MRS-MST} \). Conventional MRS-MST methods have been used to monitor the cardiac ATP hydrolysis rate in dog hearts with ischemia-reperfusion injury \( (13) \), when the amount of myocardial \( \text{Pi} \) increases to measurable levels, but measurements in healthy hearts or in hearts with regional ischemia have been rare and generally unsuccessful \( (1, 3, 15) \). Nevertheless, the rate reported here for animals in the Normal group under baseline conditions \( (k_{\text{ATP} \rightarrow \text{Pi}} = 0.16 \pm 0.03 \text{ s}^{-1}) \) is consistent with observations in another mammalian study by Portman \( (12) \). Using conventional methods, the author determined that the rate constant for the \( \text{Pi} \rightarrow \text{ATP} \) reaction in lamb hearts was 0.49 \( \pm 0.09 \text{ s}^{-1} \), and that the \( \text{Pi}/\text{ATP} \) was 0.4, which suggests that \( k_{\text{ATP} \rightarrow \text{Pi}} \) was \( -0.2 \text{ s}^{-1} \), although the author acknowledged that the study was limited by a lengthy scan time and by a relatively poor signal-to-noise ratio (S/N) for \( \text{Pi} \). The measurements of \( k_{\text{ATP} \rightarrow \text{Pi}} \) and \( \text{Flux}_{\text{ATP} \rightarrow \text{Pi}} \), that are obtained via MRS-MST incorporate the sum of all cellular activities that consume ATP; thus the technique cannot be used to measure the specific contribution of an individual enzyme, enzyme
system (e.g., the glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase enzyme pair), or enzyme pathway activity.

Because the scan time and S/N of an MR spectrum are dependent on T1/M02, the T1 for Pi is longer than the T1 for ATP or PCr, and myocardial free Pi is low; therefore, the myocardial free Pi is difficult to measure by NMR. Thus, by eliminating the need to measure Pi levels, our 31P-MRS-MST method made it possible to measure kATP→Pi, while simultaneously improving the S/N. In the studies reported here, the total scan time for our initial assessment of each heart was 30 min, and most of that time was required for the measurement of T1. Subsequent measurements in the same heart (e.g., under high-workload conditions) could be performed in just 8 min, because the T1 values were already known. Furthermore, the advantages associated with this improvement in scan time will likely be useful for investigations of many physiological systems, because even when Pi can be quantified, as in the brain and skeletal muscle, it is much less abundant, and has a much longer T1, than PCr or ATP (3). We have also recently developed a T1 nominal method that is readily compatible with the 31P-MRS-MST approach described here and may enable the scan time to be reduced by yet another order of magnitude, because the ATP hydrolysis rate can be quantified under partially relaxed conditions (18).

In conclusion, this report is the first to confirm, experimentally, that the rate of ATP hydrolysis increases during high cardiac work states in both normal and LVH hearts, whereas the rate of CK-mediated ATP production remains constant, despite a more than twofold increase in RPP. We also show that the slope of the linear relationship between the rate of ATP hydrolysis and RPP are similar between normal and LVH hearts, the rate constant of ATP hydrolysis rate increases linearly related to cardiac workload increases, and the maximal date constant of ATP hydrolysis rate achieved is significantly lower in failing hearts.

**Limitations.** It is noted that the RPPs in Table 2 are measured during the open chest. In response to catecholamine stimulation, the LVSP increased significantly more in AOB animals (Table 2), indicating a significant aortic stenosis that these animals are exposed to during the awake conditions. The 31P-MR spectroscopic assessments were performed under an open-chest preparation in the present study due to instrumentation limitations. Further work is warranted to implement the noninvasive version of MRS-MST protocol. However, because of the high field magnet, we can only use fluid-filled catheter for LV pressure measurements. The dP/dt signal was too noisy from the fluid-filled catheter preparation under the general anesthesia, which significantly suppress the cardiac systolic function of the diseased heart. This cardiac suppressive effect

Table 3. Myocardial perfusion measurements

<table>
<thead>
<tr>
<th>MBF by Microspheres, Baseline</th>
<th>MBF by Perfusion MRI Baseline</th>
<th>HWL</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Normal</td>
<td>0.99 ± 0.11</td>
<td>1.19 ± 0.17</td>
</tr>
<tr>
<td>AOB, week 8</td>
<td>1.06 ± 0.16</td>
<td>1.25 ± 0.16</td>
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</tbody>
</table>

Values are means ± SD in ml·g⁻¹·min⁻¹; n, no., of animals. MBF: myocardial blood flow. Baseline, animals were anesthetized with 2% inhaled isoflurane. HWL condition was achieved with intravenous administration of dopamine/dobutamine (10 μg·kg⁻¹·min⁻¹). *P < 0.05 vs. baseline.
of general anesthesia can result in a reduced LVSP, particularly at week 1 after AOB.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Q.X. and J.Z. conception and design of research; Q.X., P.Z., J.G., C.S., and A.J. performed experiments; Q.X. analyzed data; P.Z., J.Z., P.Z., interpreted results of experiments; Q.X. prepared figures; Q.X. and J.Z. drafted manuscript; Q.X. and J.Z. edited and revised manuscript; Q.X. and J.Z. approved final version of manuscript.

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