Mechanoenergetic Alterations During the Transition From Cardiac Hypertrophy to Failure in Dahl Salt-Sensitive Rats

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Background—The time course and mechanisms of altered mechanoenergetics and depressed cross-bridge cycling in hypertrophied and failing myocardium are uncertain.

Methods and Results—We studied mechanoenergetics in Dahl salt-sensitive (DS) rats fed high-salt diet (HS) for 6 (HS-6) and 12 (HS-12) weeks to produce compensated hypertrophy and failure. The slope of the end-systolic pressure-volume relation (E′ max) was similar in HS-6 and low-salt controls (LS-6), but reduced in HS-12 compared with controls (LS-12). Efficiency [1/slope of oxygen consumption (V O2)–pressure-volume area (PVA) relation] was similar in HS-6 and LS-6 but higher in HS-12 versus LS-12 (59±16% versus 44±7%, P<0.05). Economy [1/slope of the force-time integral (FTI)–V O2 relation] was similar in HS-6 and LS-6 but higher in HS-12 versus LS-12 (218±123 versus 74±39×103 g·s·mL O2⁻¹·g; P<0.05). Compared with controls, myofibrillar ATPase activity was reduced by 24% in HS-6 and 44% in HS-12. V3 Isomyosin was increased in HS-6 (40±12% versus 9±8%; P<0.05) and further increased in HS-12 (76±10% versus 22±18%; P<0.05). Hypothyroid LS-12 rats had 100% V3 isomyosin, yet efficiency, economy, and ATPase values were intermediate between LS-12 and HS-12. HS-12 rats demonstrated increased troponin T isoform (17±2 versus 23±2%, P<0.05). There were no changes in troponin I or tropomyosin isoforms. However, the proportion of phosphorylated troponin T was reduced in HS-12 versus LS-12 hearts (P<.001).

Conclusions—In DS rats, the transition to failure is associated with depressed E′ max and increased efficiency and economy. These findings are linked to myofibrillar ATPase activity and suggest that mechanisms other than isomyosin switching are important determinants of ventricular energetics. A troponin T isoform switch is one potential mechanism.

Key Words: myocardium ■ hypertrophy ■ myofibrillar ATPase activity ■ myosin ■ troponin T

Depressed cross-bridge cycling is a hallmark of overloaded myocardium.1–4 The time course of this derangement, its relation to the transition to failure, manifestations in the ventricle and mechanism are poorly understood. In small mammals, myosin isoform switching from V1 to the slower V3 form accounts only in part for depressed cross-bridge cycling.2,4 Myosin isoform switching during overload has not been thought to occur in large mammals, including humans,5 who have been considered to have exclusively V3 myosin. Thus, the mechanism of depressed cross-bridge cycling in these species is especially poorly understood.2,6,7 Recently, mRNA for V1 myosin was detected in nonfailing human myocardium, with a decrease in failure.8 The latter report pointed out that technical difficulties make it difficult to separate human V1 and V3 isoforms. Thus, myosin isoform switching has reemerged as a possible mechanism of depressed cross-bridge cycling in human myocardium. A proposed mechanism of non–isomyosin mediated depressed cross-bridge cycling in failing myocardium is an alteration in thin filament regulatory proteins,9 but supporting evidence is scant.

The goals of this study were (1) to delineate the time course of depressed cross-bridge cycling in a small-animal model of cardiac hypertrophy and the transition to failure, (2) to test the hypothesis that, in the ventricle, depressed cross-bridge cycling is manifest as alterations in parameters proposed to reflect the mechanoenergetic behavior of the contractile machinery, and (3) to test preliminarily the hypothesis that thin-filament isoform shifts or altered phosphorylation state contributes to depressed cross-bridge cycling in overloaded myocardium.

We used Dahl salt-sensitive (DS) rats,10 which develop combined pressure and volume overload when fed a high-salt diet (HS). A renin gene polymorphism11 and a mutation of the Na+–K+ ATPase gene12 have been identified in this strain. With HS, DS rats develop compensated hypertrophy and

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progress to a dilated, hypokinetic left ventricle (LV) without significant myocardial cell loss and/or extensive fibrosis. With failure, there is increased mortality, pulmonary congestion, increased plasma ANP and norepinephrine concentration, and decreased β-adrenergic responsiveness.

Methods

Animal Model

Procedures were approved by the Institutional Animal Care and Use Committee of the University of Vermont. We used 148 male DS rats (Brookhaven National Laboratory, Upton, NY). At 6 weeks of age, rats were divided into populations receiving either HS (8% NaCl) or a low-salt diet (LS, 0.3% NaCl) (Harlem Teklad). Each group was divided into subgroups that underwent isolated heart studies at either 6 or 12 weeks after starting diet. Thus, the rats were divided into 4 groups by diet and duration (in weeks): those given HS for 6 or 12 weeks (HS-6 [n=11] or HS-12 [n=16]) or LS for 6 or 12 weeks (LS-6 [n=15] or LS-12 [n=15]). Because of high mortality, 109 rats were randomly assigned to the HS-12 group. Blood pressure was measured (tail cuff) every 2 weeks after starting diet. In an additional group, 9 weeks after beginning LS, hypothyroidism was induced by adding 0.8 mg/mL propylthiouracil (PTU) to drinking water for 3 weeks (n=9), with isolated heart studies at 12 weeks. PTU treatment was included to convert from predominantly V1 to V3 myosin, allowing comparisons to HS groups with similar shifts.

Isolated Heart Preparation

Rats were anesthetized (sodium pentobarbital, 90 mg/kg IP), a tracheotomy was performed, and ventilation was initiated with a Harvard respirator. The chest was opened at the midline, pulmonary hilum and superior and inferior venae cavae were ligated, and a perfusion tube was inserted into the ascending aorta. Blood pressure was measured (tail cuff) every 2 weeks after starting diet. In an additional group, 9 weeks after beginning LS, hypothyroidism was induced by adding 0.8 mg/mL propylthiouracil (PTU) to drinking water for 3 weeks (n=9), with isolated heart studies at 12 weeks. PTU treatment was included to convert from predominantly V1 to V3 myosin, allowing comparisons to HS groups with similar shifts.

Perfusate

Perfusate composition (mmol/L) was 108.0 NaCl, 4.0 KCl, 1.4 KH₂PO₄, 25.0 NaHCO₃, 11.0 dextrose, 10.0 Na-pyruvate, and 2.5 CaCl₂ (Sigma Chemical Co). Perfusate was filtered, equilibrated with 95% O₂, 5% CO₂, warmed to 37°C, and adjusted to pH 7.4 by changing percent CO₂. Perfusion pressure was controlled by a pressurized reservoir connected to a pressure regulator and compressed air. Perfusion temperature was maintained at 35°C to 37°C with water jackets around the reservoir.

LV Balloon

Balloons were constructed by stretching a small piece of HDPE with a round-tip device, which was then mounted on a 15-gauge Luer adapter with a thin latex seal. At LV volume 0.03 to 0.05 mL, peak isovolumic pressure was always >0. Before each experiment, the pressure-volume (PV) relation of the balloon was measured to ensure that pressure was 0 at volume >0.2 mL. To test the accuracy of balloon volume, we measured residual volume between the balloon and the inner LV surface in 5 hearts fixed in 10% formalin using methods similar to those in canine and rabbit hearts. Residual volume was 7.1±1.7 μL (±SD) (8.6±1.5% of total LV volume, determined as the volume of water within the balloon plus volume of the balloon walls and connector within the LV plus residual volume) at an intraventricular pressure of 120 mm Hg, 9.2±1.9 μL (11.7±3.3%) at 40 mm Hg, 16.9±6.9 μL (19.8±4.0%) at 10 mm Hg, and 23.3±7.3 μL (27.9±5.8%) at 5 mm Hg. The effects of these errors on mechanoenergetics are discussed subsequently. This system showed nearly optimal damping (coefficient, 0.541) with a high natural frequency (undamped frequency, 222 Hz).

Experimental Protocol

Hearts were stimulated at 240 bpm (LV pacing). Three PTU hearts were paced at 180 bpm due to pulsus alternans at 240 bpm. Measurements of LV and perfusion pressure, coronary flow, and AV O₂ were made at various balloon volumes during steady-state isovolumic contractions. LV volume was varied between that at peak pressure was 0 and maximal volume (0.15 mL or diastolic pressure >25 mm Hg). Perfusion pressure was maintained constant and as close as possible to 90 mm Hg for LS and PTU hearts and 120 mm Hg for HS hearts, simulating in vivo diastolic values. Oxygen consumption (VO₂) for basal metabolism was measured after KCl arrest. Then, LV, RV, and organs were weighed as rapidly as possible, and LVs were quick-frozen and stored at −70°C until ATPase, protein, or phosphorylation analyses were performed.

Mechanoenergetic Parameters

LV and coronary perfusion pressure and AV O₂ were measured using a Spinco 12C model 80a ultracentrifuge at 120 000 × g for 1 hour. AV O₂ was calculated as oxygen uptake by the heart from non–RBC containing perfusate.

LV Systolic and Diastolic Function Analyses at Matched Stress

We compared systolic and diastolic function at matched stress. Fiber stress (σ) was calculated at end-systolic (ES) and end-diastolic (ED) assuming a thick-walled spherical chamber.

$$\sigma(r) = \sigma_0 r^2 \left(1 - \frac{r^2}{r_i^2} \right)$$

where \( r \) and \( r_i \) are internal and external radii, respectively. Radii were determined from the equation for the volume of the respective sphere of interest: \( V = \frac{4}{3} \pi r^3 \). V is LV (or wall) volume, and LVP is left ventricular pressure. LV wall volume was set equal to LV mass divided by specific gravity (1.04 mL/g).

Systolic function was quantified as LV developed pressure and maximal dP/dt at matched ED stress of 10 g/cm². Diastolic function was quantified by EDP and minimal dP/dt at matched ES stress of 100 g/cm².
End-Systolic PV Relation, \( \dot{V}O_2 \) PV Area Relation, and Efficiency

LV systolic function was also quantified as slope of the nonlinear ES PV relation (ESPVR), \( E'(\dot{V}_0) \) (in mm Hg • mL \(^{-1} \) • g \(^{-1} \)), \( P = E'(\dot{V}_0) (V-V_0)+\alpha (V-V_0) \), where \( P \) and \( V \) are LV pressure and volume, \( V_0 \) is the volume axis intercept, and \( \alpha \) is a constant.

Total energy output was quantified as PV area (PVA), below the area circumscribed by ESPVR, EDPVR, and the systolic PV trajectory, normalized per gram LV (in mm Hg • mL • beat \(^{-1} \) • g \(^{-1} \)). \( \dot{V}O_2 \) was plotted as a function of PVA as LV volume was varied and a linear regression analysis \( (\dot{V}O_2 = aPVA+b) \) performed. Slope \( a \) is \( \dot{V}O_2 \) cost of PVA, and intercept \( b \) is \( \dot{V}O_2 \) at 0 PVA (unloaded \( \dot{V}O_2 \)). Slope \( a \) is the efficiency of conversion of \( \dot{V}O_2 \) to PVA (contractile efficiency) after conversion of PVA and \( \dot{V}O_2 \) to joules.

The effects of the error in volume measurement on ESPVR (8.3% to 10.0% overestimation of \( E'(\dot{V}_0) \) and 9.1% to 14.0% underestimation of \( V_0 \), based on average ESPVR of each group) and \( \dot{V}O_2 \)-PVA (10.7% to 12.4% overestimate of slope and estimation error of \(-4.0\% \) to 0.2% of \( \dot{V}O_2 \) intercept, based on average \( \dot{V}O_2 \)-PVA relation of each group) were small and similar among the groups.

Force-Time Integral and Economy

Force-time integral (FTI) is the time integral of developed force per cycle. Based on the force equilibrium equation for a sphere, total developed force was calculated as follows:

\[
F = 1.36 \times P \times A = 1.64 \times P \times V^{0.61}
\]

where \( P \) is LV developed pressure, \( A \) is lumen cross-sectional area, and \( V \) is LV volume. \( F \) was integrated throughout 1 cardiac cycle to obtain FTI.\(^{24} \) Economy was determined as slope \( z \) of the linear \( \dot{V}O_2 \)-FTI relation (g • s \(^{-1} \) • mL, \( O_2 \) • g) and is analogous to thermoeconomic efficiency in myothermal studies.\(^{15,23} \)

Myofibrillar ATPase Activity

Maximal myofibrillar ATPase activity (pCa5) was determined by the method of White\(^{26} \) in 6 hearts of each group. Protein concentration was determined by the Lowery method.\(^{27} \)

Myosin Isomorphs

Myosin isoforms were determined with pyrophosphate gel electrophoresis\(^{15} \) using frozen tissue. Fifteen gels were stained with Coomassie Blue R-250 and destained in acetic acid. Destained gels were scanned with a PDI laser densitometer. Areas of each protein peak were determined by analysis of total peak area using Peakfit curve-fitting software (Jandel Scientific, Inc) and fitting with a gaussian shape.

Thin-Filament Regulatory Proteins

Troponin T (TnT) isoforms were determined in LV myocardium from 6 hearts of each group and 1 heart from a neonatal DS rat, as described previously.\(^{26} \) One-dimensional SDS-PAGE (30% acrylamide, 1.1% bis-acrylamide) was used for 1-dimensional SDS-PAGE was performed with 0.8% rather than 1.5% acrylamide gels stained with 0.025% Coomassie Blue. For immunoblotting, primary antibody was a C5 monoclonal specific for both cardiac and skeletal TnI (Advanced ImmunoChemical Inc) and secondary was goat anti-mouse (Promega). Tropomyosin (Tm) isoforms were assessed by scanning Coomassie stained myofibrillar gels for proteins of MW range 33 to 34 kDa. Tm appears as a discrete band(s) at this MW.\(^{32} \)

TnT Phosphorylation

We modified the methods of Anderson et al\(^{33} \) to assess TnT phosphorylation. Paired samples of myofibrils prepared as for ATPase assays from each of 6 LS-12 and 6 HS-12 hearts were dephosphorylated with alkaline phosphatase (AP)\(^{34} \) or subjected to the same protocol without AP. Two-dimensional electrophoresis of myofibrillar gels was performed, followed by Coomassie Blue staining. Anderson et al\(^{33} \) demonstrated that TnT appears as 2 spots on 2-dimensional electrophoresis. After AP, only the more basic spot remains. We reproduced this in each specimen. Accordingly, we assumed the more basic spot was nonphosphorylated and performed laser densitometry of the 2 spots in gels not subjected to AP to quantify phosphorylated versus nonphosphorylated TnT.

Statistics

Data are reported as mean±SD. The least squares method was used to estimate ESPVR and \( \dot{V}O_2 \)-PVA and \( \dot{V}O_2 \)-FTI relations. One-way ANOVA was used to detect differences in BP, body weight, organ weight, renal function, mechanoenergetics, myofibrillar ATPase, and contractile proteins. A Student-Newman-Keuls test was used for multiple comparisons. Relations between contractile efficiency or economy and ATPase were tested by linear regression analysis. Survival curves were compared by the generalized Wilcoxon test. A value of \( P<0.05 \) was taken to indicate significance.

**Effect of Dietary Salt on Blood Pressure, Organ Weight, Renal Function, and Mortality**

<table>
<thead>
<tr>
<th>SBP, mm Hg</th>
<th>BW, g</th>
<th>LV/BW, g/kg</th>
<th>Lung/BW, g/kg</th>
<th>BUN, mg/dL</th>
<th>Creatinine, mg/dL</th>
<th>Mortality, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS-6</td>
<td>14 619</td>
<td>392±36‡</td>
<td>3.45±0.44</td>
<td>6.1±1.6</td>
<td>18±1</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>HS-6</td>
<td>234±35*</td>
<td>362±35*</td>
<td>4.79±0.49*</td>
<td>6.0±1.5</td>
<td>24±6†</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>LS-12</td>
<td>150±6</td>
<td>455±22</td>
<td>3.16±0.71</td>
<td>4.8±1.5</td>
<td>18±1</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>HS-12</td>
<td>273±7*</td>
<td>365±45*</td>
<td>5.20±0.71*</td>
<td>7.8±5.1*</td>
<td>34±11*</td>
<td>1.0±0.3*</td>
</tr>
<tr>
<td>PTU</td>
<td>n/a</td>
<td>363±18*</td>
<td>3.27±0.24†</td>
<td>5.8±1.2</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; BW, body weight; LV/BW, left ventricular weight/body weight ratio; Lung BW, lung weight/body weight ratio; Creatinine, serum creatinine; and n/a, not assessed.

* \( P<0.05 \) vs age-matched control; † \( P<0.05 \) vs HS-12; ‡ \( P<0.05 \) vs LS-12.
Results

Blood Pressure, Mortality, Organ Weight, and Renal Function

The Table shows effects of dietary salt on systolic BP, body weight, organ weight, renal function, and mortality. Hypertension developed with HS. At 6 weeks, there was a marked increase in LV/body weight ratio, with only a modest additional increase at 12 weeks. Between 6 and 12 weeks, HS rats showed increased mortality, lung weight, blood urea nitrogen, and creatinine, consistent with a transition to failure. Diastolic BP averaged 83 ± 9 mm Hg in LS and 122 ± 31 mm Hg in HS rats, with no differences at 6 versus 12 weeks.

LV Mechanics

Figure 1 shows representative PV, VO₂-PVA, and VO₂-FTI relations from one LS-12 and one HS-12 rat. As expected, the ESPVR was curvilinear. VO₂-PVA and VO₂-FTI relations were highly linear (for HS-6, $r=0.92±0.08$ and $r=0.94±0.07$; LS-6, $r=0.91±0.06$ and $r=0.93±0.06$; HS-12, $r=0.81±0.19$ and $r=0.89±0.09$; LS-12, $r=0.88±0.11$ and $r=0.91±0.08$, and PTU, $r=0.90±0.08$ and $r=0.89±0.07$, respectively [mean ± SD]).

At perfusion pressure averaging 91 ± 5 mm Hg for LS and PTU and 119 ± 12 mm Hg for HS, coronary flow (mL/min · g⁻¹) was 12.9 ± 2.5 in LS-6, 13.2 ± 1.7 in LS-12, and 12.8 ± 2.7 in PTU rats. Flow was modestly decreased in HS-6 (11.5 ± 3.1) and HS-12 (10.0 ± 2.3) rats. The decrease in HS-12 was significant versus LS-12 or PTU ($P<0.05$), but the decrease for HS-6 was not compared with LS-6. Compared with LS controls, developed pressure, maximum dP/dt (at matched diastolic stress), and $E'_{max}$ were significantly decreased in the HS-12 group (Figure 2). These parameters were also reduced in the PTU group versus age-matched LS-12 controls. Maximum dP/dt was decreased in HS-6 versus LS-6 rats, but developed pressure and $E'_{max}$ were not significantly different. $E'_{max}$ was decreased in HS-12 versus HS-6 rats ($P<0.05$).
Compared with age-matched LS controls, EDP at matched ES stress was increased in HS rats at 6 (11.0 ± 6.4 versus 5.2 ± 3.5 mm Hg, P < 0.05) and 12 (12.3 ± 4.7 versus 5.0 ± 5.5 mm Hg, P < 0.05) weeks, respectively. Minimum dP/dt at matched ES stress was decreased in HS-6 (909 ± 295 versus 1327 ± 598 mm Hg/s, P < 0.05) and HS-12 (660 ± 201 versus 959 ± 399 mm Hg/s, P < 0.05) rats. Minimum dP/dt was also decreased in PTU (344 ± 74 mm Hg/s) rats versus controls (P < 0.05) and HS-12 (P < 0.05). There was a trend toward higher EDP in PTU rats (9.5 ± 7.6 mm Hg), but this was not statistically significant.

LV Energetics

Figure 3 shows values for VO₂ intercept of the VO₂-PVA relation, efficiency, and economy. The intercept was decreased in PTU versus age-matched LS-12 controls. There was a trend toward a decrease in HS-12 versus LS-12 (P = 0.11). Efficiency was significantly increased in HS-12 versus LS-12. There were no differences between LS-6 and HS-6. Efficiency was not significantly different in PTU compared with other groups, although there was a trend toward a significantly lower value versus HS-12 (P = 0.08). Economy was strikingly increased in HS-12 versus age-matched controls and the HS-6 group (P < 0.01). In the PTU group, economy was significantly increased compared with LS-12. Economy in PTU was lower than in HS-12 but this difference was not statistically significant (P = 0.21). Basal metabolism during KCl arrest was similar among the groups (HS-6 3.07 ± 0.84, LS-6 3.10 ± 1.15, HS-12 2.59 ± 0.34, LS-12 3.20 ± 1.15, PTU 2.35 ± 0.86 × 10⁻³ mL O₂ · min⁻¹ · g⁻¹). In hearts perfused with RBCs, HS-12 hearts also revealed increased efficiency (0.62 ± 0.07) and economy (132 ± 15 × 10⁻³ g · s · mL O₂⁻¹ · g⁻¹) versus LS-12 (0.44 ± 0.07 and 69 ± 28 × 10⁻³ g · s · mL O₂⁻¹ · g⁻¹, respectively).

ATPase Activity and Myosin Isozymes

Values for myofibrillar ATPase activity and isomyosins are shown in Figure 4. ATPase activity was decreased in HS-6, HS-12, and PTU groups versus age-matched controls. ATPase in HS-12 was also decreased versus the HS-6 group. There was a trend toward higher activity in PTU versus HS-12, but the difference was not statistically significant (P = 0.10). PTU resulted in conversion to 100% V3 isomyosin. The percent of V3 isomyosin (%V3) was increased in HS-6, HS-12, and PTU groups compared with age-matched controls. %V3 was also significantly increased in HS-12 and PTU versus HS-6 and in PTU versus HS-12. Across all groups, ATPase activity was significantly correlated with %V3 (r = 0.75, P < 0.001).

Thin-Filament Isoforms

Four TnT isoforms, TnT₁ through TnT₄, were detected in the neonatal DS rat (Figure 5, left). TnT₁ and TnT₄ are dominant; TnT₂ and TnT₃ are evident as faint bands. In LS and HS, 2 isoforms (TnT₁ and TnT₄) were detected, and TnT₄ was dominant. Group mean percent TnT₁ is shown in Figure 5, right. TnT isoforms were virtually identical in LS-6, LS-12, HS-6, and PTU (17% to 18% TnT₁). In HS-12, percent TnT₁ increased to 23%, significantly greater than in any other group. Across all groups, ATPase activity was inversely correlated with percent TnT₁ (r = -0.483, P = 0.003).

TnI appeared as a single band corresponding to the cardiac isoform (MW ≈ 24 kDa) in each LS-12 and HS-12 heart, with
no skeletal TnI detectable. Tm appeared as a single band in the 33 to 34 kDa range in each LS-12 and HS-12 heart.

**TnT Phosphorylation**

Examples of 2D gels are shown in Figure 6. Without AP, TnT appears as 2 spots. After AP, only the more basic spot remains. The percent phosphorylated TnT was 51 ± 10 in LS-12 and 27 ± in HS-12 hearts (P < 0.001).

**Contractile Proteins and Energetics**

Figure 7 shows the relation between mean values of percent V3 myosin and ATPase activity and efficiency (left) and economy (right). In hearts not subjected to overload, the influence of isomyosin content on these parameters was relatively modest, and can be appreciated by comparing the 2 LS and PTU groups. Increases in efficiency and economy in HS-12 were more striking and appear greater than can be accounted for by isomyosin content alone, especially considering the fact that values were lower in the PTU group despite higher %V3. Correspondingly, ATPase activity was more depressed in HS-12 than would be expected based on isomyosin content alone (Figure 8). (As noted, there were trends toward differences in efficiency, economy, and ATPase activity between HS-12 and PTU hearts, but none were significant at P < 0.05.)

As shown in Figure 7, bottom, there was an inverse correlation between ATPase activity and efficiency (r = 0.441, P = 0.002) and economy (r = 0.576, P < 0.001). In contrast to relationships between %V3 and efficiency/economy and %V3 and ATPase activity (Figure 8), when ATPase activity was related to efficiency and economy, no group appeared anomalous.

**Discussion**

As expected, DS rats developed compensated hypertrophy after 6 weeks of HS, and then underwent a transition to failure, with increased mortality and lung/body weight ratio, depressed amount and rate of LV pressure development, and reduced E’ max. Concomitant with this transition, efficiency and economy increased. These mechanoeenergetic alterations are correlated with myofibrillar ATPase activity and occur in conjunction with alterations in myosin and TnT isoforms and TnT phosphorylation.
course and nature of ventricular mechanoenergetic alterations during hypertrophy and failure are uncertain, as is the pathophysiological significance of the aforementioned isolated muscle experiments. Our studies reveal favorable mechanoenergetic alterations when decompensation occurs and are consistent with increased economy reported in isolated muscle studies.

The VO\(_2\) intercept of the VO\(_2\)-PVA relation is considered to represent VO\(_2\) used for excitation-contraction coupling (mainly sarcoplasmic reticulum Ca\(^{2+}\) reuptake) and basal metabolism. We observed a trend toward a decrease in HS-12 rats. Because basal VO\(_2\) was unchanged, this likely reflects depressed Ca\(^{2+}\) cycling, as observed in failing Dahl rats.

**Determinants of Efficiency and Economy**

Efficiency and economy are markers of altered cross-bridge cycling because they relate mechanical output of the contractile machinery to chemical energy input. Other measures of ventricular performance vary with changes in transsarcolemmal signaling and excitation-contraction coupling as well as cross-bridge cycling. PVA is appropriate to estimate thermodynamic efficiency because it represents total mechanical energy output. FTI does not have units of energy. However, during isovolumic contractions, most mechanical energy is used to produce force and both parameters should therefore change in parallel. A practical difference is that efficiency ranges between 0% and 100%, whereas economy has no upper limit. In calculating efficiency, recovery metabolism
Mechanoenergetics During Hypertrophy and Failure

(energy required to regenerate high-energy phosphates) is neglected and assumed to be equal for all energy consuming processes. This results in systemic underestimation of the thermodynamic efficiency of the contractile machinery and may make it relatively difficult to detect increases in efficiency and explain the larger increases in economy in DS rats.

Efficiency and economy are determined by factors that modify the energy requirements of the cross-bridge cycle or its mechanical output. It is useful to consider these systemically. A priori, it seems unlikely that ATPase energy requirements (1 ATP per cycle) decrease during failure. This would require a fundamental change in the thermodynamics of the enzyme. An increase in force produced by each cross-bridge would require a change in the force-producing mechanism, thought to reside in the S1 subunit of myosin (and to which the light chains are attached). In this regard, expression of atrial light chains in the right ventricle is observed in congenital heart disease. Margossian et al reported changes in the stoichiometry of regulatory to essential light chains in failing human myocardium in association with depressed ATP splitting. Although the latter result has not been confirmed, alterations in light chains offer potential explanations for our results. Arguing against this is the fact that actomyosin ATPase activity is normal in failing human myocardium. Another possibility is an increased cross-bridge duty cycle (percent time/cycle force is generated), resulting in more force producers acting in concert and more PVA/FTI per ATP split. Duty cycle is determined by the rate constants of the biochemical reactions underlying the cross-bridge cycle. An increase could occur as a result of altered cross-bridge kinetics in failing myocardium.

An alternative mechanism is myocardial remodeling resulting in a mechanical advantage for myofibrils in the hypertrophied/failing LV. This seems an unlikely explanation for mechanoenergetic alterations that are most marked during failure, when parameters of contractile performance become depressed. Another possibility is an alteration in energy metabolism. Hypertrophied or failing myocardium is deficient in energy reserves and shifts from aerobic to glycolytic metabolism. A decrease in reserves should not affect efficiency or economy because it should not alter the energy input/output ratio of the contractile machinery. However, a switch toward glycolytic metabolism would increase efficiency and economy because of increased ATP production without additional O2 requirements. While increased glycolysis may have contributed to our results, several considerations argue against this as the predominant factor. Glycolytic ATP production is modest compared with aerobic pathways. In failing myocardium, the ratio of initial heat (excitation-contraction coupling plus cross-bridge cycling energy) to recovery heat is unchanged. This ratio reflects the efficiency of conversion of O2 to ATP and should be altered if there is a quantitatively important increase in ATP production from glycolysis. Finally, the inverse relationship between ATPase activity and efficiency and economy itself suggests that depressed cross-bridge cycling is the main mechanism of our results.

Modeling studies predict that efficiency should reflect cross-bridge level events. We reported decreased efficiency in hyperthyroidism, but there has been little further testing of this concept. The present studies combined with myothermal studies cited earlier support the idea that alterations in ventricular mechanoenergetic parameters reflect underlying changes in cross-bridge kinetics.

Significance of Changes in Contractile Proteins

With overload, rats switch from V1 to mainly V3 myosin. Parallels between isomyosin distribution, ATPase activity, and myothermal economy provide evidence of causal links between structural changes in myosin and mechanical performance of the myocardium. In vitro motility studies with varying myosin isoforms confirm these findings at the molecular level. As noted previously, however, increases in myothermal economy appear out of proportion to isomyosin content. In humans, if V1 myosin is present in significant amounts, this would make the higher than predicted economy in failure more striking because of the inverse relationship between economy and V1 content. Thus, an unresolved question in heart failure is why cross-bridge cycling and mechanoenergetics are altered independent of myosin isoforms.

In this study, %V3 myosin increased progressively with compensated hypertrophy and failure and was correlated with ATPase activity. A larger increase in %V3 ascribable to overload occurred during compensated hypertrophy than failure (a portion of the latter is related to aging, as indicated by increased V3 in LS-12 versus LS-6). To better understand the influence of isomyosin content, we studied hypothyroid rats, with 100% V3 myosin. The only other known effect of thyroid status on contractile proteins is a very small change in TnI isoforms confined to the neonate. Several aspects of our results suggest that altered ventricular mechanoenergetics and ATPase activity in DS rats cannot solely be explained by isomyosin content, analogous to human heart failure. Efficiency and economy were higher and ATPase lower in HS-12 hearts than would be expected on the basis of isomyosin content (Figures 7 and 8). For each parameter, there was a trend toward a statistically significant difference between HS-12 and PTU, despite the lower %V3 in HS-12 hearts (average 75% versus 100%). Thus, 2 mechanoenergetic and biochemical parameters were internally consistent in this regard. In addition to the relation between isomyosin content and mechanoenergetics provided by LS-6, LS-12, and PTU rats, the temporal sequence of altered mechanoenergetics and isomyosins also argues for a non–isomyosin mediated effect. Thus, the %V3 in HS-6 averaged 44%, versus 9% in LS-6 rats, amounting to a 35% increment, whereas the %V3 in HS-12 rats was 75%, for an additional 30% increment during failure. Despite these comparable increments in V3 content in compensated versus decompensated hearts, increases in efficiency and economy occurred exclusively during decompensation. In contrast, ATPase activity did decrease significantly during compensated hypertrophy. This suggests that non–isomyosin mediated depression in cross-bridge cycling occurring during decompensation is associated with a more important effect on mechanoenergetics than depressed ATPase related to isomyosin switching.
To explain altered cross-bridge cycling and mechanoenergetics in failure, changes in thin-filament regulatory proteins have been proposed. As a preliminary investigation, we assessed TnT, TnI, and TnM isoforms and TnT phosphorylation. The fact that neither TnI nor TnM manifested isoform shifts during overload is consistent with previous reports. In contrast, there are reports of reexpression of a fetal TnT isoform in human failure, and we recently demonstrated a modest TnT isoform switch in hypothyroid rabbit hearts.

TnT exists as multiple, developmentally regulated isoforms. Isoform variation is correlated with developmental changes in myofibrillar Ca\(^{2+}\) sensitivity. We observed an increase in TnT, in HS rats that, in contrast to isomyosin switching, was temporally correlated with both the transition to failure and concomitant increases in efficiency and economy. The isoform pattern (percent TnT \(_1\) greater than percent TnT \(_3\)) is similar to that reported by Reiser et al in adult Wistar rats. Results in the neonatal DS rat indicate that TnT, is detectable at this stage. Thus, a TnT isoform switch may explain the component of altered mechanoenergetics and ATPase activity not accounted for by myosin content. Against this is its small magnitude. However, TnT isoform variation occurs in its highly charged, amino-terminal portion, and TnT has been considered to have a role in cooperativity. Thus, a relatively small isoform shift could possibly have a large functional effect.

Changes in phosphorylation may also contribute to depressed cross-bridge cycling in heart failure. With respect to thin-filament proteins, protein kinase A (PKA) phosphorylation of TnI is reduced in failing myocardium. However, PKA phosphorylation of TnI influences TnC-calcium affinity without changing maximal force or ATPase activity and hence is not a good explanation for our results. Protein kinase C (PKC) phosphorylates TnI and TnT, with multiple, isoform-specific phosphorylation sites on both. Phosphorylation of TnI is dominated quantitatively by PKA, but TnT is phosphorylated exclusively by PKC. Thus, TnT phosphorylation should reflect PKC activity. Depending on sites and isoform specificity, increased PKC phosphorylation of TnT can either decrease both maximal ATPase activity and its Ca\(^{2+}\) sensitivity or slightly increase Ca\(^{2+}\) sensitivity without altering maximal activity. We found decreased percent phosphorylated TnT in failing hearts. If anything, this would be expected to increase maximal ATPase activity and thus does not provide an explanation for depressed cross-bridge cycling. Although we did not assess TnI, decreased PKC phosphorylation would also be expected to increase ATPase activity. Assuming that decreased TnT phosphorylation reflects PKC, this result was surprising in view of reports of increased PKC activity in heart failure. Once again, however, effects on contractile protein phosphorylation are highly isoform-specific, and some studies have shown differing results.

Altered thin-filament regulatory proteins are not the only potential mechanisms for non–isomyosin mediated depressed cross-bridge cycling: we have already mentioned light chain modifications. Protein C can be phosphorylated but is thought to have relatively minor effects on ATPase activity. Thus, our results support but do not prove a role for TnT isoform shifts and suggest that thin-filament regulatory protein phosphorylation does not cause depressed cross-bridge cycling.

Limitations We used crystalloid perfusate in most experiments, which is known to decrease O\(_2\)-carrying capacity and could cause ischemia. Decreased coronary perfusion pressure has been reported to decrease the slope of the Vo\(_2\)-PVA relation in the normal canine heart, finding attributed to progressively decreasing nonmechanical Vo\(_2\) caused by decreased contractility related to cardiac ischemia during increased load. To minimize ischemia, we used an RBC suspension to increase O\(_2\)-carrying capacity. Increased efficiency and economy in failing hearts were also evident in this subset of experiments.

We used a constant perfusion pressure of \(\approx90\) mm Hg in LS and PTU hearts and 120 mm Hg in HS hearts to approximate in vivo diastolic blood pressure. However, decreased coronary flow was observed in HS rats. This may be explained by endothelial dysfunction. Because rat hearts have a detectable Gregg effect, the lower coronary flow may have contributed to the mechanoenergetic alterations. However, the Gregg effect influences the Vo\(_2\) intercept but not the slope of the Vo\(_2\)-PVA relation. Thus, it is unlikely that perfusion pressure per se influenced the latter results.

Clinical Implications Since the failing heart has limited energy reserves, increased efficiency and economy should be beneficial adaptations. Thus, in failing myocardium, depressed cross-bridge cycling may be viewed as having dual effects. It contributes to abnormal contractile performance but results in reduced energy expenditure out of proportion to the decrease in performance.

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References


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