Effects of Cardiac Myosin Isoform Variation on Myofilament Function and Crossbridge Kinetics in Transgenic Rabbits

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Background—The left ventricles of both rabbits and humans express predominantly β-myosin heavy chain (MHC). Transgenic (TG) rabbits expressing 40% α-MHC are protected against tachycardia-induced cardiomyopathy, but the normal amount of α-MHC expressed in humans is only 5% to 7% and its functional importance is questionable. This study was undertaken to identify a myofilament-based mechanism underlying tachycardia-induced cardiomyopathy protection and to extrapolate the impact of MHC isoform variation on myofilament function in human hearts.

Methods and Results—Papillary muscle strips from TG rabbits expressing 40% (TG40) and 15% α-MHC (TG15) and from nontransgenic (NTG) controls expressing ≈100% β-MHC (NTG40 and NTG15) were demembranated and calcium activated. Myofilament tension and calcium sensitivity were similar in TGs and respective NTGs. Force-clamp measurements revealed ≈50% higher power production in TG40 versus NTG40 (P<0.001) and ≈20% higher power in TG15 versus NTG15 (P<0.05). A characteristic of acto-myosin crossbridge kinetics, the “dip” frequency, was significantly higher in TG40 versus NTG40 (0.70±0.04 versus 0.39±0.09 Hz, P<0.01) but not in TG15 versus NTG15. The calculated crossbridge time-on was also significantly shorter in TG40 (102.3±14.2 ms) versus NTG40 (175.7±19.7 ms) but not in TG15 versus NTG15.

Conclusions—The incorporation of 40% α-MHC leads to greater myofilament power production and more rapid crossbridge cycling, which facilitate ejection and relengthening during short cycle intervals, and thus protect against tachycardia-induced cardiomyopathy. Our results suggest, however, that, even when compared with the virtual absence of α-MHC in the failing heart, the 5% to 7% α-MHC content of the normal human heart has little if any functional significance. (Circ Heart Fail. 2009;2:334-341.)

Key Words: diastole ■ myosin isoforms ■ myofilament ■ heart failure ■ transgenic rabbits

Expression of the 2 cardiac myosin heavy chain (MHC) isoforms, α and β, is regulated developmentally and hormonally and in a species-dependent manner.1–3 Rodents express predominantly α-MHC, whereas larger adult mammals, including rabbits and humans, express predominantly β-MHC. Structurally, the 2 MHC isoforms are 93% identical,4 yet the biochemical and biomechanical properties are markedly different.5 Using the laser trap, crossbridge attachment time (ton) for α-MHC was ≈60% that of β-MHC.6 Using the in vitro motility assay, unloaded actin velocity with α-MHC was 2 to 3 times greater than with β-MHC5,8 while generating half the isometric force.5 Finally, the ATPase activity of α-MHC is 2 to 3 times greater than that of β-MHC.9,10 Despite these major differences, the influence of the 2 isoforms on crossbridge kinetics and power generation in the intact myofilament is incompletely understood.

Clinical Perspective on p 341

Myosin isoform shifts have been reported in end-stage, human heart failure. Miyata et al,11 Reiser et al,12 and Noguchi et al13 reported reduced expression of α-MHC in failing hearts, from 5% to 7% of total MHC to virtually undetectable levels. It has been suggested that these modest shifts are functionally significant.11 Moreover, Herron and McDonald14 manipulated thyroid state in rats to produce cardiac myofilaments with either 12% or undetectable levels of α-MHC, simulating differences observed in nonfailing versus failing human hearts. Using demembranated cardiomyocyte fragments, they reported higher power output in linear proportion to the fractional content of α-MHC, suggesting that small shifts in isoform content within an intact sarcomere have significant functional consequences.15–17
However, although small, the average amount of α-MHC detected in these cardiomyocyte preparations was about twice that in normal human myocardium.11–13 Moreover, manipulation of thyroid state induces various other changes in the myocardium (eg, alterations in protein phosphorylation18) besides MHC isoform shifting17 that could independently influence myofilament function. Arguing against functional significance of MHC isoform shifts in heart failure, we reported that in vitro actin velocity and force generating capacity were similar for cardiac myosin from end-stage, failing human hearts as compared with myosin from nonfailing hearts,13 but these studies were performed using isolated proteins. Thus, the question of whether MHC isoform shifting is functionally significant in failing human hearts remains unresolved.

We recently produced a transgenic (TG) rabbit that expresses increased α-MHC content in the β-MHC background, thus obviating the need for pharmacological or thyroid manipulation to modify MHC isoforms.19,20 These animals do not have detectable changes in other proteins that modulate cardiac contraction. With fractional α-MHC expression levels of ~40% and ~15%, echocardiograms in the resting state did not reveal differences in cardiac dimensions or shortening compared with nontransgenic (NTG) controls, but the presence of 40% α-MHC partially protected these animals from pacing tachycardia-induced cardiomyopathy.20

The present study was undertaken in demembranated myocardial strips to demonstrate whether an identifiable mechanism at the myofilament level could explain protection from tachycardia-induced cardiomyopathy and, more generally, to delineate the effects of MHC isoform variation on myofilament contractile function. By extrapolation, we sought to shed additional light on whether MHC isoform shifting is functionally significant in human heart failure.

Methods

Animals

All procedures were reviewed and approved by the Institutional Animal Care and Use Committees of the University of Vermont and Vermont Children’s Hospital. TG rabbits with ~40% α-MHC content (TG40) and ~15% α-MHC content (TG15) and the age- and genotype-matched NTG controls containing ~97% β-MHC (NTG40 and NTG15, respectively) were examined. Because of the limited availability of TG rabbits, there was a significant age difference between the TG40/NTG40 pair (22.1±0.5 months) and the TG15/NTG15 pair (39.3±1.5 months), but there were no age differences between the TG and genotype-matched controls. All procedures and experiments were performed in a blinded fashion. Rabbits were anesthetized with isoflurane (3% to 4% induction, 2% to 3% maintenance) and maintained on a respirator during echocardiographic measurement of left ventricular end-systolic diameter, end-diastolic diameter, interventricular septum and posterior wall thickness, and fractional shortening.

Solutions

Concentrations (mmol/L) were formulated by solving equations describing ionic equilibria.21 All reagents were purchased from Sigma (St. Louis, Mo) except when noted. Relaxing solution (pCa 8) consisted of 5 MgATP, 40 phosphocreatine, 240 μM creatine kinase, 1 free Mg2+, 0.11 CaCl2, 5 EGTA, and 20 BES buffer with pH 7.0 and 190 mg/mL ionic strength. Activating solution was the same as relaxing solution with pCa 4.5. Storage solution was the same as relaxing solution with 10 μg/mL leupetin and 50% (wt/vol) glycerol added. Skinning solution was the same as storage solution with 1% (vol/vol) Triton X-100.

Muscle Strip Preparations

Muscle strip preparation has been described elsewhere.22 Briefly, papillary muscle strips were demembranated in skinnning solution for 2 hours at room temperature, dissected to 140 to 200 μm diameter and 600 to 800 μm length, and stored at ~20°C for fewer than 4 days. Strips were attached between a length motor and force gauge using aluminum T-clips, lowered into a 30-μL droplet of relaxing solution maintained at 17°C, stretched to a sarcomere length of 2.2 μm detected by Fourier analysis of video image (IonOptix Corp., Milton, MA), and calcium activated incrementally between pCa 8.0 and 4.5. Recorded forces were normalized to cross-sectional area to provide isometric tension (T). Recorded T minus relaxed tension (Tmin) was normalized to maximum developed tension (Tmax–Tmin) and fit to the Hill equation:

\[
(T – T_{min})/(T_{max} – T_{min}) = [Ca^{2+}]^{n}/([Ca^{2+}]_{50}^{n} + [Ca^{2+}]^{n})
\]

where [Ca^{2+}]_{50}, calcium concentration at half-activation; pCa_{50} = –log [Ca^{2+}]_{50}; and n, Hill coefficient using a nonlinear least squares algorithm (Sigma Plot 8.0, SPSS, Chicago, Ill.).

Force-Clamp Technique

The force-clamp technique was applied at maximal calcium activation. Various mechanical loads were expressed as a fraction of Tmax. Force was maintained constant by feedback control of muscle length.23 The tension-velocity (T-V) relationship was fit to a hyperbolic Hill equation normalized to Tmax:

\[
(T’ + a’)(V + b) = (1 + a’)/b
\]

where T’=T/T_{max}, a’=a/T_{max}, and a and b are the parameters of the non-normalized hyperbolic Hill equation using a nonlinear least squares algorithm (Sigma Plot 8.0). The physiological characteristics maximum unloaded shortening velocity (V_{max}), velocity at maximum power (V_{opt}), tension at maximum power (T’_{opt}), and maximum power production (P_{max}) were calculated from a’ and b, as follows:24

\[
V_{max} = b/a’ (ML/s),
\]

\[
V_{opt} = b(1 + a’/2)^{1/2} - a’ (ML/s),
\]

\[
T’_{opt} = (a’/2 + a’)^{1/2} - a’ (fraction of T_{max}),
\]

\[
P_{max} = (1 – T’_{opt})T’_{opt}(a’ + T’_{opt})(ML/s \times fraction of T_{max}).
\]

Length Perturbation Analysis

Length perturbations of 0.125% strip length were applied at discrete frequencies over the range 0.1 to 250 Hz using a microcomputer and custom-made software (Igor-Pro 5.0, WaveMetrics, Lake Oswego, Ore.)21,22,23,26. Length and force signals were digitized, and the elastic and viscous moduli were calculated as the magnitudes of the in-phase and out-of-phase components of the tension response at each frequency divided by the magnitude of the normalized length perturbation. The complex modulus was defined as the elastic and viscous moduli taken as its real and imaginary parts and fitted to the following empirically determined equation using custom software (IDL 5.5, ITT, Boulder, Colo.)27,28:

\[
Y_{iso} = A_{iso}(ω) = B_{iso}(2 \pi \bar{g} + iω + C_{iso}(2 \pi + iω),
\]

where Y_{iso} is a complex modulus, and ω=2πx frequency of perturbation. The mean crossbridge attachment time, t_{iso}, was calculated as 1/(2πω), as demonstrated by Palmer et al.29

Statistical Analysis

All data are presented as mean±SEM. Results from at least 2 muscle strips from each rabbit heart were averaged together to provide a single value for each heart used in statistical comparisons. Because of the difference in age between TG40/NTG40 and TG15/NTG15,
Table 1. Rabbit Heart Characteristics Measured by Echocardiography

<table>
<thead>
<tr>
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<th>TG40</th>
<th>NTG40</th>
<th>TG15</th>
<th>NTG15</th>
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</thead>
<tbody>
<tr>
<td>No. of rabbits</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>LVES, mm</td>
<td>73±15</td>
<td>81±4</td>
<td>91±4</td>
<td>95±4</td>
</tr>
<tr>
<td>LVED, mm</td>
<td>119±24</td>
<td>132±7</td>
<td>136±4</td>
<td>140±7</td>
</tr>
<tr>
<td>FS, %</td>
<td>37.7±7.4</td>
<td>38.8±2.1</td>
<td>32.7±0.8</td>
<td>32.2±0.8</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>32.2±0.6</td>
<td>28.4±3.3</td>
<td>34.9±1.1</td>
<td>31.7±0.8</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>34.0±2.1</td>
<td>25.1±2.6</td>
<td>29.9±0.7</td>
<td>33.0±3.0</td>
</tr>
</tbody>
</table>

LVES indicates left ventricular end-systolic diameter; LVED, left ventricular end-diastolic diameter; FS, fractional shortening; IVS, interventricular septal thickness; LVPW, posterior wall thickness.

Significantly different from respective NTG (P<0.05).

only comparisons between TG and NTG groups (ie, TG40 versus NTG40 and TG15 versus NTG15) were made. The unpaired Student t test was used to compare continuous variables. For force-clamp and sinusoidal analysis studies, a repeated-measures analysis of variance was conducted: fraction of Tmax and frequency of perturbation, respectively, were used as the repeated trial factor. A significant group main effect was followed by a comparison of the TG versus NTG groupings at each trial factor using a Fisher least significant difference approach to protect against inflated type I errors. Statistical tests were performed using SPSS 14.0 (Chicago, Ill) and were considered significant at the 0.05, 0.01, and 0.001 levels.

Results

Characteristics of Rabbit Hearts

Functional evaluation by echocardiography did not reveal any significant differences in ventricular function, fractional shortening, or wall thickness between TG and respective NTG groups (Table 1) as reported previously. Age and left and right ventricular masses (Table 2) were similar between the TG and NTG pairs. Previously, it was shown that there are no significant differences in transcript levels of atrial natriuretic factor, phospholamban, and SERCA2a in these TG rabbits, and histologic investigation did not reveal any differences in cell size, fibrosis, or cardiomyocyte orientation. The fractional content of α-MHC in the papillary muscles of the TG40 (40.7±4.9%), TG15 (12.5±0.5%), and NTG (2.5±0.9%) hearts was not different from that in the free wall of the same TG40 (39.8±4.4%), TG15 (12.5±3.5%), and NTG (4.3±1.5%) hearts.

Table 2. Characteristics of Rabbit Hearts Used in Myofilament Experiments

<table>
<thead>
<tr>
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<th>TG40</th>
<th>NTG40</th>
<th>TG15</th>
<th>NTG15</th>
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<tbody>
<tr>
<td>No. of rabbits</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Age, m</td>
<td>21.9±0.2</td>
<td>22.4±1.0</td>
<td>40.5±1.5</td>
<td>37.8±4.2</td>
</tr>
<tr>
<td>Female/male</td>
<td>3/1</td>
<td>2/2</td>
<td>4/1</td>
<td>3/1</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>4.3±0.5</td>
<td>4.9±0.4</td>
<td>3.8±0.2*</td>
<td>4.8±0.3</td>
</tr>
<tr>
<td>LV mass, g</td>
<td>8.3±1.2</td>
<td>7.7±0.9</td>
<td>4.9±0.3</td>
<td>5.2±0.3</td>
</tr>
<tr>
<td>RV mass, g</td>
<td>2.3±0.3</td>
<td>2.8±0.5</td>
<td>1.2±0.2</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>LV/BODY mass, 10^-3</td>
<td>2.0±0.2</td>
<td>1.6±0.1</td>
<td>1.3±0.1</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>RV/BODY mass, 10^-3</td>
<td>0.5±0.05</td>
<td>0.6±0.06</td>
<td>0.3±0.04</td>
<td>0.3±0.03</td>
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</table>

*Significantly different from respective NTG (P<0.05).

Tension-pCa and Tension-Velocity Relationships

The normalized tension-pCa relationships for each TG/NTG pair were very similar, as shown in Figure 1. There were no statistically significant differences in Tmin, Tdev, pCa50, or n (Hill coefficient of cooperativity) between the TG and respective NTG groups (Table 3). Results from the force-clamp experiments are shown in Table 3, and tension-velocity and tension-power relationships are shown in Figure 2. Comparisons of force-clamp measurements between TG40 and NTG40 revealed a significant group main effect (P<0.001) and a significant group×fraction of Tmax interaction (P<0.001), indicating an upward shift (greater velocity and power production) in TG40 compared with NTG40 and a change in the shape of the tension-velocity and tension-power relationships (Figure 2A and 2C). Maximum power production, Pmax, and the fraction of tension at Pmax, T’opt, were significantly higher in the TG40 group compared with the NTG40 group (Table 3). Further post hoc analyses at each fraction of Tmax delineated significant differences in velocity and power between the TG40 and NTG40 groups at fractions of Tmax between 0.3 and 0.7 (Figure 2C). However, no significant differences in the Vmax and Vopt were observed between TG40 and NTG40 (Table 3).

Repeated-measures comparisons of force-clamp measurements between TG15 and NTG15 revealed a significant group main effect (P<0.05) for velocity and power, indicating an overall increase in velocity and power over the entire tension-velocity and tension-power relationships for TG15 compared with NTG15 (Figure 2B and 2D). This increase in velocity and power, however, was subtle, and post hoc analyses did not reveal significant differences between TG15 and NTG15 at any specific fraction of Tmax. There were also no significant differences in the Vmax, Vopt, T’opt, or Pmax between the TG15 and NTG15 groups (Table 3).

Complex Moduli and Crossbridge Kinetics

Figure 3 illustrates the elastic and viscous moduli recorded at maximum calcium activation pCa 4.5. Using repeated-measures analysis, elastic modulus demonstrated a significant group×frequency interaction for both TG40/NTG40 (P<0.01; Figure 3A) and TG15/NTG15 comparisons (P<0.05; Figure 3C). Post hoc analysis showed that elastic modulus was lower in TG40 compared with NTG40 over the frequency range 0.9 to...
3.8 Hz (Figure 3A) and lower in TG15 compared with NTG15 over 1.8 to 2.8 Hz (Figure 3C). The viscous modulus likewise demonstrated group×frequency interaction for the TG40/NTG40 comparison (P<0.01, Figure 3B), particularly over 0.3 to 1.2 Hz (Figure 3B), but not TG15/NTG15 (Figure 3D). The frequency at which the magnitude of the complex modulus is a minimum, or “dip” frequency, characterizes acto-myosin crossbridge kinetics and was significantly higher in TG40 as compared with NTG40 (0.70±0.04 versus 0.67±0.10 Hz, P<0.01). In contrast, there was no significant difference between TG15 and NTG15 in dip frequency (0.71±0.09 versus 0.67±0.10 Hz, P=NS). The above results for model-independent indices of myofilament performance demonstrate that α-MHC incorporated in the TG40 myocardium significantly enhances crossbridge kinetics.

Table 4 presents model-dependent parameters obtained from fitting the measured elastic and viscous moduli at pCa 4.5 to Equation 3. Parameters A and k, which respectively describe the magnitude and the relative viscosity of the passive viscoelastic response of the muscle, were not different between TG and NTG groups. Parameters B and C, which respectively describe the magnitudes of the work-generating and work-absorbing responses of the acto-myosin crossbridges were also not different between TG and NTG groups. Parameters b and c, which respectively represent the characteristic frequencies of mechanical work-generating and work-absorbing responses were significantly higher in TG40 as compared with NTG40 (Table 4). The mean time period during which myosin crossbridges remain attached to actin, t_on, was significantly shorter in TG40 compared with NTG40. These kinetic parameters b, c, and t_on were not significantly different between TG15 and NTG15.
Table 4. Model Parameters of Equation 3 Describing Myofilament Mechanical Properties and Crossbridge Kinetics Measured by Sinusoidal Length Perturbation Analysis

<table>
<thead>
<tr>
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<th>TG40</th>
<th>NTG40</th>
<th>TG15</th>
<th>NTG15</th>
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<tbody>
<tr>
<td>A, mN·mm⁻²</td>
<td>267±28</td>
<td>289±32</td>
<td>235±19</td>
<td>259±12</td>
</tr>
<tr>
<td>B, mN·mm⁻²</td>
<td>2994±806</td>
<td>4769±696</td>
<td>846±98</td>
<td>792±236</td>
</tr>
<tr>
<td>C, Hz</td>
<td>1.33±0.12*</td>
<td>0.84±0.09</td>
<td>1.03±0.10</td>
<td>0.78±0.11</td>
</tr>
<tr>
<td>tᵦ, ms</td>
<td>102.3±14.2*</td>
<td>175.7±19.7</td>
<td>97.7±12.9</td>
<td>105.0±7.7</td>
</tr>
</tbody>
</table>

Values reported as mean±SEM. A indicates magnitude of viscoelastic stiffness of passive elements; k, relative degree of viscous (k→1) vs elastic (k→0) quality of passive elements; B, magnitude of work-generating process of crossbridges; C, characteristic frequency of work generation; c, magnitude of work-absorbing process of crossbridges; c, characteristic frequency of work absorption; tᵦ, mean crossbridge attachment time during a crossbridge cycle.

*Significantly different from respective NTG (P<0.01).

Figure 4 illustrates the elastic and viscous moduli recorded under relaxed conditions (pCa 6.5). The elastic modulus was not different between either TG40/NTG40 or TG15/NTG15 pairs (Figure 4A and 4C). However, the viscous modulus was significantly lower in TG40 as compared with NTG40 over the frequency range of 0.3 to 0.7 Hz (Figure 4B) and lower in the TG15 as compared with NTG15 over the frequency range of 0.25 to 0.4 Hz (Figure 4D). These data suggest that even at very low calcium activation conditions, as would be the case during late diastole, crossbridges form, and myosin isoform myofilaments are relengthened. However, those crossbridges formed at peak shortening and still attached later in diastole would impart a resistance to relengthening as indicated most obviously by the negative stress that arises for the NTG40 at time ≈120 ms in Figure 5A.

Figure 5B displays power, ie, the rate of mechanical energy transfer to the myofilaments, over a cardiac cycle. Power is initially positive during shortening, indicating that mechanical energy is transferred to the myofilaments and then mostly negative during relengthening, indicating recovery of mechanical energy from the myofilaments and facilitating relaxation. As indicated in both Figure 5A and 5B, myofilaments of the TG40 resist shortening less and recover more mechanical energy during relengthening than those of the NTG40. Furthermore, a positive power arises during diastole only in the NTG40 (eg, time ≈120 ms in Figure 5B), which indicates a loss of energy during this late portion of the cardiac cycle. This loss of energy is caused by the viscous drag associated with those crossbridges formed during peak shortening and still attached during diastole. Figure 5C depicts a quantitative prediction of the total mechanical energy lost during a single 158-ms cardiac cycle. The energy loss in the TG40 is about half that of NTG40 at all pCa values.

Discussion

To the best of our knowledge, this is the first study to investigate the mechanical and kinetic properties of intact myofilaments in a TG rabbit. A previous study of TG mouse cardiomyocytes found that the contraction-relaxation function was not affected by an elevation in β-MHC content; however, the effects of MHC isoform profile on myofilament function cannot be easily inferred from the intact cardiomyocyte. Other studies performed in rodents assessed the effects of MHC isoform variation on myofilament function after modification of thyroid status to convert the predominantly α-MHC rodent heart to variable amounts of β-MHC. Extrapolation of these results to large mammalian hearts is problematic because altering thyroid status modifies other aspects of myofilament and nonmyofilament function.

The TG rabbit model has the advantage of allowing examination of a relatively physiological condition, including...
normally expressed β-MHC with less possibility of non-MHC-mediated alterations in myofilament function.

The primary aims of this study were to identify the mechanism at the myofilament level that explains the protection against tachycardia-induced cardiomyopathy in the TG rabbits expressing ~40% α-MHC as compared with NTG rabbits and to delineate the impact of variation in the 2 cardiac MHC isoforms on myocardial performance. In conjunction with the latter aim, we also studied TG rabbits with ~15% α-MHC in the papillary muscles to provide data points between the extremes of 40% α-MHC and ~2.5% α-MHC in NTGs and thus allow inferences in regard to the functional significance of the small MHC isoform shifts that occur in failing human hearts.

Force-clamp measurements were used to assess the myofilament contribution to systolic function and revealed that myofilament power production was significantly enhanced on the order of 50% (Figure 2) in the TG40 versus the NTG40. This amounts to power enhancement of ~1.33× per % α-MHC content. There was no difference in maximal isometric tension between TG40 and NTG40 (Table 3); therefore, the enhanced myofilament power production is solely the result of the enhanced velocity of loaded shortening (Figure 2A). This enhanced velocity of loaded shortening in the TG40 would assist in ejection over a shorter period of time, as would be necessary to accommodate tachycardia. We did not, however, detect a higher value for unloaded velocity, V\text{max}, in the TG40 as compared with NTG40. This negative finding is not uncommon when V\text{max} must be extrapolated from the tension-velocity relationship even when other assays, such as the slack test, demonstrate differences in unloaded shortening velocities.

There was also a significant enhancement of the tension-power relationship in TG15 as compared with NTG15, but this change was considerably smaller, ie, on the order of 20% at maximum power (Figure 2). This amounts to power enhancement of ~2× per percentage α-MHC content. The combined results for power production in the TG40 and TG15 are reasonably consistent with previous studies that showed a linear relationship between α-MHC content and power output. If we assume a linear relationship between α-MHC and power output, our results would predict a percent power enhancement of ~1.33 to ~2× per percentage α-MHC content. The incorporation of 5% to 7% α-MHC in the normal human left ventricle would thus result in 7% to 14% more power as compared with no α-MHC in failing hearts.

It is unlikely that such a small difference in power production is physiologically meaningful.

The C-process of our sinusoidal length perturbation analysis demonstrated that increasing the proportion of α-MHC to 40% resulted in a significantly shorter ton, which reflects a more rapid myosin off-rate, ie, g\text{app}, of a conventional 2-state model. These results are consistent with the higher actin velocity observed for α-MHC in the myosin motility assay, which is thought to be proportional to the reciprocal of ton and the higher ATPase activity for α-MHC as compared with β-MHC. A shorter ton with the addition of α-MHC was expected; using the laser trap, ton for rabbit α-MHC was measured to be ~60% that of rabbit β-MHC. However, a shorter ton for α-MHC cannot account for the lower elastic and viscous moduli in some frequency ranges in the TG populations shown in Figures 3 and 4. The lower values for these moduli in the TG must arise from differences in the respective B-processes.

The characteristic frequency b of the B-process was significantly higher for activated myofilaments of the TG40 as compared with NTG40 (Table 4), and this result reflects the
higher range of frequencies over which α-MHC lowers the elastic and viscous moduli. The molecular mechanisms underlying the B-process are not well understood, although Kawai and colleagues have attributed the value 2πβ to a phosphate-dependent, weighted sum of the forward and reverse rates of the myosin power stroke.\(^{25,27}\) Regardless of this or any other interpretation of the B-process, the phenomenon underlying the B-process clearly lowers the elastic and viscous moduli over physiologically significant frequencies. The incorporation of a significant proportion of α-MHC furthermore protects the myofilaments from the high stresses and energy losses at higher pacing frequencies, as illustrated in Figure 5A through 5C, and would be expected to protect against tachycardia-induced cardiomyopathy.

There are limitations to our study. First, there was an age difference between TG40-NTG40 group and TG15-NTG15 group. However, age was comparable within each group (ie, TG versus NTG), and therefore it is reasonable to compare each TG group with its control NTG group. Indeed, our results suggest that age may significantly reduce myofilament performance and explain some of the apparent differences between the NTG40 and NTG15 groups. For example, we found lower measures of velocity, power production, and \(I_{\text{Vmax}}\) in the older NTG15 as compared with the younger NTG40.

Second, our TG rabbits have greater α-MHC contents (40% and 15%) than nonfailing human myocardium. However, as discussed earlier, we believe it is reasonable to use our findings to infer the effects of variations in the 2 cardiac MHCs in failing human myocardium. Third, we recognize the relatively low statistical power of this study; nevertheless, we believe that these data demonstrate the importance of MHC isoform on myofilament mechanical characteristics affecting diastolic function independent of calcium regulation.

In summary, we showed that increasing α-MHC content to \(\approx 40\%\) on a β-MHC background in the rabbit results in greater myofilament power production, more rapid rates of crossbridge cycling and lower elastic and viscous moduli at physiologically significant frequencies. In contrast, increasing α-MHC content to \(\approx 12.5\%\) does not result in detectable differences in crossbridge cycling kinetics and causes only modest increases in power production. These effects contribute toward protection against functional consequences of prolonged tachycardia in the TG40 rabbits, but the smaller α-MHC content in failing human myocardium is unlikely to have functional significance.

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Disclosures
None.

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**CLINICAL PERSPECTIVE**

Shifting of the 2 cardiac myosin heavy chain (MHC) isoforms has been recognized for many years in failing myocardium. This change consists of a larger proportion of the β-MHC isoform at the expense of the α-MHC isoform. Because β-MHC has a slower ATPase rate than α-MHC, this also results in slower crossbridge cycling kinetics and slower contraction and relaxation. MHC isoform shifting is pronounced in failing rodent myocardium. Rodents normally have predominantly α-MHC and undergo shifting to predominantly β-MHC, a change with major functional consequences. In contrast, normal human myocardium consists of 90% to 95% β-MHC. Shifting to even higher proportions of β-MHC occurs in failing myocardium, but the magnitude of the shift is necessarily much smaller than that in rodents, and its functional significance is uncertain. In the present study, we used adult transgenic rabbits who express variable amounts of α-MHC (15% and 40%) and wild-type rabbits who express minimal amounts of α-MHC to determine how α-MHC content affects crossbridge kinetics (examined by sinusoidal analysis) and myofilament power output (force-velocity relations). The 40% α-MHC rabbit has been shown to be partially protected from the deleterious effects of prolonged pacing tachycardia. Our results indicate that there is a linear relationship between crossbridge kinetics and power output and α-MHC content. The relationship predicts that the magnitude of isoform shifting observed in failing human myocardium should result in minimal functional consequences. In the 40% α-MHC rabbit, optimal power output was shifted to higher frequencies, suggesting a possible mechanism for protection from tachycardia.
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Takeki Suzuki, Bradley M. Palmer, Jeanne James, Yuan Wang, Zengyi Chen, Peter VanBuren, David W. Maughan, Jeffrey Robbins and Martin M. LeWinter

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