Power output is linearly related to MyHC content in rat skinned myocytes and isolated working hearts

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Korte, F. Steven, Todd J. Herron, Michael J. Rovetto, and Kerry S. McDonald. Power output is linearly related to MyHC content in rat skinned myocytes and isolated working hearts. Am J Physiol Heart Circ Physiol 289: H801–H812, 2005. First published March 25, 2005; doi:10.1152/ajpheart.01227.2004.—The amount of work the heart can perform during ejection is governed by the inherent contractile properties of individual myocytes. One way to alter contractile properties is to alter contractile proteins such as myosin heavy chain (MyHC), which is known to demonstrate isoform plasticity in response to disease states. The purpose of this study was to examine myocyte functionality over the complete range of MyHC expression in heart, from 100% α-MyHC to 100% β-MyHC, using euthyroid and hypothyroid rats. Peak power output in skinned cardiac myocytes decreased as a nearly linear function of β-MyHC expression during maximal ($r^2 = 0.85, n = 44$ myocyte preparations) and submaximal ($r^2 = 0.82, n = 31$ myocyte preparations) Ca$^{2+}$ activation. To determine whether single myocyte function translated to the level of the whole heart, power output was measured in working heart preparations expressing varied ratios of MyHC. Left ventricular power output of isolated working heart preparations also decreased as a linear function of increasing β-MyHC expression ($r^2 = 0.82, n = 34$ myocyte preparations). These results demonstrate that power output is highly dependent on MyHC expression in single myocytes, and this translates to the performance of working left ventricles.

The capability of the heart to pump blood is ultimately determined by the extent and velocity of myocardial shortening during the ejection phase of the cardiac cycle. One primary determinant of myocardial shortening and thus stroke volume is likely the type of myosin heavy chain (MyHC) present in myocytes. In mammalian ventricles, there are two cardiac MyHC isoforms, α- and β-MyHC, which are products of two closely related genes whose expression is tightly regulated during development (25). During prenatal development, β-MyHC is the predominant isoform in the ventricles of all mammals. However, in rodents, the expression pattern shifts from β- to α-MyHC around birth, and α-MyHC remains high in adult rodent ventricles until later stages of adult life, when β-MyHC expression increases (10). The situation differs in humans, where β-MyHC is thought to be high at birth and remain high throughout adult life. In normal adult human hearts, α-MyHC mRNA levels represent ~35% of total ventricular MyHC mRNA (27, 38), but α-MyHC isoform expression has been reported to be limited to ~10% of total MyHC protein (34, 47, 51). However, recent evidence (15) suggests that α-MyHC may be as high as 70% in human neonates and gradually decrease with age.

Several other stimuli can also shift MyHC composition in adult mammalian hearts. It appears that the most potent stimulus of MyHC shifts is thyroid hormone, which induces α-MyHC expression, whereas hypothyroidism results in significant downregulation of α-MyHC and greater β-MyHC expression (5, 35). Pathophysiological conditions such as pressure overload (14, 24, 26, 29) and diabetes (8, 12) also lead to downregulation of α-MyHC and increased β-MyHC expression at least in rodents. Interestingly, recent evidence (34) indicated downregulation of α-MyHC protein and upregulation of β-MyHC expression in failing human hearts. The loss of α-MyHC may have marked effects on myocardial power output and thus ejection due to slowed rates of rise of force (and thus slowed pressure development during systole, which reduces time for ejection; Refs. 10, 44, 50) and because myocardial shortening velocities are significantly slower when β-MyHC predominates (7, 10, 11, 16, 17, 23, 40, 52). Thus a primary objective of this study was to systematically determine how varied α-to-β-MyHC ratios affect force, shortening velocity, and power output of ventricular cardiac myocytes. For these experiments, MyHC was manipulated over the entire range from 100% α-MyHC to 100% β-MyHC by thyroid hormone-dependent expression of MyHC isoforms in rats. Functional measurements were performed using skinned cardiac myocyte preparations that yield mechanical records free of artifactual effects of extracellular viscoelastic structures and thereby allow direct study of the mechanisms regulating myofibrillar function. Definitive relationships between myofibrillar function and MyHC content were achieved by SDS-PAGE analysis of protein composition of the same myocytes from which functional measurements were made.

A second objective was to determine whether MyHC functionality at the myofibrillar level translates to whole heart function. For these experiments, left ventricular power output was assessed as a function of α-to-β-MyHC ratios in isolated working heart preparations.

MATERIALS AND METHODS

Experimental animals. All procedures involving animal use were performed according to the Animal Care and Use Committee of the University of Missouri. Male Sprague-Dawley rats (6 wk of age) were obtained from Harlan (Madison, WI), housed in groups of two or three individuals, and provided with access to food and water ad libitum. Rats were made hypothyroid either by surgical removal of the thyroid gland (thyroidectomy) or by addition of propylthiouracil (PTU; 0.8

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Fig. 1. Identification of \( \alpha \)- and \( \beta \)-myosin heavy chain (MyHC) isoforms in skinned cardiac myocytes using 12% SDS-PAGE. Skinned ventricular myocyte preparations contained \( \sim 100\% \) \( \alpha \)-MyHC from 2-mo-old rats before induction of hypothyroidism. Myocytes contained \( \sim 50\% \) \( \alpha \)-MyHC and 50% \( \beta \)-MyHC after \( \sim 14 \) days of induction of hypothyroidism and nearly 100% \( \beta \)-MyHC after 21 days of hypothyroidism.

mg/ml) to the animals’ drinking water. PTU treatment shifted cardiac MyHC isoform content from 100% \( \alpha \)-MyHC to an \( \sim 50:50 \) \( \alpha \)-to-\( \beta \)-MyHC ratio over a 2-wk period, whereas thyroidectomy caused shifts from a 50-50 \( \alpha \)-to-\( \beta \)-MyHC ratio (1 wk postsurgery) to 100% \( \beta \)-MyHC (3 wk postsurgery). Figure 1 shows a composite of MyHC images from silver-stained SDS-PAGE gels that were collected throughout the time course of the study that demonstrate the shift in MyHC content during hypothyroidism. Thyroidectomy, PTU administration, or the combination of thyroidectomy and PTU administration have been shown to alter MyHC isoform expression without altering the expression of other key myofilament proteins (11, 16, 33).

Solutions. The compositions of relaxing and activating solutions were as follows (in mmol/l, obtained from Sigma at highest possible purity): 1 free Mg\(^{2+}\), 7 EGTA, 4 Mg-ATP, 20 imidazole, and 14.5 creatine phosphate (pH 7.0); 0.1% bovine serum albumin (dialyzed against 40 –50 volumes of the preceding buffer salt solution). Myocardial preparations. Skinned cardiac myocytes were obtained by mechanical disruption of hearts from Sprague-Dawley rats as described previously (31). Briefly, rats were anesthetized by inhalation of isoflurane (20% vol/vol in olive oil), and hearts were excised and rapidly placed in ice-cold relaxing solution. The ventricles were dissected away from the atria, cut into 2–3-mm pieces, and further dissected for 5 s in a Waring blender. The resulting suspension of cells was centrifuged for 105 s at 165 \( g \), after which the supernatant was discarded. The myocytes were skinned by suspending the cell pellet for 5 min in 0.3% ultrapure Triton X-100 (Pierce Chemical) in cold relaxing solution. The skinned cells were washed twice with cold relaxing solution, suspended in 10–15 ml of relaxing solution, and kept on ice during the day of the experiment.

For whole heart experiments, hearts were quickly removed, and the aorta was cannulated and perfused with oxygenated perfusion buffer for 10 min in a Langendorff apparatus. The pulmonary vein was then cannulated, and hearts were switched to a working heart system (39). Because the hearts from hypothyroid rats could not be electrically paced at the high endogenous rates (260–360 beats/min) found in the normal perfused rat hearts at 37°C, the temperature of the perfusate was set at 32°C for all hearts. This allowed electrical pacing of all hearts at 180 beats/min. Heart rate, blood pressure, aortic flow, and coronary flow were constantly monitored at a preload of 10 cmH\(_2\)O both before and after administration of epinephrine, which was added to perfusion buffer at a final concentration of 0.1 mM. Afterload was kept constant at 80 cmH\(_2\)O throughout the experiments.

Experimental apparatus. The experimental apparatus for physiological measurements of myocyte preparations was similar to one previously described in detail (36) and modified specifically for cardiac myocyte preparations (30). Briefly, myocyte preparations were attached between a force transducer and a torque motor by gently placing the ends of the myocyte into stainless steel 25-gauge trophys. The ends of the myocyte were secured by overlaying a 0.5-mm long piece of 3.0 monofilament nylon suture (Ethicon) onto each end of the myocyte and then tying the suture into the trophys with two loops of 10-0 monofilament suture (Ethicon). The attachment procedure was performed under a stereomicroscope (approximately \( \times 100 \) magnification) using finely shaped forceps. The myocyte preparations appeared to have comparable sarcomere integrity upon visual inspection independent of MyHC content. In Fig. 2A, photomicrographs show the attached skinned cardiac myocytes in relaxing and activating solutions.

Before mechanical measurements were obtained, the experimental apparatus was mounted on the stage of an inverted microscope (model IX-70; Olympus Instruments), which rested on a pneumatic antivibration table with a cutoff frequency of \( \sim 1 \) Hz. Force measurements were made using a capacitance-gauge transducer (model 403; sensitivity, 20 mV/mg plus a \( \times 10 \) amplifier; resonant frequency, 600 Hz; Aurora Scientific; Aurora, Ontario, Canada). Length changes during mechanical measurements were introduced at one end of the preparation using a DC torque motor (model 308; Aurora Scientific) driven by voltage commands from a personal computer via a 12-bit digital-to-analog converter (AT-MIO-16E-1; National Instruments; Austin, TX). Force and length signals were digitized at 1 kHz using a 12-bit analog-to-digital converter, and each was displayed and stored on a personal computer using custom software based on LabView for Windows (National Instruments).

Images of the myocyte preparations were recorded digitally on a personal computer while relaxed and during activation using a Hamamatsu charge-couple device camera (model 2400) and video snapshot software (Fig. 2). Videomicroscopy was completed using a \( \times 40 \) objective (model UWD 40; Olympus) and \( \times 25 \) intermediate lenses. During and after each experiment, the images were reviewed to obtain sarcomere length measurements from the myocyte while it was relaxed and activated; myocyte length and width for cross-sectional area calculations also were obtained from these images. Sarcomere lengths of these preparations were set to yield passive forces near zero. The dimensions of the skinned cardiac myocyte preparations were as follows: length, \( 163 \pm 39 \) \( \mu \)m; width, \( 21 \pm 4 \) \( \mu \)m; relaxed sarcomere length, \( 2.26 \pm 0.08 \) \( \mu \)m; and \( Ca^{2+} \)-activated sarcomere length, \( 2.26 \pm 0.08 \) \( \mu \)m (values are means \( \pm \) SD; \( n = 56 \) myocyte preparations). The calculation for cross-sectional area was based on an assumption of an elliptical cross-section (56), and the myocyte mass calculation was based on the assumption of muscle density being 1.06 mg/mm\(^3\).

Force-velocity and power-load measurements. The protocol for obtaining force-velocity and power-load measurements has been described in detail (30), and all measurements were made at 13 ± 1°C. The attached myocyte was first transferred into maximal \( Ca^{2+} \)-activating solution (pCa 4.5) and allowed to obtain steady-state isometric force, after which a series of subisometric force clamps were applied to determine isotonic shortening velocities. The isotonic force was maintained using a servo system for 150–250 ms while length changes during this time were monitored. Figure 2B illustrates a series of force clamps and monitored length changes. After the force clamp, the myocyte was slackened to near zero force to estimate the relative load sustained during the isotonic shortening, after which the myocyte was reextended to its starting length. Owing to the short lengths of
myocyte preparations, the rapid slackening after the isotonic shortening did not always result in an accurate baseline force value. When this occurred, it resulted in an underestimation of peak force and thus of relative force during loaded contractions. In these cases, more accurate estimates of the relative forces during isotonic shortening were obtained by interpolating between the peak forces in isometric contractions that were performed before and after every series of loaded contractions. The myocytes were kept in maximal Ca$^{2+}$-activating solution for 2–3 min, during which 10–20 force clamps were performed without significant loss of force. If the maximal Ca$^{2+}$-activated force decreased below 80% of initial force, data from that myocyte were discarded. Force-velocity measurements also were obtained during half-maximal Ca$^{2+}$ activations following the same protocol as above.

Unloaded shortening velocity measurements. Unloaded shortening velocity ($V_0$) was measured during maximal Ca$^{2+}$ activations using the slack-test method (9, 19, 56). Once steady-state force was reached, the myocyte preparation was rapidly (<2 ms) slackened to a predetermined value between 5 and 20% of its initial length. The time between the imposition of the slack step and the onset of force redevelopment was measured from the intersection of two lines fitted by eye through the zero-force baseline and the initial phase of force redevelopment. The length of release was plotted against the duration of unloaded shortening, and $V_0$ was determined from the slope of a line fitted to the data by linear regression analysis.

Data analyses. Single myocyte length traces, force-velocity curves, and power-load curves were analyzed as previously described (30). Myocyte preparation length traces during loaded shortening were fit to a single decaying exponential equation

$$L = Ae^{-kt} + C$$  \hspace{1cm} (1)

where $L$ is cell length at time $t$, $A$ and $C$ are constants with dimensions of length, and $k$ is the rate constant of shortening ($k_{shortening}$). Velocity of shortening at any given time, $t$, was determined as the slope of the tangent to the fitted curve at that time point. In this study, velocities of shortening were calculated by extrapolation of the fitted curve to the onset of the force clamp (i.e., $t = 0$) so as to reflect the velocity
of shortening at the initial sarcomere length as previously discussed (58).

Hyperbolic force-velocity curves were fit to the relative force-velocity data using the Hill equation (18)

\[(P + a)(V + b) = (P_o + a)b\]  

where \(P\) is force during shortening at velocity \(V\), \(P_o\) is the peak isometric force, and \(a\) and \(b\) are constants with dimensions of force and velocity, respectively. Power-load curves were obtained by multiplying force by velocity at each load on the force-velocity curve. The optimum force for mechanical power output (\(F_{opt}\)) was calculated using the equation (60)

\[F_{opt} = (a^2 + aP_o)^{1/2} - a\]  

Curve fitting was performed using a customized program written in QBasic as well as commercial software (SigmaPlot). Peak normalized power output was plotted as a function of relative MyHC isoform expression, and the data were analyzed as a linear regression to determine the relationship between power output and relative MyHC isoform expression.

For whole heart experiments, power was calculated using the following equation (48):

\[P = \frac{\text{mean arterial pressure} \times \text{left atrial pressure}}{2} \times \text{cardiac output}\]

SDS-PAGE and silver staining. After the measurement of power output, the relative expression of each MyHC isoform was determined for each myocyte preparation. The myocyte was removed from the experimental apparatus using a micropipette and stored in 10% SDS sample buffer at -80°C for subsequent SDS-PAGE analysis. The gel electrophoresis procedure was similar to one previously described (16, 17). The gels were prepared with 3.5% acrylamide in the stacking gel and 12% acrylamide in the resolving gel. Myocyte proteins were separated at constant current (12 mA) for 8.5 h. The separated proteins were fixed in an acid-alcohol solution and then in glutaraldehyde. MyHC isoforms were visualized by ultrasensitive silver staining, and then gels were dried and stored between Mylar sheets (see Fig. 1). The relative expression of each MyHC isoform was determined using QuantiScan (Biosoft) software and an Epson scanner to measure the relative intensity and area of each MyHC band.

Likewise, the relative expression of each MyHC isoform was determined for each whole heart after power output measurements were obtained. Once perfusion was completed, hearts were removed from cannulas and the ventricles were isolated, weighed, freeze-clamped, and stored at -80°C. Frozen ventricular samples were used for SDS-PAGE analysis of relative MyHC isoform expression.

RESULTS

Effects of thyroidectomy and treatment on MyHC composition. Thyroidectomy and PTU treatment in rats resulted in decreased \(\alpha\)-MyHC and commensurate increased \(\beta\)-MyHC content as determined by SDS-PAGE silver staining. Rats were killed at intermediate time points from the onset of treatment to harvest cardiac myocytes expressing varied \(\alpha\)-to-\(\beta\)-MyHC ratios, from which mechanical performance was characterized. Figure 1 shows a composite of MyHC images from silver-stained SDS-PAGE gels collected throughout the time course of the study that demonstrate the shift in MyHC content during hypothyroidism.

Effects of varied ratios of MyHC composition on skinned myocyte contractile properties. There was no apparent difference in the force-generating capacity of skinned myocyte preparations that expressed MyHC ranging from 100% \(\alpha\)-MyHC to 100% \(\beta\)-MyHC. Figure 3 shows the relationship between MyHC ratio and force per cross-sectional area of skinned cardiac myocyte preparations. There was no correla-

tion between myocyte maximal Ca\(^{2+}\)-activated force development and MyHC content (\(r^2 = 0.06\)). This would be consistent with \(V_1\) and \(V_3\) molecules having equivalent unitary forces and similar cross-bridge forces averaged over time (\(F_{avg}\)) as assessed by optical tweezers (57) and in vitro motility assays (1), respectively, assuming the number of cycling cross-bridges is similar between myocytes during maximal Ca\(^{2+}\) activation.

This implies that both the unitary force and the duty cycle (i.e., the fraction of cross-bridge cycle time that the myosin is generating force) are similar between \(V_1\) and \(V_3\) myosin molecules in smaller mammals, which may not be the case in some larger mammals, e.g., rabbits (41, 59). It is important to note, however, that rodent \(\beta\)-MyHC appears to produce nearly twice as much force per cross-bridge cycle, because tension cost is half that of preparations containing \(\alpha\)-MyHC (21, 49).

We next examined the relationship between MyHC isoforms and loaded shortening/power output in rat skinned cardiac myocyte preparations. Figure 4A shows force clamps and length traces obtained during maximal Ca\(^{2+}\) activation of three preparations that contained varied MyHC ratios. The traces demonstrate that increased amounts of \(\beta\)-MyHC slowed cell shortening over a wide range of loads. Figure 4B shows force-velocity and power-load curves for five different myocyte preparations, each of which contained a different \(\alpha\)-to-\(\beta\)-MyHC ratio (Fig. 4B, inset, shows SDS-PAGE silver staining for each of these preparations). Increased amounts of \(\beta\)-MyHC resulted in a downward shift and increased curvature of the force-velocity relationships, both of which led to significantly decreased power output at all loads less than isometric (see Table 1 and Fig. 4B). The increased curvature of the force-velocity relationship yielded lower \(F_{opt}\) values (the relative force at which power is optimal) as \(\beta\)-MyHC increased. This is consistent with studies that reported greater curvature of force-velocity relationships in slow-twitch skeletal muscle (which contains \(\beta\)-MyHC) than in fast-twitch muscle (60). The power output-generating capacity of myocytes was tightly correlated with \(\beta\)-MyHC content. Figure 4C shows the relationship between \(\beta\)-MyHC and peak normalized power output. Regression analysis yielded a significant relationship (\(P < 0.0001\) with a slope of -0.0007 and \(r^2 = 0.85\) (\(n = 44\) myocyte preparations), which implicates an inverse linear relationship between \(\beta\)-MyHC and peak normalized power output over the entire range of \(\alpha\)-to-\(\beta\)-MyHC ratios during maximal Ca\(^{2+}\) activation.

Fig. 3. Relationship between skinned myocyte preparation force and \(\beta\)-MyHC content. Myocyte preparation force was independent of MyHC isoform content (\(r^2 = 0.06\) for linear regression).
Because it is unlikely that left ventricular myocytes are maximally activated by Ca\(^{2+}\) in vivo, the relationship between β-MyHC content and force-velocity/power output was also determined at half-maximal Ca\(^{2+}\) activations. Figure 5 shows force-velocity and power-output curves from myocytes at half-maximal Ca\(^{2+}\) activations expressing varied MyHC isoform ratios. Increased β-MyHC resulted in a downward shift and greater curvature of force-velocity relationships during submaximal Ca\(^{2+}\) activations. Concordantly, the power-load curves of myocytes that contained increased β-MyHC had lower power output at all loads, and F\(_{\text{opt}}\) shifted to the left toward lighter loads (Table 2). The peak normalized power output also was highly correlated with β-MyHC content during submaximal Ca\(^{2+}\) activations (Fig. 5C). This relationship also was determined to be significant (P < 0.0001) with a slope of $-0.0007$ and $r^2$ of 0.85 ($n = 44$ myocyte preparations). Overall, these results implicate MyHC isoform content as a key determinant of myocyte power output and perhaps ventricular performance.

**Effects of varied MyHC ratios on V\(_0\) of skinned myocyte preparations.** We also examined the relationship between MyHC ratios and V\(_0\). Slack-test analysis was used to quantify V\(_0\), because extrapolation of force-velocity relationships tends to underestimate maximum shortening velocity. Figure 6
The primary focus of this study was to examine the relationship between power output-generating capacity and \(\beta\)-MyHC content in rat skinned cardiac myocyte preparations. Skinned myocyte preparations were used to directly study how MyHC isoforms regulate myocyte cross-bridge-cycling kinetics during loaded shortening. We observed an inverse linear relationship between the amount of \(\beta\)-MyHC protein and the power output-generating capacity of rat skinned cardiac myocyte preparations, whereby increased amounts of \(\beta\)-MyHC progressively decreased peak power output. A similar inverse relationship was observed between \(\beta\)-MyHC and \(V_0\). These results are consistent with the idea that myosin cross-bridges work independently during both loaded and unloaded shortening. Furthermore, the inverse linear relationship between power output and MyHC composition of individual myocytes translated to working heart preparations both with and without adrenergic receptor agonist. Although other confounding factors may exist in whole heart preparations, data presented here underscore the importance of MyHC working independently to determine ventricular function.

**Effects of MyHC isoform on myocardial functional properties.** The effects of cardiac MyHC isoforms on mechanical function have been studied using a number of different myocardial preparations. Initial observations indicated that either hypothyroidism or pressure overload decreased force-development rates and slowed shortening velocities in electrically stimulated papillary muscle preparations from cats (4) and rats (54). Each of these manipulations was subsequently shown to progressively shift the myosin isoenzyme expression from \(V_1\) to \(V_3\) (20, 25), which is the isoform with the slowest ATP hydrolytic activity (1, 26, 43, 59). Several other studies have correlated cardiac MyHC protein with maximum shortening velocity. Schwartz et al. (52) reported a nearly linear decline in maximum shortening velocity with increased \(\beta\)-MyHC content in rat papillary muscle preparations. Similarly, maximum velocity of shortening has been reported to decline in a nearly linear fashion with increased amounts of \(\beta\)-MyHC in rabbit papillary muscles (40), rat skinned ventricular (multicellular) preparations (10), rat skinned ventricular myocyte preparations (55), and the data obtained in this study (see Fig. 6). Consistent with these results, the relationship between \(V_0\) and MyHC isoform expression in skinned skeletal muscle fibers from rabbit soleus muscle also was found to be nearly linear (45, 46). It is of interest, however, that the maximum shortening velocity has been shown to vary from \(\sim1.8\)-fold (7) to \(\sim4.5\)-fold (10, 40) between preparations that contained 100% \(\alpha\)-MyHC vs. 100% \(\beta\)-MyHC. The reason(s) for the quantitative variability is unknown but likely involves varied species, preparations (e.g., intact vs. skinned), and temperatures.

Linear correlations also have been reported for MyHC isoform content and many other myocardial contractile properties. For instance, the velocity of actin filament sliding was linearly related to the ratio of \(\alpha\)-to-\(\beta\)-MyHC during an in vitro motility
mixture assay (1). Additionally, the rate of force development was inversely related to MyHC isoform content (10, 44, 50). A linear relationship between MyHC and tension cost was also reported for rat right ventricular trabeculae (49). Pagani and Julian (40) also reported that loaded shortening velocity (at 50% of isometric load) decreased linearly with increased β-MyHC expression. A main finding in our study was that peak normalized power output varied as an inverse linear function of β-MyHC content in skinned cardiac myocyte preparations. This is quantitatively consistent with the decrease in power output in left ventricular strips as β-MyHC was increased either by lowered thyroid hormone levels or transgenic expression (23).

It is quite striking that an inverse linear relationship exists between β-MyHC content and so many mechanical properties regardless of the preparation (e.g., whole hearts, muscle strips, single myocytes, in vitro motility assays). Cumulatively, these results suggest that shortening velocity, force development rates, and economy (i.e., force/ATPase) are all a function of the arithmetic sum of the number of myosin molecules that contain α- and β-MyHC, which seem to act independent of one another. Regarding muscle shortening, Huxley (22) originally proposed that as myosin propels actin filaments, some cross-bridges become compressed and thereby impose a load against which force-generating cross-bridges operate. The model dictates that maximum shortening velocity occurs when the force...
produced by positively strained cross-bridges equals the opposing force provided by compressively (negatively) strained cross-bridges. Siemankowski et al. (53) measured the rate of ADP dissociation from actomyosin preparations and observed MyHC-specific differences in ADP dissociation rates. They concluded that ADP dissociation was sufficiently slow to limit maximum shortening velocity by determining the rate of myosin detachment from actin. The slower $V_0$ in response to $\beta$-MyHC most likely results from slower kinetics of cross-bridge detachment thus imposing a greater internal load that $\alpha$-MyHC most likely results from slower kinetics of cross-bridge detachment over most of the load range. This results in less accumulation of compressively strained cross-bridges as $\beta$-MyHC is able to detach before becoming compressed due to slow thin filament sliding velocity. The question arises as to whether force-generating transitions or cross-bridge detachment rates limit power output in the physiologically relevant range, i.e., near $F_{\text{opt}}$. Progressively greater $\beta$-MyHC content shifted the force-velocity curve downward such that there was slower shortening velocity at any given relative load, which implies that force-generating transitions were sufficiently slowed to attenuate the number of force-generating cross-bridges during a given velocity of filament sliding. On the other hand, if detachment were limiting over most of the load range, slower detachment with $\beta$-MyHC would result in a greater proportion of force-generating cross-bridges at a given relative velocity and yield less force-velocity curvature with $\beta$-MyHC, which was not observed. Rather, we saw an increase in force-velocity curvature with $\beta$-MyHC, which implies that power output is limited by force-generating transitions rather than cross-bridge detachment over most of the load range. This is also consistent with recent findings (19) in which added $P_i$, which speeds force generation, increased normalized power at all loads $>10\%$ isometric force, whereas $V_0$ was unaffected.

### Structural basis for functionality of cardiac MyHC isoforms

The structural basis for the functional differences between $\alpha$- and $\beta$-MyHC remain unknown. Rat $\alpha$- and $\beta$-MyHC have 93% amino acid homology (32) with the majority of amino acid differences localized to $\beta$-MyHC: 1) the base of the catalytic domain near the essential light chain; 2) the mouth of the nucleotide-binding pocket (loop 1); 3) surface loop 2, which spans the actin-binding cleft; 4) the neck region of S1; and 5) the S2 segment (1). Transgenic replacement of loops 1 and 2 from $\beta$-MyHC into $\alpha$-MyHC did not affect in vitro filament sliding or myofibrillar ATPase activity (23). Additionally, rat and pig $\beta$-MyHC have virtually identical nucleotide-binding pockets (i.e., loop 1), yet rat $\beta$-MyHC myocardial preparations exhibited much faster $V_0$ values and ADP dissociation rates (42). These results imply that differences besides those in these

### Table 2. Mechanical properties of rat skinned myocyte preparations containing varied $\beta$-MyHC levels during submaximal Ca$^{2+}$ activation

<table>
<thead>
<tr>
<th>$\beta$-MyHC, %</th>
<th>$F_{\text{opt}}$</th>
<th>$V_{\text{opt}}$, ML/s</th>
<th>$\alpha P_0$</th>
<th>Absolute, $\mu$W/mg</th>
<th>Peak Normalized, $P/P_0$, ML/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>4±4 (4)</td>
<td>0.39±0.07</td>
<td>0.24±0.05</td>
<td>0.66±0.35</td>
<td>1.60±0.98</td>
<td>0.090±0.015</td>
</tr>
<tr>
<td>17±3 (6)</td>
<td>0.32±0.08</td>
<td>0.25±0.07</td>
<td>0.40±0.35</td>
<td>0.97±0.64</td>
<td>0.077±0.018</td>
</tr>
<tr>
<td>38±4 (3)</td>
<td>0.32±0.07</td>
<td>0.18±0.05</td>
<td>0.44±0.47</td>
<td>0.86±0.36</td>
<td>0.056±0.012</td>
</tr>
<tr>
<td>54±5 (4)</td>
<td>0.24±0.04</td>
<td>0.16±0.04</td>
<td>0.11±0.06</td>
<td>0.53±0.19</td>
<td>0.039±0.015</td>
</tr>
<tr>
<td>76±5 (4)</td>
<td>0.24±0.06</td>
<td>0.13±0.03</td>
<td>0.13±0.10</td>
<td>0.31±0.05</td>
<td>0.030±0.005</td>
</tr>
<tr>
<td>88±2 (4)</td>
<td>0.20±0.11</td>
<td>0.16±0.04</td>
<td>0.07±0.01</td>
<td>0.28±0.11</td>
<td>0.031±0.007</td>
</tr>
<tr>
<td>100 (6)</td>
<td>0.22±0.07</td>
<td>0.09±0.05</td>
<td>0.10±0.08</td>
<td>0.26±0.18</td>
<td>0.020±0.005</td>
</tr>
</tbody>
</table>

Values are means ± SD; $n$, no. of myocyte preparations shown in parentheses.
regions of the nucleotide-binding loop and actin-binding domain account for diversity in mechanical function. Alpert et al. (1) have narrowed the functional differences (at least for actin sliding in vitro) between small and large mammal γ-MyHC to only two nonconservative amino acid differences (i.e., 424 and 573). Residue 424 is in the upper 50-kDa segment of myosin; it is part of the long α-helix that borders the cleft separating the upper and lower 50-kDa domains. Residue 573 is localized to a surface loop that may interact with actin monomers. Thus these two amino acid substitutions are likely candidates for the functional difference between V1 and V3 myosin across species. However, definitive assessment of the role that these and other nonconservative residues play in conferring functional differences between α- and β-MyHC (including power output) awaits studies using chimeric myosins.

Role of MyHC in left ventricular function. The switch from α- to β-MyHC by hypothyroidism also was correlated with a decrease in left ventricular power output using a working heart model ($r^2 = 0.82$) thus implicating MyHC-dependent myocyte power output to ventricular function. Power output decreased ~65% in left ventricles that ranged from ~100% α-MyHC to ~100% β-MyHC, which was quantitatively very similar to the decrease in power in myocyte preparations. A similar decrease (37 vs. our 47%) was observed in stroke volume in isolated rat hearts that contained ~0 vs. ~65% β-MyHC (3). The high expression of β-MyHC in those rats arose from a natural

![Image](image_url)
heritable variation in MyHC expression as opposed to variations that occur secondary to environmental alterations such as thyroidectomy, exercise conditioning, pressure overload, and diabetes. Additionally, similar changes have been observed in working mouse heart preparations in response to hyper- and hypothyroidism whereby contractility, as assessed by the first derivative of pressure development ($\frac{dP}{dt_{\text{max}}}$), decreased by 60% in hypothyroid mice (13). A smaller response was recently reported (23) in transgenic mice that expressed $\sim 70\%$ $\beta$-MyHC. There was a 12% decrease in $\frac{dP}{dt_{\text{max}}}$ in working hearts from transgenic mice (i.e., hearts expressing $\sim 70\%$ $\beta$-MyHC) compared with our nearly 50% decrease in power in hearts expressing $\sim 70\%$ $\beta$-MyHC. The reasons for this quantitative difference include different species, different parameters (i.e., $\frac{dP}{dt_{\text{max}}}$ vs. power), and the fact that altered thyroid hormone levels likely affect the expression of many genes in heart. Thyroid hormone alters the transcription of the sarcoplasmic reticulum Ca$^{2+}$-ATPase and phospholamban genes (2, 37). Alterations of these genes with hypothyroidism may modify intracellular Ca$^{2+}$ concentration (28), which is known to modulate myocyte force-development rates (61). Although hypothyroidism also has been reported to downregulate $\beta$-adrenergic receptors (6), we observed a similar increase in power after $\beta$-adrenergic receptor stimulation in hearts from...
hypothyroid rats that expressed primarily β-MyHC. Grupp et al. (13) also found that isoproterenol elevated contractility similarly in working hearts from both hyper- and hypothyroid mice (13). These combined results suggest that β-adrenergic receptor number remains sufficient or β-adrenergic responsiveness is slightly enhanced after hypothyroidism.

Overall, there appears to be strong evidence for a causal link between cardiac MyHC isoform and mechanical performance at the molecular, cellular, and whole heart levels. Elevations in α-MyHC ratios likely increase contractility; this is known to occur in larger mammals that typically express β-MyHC [see Morkin et al. (35) for review] by speeding the rate of myocyte force generation and thus pressure development during phase 2 of the cardiac cycle. This would allow more time for ejection during systole. Additionally, higher power-generating capacity would elicit greater shortening during phase 3, which would effectively increase stroke volume. These ideas are consistent with the findings that small increases in α-MyHC content against a predominant β-MyHC background speed force development (10, 44, 50) and loaded shortening (17, and this study). The functional consequences of varied α-to-β-MyHC content likely play an important role in the progression of human heart failure, as left ventricular myosin was shown to shift from ~10% α-MyHC in normal hearts to ~20% α-MyHC in diseased hearts (34), and recent evidence suggests this shift may be even greater (15). This shift to β-MyHC may be compensatory to reduce oxygen demand, but it also most likely reduces cardiac functional reserve that is beneficial in times of increased peripheral demand such as hemorrhage, stress, or exercise.

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