Published in final edited form as:

J Mol Cell Cardiol. 2007 January ; 42(1): 214–221. doi:10.1016/j.yjmcc.2006.08.116.

Shifts in the myosin heavy chain isozymes in the mouse heart result in increased energy efficiency

Kirsten Hoyer*, Maïke Krenz†, Jeffrey Robbins†, and Joanne S. Ingwall*

* NMR Laboratory for Physiological Chemistry, Brigham and Women's Hospital, Boston, MA 02115, USA

† Cincinnati Children's Hospital Medical Center, Division of Molecular Cardiovascular Biology, MLC 7020, Cincinnati, OH 45229, USA

Abstract

Cardiac-specific transgenesis in the mouse is widely used to study the basic biology and chemistry of the heart and to model human cardiovascular disease. A fundamental difference between mouse and human hearts is the background motor protein: mouse hearts contain predominantly the $\alpha\alpha$ -myosin heavy chain (MyHC) isozyme while human hearts contain predominantly the $\beta\beta$ -MyHC isozyme. Although the intrinsic differences in mechanical and enzymatic properties of the $\alpha\alpha$ - and $\beta\beta$ -MyHC molecules are well known, the consequences of isozyme shifts on energetic of the intact beating heart remain unknown. Therefore, we compared the free energy of ATP hydrolysis ($|G_{\sim\text{ATP}}|$) determined by ^{31}P NMR spectroscopy in isolated perfused littermate mouse hearts containing the same amount of myosin comprised of either >95% $\alpha\alpha$ -MyHC or ~83% $\beta\beta$ -MyHC. $|G_{\sim\text{ATP}}|$ was ~2 kJ mol⁻¹ higher in the $\beta\beta$ -MyHC hearts at all workloads. Furthermore, upon inotropic challenge, hearts containing predominantly $\beta\beta$ -MyHC hearts increased developed pressure more than $\alpha\alpha$ -MyHC hearts whereas heart rate increased more in $\alpha\alpha$ -MyHC hearts. Thus, hearts containing predominantly the $\beta\beta$ -MyHC isozyme are more energy efficient than $\alpha\alpha$ -MyHC hearts. We suggest that these fundamental differences in the motor protein energy efficiency at the whole heart level should be considered when interpreting results using mouse-based cardiovascular modeling of normal and diseased human heart.

Keywords

^{31}P -NMR spectroscopy; biophysics; cardiac energy; myosin heavy chain isozyme; mouse heart; ATP; $|G_{\sim\text{ATP}}|$; cardiac-specific transgenesis

Address for correspondence Kirsten Hoyer, Ph.D., NMR Laboratory for Physiological Chemistry, Brigham and Women's Hospital, Harvard Medical School, 221 Longwood Ave., BLI 247, Boston, 02115 MA USA, email: khoyer@rics.bwh.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Myosin is the primary mechanoenzyme responsible for muscle contraction, coupling the energy released from ATP hydrolysis ($|G_{ATP}|$) to force generation. It is also the primary ATPase in muscle, consuming most of the ATP used for contraction. Myosin is a highly conserved protein composed of two myosin heavy chains (MyHC) and four light chains [1]. In vertebrate hearts, two isozymes of MyHC exist. The $\alpha\alpha$ -homodimer predominates in cardiac ventricles of small mammals [1], the $\beta\beta$ -homodimer in ventricles of large mammals and man [2]. Despite the fact that the two isozymes are highly homologous, with 93% amino acid identity [3], their physiologic and biochemical properties differ. Actin- and Ca^{2+} -activated ATPase activities of the different myosin isozymes determine the velocity of sarcomere contraction [4].

In cardiac ventricles, developmental stage, thyroid status, intermittent exercise, chronic work overload, and diseased states all alter MyHC composition. In end-stage failing human heart, for example, $\alpha\alpha$ -MyHC decreases until it is not detectable, leading to a relative increase in $\beta\beta$ -MyHC [5]. As $\beta\beta$ -MyHC is the more economical ATPase, this isoform shift may be energetically beneficial for the diseased heart. In mouse hearts, thyroid hormones induce $\alpha\alpha$ -MyHC expression whereas hypothyroidism and pressure overload result in an increase in $\beta\beta$ -MyHC composition. An *in vitro* mobility assay has been used to demonstrate that $\alpha\alpha$ -MyHC has twice the actin-activated and Ca^{2+} -stimulated ATPase activity and three-times the actin filament sliding velocity whereas it produces only one-half the average cross-bridge force when compared with $\beta\beta$ -MyHC [6]. Consistent with this, myocyte shortening and relengthening are slower for hypothyroid rat myocytes containing more $\beta\beta$ -MyHC [7] and maximal velocity of unloaded shortening is slower in papillary muscles with more $\beta\beta$ -MyHC [8].

One limitation of these studies is that the cardiac isozyme shift was achieved by endocrine intervention, changing the expression of many proteins ([9] and references therein). Therefore, whether these functional changes are induced solely by the MyHC isozyme shift or by other biochemical and structural changes is uncertain. A different strategy for altering the composition of MyHC isozymes is to use transgenesis in mice. Cardiac-specific expression of the protein of interest has the advantage that any cardiac phenotype must be due, either directly or indirectly, to that protein. Tardiff et al. [10] investigated the functional significance of cardiac myosin isozyme diversity using transgenic mice in which 12% of the ventricular $\alpha\alpha$ -MyHC was replaced by rat- $\beta\beta$ -MyHC. Krenz et al. [11] studied mice with 73% of ventricular $\alpha\alpha$ -MyHC replaced by mouse- $\beta\beta$ -MyHC. Both studies demonstrated no overt morphological phenotype, but systolic function and the rate of relaxation in hearts containing more $\beta\beta$ -MyHC were decreased compared with non-transgenic hearts.

These results support the classic view that the mechanical properties of hearts containing different MyHC isozymes are due to innate differences in the mechanoenzyme. However, the energetic consequences of replacing one MyHC isozyme with the other in the intact beating heart remain undefined. Here we compare the energetics of non-transgenic mouse hearts containing >95% $\alpha\alpha$ -MyHC [2] to genetically engineered mouse hearts with ~83% $\beta\beta$ -MyHC. We defined the energetic state of the isolated perfused heart using the non-

invasive tool of ^{31}P NMR spectroscopy to determine $|G_{\sim\text{ATP}}|$ while simultaneously measuring contractile performance. We found that mouse hearts bearing predominantly $\beta\beta$ -MyHC have an energetic phenotype that is distinct from hearts with native $\alpha\alpha$ -MyHC.

2. Materials and methods

2.1. Mouse model

Female mice (FVB/N) with ~83% replacement of ventricular $\alpha\alpha$ -MyHC with mouse $\beta\beta$ -MyHC were generated as described [12]. The isozyme content of MyHC was determined using glycerol gels as described in [11]. Four- to six-month-old female non-transgenic littermates were used as controls. Animals were maintained in accordance with NIH guidelines for the use and care of laboratory animals. The experimental protocols of this study were all in compliance with the recommendations of current NIH and American Physiological Society guidelines.

2.2. Isolated perfused mice hearts

Hearts were isolated and perfused in the Langendorff mode with phosphate-free Krebs-Henseleit buffer as described [13]. Systolic function was assessed as systolic pressure (SP), developed pressure (DevP), rate pressure product (RPP), and wall stress as global average stress [14]. Contractility was estimated as $+dP/dt$ (the maximum value of the first derivative of LV pressure). Diastolic function was assessed as EDP (end-diastolic pressure) and $-dP/dt$ (the minimum value of the first derivative of LV pressure). EDP was set to 10 ± 1 mm Hg at baseline conditions and then allowed to change with changing conditions. For a description of the measurement of indices of isovolumic contractile performance, see [13].

2.3. Experimental protocols

Hearts containing >95% $\alpha\alpha$ -MyHC (referred to as $\alpha\alpha$ -MyHC hearts) and ~83% $\beta\beta$ -MyHC (referred to as $\beta\beta$ -MyHC hearts) were used for ^{31}P NMR spectroscopy experiments; measures of contractile performance were made throughout. Hearts were paced at 7 Hz for 20 min, and then challenged with dobutamine (300 nM; unpaced) for 11 min, followed by 30 min recovery without dobutamine delivery. At the end of each protocol hearts were blotted dry, weighed and stored at -80°C .

2.4. ^{31}P NMR spectroscopy

^{31}P NMR free induction decays (FIDs) were acquired at 161.4 MHz using a Varian Inova spectrometer (Varian, Palo Alto, CA), averaging 200 FIDs (60° pulse, 2.5 sec recycle time). The resonance areas and chemical shifts (intracellular pH) were quantified using the MacNuts–Utility transform software (Acorn NMR, Livermore, CA). Values of $G_{\sim\text{ATP}}$ are expressed as their absolute numbers and were calculated as shown in the following equation:

$$|\Delta G_{\sim\text{ATP}}|(\text{kJ/mol}) = |\Delta G^0_{\sim\text{ATP}} + RT \ln ([\text{ADP}][\text{Pi}]/[\text{ATP}])|$$

where G^0 is the standard free energy change of ATP hydrolysis under standard conditions of molarity, temperature, pH, and $[Mg^{2+}]$; R is the gas constant (8.3 J/mol K), and T is in Kelvin. Free cytosolic [ADP] was calculated using the equilibrium expression for the creatine kinase (CK) reaction [13] and measured metabolite contents.

2.5. Myocardial oxygen consumption (MVO₂)

MVO₂ was measured using Clark type microelectrodes (Microelectrodes Inc., Bedford, NH). The pulmonary artery was cannulated with a PE-10 catheter connected via a T-piece to an electrode. Perfusate was withdrawn from the pulmonary artery with a constant flow of 0.1 ml/min by a pulsation-free pump. The T-piece in front of the O₂ electrode allowed the outflow of excessive pulmonary flow. Inflowing O₂ tension was measured with a second electrode at the same flow rate. Both electrodes were kept at 37°C. MVO₂ was calculated using the difference of inflowing and outflowing O₂ tensions, coronary flow rate, heart weight, and the molar O₂ solubility coefficient [15].

2.6. Biochemical measurements

Five to 10 mg of ventricular tissue was homogenized for 10 sec at 4°C in K-phosphate buffer (0.1 M) containing 1 mM EDTA and 1 mM β-mercaptoethanol, pH 7.4 (final concentration of 5 mg wet weight/ml). Aliquots were removed to determine protein [16] and total creatine contents [17]. Triton X-100 was added to the homogenate at a final concentration of 0.1 % for analysis of activities of CK [18]; of the major glycolytic enzymes phosphofructokinase (PFK) [19] and lactate dehydrogenase (LDH) [20]; and of the mitochondrial markers citrate synthase (CS) [21] and cytochrome C oxidase (COX, measured with a Sigma assay kit, [22]).

2.7. Statistical analysis

Results are expressed as means ± SEM. Comparisons were tested with one-way ANOVA followed by a multiple comparison test using Fisher's protected least significant difference; P<0.05 was considered significant. Linear regressions and tests whether slopes and intercepts differed were calculated with Prism (GraphPad, San Diego, CA).

3. Results

3.1. Properties of the hearts

To determine whether whole heart energetics differed or were the same in αα- and ββ-MyHC hearts, it was necessary to make the comparison under identical experimental conditions, using pairs of 4–6 month-old littermate mouse hearts, one from each group, perfused in the Langendorff mode. Cardiomyocytes rigidly control the stoichiometry of the contractile proteins within the sarcomere, and the total amount of myosin in the two types of hearts was the same [11]. Furthermore, previously analyzed transcript levels of a number of heart failure/hypertrophy related genes such as atrial natriuretic factor, brain natriuretic peptide, alpha-skeletal actin, and phospholamban in right- and left-ventricular tissue samples from αα- and ββ-MyHC mice did not show discernable differences (Krenz & Robbins, unpublished data). Body weights of 25.9 ± 0.2 g and 26.8 ± 0.3 g and heart weights of 94.8 ± 1.6 mg and 105.3 ± 1.5 mg were indistinguishable for the αα- and ββ-MyHC mice (n=13

for both groups), respectively. Left and right ventricular weights were also comparable (data not shown); atrial weights were higher in the $\beta\beta$ -MyHC hearts (10.2 ± 0.35 mg vs. 6.2 ± 0.06 mg, $P < 0.001$). Basal perfusion conditions were identical for both heart groups: perfusion pressure of 80 mmHg, pacing rate of 7 Hz, placing and filling the balloon (volume for $\alpha\alpha$ -MyHC: 13.8 ± 1.3 μ l; for $\beta\beta$ -MyHC: 15.3 ± 1.0 μ l, $n=8$) in the same way to set the EDP at ~ 10 mmHg, and coronary flow of ~ 2 ml min^{-1} . The stimulus to increase workload was the inotropic drug dobutamine, delivered to a final concentration of 300 nM for each heart.

3.2. Energetics

To determine whether the changes in the MyHC isozyme composition lead to differences in the energetic state of the intact beating heart, we obtained sequential ^{31}P NMR spectra at three time points. We determined ATP, ADP, phosphocreatine (PCr), and inorganic phosphate (Pi) concentrations in the hearts at baseline, during high workload, and during recovery from the high work. Figure 1 shows summed ^{31}P NMR spectra acquired during baseline perfusion and inotropic challenge from $\alpha\alpha$ - and $\beta\beta$ -MyHC hearts and Table 1 summarizes the results.

During baseline perfusion, [ATP], [ADP] and pH_i for $\beta\beta$ - and $\alpha\alpha$ -MyHC hearts were indistinguishable. However, [PCr] was 10% higher ($P < 0.05$) while [Pi] was 56% lower ($P < 0.05$) in the $\beta\beta$ -MyHC hearts. Based on previous experiments comparing baseline and inotropic challenge in isolated mouse hearts [23], we expected [PCr] to decrease, [Pi] and [ADP] to increase, and [ATP] and intracellular pH (pH_i) not to change with high workload. This pattern demonstrating increased energy utilization with increased workload at the whole heart level was observed here, i.e. the CK reaction supplied sufficient phosphoryl groups to match ATP utilization at high workload by hydrolyzing PCr. Of interest, the greater [PCr] observed in the $\beta\beta$ -MyHC hearts at baseline persisted even at high workloads.

Changes in [ATP], [ADP], and [Pi] can be expressed as $|G_{\sim\text{ATP}}|$, representing the chemical driving force for the ATPase reactions in the cell and describing its energy state. $|G_{\sim\text{ATP}}|$ was higher in $\beta\beta$ -MyHC hearts ($n=8$ for both groups) at baseline (61.6 vs. 59.3 kJ mol^{-1} , $P=0.03$, Figure 2), during high workload (55.7 vs. 53.7 kJ mol^{-1} , $P < 0.001$, Figure 2) and for recovering hearts (63.5 vs. 60.4 kJ mol^{-1} , $P < 0.009$). Note that the difference in $|G_{\sim\text{ATP}}|$ of $2\text{--}3$ kJ mol^{-1} requires large differences in [ATP], [ADP], and/or [Pi]. Since the maximum difference in $|G_{\sim\text{ATP}}|$ observed for the intact heart caused by an abrupt increase in workload is ~ 6 kJ mol^{-1} within each group, the difference of $\sim 2\text{--}3$ kJ mol^{-1} between the two groups is $\sim 33\text{--}50$ % of the maximum.

3.3 Isovolumic contractile performance

To determine whether switching the MyHC isozyme composition from $\alpha\alpha$ - to $\beta\beta$ -MyHC alters cardiac function, we measured isovolumic contractile performance during baseline, high workload, and recovery. Representative pressure tracings of $\alpha\alpha$ - and $\beta\beta$ -MyHC hearts and mean values for indices of systolic and diastolic function are shown in Figure 3.

During low work conditions (baseline), all indices of systolic contractile performance, namely SP, DevP, $+\text{dP}/\text{dt}$, and RPP, were similar for the two groups. Diastolic function,

assessed as the rate of relaxation ($-dP/dt$) was $>30\%$ slower in the $\beta\beta$ -MyHC hearts, resulting in a slower half time of relaxation.

To determine if the $\beta\beta$ -MyHC hearts respond similarly to high workload conditions as the $\alpha\alpha$ -MyHC hearts, we measured contractile performance in hearts challenged with 300 nM dobutamine, a dose producing maximal change in contractile performance. This resulted in increases in RPP in both groups (to $82,600 \text{ mmHg min}^{-1}$ in the $\beta\beta$ - and $74,000 \text{ mmHg min}^{-1}$ in the $\alpha\alpha$ -MyHC hearts), but how it was achieved differed: In the $\alpha\alpha$ -MyHC hearts, the heart rate (HR) increased by 24% and DevP by 71%; in the $\beta\beta$ -MyHC HR increased by only 15% while DevP increased by 128%. Thus, increases in HR and DevP both contributed to the increased RPP for $\alpha\alpha$ -MyHC hearts whereas increased DevP was the main contributing factor for the increase in $\beta\beta$ -MyHC hearts. The increase in wall stress (to 176 ± 12 and $150 \pm 10 \text{ kdynes (cm}^2\text{)}^{-1}$ for $\beta\beta$ -MyHC hearts vs. $\alpha\alpha$ -MyHC hearts) was also substantially greater in $\beta\beta$ -MyHC than in the $\alpha\alpha$ -MyHC hearts (123% vs. 70%). Negative dP/dt increased so much during inotropic challenge in the $\beta\beta$ -MyHC hearts (248% vs. 150% for the $\alpha\alpha$ -MyHC hearts) that the large difference in this parameter between the groups at baseline was abolished.

3.4. Relationship between energetics and contractile performance

The relationships between physiologic performance assessed as RPP and the energetic state assessed as $|G_{\sim ATP}|$ for the $\alpha\alpha$ - and $\beta\beta$ -MyHC hearts using data obtained during baseline, inotropic challenge, and recovery are shown in Figure 4. Both groups demonstrated the expected decrease in $|G_{\sim ATP}|$ as RPP increased in response to the inotropic challenge and $|G_{\sim ATP}|$ returned to baseline values during recovery from high workload. Data are well fit by linear relationships. The slopes are indistinguishable for the two groups, but the y-intercept is higher ($P < 0.001$) for the $\beta\beta$ -MyHC hearts. Thus, for any given RPP, the energetic state is $\sim 2\text{--}3 \text{ kJ mol}^{-1}$ higher for $\beta\beta$ -MyHC hearts, and for any given $|G_{\sim ATP}|$, the $\beta\beta$ -MyHC hearts yield more isovolumic contractile work.

3.5. ATP supply

To rule out any differences in the ATP synthesis pathways in hearts in which the $\alpha\alpha$ -MyHC were replaced by $\beta\beta$ -MyHC, we performed two sets of experiments. The first was to determine the relationship between RPP and MVO_2 in the intact heart and the second was to determine $V_{\max}$ of key enzymes of ATP synthesis pathways in extracts of hearts. There were no differences in the RPP/ MVO_2 relationships or in the tissue activities of glycolytic enzymes, and key phosphoryltransferases (Table 2) between the two study groups. One marker of the capacity for oxidative phosphorylation, cytochrome C oxidase, was slightly lower (19%) in the $\beta\beta$ -MyHC than in the $\alpha\alpha$ -MyHC hearts.

4. Discussion

In this study, we defined the energetic consequences of a bioengineered isozyme shift from 95% $\alpha\alpha$ -MyHC/5% $\beta\beta$ -MyHC to 17% $\alpha\alpha$ -MyHC/83% $\beta\beta$ -MyHC in intact beating mouse hearts. We found that the energetic phenotype of these two hearts differed. The $\beta\beta$ -MyHC hearts exhibited a higher energetic driving force expressed as the free energy available from

ATP hydrolysis at all workloads: at baseline, during inotropic challenge and during recovery from high workloads. Since isovolumic contractile performance was greater in the $\beta\beta$ -MyHC than for $\alpha\alpha$ -MyHC hearts for the same change in chemical driving force, we conclude that hearts containing predominantly $\beta\beta$ -MyHC are more efficient.

The different energetic phenotype at the whole heart level for $\beta\beta$ -MyHC hearts is due to the innate differences between the $\alpha\alpha$ - and $\beta\beta$ -MyHC isozymes, and not due to differences in sarcomere length [11], myosin light chain composition [24], local pH or source of ATP used for contraction. Although filament sliding velocity is reduced under both acidic and alkaline pH [25], differences in pH cannot explain the mechanical differences observed here as pH_i was the same in $\alpha\alpha$ - and $\beta\beta$ -MyHC hearts. Finally, neither the rate of oxygen consumed nor the capacity for ATP synthesis differed for $\alpha\alpha$ - and $\beta\beta$ -MyHC hearts. Note that the slightly lower V_{max} observed for COX in the $\beta\beta$ -MyHC hearts does not effect the oxygen uptake.

4.1. Basis for energetic phenotype of $\beta\beta$ -MyHC mouse hearts

In this study of the intact heart using the non-invasive tool of NMR spectroscopy, we found that under identical conditions of perfusion and oxygen consumed, $\beta\beta$ -MyHC hearts consistently had a higher $|G_{ATP}|$. In the two-state cross-bridge model of muscle contraction [26], the $\beta\beta$ -MyHC cross-bridge remains attached to the thin filament for a longer period of time [6, 7]. Thus, if whole heart contractile performance is determined primarily by the properties of the major motor protein, intact hearts containing more $\beta\beta$ -MyHC should have slower rates of relaxation and develop more force (systolic pressure) for the same energy cost, or equivalently have higher $|G_{ATP}|$ for a given SP, DevP or RPP. Here we show that this is the case. In general agreement with results of others using smaller MyHC replacements [10, 11], we also found large differences in the rate of relaxation ($-dP/dt$, >30% slower) and the half-time of relaxation (~25% longer) for $\beta\beta$ -MyHC hearts. Importantly, the change in relaxation rate cannot explain the greater efficiency or the greater $|G_{ATP}|$ observed in $\beta\beta$ -MyHC hearts because the differences in relaxation rate were abolished during inotropic challenge while the differences in $|G_{ATP}|$ between the two types of hearts remained.

Upon inotropic challenge, increases in SP and DevP were greater in the $\beta\beta$ -hearts compared to the $\alpha\alpha$ -hearts (123% vs. 74% and 128% vs. 71%, respectively, as the percentage change over baseline). Heart rate did not increase as high in the $\beta\beta$ -hearts as it did in the $\alpha\alpha$ -hearts (15% vs. 24%). These results on the whole heart level can be explained by the intrinsic properties of the $\alpha\alpha$ - and $\beta\beta$ -MyHC: the hearts of the $\beta\beta$ -MyHC isozyme have a higher force-generation associated with a lower heart rate. While cardiac performance is influenced by neurohumoral factors and by variations in Ca^{2+} regulation, we suggest that the major determinant of whole heart energetics when these factors are held constant is the nature of the motor protein.

The correlation between HR and the content of MyHC isozyme merits further comment. A trend towards lower HR has also been observed in other isolated heart studies [10, 11] as well as in propranolol-treated mice with predominantly $\beta\beta$ -MyHC [11]. In small animals, accumulating high levels of $\alpha\alpha$ -MyHC is considered to be an adaptation because its faster hydrolysis rate facilitates the extremely rapid rates of cardiac contraction and relaxation

[27]. Therefore, the lower heart rate found in the $\beta\beta$ -MyHC hearts may be the result of a long-term adaptation to the presence of slow MyHC isozyme.

In studies using hypothyroidism to shift the cardiac MyHC composition, it has not been possible to distinguish whether differences in tension development and relaxation are caused by the $\alpha\alpha$ -to $\beta\beta$ -MyHC shift in the sarcomere or are due to altered Ca^{2+} handling properties changed by the thyroid state, or both [7, 9]. Here, using transgenesis to alter MyHC composition, we found that the different intrinsic rates of cross-bridge detachment and ATPase activities of the MyHC isozymes are sufficient to explain the differences in rates of tension development and relaxation and in $|G_{\sim\text{ATP}}|$ observed for intact beating hearts containing either predominantly $\alpha\alpha$ -MyHC or $\beta\beta$ -MyHC. This conclusion is also supported by the findings of others showing that the $[\text{Ca}]$ -myofibrillar ATPase activity curve does not shift for $\beta\beta$ -MyHC [11] compared to $\alpha\alpha$ -MyHC, that calcium transients were not altered in rat ventricular myocytes expressing human $\beta\beta$ -MyHC [28], and that there is no shift in MyHC isozyme composition in mouse cardiomyocytes with a decreased SERCA pump level with demonstrated changes in intracellular Ca^{2+} homeostasis and contractility [29].

4.2. $\beta\beta$ -MyHC mouse model

In all studies using mouse models with MyHC replacements, 12% [10], 73% [11, 12] and 83% (this study, [12]), the isozyme switch was found to be well tolerated and did not induce disease. However, the ability of hearts with high percentage replacement of endogenous $\alpha\alpha$ -MyHC with $\beta\beta$ -MyHC (73 and 83%) to tolerate stress differs depending on whether the stress is acute, intermittent or chronic. The consequence of acute stress was investigated in both isolated hearts (this study) and in *in vivo* anesthetized animals [12]. Both $\alpha\alpha$ - and $\beta\beta$ -MyHC hearts increased systolic performance in response to a treatment of the inotrope dobutamine. In the absence of neural and humoral control, isolated $\beta\beta$ -MyHC hearts responded with an even higher increase in systolic performance: RPP increased by 161% compared to 112% for the $\alpha\alpha$ -MyHC hearts. In anesthetized intact mice, infusing dobutamine elicited directionally similar but much smaller increases (~25%) in systolic function. With intermittent stress caused by two swimming sessions of 90 min/day for two weeks, both $\alpha\alpha$ - and $\beta\beta$ -MyHC hearts showed comparable hypertrophic response without signs of heart failure [12].

In contrast to tolerating acute and intermittent stresses, $\beta\beta$ -MyHC hearts did not tolerate chronic severe stress as well as $\alpha\alpha$ -MyHC hearts [12]. In response to continuous infusion of the inotrope isoproterenol to mice for two weeks, SP was lower in mice with $\beta\beta$ -MyHC (130 vs. 150 mm Hg). Moreover, $\beta\beta$ -MyHC mice developed more pronounced cardiac dysfunction in a post-infarction failure model. Since high levels of $\alpha\alpha$ -MyHC with fast ATP hydrolysis rate support rapid rates of contraction and relaxation of hearts of small animals [27], we suggest that replacing fast myosin with slow myosin leads to a chronic mismatch between enzyme kinetics and demand to increase work. This would contribute to the reduced ability of the hearts to withstand chronic severe stress despite being more energy efficient on a beat-to-beat basis.

4.3. $\beta\beta$ -MyHC mouse model and human disease

In end-stage failing hearts, the low $\alpha\alpha$ -MyHC protein content decreases until it is undetectable [5], resulting in a relatively increased $\beta\beta$ -MyHC content. While the decrease in $\alpha\alpha$ -MyHC composition may contribute to systolic dysfunction [30], based on results presented here, we suggest that hearts containing a greater fraction of the more energy efficient $\beta\beta$ -MyHC isozyme would be compensatory as long as the workload of the heart remains relatively low. This is supported by studies showing that pharmacological intervention having a negative inotropic effect (such as ACE inhibitors, angiotensin blockers, and β -blockers) improves outcome in heart failure ([31], references therein).

Genetic modification in the mouse is commonly used as a tool in the investigation of cardiac disease. One potentially important limitation of these animal models is that the primary energy consuming ATPase is not the isozyme present in the human heart [32]. The amino acid composition of mouse and rat $\alpha\alpha$ - and $\beta\beta$ -MyHC differ in the same five functionally important domains of the myosin head and rod (for details see [1, 3]). Importantly, missense mutations resulting in Familial Hypertrophic Cardiomyopathy (FHC) are also found in these same domains [1, 33]. These mutations have unpredictable effects on mechanical properties of human as well as mouse hearts ([34–36], references therein). Although the underlying molecular mechanisms are not completely understood ([37], references therein), it has been proposed that altered cardiac energetics is a general mechanism underlying the phenotypic expression of malignant FHC-associated mutations ([38], references therein). We suggest that it may be informative to study this disease using a mouse model with a high replacement of $\alpha\alpha$ -MyHC by $\beta\beta$ -MyHC.

5. Conclusion

Using intact beating mouse hearts genetically modified to replace $\alpha\alpha$ -MyHC with $\beta\beta$ -MyHC, we found that hearts containing predominantly $\beta\beta$ -MyHC have higher mechanical-energetic efficiency. Thus, the mechanical and energetic properties of the whole heart differ depending on only a few amino acid changes in MyHC. We suggest that this should be taken into account when interpreting results using mouse-based cardiovascular modeling of normal and diseased hearts.

Acknowledgments

We thank Dr. Paul D. Allen for helpful advice. This work was supported by a grant from the National Institutes of Health (HL075619) and a Post-Doctoral fellowship from the American Heart Association (M.K.).

References

1. Alpert NR, Brosseau C, Federico A, Krenz M, Robbins J, Warshaw DM. Molecular mechanics of mouse cardiac myosin isoforms. *Am J Physiol Heart Circ Physiol*. 2002; 283:H1446–54. [PubMed: 12234796]
2. Reiser PJ, Portman MA, Ning XH, Schomisch Moravec C. Human cardiac myosin heavy chain isoforms in fetal and failing adult atria and ventricles. *Am J Physiol Heart Circ Physiol*. 2001; 280:H1814–20. [PubMed: 11247796]
3. McNally EM, Kraft R, Bravo-Zehnder M, Taylor DA, Leinwand LA. Full-length rat alpha and beta cardiac myosin heavy chain sequences. *J Mol Biol*. 1989; 210:665–71. [PubMed: 2614840]

4. Barany M. ATPase activity of myosin correlated with speed of muscle shortening. *J Gen Physiol.* 1967; 50 (Suppl):197–218. [PubMed: 4227924]
5. Miyata S, Minobe W, Bristow MR, Leinwand LA. Myosin heavy chain isoform expression in the failing and nonfailing human heart. *Circ Res.* 2000; 86:386–90. [PubMed: 10700442]
6. VanBuren P, Harris DE, Alpert NR, Warshaw DM. Cardiac V₁ and V₃ myosins differ in their hydrolytic and mechanical activities in vitro. *Circ Res.* 1995; 77:439–44. [PubMed: 7614728]
7. Fitzsimons DP, Patel JR, Moss RL. Role of myosin heavy chain composition in kinetics of force development and relaxation in rat myocardium. *J Physiol.* 1998; 513 (Pt 1):171–83. [PubMed: 9782168]
8. Cappelli V, Bottinelli R, Poggesi C, Moggio R, Reggiani C. Shortening velocity and myosin and myofibrillar ATPase activity related to myosin isoenzyme composition during postnatal development in rat myocardium. *Circ Res.* 1989; 65:446–57. [PubMed: 2526695]
9. Kiss E, Jakab G, Kranias EG, Edes I. Thyroid hormone – induced alterations in phospholamban protein expression. *Circ Res.* 1994; 75:245–51. [PubMed: 8033338]
10. Tardiff JC, Hewett TE, Factor SM, Vikstrom KL, Robbins J, Leinwand LA. Expression of the β (slow)-isoform of MHC in the adult mouse heart causes dominant-negative functional effects. *Am J Physiol Heart Circ Physiol.* 2000; 278:H412–19. [PubMed: 10666070]
11. Krenz M, Sanbe A, Bouyer-Daloz F, Gulick J, Klevitsky R, Hewett TE, et al. Analysis of myosin heavy chain functionality in the heart. *J Biol Chem.* 2003; 278:17466–74. [PubMed: 12626511]
12. Krenz M, Robbins J. Impact of beta-myosin heavy chain expression on cardiac function during stress. *J Am Coll Cardiol.* 2004; 44:2390–97. [PubMed: 15607403]
13. Saupe KW, Spindler M, Tian R, Ingwall JS. Impaired cardiac energetics in mice lacking muscle-specific isoenzymes of creatine kinase. *Circ Res.* 1998; 82:898–907. [PubMed: 9576109]
14. Mirsky I, Krayenbuehl HP. The role of wall stress in the assessment of ventricular function. *Herz.* 1981; 6:288–99. [PubMed: 6457780]
15. Saupe KW, Spindler M, Hopkins JC, Shen W, Ingwall JS. Kinetic, thermodynamic, and developmental consequences of deleting creatine kinase isoenzymes from the heart. Reaction kinetics of the creatine kinase isoenzymes in the heart. *J Biol Chem.* 2000; 275:19742–6. [PubMed: 10867023]
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951; 193:265–75. [PubMed: 14907713]
17. Kammermeier H. Microassay of free and total creatine from tissue extracts by combination of chromatographic and fluorometric methods. *Anal Biochem.* 1973; 56:341–5. [PubMed: 4765631]
18. Rosalki SB. An improved procedure for serum creatine phosphokinase determination. *J Lab Clin Med.* 1967; 69:696–705. [PubMed: 4381359]
19. Oblinger MM, Foe LG, Kwiatkowska D, Kemp RG. Phosphofructokinase in the rat nervous system: regional differences in activity and characteristics of axonal transport. *J Neurosci Res.* 1988; 21:25–34. [PubMed: 2464070]
20. Bernstein LH, Everse J. Determination of the isoenzyme levels of lactate dehydrogenase. *Methods Enzymol.* 1975; 41:47–52. [PubMed: 165387]
21. Srere PBH, Gowen L. The citrate condensing enzyme of pigeon breast muscle and moth flight muscle. *Acta Chem Scand.* 1963; 17 (Suppl 1):S129–34.
22. Storrie B, Madden EA. Isolation of subcellular organelles. *Methods Enzymol.* 1990; 182:203–25. [PubMed: 2156127]
23. Ingwall, JS. *ATP and the Heart.* 1. Boston: Kluwer Academic Publishers; 2002.
24. Pagani ED, Julian FJ. Rabbit papillary muscle myosin isozymes and the velocity of muscle shortening. *Circ Res.* 1984; 54:586–94. [PubMed: 6723002]
25. Yamashita H, Sugiura S, Serizawa T, Sugimoto T, Iizuka M, Katayama E, et al. Sliding velocity of isolated rabbit cardiac myosin correlates with isozyme distribution. *Am J Physiol.* 1992; 263:H464–72. [PubMed: 1387292]
26. Huxley AF. Muscle structure and theories of contraction. *Prog Biophys Biophys Chem.* 1957; 7:255–318. [PubMed: 13485191]

27. Hasenfuss G, Mulieri LA, Blanchard EM, Holubarsch C, Leavitt BJ, Ittleman F, et al. Circ Res. 1991; 68:836–46. [PubMed: 1742869]
28. Herron TJ, Fomicheva E, Vandenboom R, Metzger JM. Human β -myosin heavy chain gene expression directly attenuates cardiac contractility in α -myosin heavy chain dominant single myocytes. Circulation. 2005; 112 (Suppl II):185. [PubMed: 15998685]
29. Ji Y, Lalli MJ, Babu GJ, Xu Y, Kirkpatrick DL, Liu LH, et al. Disruption of a single copy of the SERCA2 gene results in altered Ca^{2+} homeostasis and cardiomyocyte function. J Biol Chem. 2000; 275:38073–80. [PubMed: 10970890]
30. Carniel E, Taylor MR, Sinagra G, Di Lenarda A, Ku L, Fain PR, et al. α -Myosin heavy chain: a sarcomeric gene associated with dilated and hypertrophic phenotypes of cardiomyopathy. Circulation. 2005; 112:54–9. [PubMed: 15998695]
31. Ingwall JS, Weiss RG. Is the failing heart energy starved? On using chemical energy to support cardiac function. Circ Res. 2004; 95:135–45. [PubMed: 15271865]
32. Lowey S. Functional consequences of mutations in the myosin heavy chain at sites implicated in familial hypertrophic cardiomyopathy. Trends Cardiovasc Med. 2002; 12:348–54. [PubMed: 12536121]
33. Rayment I, Holden HM, Sellers JR, Fananapazir L, Epstein ND. Structural interpretation of the mutations in the β -cardiac myosin that have been implicated in familial hypertrophic cardiomyopathy. Proc Natl Acad Sci USA. 1995; 92:3864–8. [PubMed: 7731997]
34. Alpert NR, Mohiddin SA, Tripodi D, Jacobson-Hatzell J, Vaughn-Whitley K, Brosseau C, et al. Molecular and phenotypic effects of heterozygous, homozygous, and compound heterozygote myosin heavy-chain mutations. Am J Physiol Heart Circ Physiol. 2005; 288:H1097–102. [PubMed: 15528230]
35. Keller DI, Coirault C, Rau T, Cheav T, Weyand M, Amann K, et al. Human homozygous R403W mutant cardiac myosin presents disproportionate enhancement of mechanical and enzymatic properties. J Mol Cell Cardiol. 2004; 36:355–62. [PubMed: 15010274]
36. Ertz-Berger BR, He H, Dowell C, Factor SM, Haim TE, Nunez S, et al. Changes in the chemical and dynamic properties of cardiac troponin T cause discrete cardiomyopathies in transgenic mice. Proc Natl Acad Sci USA. 2005; 102:18219–24. [PubMed: 16326803]
37. Fatkin D, Graham RM. Molecular Mechanisms of inherited cardiomyopathies. Physiol Rev. 2002; 82:945–80. [PubMed: 12270949]
38. Ingwall JS. Transgenesis and cardiac energetics: new insights into cardiac metabolism. J Mol Cell Cardiol. 2004; 37:613–23. [PubMed: 15350834]

Abbreviations

CK	creatine kinase
COX	cytochrome C oxidase
CS	citrate synthase
 G_{-ATP} 	free energy of ATP hydrolysis
DevP	developed pressure
– dP/dt	minimum value of the first derivative of LV pressure
+ dP/dt	maximum value of the first derivative of LV pressure
EDP	end-diastolic pressure
FHC	familial hypertrophic cardiomyopathy
FIDs	free induction decays

HR	heart rate
LDH	lactate dehydrogenase
LV	left ventricle
MVO₂	myocardial oxygen consumption
MyHC	myosin heavy chain
PCr	phosphocreatine
PFK	phosphofructokinase
pH_i	intracellular pH
Pi	inorganic phosphate
³¹P-NMR	phosphorus-31 nuclear magnetic resonance spectroscopy
SP	systolic pressure
RPP	rate pressure product

Figure 1.

Summed ^{31}P NMR spectra from four $\alpha\alpha$ -MyHC (A and B) and $\beta\beta$ -MyHC (C and D) hearts. A and C show spectra at baseline; B and D during inotropic challenge. Resonances correspond to (from left to right) Pi, PCr, and the three phosphate groups of ATP (γ , α , β). The height of the PCr resonance for $\alpha\alpha$ -MyHC hearts at baseline is set to 1.0 to allow comparisons. Note that the PCr resonance for $\beta\beta$ -MyHC hearts at baseline is higher than for the $\alpha\alpha$ -hearts. During dobutamine treatment, areas of ATP resonances do not change, but PCr resonance area decreases while Pi increases more in the $\alpha\alpha$ -MyHC than in the $\beta\beta$ -

MyHC hearts. *: $P < 0.05$ for $\alpha\alpha$ -MyHC vs. $\beta\beta$ -MyHC, +: $P < 0.05$ for baseline vs. inotropic challenge within the groups.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2.

The free energy of ATP hydrolysis ($|\Delta G_{\sim ATP}|$) at baseline and during dobutamine treatment for individual hearts. Also shown is the average value marked for each condition (solid line). Note that $|\Delta G_{\sim ATP}|$ is higher for $\beta\beta$ -MyHC hearts ($n=8$) than for $\alpha\alpha$ -MyHC hearts ($n=8$) at both work states. Means $\pm$ SEM.

Figure 3.

Representative tracings of left ventricular isovolumic contractile performance. A, $\alpha\alpha$ -MyHC and B, $\beta\beta$ -MyHC hearts at baseline; C, $\alpha\alpha$ -MyHC and D, $\beta\beta$ -MyHC hearts during increased cardiac work. Balloon volume was set at an EDP of 10 mmHg at baseline and then allowed to vary. Hearts were paced at 420 bpm (7 Hz) only during baseline. DevP is the difference between the EDP and SP (dashed line); RPP (the product of HR times DevP). Also shown are the maximum and the minimum values of positive and negative dP/dt (solid line) as well as their ratio ($\pm$ dP/dt) and the half time of relaxation at baseline. * $P < 0.05$ basal $\alpha\alpha$ -

MyHC vs. $\beta\beta$ -MyHC, $^+P<0.05$ inotropic challenged $\alpha\alpha$ -MyHC vs. $\beta\beta$ -MyHC, RPP during inotropic challenge in $\alpha\alpha$ -MyHC vs. $\beta\beta$ -MyHC differ with a significance of $^{\#}P=0.06$. The quantitative changes within the groups after adding dobutamine are all significant except for the $+/-$ dP/dt in the $\alpha\alpha$ -MyHC mouse hearts. Note that the x-axes differ between C and D. Means $\pm$ SEM (n=8 for both groups).

Figure 4.

Relationship between RPP and $|\Delta G_{ATP}|$ for $\alpha\alpha$ -MyHC and $\beta\beta$ -MyHC mouse hearts. Linear regressions are: $y = -6267x + 406,700$ with $R^2 = 0.82$ ($\alpha\alpha$ -MyHC, circles) and $y = -7140x + 476,600$ with $R^2 = 0.91$ ($\beta\beta$ -MyHC, squares). While the slopes are not different, y-intercepts differ ($P < 0.0001$). Filled symbols: baseline and during inotropic challenge; open symbols: during recovery.

Table 1
Cardiac energetics

	$\alpha\alpha$ -MyHC		$\beta\beta$ -MyHC	
	baseline	dobutamine	baseline	dobutamine
Pi (mM)	2.8 [*] $\pm$ 0.54	10.1 ⁺ $\pm$ 0.58	1.2 $\pm$ 0.30	5.7 $\pm$ 0.39
PCr (mM)	18.6 [*] $\pm$ 0.34	12.5 ⁺ $\pm$ 0.56	20.5 $\pm$ 0.89	14.9 $\pm$ 0.40
pH _i	7.13 $\pm$ 0.20	7.10 $\pm$ 0.01	7.14 $\pm$ 0.01	7.10 $\pm$ 0.01
ATP (mM)	10.1 $\pm$ 0.01	9.7 $\pm$ 0.27	10.3 $\pm$ 0.32	9.6 $\pm$ 0.41
ADP (μ M)	64 $\pm$ 5	123 $\pm$ 11	61 $\pm$ 6	101 $\pm$ 8
G _{-ATP} (kJ mol ⁻¹)	59.3 [*] $\pm$ 0.7	53.7 ⁺ $\pm$ 0.3	61.6 $\pm$ 0.8	55.7 $\pm$ 0.2

All results are given as mean $\pm$ SEM (n=8) at baseline and during inotropic challenge (dobutamine) in both MyHC-groups.

^{*} P<0.05 baseline $\alpha\alpha$ -MyHC vs. $\beta\beta$ -MyHC,

⁺ P<0.05 inotropic challenged $\alpha\alpha$ -MyHC vs. $\beta\beta$ -MyHC.

TABLE 2
ATP supply

	$\alpha\alpha$ -MyHC		$\beta\beta$ -MyHC	
PFK	339 $\pm$ 13		351 $\pm$ 10	
LDH	669 $\pm$ 16		698 $\pm$ 20	
CS	396 $\pm$ 19		420 $\pm$ 20	
COX	160* $\pm$ 8		130 $\pm$ 4	
total CK	4294 $\pm$ 164		4248 $\pm$ 111	
Creatine (mM)	31.0 $\pm$ 0.7		32.5 $\pm$ 1.3	
MVO ₂ (μ mol O ₂ /g _{ww} min)	Baseline 10 $\pm$ 1	Dobutamine 16 $\pm$ 1	Baseline 10 $\pm$ 2	Dobutamine 17 $\pm$ 3

Representative enzymes of the glycolytic pathway (PFK, LDH) and oxidative phosphorylation (CS, COX), and total CK activity and creatine content were measured in tissue homogenates of $\alpha\alpha$ -MyHC (n=8) and $\beta\beta$ -MyHC (n=8) hearts. Activities presented as mIU/mg protein. Oxygen consumption (MVO₂) was monitored during baseline and during treatment with dobutamine in both heart groups (n=5) as a measure of ATP synthesis rate. Means $\pm$ SE.

*
P<0.05