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Impact of Beta-Myosin Heavy Chain Expression on Cardiac Function During Stress

Maike Krenz, MD, Jeffrey Robbins, PHD

Cincinnati, Ohio

OBJECTIVES In failing mouse and human hearts, a shift in myosin heavy chain (MHC) isoform content from alpha to beta can occur. However, the impact of this phenomenon on disease progression is not well understood. Therefore, using transgenic (TG) mice, we tested how a pre-existing shift from alpha- to beta-MHC affects cardiac function under chronic mechanical or pharmacologic cardiovascular stress.

BACKGROUND Expression of beta-MHC is considered to be energetically favorable, but this might be offset by depressed cardiac function.

METHODS Transgenic mice with near-complete replacement of the normally predominant alpha- with beta-MHC were subjected to cardiac stress.

RESULTS At baseline, TG mice show moderately reduced cardiac contractile function but are otherwise healthy with normal ventricular morphology. After four weeks of swimming, both TG and non-TG animals showed a 20% increase in left ventricular (LV)/body weight ratios. The TG hearts displayed mildly greater end-diastolic and end-systolic LV diameters than nontransgenic hearts after training, but no signs of LV failure were observed. However, chronic stimulation with isoproterenol resulted in augmented LV hypertrophy with signs of LV decompensation in TG mice. Furthermore, in a post-infarction failure model, TG hearts displayed accelerated LV dilation and a faster decline of shortening fraction.

CONCLUSIONS Expression of beta-MHC appears to be disadvantageous to the mice under severe cardiovascular stress, implying that the alpha→beta-MHC isoform shift observed in cardiac disease may be a maladaptive response. (J Am Coll Cardiol 2004;44:2390–7) © 2004 by the American College of Cardiology Foundation

Expression of alpha- and beta-myosin heavy chain (MHC), the two functionally distinct cardiac MHC isoforms is species-dependent and tightly controlled by developmental and hormonal factors (1,2). Relative expression levels of these isoforms can be altered in disease states such as cardiac failure or hypertrophy (3). For example, in failing adult mouse hearts, a shift from the normally predominant alpha-MHC toward beta-MHC is often observed (4). Similarly, up-regulation of beta-MHC transcription can serve as an early and sensitive marker of cardiac hypertrophy (5). Importantly, recent evidence shows that alpha-MHC expression is down-regulated in human hearts as well (6–8). Although beta-MHC is the predominant isoform in normal adult human hearts, it has been suggested that even small shifts in relative isoform expression can significantly alter cardiomyocyte power output (9). Beta-MHC is characterized by lower adenosine triphosphatase activity and lower filament sliding velocity, but can generate cross-bridge force with a higher economy of energy consumption than alpha-MHC (10–12). This suggests that a shift from alpha- to beta-MHC might be an adaptative response in order to preserve energy. On the other hand, depressed contractile function can promote disease progression (13,14). Therefore, it is conceivable that the decrease in contractile function due to increased beta-MHC might outweigh the benefits of improved economy and ultimately dictate clinical outcome. It has recently been shown that improvement of left ventricular (LV) function is associated with a coordinate increase in alpha- and a decrease in beta-MHC messenger ribonucleic acid (mRNA) expression in patients with idiopathic dilative cardiomyopathy (15). These findings support the notion that increased beta-MHC expression may have a detrimental effect on failing hearts. However, apart from circumstantial evidence, there are no direct data available to resolve this controversy.

The present study was designed to investigate the impact of the two cardiac MHC isoforms on cardiac function and their role during cardiovascular stress. Our aim was to address the critical question of whether increased beta-MHC expression has a beneficial or detrimental effect on the failing heart. Although not directly comparable to MHC isoform shifts in failing human hearts because of the isoform differences between the two, we decided to use a mouse model with near-complete replacement of alpha-MHC with beta-MHC, because this would allow us to test a proof-of-principle in a well-defined animal model with altered cardiac isoform content. Previously, we generated transgenic (TG) mice expressing predominantly beta-MHC instead of alpha-MHC in the heart to study how the reduced mechanical function of beta-MHC observed in vitro was reflected in vivo (16). As expected, cardiac contractile function was significantly reduced in vivo, but animals were healthy, had a normal life span, and showed no

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Abbreviations and Acronyms

dP/dt_{max} = first derivative of left ventricular pressure
LV = left ventricle/ventricular
MHC = myosin heavy chain
NTG = nontransgenic
TG = transgenic

cardiac pathology at the light or electron microscope levels. These data indicated that the alpha→beta-MHC isoform shift is well tolerated and by itself does not induce disease. In the present study, these TG mice were used to investigate how a pre-existing shift from alpha- to beta-MHC affects cardiac function under chronic mechanical or pharmacologic cardiovascular stress.

METHODS

Transgenic animals. All animal procedures were in accordance with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee. We previously generated TG mice with cardiac-specific expression of beta-MHC (16). The cardiomyocyte rigidly controls overall stoichiometry of the sarcomeric protein pool, such that increased TG at the mRNA level does not lead to increases in total protein (17); that is, there is no "overexpression." The steady-state levels of endogenous protein are down-regulated and replaced proportionally by the TG protein. Therefore, we achieved near-complete replacement of endogenous alpha-MHC with the TG protein. Normally, adult mouse cardiomyocytes contain nearly 100% alpha-MHC. In this study, two different FVB/N mouse lines were used: line 102 bred to homozygosity with 73% replacement of alpha- with beta-MHC, and line 137 heterozygotes with 84% protein replacement. Apart from mild left atrial enlargement in line 137 (but not in line 102), the morphology of the TG hearts was normal (16), and the life spans in both lines did not differ from those of NTG animals.

Post-infarction failure model. Three-month-old male mice under isoflurane anesthesia were intubated and ventilated. After lateral thoracotomy, the anterior descending branch of the left coronary artery was ligated 1 to 2 mm below the left auriculum. Cyanosis and akinesia of the affected area confirmed complete ligation of the vessel. In sham operations, the suture was passed under the artery at the same location. The chest was closed, and the animal allowed to recover. Serial echocardiographic studies under isoflurane anesthesia were performed to monitor heart function. After sacrifice, endocardial areas of infarcted and non-infarcted myocardium were planimetered (18).

Chronic isoproterenol challenge. Under isoflurane anesthesia, subcutaneous osmotic pumps (Durect Corp., Cupertino, California) were implanted between the scapulae in 2- to 3-month-old female mice and 60 mg/kg/day isoproterenol in 0.02% ascorbic acid (Sigma, St. Louis, Missouri), or vehicle only was delivered. Systemic blood pressure was measured in conscious mice using a tail-cuff device (Visitech Systems, Apex, North Carolina).

Involuntary exercise. Male 2-month-old mice were adapted by beginning with 10-min swimming sessions twice daily, 7 days a week. Session length was increased by 10 min/day until reaching 90 min, then continued at twice 90 min through to day 28. Mice swam in tanks with a surface area of 225 cm^2 per mouse and a water temperature of 30 to 32°C. Mice were continuously monitored to ensure that mice could not rest by floating or climbing onto weaker animals. Exercise capacity was evaluated using treadmills (Omnipacer LC4, Omnitech Electronics, Columbus, Ohio) (19). On days 24 to 26 of the swim training, mice were adapted to the treadmills. On days 27 to 29, treadmills were set at a 7° incline, and speed was increased in 10-min intervals from 15 to 20, 25, and 30 m/min. Beam breaks of the infrared detection system installed at the end of the belt before the shock grid were counted, and data of days 27 to 29 were averaged. Echocardiography was performed before and after swim training.

Acute dobutamine challenge. Cardiac hemodynamics were measured in intact, closed-chest anesthetized mice of either gender (20). Dobutamine was administered in increasing concentrations (1 to 32 ng/min/g body weight), and LV pressures were recorded.

Statistics. Data are presented as the mean value ± SEM and were analyzed using InStat Version 2.03 (default settings). For comparisons of data from two groups, *t* tests were used. For comparisons of multiple groups, one-way analysis of variance followed by the Tukey-Kramer multiple comparisons test was used. For all tests, $p < 0.05$ was considered significant.

RESULTS

Post-infarction failure model. Thirty-one beta-TG mice (13 from line 102 and 18 from line 137) and 33 nontransgenic (NTG) littermates underwent coronary ligation. The

Table 1. Post-infarction Body Weights, Organ Weights, and Infarct Sizes

	Body Weight (g)	LV Weight (mg)	LV/Body Weight Ratio (mg/g)	Lung Weight (mg)	Liver Weight (g)	Infarct Size (%)
NTG sham	32.8 ± 0.8	100 ± 2	3.0 ± 0.1	174 ± 10	1.5 ± 0.1	
TG sham	32.2 ± 0.8	101 ± 2	3.1 ± 0.1	174 ± 6	1.6 ± 0.1	
NTG infarct	32.4 ± 0.8	111 ± 3†	3.4 ± 0.1	175 ± 6	1.7 ± 0.1	30.3 ± 2.6
TG infarct	28.8 ± 1.2	118 ± 4†	4.1 ± 0.3*†	285 ± 33*†	1.5 ± 0.1	29.6 ± 1.9

*$p < 0.05$ NTG infarct versus TG infarct. †$p < 0.05$ infarct versus sham. n = 8 to 10 in sham groups; n = 11 in infarct groups. Data are presented as the mean value ± SEM. LV = left ventricular; NTG = nontransgenic; TG = transgenic.

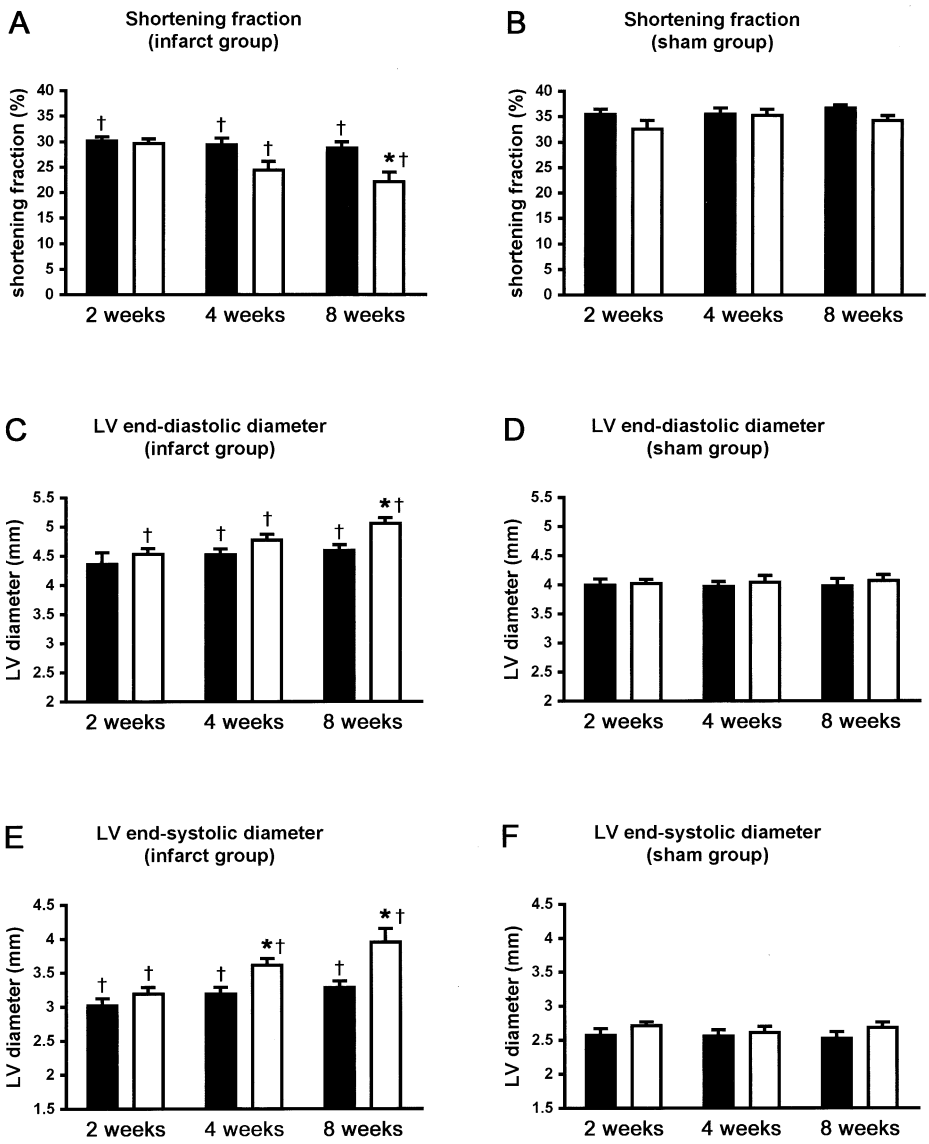

Figure 1. Echocardiographic assessment of left ventricular (LV) function after coronary ligation. **Solid bars** = nontransgenic (NTG); **open bars** = transgenic (TG). *$p < 0.05$ TG versus NTG. †$p < 0.05$ infarct group versus respective sham group (n = 11 in NTG infarct group; n = 10 in TG infarct group; n = 10 in NTG sham group; n = 8 in TG sham group).

data gathered from both line 102 and line 137 were similar and subsequently were grouped. Postoperatively, there were no differences in survival between TG and NTG animals. Within the first 10 days after surgery, 54% of the TG mice and 60% of the NTG mice died due to either LV failure or LV rupture, whereas 90% of the 20 sham-operated animals survived. There were no further deaths during the protocol.

In the surviving animals, infarct size was no different between groups (30.3% of total LV endocardial area in NTG, 29.6% in TGs). Left ventricular weight was increased in both groups but did not differ between NTG and TG animals after infarct (Table 1). Because TG mice lost ~10% of their body weight, this resulted in an increased LV/body weight ratio in TG mice. There was no difference in tibial length (NTG sham: 17.7 ± 0.1 mm: TG sham: 17.7 ± 0.1 mm: NTG infarct: 17.8 ± 0.1 mm: TG infarct: 17.8 ± 0.1 mm). Importantly, lung weights were significantly higher in TG animals with infarcts, indicating substantial LV congestion (Table 1). In contrast, liver weights did not differ, indicating that congestion was restricted to the left side of the heart.

Left ventricular chamber size and function was studied in two-week intervals after surgery using echocardiography (Fig. 1). There were no significant differences between the sham groups at any time. The NTG hearts displayed only a very mild loss in shortening fraction postoperatively, whereas shortening fraction significantly and progressively declined in TG animals (Fig. 1A). Left ventricular chamber size significantly increased over time, but at a faster rate in TG mice (Figs. 1C and 1E). The septum and free wall thickness did not change (data not shown). That we observed in NTG mice a much more subtle LV functional decline, as reported by other groups using different mouse

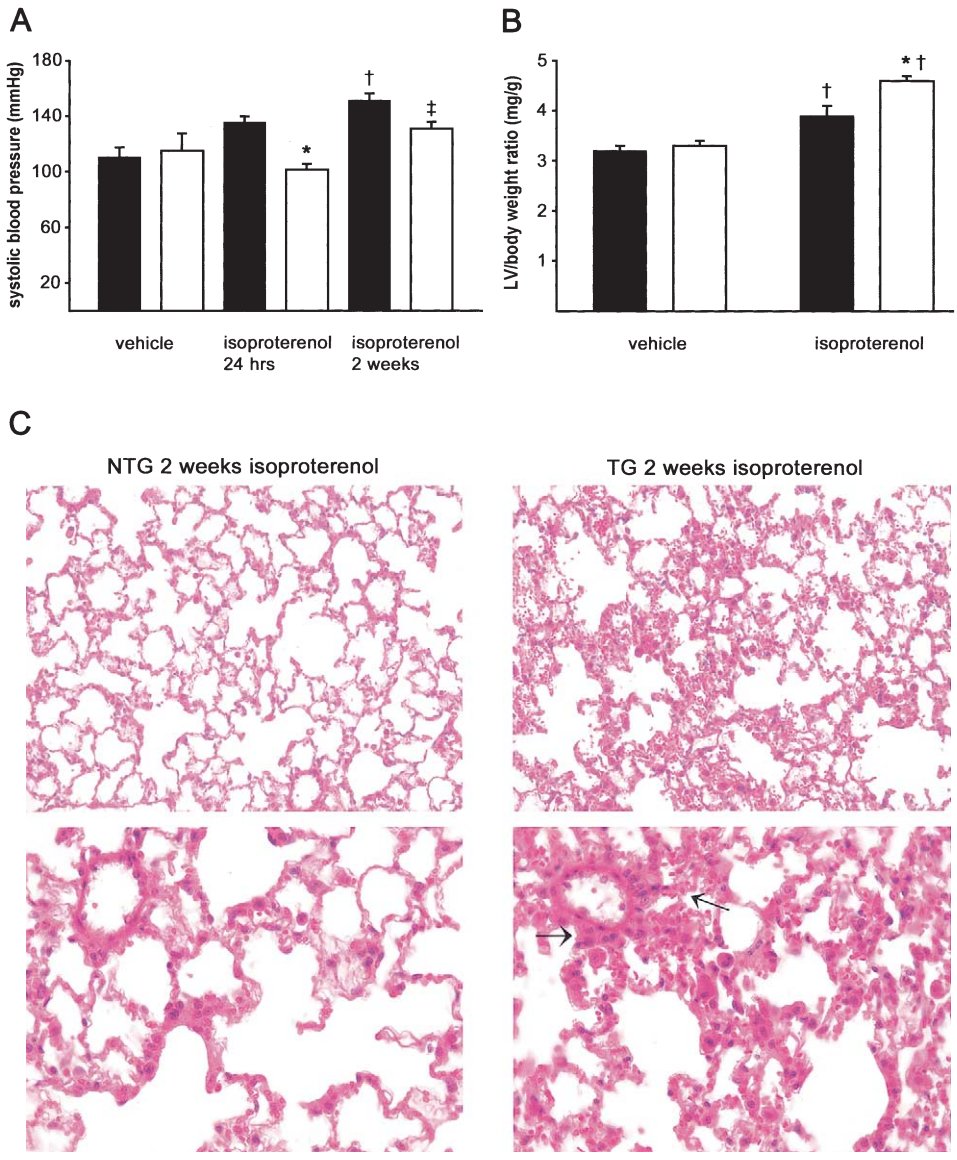

Figure 2. **(A)** Systolic blood pressure after osmotic pump implantation (n = 9 to 11). **(B)** Ratio of left ventricular weight to body weight two weeks after pump implantation. **Solid bars** = nontransgenic (NTG); **open bars** = transgenic (TG). *p < 0.05 TG versus NTG. †p < 0.05 versus respective vehicle group. ‡p < 0.05 24 h versus 2 weeks (n = 8 to 12). **(C)** Hematoxylin-eosin–stained 5-μm sections of lung tissue. Note perivascular edema and engorged alveolar capillaries filled with erythrocytes in the TG lung **(arrows)**.

strains (3,18,21), is most likely due to the decreased susceptibility toward heart failure exhibited by the FVB/N strain (22). We noted that NTG hearts showed only a minor increase of beta-MHC protein (≤5%) at the end of the two-month follow-up period after infarction. In the TG hearts, the relative amount of beta-MHC was also decreased at this time point. The decrease was somewhat dependent on the individual infarct size and, in some instances, dropped from 80% to 40% to 60% of total MHC. Even in the face of this decrease, the beta-MHC isoform shift resulted in faster functional deterioration as compared with the NTG hearts.

Chronic isoproterenol infusion. As an alternative way of stressing the cardiovascular system, TG and NTG animals from line 137 underwent two weeks of continuous infusion of the beta-agonist isoproterenol. Blood pressure measurements demonstrated that systemic blood pressure was lower in TG compared with NTG animals after the first 24 h of isoproterenol infusion (Fig. 2A). At the end of the two-week infusion period, blood pressure recovered but did not rise above baseline level. In contrast, the NTG animals responded with an increase in blood pressure within 24 h. Over the two-week infusion period, blood pressure continued to rise in the NTG animals (Fig. 2A). The NTG animals showed a 22% increase in LV/body weight ratio, whereas this ratio increased by 40% in TG mice (Table 2, Fig. 2B). Importantly, despite the more pronounced cardiac hypertrophy exhibited by the TG mice, LV mass was not sufficient to meet the hemodynamic demands under isoproterenol, resulting in increased wet lung weight (Table 2). In

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Table 2. Body and Organ Weights After Chronic Isoproterenol Challenge

	Body Weight (g)	LV Weight (mg)	RV Weight (mg)	Atrial Weight (mg)	LV/Body Weight Ratio (mg/g)	Lung Weight (mg)
NTG vehicle	25.1 ± 0.3	81 ± 2	22 ± 1	8 ± 1	3.2 ± 0.1	153 ± 2
TG vehicle	25.8 ± 0.8	85 ± 2	22 ± 1	14 ± 1*	3.3 ± 0.1	148 ± 5
NTG isoproterenol	26.8 ± 0.4	106 ± 4†	26 ± 2†	13 ± 1†	3.9 ± 0.2†	168 ± 5
TG isoproterenol	25.6 ± 0.5	118 ± 2*†	30 ± 2*†	23 ± 2*†	4.6 ± 0.1*†	207 ± 14†

*$p < 0.05$ TG versus NTG. †$p < 0.05$ vehicle versus isoproterenol. n = 8 to 12 animals per group. Data are presented as the mean value ± SEM.
RV = right ventricular; other abbreviations as in Table 1.

histologic sections, the lungs of the isoproterenol-treated TG mice showed areas with acute pulmonary congestion and edema (Fig. 2C). There was no change in the alpha-/beta-MHC protein ratio induced by isoproterenol.

Involuntary exercise. Both NTG and TG mice from line 137 tolerated the exercise regimen very well. During the swimming sessions, no obvious differences in performance could be observed between TG and NTG animals. For quantitative assessment of exercise capacity, treadmills were used. The number of beam breaks at various treadmill speeds was no different between TG and NTG animals (Fig. 3A). Consistent with these data, no signs of LV congestion were detectable (wet lung weights in NTG non-exercised 155 ± 3 mg, TG non-exercised 145 ± 6 mg, NTG exercised 150 ± 5 mg, TG exercised 142 ± 4 mg). Over the four-week training period, mice lost 15% to 17% of their body weight (NTG non-exercised 31.3 ± 1.3 g, TG non-exercised 31 ± 1.4 g, NTG exercised 26.8 ± 0.6 g, TG exercised 25.8 ± 0.6 g*, *$p < 0.05$ vs. respective non-exercised group). The LV weights were no different between groups (NTG non-exercised: 85 ± 3 mg: TG non-exercised: 86 ± 1 mg: NTG exercised: 90 ± 5 mg: TG exercised: 88 ± 2 mg). Consequently, the ratio of LV to body weight increased by ~22% in NTG and TG animals (Fig. 3B).

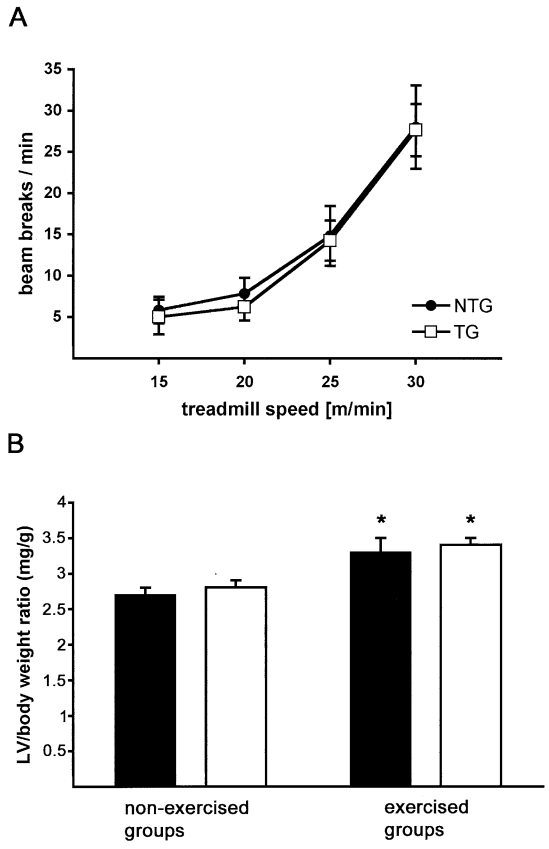

Figure 3. **(A)** Exercise capacity on the treadmill of transgenic (TG) **(open squares)** and nontransgenic (NTG) **(solid circles)** mice at the end of the four-week swim training. **(B)** Ratio of left ventricular (LV) weight to body weight after four weeks in exercised and non-exercised groups. **Solid bars** = NTG; **open bars** = TG. *$p < 0.05$ versus respective non-exercised group (n = 4 to 6 in each group).

Left ventricular function before and after training was compared using echocardiography (Fig. 4). Minor but not significant differences in shortening fraction were found: NTG but not TG hearts improved slightly due to exercise training. Exercise training moderately increased end-systolic thickness of the LV free wall in NTG but not in TG mice. The diameters of TG LVs were slightly larger after training, but these differences did not reach statistical significance. The relative amounts of alpha-/beta-MHC protein did not change in exercised NTG and TG animals.

Acute dobutamine challenge. As previously reported (16), contractile function in TG hearts (line 102) was moderately depressed at baseline. Dobutamine infusion increased LV contractile performance in both TG and NTG mice (Fig. 5). However, the positive inotropic response was less pronounced in TG compared with NTG animals: dP/dt_{max} increased in NTGs by 50% at the highest dobutamine dose, but only by 30% over baseline in TGs (Fig. 5B). The LV peak pressure increased at a similar rate in both groups (Fig. 5A).

DISCUSSION

To gain insight into the impact that MHC isoform shifts might have on cardiac disease, we subjected TG mice stably expressing beta-MHC in the heart to chronic cardiovascular stress. The data demonstrate that beta-TG hearts were at a disadvantage under chronic isoproterenol challenge or in a post-infarction failure model. Therefore, the alpha→beta-MHC isoform shift associated with cardiac disease appears to be detrimental rather than beneficial under these conditions.

It has been suggested that expression of beta-MHC would save intracellular energy due to the higher economy of energy consumption (10,11). Considering that 75% of all ATP consumed in the cardiomyocyte is used for cross-bridge cycling (23), even small changes in economy could

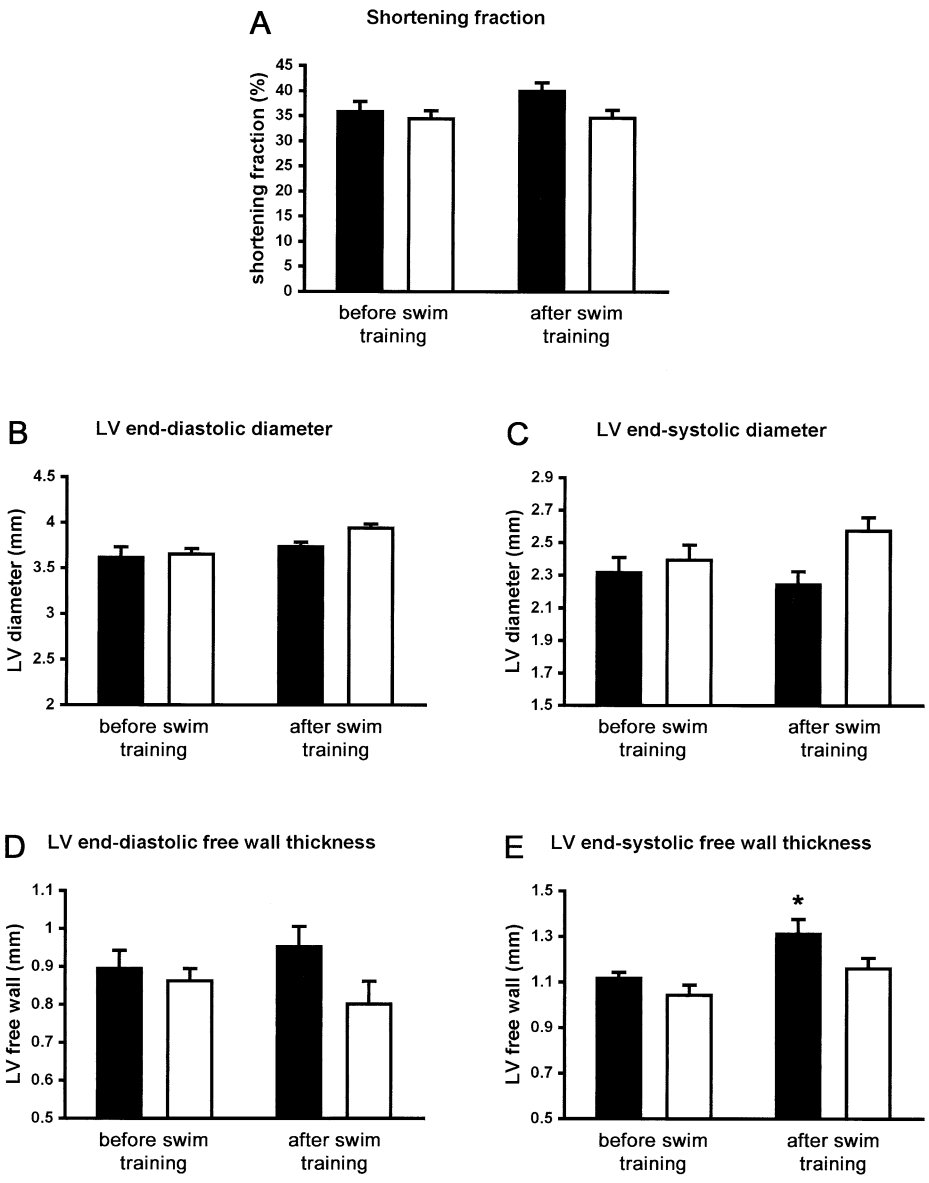

Figure 4. Left ventricular (LV) function and chamber dimensions before and after swim training, as assessed by echocardiography. **Solid bars** = nontransgenic (NTG); **open bars** = transgenic (TG). *$p < 0.05$ before versus after swim training ($n = 5$ or 6 in each group).

therefore lead to substantial alterations in overall energetic homeostasis. Consequently, hearts predominantly expressing beta-MHC should be better equipped to handle the increased energy demand under cardiovascular stress. However, the present data demonstrate that this postulated advantage has no significant bearing on the heart's ability to tolerate chronic stress. This is consistent with a recent study by Crilley et al. (24) on the cardiac bioenergetic deficit in patients with familial hypertrophic cardiomyopathy. They showed that energy metabolism was impaired in individuals carrying three different mutations in the beta-MHC, cardiac troponin T, or myosin-binding protein C genes. Importantly, even in gene mutation carriers without hypertrophy, a bioenergetic deficit could be observed. Therefore, although there is strong evidence for a link between cardiac energetics and development of a disease phenotype in humans and animals (25,26), energy availability alone cannot account for disease penetrance. Although beta-MHC may save intracellular energy, it appears that this is not the determining factor for the clinical outcome in the mice.

If not energy homeostasis, which factor then is responsible for the accelerated disease progression observed in beta-MHC–expressing mouse hearts? There is currently much controversy over the hypothesis that abnormalities in contractility initiate the heart failure syndrome and drive its progression (27). Although most studies agree that the contractility of failing human hearts is depressed, we lack direct evidence as to whether this is a primary or secondary event. Clearly, contractile function is already depressed in beta-TG mice under normal husbandry conditions, but the MHC isoform shift is well tolerated and does not induce cardiac disease (16). However, in our post-infarction failure

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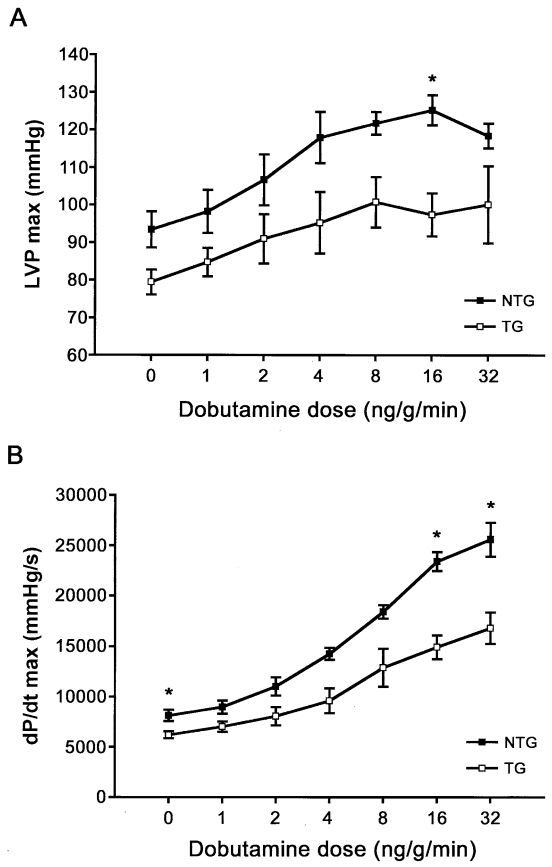

Figure 5. Hemodynamic parameters under acute dobutamine challenge in increasing doses in the intact, closed-chest animal. **(A)** Left ventricular systolic peak pressure (LVP). **(B)** Maxima of the first derivative of left ventricular pressure (dP/dt_{max}). **Solid squares** = nontransgenic (NTG); **open squares** = transgenic (TG). *$p < 0.05$ TG versus NTG ($n = 4$ or 5 per group).

model, contractile function of TG hearts deteriorates at a faster rate and to a greater extent than in NTG controls, implying that depressed contractility may be a primary event. This interpretation is supported by numerous other animal studies showing that depressed contractility significantly contributes to the initiation and progression of heart failure (13,14).

Interestingly, beta-TG hearts respond to chronic catecholamine challenge not with a dilative phenotype as in the post-infarction failure model but with increased cardiac hypertrophy. However, these differences were not reflected at the molecular level, and the changes that did occur in levels of the usual indicators, such as atrial natriuretic factor or phospholamban, did not differ significantly between the two groups (data not shown). However, the adaptative response clearly was insufficient to meet hemodynamic demands under isoproterenol stimulation in beta-TG mice. The interpretation of these experiments is limited by the fact that we cannot exclude that the peripheral vascular sensitivity to isoproterenol is different in TG animals. However, if there were substantial differences in the response of the peripheral vasculature to catecholamines, these should have become apparent in our endurance exercise experiments. We could not detect any differences in exercise capacity in mice running on treadmills.

The chronic isoproterenol challenge and the post-infarction studies indicate that the presence of a high level of beta-MHC is detrimental under chronic cardiovascular stress, presumably due to the decreased contractile performance of the cardiomyocytes. However, a direct mechanistic link between contractility and the development of heart failure is still lacking. It has been postulated that cellular stress sensors located at or linked to the cytoskeleton are important for the modulation of the cellular response to mechanical stress (28,29). It may be that such stress sensors also play an important role in modulating disease progression. Because these sensors are linked to the cytoskeleton, their signaling output would depend not only on external forces but also on the ability of the cell to contract under these conditions. It is therefore conceivable that the readout of mechanical stress sensors is altered in beta-TG mice, which may result in more pronounced remodeling, as observed both under isoproterenol challenge and in the post-infarction failure model. However, as the nature and downstream pathways of the postulated stress sensors remain to be identified, the hypothesis is difficult to test at this point.

Furthermore, it is not known whether intracellular stress sensors can distinguish between "physiologic" and "pathologic" stresses, provided that these indeed are two distinct entities. Under mild, physiologic stress, both TG and NTG hearts showed a similar hypertrophic response without signs of heart failure. It appears that the shift from alpha- to beta-MHC can be compensated for under physiologic endurance exercise, but counterbalancing mechanisms become overwhelmed under more intense cardiovascular strain. Whether this is universally true for all forms of pathologic cardiovascular stress remains to be determined.

We were interested in quantifying the amount of cardiac reserve available in beta-TG mice and measured the hemodynamic response to increasing doses of dobutamine in a closed-chest preparation. As expected, the TG hearts displayed a considerable amount of cardiac reserve, but at high dobutamine doses, failed to increase dP/dt_{max} to the same extent as NTG hearts. As is often seen in heart disease patients undergoing stress echocardiography, the acute dobutamine stress test was able to unmask the limitations in cardiac reserve. However, we could not observe a discrete threshold, but rather a gradual response: the higher the dobutamine dose, the more pronounced the difference in performance between TG and NTG hearts. This suggests that there might be intermediate stages between a compensated and an uncompensated response. We suspect that, indeed, such an intermediate state occurred in our exercised mice: we observed a trend toward LV dilation, but no signs of restricted exercise capacity or cardiac decompensation.

Conclusions. The present study shows that cardiac expression of beta-MHC is disadvantageous under chronic isoproterenol challenge and in a post-infarction failure model.

Although we do not believe that the murine data are directly applicable to human heart failure, they do imply that the alpha→beta-MHC shift observed in human heart disease may be more than just a marker for the severity of the disease state and might actually contribute to its development. Our data validate the general approach and justify the extension of carrying out MHC isoform shifts in models that more closely resemble the human heart such as the rabbit (30). The present findings have important implications for the development of future therapeutic strategies aimed at altering the mechanical characteristics of the MHC molecule.

Reprint requests and correspondence: Dr. Jeffrey Robbins, Molecular Cardiovascular Biology, MLC 7020, Children's Hospital, 3333 Burnet Avenue, Cincinnati, Ohio 45229-3039. E-mail: jeff.robbins@cchmc.org.

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