Ablation of Cardiac Myosin-Binding Protein-C Accelerates Stretch Activation in Murine Skinned Myocardium

Julian E. Stelzer, Sandy B. Dunning, Richard L. Moss

Abstract—Cardiac myosin binding protein-C (cMyBP-C) is a thick filament accessory protein that binds tightly to myosin, but despite evidence that mutations in the cMyBP-C gene comprise a frequent cause of hypertrophic cardiomyopathy, relatively little is known about the role(s) of cMyBP-C in myocardium. Based on earlier studies demonstrating the potential importance of stretch activation in cardiac contraction, we examined the effects of cMyBP-C on the stretch activation responses of skinned ventricular preparations from wild-type (WT) and homozygous cMyBP-C knockout mice (cMyBP-C−/−) previously developed in our laboratory. Sudden stretch of skinned myocardium during maximal or submaximal Ca2+ activations resulted in an instantaneous increase in force that quickly decayed to a minimum and was followed by a delayed redevelopment of force (ie, stretch activation) to levels greater than prestretch force. Ablation of cMyBP-C dramatically altered the stretch activation response, ie, the rates of force decay and delayed force transient were accelerated compared with WT myocardium. These results suggest that cMyBP-C normally constrains the spatial position of myosin cross-bridges, which, in turn, limits both the rate and extent of interaction of cross-bridges with actin. We propose that ablation of cMyBP-C removes this constraint, increases the likelihood of cross-bridge binding to actin, and speeds the rate of delayed force development following stretch. Regardless of the specific mechanism, acceleration of cross-bridge cycling in cMyBP-C−/− myocardium could account for the abbreviation of systolic ejection in this mouse as a direct consequence of premature stretch activation of ventricular myocardium. (Circ Res. 2006;98:1212-1218.)

Key Words: cross-bridge kinetics ■ cooperativity ■ contractile proteins ■ regulation of contraction

Familial hypertrophic cardiomyopathy (FHC) in humans is an inherited autosomal dominant disease that, in most cases, is caused by mutations in sarcomeric proteins. Mutations in one sarcomeric protein, cardiac myosin binding protein-C (cMyBP-C), have been reported to be among the most common causes of FHC,1–3 and yet relatively little is known about its function. To better understand the role of cMyBP-C in myocardial contraction, we previously developed a cMyBP-C-null mouse (cMyBP-C−/−) specifically lacking cMyBP-C.4 cMyBP-C−/− mice exhibit severe ventricular hypertrophy, systolic dysfunction evident in abbreviation of ejection and reduced stroke volume, and diastolic dysfunction seen as a decreased rate of isovolumic relaxation.4,5 The present study was undertaken to determine the effects of ablation of cMyBP-C on the dynamic mechanical properties of myocardium to understand the basis for functional deficits in the cMyBP-C−/− mouse.

During both the isovolumic and ejection phases of systole, the left ventricle undergoes significant torsional deformation as the apex twists counterclockwise (as viewed from the apex) relative to the base,6 with the endocardium exhibiting greater torsional strain than the epicardium.7 Such a differential in torsional strain across the ventricular wall might be expected to modulate systolic force generation and the timing of force production in different parts of the wall. Because higher force regions would stretch lower force regions during torsional twist, there should be stretch activation (delayed development of force) of subendocardial myocardium, similar to the delayed development of force developed by isolated cardiac muscle following stretch.8 Stretch activation may thus play a role in myocardial power generation and systolic ejection.9 In this regard, a recent study showed that the rate and amplitude of the stretch activation response in mouse myocardium varies with the level of activation, suggesting that the response to stretch likely is regulated on a beat-to-beat basis.10,11

Given the systolic dysfunction evident in the cMyBP-C−/− mouse, we hypothesized that the stretch activation response in cMyBP-C−/− myocardium would be reduced or absent and thereby account for reduced systolic ejection. Myosin binding protein-C (MyBP-C) is bound to thick filaments in distinct stripes (between 7 to 11) in the C-zone of the A-bands in each half sarcomere.12 The C terminus of MyBP-C contains a binding domain,13 with high affinity for myosin subfragment-214 and the myosin molecule contains sites for binding MyBP-C within the S2 and light meromyosin (LMM) fragments.15,16 Because the periodicity of MyBP-C17 is very similar to that of myosin (42.9 nm18) and it binds to myosin...
in a functionally important region, it is plausible that cMyBP-C plays an important role in the regulation of cross-bridge movement and interaction with actin, as suggested previously.10–21

To explore whether the reduced ejection fraction observed in cMyBP-C−/− hearts is attributable to alterations in stretch activation, we compared stretch activation responses in wild-type (WT) and cMyBP-C−/− myocardium. Contrary to our initial hypothesis that stretch activation in cMyBP-C−/− myocardium is reduced or absent, we observed no change in the amplitude of stretch activation, which by itself is not consistent with depressed systolic function in the null mouse. However, the rate of rise of force during delayed force development was substantially faster than in WT myocardium, such that the earlier than normal stretch activation response could account for the premature cessation of ejection observed in cMyBP-C−/− mice.

Materials and Methods

Transgenic Mice

Homozygous cardiac MyBP-C–null (cMyBP-C−/−) mice were generated previously24 and maintained in a breeding colony. For experimental measurements, adult mice (3 to 6 months of age) of either sex were used. Aged-matched WT mice of the same background strain as cMyBP-C−/− mice (SV/129; Taconic Farms, Germantown, NY) were used as controls. All procedures involving animal use were performed according to institutional guidelines approved by the Association for Assessment and Accreditation of Laboratory Care International (AAALAC).

Solutions

Solution compositions were calculated using the computer program developed by Fabiato22 and the stability constants listed by Godt and Lindley23 corrected to pH 7.0 and 22°C. All solutions contained (in mmol/L) 100 N,N-bis(2 hydroxy-ethyl)-2-aminoethanesulfonic acid (BES), 15 creatine phosphate, 5 dithiothreitol, 1 free Mg2+, and 4 MgATP. In addition, pCa 9.0 solution contained (in mmol/L) 7 EGTA and 0.02 CaCl2, pCa 4.5 contained 7 EGTA and 7.01 CaCl2, and preactivating solution contained 0.07 EGTA. Ionic strength of all solutions was adjusted to 180 mmol/L with potassium propionate. Solutions containing different amounts of [Ca2+]o were prepared by mixing appropriate volumes of stock solutions of pCa 9.0 and pCa 4.5.

Skinned Myocardial Preparations

Skinned ventricular myocardium for stretch activation experiments was prepared as previously described.24 Briefly, following IP injection of 5000 U heparin/kg body weight, S129 WT mice (3 to 6 months old) were anesthetized with inhaled isoflurane (15% isoflu-rane in mineral oil) in accordance with institutional animal care guidelines. Their hearts were excised and right and left ventricles were dissected at room temperature in a relaxing solution containing (in mmol/L) 100 KCl, 20 imidazole, 7 MgCl2, 2 EGTA, and 4 MgATP (pH 7.0) and were then rapidly frozen in liquid nitrogen. To prepare skinned myocardial preparations, the frozen ventricles were thawed and homogenized in relaxing solution containing (in mmol/L) 250 NaCl, 1% Triton X-100. After 30 minutes, the skinned preparations were washed with fresh relaxing solution and resuspended in relaxing solution containing 250 µg/mL saponin and 1% Triton X-100. After 30 minutes, the skinned preparations were washed with fresh relaxing solution and were dispersed in ~50 mL of relaxing solution in a glass Petri dish. The dish was kept on ice at all times, except during the selection of individual preparations for mechanical experiments.

Figure 1. Stretch activation response in murine WT myocardium. The force transient shown (bottom) is typical of typical stretch activation responses of WT myocardium following a stretch of 1% of muscle length (top). Once a steady-state isometric force of ~50% of maximal was achieved in the presence of Ca2+, the muscle was stretched and then held at the longer length for 5 seconds. The stretch activation response was multiphasic, as reported previously.10 The recorded variables are labeled on the force record and described in the text.

Apparatus and Experimental Protocol

Skinned preparations with well-defined edges and no evident free ends in the middle region were transferred from the Petri dish to a stainless steel experimental chamber25 containing relaxing solution. The ends of a preparation were attached to the arms of a motor (model 312B, Aurora Scientific Inc) and force transducer (model 403; Aurora Scientific Inc), as described earlier.25 The chamber assembly was then placed on the stage of an inverted microscope (Carl Zeiss) fitted with a ×40 objective and a closed-circuit television camera (model WV-BL600, Panasonic). Bitmap images of the preparations were acquired using an AGP 4X/2X graphics card and associated software (ATI Technologies Inc) and were used to assess mean sarcomere length during the course of each experiment. Changes in force and motor position were sampled (16-bit resolution, DAP5216a; Microstar Laboratories, Bellevue, Wash) at 2 kHz using SLControl software developed in our laboratory26 and saved to computer files for later analysis. Fiber force during the experiments was also recorded on a digital oscilloscope (Nicolet Instrument Corporation, Madison, Wis).

At the start of each experiment, the preparations were stretched to a mean sarcomere length of ~2.12 µm, such that resting force was minimal for measurements of steady-state Ca2+-activated force and stretch activation. The preparations were initially activated at pCa 4.5 to establish the maximal tension (Pm), and then force was recorded in pCa 9.0 to establish the resting force, which was then subtracted from the total force in activating solutions to yield the Ca2+-activated force (P). For stretch activation experiments, fibers were activated in pCa solutions, yielding maximum force (pCa 4.5), ~50% of maximum force, and ~25% of maximum force. When steady-state tension was reached, a rapid stretch of 1% fiber length (L0) was imposed and held for 5 seconds before returning the fiber to pCa 9.0.

The stretch activation variables measured are shown in Figure 1. All amplitudes were normalized to prestretch isometric tension to allow comparisons between different levels of activation. Amplitudes were measured as follows: P1, measured from prestretch steady-state force to the peak of phase 1; P2, measured from prestretch steady-state force to the minimum force decay; P3,
measured from prestretch steady-state force to the peak value of delayed force; and \( P_{\text{d}} \), difference between \( P_1 \) and \( P_2 \).

Apparent rate constants were derived for phase 2 \( (k_{\text{d2}}, \text{sec}^{-1}) \) from the force decay following the peak of phase 1 and for phase 3 \( (k_{\text{d3}}, \text{sec}^{-1}) \) from the point of force reuptake following phase 2 to the completion of delayed force development.

**Data Analysis**

Cross-sectional areas of skinned preparations were calculated by assuming that the preparations were cylindrical and by measuring width of the mounted preparation. Submaximal \( \text{Ca}^{2+} \)-activated force (\( P \)) was expressed as a fraction of the maximum \( \text{Ca}^{2+} \)-activated force (\( P_0 \)) generated at pCa 4.5, ie, \( P/P_0 \). Rate constants of force decay were obtained by fitting a single exponential to the time course of decay, ie, \( \text{y} = \text{a}(1 - \exp(-k_1x)) \), where \( a \) is the amplitude of the single exponential phase and \( k_1 \) is the rate constant of decay. Rate constants of delayed force development were obtained by a double exponential fit, \( \text{y} = \text{aexp}(-k_1x) + \text{bexp}(-k_2x) \), where \( a \) is the amplitude of the first exponential phase that rises with rate constant \( k_1 \) and \( b \) is the amplitude of the second exponential phase rising with rate constant \( k_2 \), or were estimated by linear transformation of the half-time of force redevelopment, ie, \( k_2 = -\ln0.5 \times (t_{1/2})^{-1} \).

All data are reported as means±SEM. Comparisons of stretch activation variables between WT and cMyBP-C\(^{-/-}\) myocardium at different levels of activation were performed using a 1-way ANOVA with a Tukey post hoc test, with statistical significance set at \( P<0.05 \).

**Results**

**Response of WT and cMyBP-C\(^{-/-}\) Mouse Ventricular Myocardium to Stretch**

Figure 1 illustrates a typical response of WT mouse skinned myocardium to a rapid stretch of 1% of initial muscle length during \( \text{Ca}^{2+} \) activation that yielded a prestretch force of \( \approx 50\% \) maximal. The initial rapid increase in force (phase 1) is coincident with stretch and is caused by strain of attached cross-bridges\(^27\); phase 1 is followed by a rapid decline in force (phase 2) and a delayed force recovery (phase 3) that persists for several seconds before ultimately decaying to the original isometric force. Amplitudes of these phases are indicated in Figure 1 as \( P_0 \), \( P_1 \), and \( P_2 \). Note that \( P_1 \) (the minimum at the end of force decay) can fall below prestretch isometric force and can have negative values, whereas \( P_1 \) (phase 1 amplitude) and \( P_2 \) (phase 3 amplitude) are always positive. The values of \( P_1 \) in this study were likely underestimated because our data sampling rate was limited, but this variable was not studied in this work.

To allow comparisons of stretch activation responses in WT and cMyBP-C\(^{-/-}\) myocardium at different levels of activation, stretch activation amplitudes were normalized to prestretch isometric force (Figures 2 and 3). The amplitude of phase 1 \( (P_1) \) increased as prestretch isometric force increased and did not differ significantly between WT and cMyBP-C\(^{-/-}\) myocardium (Figure 2) within the time resolution of our force transducer. In WT myocardium \( P_2 \) amplitude was lowest at maximal activation and highest (most positive) at low levels of activation, whereas \( P_2 \) values in cMyBP-C\(^{-/-}\) myocardium were almost always negative and dipped below isometric force at all levels of activation (Figure 3). The dramatic differences in \( P_2 \) amplitude at submaximal levels of activation can be clearly seen when the stretch activation responses of WT and cMyBP-C\(^{-/-}\) myocardium are superimposed at activation levels of \( \approx 50\% \) (Figure 4). The amplitudes of \( P_1 \) and \( P_2 \) were not corrected for resting force, which in each preparation was less than 5% of the maximum active force at pCa 4.5.

The rapid force decay in phase 2 proceeds with an apparent rate constant \( k_{\text{d1}} \) and is thought to represent detachment of cross-bridges that are strained by stretch and their rapid replacement by unstrained cross-bridges. The apparent rate constant of force decay \( (k_{\text{d2}}) \) was slightly faster as the level of activation decreased for both WT and cMyBP-C\(^{-/-}\) (Figure 5) and was dramatically faster in cMyBP-C\(^{-/-}\) myocardium at submaximal levels of activation (Figure 4).

The net delayed recruitment of cross-bridges following stretch can be determined from the amplitude of \( P_3 \), which has been shown to be highly dependent on prestretch isometric force. Normalized values of \( P_3 \) decreased as a function of increased activation level such that proportionately fewer cross-bridges were recruited by stretch at higher levels of activation, a phenomenon that did not differ between WT and cMyBP-C\(^{-/-}\) myocardium at any level of activation. How-
ever, the negative amplitudes of \( P_2 \) in cMyBP-C myocardium increased the overall amplitude of the phase 3 tension transient compared with WT, as indicated by the large increase in \( P_{df} \) for cMyBP-C myocardium (Figure 6).

The stretch activation response (phase 3) does not occur as a simple exponential process\(^{10,30}\) and thus does not fit well with a single exponential equation, especially at low levels of activation. To facilitate comparisons of phase 3 rates of force development (\( k_{df} \)) at different levels of activation, apparent rate constants were derived from the half-times of force development.\(^{10,30}\) As previously reported,\(^{10}\) apparent rate constants of force development (\( k_d \)) in murine myocardium are activation dependent, increasing with level of pre-stretch isometric force (Table). Because \( k_d \) is generally thought to involve the cooperative recruitment of cross-bridges into strongly bound states,\(^{29,31,32}\) the acceleration of \( k_{df} \) at high levels of activation can be understood in terms of an increased number of cross-bridges bound to the thin filament, leaving fewer cross-bridges available for recruitment following stretch, thereby reducing the amplitude of stretch activation (as a fraction of pre-stretch force) and increasing its rate. In the present study, there was no difference in \( k_d \) between WT and cMyBP-C myocardium at maximal activation, but \( k_d \) was significantly accelerated at submaximal levels of activation in cMyBP-C myocardium compared with WT myocardium (Figure 4 and the Table), suggesting that cMyBP-C ablation accelerated the recruitment of cross-bridges into force generating states, thereby accelerating the overall rate of the stretch activation response.

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**Figure 3.** Effect of activation on \( P_2 \) in WT and cMyBP-C myocardium. \( P_2 \) values normalized to pre-stretch isometric force were measured as a function of activation from the force responses to stretches of 1% of muscle length in both WT (●) (\( n=10 \)) and cMyBP-C (▲) (\( n=10 \)) myocardium. Data are means±SEM. *Significantly different from WT, \( P<0.05 \).

**Figure 4.** Stretch activation responses in WT and cMyBP-C myocardium. Force transients following a stretch of 1% of muscle length were recorded from WT and cMyBP-C myocardium activated with \( Ca^{2+} \) to a pre-stretch isometric force of ~50% maximal. The transients are normalized to pre-stretch force, which corresponds to the 0 relative force baseline. Apparent rate constants of force decay (\( k_{rd} \)) and delayed force development (\( k_d \)) were 241 sec\(^{-1}\) and 165 sec\(^{-1}\) for WT myocardium and 378 sec\(^{-1}\) and 29.6 sec\(^{-1}\) for cMyBP-C myocardium, respectively.

**Figure 5.** Effect of activation on \( k_{rd} \) in WT and cMyBP-C myocardium. \( k_{rd} \) values were normalized to pre-stretch isometric force and are plotted as a function of activation level for WT (●) (\( n=10 \)) and cMyBP-C (▲) (\( n=10 \)) myocardium. Data shown were obtained following stretches of 1% of muscle length. Data are means±SEM. *Significantly different from WT, \( P<0.05 \).

**Figure 6.** Effect of activation on \( P_{df} \) in WT and cMyBP-C myocardium. \( P_{df} \) values were normalized to pre-stretch isometric force and are plotted as a function of activation level for WT (●) (\( n=10 \)) and cMyBP-C (▲) (\( n=10 \)) myocardium. The data shown were obtained following stretches of 1% of muscle length. Data are means±SEM. *Significantly different from WT, \( P<0.05 \).
Activation Dependence of Phase 3 Delayed-Force Development in WT and cMyBP-C−/− Myocardium

<table>
<thead>
<tr>
<th>Group</th>
<th>Activation Level (P/P₀)</th>
<th>kₐ (sec⁻¹)</th>
<th>a</th>
<th>kᵢ (sec⁻¹)</th>
<th>b</th>
<th>kᵣ (sec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>1.00</td>
<td>40.8±1.8</td>
<td>1.00</td>
<td>41.4±2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cMyBP-C−/−</td>
<td>1.00</td>
<td>39.6±1.4</td>
<td>1.00</td>
<td>41.1±2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>0.52±0.02</td>
<td>18.5±1.2</td>
<td>0.66±0.03</td>
<td>30.5±2.0</td>
<td>0.34±0.03</td>
<td>4.2±0.8</td>
</tr>
<tr>
<td>cMyBP-C−/−</td>
<td>0.52±0.02</td>
<td>30.9±1.3*</td>
<td>1.00*</td>
<td>31.6±2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>0.24±0.01</td>
<td>10.5±0.9</td>
<td>0.52±0.03</td>
<td>20.5±1.8</td>
<td>0.48±0.03</td>
<td>2.3±0.4</td>
</tr>
<tr>
<td>cMyBP-C−/−</td>
<td>0.27±0.02</td>
<td>21.2±1.3*</td>
<td>1.00*</td>
<td>21.9±1.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rate and amplitude data were obtained from force transients in response to stretches of 1% of muscle length at each of the indicated levels of activation (adjusted by varying free [Ca²⁺]). Data in each case are reported as means±SEM from 10 preparations. As described in Materials and Methods, the apparent rate constants for delayed force recovery were obtained either from conversion of half-times of force recovery (yielding kᵢ) or by fitting each record with a double exponential equation, y = a · exp(−k₁ · x) + b · exp(−kᵢ · x), where a is the amplitude of the first exponential phase with rate constant k₁ and b is the amplitude of the second exponential phase with rate constant kᵢ. *Significantly different from WT, P<0.05.

Discussion

This study was undertaken to investigate the role of cMyBP-C in cardiac contraction and to specifically determine how ablation of cMyBP-C leads to impairment of cardiac function. Earlier in vivo measurements of left ventricular function showed that cMyBP-C−/− mouse hearts have reduced ejection, an abbreviated ejection phase, and decreased rates of relaxation compared with WT controls.4,5 The overall depression of systolic function in null mice occurs despite a normal initial rise in maximal pressure development (dP/dtmax), presumably because the period of ejection is shortened, thereby reducing the volume of blood ejected. Paradoxically, cMyBP-C−/− myocardium exhibits accelerated rates of force development and increased power at submaximal levels of activation,33 possibly as a consequence of enhanced levels of thin filament activation. We have previously suggested that ablation of cMyBP-C increases cooperative activation of the thin filament because of increased likelihood of cross-bridge binding.44 Structural models for such a mechanism emerge from consideration of possible interactions of cMyBP-C with myosin because cMyBP-C has been proposed to restrict myosin interaction with actin by tethering the myosin heads to the thick filament19 or by forming a collar around the backbone of the thick filament.5 Ablation of cMyBP-C would alleviate the constraints on the position of myosin and lead to an increased probability of cross-bridge formation and acceleration of the transition of cross-bridges to force generating states. The resulting upregulation of thin filament activation, or a reduced ability to inactivate the thin filament, can lead to slowed relaxation, which would decrease both the diastolic filling time and the end diastolic volume, ultimately decreasing ejection fraction and depressing overall systolic function.

The stretch activation response is critical to the generation of oscillatory power required for the beating of insect wings35 and is also a prominent process in heart muscle,8,31,36 where it is thought to play an important role in modulating pump function and power output.9,37 In this respect, it has been observed that some mutations of sarcomeric proteins in insect muscles38 and mouse myocardium36 alter the stretch activation response and power output. From such studies, it appears that both the rate of force development (kₐ) and the amplitude of the additional force recruited by stretch activation (P₃) are associated with modulation of power output, an important determinant of systolic function.

The present study shows that the stretch activation response in myocardium is dramatically altered by ablation of cMyBP-C. When isometrically contracting myocardium is suddenly stretched, there is a corresponding increase in force caused by strain of attached cross-bridges. In turn, strain results in detachment of some cross-bridges and a decrease in force, a process that is quickly reversed by attachment of new cross-bridges and redevelopment of force to greater than presstretch isometric levels. cMyBP-C−/− myocardium exhibits increases in the apparent rate constant and amplitude of the phase 2 force decay compared with WT. The N terminus of cMyBP-C binds to myosin S2 and/or actin and thus might modulate the interaction between myosin and actin,3,39–41 so that removal of cMyBP-C could alter rates of cross-bridge attachment or detachment or both. Genetic deletion or acute biochemical extraction of cMyBP-C−/− from myocardium increases shortening velocity.19,33 presumably because of increased rates of cross-bridge detachment. Accelerated detachment would explain the observed increase in kᵢ and the greater decline in force immediately following stretch of cMyBP-C−/− myocardium. It is also possible that accelerated transitions to force generating states (kᵢ) in cMyBP-C−/− myocardium contribute to increased cross-bridge detachment as a consequence of an increased flux of cross-bridges into and through the force-producing steps preceding detachment.

Changes in the amplitude of P₃ have also been interpreted as indicating reversal of force-producing steps in response to stretch,42 such that more negative values of P₃ represent greater reversal of steps such as the phosphate release step.43 A possible adaptive advantage of such reversal is improved efficiency in myocardium subjected to stretch, because cross-bridges could conceivably detach from actin and quickly reattach without consuming ATP.42 Delayed force redevelopment (phase 3) in the response to stretch, ie, stretch activation, is thought to be mediated by recruitment of additional cross-bridges to force generating states.10,30,31 Here, there was no difference in amplitude of P₃.
between WT and cMyBP-C<sup>−/−</sup> myocardium, suggesting that the number of cross-bridges recruited by stretch was similar in the 2 cases; however, the rate constant of force redevelopment (k<sub>df</sub>) was dramatically accelerated in cMyBP-C<sup>−/−</sup> myocardium. Because stretch activation (phase 3) in cardiac muscle involves both cooperative and direct stretch-induced recruitment of cross-bridges,10,31 the acceleration of k<sub>df</sub> in cMyBP-C<sup>−/−</sup> myocardium suggests that the rates of force-generating transitions are accelerated and that the contributions resulting from cooperative recruitment of cross-bridges (which tend to slow force generation)<sup>44</sup> are reduced in the absence of cMyBP-C. Thus, the increased power output reported in cMyBP-C<sup>−/−</sup> myocytes<sup>32</sup> can be explained as a consequence of faster cross-bridge cycling kinetics.

From the stoichiometric ratio of cMyBP-C molecules to myosin heads,<sup>45</sup> cMyBP-C binds to only a small fraction of myosin heads in WT hearts, and, thus, ablation would directly affect relatively few myosin heads. If cMyBP-C behaves as a tether on myosin, ablation may remove a spatial constraint on these heads<sup>3,4,19,34,46</sup> and thereby increase the probability of binding to actin. Increased binding of small numbers of cross-bridges might not significantly increase overall force (there is no increase in maximum isometric force in cMyBP-C<sup>−/−</sup> myocardium) but could still accelerate the rate of force development and cross-bridge cycling kinetics attributable to the sensitivity of the cardiac thin filament to the activating effects of even small numbers of attached cross-bridges (reviewed by Moss et al<sup>47</sup>.

Ablation of cMyBP-C accelerates cross-bridge kinetics (Table and Figure 4) but, seemingly paradoxically, results in diminished stroke volume and cardiac hypertrophy.<sup>4</sup> However, the apparent acceleration of rate constants of cross-bridge detachment and attachment in cMyBP-C<sup>−/−</sup> myocardium can account for depressed pump function. In the context of cardiac contraction in which stronger regions of myocardium stretch weaker parts to induce delayed stretch activation,<sup>9,10</sup> accelerated turnover kinetics would predict a shorter delay in stretch activation, an earlier peak for the cardiac twitch, and earlier completion of the ejection phase. Such a phenomenon might be difficult to appreciate from our recordings because [Ca<sup>2+</sup>] is maintained constant and the force transient is slow to decay. In contrast, in living muscle the transient increase in intracellular Ca<sup>2+</sup> is over quickly, so that the faster stretch activation response in cMyBP-C<sup>−/−</sup> myocardium would result in an earlier early onset of relaxation of force. In effect, the accelerated kinetics of stretch activation are no longer tuned to the twitch kinetics required for a normal ejection period.

Taken together, our results support the idea that cMyBP-C normally constrains the interaction of myosin heads with actin and suggest that the acceleration of cross-bridge cycling kinetics attributable to ablation of cMyBP-C contributes to reduced ejection by abbreviating the period of ejection. Earlier work<sup>6</sup> has shown that the in vivo, functional effects of cMyBP-C ablation are very similar to those caused by a C-terminal truncation of cMyBP-C<sup>−</sup> C<sup>−</sup> a common form of cMyBP-C mutations leading to hypertrophic cardiomyopathy in humans. Thus, results from this study may have applicability to the mechanism of contractile dysfunction in cMyBP-C cardiomyopathies, which are among the most common inherited myocardial diseases.<sup>1−3</sup>

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