Effect of Cardiac Myosin Binding Protein-C on Mechanoenergetics in Mouse Myocardium

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Abstract—We examined the effect of cardiac myosin binding protein-C (cMyBP-C) on contractile efficiency in isovolumically contracting left ventricle (LV) and on internal viscosity and oscillatory work production in skinned myocardial strips. A 6-week diet of 0.15% 6-n-propyl-2-thiouracil (PTU) was fed to wild-type (+/+PTU) and homozygous-truncated cMyBP-C (t/PTU) mice starting at age 7 weeks and leading to a myosin heavy chain (MHC) isoform profile of 10% α-MHC and 90% β-MHC in both groups. Western blot analysis confirmed that cMyBP-C was present in the +/+PTU and effectively absent in the t/PTU. Total LV mechanical energy per beat was quantified as pressure-volume area (PVA). O₂ consumption (Vₒ₂) per beat was plotted against PVA at varying LV volumes. The reciprocal of the slope of the linear Vₒ₂–PVA relation represents the contractile efficiency of converting O₂ to mechanical energy. Contractile efficiency was significantly enhanced in t/PTU (26.1±2.6%) compared with +/+PTU (17.1±1.6%). In skinned myocardial strips, maximum isometric tension was similar in t/PTU (18.7±2.1 mN⋅mm⁻²) and +/+PTU (21.9±4.0 mN⋅mm⁻²), but maximum oscillatory work induced by sinusoidal length perturbations occurred at higher frequencies in t/PTU (7.3±1.17 Hz) compared with +/+PTU (4.48±0.60 Hz) and was significantly more sensitive to phosphate concentration in the t/PTU. Under rigor conditions, the internal viscous load was significantly lower in the t/PTU compared with +/+PTU, ie, ~40% lower at 1 Hz. These results collectively suggest that contractile efficiency is enhanced in the t/PTU, probably through a reduced loss of mechanical energy by a viscous load normally provided by cMyBP-C and through a gain of phosphate-dependent oscillatory work normally inhibited by cMyBP-C. (Circ Res. 2004;94:1615-1622.)

Key Words: contractile efficiency □ C-protein □ compliance □ stiffness □ viscosity

The structural and functional roles of cardiac myosin binding protein-C (cMyBP-C) have recently attracted considerable attention, particularly because mutations of its allele have been associated with familial hypertrophic cardiomyopathy (FHC). It has become increasingly apparent that the C-terminus of cMyBP-C plays a structural role in reinforcement of the thick filament by binding to the myosin rod, to titin, and to other cMyBP-C isoforms, such as myosin heavy chain (MHC) isoform from mouse myocardium inhibits, 19 does not affect, 11 or enhances 9 calcium activation, whereas the absence of cMyBP-C in mouse myocardium inhibits, 19 does not affect, 11 or enhances 9 calcium activation. Some of the discrepancies in these observations may be caused by differing experimental preparations and methods and/or the variable shifts of sarcomeric protein isoforms, such as myosin heavy chain (MHC) isoform from α-MHC to β-MHC, that occur in mouse models deficient in cMyBP-C. 19,20 Curiously, in both mouse models so far demonstrated to lack sarcomeric cMyBP-C, the LV is dilated, systolic elastance is dramatically reduced compared with normal, and the duration of ejection phase is similarly abbreviated. 11,19,20 It...
would seem likely that a common cMyBP-C-dependent mechanism by which these hearts are triggered to develop a similarly atypical dilated cardiomyopathy (DCM) should be anticipated and may also underlie, to a degree, some forms of cMyBP-C-dependent FHC.

One consistent observation across the various experimental models and methods has been the increase in unloaded shortening velocity or force redevelopment with the absence of cMyBP-C, which is thought to provide a viscous load during shortening.11,15,21,22 Because a viscous load would be expected to cause an irreversible loss of mechanical energy during myocardial contraction and relaxation, we hypothesized that a lack of cMyBP-C would elevate myocardial contractile efficiency in the mouse LV independent of MHC isoform. We report here that contractile efficiency in the isovolumic mouse LV lacking cMyBP-C is indeed higher than in the control and attribute this finding to a significantly lower viscous load as measured in skinned myocardium lacking cMyBP-C.

Another consistent finding in myocardium lacking cMyBP-C has been a significantly reduced myofilament stiffness.9,11 Because acto-myosin crossbridge kinetics can be influenced by myofilament stiffness,23 and because characteristic frequencies of crossbridge kinetics are particular sensitive to changes in inorganic phosphate ([Pi]),24 we further hypothesized that the absence of cMyBP-C in the thick filament would significantly change the phosphate dependency of the characteristic frequency, at which mechanical work was generated by cycling crossbridges. We report here that the maximum oscillatory work in cMyBP-C−deficient skinned myocardium occurs at higher frequencies and is more sensitive to [Pi].

**Methods**

**Mouse Model**

Mice of either sex were fed an iodine-deficient, 0.15% 6-n-propyl-2-thiouracil (PTU) diet (Harlan-Teklad #TD97061) for 6 weeks before study. Wild-type mice fed PTU (+/+/PTU) and truncated cMyBP-C mice fed PTU (t/+/PTU) were aged 12 to 16 weeks at the time of study and were of the SvEv129 background.20 All procedures were reviewed and approved by Institutional Animal Care and Use Committees.

cMyBP-C Content and MHC Isoform

LVs from each group were homogenized, lyophilized, and stored at −20°C. Homogenized LV was loaded onto 4% to 15% gradient SDS-PAGE gels, transferred to nitrocellulose, exposed to a polyclonal antibody for cMyBP-C,25 and detected by chemiluminescence. Homogenized LV was also loaded onto gels composed of 5% gelatin and 8% acrylamide, which were performed at 75 to 200 V for 24 hours at 4°C to 6°C,26 and subsequently fixed and silver stained. Optical densities of α-MHC and β-MHC bands above background were fit to Gaussian curves for estimating relative content.

**LV Mechanics**

The isovolumic mouse heart preparation and associated data analyses have been reported in detail previously.27 Briefly, after anesthesia and mechanical ventilation the heart was excised, and the aorta cannulated and perfused with buffer (2.5 mmol/L Ca²⁺, 35°C to 37°C, pH 7.35 to 7.45). A custom-made balloon mounted on a catheter was placed in the LV via the mitral orifice. A micromanometer catheter was introduced just above the mitral orifice via a side port. Pacing electrodes were attached to the LV and heart rate maintained at 240 beats per minute. The heart was then placed in a chamber maintained at 35°C to 37°C. Coronary perfusion pressure was controlled by a pressurized arterial reservoir at ~100 mm Hg. Coronary flow was measured directly by timed collections of right ventricle (RV) drainage. Coronary arteriovenous O₂ content difference (AVO₂) was measured continuously with a platinum O₂ electrode system.27

After a 30-minutes stabilization period, balloon volume was varied from 0 to 30 or 40 μL in increments of 2 to 4 μL using a manual micrometer syringe driver. LV pressure (P), coronary flow, and arterio-venous O₂ content difference (AVO₂) were measured under steady-state conditions at each volume.27,28 Total VO₂ (mL·beat⁻¹) was calculated as coronary flow (mL·min⁻¹) × AVO₂ (volume fraction of O₂) divided by heart rate. RV VO₂ in mL·beat⁻¹ was estimated at zero balloon volume and assumed to be constant at all LV volumes, and LV VO₂ = (total VO₂ − RV VO₂ at zero balloon volume).27

LV function was assessed using pressure-volume (PV) data at a common volume of 40 μL. Maximum elastance (E₉₀) was estimated by fitting the end-systolic PV data to the equation ESP=Eₑ₀+ESᵥ₀ ln(V) + c, where ESV₀ is the volume-axis intercept and c is a constant. Diastolic elastance was estimated by fitting the end-diastolic PV data to the equation EDP=β(exp(y) − exp(yEDV₀)), where β (mm Hg) and γ (mL⁻¹) are directly proportional to diastolic elastance at EDV₀, the volume-axis intercept.

Total mechanical energy output per beat was quantified as pressure-volume area (PVA), the area circumscribed by the end-systolic PV relation, the end-diastolic PV relation, and the systolic portion of the PV trajectory of each beat, and was normalized per gram LV.27–29 VO₂ was plotted against PVA at varying LV volumes and a linear regression (VO₂=aPVA+b) performed, where a=O₂ cost of PVA and intercept b=VO₂ at 0 PVA, i.e., unloaded VO₂. The reciprocal of the slope, 1/a, is the dimensionless contractile efficiency of converting O₂ to mechanical energy by the contractile machinery after expressing PVA and VO₂ in units of Joules.27–29 PVA-independent VO₂ represents energy consumed mainly for basal metabolism and excitation-contraction (EC) coupling.27–29

**Skinned Myocardium Oscillatory Work**

Concentrations are expressed in mmol/L unless otherwise noted. Relaxing solution: pCa 8.0, 5.0 EGTA, 5.0 ATP, 1.0 Mg²⁺, 0.25 P, 20 BES, 35 phosphocreatine (PCr), 250 μM creatine kinase (CK), ionic strength 200, and pH 7.0. Activating solution: same as relaxing solution with pCa 4.0. Rigor solution: same as activating with 0 ATP, 0 PCR, and 0 CK. Storage solution: same as relaxing with 10 μg/mL leupeptin and 50% wt/vol glycerol. Skinning solution: same as relaxing with 30 2,3-butanedione monoxime (BDM). 10 μg/mL leupeptin, 1% wt/vol Triton X-100, and 50% wt/vol glycerol.

LV skinned myocardial strips were prepared using methods described previously.30 Mice were killed by cervical dislocation, and hearts were rapidly excised and placed in Krebs-Ringer +30 BDM bubbled with 95% O₂ + 5% CO₂. Papillary muscles were dissected to yield at least 4 thin strips (~140 μm diameter, ~800 μm length) with longitudinally oriented parallel fibers, skinned at 22°C for 2 hours, and stored at −20°C for no more than 5 days. At the time of study, a strip was attached to aluminum T-clips ~150 μm apart, mounted between a piezoelectric motor and a strain gauge, lowered into 30 μL relaxing solution, maintained at 37°C, and stretched and maintained at 2.2 μm sarcomere length detected by videography and digital Fourier transform (IonOptix). Although some myocyte disarray has been reported for the t/t myocardium,29,30 visual inspection suggested comparable sarcomere integrity in the skinned t/+/PTU and t/+/PTU myocardium.

Two strips from each heart were calcium activated from pCa 8.0 to pCa 4.5 and then exposed to rigor solution. Two additional strips from each heart were maximally activated at pCa 4.5 and exposed to varying [P] ranging from 0.25 to 4 mmol/L. At each pCa and [P] condition, sinusoidal perturbations of amplitude Lₘₚ=0.125% strip length were applied over the frequencies 0.125 to 250 Hz. The elastic and viscous (E₉₀) moduli were calculated from the recorded tension.
transient as the relative magnitudes of the in-phase and 90-degree out-of-phase components with respect to the length perturbation. 24,25

Analysis
All data are presented as mean±SEM. Repeated-measures ANOVA were performed for all variables measured over multiple frequencies, pCa, or [P_i], with cMyBP-C as a group identifier. Normalized isometric tension–pCa relations were fit to the Hill equation using a 2-parameter nonlinear least squares algorithm (Sigma Plot 11.0, SPSS). Statistical significance is reported at the P<0.05 and P<0.01 levels.

Results
Mouse Characteristics, cMyBP-C Content, and MHC Isoform
Morphological characteristics of a subset of +/+PTU and t/tPTU mice are presented in Table 1. The t/tPTU hearts demonstrated greater RV and LV mass as reported previously for t/t mice not treated with PTU. 9,11,20 Figure 1A and 1B, respectively, illustrate an electrophoresis gel of LV sarcomeric proteins and a Western blot of a polyclonal antibody for cMyBP-C. 25

The wild-type cMyBP-C was detected at ~150 kDa in the +/+PTU myocardium, whereas cMyBP-C was not detected in the t/tPTU myocardium at any molecular weight. Any cMyBP-C present in the t/tPTU LV was therefore present at a level under the detection sensitivity of these assays. Figure 1C and 1D display, respectively, an electrophoresis gel and corresponding optical densities of MHC isoform distributions in the t/tPTU and +/+PTU LVs. The content of α-MHC was 9% to 10% in both groups (Table 1).

LV Mechanics
LV pressure-derived indexes are shown in Table 2. At 40 μL chamber volume, t/tPTU LV generated a lower ESP but a normal dP/dtmax compared with +/+PTU. The t/tPTU LV also showed significantly slowed relaxation as reflected by dP/dtmin and τ (Table 2). Representative end-systolic and end-diastolic PV relationships in t/tPTU and +/+PTU LVs are shown in Figure 2A. Note that the slopes of the end-systolic PV relationship, ie, Emax, and the end-diastolic PV relationship, which is directly proportional to estimated parameters β and γ, were significantly reduced in t/tPTU LVs. In addition, higher values for ESVo and EDVo in the t/tPTU LV

Figure 1. cMyBP-C content and MHC isoform. A, Gel electrophoresis detected wild-type cMyBP-C (~150 kDa) in the +/+PTU myocardium, but not in the t/tPTU. B, Western blot analysis by polyclonal antibody did not detect truncated cMyBP-C at any molecular weight in the t/tPTU myocardium. C, α-MHC (α) and β-MHC (β) could be identified separately after silver stain. D, The relative optical densities of the α-MHC and β-MHC isoforms were measured to be ~10% and 90%, respectively, in both the +/+PTU and t/tPTU myocardium.
indicate chamber dilatation. These findings are similar to those reported for t/t mice not treated with PTU.11,20

Representative VO$_2$-PVA relations are shown in Figure 2B. In this example, the VO$_2$ intercept was greater and the slope of the VO$_2$-PVA relation was lower in the t/t PTU LV compared with +/-PTU (Figure 2C and 2D). The VO$_2$ intercept averaged 0.725±0.041 (mL O$_2$ · g$^{-1}$ · beat$^{-1}$) in t/t PTU and 0.525±0.036 (mL O$_2$ · g$^{-1}$ · beat$^{-1}$) in +/-PTU (P<0.05), whereas contractile efficiency averaged 26.1±2.6% in t/t PTU and 17.1±1.4% in +/-PTU (P<0.05).

Assuming that basal metabolism is not different between groups, the higher VO$_2$ intercept in the t/t PTU is caused by increased energy costs of EC coupling rather than the lack of cMyBP-C.27–29,31 The contractile efficiency in the t/t PTU, including effects of the force-producing sarcomere, including effects of cMyBP-C deficiency.27–29 We chose to focus our further investigations on the effects of cMyBP-C deficiency on myofilament stiffness and viscous load and on calcium- and phosphate-dependence of acto-myosin kinetics reflected by isometric tension and oscillatory work production in skinned myocardial strips.

**Myofilament Stiffness and Viscosity**

In skinned myocardial strips at maximum calcium activation (pCa 4.5), the myofilament elastic modulus measured at any frequency up to 250 Hz was comparable between the t/t PTU and +/-PTU groups (Figure 3A). However, repeated-measures ANOVA for the elastic modulus revealed a highly significant (P<0.01) cMyBP-C × frequency interaction, which indicated a difference in the frequency dependence of the elastic modulus between the groups. There was also a significant difference in the frequency dependence of the viscous modulus between the groups (cMyBP-C × frequency interaction, P<0.01) and a highly significant cMyBP-C main effect (P<0.01), which was reflected in the viscous modulus being significantly (P<0.05) lower in the t/t PTU at frequencies 5 to 9 Hz and ≥65 Hz (Figure 3B). It should be noted that negative values for the viscous modulus over the frequency range 4 to 12 Hz indicate a net production (rather than absorption) of mechanical energy during the length perturbations.24

The elastic and viscous moduli under activated conditions are known to be dependent on the mechanical properties of the myofilaments and the kinetic properties of the acto-myosin crossbridge.24 We elucidated the effects of a lack of cMyBP-C on the mechanical properties of the myofilaments by imposing rigor conditions, which suppressed crossbridge kinetics. Repeated-measures ANOVA for the elastic and viscous moduli under rigor conditions revealed significant (P<0.05) cMyBP-C main effects. Specifically, the elastic and viscous moduli of the myofilaments under rigor conditions were significantly lower in the t/t PTU lacking cMyBP-C (Figure 3C and 3D).

**Calcium-Dependence of Isometric Tension and Oscillatory Work**

Maximum calcium-activated isometric tension in the t/t PTU (18.7±2.1 mN/mm$^2$, n=8) was not statistically different from that in +/-PTU (21.9±4.0 mN/mm$^2$, n=6). Figure 4A illustrates isometric tension-pCa relationships, which were similar in calcium sensitivity between the t/t PTU (pCa$_{50}$=5.59±0.04) and +/-PTU (5.59±0.06) and in Hill coefficient between the t/t PTU (n=4.1±0.5) and +/-PTU (4.1±0.3).

At maximum calcium activation, the oscillatory work (W) was higher in the t/t PTU for frequencies 5 to 9 Hz (Figure 4B). This particular frequency range is physiologically relevant, because the heart rate of the mouse is 8 to 12 Hz. Maximum oscillatory work (W$_{max}$) was not found to be statistically different between the groups over the activating calcium concentrations of pCa 5.75 to 4.5 (Figure 4C). However, the frequency of maximum oscillatory work (f$_{w_{max}}$) was signifi-
sstantly higher in the t/tPTU, as demonstrated by a statistically significant cMyBP-C main effect ($P<0.05$) and statistically higher values for $f_{\text{max}}$ at pCa 5.0 and 4.5 (Figure 4D). There was no statistically significant calcium-dependence for $f_{\text{max}}$, however, as indicated by the lack of a statistically significant ($P>0.1$) pCa main effect or interaction for $f_{\text{max}}$. Nevertheless, these results for $f_{\text{max}}$ suggest that a lack of cMyBP-C increases the characteristic frequency at which acto-myosin crossbridges can generate oscillatory work.

**Phosphate-Dependence of Isometric Tension and Oscillatory Work**

Figure 5A illustrates the phosphate-dependence of isometric tension. Continuous activation over the 30 minutes required
to vary [P] from 0.25 to 4 mmol/L resulted in ~15% “run down” of isometric tension. However, the isometric tension in the t/tPTU was sensitive to [P] over this range whereas the +/+PTU was not, as indicated by a statistically significant cMyBP-C main effect (P<0.05) and significantly lower values for normalized isometric tension in the t/tPTU at [P] ≥ 0.25 mmol/L.

At 4 mmol/L [P], W was significantly higher in the t/tPTU over the frequencies 6 to 12 Hz (Figure 5B). The phosphate sensitivity of W max was enhanced in the t/tPTU, as demonstrated by a significant cMyBP-C X [P] interaction (P<0.05) and statistically higher values for W max in the t/tPTU at [P] ≥ 2 mmol/L (Figure 5C). The value for W max was higher in the t/tPTU at all [P] conditions examined (Figure 5D). These results for W max across varying [P] suggest that a lack of cMyBP-C increases the phosphate-dependency of the characteristic frequency at which acto-miosin crossbridges generate oscillatory work.

**Discussion**

In the present study, an effective lack of cMyBP-C was shown to underlie an increase in the contractile efficiency of the LV, a decrease in the elastic stiffness and viscous load of the sarcomere, and an increase in the magnitude and frequency of myofilament oscillatory work generation at [P] ≥ 2 mmol/L. These findings at the myofilament level suggest that the enhanced LV contractile efficiency in the t/tPTU lacking cMyBP-C was caused in part by a reduced viscous load normally provided by cMyBP-C and possibly a gain of phosphate-dependent oscillatory work normally inhibited by cMyBP-C.

Considered by itself, the higher contractile efficiency in LV lacking cMyBP-C suggests that cMyBP-C is somewhat deleterious to cardiac function. This is clearly not the case, however, because hearts lacking cMyBP-C develop DCM. Thus, changes in LV contractile efficiency do not directly reflect the functional significance of cMyBP-C. Based on the data presented here and previously, cMyBP-C serves at least one important purpose, maintenance of LV systolic stiffness during ejection,\(^1\) partly because (2) cMyBP-C contributes proportionally to internal elastic stiffness\(^9,11\) and viscous load,\(^1,11\) which (3) decreases shortening and relaxation velocities.\(^1,11,15,21,22\)

Our findings in the isovolumic t/tPTU LV are consistent with those reported by McConnell et al,\(^2\) who assessed t/t LV function using a conductance catheter to construct PV loops. The normal dP/dt max in cMyBP-C–deficient LV is atypical for DCM and is reflected in the increased shortening velocity of skinned myocardial strips and myocytes lacking cMyBP-C.\(^1,11,15,21,22\) The reduced E max in cMyBP-C–deficient LV is consistent with reduced stiffness of activated myofilaments lacking cMyBP-C, as shown in the present study and previously.\(^1,11\) The reduced diastolic elastance in the t/tPTU LV is a new finding for LV lacking cMyBP-C but is consistent with the reduced stiffness in skinned t/t myocardium at diastolic calcium concentrations.\(^11\) Finally, the reduced rate of LV relaxation in cMyBP-C–deficient LV is likely a result of the downregulation of sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA2), which would be expected in heart failure\(^31\) and has been reported in the t/t mouse.\(^32\)

Because SERCA2 is likely downregulated in the t/tPTU compared with +/+PTU,\(^33\) cytosolic Ca\(^{2+}\) removal in the t/tPTU would rely more on Na\(^+-\)Ca\(^{2+}\) exchange, which is energetically linked to Na\(^+-\)K\(^{-}\)-ATPase. Cytosolic Ca\(^{2+}\) removal via...
Na⁺–Ca²⁺ exchange indirectly requires more ATP than that via SERCA2; therefore, the energy costs of calcium handling during progression to heart failure may increase despite reduced SERCA2 activity. Although we have no direct evidence for this specific explanation, the higher unloaded $V_O_2$ observed in the t/PTU most likely reflects the higher energy cost of calcium handling and not the lack of cMyBP-C.

Myofilaments lacking cMyBP-C possessed significantly reduced elastic and viscous moduli of MHC isoform and conditions of both activation and rigor. The lower elastic and viscous moduli therefore directly reflect the mechanical characteristics of molecules in close proximity to each other and affecting stretch, compression, and friction on each other during length perturbation. Because cMyBP-C is known to bind strongly to myosin rods and to titin, a lack of cMyBP-C would facilitate the sliding of myosin rods in relation to titin molecules within the thick filament as the myosin heads move with the thin filament. In addition, with no cMyBP-C to anchor the relative positions of myosin rods and titin molecules at the usual 43 nm intervals within the thick filament, that portion of the titin molecule, which is encompassed by myosin, would effectively have increased its segment length from 43 nm to the entire length of the thick filament within a half sarcomere, $\sim 600$ nm. Assuming individual titin molecules maintain their stiffness properties, the increase in segment length would dramatically reduce the functional stiffness of titin in the thick filament lacking cMyBP-C. The sarcomere lacking cMyBP-C would then also possess a reduced stiffness during both activation and relaxation.

We found that a lack of cMyBP-C did not significantly change the isometric tension–pCa relationship in skinned myocardium. Although cMyBP-C deficiency has been shown to reduce or enhance myofilament calcium sensitivity, we propose that these reports may reflect differences in thin filament protein content or isoform, such as skeletal troponin-C used to replace cardiac or variable troponin-T isoforms as occur in heart failure. The 6-week PTU treatment used for both the t/PTU and +/+ PTU mice would be expected to induce similar protein isoform profiles in both the thick and thin filaments. The strikingly similar calcium sensitivities in the t/PTU and +/+ PTU report here may therefore indicate that cMyBP-C does not significantly affect calcium sensitivity of isometric tension, just as cMyBP-C phosphorylation also does not affect calcium sensitivity.

A reduction in isometric tension with increasing [P_i], as observed in the myofilaments lacking cMyBP-C, can be explained by a higher probability of reversal of the force-producing power stroke of the acto-myosin crossbridge, which would occur with a higher probability of a back-reaction of the phosphate-release step. Our finding that a lack of cMyBP-C furthermore increases the characteristic frequency and the phosphate-dependence of oscillatory work production is intriguing. According to the scheme of the crossbridge cycle proposed by Kawai et al., the characteristic frequency of oscillatory work production is directly proportional to the sum of the forward and reverse rates of the force-producing power stroke of the acto-myosin crossbridge. Because the characteristic frequency of oscillatory work production increases with removal of cMyBP-C, cMyBP-C conceivably reduces the forward and/or reverse rates of the force-producing power stroke.

**Limitations**

Six weeks of PTU treatment usually suppress $\alpha$-MHC content in adult mice, but did not completely suppress $\alpha$-MHC in our mice aged $\sim 8$ weeks. A lower $\alpha$-MHC content would be expected to increase LV contractile efficiency and may underlie some reports of increased contractile efficiency in other models of heart failure. Although small amounts of $\alpha$-MHC may influence sarcomeric function, the proportions of $\alpha$-MHC in t/PTU and +/+ PTU LVs were virtually identical; therefore, its presence was not likely responsible for group differences we observed. Other unknown conditions, however, were not controlled in our experiments. For example, we did not specifically control for possible differential phosphorylation states or tension–length relationships, although lattice spacing in the t/PTU and +/+ PTU are similar. Despite these possible limitations, all LVs and skinned strips were prepared similarly, and our results most likely reflect the lack of cMyBP-C in the t/PTU LV.

**Conclusions**

The most succinct explanation for the data associated with the phosphate-dependency of isometric tension and of oscillatory work production would be that cMyBP-C and/or the enhanced thick filament stiffness mechanically stabilizes the post-power stroke state of the acto-myosin crossbridge and thereby inhibits the reversal of the force producing power stroke. In the context of LV systolic function, we furthermore conclude that the presence of cMyBP-C maintains or extends the duration of the ejection phase by stabilizing the post-power stroke state of the acto-myosin crossbridge and by providing an energy-consuming viscous load (through power stroke stabilization or direct interaction with actin) that inhibits premature mechanical relaxation of the L.V. Although the current study does not elucidate the mechanism(s) by which cMyBP-C deficiency or mutation may lead to DCM in mice or FHC in humans, these results do reinforce some roles for cMyBP-C in normal cardiac function.

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**References**


22. Korte FS, McDonald KS, Harris SP, Moss RL. Load shortening, power output, and rate of force redevelopment are increased with knockout of cardiac myosin binding protein-C. Circ Res. 2003;93:752–758.


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