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 ventricles (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 (+/+) and homozygous-truncated cMyBP-C (t/t) mice starting at age ∼8 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 +/+ and effectively absent in the t/t. 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/t (26.1±2.6%) compared with +/+ (17.1±1.6%). In skinned myocardial strips, maximum isometric tension was similar in t/t (18.7±2.1 mN·mm⁻²) and +/+ (21.9±4.0 mN·mm⁻²), but maximum oscillatory work induced by sinusoidal length perturbations occurred at higher frequencies in t/t (7.31±1.17 Hz) compared with +/+ (4.48±0.60 Hz) and was significantly more sensitive to phosphate concentration in the t/t. Under rigor conditions, the internal viscous load was significantly lower in the t/t compared with +/+ blur, i.e., ∼40% lower at 1 Hz. These results collectively suggest that contractile efficiency is enhanced in the t/t, 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 reinforcing the thick filament by binding to the myosin rod, to titin, and to other cMyBP-C. By mechanically stabilizing the thick filament cMyBP-C facilitates force transmission between the acto-myosin crossbridge and the M-line. Analyses of X-ray diffraction patterns suggest that cMyBP-C may provide a mechanical link between the thick and thin filaments, further strengthening the myofilament lattice. Through either or both of these means, cMyBP-C contributes significantly to the structural integrity and associated mechanical properties of the sarcomere and left ventricle (LV). The N-terminus of cMyBP-C has 4 phosphorylation sites and binding sites to myosin S2 and to actin, all of which have been implicated in the modulation of myosin head arrangement and acto-myosin crossbridge kinetics. The possible effects of cMyBP-C and of its phosphorylation on acto-myosin crossbridge kinetics are not yet clear, although its phosphorylation does not apparently affect the tension–pCa relationship. The effect of cMyBP-C itself on the tension–pCa relationship has so far proved equivocal: the chemical extraction of cMyBP-C from rat cardiomyocytes enhances calcium activation, whereas the absence of cMyBP-C in mouse myocardium inhibits, does not affect, or enhances 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. 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.
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 HFC.

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. 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. Because acto-myosin crossbridge kinetics can be influenced by myofilament stiffness, and because characteristic frequencies of crossbridge kinetics are particular sensitive to changes in inorganic phosphate ([Pi]), 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-thioauracil (PTU) diet (Harlan-Teklad #TD979061) for 6 weeks before study. Wild-type mice fed PTU (+/−ptu) and truncated cMyBP-C mice fed PTU (ttptu) were aged 12 to 16 weeks at the time of study and were of the SvEv129 background. 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, and detected by chemiluminescence. Homogenized LV was also loaded onto gels composed of 5% glycerol and 8% acrylamide, which were performed at 75 to 200 V for 27 hours at 4°C to 6°C, 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. 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.

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. 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 = (VO2−RV VO2) at zero balloon volume.

LV function was assessed using pressure-volume (PV) data at a common volume of 40 μL.27 Maximum elastance (Emax) was estimated by fitting the end-systolic PV data to the equation ESP=E0+ESVγ+ΔV, where ESVγ is the volume-axis intercept and γ is a constant. Diastolic elastance was estimated by fitting the end-diastolic PV data to the equation EDP=β[exp(γβ)−exp(γβEDVγ)], where β (mm Hg) and γ (mL−1) are directly proportional to diastolic elastance at EDVγ 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, a−1, 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 U/mL 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 leupetin and 50% wt/vol glycerol. Skinning solution: same as relaxing with 30 μg/mL leupetin, 1% wt/vol Triton X100, and 50% wt/vol glycerol.

LV skinned myocardial strips were prepared using methods described previously.28 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 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, visual inspection suggested comparable sarcomere integrity in the skinned ttptu and +/−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 [Pi] ranging from 0.25 to 4 mmol/L. At each pCa and [Pi] condition, sinusoidal perturbations of amplitude Iamp=0.125% strip length were applied over the frequencies 0.125 to 250 Hz. The elastic and viscous (Ee) 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.\textsuperscript{24} The oscillatory work (W) generated by a strip within one sinusoidal cycle was $W = -\pi \cdot E_{\max}^2 \cdot E_{\min}^2$. \textsuperscript{24}

**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 $t/t_{PTU}$ mice are presented in Table 1. The $t/t_{PTU}$ hearts demonstrated greater RV and LV mass as reported previously for $t/t$ mice.\textsuperscript{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.\textsuperscript{25} The wild-type cMyBP-C was detected at $\sim$150 kDa in the $+/+_{PTU}$ myocardium, whereas cMyBP-C was not detected in the $t/t_{PTU}$ myocardium at any molecular weight. Any cMyBP-C present in the $t/t_{PTU}$ 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 $t/t_{PTU}$ and $+/+_{PTU}$ LVs. The content of $\alpha$-MHC was 9% to 10% in both groups (Table 1).

**LV Mechanics**

LV pressure-derived indexes are shown in Table 2. At 40 $\mu$L chamber volume, $t/t_{PTU}$ LV generated a lower ESP but a normal $dP/dt_{max}$ compared with $+/+_{PTU}$. The $t/t_{PTU}$ LV also showed significantly slowed relaxation as reflected by $dP/dt_{min}$ and $\tau$ (Table 2). Representative end-systolic and end-diastolic PV relationships in $t/t_{PTU}$ and $+/+_{PTU}$ LVs are shown in Figure 2A. Note that the slopes of the end-systolic PV relationship, ie, $E_{max}$, and the end-diastolic PV relationship, which is directly proportional to diastolic elastance, $E_{\min}$, were significantly reduced in $t/t_{PTU}$ LVs. In addition, higher values for ESV and EDV in the $t/t_{PTU}$ LVs are presented in Table 1. The $t/t_{PTU}$ hearts demonstrated greater RV and LV mass as reported previously for $t/t$ mice.\textsuperscript{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.\textsuperscript{25} The wild-type cMyBP-C was detected at $\sim$150 kDa in the $+/+_{PTU}$ myocardium, whereas cMyBP-C was not detected in the $t/t_{PTU}$ myocardium at any molecular weight. Any cMyBP-C present in the $t/t_{PTU}$ 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 $t/t_{PTU}$ and $+/+_{PTU}$ LVs. The content of $\alpha$-MHC was 9% to 10% in both groups (Table 1).

![Figure 1](https://circres.ahajournals.org/)

**Figure 1.** cMyBP-C content and MHC isoform. A, Gel electrophoresis detected wild-type cMyBP-C ($\sim$150 kDa) in the $+/+_{PTU}$ myocardium, but not in the $t/t_{PTU}$. B, Western blot analysis by polyclonal antibody did not detect truncated cMyBP-C at any molecular weight in the $t/t_{PTU}$ myocardium. C, $\alpha$-MHC ($\alpha$) and $\beta$-MHC ($\beta$) could be identified separately after silver stain. D, The relative optical densities of the $\alpha$-MHC and $\beta$-MHC isoforms were measured to be $\sim$10% and 90%, respectively, in both the $+/+_{PTU}$ and $t/t_{PTU}$ myocardium.
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/tPTU 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/tPTU 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/tPTU lacking cMyBP-C (Figure 3C and 3D).

Calcium-Dependence of Isometric Tension and Oscillatory Work

Maximum calcium-activated isometric tension in the t/tPTU (18.7±2.1 mN/mm², n=8) was not statistically different from that in +/+PTU (21.9±4.0 mN/mm², n=6). Figure 4A illustrates isometric tension–pCa relationships, which were similar in calcium sensitivity between the t/tPTU (pCa0=5.59±0.04) and +/+PTU (5.59±0.06) and in Hill coefficient between the t/tPTU (n=4.1±0.5) and +/+PTU (4.1±0.3).

At maximum calcium activation, the oscillatory work (W) was higher in the t/tPTU 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 (Wmax) 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 (fWmax) was signif-

| Table 2. Characteristics of LV mechanoenergetics. A, Representative LV pressure-volume (PVA) relationships illustrate a lower end-systolic (solid lines) and lower end-diastolic (dotted lines) PV slope in the t/tPTU group. The PV area (PVA) bounded by the end-systolic PV relation, the end-diastolic PV relation, and the developed pressure (P) at a prescribed LV volume represents the mechanical energy generated by a single beat in the isovolumic LV. B, Representative VO2-PVA relationships demonstrate that the rate of O2 consumption is linearly related to PVA. C, Nonmechanical O2 consumption was greater in the t/tPTU, indicating a greater basal metabolism and/or greater energy requirement for EC coupling. D, Contractile efficiency, measured as the inverse of the VO2–PVA slope, was enhanced in the t/tPTU, probably because of a reduced loss of mechanical energy by the viscous load normally provided by cMyBP-C. n=4. PTU, S +/+PTU. *P<0.05.
cantly higher in the t/tPTU, as demonstrated by a statistically significant cMyBP-C main effect ($P<0.05$) and statistically higher values for $f_{w_{\text{max}}}$ at $pCa$ 5.0 and 4.5 (Figure 4D). There was no statistically significant calcium-dependence for $f_{w_{\text{max}}}$, however, as indicated by the lack of a statistically significant ($P>0.1$) $pCa$ main effect or interaction for $f_{w_{\text{max}}}$ Nevertheless, these results for $f_{w_{\text{max}}}$ 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/t PTU 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/t PTU at [P] > 0.25 mmol/L.

At 4 mmol/L [P], W was significantly higher in the t/t PTU over the frequencies 6 to 12 Hz (Figure 5B). The phosphate sensitivity of Wmax was enhanced in the t/t PTU, as demonstrated by a significant cMyBP-C X [P] interaction (P<0.05) and statistically higher values for Wmax in the t/t PTU at [P] ≥2 mmol/L (Figure 5C). The value for fWmax was higher in the t/t PTU at all [P] conditions examined (Figure 5D). These results for fWmax across varying [P] suggest that a lack of cMyBP-C increases the phosphate-dependency of the characteristic frequency at which acto-myosin 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/t PTU 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,11 by at least 3 related mechanisms: (1) cMyBP-C inhibits a premature transition to subnormal diastolic stiffness at the end of the ejection phase,11 partly because (2) cMyBP-C contributes proportionally to internal elastic stiffness8,11 and viscous load,11 which (3) decreases shortening and relaxation velocities.11,15,21,22

Our findings in the isovolumic t/t PTU LV are consistent with those reported by McConnell et al,20 who assessed t/t LV function using a conductance catheter to construct PV loops. The normal dp/dtmax 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.11,15,21,22 The reduced Emax in cMyBP-C–deficient LV is consistent with reduced stiffness of activated myofilaments lacking cMyBP-C, as shown in the present study and previously.11,20 The reduced diastolic elastance in the t/t PTU 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 Ca2+-ATPase (SERCA2a), which would be expected in heart failure31 and has been reported in the t/t mouse.32

Because SERCA2 is likely downregulated in the t/t PTU compared with +/+PTU,32 cytosolic Ca2+ removal in the t/t PTU would rely more on Na+–Ca2+ exchange, which is energetically linked to Na+–K+–ATPase. Cytosolic Ca2+ removal via
Na\textsuperscript+-Ca\textsuperscript{2+} 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.\textsuperscript{31} Although we have no direct evidence for this specific explanation, the higher unloaded Vo\textsubscript{2} observed in the t/t PTU most likely reflects the higher energy cost of calcium handling\textsuperscript{27–29} and not the lack of cMyBP-C.

Myofilaments lacking cMyBP-C possessed significantly reduced elastic and viscous moduli independent of MHC isoform and during 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,\textsuperscript{3,8} 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,\textsuperscript{3,8} 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, \(\approx 600\) nm.\textsuperscript{33} 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 isotropic tension–pCa relationship in skinned myocardium. Although cMyBP-C deficiency has been shown to reduce\textsuperscript{19} or enhance\textsuperscript{9,16} 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\textsuperscript{16} or variable troponin-T isoforms as occur in heart failure.\textsuperscript{27,28} Although small amounts of \(\alpha\)-MHC may influence sarcomeric function, the proportions of \(\alpha\)-MHC in t/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/t PTU and +/-PTU are similar.\textsuperscript{9} 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/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)\textsuperscript{10} that inhibits premature mechanical relaxation of the LV. 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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