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Arch Biochem Biophys. Author manuscript; available in PMC 2020 February 15.

Published in final edited form as:

Arch Biochem Biophys. 2019 February 15; 662: 213–218. doi:10.1016/j.abb.2018.12.021.

Lost in translation: interpreting cardiac muscle mechanics data in clinical practice

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Abstract

Current inotropic therapies improve systolic function in heart failure patients but they also elicit undesirable side effects such as arrhythmias and increased intracellular Ca^{2+} transients. In order to maintain myocyte Ca^{2+} homeostasis, the increased cytosolic Ca^{2+} needs to be actively transported back to sarcoplasmic reticulum leading to depleted ATP reserves. Thus, an emerging approach is to design sarcomere-based treatments to correct impaired contractility via a direct and allosteric modulation of myosin's intrinsic force-generating behavior –a concept that potentially avoids the "off-target" effects. To achieve this goal, various biophysical approaches are utilized to investigate the mechanistic impact of sarcomeric modulators but information derived from diverse approaches is not fully integrated into therapeutic applications. This is in part due to the lack of information that provides a coherent connecting link between biophysical data to *in vivo* function. Hence, our ability to clearly discern the drug-mediated impact on whole-heart function is diminished. Reducing this translational barrier can significantly accelerate clinical progress related to sarcomere-based therapies by optimizing drug-dosing and treatment duration protocols based on information obtained from biophysical studies. Therefore, we attempt to link biophysical mechanical measurements obtained in isolated cardiac muscle and *in vivo* contractile function.

Keywords

failing myocardium; myosin modulators; sarcomere-based therapies; biophysical measurements; cross-bridge behavior; *in vivo* function

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Disclosures

There are no conflicts of interest.

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Introduction:

Despite years of intense research on cardiovascular disease and therapies, most clinically practiced drug-therapies use decades-old symptom-targeting approaches that manipulate downstream neurohormonal and Ca^{2+} signaling pathways [1, 2]. As a result, these therapies induce several disruptive side effects and the prognosis for cardiac patients remains poor [3]. For conditions such as heart failure (HF), hypertrophic cardiomyopathy (HCM), and dilated cardiomyopathy (DCM) where abnormal contractility manifests from myofilament dysfunction [4], advances in molecular development may provide mechanistic-based drug therapies that directly target the cardiac sarcomere [2, 5]. To help identify these new drug targets and their impact on cardiac contractility, several biophysical approaches are used that include single molecule [6], *in vitro* motility assays [7, 8], solution-based assays [9], and detergent-skinned multicellular cardiac preparations [10–12]. Although these biophysical approaches have been instrumental in elucidating the actions of potential drug molecules on cardiac myocytes, implementation of the information derived from these experiments into therapeutic application has been slow. Part of the reason for this delay is that many of the cardiac muscle parameters measured by biophysical studies lack a clear translation to whole heart function, hindering the ability to better design and guide human clinical trials. Thus, the aim of this review is to relate biophysical parameters of cardiac muscle function with important contractile indices of *in vivo* heart performance, and bridge the translational gap between basic preclinical studies, clinical trials, and practice. Due to space limitations, we will focus mainly on the translational relevance of mechanical experiments using detergent-skinned multicellular myocardial preparations because they provide unique mechanistic insights that other biophysical assays cannot. Three such measurements we highlight are the rate of tension redevelopment (k_{tr}), stretch activation (SA), and force-velocity (FV) measurements as they are commonly performed by many laboratories including our own.

A key advantage of skinned cardiac preparations is that they allow researchers to probe drug effects on cross-bridge (XB) behavior in a fully assembled sarcomeric lattice. In general, XBs produce force by cycling through weakly- and strongly-bound states with the thin filament (for an in-depth review, please see [13–15]). While molecular assays can identify drug effects on specific states of the myosin XB cycle [6, 7, 16–18], potential drugs may also function by targeting other sarcomere proteins that mediate XB behavior [19]. These may include both thin and thick filament proteins such as the troponin complex, tropomyosin, titin, and cardiac myosin binding protein C (cMyBPC) [19]. Additionally, experiments on skinned cardiac preparations allow researchers to characterize important cooperative effects that mediate XB behavior and cardiac muscle force generation [20]. For example, one measurement used to assess drug effects on global XB behavior is k_{tr} [10] where a slack-restretch protocol mechanically disrupts XBs causing a force redevelopment as they reenter force-bearing states by repeatedly traversing the XB cycle [21]. However, k_{tr} incorporates multiple processes of XB cycling, and is an index of the sum of all forward and reverse rate constants of XB cycling following a large stretch maneuver [21]. Thus, it is often useful to further dissect the elements of XB behavior as drugs commonly impact a particular state of myosin XB cycle. For this, researchers frequently use SA to record force responses of cardiac preparations to small step-length perturbations (for details, refer to [22,

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23]). Specifically, SA transients permit measurement of the rates of XB detachment (k_{rel}) and recruitment (k_{df}) (Figure 1), reflecting XB transitions from their attached to detached and detached to attached states [23]. It is likely that both XB detachment and recruitment occur simultaneously during k_{rel} and k_{df} phase, however XB detachment predominates during k_{rel} phase leading to a net force decay and XB recruitment predominates during k_{df} phase leading to a net force development [22, 24]. SA also enables researchers to estimate XB detachment and recruitment magnitudes, permitting a more detailed understanding of drug-induced modulations of XB behavior [10–12]. Besides k_{tr} and SA, FV relationships, which assess myocardial power output, can be determined from measuring loaded shortening velocity at various force clamps [25]. Since the rate of loaded muscle shortening is most dependent on the rate of XB detachment [26], it can be used to characterize length-mediated cooperative deactivation mechanisms [25] which play important roles during *in vivo* ventricular relaxation [15].

Finally, it should be noted that there are inherent caveats to translating results from skinned cardiac preparations to whole-heart function. For one, they cannot fully simulate the dynamic interplay between other regulatory mechanisms that prevail *in vivo* [27, 28]. Secondly, experiments on skinned cardiac preparations are generally performed in constant Ca^{2+} environments as the Ca^{2+} transients present *in vivo* are difficult to replicate. Instead, the contractile behavior of cardiac fibers is often studied at several different steady state Ca^{2+} concentrations ranging from pCa 9.0 to pCa 4.5. Doing so allows experimenters to build a more complete picture of how XB behavior changes as a function of Ca^{2+} activation level than would otherwise be possible. This approach has proven useful for studying XB kinetics and drug effects at submaximal Ca^{2+} levels, which have important physiological implications concerning *in vivo* force development and relaxation [11, 12]. It is also notable that much of skinned or intact fiber experiments are conducted at sub-physiologic temperatures (~15–25°C) to preserve myofilament integrity and minimize force rundown [29, 30]. While the absolute values of XB kinetics decrease as temperature decreases, their responsiveness to post-translational modifications and other regulatory mechanisms remain intact and correlate well with measurements made *in vivo* [31, 32]. This extrapolation allows for the study of critical phases of XB cycling and the underlying myofilament regulatory mechanisms. Thus, these experiments provide a flexible way to generate crucial preclinical data on drugs that could accelerate the translation of basic science research findings to clinical studies.

Relating contractile measurements in isolated cardiac muscle preparations to *in vivo* whole organ function:

There is an apparent gap between basic and clinical science methods and measurements investigating cardiac contractile function, making it difficult to directly relate specific parameters of a XB cycle to the events in a cardiac cycle. But it is clear that ventricular contractile function is heavily dependent on XB kinetics [15]. Thus, understanding the pathophysiologic link between sarcomeric processes and whole heart function can provide useful prognostic information and predict response to therapies.

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Invasive left ventricular (LV) catheterization was among the earliest and most direct way of assessing *in vivo* LV pathophysiology, providing important indices of pressure and volume (PV) hemodynamic function. Though it is now rarely used in the diagnosis or clinical evaluation of cardiomyopathies, PV loop analysis still provides a basic physiological framework in relating XB mechanics to ventricular contractility. The effect of ventricular contractility on *in vivo* function has been well studied in models of familial HCM, as it is predominantly caused by autosomal dominant mutations in sarcomeric proteins [33]. Although experimental models using these mutations are not a direct representation of clinical function, they do recapitulate key phenotypic characteristics and fundamental effects of myofilament abnormalities on *in vivo* contractility. In particular, mutations in the gene encoding cardiac myosin binding protein-C (cMyBPC), a regulatory protein found within the C zone of thick filaments, are among the most common causes of familial HCM [34, 35]. While the complex regulatory effects of cMyBPC on XB kinetics is beyond the scope of this review (reviewed in [20]), there are numerous cMyBPC studies that can provide fundamental insights regarding changes in XB kinetics in relation to whole heart function [36–39].

Briefly, cMyBPC mediates acto-myosin interactions in response to neurohormonal adrenergic drive, a mechanism that phosphorylates cMyBPC. cMyBPC phosphorylation has been shown to promote acto-myosin interactions [20] and contribute to thin filament cooperative activation, thereby accelerating XB kinetics [39, 40]. The effects of cMyBPC on rates of XB recruitment (k_{df}) and detachment (k_{rel}) are a measure of the intrinsic myocardial contractility and can be related to specific events of the cardiac cycle including ventricular filling, isovolumic contraction (IVC), ejection, and isovolumic relaxation (IVR) (Figure 2). In skinned fiber stretch activation (SA) experiments, SA elicits a characteristic XB detachment and recruitment kinetics. At the whole-heart level, SA is analogous to the ventricular wall stretch during ventricular filling and IVC phases. Following the closure of mitral valve, myofilaments develop force during IVC at a constant muscle length as a result of XB recruitment (k_{df}). In addition, the timing of myocardial activation during IVC varies across different regions of the heart, first in the endocardium and later in the epicardium, such that at a given time there are some regions of the heart that undergo a stretch activation response [41]. Studies have demonstrated that cMyBPC-mediated XB recruitment is important during low [Ca^{2+}] when thin filament cooperative activation is enhanced, in part, by cMyBPC binding to actin [42]. Thus, the kinetic measurements in fiber SA experiments are expected to play a role throughout IVC pressure development. Fiber and *in vivo* experiments in mice have demonstrated that the rate (k_{df}) and magnitude (P_{df}) of XB recruitment of force generating states show a good correlation to the rate (i.e., dP/dt_{max}) and magnitude (i.e. maximal pressure) of pressure development during IVC [15, 43] (Figure 2). Overall, the presence of cMyBPC in the C zone serves to tune the timing of myofilament activation and slow the rate of pressure development. This slowing or braking role is thought to normalize the timing of contraction as well as prolong the force generation [44].

The rate of XB detachment, k_{rel}, is a measure of XB turnover and contributes to XB on-time and duty ratio. The effect of k_{rel} on *in vivo* contractility is dependent on the number of times XBs turnover during a cardiac cycle [15]. Given what is currently known of sarcomere structure, XB turnover per heart beat is most likely between 1–3 cycles [15, 45]. Thus, as the

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number of times XBs turnover during one cardiac cycle is more than once per cardiac cycle, acceleration of XB detachment (k_{rel}) increases the overall rate of XB turnover and therefore also increase the rate of pressure development (dP/dt) [46]. Furthermore, XB turnover also likely determines the ejection duration and early IVR because the rates of cooperative XB deactivation will determine how long XBs remain attached in late systole [43, 46, 47] (Figure 2). Factors that accelerate k_{rel} (e.g. increased cMyBPC phosphorylation) also accelerate myocyte loaded shortening velocity and shortens *in vivo* ejection time [46, 48]. The acceleration of ejection is an important response during periods of increase adrenergic stimulus but is also a hallmark of dysfunction in HCM, where shortened basal ejection is indicative of hypercontractile myofilament function. XB detachment rate has also been shown to impact rate of early relaxation in early diastole [27, 46, 47, 49], which is thought to involve XB-mediated processes (i.e. cooperative thin filament deactivation) (Figure 2) [15, 26]. The observed blunted diastolic thin filament deactivation in HCM models is consistent with clinical findings that diastolic dysfunction is an important pathogenic component of pre-clinical HCM [49–51].

Transthoracic 2D and Doppler echocardiography have long been the gold standard for the assessment of ventricular function and diagnosis of cardiomyopathies. Yet a positive diagnosis can encompass a wide spectrum of clinical phenotypes depending on disease progression and primary disease mechanism [52, 53]. Furthermore, many cardiomyopathy-associated mutations show incomplete penetrance, and hence there is a need to distinguish genotype positive but phenotype negative individuals who have normal contractile function or delayed-onset age-dependent disease progression [51, 54, 55]. Conventional functional parameters such as ejection fraction (EF) and fractional shortening (FS) are load dependent and are subject to factors independent of myocardium contractility. However, recent advancements in strain analysis by tissue Doppler imaging (TDI), speckle-tracking echocardiography (STE), and cardiac magnetic resonance (CMR) provide an effective method to examine contractility that is loading independent [56–58]. These techniques offer informative and noninvasive assessment of contractile indices such as regional strain rate (SR) and time-to-peak SR (Figure 3). They have been shown to correlate well with invasive PV measurements such as time-to-peak pressure development [58], and *in vitro* physiological kinetic measurements (e.g. loaded shortening velocity) [59]. In addition, XB kinetic parameters would also be expected to contribute to SR, similar to effects observed with PV indices. Physiologic and pathologic acceleration of force development, k_{df}, would also be expected to increase SR similar to the observed changes in dP/dt_{max} in PV analysis. The relationship between the timing for force generation (e.g. early truncation of ejection) observed in PV analysis of the cMyBPC$^{-/-}$ mouse model [60] is also recapitulated in STE measurements (unpublished data), correlating well to both time-to-peak strain and time-to-peak SR measurements (Figure 3). Thus, capturing regional strain can provide a potential means of translating contractility changes at the fiber level to observed localized contractile dysfunction at the whole-heart level, bridging the translational disconnect between basic and clinical indices. Notably, in conditions such as HCM, early detection of subclinical contractility changes within specific cardiac regions (i.e. septum) can be of great prognostic importance [51].

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Skinned cardiac muscle fiber measurements provide mechanistic insights into effects of drugs in clinical trials:

It is now well established that current inotropic therapies enhance cardiac contractility but concurrently elicit undesirable increases in intracellular [Ca^{2+}], myocardial oxygen and ATP needs –factors that ultimately cause arrhythmias and mortality [61]. Thus, a novel approach to improve cardiac performance is to develop a new class of sarcomere-based drugs that evade cellular signaling pathways and instead directly target the cardiac sarcomere [61, 62]. The importance of novel sarcomere-based therapies is highlighted by clinical trials showing promising results using drugs that correct *in vivo* myocardial function by directly modulating force-generating properties of myosin [63–66]. Specifically, omecamtiv mecarbil (OM) and mavacamten (Myk461) are two myosin modulators in clinical and preclinical trials for improving systolic function or suppressing hyperdynamic ventricular function, respectively [16, 65]. Because of their selective affinity to cardiac myosin, any *in vivo* functional changes induced by OM or Myk461 can be linked to their impact on sarcomeric force generation and myosin XB properties. In this context, biophysical measurements using chemically-skinned cardiac muscle fiber preparations can provide insights into the sarcomeric mechanisms governing *in vivo* drug-induced functional changes and as such, predictions from skinned fiber experiments may also aid in developing novel therapies for other sarcomere targets.

Of clinical importance, OM infusions improve *in vivo* systolic function in healthy and HF populations [63–66]. Specifically, OM increases stroke volume, FS, and EF along with prolonged systolic ejection time (SET) and slowed heart rate [63, 64]. A better understanding of OM's *in vivo* effects can be aided by muscle fiber experiments. For example, systolic enhancement can be explained by OM-induced force enhancement at submaximal Ca^{2+} activations in skinned myocardial preparations [10, 12] –suggesting that OM recruits additional XBs to actin during early stages of IVC when sarcomeric Ca^{2+} levels are not at their peak. Interestingly, despite enhancing force generation, OM significantly slows both the rates of XB recruitment (k_{df}) and force redevelopment (k_{tr}), indicating that OM promotes cooperative XB-mediated XB recruitment to actin, a time-consuming process that slows and also sustains the overall force development [10, 12]. This sarcomeric mechanism supports OM-induced *in vivo* SET prolongations. SET prolongation is further supported by the finding that OM extends the overall time to achieve the peak of force development (T_{pk}) in myocardial preparations [12]. Additionally, OM also slowed XB detachment (k_{rel}) suggesting a net increase in XB duty time [10, 12], a finding supported by FV (loaded shortening) experiments (Figure 4) and other biophysical approaches [8, 67, 68]. This OM-induced FV slowing suggests that *in vivo*, OM maintains myocardial power output and contractility for a longer duration. Interestingly, despite a prolonged SET, diastolic duration was only slightly reduced which is likely attributed to a decreased heart rate following OM infusion [63], –thus plasma OM infusions (200–500ng/mL) caused only non-significant decreases in end diastolic volumes (EDVs) [63]. However, in the same study it was observed that EDVs were significantly decreased when [OM] exceeded 500ng/mL. This observation can be explained by results from skinned myocardial experiments which showed that high OM doses not only excessively prolong the T_{pk} but also slow k_{rel} in HF

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myocardium [12], an additive effect that potentially impairs ventricular relaxation and end-diastolic filling as observed clinically [63] –thus, indicating a need for dose optimization such that only the beneficial effects are elicited. Another aspect to be considered for sarcomere-based therapies is that HF myocardium likely undergoes some degree of remodeling and as a result, myosin modulators may behave differently in HF myocardium. For example, skinned fiber studies revealed a substantial time delay in XB recruitment (T_{rec}) which subsequently delayed the T_{pk} in OM-treated HF myocardial preparations [12] – suggesting that OM-induced force enhancements may be delayed in remodeled myocardium.

Another class of myosin modulators are myosin inhibitors (e.g., Myk461), in development to correct hyperdynamic systolic function in HCM patients. Preclinical studies using mouse and feline HCM models show that Myk461 effectively reduces hyperdynamic systolic function, and also reverses pathological remodeling [16, 69]. Supporting evidence from kinetic assays showed that Myk461-mediated decreases in *in vivo* contractility are perhaps related to its ability to inhibit phosphate release from myosin heads, and thereby prevent XB transitions to their strongly-bound states [16, 17]. Using skinned fiber experiments, we recently showed that Myk461-mediated reductions in hypercontractility are primarily due to decreased force development by slowing the net transition of cooperatively recruited XBs to their force-bearing state [11]. Interestingly it appears that Myk461 may affect XB function differently depending on whether cMyBPC is present or absent in the sarcomere. Specifically, we observed that the Myk461-mediated force reductions are attenuated when myocardium lacks cMyBPC, which may be due to a preferential Myk461-induced slowing of k_{rel} in myocardium lacking cMyBPC [11], although more studies will be required to understand the precise contributions of cMyBPC expression on Myk461-mediated modulation of XB behavior. Moreover, this finding is clinically relevant because cMyBPC-related HCM is very common (~40% of all hereditary HCM cases [34, 35]) and largely leads to reduced cMyBPC expression via haploinsufficiency [70–73]. Collectively, in addition to providing key mechanistic insights into *in vivo* clinical data, skinned fiber studies may also be valuable for elucidating mechanisms that are otherwise hard to isolate using *in vivo* techniques, and thus help explain unexpected consequences of therapeutic drugs or dose-dependent effects in a complex whole organ system.

Summary:

Advancement of cardiovascular medicine requires that clinical trials leverage the molecular insights derived from biophysical measurements in isolated cardiac muscle. Integration of unique findings at the sarcomeric level will lead to a better understanding of drug function *in vivo* and optimization of clinical trials to achieve improved patient outcomes.

Acknowledgments

Sources of Funding

This work was supported by the National Institutes of Health (HL-114770 to Stelzer; T32-HL007567 and GM007250 to Li), American Heart Association (16POST30730000 to Mamidi), and National Institutes of Health (P30 EY011373) grants.

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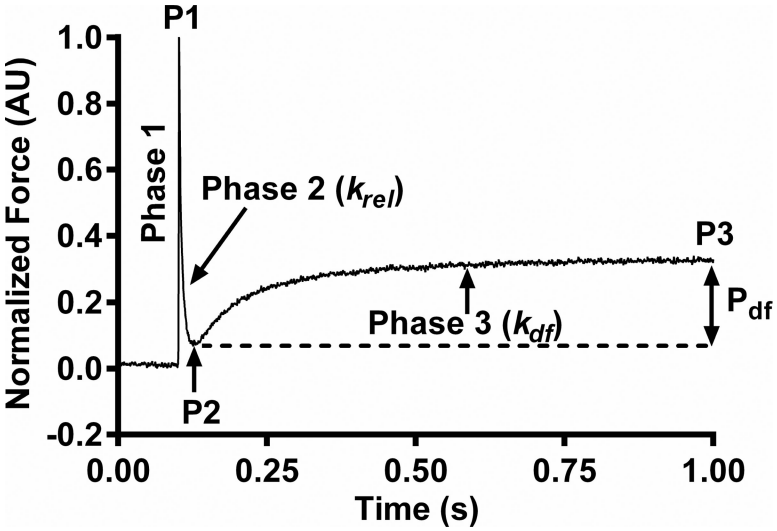

Figure 1. Representative stretch activation response in wild-type (WT) mouse myocardium.

The important phases of the force transient and various stretch activation parameters are highlighted. In an isometrically contracting mouse myocardial preparation, a sudden 2% stretch in muscle length (Phase 1) induces an abrupt spike in force (P1) due to strained elastic elements within the strongly bound XBs. Force then rapidly decays (Phase 2), with a rate constant k_{rel} and magnitude P2, due to the detachment of the strained XBs into a non-force bearing state. Next, there is a delayed force redevelopment (Phase 3), with a rate constant k_{df}, as XBs reenter force-bearing states via stretch-induced cooperative recruitment to reach a higher steady state force level (P3). P_{df} denotes the magnitude of XB recruitment and is the difference between P3 and P2. For a detailed description of various phases, refer to [22, 23].

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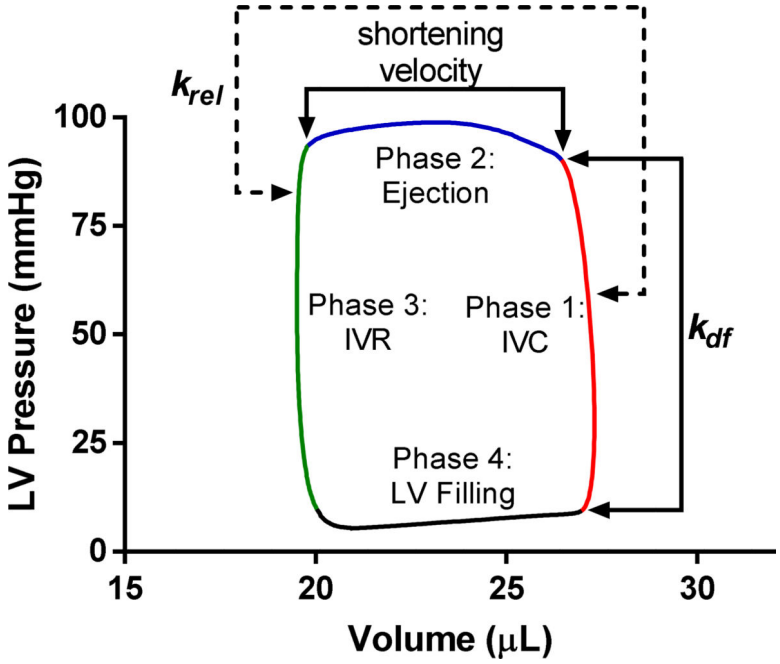

Figure 2. Representative pressure-volume trace (PV loop) with the phases of cardiac cycle and correlated parameters measured in skinned myocardial preparations.

Early isovolumic pressure development, dP/dt_{max}, and t_d during phase 1 is affected by the magnitude (P_{df}) as well as the rate (k_{df}) of XB recruitment. k_{df} is Ca^{2+} sensitive due to the influx of Ca^{2+} during IVC. k_{rel} is a measure of XB turnover, so it may also contribute to the isovolumic pressure development during phase 1. ET during phase 2 is influenced by k_{rel} and shortening velocity. Since Ca^{2+} is absent at the end of the ejection phase, k_{rel} determines ET by altering the timing of cooperative deactivation of thin filaments. Early isovolumic relaxation during phase 3 can be correlated with k_{rel} as well because XB detachment rates influence early diastolic relaxation. dP/dt_{max}, maximum rate of pressure development; ET, ejection time; IVC, isovolumic contraction; IVR, isovolumic relaxation; k_{df}, rate of XB recruitment; k_{rel}, rate of XB detachment or XB turnover; LV, left ventricular; t_d, time to dp/dt_{max}; and XB, cross bridge.

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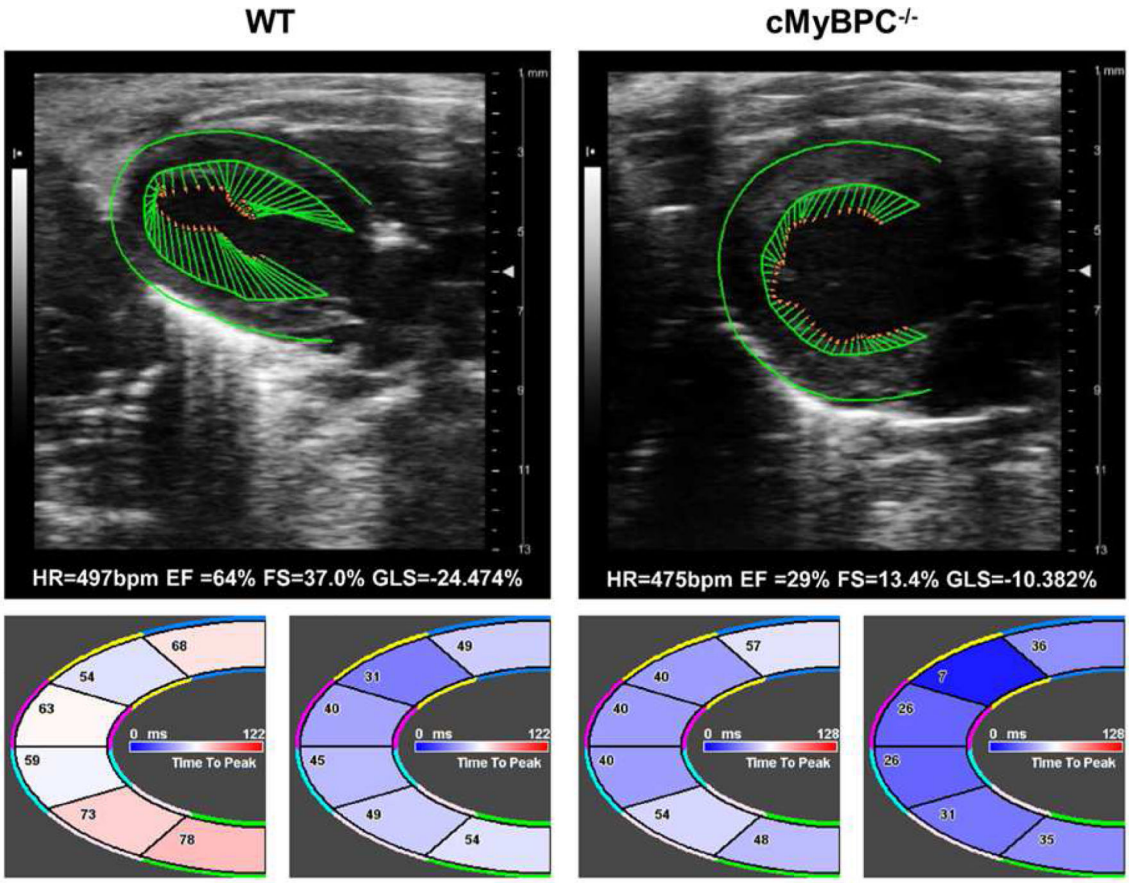

Figure 3. Representative speckle-tracking echocardiography (STE) analysis of WT and cMyBPC $^{-/-}$ mouse myocardium.

The top panel shows representative STE images along with the heart rate (HR), ejection fraction (EF), fractional shortening (FS), and global longitudinal strain (GLS) of WT (left) and cMyBPC$^{-/-}$ (right) mouse myocardium. The regional time-to-peak strain (left) and time-to-peak strain rate (right) are shown below WT and cMyBPC$^{-/-}$ STE images. Notably, cMyBPC$^{-/-}$ STE shows reduced GLS, shortened time-to-peak strain, and shortened time-to-peak strain rate.

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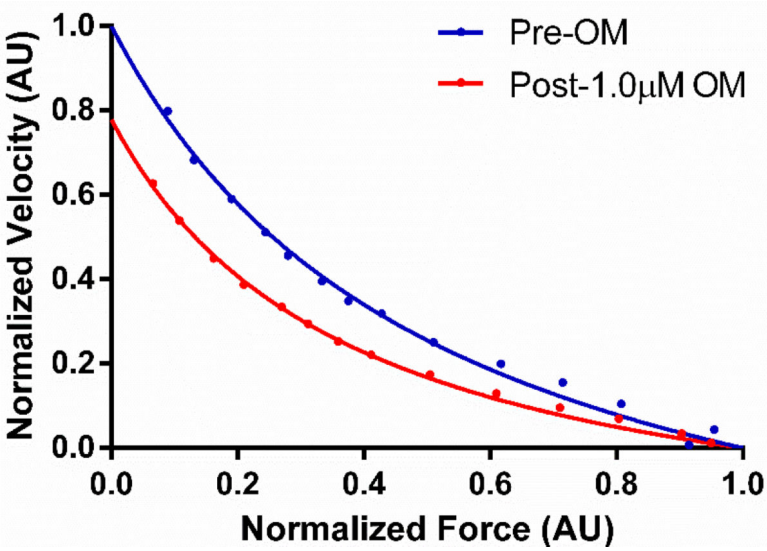

Figure 4. Representative force velocity (FV) curves demonstrating the effect of OM on loaded shortening velocity in donor human myocardium.

Representative FV curves collected at ~30% of maximal force generation and at ~25°C are shown and demonstrate a slowed loaded shortening velocity following incubation of skinned myocardial fibers with 1.0μM OM.