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Maladaptive modifications in myofilament proteins and triggers in the progression to heart failure and sudden death

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Abstract

In this review, we address the following question: Are modifications at the level of sarcomeric proteins in acquired heart failure early inducers of altered cardiac dynamics and signaling leading to remodeling and progression to decompensation? There is no doubt that most inherited cardiomyopathies are caused by mutations in proteins of the sarcomere. We think this linkage indicates that early changes at the level of the sarcomeres in acquired cardiac disorders may be significant in triggering the progression to failure. We consider evidence that there are rate-limiting mechanisms downstream of the trigger event of Ca^{2+} binding to troponin C, which control cardiac dynamics. We discuss new perspectives on how modifications in these mechanisms may be of relevance to redox signaling in diastolic heart failure, to angiotensin II signaling via β -arrestin, and to remodeling related to altered structural rigidity of tropomyosin. We think that these new perspectives provide a rationale for future studies directed at a more thorough understanding of the question driving our review.

Keywords

Troponin; Tropomyosin; Myosin-binding protein C; Posttranslational modifications

Introduction and scope

Heart failure (HF) and other disorders of the heart are commonly studied after disease progression to decompensation, especially in human heart studies [28, 71]. This makes sense in view of the need for therapies in patients in which HF is extant or clearly in progress, for example, following survival of a myocardial infarction (MI). Yet, understanding the sequence of events that underlie the transition from the compensated to the decompensated state remains an important and challenging objective. In considering the timing of the mechanisms in the transition, factors extrinsic to the heart muscle (for example, hypertension, coronary artery disease, viral infection) are common instigators of the disease process. However, prevalent cardiomyopathies (CMs) are also instigated by modifications intrinsic to the heart muscle cells, especially in the form of missense or deletion mutations in proteins of the sarcomere [20, 67]. In this case, the progression to hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) and sudden death starts at the level of the sarcomeres. Moreover, arrhythmogenic sudden death has been directly linked to altered sarcomeric properties [4]. We think that this linkage of disease progression to the sarcomeres provides lessons relevant to an understanding on acquired HF. Therefore, our review addresses the following major question: Are modifications at the level of sarcomeric proteins in acquired HF early inducers of altered cardiac dynamics and signaling leading to remodeling and progression to decompensation?

There have been excellent reviews [14, 22, 23, 37, 54, 61, 64] of the roles of cardiac sarcomeres in familial and acquired disorders of the heart leading to HF and in chapters of a recent monograph [62] on “Biophysics of the failing heart.” Mechanisms that control the heartbeat have been usefully categorized into either membrane-controlled mechanisms upstream of Ca^{2+} binding to troponin C (TnC) or sarcomere-controlled mechanisms downstream of Ca^{2+} binding to TnC [7, 39, 63]. In the present review, we focus on relatively new findings, which provide new perspectives in whether downstream myofilament modifications may be among the earliest changes leading to HF progression. Moreover, our review emphasizes the importance of fully integrating altered myofilament properties together with altered growth factor, nuclear, neurohumoral, and Ca^{2+} signaling into the consideration of mechanisms of HF progression.

Primary alterations in sarcomere proteins affect systolic and diastolic states by altering cardiac dynamics

If, as we propose, causal and early modifications in sarcomeric proteins occur in the progression to HF, then these modifications should perturb myofilament functions that are significant determinants of cardiac dynamics. While there is no doubt that levels and kinetics of Ca^{2+} transients trigger contraction and are critical determinants of the level and rate of rise and fall of systolic pressure, there appears to be a significant role for processes at the level of the myofilaments as determinants of the duration of systolic elastance, and the time course of isovolumic relaxation. We [27] have previously summarized arguments for this assertion, and since then, more supportive documentation [30, 63] has been published. In our assessment of the role of downstream sarcomere mechanisms in cardiac dynamics, we employed results of indirect evidence and modeling to predict the time course of Ca^{2+} bound

to TnC during a beat of the heart. We concluded from this speculation that the fall in bound Ca^{2+} occurred well before the fall in ventricular pressure and that control mechanisms downstream of Ca^{2+} bound to TnC have a prominent role in determining the duration of systole and the time course of fall in isovolumic pressure. We emphasized that one mechanism for this maintenance of ejection and pressure appears to be a time-dependent cooperative activation of the thin filaments. There is also abundant evidence to support the idea of control of contraction/relaxation kinetics by alterations in crossbridge kinetics [27, 63]. Gain and loss of function studies with variations in crossbridge kinetics have been relatively straight-forward. However, gain and loss of cooperative activation in the sarcomeres and determination of the impact of this modification of the dynamics of the heartbeat have been more difficult to attain with high fidelity and specificity. We summarize one approach below in the section discussing altered tropomyosin (TM) flexibility and cardiac function.

Further support for the idea that mechanisms at the level of the myofilaments may be rate limiting has come from studies of Stehle and Iorga [63], who measured the kinetics of downstream and upstream mechanisms in preparations with and without removal of the membranes. A conclusion they state from their modeling and measurements is that "...the dynamics of cardiac contraction and relaxation are determined mainly by sarcomeric downstream mechanisms, in particular by the kinetics of the cross-bridge cycle." Moreover, they emphasize that sarcomere lengthening is an essential element in cardiac relaxation and the early phase of diastolic filling. Janssen [30] also report a strong support for an essential role of downstream mechanisms in a control of the dynamics of the heartbeat. They carried out experiments at physiological temperatures in preparations with little or no diffusion barriers and have provided convincing evidence that the time course and amplitude of Ca^{2+} transients do not explain changes in force development occurring during both the force-frequency relation and length-dependent activation (Frank-Starling relation). In the case of the force-frequency relation, the data indicate that relaxation occurs despite high levels of diastolic Ca^{2+} . The mechanism appears to be an increase in troponin I (TnI) phosphorylation at sites well known to desensitize the myofilaments to Ca^{2+} . The exact mechanism relating frequency to TnI phosphorylation remains to be determined. A reduction in force with increases in frequency in the myocardium from failed human hearts has been known to occur for many years [2]. With regard to Starling's law and length-dependent myofilament activation, while the mechanism remains a topic of research and debate [12, 15, 17, 34], studies by Monasky et al. [50] have emphasized a role for downstream mechanisms in control of the prolonged relaxation occurring with preload-dependent increases in muscle length. Posttranslational modifications of myofilament proteins have been invoked as a cause for the length-dependent modulation of contractile dynamics [49]. Moreover, as pointed out by ter Keurs [66], the loss of adrenergic signaling in HF induces an increased reliance of the heart on the Frank-Starling relation with the potential of the elevated end-diastolic pressures to exacerbate coronary flow abnormalities.

Hypertension-induced redox signaling to myofilaments induces diastolic dysfunction independent of altered cellular Ca^{2+} fluxes

The release of reactive oxygen species (ROS) occurs as a general response to high angiotensin II (Ang II) levels in hypertension [38]. In this and the next section, we discuss new perspectives in acquired HF in terms of a potential role of posttranslational modifications of myofilament proteins in response to hypertension and Ang II. Figure 1 illustrates a scheme summarizing these perspectives. The role of oxidative damage to sarcomeric proteins has been reviewed before [5], and here we focus on significant recent advancements to our understanding on sarcomeric oxidative stress in the last few years. We have employed a well-established model of arterial hypertension and diastolic dysfunction to understand how the sarcomeric proteins are modified following an oxidative insult [41]. It has been shown that the deoxycorticosterone acetate (DOCA)-salt hypertension mouse model is characterized by an increase in the production of ROS from the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, leading to oxidation of tetrahydrobiopterin (BH_4), a cofactor of endothelial nitric oxide synthase (eNOS) in the production of nitric oxide from L-arginine [35]. This oxidation of BH_4 to dihydrobiopterin, and thus its depletion, leads to uncoupling of eNOS and the production of superoxide anions instead of nitric oxide [6, 18]. In this mouse model, recent work in our laboratories has identified a myofilament redox-related modification as a primary cause of diastolic abnormalities [31, 41, 51]. In fact, as indicated in Fig. 1, in the DOCA-salt mouse model, cardiac oxidation leads to cardiac diastolic dysfunction, which can be ameliorated by BH_4 treatment [31, 57]. Concomitantly, we observed that together with a reversal of diastolic dysfunction, there was a reversal of an increase in S-glutathionylation of cardiac myosin-binding protein C (cMyBP-C), and the slowed crossbridge kinetics, which occurred in hearts from DOCA-salt mice [31]. Increased S-glutathionylation is one indicator of oxidative stress since oxidative stress leads to reversible glutathione binding to thiols of proteins to protect them from irreversible oxidative damage [69].

We have also reported an improvement of the impaired ventricular relaxation in hearts of DOCA-salt mice with administration of Ranolazine [41], a late I_{Na} blocker used clinically to treat angina [24]. In this study, the time course and amplitude of Ca^{2+} transients in ventricular myocytes were not different from sham, suggesting a mechanism by which relaxation is altered on the level of the myofilaments independent of Ca^{2+} signaling [41]. Detergent-extracted fiber bundles from DOCA-salt hearts demonstrated an increased myofilament Ca^{2+} sensitivity and slowing of cross-bridge kinetics, which were also reversed with Ranolazine treatment [41]. These results strongly indicate a role for altered myofilament relaxation dynamics and/or response to Ca^{2+} as a significant factor in this model of a diastolic disorder with preserved systolic function and suggest a mechanism involving an oxidative modification on MyBP-C [31, 41].

Since oxidative stress during hypertension can lead to an increase in the posttranslational modification of S-glutathionylation and, indeed, an increase in S-glutathionylation of MyBP-C in the hypertensive DOCA-salt mouse model has been observed, a later study probed the role of MyBP-C and the different sites, which may be involved in regulating the sarcomeric response to Ca^{2+} [51]. S-glutathionylation of MyBP-C C655, C479, and C627 was

associated with an increase in myofilament Ca^{2+} sensitivity in isolated detergent-extracted force-generating fiber bundles treated with oxidized glutathione (GSSG). This increase in myofilament Ca^{2+} sensitivity and *S*-glutathionylation of MyBP-C were reversed with the reducing agent, dithiothreitol (DTT) [51]. These results provide a novel mechanism involving the sarcomeric protein MyBP-C in the regulation of the sarcomeric response to Ca^{2+} and suggest that alterations to sarcomeric proteins may occur before changes in Ca^{2+} fluxes in hypertension and the progression to acquired HF. This is a significant advancement in identifying one of the first modifications to oxidative stress and opens a new focus of investigation, since sarcomeric alterations during oxidative stress can occur before and independently of alterations in Ca^{2+} signaling. This may apply not only to hypertensive heart disease but also to other diseases in which severe oxidative stress is central to the pathology. For example, the release of ROS occurs as an early response to MI and reperfusion [76]. In a short-term response to MI in a mouse model, we concluded that titin may also be a site of *S*-glutathionylation [3]. More recent work [1], positively identified titin as a substrate for *S*-glutathionylation and determined that with stretch cryptic cysteines, become available as substrates. The effect of *S*-glutathionylation of titin was to increase its extensibility in vitro and in situ in cardiac myocytes.

Ang II induces an increase in myofilament Ca^{2+} response that is preserved by a biased ligand acting as an Ang I-type 1 receptor blocker

Studies of biased ligands, which selectively activate signal transduction pathways of a given receptor, suggest an exciting new role for myofilaments in HF (Fig. 1). These biased ligands can partially activate downstream signaling cascades of a particular receptor by activating one cascade while blocking another. Such precise signaling cascade targeting may lead to the development of pharmaceuticals with less undesired side effects [16]. Two such biased ligands, namely, TRV120023 and TRV120027, have been described previously to bind Ang II-type 1 receptors (AT1R) to block G protein signaling yet preserve β -arrestin signaling and contractility [32, 48, 73]. In this way, the biased ligand TRV120027 inhibits Ang II-mediated vasoconstriction via blockade of G protein signaling yet increases cardiomyocyte contractility via β -arrestin signaling [73]. TRV120027 can reduce blood pressure in humans [60] and dogs [8, 9] and is in development as a pharmaceutical treatment for acute HF.

When administered to cardiomyocytes, TRV120023, a drug very similar to TRV120027, did not lead to the accumulation of inositol monophosphate 1 (IP1) and diacylglycerol (DAG) [32], nor did administration of the β -arrestin-biased AT1R agonist [Sar¹, Ile⁴, Ile⁸]-angiotensin II (SII) raise intracellular Ca^{2+} levels [55], suggesting an increase in the Ca^{2+} sensitivity of sarcomeric proteins as the mechanism of the inotropic effect of TRV120023 [32]. Since sarcomeric proteins can already regulate differences in force production in healthy myocardium despite the same intracellular Ca^{2+} concentration [46, 50], it stood to reason that changes at the myofilament level independent of Ca^{2+} signaling could alter contractile parameters. In fact, even in a rabbit model of acutely altered Ca^{2+} hemodynamics, there was still a significant dissociation between the rate of force relaxation and intracellular Ca^{2+} decline, indicating that the sarcomeric proteins are the final regulators in the development of force [47].

A recent study [48] investigated the effect of Ang II and TRV120023 on myofilament Ca^{2+} sensitivity. In this study, TRV120023 increased maximal Ca^{2+} -activated isometric tension, pCa_{50} , and the *Hill* coefficient, which is a determinant of crossbridge cooperative activation. Ang II increased maximal Ca^{2+} -activated isometric tension and pCa_{50} but decreased the *Hill* coefficient, suggesting a different mechanism from TRV120023 for myofilament regulation [48]. The effect of Ang II on maximal Ca^{2+} -activated isometric tension and pCa_{50} was still evident with simultaneous treatment with TRV120023 while apparently abolished with simultaneous treatment with losartan, an AT1R antagonist [48]. This is consistent with another study in which cardiomyocyte fractional shortening was increased after administration of Ang II, TRV120027, or TRV120023 [73]. The effects of both TRV120027 and TRV120023 were blocked by coadministration of valsartan, another Ang II receptor blocker with high affinity for AT1Rs [73]. Further investigation into sarcomeric changes revealed an increase in TM phosphorylation during both Ang II and TRV120023 administrations [48]. Additionally, other effects during administration of Ang II or TRV120023 were observed on β -myosin heavy chain isoform expression and phosphorylation of cardiac TnI and myosin-binding protein C S282 [48]. These studies suggest that during Ang II signaling or biased ligand signaling by TRV120027 and TRV120023, the final regulation of contraction is mediated by early changes at the level of the sarcomeric proteins independent of Ca^{2+} signaling.

Alterations in structural flexibility of tropomyosin affect cardiac function

As summarized above, whereas the cooperative activation of the myofilaments appears to be controlled by β -arrestin signaling, its role in the control of the dynamics of the heartbeat remains unclear. TM is indispensable both in cooperative activation within a structural unit (seven actins controlled by Tn/TM) as well as in cooperative activation by interactions between structural units leading to an extended functional unit. There is evidence that the flexibility of TM might be important for the role TM plays in cooperative activation. In this section of the review, we analyze new findings on structural and functional implications of alterations in TM's structural rigidity or flexibility and discuss whether the alteration in the flexibility of TM could be an initial molecular abnormality that leads to disease phenotype. Support for this idea comes from studies indicating that alterations in structural flexibility of TM may be an initial trigger for inherited HCM and DCM that are linked to TM mutations [25, 33, 40, 43, 45, 78].

α -TM has an α -helical coiled coil structure along its entire length in which two α -helices associate to form a supercoil [13, 53, 77]. Coiled coils form a heptad repeat motif (*abcdefg*) where hydrophobic residues at the core positions *a* and *d* stabilize the molecule, while residues at positions *e* and *f* can supplement this stabilization [74]. Although destabilizing breaks in the heptad repeat structure of coiled coil proteins is a common and functionally important characteristic of these proteins, α -TM is unique as it has no discontinuity in its coiled coil repeats [10]. Alternatively, large hydrophobic residues at positions *a* and *d* are often interrupted by small hydrophobic, charged, or polar residues in α -TM. This interruption creates in TM's structurally distinct domains that differ in their structural stability, which introduces an alteration in structural flexibility [11, 29, 52, 70]. Brown et al. was first to point out poorly packed regions around Ala residues of α -TM that are scattered

along the molecule and located at *a* and *d* positions [11]. These so-called Ala clusters destabilize regions of TM to provide the molecule flexibility, which is considered important for binding of TM to actin [58, 59]. In 2008, Sumida et al. showed that a conserved Asp137 residue of α -TM in the *d* position contributes to another poorly packed region at the center of TM and provides flexibility to the molecule [65]. Mutation of this Asp137 to Leu, a hydrophobic residue, decreases the flexibility of TM and increases the ATPase activity of reconstituted filaments [65, 78]. Another flexible region around the noncanonical Gln263, which is also located at a *d* position, has been shown to have a significant function enabling complex formation between C-terminus and N-terminus of the adjacent TM molecules as well as between TM and troponin T [21]. It is previously reported that there is another poorly packed region around noncanonical and destabilizing residues (Tyr214, Glu218, Tyr221, and Tyr267) at the hydrophobic core of α -TM; nonetheless, its functional significance is not well understood [44]. These findings altogether suggest that destabilizing regions of α -TM are essential for its proper functioning in vitro and highlight the need for further studies to address functional significance of these regions in ejecting hearts.

Recent studies have suggested a possible link between alterations in TM's structural flexibility and inherited CMs. For example, TM's increased flexibility has emerged as the characteristic of HCM-linked mutations (Glu180Gly, Asp175Asn, Lys70Thr, and Ala63Val) [25, 33, 40], whereas the DCM-linked Glu40Lys and Glu54Lys mutations have been shown to decrease the flexibility of TM [45]. In support of the causal link between altered flexibilities of TM and CMs, findings from our recent study showed that an expression in transgenic (TG) mouse hearts of a decreased flexibility mutant α -TM-Asp137Leu, which is not yet associated with CMs, depresses cardiac contractility and leads to a phenotype similar to DCM [78]. In this study, depressed contractile function and mild ventricular dilation of TG mice hearts was detected with echocardiography. As an extension to this previous study, our recent pressure-volume (PV) loop measurements (Fig. 2) confirmed depressed pump function in α -TM-Asp137Leu TG mice hearts. As shown in the representative baseline PV loops, during an inferior vena cava (IVC) occlusion, there was a loss of intrinsic inotropy in TG mice hearts as evident from a significant decrease in the slope of end-systolic pressure-volume relation (ESPVR). The significant increase in end-systolic volume (ESV) and end-diastolic volume (EDV), the decrease in the slope of ESPVR and end-diastolic pressure-volume relation (EDPVR), and the decrease in dP/dt max corroborate a phenotype similar to DCM. Interestingly, this depression in contractility occurred in the absence of any changes in intracellular Ca^{2+} transients. This result suggests a mechanism intrinsic to the myofilaments.

DCM-linked sarcomeric mutations are generally considered to cause a decrease in myofilament Ca^{2+} sensitivity, whereas HCM-linked sarcomeric mutations lead to an increase. Although our laboratory findings (i.e., TG mice that expresses a decreased flexibility mutant α -TM-Asp137Leu in the heart shows a DCM phenotype and a decreased myofilament Ca^{2+} sensitivity [78]) support this observation, there are still significant exceptions. For example, one study reports that DCM-linked α -TM Asp230Asn mutation increases myofilament Ca^{2+} response. Another study shows that α -TM Glu54Lys mutation does not change Ca^{2+} sensitivity [43]. A recent study demonstrates that there is no increase

in Ca^{2+} sensitivity in a human HCM heart sample with an α -TM Ile284Val mutation [56]. Therefore, change in Ca^{2+} sensitivity may not be the major trigger in pathogenesis. Considering the findings summarized before, alterations in structural flexibility of TM may be an essential initial molecular abnormality that leads to disease phenotype. However, it is likely that additional factors contribute to the disease mechanism.

At the sarcomere level, TM regulates Ca^{2+} - and ATP-dependent interactions between thin and thick filament proteins [75]. During this process, TM transmits the information from TnC, the Ca^{2+} receptor, to the actin-myosin interaction. We argue that variations in the flexibility of α -TM affect information transmission along the length of the molecule and thus lead to dysfunction. According to a widely accepted three-state model by McKillop and Geeves [42], during muscle contraction, thin filaments exist in a rapid equilibrium between three states. Ca^{2+} binding to Tn triggers relocation of TM from a blocked thin filament state to a weak crossbridge binding or closed state, and full activation requires an open state induced by strong crossbridge binding [36, 42, 72]. In addition to its effect on information transmission along the molecule, structural flexibility of TM is considered one of the main mediators of TM fluctuations relative to actin filaments during thin filament transition from activation (systole) to relaxation (diastole). Due to a small value for the equilibrium constant between blocked and open states of the thin filaments [19], relatively small changes in the thermal fluctuations of TM could easily alter its rapid regulatory movements on the surface of actin. There is no doubt that disruptions in the molecular switch mechanism would eventually alter physiological functioning of TM and result in a disease phenotype. Findings from our previous study support this hypothesis. We showed that decreasing structural flexibility of TM depresses Ca^{2+} -dependent activation of cardiac thin filaments from TG mouse without causing any changes in the strongly bound crossbridge-dependent activation of skinned fiber preparations [78]. This result implies that decreasing flexibility of TM impedes Ca^{2+} -dependent transition of TM between blocked and closed states. Yet, we could not find any changes in strong crossbridge-dependent transition of TM between closed and open states. Therefore, the decrease in TM's flexibility is likely to have impeded Ca^{2+} -dependent relocation of TM between blocked and closed states, resulting in a delay in time-sensitive contraction and relaxation processes of a cardiac myocyte. This proposed delay could eventually induce cardiac dysfunction, as was observed in TG α -TM-Asp137Leu mouse hearts.

Cooperative activation process of cardiac myofilaments involves Ca^{2+} binding to regulatory units, cooperative interactions between thin and thick filament proteins within a single regulatory unit, and long-range cooperative interactions between neighbor regulatory units along the filaments [19, 26, 68]. Although individual contribution of each component has not been clearly identified, TM molecules are known to play a crucial role in this cooperative activation process, possibly through end-to-end interactions between adjacent molecules leading to the spread of activation signal [19, 26]. However, little is known about the likely influence of changes in structural flexibility of TM on this cooperative activation process. Loong et al. recently argue that alterations in TM's rigidity affect the cooperative spread of activation signal along TM molecule and across thin filaments [39, 40]. In their model, they particularly propose that increased flexibility of the HCM mutant α -TM-Glu180Gly significantly impedes signal transmission along the molecule, causing a higher

Ca²⁺ sensitivity as well as a reduction in cooperative transmission of activation signal along the thin filaments. This explains prolonged systolic activation and diastolic relaxation of cardiac thin filaments from HCM hearts that express a more flexible TM. In support of these studies, our recent findings demonstrated an increase in Ca²⁺-dependent cooperative activation of thin filaments from TG mouse hearts expressing a decreased flexibility mutant α -TM-Asp137Leu [78]. On the contrary, a previous in vitro study by Heller et al. shows that HCM mutations Lys70Thr and Ala63Val increase N-domain flexibility of TM while having no effect on Ca²⁺-dependent cooperative activation of thin filaments [25]. Therefore, more studies are needed to reveal the effect of alterations in structural flexibility of TM on cooperative activation of thin filaments.

Future directions in investigating integration of modifications in myofilament proteins with cardiac dynamics

A critical question for the future is the time course of the relation between Ca²⁺ bound to the regulatory site of TnC and the time course of pressure and volume in heartbeats in various states. Probes for this determination in real time under load would serve to answer many questions concerning the relative role of upstream and downstream control of cardiac function (for example, those illustrated in Fig. 1). A related future direction is the determination of the dynamics of posttranslational modifications of sarcomeric proteins in relation to altered functional state of the myocardium. The development of probes to address this question should be a major consideration. While much has been theorized regarding the importance of cooperative activation of the sarcomeres, little is known regarding the in situ role for this mechanism or the relevance of variations in cooperative activation on cardiac function. Exciting future directions of research also involve continual unraveling of the many functions of the sarcomeric proteins in mechano-signaling including the functional effects of altered flexibility, and the influence of folded state on posttranslational modifications.

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Fig. 1.

Angiotensin II signaling and control of cardiac myofilament kinetics and response to Ca^{2+} . The Ang II signaling cascade on the *left* emphasizes evidence that in the presence of Ang II, (1) redox signaling (reversed by BH_4) via S-glutathionylation of myosin binding protein C slows crossbridge (XB) kinetics and may be significant in diastolic heart failure, and (2) kinase/phosphatase modifications alter myofilament protein phosphorylation and response to Ca^{2+} . The signaling scheme on the *right* emphasizes evidence that the biased ligand, TRV120023, which binds to the Ang II receptor type 1 (AT1R), unlike conventional blockers, blocks only G protein signaling and activates β -arrestin 2-mediated signaling. Thus, Ang II effects on contractility are increased and preserved, whereas hypertrophy is blocked. Also shown in the lower *right hand panel* is the influence of tropomyosin (TM) flexibility on cooperative activation and cooperativity. See text for further discussion

Fig. 2. Representative left ventricular pressure-volume loops under baseline conditions, following an inferior vena cava occlusion. The *black solid loops and solid slope line* represent the ESPVR in the non-transgenic (NTG) group. The *dotted black loops and dotted slope* represent the depressed ESPVR in the TG-TM-D137L group