

Diastolic myofilament dysfunction in the failing human heart

Jolanda van der Velden

Received: 17 March 2011 / Revised: 23 March 2011 / Accepted: 24 March 2011 / Published online: 13 April 2011
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Abstract In recent years, it has become evident that heart failure is not solely due to reduced contractile performance of the heart muscle as impaired relaxation is evident in almost all heart failure patients. In more than half of all heart failure patients, diastolic dysfunction is the major cardiac deficit. These heart failure patients have normal (or preserved) left ventricular ejection fraction, but impaired diastolic function evident from increased left ventricular end-diastolic pressure. Perturbations at the cellular level which cause impaired relaxation of the heart muscle involve changes in Ca^{2+} -handling proteins, extracellular matrix components, and myofilament properties. The present review discusses the deficits in myofilament function observed in human heart failure and the most likely underlying causal protein changes. Moreover, the consequences of impaired myofilament function for in vivo diastolic dysfunction are discussed taking into account the reported changes in Ca^{2+} handling.

Keywords Diastole · Myocardial contractility · Muscle stiffness · Myofilament · Phosphorylation · Heart

Systolic and diastolic function of the heart: role of the myofilaments

Every heart beat, the ventricles eject blood into the small and large circulation to provide organs with sufficient oxygen. Cardiac output depends on the amount of blood

ejected per heart beat (i.e., stroke volume) and heart rate. Although myocardial muscle contraction is indispensable for proper cardiac output during the systolic (activation) phase of the cardiac cycle, filling of the ventricles during the diastolic (relaxation) phase heavily depends on proper cardiac muscle relaxation. The latter is even more important during increased cardiac stress as occurs during exercise. To match cardiac output to increased demands of the body, heart rate is increased by enhanced sympathetic drive. The magnitude of contraction is increased by increased Ca^{2+} -induced Ca^{2+} -release from the sarcoplasmic reticulum (SR) within the heart muscle cells. To match the increase in heart rate, a faster relaxation of the heart muscle is required which is achieved by increased re-uptake of Ca^{2+} into the SR and desensitization of the myofilaments to Ca^{2+} [6, 56].

Upon depolarization of the heart muscle cells, L-type Ca^{2+} -channels are opened, which causes Ca^{2+} entry into the cytosol and triggers Ca^{2+} release from the SR via the ryanodine receptors (RyR2; so-called Ca^{2+} -induced Ca^{2+} -release). Subsequently, Ca^{2+} binds to troponin C and initiates myofilament contraction via interactions between the thick filament myosin heads and the thin filament component actin. Relaxation of the heart muscle cells occurs upon detachment of Ca^{2+} from the troponin complex and subsequent re-uptake of Ca^{2+} into the SR via the SR Ca^{2+} -ATPase (SERCA2), which activity depends on the phosphorylation status of phospholamban (i.e., unphosphorylated phospholamban blocks SERCA2 activity). SERCA2 is responsible for re-uptake of ~70% of the Ca^{2+} involved in the Ca^{2+} transient and approximately 30% of the cytosolic Ca^{2+} is removed out of the cell via the Na^{+} - Ca^{2+} exchanger (NCX) [6, 7]. Changes in cellular Ca^{2+} cycling and myofilament properties are under the tight control of kinases and phosphatases within the heart muscle cells, which respectively phosphorylate and dephosphory-

J. van der Velden (✉)
Laboratory for Physiology, Institute for Cardiovascular Research (ICaR-VU), VU University Medical Center, van der Boechorststraat 7, 1081 BT, Amsterdam, the Netherlands
e-mail: j.vandervelden@vumc.nl

late cellular target proteins that regulate contraction and relaxation [29, 51]. Upon increased sympathetic activation, β_1 -adrenergic receptors are activated which initiates protein kinase A (PKA)-mediated phosphorylation of proteins involved in Ca^{2+} handling (RyR2, phospholamban) and of the myofilament target proteins troponin I (cTnI), myosin binding protein C (cMyBP-C), and titin [6, 28, 40, 56, 75]. The predominant role of the myofilaments during increased β_1 -adrenergic receptor stimulation is enhancement of relaxation, which is caused by desensitization of the myofilaments to Ca^{2+} (Fig. 1a) and faster kinetics of

cross-bridge cycling [17, 77]. The PKA-mediated phosphorylation of cTnI is thought to be the major contributor to myofilament Ca^{2+} -desensitization [56]. More recent studies have indicated a modulating role for cMyBP-C in the PKA-mediated reduction of myofilament Ca^{2+} -sensitivity [16, 17, 37], although the major effect exerted by phosphorylated cMyBP-C seems to be enhancement of the rate of contraction and relaxation [39, 44, 58, 59]. The third protein phosphorylated upon β_1 -adrenergic receptor activation is the giant protein titin [75], which, upon PKA-mediated phosphorylation, reduces passive stiffness of cardiac muscle cells [11, 40, 75]. Overall, the PKA-mediated enhancement of myofilament relaxation is critical to maintain proper cardiac performance at increased heart rates associated with β -adrenergic stimulation.

Apart from phosphorylation-induced changes in myofilament function, a change in sarcomere length upon increased filling of the ventricles during diastole increases the maximal force-generating capacity and the Ca^{2+} -sensitivity of the myofilaments (Fig. 1b). This length-dependent activation is called the Frank–Starling mechanism of the heart and underlies increased cardiac output at increased left ventricular (LV) end-diastolic volumes. The exact mechanisms underlying the increased force-generating capacity of the myofilaments at higher sarcomere lengths are still controversial and have been extensively discussed in previous reviews [33, 36].

Lastly, changes in heart rate adjust myofilament properties to cardiac pump performance [2, 42, 43, 69]. Under physiological conditions, an increase in cardiac stimulation frequency results in enhanced systolic function (so-called positive force–frequency relation), which has been attributed to an increased Ca^{2+} influx into the cardiomyocytes. The increase in Ca^{2+} influx increases SR Ca^{2+} content and promotes the Ca^{2+} -induced Ca^{2+} -release. Varian and Janssen [69] observed a decrease in myofilament Ca^{2+} -sensitivity with increased frequency in the healthy myocardium and suggested that, similar to β -adrenergic PKA-mediated Ca^{2+} -desensitization, the frequency-induced myofilament Ca^{2+} -desensitization accelerates relaxation of the heart muscle. The frequency-mediated alteration in myofilament Ca^{2+} -sensitivity most likely involves changes in protein phosphorylation caused by Ca^{2+} -activated kinases [42, 61, 70].

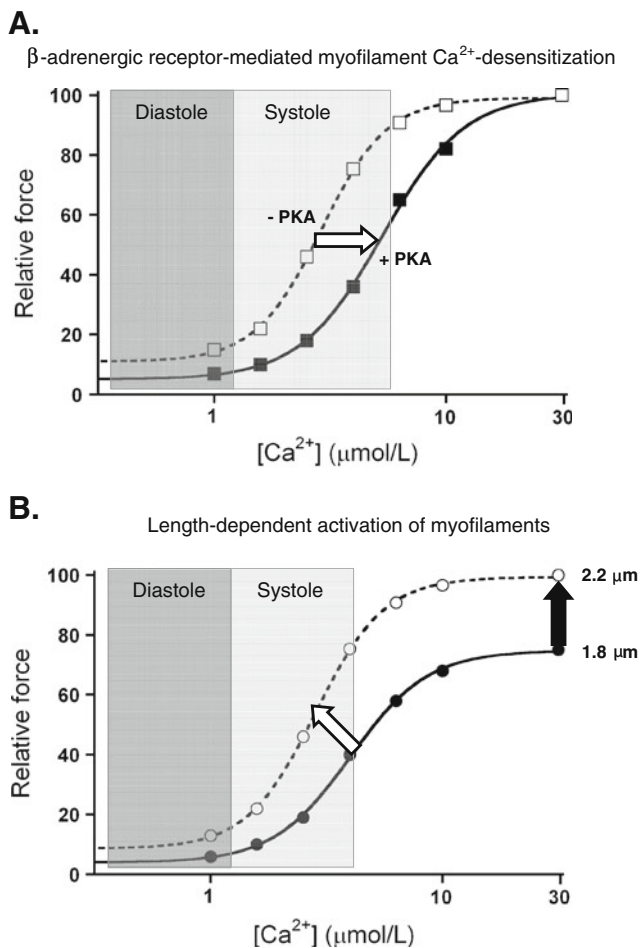


Fig. 1 Myofilament responses to increased sympathetic activation and increased left ventricular filling during diastole (Frank–Starling mechanism). **a** Activation of protein kinase A (PKA) upon β -adrenergic receptor stimulation increases phosphorylation of myofilament proteins (troponin I, myosin binding protein C and titin) and thereby reduces myofilament Ca^{2+} -sensitivity (indicated by the white arrow), enhances cross-bridge kinetics and lowers passive stiffness. The PKA-mediated changes in myofilament properties contribute to enhanced muscle relaxation, which is required for proper filling of the heart during diastole. **b** An increase in left ventricular filling (increased end-diastolic left ventricular volume) increases the maximal force-generating capacity (black arrow) and myofilament Ca^{2+} -sensitivity (white arrow) and underlies increased cardiac output during the subsequent systolic phase

Systolic and diastolic heart failure

The amount of blood ejected as a fraction of total blood in the ventricles at the end of the diastolic phase is called the ejection fraction. In clinical practice, LV ejection fraction (LVEF) is used as a measure to define systolic cardiac performance. A patient with a LV ejection fraction <45%

has heart failure with reduced ejection fraction (HFREF) or systolic heart failure. Over the last two decades, it became evident that more than 50% of all heart failure patients suffer of heart failure with normal (or preserved) ejection fraction (HFNEF) [53]. Compared to HFREF patients, these so-called HFNEF patients have a higher mortality and morbidity [1, 8]. The main perturbation in HFNEF patients is diastolic dysfunction. Moreover, frequently, patients with systolic dysfunction also show impaired diastolic function. The epidemiologic evidence that diastolic rather than systolic dysfunction is a major cause of cardiac failure in Western society has triggered scientists to investigate the underlying mechanisms of diastolic dysfunction in humans.

Perturbations at the cellular level which are thought to underlie diastolic dysfunction in human heart failure are: impaired Ca^{2+} -handling [7], extracellular matrix modifications [11, 63], and myofilament dysfunction [29, 30, 67, 73]. In heart failure, the decline in the cytosolic Ca^{2+} -transient is slowed, which is most likely caused by reduced re-uptake of Ca^{2+} into the SR due to reduced SERCA2 expression and reduced PKA-mediated phosphorylation of phospholamban. The reduced Ca^{2+} -transient decline contributes to diastolic dysfunction and hampers ventricular filling during the relaxation phase of the heart. The reduced Ca^{2+} re-uptake also lowers SR Ca^{2+} content and thereby reduces the amount of Ca^{2+} available for the subsequent contraction. Thus, perturbations in Ca^{2+} handling contribute to both systolic and diastolic dysfunction of the heart. Cardiac dysfunction has been ascribed to alterations in the extracellular matrix. Stiffness of the extracellular matrix is largely determined by collagen through regulation of its total amount, the relative abundance of collagen type I and the degree of collagen cross-linking. Excessive collagen type I deposition results from an imbalance between an exaggerated synthesis and a depressed degradation. Comparison of myocardial structure of LV endomyocardial catheter biopsies from HFNEF and HFREF patients showed similar increases in collagen volume fraction in both groups (respectively, $12.2 \pm 1.4\%$ and $14.4 \pm 1.5\%$) compared to normal values ($5.4 \pm 2.2\%$) [63]. In the HFREF group, collagen deposition was significantly enhanced by diabetes mellitus and associated with increased deposition of AGEs (advanced glycation end products) [64]. AGEs result from long-standing hyperglycemia and augment passive stiffness via cross-linking and enhanced collagen formation. Recently, Westermann et al. [72] provided evidence that enhanced deposition and remodelling of the extracellular matrix in HFNEF patients may involve myocardial inflammation. In addition to collagen deposition, intrinsic cardiomyocyte stiffness contributes to LV diastolic dysfunction evident from the high myofilament passive force observed in patients with HFNEF and HFREF [63].

Diastolic heart failure: role of the myofilaments

The first studies on myofilament function in membrane-permeabilized single cell preparations were already performed more than 30 years ago [22, 26]. Myofilament function is commonly measured in Triton-permeabilized cardiac muscle preparations, which allows investigation of myofilament properties without interference of extracellular matrix components and under well-controlled conditions (e.g., fixed sarcomere length and calcium concentration) [65]. Nowadays, single cells can be isolated from small needle biopsies, which are obtained during cardiac surgery or cardiac catheterization [11, 18]. The major limitation of the method may be the small size of the human cardiac tissue samples available for research, as throughout the heart, regional and transmural differences may exist in myofilament properties. Heterogeneity in myofilament function and protein phosphorylation may be larger in cardiac disease as the disease trigger may be localized to a certain area of the heart. Transmural differences in myofilament properties have been reported in rodent studies [15, 21]. To assess regional differences in myofilament properties in the human heart, LV subepi- and subendocardial biopsies were obtained during valve replacement surgery from patients with mitral valve or aortic valve stenosis or insufficiency [66]. In the latter study, we did not find evidence for regional differences in myofilament function and protein composition within the human ventricle. In addition, recent analysis of variability of the phosphorylation of the PKA target proteins cTnI and cMyBP-C showed that the intra-patient variability in protein phosphorylation was comparable between donor and cardiomyopathy samples [62]. Thus, our data indicate that within the precision of the measurements small, biopsy-sized cardiac human tissue samples are representative for the region of the free LV wall from which they are obtained.

The initial studies in humans were done in samples obtained during heart transplantation surgery from end-stage failing patients with idiopathic (IDCM) or ischemic (ISHD) cardiac disease [67, 73]. A comparison was made with cells isolated from non-failing donor hearts. Myofilament Ca^{2+} -sensitivity was increased in end-stage failing compared to donor hearts as illustrated in Fig. 2a. The higher sensitivity for Ca^{2+} in end-stage failing hearts may exert a beneficial effect on systolic cardiac performance, but it may limit relaxation during diastole. Diastolic function may be further impaired by the combined changes in myofilament properties and perturbations in Ca^{2+} handling as discussed above. The blunted decline in Ca^{2+} -transient decay in failing myocardium will increase diastolic Ca^{2+} levels and exaggerate diastolic dysfunction as shown in Fig. 2b.

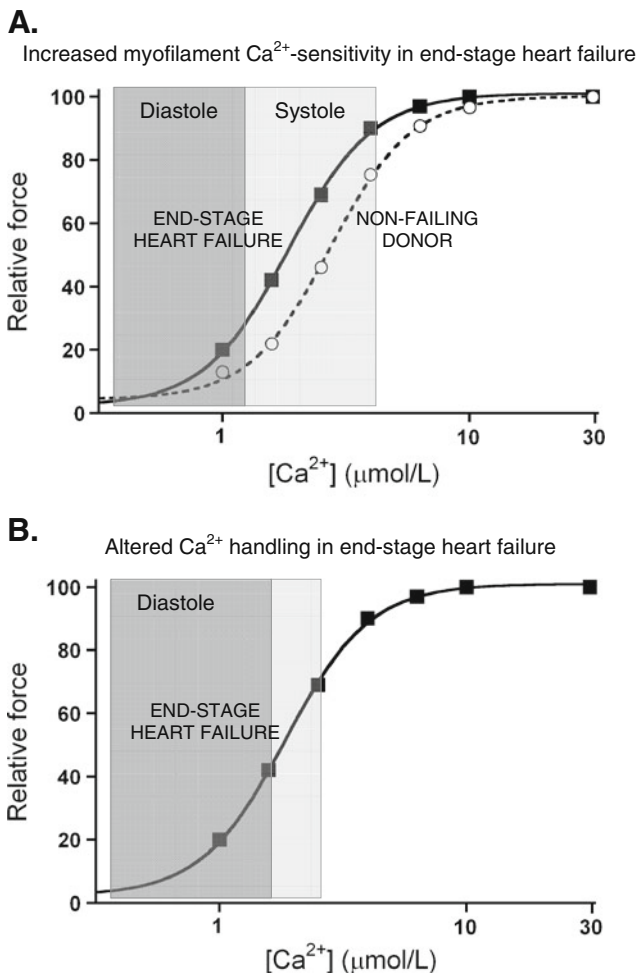


Fig. 2 **a** Force measurements in single Triton-permeabilized cardiomyocytes from end-stage failing patients with ischemic and idiopathic cardiomyopathy showed increased myofilament Ca^{2+} -sensitivity compared to non-failing donor hearts [67]. Translation of the altered responsiveness to Ca^{2+} to cardiac pump function suggests that the force-generating capacity of myofilaments will be higher at systolic $[\text{Ca}^{2+}]$ and improves cardiac output, while the enhanced myofilament Ca^{2+} -sensitivity may impair relaxation of the heart muscle during diastole. **b** Taking into account the reported changes in the Ca^{2+} transient in human heart failure (i.e. reduced systolic peak Ca^{2+} and slowed diastolic Ca^{2+} decline [7]) would further worsen diastolic function. The combination of enhanced myofilament Ca^{2+} -sensitivity and increased diastolic Ca^{2+} levels is illustrated in **b**

The enhanced myofilament Ca^{2+} -sensitivity has been ascribed to defects in the β -adrenergic receptor pathway as reduced phosphorylation of the PKA target proteins, cTnI and cMyBP-C, has been reported in end-stage failing compared to non-failing donor myocardium [9, 20, 25, 48, 67]. In further support for defective β -adrenergic signaling was the observation that myofilament Ca^{2+} -sensitivity was normalized to donor values after treatment of cells with exogenous PKA [67]. Enhanced myofilament Ca^{2+} -sensitivity and correction to control values with PKA treatment have been observed in different animal models as

well (e.g., post myocardial infarction or pressure overload) [23, 30, 68]. However, in all animal models, it has been difficult to find proof for reduced PKA-mediated phosphorylation of cTnI and cMyBP-C [30]. Only recently, we have observed a blunted cTnI phosphorylation at the PKA sites (Serines 23/24) upon dobutamine infusion in post-infarction compared to sham pigs [10]. In contrast, the dobutamine-induced phosphorylation of cMyBP-C at Ser282 (one of the PKA sites) was preserved in post-infarction hearts, and coincided with increased autophosphorylation of the cytosolic Ca^{2+} -dependent calmodulin kinase II (CaMKII- δ C) [10]. The exact cause of the enhanced myofilament Ca^{2+} -sensitivity in cardiac disease models needs to be further investigated and requires analysis of site-specific protein phosphorylation using mass spectrometry as in addition to reduced PKA activity changes in other kinase (protein kinase C, CaMKII) and in phosphatases have been documented in cardiac disease development [4, 5, 13, 51]. In a recent study [32], we have observed that alterations in the β -adrenergic receptor pathway are more pronounced in human IDCM than in ISHD and may reflect sequential changes in cellular protein composition and function and indicates the need to evaluate changes in myofilament properties in the acute phase after the initial disease trigger (e.g., infarction, valve rupture) and at later stages during remodelling of the heart muscle.

The increased myofilament Ca^{2+} -sensitivity reported in end-stage human heart failure is not a consistent observation in different experimental models of heart failure. Similar to humans, an increased myofilament Ca^{2+} -sensitivity has been observed in pig and mice with a myocardial infarction [10, 23], while a reduction in Ca^{2+} -sensitivity was found in rat models with congestive heart failure due to pressure overload or myocardial infarction [4, 5]. The direction of the Ca^{2+} -sensitivity shift may involve the stage of cardiac disease (i.e., period after the initial cardiac insult). Possible explanations for the opposite changes in myofilament Ca^{2+} -sensitivity in cardiac disease models have been discussed in recent papers [30, 47, 57].

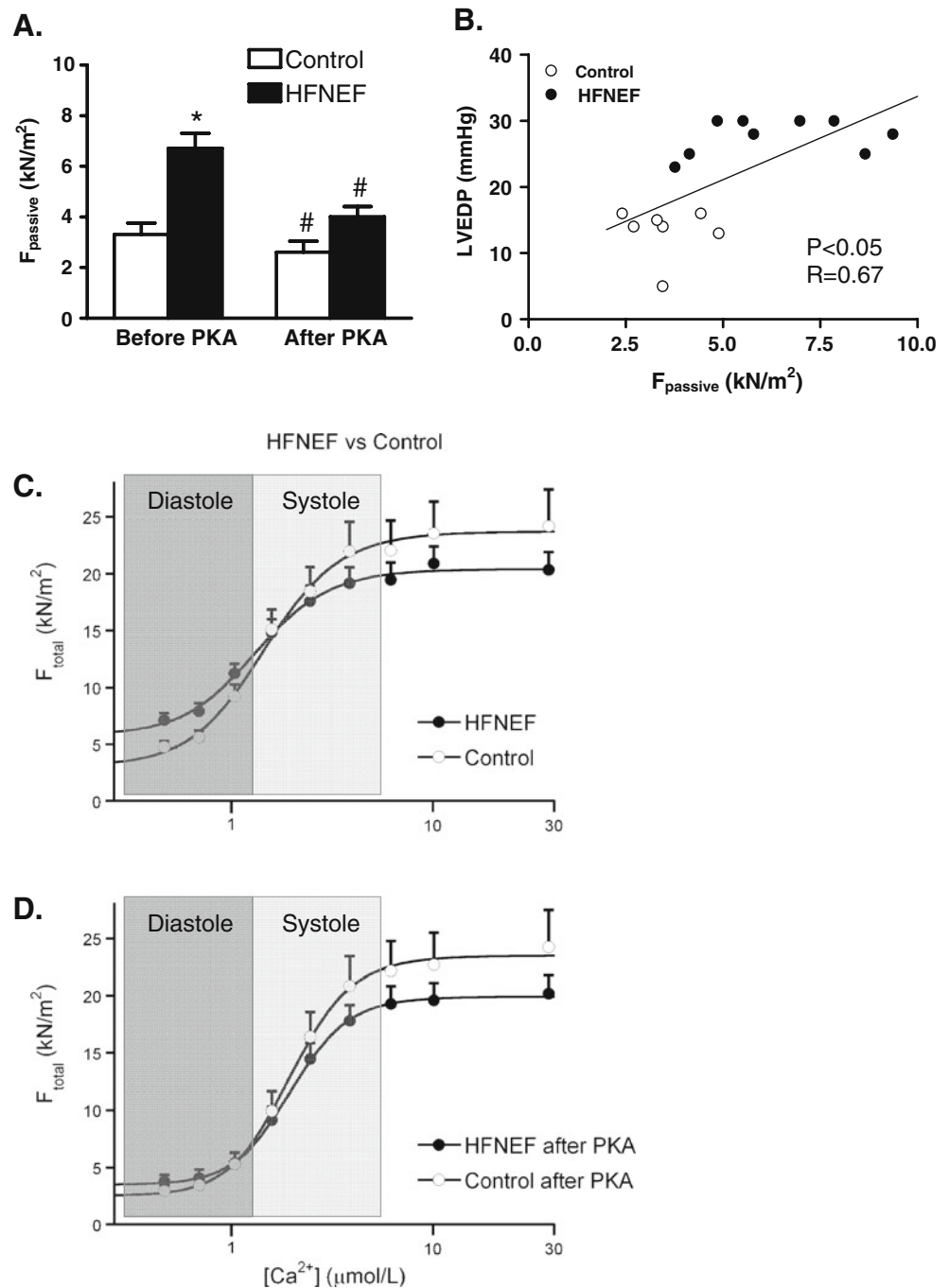
Apart from reduced PKA-mediated protein phosphorylation other myofilament protein modifications have been reported which may underlie impaired diastolic function of the heart. Varian et al. [70] reported a lack of frequency-dependent Ca^{2+} -desensitization in a rabbit model of pressure overload, which was attributed to lack of frequency-dependent cTnI phosphorylation. A reduction in myosin light chain 2 (MLC-2) phosphorylation has been reported in human end-stage heart failure [67], while loss of a transmural MLC-2 phosphorylation gradient has been described in rodent models [15, 21]. Phosphorylation of MLC-2 has been shown to enhance cross-bridge kinetics and force production per unit Ca^{2+} [55], while ablation of MLC-2 phosphorylation in mice resulted in a blunted

response to β -adrenergic receptor stimulation [54]. Specific and selective proteolysis of cTnI at its C-terminus has been proposed to play a key role in human myocardial ischemic disease, including stunning [27, 52]. The C-terminally truncated cTnI protein has been reported to reduce the force-generating capacity upon ischemia-reperfusion in rodent studies. Incorporation of C-terminal truncated cTnI in rat cardiac muscle depressed maximal force and increased cross-bridge kinetics [60]. However, exchange of C-terminal truncated cTnI in human cardiomyocytes had no effect on maximal force development and increased Ca^{2+}

$^{+}$ -sensitivity of the myofilaments [49]. This indicates that cTnI truncation at the C-terminus in human cardiomyocytes impairs diastolic rather than systolic function. In contrast, truncation of cTnI at the N-terminus has been shown to enhance ventricular diastolic function [3, 76].

Apart from the increased myofilament responsiveness to Ca^{2+} in end-stage failing human hearts, studies in cardiomyocytes isolated from cardiac catheter biopsies from HFNEF patients revealed significantly elevated passive stiffness compared to control cells (Fig. 3a) [11, 63]. The unique advantage of the catheter biopsies is that in vivo

Fig. 3 a Isolation of single Triton-permeabilized cardiomyocytes from biopsies taken during cardiac catheterization from patients with a diastolic dysfunction evident from increased left ventricular (LV) end-diastolic pressures (>16 mmHg; heart failure patients with normal (or preserved) LV ejection fraction, HFNEF) revealed increased passive stiffness compared to control cells from patients with normal LV ejection fraction and normal LV end-diastolic pressure [11]. The high passive stiffness was corrected to control values upon treatment with protein kinase A (PKA), suggesting that the high passive force is largely caused by hypophosphorylation of a sarcomeric protein (most likely titin [7]). **b** A significant relation was found between in vivo LV end-diastolic pressure and cardiomyocyte stiffness, indicating that the in vivo diastolic dysfunction in HFNEF patients is at least partly due to an intrinsic defect of the myofilaments. **c** Illustrates that the increased myofilament passive stiffness would impair diastolic function of the heart of HFNEF patients compared to controls, while the force-generating capacity of the myofilaments during systole may be somewhat lower compared to controls. **d** PKA treatment corrected myofilament diastolic dysfunction to values observed in controls



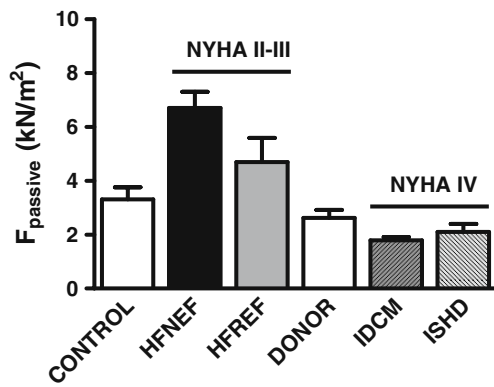


Fig. 4 Comparison of passive force measurements in cells isolated from cardiac biopsies taken during cardiac catheterization (*control*, *HFNEF*, and *HFREF*) and from heart tissue obtained during heart transplantation surgery (*donor*, *IDCM*, and *ISHD*) illustrates high passive stiffness in *HFNEF* and *HFREF* patients, similar values in control and donor hearts, and low passive force in idiopathic and ischemic end-stage failing cardiomyopathy

hemodynamic data are collected at the time of biopsy procurement and samples are directly frozen in liquid nitrogen, which will fix phosphorylation status of the myofilament proteome. This allowed us to show that the high intrinsic passive stiffness of the myofilaments correlated well with LV end-diastolic pressure (Fig. 3b) [11], which indicates that increased passive myofilament stiffness is an important contributor to diastolic dysfunction in *HFNEF* patients. Compared to control cells from individuals with normal LVEF and normal LV end-diastolic pressure, force development by the myofilaments was higher at low “diastolic” Ca^{2+} concentrations and slightly lower at higher “systolic” Ca^{2+} concentrations in *HFNEF* as illustrated in the force-calcium relations in Fig. 3c. Treatment with exogenous PKA significantly reduced passive stiffness and abolished the difference in myofilament passive force between *HFNEF* and control cells (Fig. 3a, d), while force development at systolic Ca^{2+} concentrations

remained somewhat lower in *HFNEF* compared to the control group.

Correction of passive stiffness in *HFNEF* with PKA indicated that the myofilament dysfunction is caused by protein hypophosphorylation. As mentioned above, the main protein involved in myofilament stiffness is the giant sarcomeric protein titin. Previous studies have shown that titin is a target of PKA, PKG, and PKC [34, 41, 75]. Phosphorylation by PKA and PKG have been shown to reduce passive stiffness [41, 75], while PKC α treatment increased passive stiffness in mouse and pig myocardium [34]. In addition to reduced cTnI and cMyBP-C phosphorylation, end-stage failing human hearts showed a deficit in titin phosphorylation compared to non-failing donor hearts [40]. A study in catheter biopsies from *HFNEF* and *HFREF* patients indicated relative hypophosphorylation of the stiff N2B isoform compared to control samples [12]. These data support the hypothesis that hypophosphorylated titin causes increased passive stiffness in cardiac disease. Until present, no evidence for a detrimental effect of PKC-mediated titin phosphorylation has been found in human cardiac samples. Opposite to the expected increase in passive stiffness, PKC treatment of end-stage failing cardiomyocytes slightly reduced passive force [38]. However, the exact modulating role of PKC-mediated titin phosphorylation on passive stiffness in human myocardium should be more carefully assessed in cardiac tissue which is obtained after different stimuli, e.g., after alpha-adrenergic receptor stimulation, which is known to activate downstream PKC.

Phosphorylation deficits of titin may be counterbalanced by adaptations in titin isoform composition possibly aimed to lower passive myofibrillar stiffness [14, 35]. Titin isoform switching has been demonstrated in end-stage failing myocardium: a shift from the stiff N2B isoform to the compliant N2BA isoform coincided with lower passive stiffness [46, 50] and may rescue diastolic dysfunction. Alternatively, a maladaptive shift towards the stiff N2B

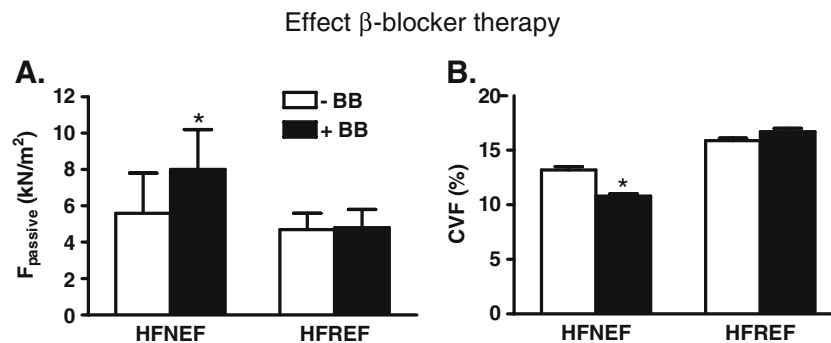


Fig 5 Comparison of heart failure patients with normal (*HFNEF*) and reduced (*HFREF*) left ventricular ejection fraction which received chronic β -blocker therapy +BB compared to patients untreated with β -blockers -BB showed higher passive stiffness in *HFNEF* patients

treated with β -blockers a, while the percentage of collagen volume fraction (CVF) was significantly lower in the *HFNEF* patients with β -blockers b. Passive force was higher in *HFNEF* compared to *HFREF* patients, while CVF was higher in *HFREF* than in *HFNEF*

isoform has been reported in human samples from patients with aortic stenosis and LV hypertrophy [71], which would exert a detrimental effect on diastolic function.

Figure 4 depicts passive stiffness measured in single cells isolated from catheter biopsies and from hearts which were obtained during heart transplantation surgery. Passive stiffness was highest in HFNEF and HFREF patients which were classified as relatively moderate forms of heart failure (NYHA II to III). Passive stiffness was lowest in end-stage failing human hearts classified as NYHA IV. Noteworthy, passive force development in control cardiac catheter biopsies and donor hearts are similar. The changes in passive stiffness in patient groups compared to the control groups may reflect altered passive stiffness during the progression of cardiac disease, characterized by an enhanced passive stiffness at relatively early stages of the disease where titin phosphorylation deficits are predominant, and lower passive stiffness in end-stage failing hearts in which the shift to more compliant titin balances the perturbations in titin phosphorylation.

Clinical perspectives

Large clinical trials have convincingly shown that β -blocker therapy reduces mortality and improves LV function in HFREF patients. In HFNEF patients, favourable effects of β -blocker therapy on mortality and LV function have not been convincingly demonstrated. Comparison of patients who received β -blocker therapy and patients untreated with β -blockers showed higher maximal force-generating capacity of myofilament in patients with β -blocker therapy, which may underlie improved systolic performance [31]. However, β -blocker therapy increased passive force in HFNEF patients (Fig. 5a), which may even worsen diastolic dysfunction. A positive effect of the β -blockers was observed on the extracellular matrix as the collagen volume fraction was significantly lower in HFNEF with β -blockers (Fig. 5b). The opposite effects of β -blocker therapy on two important determinants of cardiac diastolic function may partly explain the inconsistent results of β -blocker trials in HFNEF patients. Moreover, although collagen deposition may underlie diastolic dysfunction, within the physiologic sarcomere lengths, stiffness is largely determined by titin. In a recent study, Chung and Granzier [19] have shown that titin is the dominant contributor to LV passive pressure within physiological volumes, while the extracellular matrix exerts a dominant effect on LV pressure at larger volumes. As diastolic dysfunction is present in both HFNEF and HFREF patients and is also a major problem in the growing population of patients with diabetic cardiomyopathy [64], it is of great relevance to design a targeted treatment to titin-mediated

passive stiffness. Benefits of therapy targeted at myofilament function may depend on the stage of cardiac disease. In pigs, β -blocker therapy directly initiated after a myocardial infarction reversed the increased myofilament Ca^{2+} -sensitivity to values observed in sham animals and significantly reduced passive stiffness in infarct animals [24]. The latter observations are in contrast with the data from our human studies, which showed an increase in passive force in β -blocker treated HFNEF patients (Fig. 5a) and a minor increase in myofilament Ca^{2+} -sensitivity (not shown) [31]. Although there may be species differences, these conflicting data may also be explained by timing at which therapy is initiated. Longitudinal studies in large animal models [45, 74] are warranted to disentangle adaptive from maladaptive myofilament protein changes in the initiation and progression of diastolic dysfunction in heart failure.

Acknowledgment I would like to thank Diederik Kuster for his excellent advice on the design of the figures.

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