

Pathomechanisms in heart failure: the contractile connection

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Abstract Heart failure is a multi-factorial progressive disease in which eventually the contractile performance of the heart is insufficient to meet the demands of the body, even at rest. A distinction can be made on the basis of the cause of the disease in genetic and acquired heart failure and at the functional level between systolic and diastolic heart failure. Here the basic determinants of contractile function of myocardial cells will be reviewed and an attempt will be made to elucidate their role in the development of heart failure. The following topics are addressed: the tension generating capacity, passive tension, the rate of tension development, the rate of ATP utilisation, calcium sensitivity of tension development, phosphorylation of contractile proteins, length dependent activation and stretch activation. The reduction in contractile performance during systole can be attributed predominantly to a loss of cardiomyocytes (necrosis), myocyte disarray and a decrease in myofibrillar density all resulting in a reduction in the tension generating capacity and likely also to a mismatch between energy supply and demand of the myocardium. This leads to a decline in the ejection fraction of the heart. Diastolic dysfunction can be attributed to fibrosis and an increase in titin stiffness which result in an increase in stiffness of the ventricular wall and hampers the filling of the heart with blood during diastole. A large number of post translation modifications of regulatory

sarcomeric proteins influence myocardial function by altering calcium sensitivity of tension development. It is still unclear whether in concert these influences are adaptive or maladaptive during the disease process.

Keywords Cardiomyocyte · Crossbridge · Stiffness · Calcium sensitivity · Phosphorylation · ATP

Introduction

Heart failure is a progressive disease in which the contractile performance of the heart is insufficient to meet the demands of the body, resulting in limited perfusion of vital organs in the body. This limits the supply of oxygen and nutrients to the organs and the removal of waste products. In the final stage these limitations are apparent also at rest and the only treatment options remaining are a cardiac assist device or heart transplantation.

A distinction between the different forms of heart failure can be made: (1) on the basis of the cause of the disease between familial i.e. genetic forms and acquired forms such as coronary artery disease and valve stenosis and (2) at the functional level, between systolic and diastolic heart failure. At the structural level a distinction can be made between concentric hypertrophy, in which wall thickness increases as a result of pressure overload (e.g. hypertension) while the volume of the chamber remains constant and eccentric hypertrophy as a result of volume overload (e.g. valve regurgitation) in which the end diastolic volume increases (and the ventricle dilates) while wall thickness remains constant. These forms of hypertrophy are generally considered as precursors of heart failure and ultimately a transition may occur from concentric to eccentric hypertrophy, i.e. the hypertrophied ventricle starts to dilate.

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The focus of this review is on the defects in contractile function which lead to heart failure as well as the alterations in contractile function which result from heart failure. Since familial hypertrophic cardiomyopathy (HCM; frequency 1:500) is predominantly caused by mutations in genes encoding for proteins inside myocardial cells the disease is considered a disease of the contractile unit, the sarcomere. During the last decade the frequency of genetic screening of patients with heart failure has grown progressively, and a large number of mutations (>1,000) associated with HCM have been identified. It is a daunting task to reveal the link between the protein defect and the myocardial disease process not only because of the vast number of mutations involved, but also because the protein defect may trigger cardiac malformation during cardiac development. For example, the causes of the defect may be contractile by nature but mainly operate during development and result in cardiac dysfunction because of altered cardiac structure (Olivotto et al. 2009). Such a ‘silent’ structural defect may predispose to heart failure with aging or may trigger the transition from adaptive to maladaptive hypertrophy. Moreover, adaptive post translational processes may be beneficial for the patient, but they may disguise the true origin and nature of the disease.

The processes involved in acquired forms of heart failure are equally complex. For instance coronary artery disease may result in myocardial infarction, cell necrosis and scar formation but also triggers a hypertrophic response in the remaining viable myocardial cells that can be either adaptive or in the long run, maladaptive.

Realising that in the human body the pathomechanisms will be highly complex and intertwined the aim of this review is to describe the basic determinants of contractile function of myocardial cells and to elucidate their role in the development of heart failure. In this context the following topics will be addressed:

- The *tension generating capacity* of the cardiomyocytes, for which we will refer to the maximum tension generated by the cardiomyocyte i.e. the maximum force per cross-sectional area of the cardiomyocyte (T_{\max});
- The *passive tension* of the cardiomyocyte (T_{pas}), which depends on the length of the sarcomere, i.e. the extent of filling of the heart with blood during diastole;
- The *rate of tension redevelopment* during an isometric contraction (k_{tr}) which is a measure of the intrinsic speed of the formation (f_{app}) and breaking (g_{app}) of force producing bonds between myosin and actin, the crossbridge cycle;
- The *rate of ATP utilisation* during contraction, which is determined by the rate limiting transition in the crossbridge cycle;
- The *calcium sensitivity of tension development* (EC_{50}), the intracellular free Ca^{2+} concentration at which half of the maximum tension generating capacity is reached;
- The *regulatory aspects of phosphorylation of contractile proteins* of the thin (actin) and thick (myosin) filament;
- *Length dependent activation*, the process involved in the Frank-Starling effect, which is mainly based on the relation between T_{\max} and EC_{50} on sarcomere length and finally
- *Stretch activation*, the dynamic process in which tension production transiently increases as a result of an increase in sarcomere length.

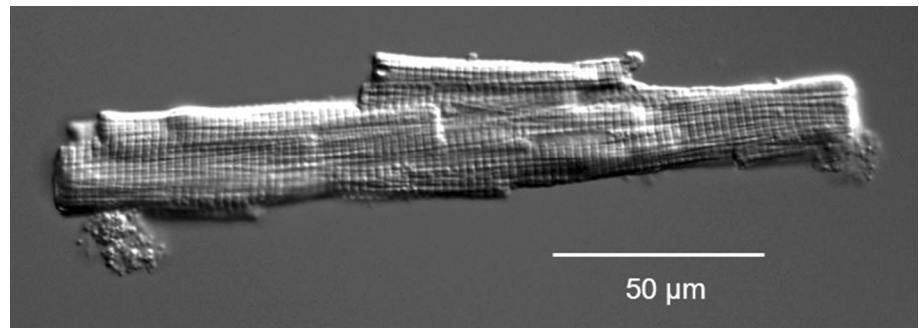
Tension generating capacity

Number of active force producing crossbridges

Force is developed in a cardiomyocyte upon binding of a myosin head which extends from the thick (myosin) filament to a myosin binding site on the thin (actin) filament (Huxley 1957). This is a cyclic process in which the myosin head binds, subsequently performs a power stroke and thereafter detaches from actin (Huxley and Simmons 1971). During the isovolumic contraction phase of the cardiac cycle force builds up but the length of the cardiomyocyte remains constant, the contraction of the cardiomyocyte is called isometric. During this phase the power stroke results in force production, in which typically each attached myosin head (or crossbridge) generates a force (F_{cb}) of 10 pN and the size of the working stroke amounts to 10 nm (Finer et al. 1994; Takagi et al. 2006). The maximum isometric force produced by a cardiomyocyte (Fig. 1) of 20 μm in diameter is approximately 35 μN . This indicates that—within a slice of a half sarcomere in width of such a cardiomyocyte— 3.5×10^6 myosin heads need to generate force simultaneously. The relative change in sarcomere length during the ejection phase of the heart is about 10 % or 0.1 μm (100 nm) per half sarcomere. Hence each myosin head needs to undergo around 10 cycles of attachment and detachment, and reattachment at a new shifted position along the actin filament. This suggests that each myosin head in the human heart undergoes only a small number of cycles during each heartbeat which lasts ~ 350 ms.

In a simplified two-state scheme of the crossbridge cycle the attachment process takes place with a rate f_{app} and the detachment process takes place with a rate g_{app} (Brenner 1988). This implies that the number of myosin heads attached equals the total number of available myosin heads

Fig. 1 Single cardiomyocyte from the rat. Courtesy of Dr. E. van Deel and Dr. R. Musters (VUmc, Amsterdam, The Netherlands)



(N), multiplied by the fraction of attached crossbridges [$f_{app}/(f_{app} + g_{app})$]. The number of force generating crossbridges thus depends on f_{app} and g_{app} . These rates vary with the type of myosin heavy chain (MHC) expressed; the rates are faster for crossbridges consisting of the fast (alpha) MHC than those consisting of the slow (beta) MHC. In rodent models of heart failure a shift from the fast MHC to the slow MHC is observed, which—if f_{app} and g_{app} do not change in proportion—influences $f_{app}/(f_{app} + g_{app})$ and therefore T_{max} .

The myosin head concentration inside intact fast skeletal muscle fibres amounts to approximately 0.25 mmol/liter cell volume and the fraction of attached myosin heads is approximately 0.3 (Barclay et al. 2010), but this estimate may well vary between species and depend on experimental conditions. Intact cardiomyocytes have a lower myosin head concentration (~ 0.2 mM) than fast skeletal muscle fibres because mitochondrial density in cardiac myocytes is larger. This latter figure is in agreement with a myosin head concentration in permeabilised trabeculae from guinea pig of 157 pmol of myosin heads/mg wet weight (Barsotti and Ferenczi 1988) and implies that during a maximum isometric contraction only a small fraction of the myosin heads is in the force generating state. This is probably less than the fraction of force generating crossbridges mentioned earlier (0.3), because a portion of the available myosin heads are in an unfavourable position relative to the adjacent actin binding sites (Steffen et al. 2001).

Overlap between thick and thin filament

The isometric tension at saturating Ca^{2+} -ion concentration in striated muscle tissue depends on the degree of overlap between the actin and myosin filaments (Gordon et al. 1966) and thus on sarcomere length. This overlap is optimal near the resting sarcomere length ($\sim 2 \mu m$) and decreases when sarcomere length increases. This gives rise to the descending limb of the isometric tension–length relation. At sarcomere lengths below resting length, the ascending limb of the isometric tension–length relation, the

isometric tension declines when sarcomere length is reduced because of an increase in overlap between the actin filaments of opposite polarity ($< 2 \mu m$) and the increase in folding of the myosin filaments between the z-disks ($< 1.6 \mu m$). Studies of the sarcomere length changes during the cardiac cycle indicate active shortening from approximately $2 \mu m$ towards $1.8 \mu m$ (Spotnitz et al. 1966). This indicates that cardiac muscle operates on the ascending limb of the isometric tension–length relation, where active tension increases with an increase in sarcomere length.

In skeletal muscle tissue it has been proposed that the thin filament associated protein nebulin acts as a molecular ruler and determines the length of the thin (actin) filament. Actin filament length in cardiac muscle is somewhat variable (Burgoyne et al. 2008). As a result the tension–sarcomere length relation could change less abruptly in comparison to skeletal muscle at the edges of the region ranging from 2.0 to $2.2 \mu m$ (where overlap between thick and thin filaments is optimal). In addition, the final descending part of the relation near zero overlap could become concave. The cardiac tissue specific homolog of nebulin is nebullette, which is considerably shorter than nebulin (Witt et al. 2006), and it is unclear whether it functions as a molecular ruler determining the actin filament length or not.

Myosin content

The heart is capable of remodelling in response to environmental demands, and a variety of stimuli can induce it to grow or shrink (Hill and Olson 2008). In order to preserve the tension generating capacity of the cardiomyocyte during hypertrophy or atrophy, myosin content needs to keep up with the changes in volume, i.e. myosin concentration and its structural arrangement need to be maintained. Hence the number of actin and myosin filaments in parallel needs to increase when the cell becomes thicker (concentric hypertrophy) and the number of sarcomeres in series needs to increase when the cells lengthen (eccentric hypertrophy, dilatation).

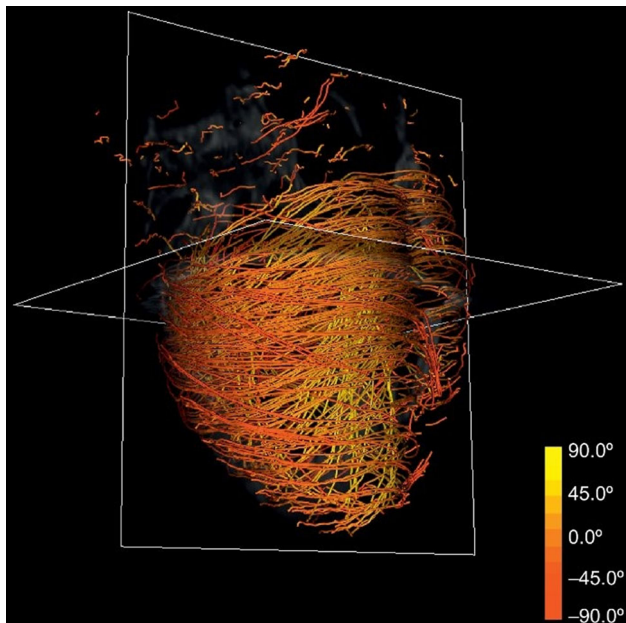


Fig. 2 Diffusion tensor magnetic resonance imaging in canine heart. The different colours indicate the angle of strands of cardiomyocytes relative to the horizontal plane. Published with permission of the author and the Publisher. Original source: (Poveda et al. 2013). (Color figure online)

Myofibrillar density

Cardiomyocytes consist of bundles of myofibrils of approximately 1 μm in diameter, which are surrounded by the sarcoplasmic reticulum, mitochondria and one nucleus or occasionally multiple nuclei and are embedded in interstitial fluid. The volume occupied by the nuclei, the sarcoplasmic reticulum (SR), the mitochondria and myofibrils amounts to 2, 3.5, 36 and 46.7 % (Page 1978) and the extracellular volume fraction (mainly capillaries) amounts to 18.5 % (Anversa et al. 1983). This indicates that the myofibrils which participate in force production occupy around 40 % of the total volume and cross-sectional area of the myocardial wall. If for instance in a specific disease state the myofibrillar density is decreased because the number of mitochondria or the interstitial volume is increased (for instance by increased fibrosis), the maximal force per cross-sectional area and wall tension will be decreased.

It should be noted that measurements on cardiac tissue are mainly performed in intact multicellular preparations i.e. thin strips of cardiac tissue or cardiac trabeculae or in permeabilised cardiac trabeculae or cardiomyocytes. Measurements in intact Tyrode (super)perfused preparations may be influenced by osmotic swelling. Permeabilised preparations also swell but the detergent used to permeabilise the surface membrane may also disrupt the SR and the mitochondria, and the effects of internal reorganisation on myofibrillar density are less clear.

Myocyte and myofibrillar disarray

Other factors that can influence tension generation inside the cardiac wall relate to structural derangements such as myocyte disarray, observed in hypertrophic cardiomyopathy (Maron et al. 1992; Varnava et al. 2001). Diffusion tensor magnetic resonance imaging has provided images of the intricate structure of the myocardium illustrating the approximately perpendicular arrangement of the epi- and endocardial strands of cardiomyocytes (Fig. 2).

This arrangement gives rise to torsional “wringing” motion of the ventricular wall during the ejection phase which promotes emptying of the ventricles. Sarcomere disarray would lead to reduced efficiency of myocyte force transmission in the myocardial wall and to a disruption of this wringing pattern.

Implications for systolic/diastolic function

The overall change (generally reduction) in the tension generating capacity of failing myocardium can be viewed as a scaling factor limiting cardiac performance. Its main effects will emerge during systole, at the lower submaximal physiological Ca^{2+} -concentrations, since developed pressure will be altered in proportion to the change in the tension generating capacity. If everything else (ventricular wall thickness, resting sarcomere length, peak systolic and end-diastolic Ca^{2+} -concentrations, etc.) remains constant, this will reduce stroke volume and the ejection fraction of the heart, an important clinical parameter of cardiac function, will decrease.

Passive tension

Role of titin, collagen and fibrosis

The cardiomyocytes are embedded in interstitial fluid and a mesh of capillaries and extracellular matrix proteins. This network of collagen (and elastin) provides support to the cardiomyocytes and gives rise to passive stiffness of the heart. Passive stiffness within the cardiomyocyte originates predominantly from titin, which spans both half sarcomeres from the Z-line towards the M-line. The resting sarcomere length is approximately 1.8 μm . Since titin is supposed to act as a bi-directional spring, it will be involved in the elastic recoil of the heart when lower sarcomere lengths ($\sim 1.6 \mu\text{m}$) would be reached. During the diastolic filling phase the quiescent cardiomyocytes will be stretched to a sarcomere length of approximately 2.1 μm , where active tension development is optimal. The effects of collagen kick in when a sarcomere length around 2.2 μm is reached and most likely limit further extension of the ventricle.

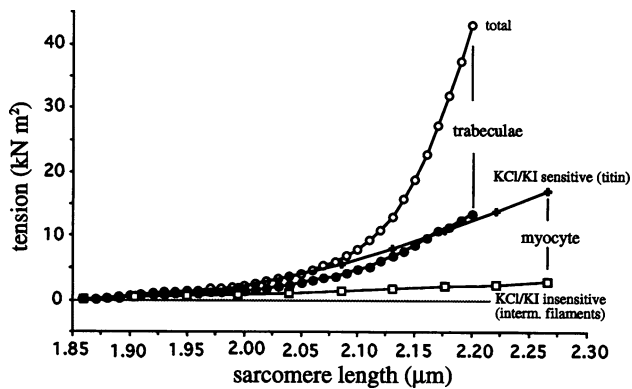


Fig. 3 Passive tension in trabeculae and single cardiomyocytes from rat during a slow stretch at a sarcomere velocity of 0.005 $\mu\text{m/s}$. Total tension (including collagen, *open circles*) and KCl/KI-sensitive tension (titin, *filled circles*) in trabeculae and KCl/KI-sensitive (titin, *crosses*) and the KCl/KI-insensitive tension (intermediate filaments, *squares*) in single cardiomyocytes (Granzier and Irving 1995). Note that the concentrated KCl/KI cocktail used in these experiments dissolved myosine filaments but presumably left the cytoskeletal network of interfilaments intact. Passive stiffness in untreated single cardiomyocytes consists of the contribution of titin (bound to the myosin filaments) and of the interfilaments, whereas in trabeculae an additional contribution is observed that can be attributed to collagen. Published with permission of the authors and the Publisher

The passive properties of the myocardium can be studied in single cardiomyocytes and in thin (intact) multicellular strips of longitudinally arranged cardiac cells. The technique to glue intact cardiomyocytes to a sensitive force transducer and a motor to study contractile properties has recently been improved (Prosser et al. 2011). The multicellular preparations of choice are cardiac trabeculae which extend from the free wall of the left ventricle to parts of the tricuspidal valve.

Passive stiffness (titin-plus collagen-based) is estimated to amount to approximately 10 kN/m^2 at a sarcomere length of approximately 2.2 μm (Fig. 3), while the developed tension (in 2.5 mM external Ca^{2+}) would be around 120 kN/m^2 (ter Keurs et al. 1980). Within the working range of the cardiomyocyte in vivo (1.8–2.2 μm), passive tension would thus be maximally $\sim 8\%$ of the active tension.

It is illustrative to translate diastolic and systolic pressures inside the ventricle to cardiomyocyte tension and the ensuing wall tension. Diastolic pressures range typically from 5 mm Hg in healthy hearts up to 45 mm Hg in severe diastolic dysfunction. Systolic pressures range from around 100 mm Hg in the normal heart to around 180 mmHg in severe hypertension. Various equations describing the pressure–volume relation have been derived, using the LAW of La place, in which wall thickness, the rather complex ellipsoidal shape of the left ventricle and the direction of the cardiomyocytes are taken into consideration. Using this type of model the pressures given above

may be converted to wall tension (and cardiomyocyte tension) by using a conversion factor of approximately 40 kN/m^2 per 100 mmHg (Arts et al. 2003).

The mechanical properties of titin (and collagen) vary with cardiac development and during disease. Titin stiffness depends on titin isoform expression achieved by alternative splicing of the TNN-gene: in adults a shorter and therefore more stiff N2B isoform and a longer, more compliant N2BA isoform, which has a N2A extension in the I-band of the sarcomere are coexpressed. See for a recent review: (Linke and Hamdani 2014). In addition, titin stiffness is influenced by post translational modifications such as phosphorylation, oxidation, S-glutathionylation and glycosylation (Borbély et al. 2005; van Heerebeek et al. 2008; Borbély et al. 2009; Falcão-Pires et al. 2011; Hidalgo et al. 2013; Kötter et al. 2013; Alegre-Cebollada et al. 2014). Titin is also considered to play a role in mechanosensing, atrophic and hypertrophic remodelling and stress responses. Likewise, the stiffness of the collagen network depends on collagen expression and the degree of crosslinking (Spinale 2007). It is also influenced by posttranslational modifications such as oxidation, glycosylation and by metalloproteinases and advanced glycation end products (AGE's). Altogether titin-and collagen-based myocardial stiffness are highly dynamic, and adaptive to changes in (cardiac) load.

Fibrosis, the consequence of extracellular cardiac matrix remodelling (Zannad 2014), is important as well in health and disease because of its reparative as well as arrhythmogenic effects for instance after myocardial infarction.

Ca^{2+} -(in)dependent crossbridge tension development

Ca^{2+} -dependent as well as Ca^{2+} -independent crossbridge cycling may contribute to diastolic stiffness as well. A typical value for the free cytosolic Ca^{2+} -concentration during diastole is 0.1 $\mu\text{mol/liter}$ cell volume. This value is outside the range where under physiological conditions active tension development is expected to occur. However in cases where calcium sensitivity of the myocardium is increased (for instance by post translation modifications (van der Velden 2011) or upon a rise in ADP concentration) Ca^{2+} -dependent crossbridge cycling may take place and thereby limit ventricular filling in a similar way as passive tension. In addition, leakage of calcium through the calcium channels inside the sarcoplasmic reticulum (the ryanodine receptors) and calcium overload (for instance at high or irregular heart rates or after the restoration of myocardial perfusion in an ischemic region) may cause a rise in cytosolic free Ca^{2+} -concentration during diastole and promote active tension development. It has become increasingly clear that intracellular Ca^{2+} -handling underlying these processes is complicated by the presence of local concentration gradients (Bers 2014).

Ca^{2+} -independent crossbridge cycling induced by rigor crossbridges (Varma et al. 2001) might in theory also play a role but the metabolic conditions in which this might contribute to a significant extent to diastolic stiffness seem rather extreme (and also highly unfavourable for systolic cardiac function).

Implications for systolic and diastolic function

An increase in passive tension (or stiffness) potentially influences systolic function because the Frank Starling mechanism (see Length dependent activation) will be recruited to a lesser extent. It may hamper diastolic function by reducing the degree of filling of the ventricles with blood. Slowing of the relaxation phase may also affect the performance of the heart at elevated heart rates.

An increase in diastolic calcium concentration may have an impact on excitation-transcription coupling (Bers 2002) and will activate calcium-dependent proteases for instance calpain 1, which may lead to protein degradation (Papp et al. 2000) and therefore to alterations in contractile function.

Rate of tension redevelopment

Simplified crossbridge scheme

The rate constant governing the initial tension development in the cardiomyocyte (k_{act}) is determined by the speed of the activation process, i.e. membrane depolarisation and the rise of the intracellular Ca^{2+} concentration and the speed of tension development at the crossbridge level. The latter rate can be determined during the quick release and restretch manoeuvre, in which an activated cardiomyocyte is abruptly shortened until it becomes slack, and thereafter quickly restretched to its original length (Brenner and Eisenberg 1986). This manoeuvre is followed by restoration of the population of force producing crossbridges without delay because the thin filament is still in its activated state. In the simplified two-state scheme of the crossbridge cycle described above, this rate of tension redevelopment (k_{tr}) is determined by the sum of the apparent attachment and detachment rates, i.e. $f_{\text{app}} + g_{\text{app}}$. Tension redevelopment is in theory faster than the initial tension development (k_{act}) during each heartbeat, because k_{act} depends not only on the rate at which crossbridges equilibrate in the force producing states but also on the preceding activation process of calcium binding to the actin filaments triggered by calcium influx into the cardiomyocyte. Analysis of the rates of both processes in single myofibrils indicated that the activation process is considerably faster than crossbridge equilibration (Stehle et al. 2009). Hence k_{act} is similar to k_{tr} .

Calcium dependence

Moss and colleagues have studied the calcium dependence of k_{tr} in great detail, not only by studying tension redevelopment upon a quick release and restretch manoeuvre at different Ca^{2+} -concentrations but also by using NEM-S1, a strong binding derivative of myosin subfragment S1 (Fitzsimons and Moss 2007). They concluded that the calcium dependence of k_{tr} is mediated via cooperative strong binding of myosin heads along the thin filament rather than by the Ca^{2+} concentration directly as was proposed previously (Brenner 1988). However, fluorescence measurement (Brenner and Chalovich 1999) showed contributions of both calcium and of strong binding myosin heads to activation of the thin filaments.

Role of MyBP-C

Cardiac myosin binding protein C is present in the central 53 % of the thick filaments within the sarcomere in 9 bands (Luther et al. 2008). Its structural organisation is still unclear and several models still exist (Moolman-Smook et al. 2002; Squire et al. 2003). It has been proposed to form a collar around the myosin filament, which could limit the lateral movement of myosin heads, thereby reducing the probability of binding of the myosin heads to actin and thereby also f_{app} . Evidence suggests that it might bind to actin as well (Whitten et al. 2008) and thereby might restrain sliding of the actin and myosin filaments relative to each other. It is therefore proposed that the most likely arrangement of MyBP-C is one in which the three C-terminal domains run along the thick filament surface while the rest of the molecule extends out and may attach to the neighbouring thin filament (Craig et al. 2014). MyBP-C therefore is considered to act as a brake on crossbridge attachment and thereby to reduce the overall k_{tr} .

Implications for systolic/diastolic function

The direct consequences of a change in k_{tr} on cardiac function are unclear because they need to be evaluated together with the effects of changes in the energetic costs of contraction.

Rate of ATP utilisation

Simplified crossbridge scheme and the Fenn effect

In the two-state scheme outlined above, each crossbridge undergoes a cycle of attachment and detachment during which one molecule ATP is hydrolysed. In an isometrically

contracting cell in steady state, the rate of crossbridge cycling is given by $f_{app} \cdot g_{app} / (f_{app} + g_{app})$. Detachment is considered to be the rate-limiting step within the cycle ($f_{app} \gg g_{app}$). In this case the rate of crossbridge cycling and thus the rate of ATP utilisation equals g_{app} . As indicated above isometric force is equal to $F_{cb} \cdot N \cdot f_{app} / (f_{app} + g_{app})$. A measure of the economy of isometric contraction is tension cost, i.e. force divided by the rate of contraction related ATP utilisation which is proportional to g_{app} , even when g_{app} would not be rate limiting.

During the ejection phase of the cardiac contraction, the cardiomyocytes shorten and the rate of crossbridge cycling (g_{app}) increases in proportion to the externally developed mechanical work, a feature known as the Fenn effect (Fenn 1923). It has been discussed that the magnitude of the Fenn effect in cardiac tissue is smaller than in frog skeletal muscle (Rall 1982). This might at least partly be caused by the submaximal level of the free cytosolic Ca^{2+} concentration in cardiac myocytes because indications have been obtained that the Fenn effect may even be reversed in cardiac tissue at very low Ca^{2+} concentrations (Stienen et al. 1993).

Measurements of muscle energetics revealed that the mechanical efficiency of cardiac muscle contraction, i.e. external work/energy input from ATP hydrolysis, is optimal at intermediate velocities of muscle shortening. A typical figure for mechanical crossbridge efficiency is $\sim 40\%$. However, overall cardiac muscle efficiency is $\sim 22\%$ because energy is required for other processes within the cardiomyocyte such as SR Ca -uptake and mitochondrial oxidative phosphorylation (Smith et al. 2005; Barclay and Widén 2010).

Role of myosin isoforms in ATP utilisation

A very simple biochemical relationship had perplexed the field of muscle energetics for many decades (Bárány 1967). This study provided evidence for an almost universal linear relation between actin-activated myosin ATPase activity and the maximum shortening velocity throughout the mammalian kingdom. Subsequent studies revealed that ATPase activity and shortening velocity both depended on the rate of ADP release from the catalytic site in myosin S1 (Siemankowski et al. 1985; Weiss et al. 2001). This could explain Bárány's findings.

Myosin light chain isoform expression in skeletal muscle plays a modulatory role in muscle energetics. Isoform MLC3f was associated with a higher rate of ATP utilisation than the MLC1f isoform (Reggiani et al. 1997). In adult cardiac muscle two different (essential) MLC1 isoforms are found predominantly but not exclusively in the atria (MLC1a) and the ventricles (MLC1v) (Morano 1999). The regulatory light chain MLC2 can be phosphorylated by a

recently discovered cardiac-specific myosin kinase (Chan et al. 2008). It has been demonstrated that phosphorylation increases myosin binding and myosin lever arm stiffness and alters the kinetics of cross-bridge cycling to increase cross-bridge duty ratio (stroke time/cycle time) (Sheikh et al. 2012).

The free energy change associated with ATP hydrolysis (ΔG_{ATP})

The amount of free energy that becomes available with the hydrolysis of ATP into ADP and inorganic phosphate (P_i) can be derived from: $\Delta G_{ATP} = -\Delta G_{ATP}^0 + RT \cdot \ln([ATP]/([ADP] \cdot [P_i]))$. Under physiological conditions the magnitude of ΔG_{ATP} in mammalian muscle amounts to approximately 60 kJ/mol, while during cardiac ischemia this value will be reduced to around 30 kJ/mol and the activity of ion pumps such as the SR Ca^{2+} will be severely depressed (Allen and Orchard 1987).

One central question still is whether the end stage failing heart is energy starved (Ingwall and Weiss 2004) or fuel overloaded (Taegtmeier et al. 2013). ^{31}P -NMR studies indicate that the ATP/PCr ratio is decreased in failing hearts but whether this is a good proxy of the energy status and cause or consequence of the disease process is still a matter of debate (Taegtmeier and Ingwall 2013). The PCr/ATP was reduced in the HCM subjects by 30 % relative to controls (Crilly et al. 2003). Moreover, a recent study provided evidence that in the hypertrophic cardiomyopathy causing sarcomere mutations examined, tension cost was perturbed (Witjas-Paalberends et al. 2014).

Tension relaxation

Mechanical experiments in skeletal muscle, the isolated intact heart, thin strips of muscle or cardiac trabeculae and single myofibrils indicate that the isometric relaxation process occurs in 2 phases: an initial slow phase in which the individual sarcomeres stay at constant length and tension declines linearly (determined by g_{app}) followed by a fast phase in which tension declines exponentially where strong sarcomeres shorten at the expense of the weaker ones which are stretched (Huxley and Simmons 1970; Stienen and Blangé 1981; Krueger et al. 1988; Stehle et al. 2006, 2009).

Implications for systolic/diastolic function

The changes in cardiac muscle energetics will have an important consequences for both systolic and diastolic function and therefore have a major impact on cardiac performance.

Calcium sensitivity of tension development

Isometric tension–intracellular calcium relation

The relation between isometric tension and the free intracellular Ca^{2+} concentration (Ca) can be described by the modified Hill equation $T(\text{Ca}) = T_{\text{max}} \cdot \text{Ca}^{\text{nH}} / (\text{K}^{\text{nH}} + \text{Ca}^{\text{nH}})$, in which nH is a measure of the steepness of the curve and K (or EC_{50}) represents the midpoint of the sigmoidal relation observed when the free Ca^{2+} concentration is plotted on an (inverse) logarithmic scale. In the classical interpretation nH would represent the number of calcium binding sites on the regulatory protein within the regulatory unit (in our case cardiac troponin C, cTnC).

Attempts have been made to determine calcium sensitivity of isometric tension development in permeabilised preparations in which the calcium concentration can be controlled directly as well as in intact preparations by measuring the free calcium concentration in conditions where active tension development was steady (Varian et al. 2006). Sarcomere control during contraction appeared to be important for accurate measurement of EC_{50} as well as nH (Kentish et al. 1986). Typical values obtained in permeabilised cardiac trabeculae from rat at 15 °C and 2.0 μm sarcomere length are EC_{50} of 4 μM and nH of 7.3 (Dobesh et al. 2002). In intact rat trabeculae at room temperature (without sarcomere length control) an EC_{50} of 0.62 μM and a nH of 4.9 were found (Gao et al. 1994). The differences obtained are at least partly caused by differences in experimental conditions.

Figure 4 illustrates the structure of the thin filament at the molecular level with the troponin complex consisting of cTnT, cTnI and cTnC, the tropomyosin strands and the double helix of globular actin. Cardiac TnC has four metal-binding EF-hand motifs but under the prevailing conditions ($\sim 1 \text{ mM}$ free Mg^{2+}) only Ca^{2+} -binding site II in the TnC N-domain is responsible for regulating cardiac contraction (Tobacman 1996). Careful analysis of the tension-calcium relation revealed that the steepness of the relation at low calcium concentration (nH1) was larger than the steepness of the curve at high calcium concentration (nH2). These data are taken as evidence that strongly-bound (force generating) myosin heads at low calcium concentration also turn on the actin filament. On the other hand it has been argued that the cooperative mechanism underlying the high value of nH is not due to force-generating cross-bridges but that it is an intrinsic property of the thin filaments (Sun and Irving 2010).

Biochemical and structural studies indicate provided evidence for a three-state model for the regulation of myosin S1 binding to the actin filament, with a “blocked state” which is unable to bind S1, a “closed state” which can only bind S1 relatively weakly and an “open state” in

which S1 can both bind and undergo an isomerisation to a more strongly bound conformation (McKillop and Geeves 1993; Lehrer and Geeves 2014). Recently it was observed that the activation process consisted of four different components (Fusi et al. 2014) but the implications thereof need to be determined.

Implications for systolic/diastolic function

An increase in calcium sensitivity of force development will cause an increase in systolic function and the associated energy turnover, which might be beneficial on the short term but maladaptive on the long term. A positive effect could be that more force is generated while the energetic costs of calcium handling remain the same. In contrast it may exert potentially negative effects on diastolic function (decreased lusitropy) when the relaxation phase would become prolonged.

Regulatory aspects of phosphorylation of contractile proteins

Here the effect of phosphorylation of sites on the thin and thick filament which are currently known to influence calcium sensitivity in human tissue will be summarised briefly. The cardiac troponin subunits cTnI and cTnT both contain a number of phosphorylation sites which are targeted by different kinases and phosphatases (Kobayashi and Solaro 2005; Solaro and van der Velden 2010). Early studies concentrated on the Serine 23/24 sites on cTnI (human sequence) and sites on cTnT because they were substrates for protein kinase A (PKA), and protein kinase C (PKC) activated upon β - and α -adrenergic stimulation, respectively (Solaro et al. 1976; Noland et al. 1989, 1995; Mittmann et al. 1992). A number of additional phosphorylation sites on cTnI have been identified (Zhang et al. 2012), including Ser42, Ser44, Ser76 (or Thr77), Thr143, Ser150 (Buscemi et al. 2002) and Ser198 (Kooij et al. 2013b).

A recent mass spectroscopy study (Zhang et al. 2011) revealed the alterations in phosphorylation in human myocardium and identified a number of different sites. To investigate the effects of phosphorylation on tension production and its calcium sensitivity in human myocardium a technique was used in which the endogenous cTn was exchanged with recombinant cTn in permeabilised human cardiomyocytes. The unphosphorylated recombinant complex was either pre-treated with PKA, $\text{PKC}\alpha$ or $\text{PKC}\epsilon$ to phosphorylate the accessible sites or pseudo phosphorylated by mutation of the Ser or Thr sites to aspartic acid (D) (Kooij et al. 2010; Wijnker et al. 2013b, 2014a). These studies indicated that Ser23/24 needed to be

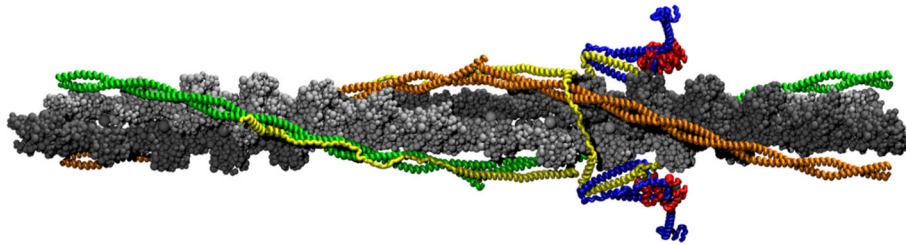


Fig. 4 Representation of the human thin filament containing human cardiac troponin (cTn), tropomyosin (Tm) and actin: *yellow* = cTnT; *blue* = cTnI; *red* = cTnC; *cyan* = calcium ion; *green/orange* = overlapping Tm; *silver/gray* = actin filament. Published

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bisphosphorylated in order to cause a reduction in calcium sensitivity (Wijnker et al. 2014a). Surprisingly this reduction was already maximal at $\sim 55\%$ pseudo bisphosphorylation. This indicates that pseudo phosphorylation (most likely random) of half of the cTn complexes along the thin filament is sufficient to induce a structural change in the position of the tropomyosin strands along the actin filament. Pseudo phosphorylation of Ser42/44 was found to decrease while Thr143 was found to increase calcium sensitivity (Wijnker et al. 2014a, b). Studies in rodents indicated that pseudo phosphorylation of Ser150 causes an increase in calcium sensitivity (Nixon et al. 2012). The endogenous level of Ser198 phosphorylation in human myocardium appeared to be low (Zhang et al. 2012), however, a level of pseudo phosphorylation of $\sim 12\%$ already caused a significant increase in calcium sensitivity (Wijnker et al. 2013a).

Human cTnT (isoform 3) contains a number of PKC phosphorylation sites including: Ser179, Ser198, Thr203 and Thr284 (Jideama et al. 1996; Noland 1996) and recently Ser179 has been identified as a substrate of PKC α (Kooij et al. 2013b). 2D gel electrophoresis in donor and end stage failing tissue human tissue revealed two major spots originating from cTnT, representing unphosphorylated (27 %) and monophosphorylated cTnT (63 %) (van der Velden et al. 2006). In rat myocardium cTnT was found to be largely monophosphorylated (Sancho Solis et al. 2008). Sumandea et al. provided evidence that among the four PKC phosphorylation sites of cTnT, Thr206 in mice, which corresponds with Thr203 in human cTnT is the functionally critical site for reduction in Ca²⁺-sensitivity, cooperativity, Mg-ATPase activity and maximum tension generation (Sumandea et al. 2003). Pseudo-phosphorylation at additional sites increased the Ca²⁺-sensitivity (Schlecht et al. 2014).

Tm phosphorylation at Ser283 (mouse sequence) results in a decrease in Ca sensitivity (Schulz et al. 2013).

MLC2 can be phosphorylated by the cardiac isoform of myosin light chain kinase at Ser15 (Chan et al. 2008). MLC2 phosphorylation increases Ca²⁺-sensitivity (Sweeney and Stull 1986) and surprisingly the effect of MLC2

dephosphorylation is enhanced in end stage failing cells relative to donor cardiomyocytes, despite a reduced level of MLC2 phosphorylation at baseline (van der Velden et al. 2003). Another intriguing finding in this study was that the MLC2 pattern on 2D gels consisted of 4 spots, suggestive of the presence of two different isoforms, with a similar phosphorylation pattern. Results in a more recent study (Scruggs et al. 2010) suggest that the doublet observed may be caused by deamination.

A reduction of MLC2 phosphorylation has been observed with pressure overload (Warren et al. 2012) implying that it might play an important role in myocardial disease. Regional differences are observed in rodent heart in MLC2 phosphorylation between subepicardial and sub-endocardial layers but this issue is still controversial (Davis et al. 2001; Cazorla et al. 2005; Ding et al. 2010).

Seventeen phosphorylation sites on cMyBP-C were identified in vivo, with the majority localized in the N-terminal domains C0-C2. The M-domain of cMyBP-C harbors several phosphorylation sites (Ser133, Ser275, Ser284, Ser304 and Ser311) that have been identified as substrates for glycogen synthase kinase 3 beta (GSK3 β), PKA, PKC or Ca²⁺-dependent calmodulin kinase II (CaMKII) (Gautel et al. 1995; Jia et al. 2010; Copeland et al. 2010). The three most abundant phosphorylated sites, Ser284, Ser286 and Thr290, are located in the regulatory M-domain of cMyBP-C. Ser284 showed a significant reduction in phosphorylation in end-stage failing hearts. (Kooij et al. 2013a). At least 3 sites on cMyBP-C can be phosphorylated in vivo by PKA: Ser275, Ser284, and Ser304 (human sequence), whereas Ser311 phosphorylation was shown to be phosphorylated by PKA in vitro (Gautel et al. 1995; Jia et al. 2010; Copeland et al. 2010). Tandem mass spectrometry analysis identified a novel phosphorylation site on serine 133 in the proline-alanine-rich linker sequence between the C0 and C1 domains of cMyBP-C. In silico kinase prediction revealed GSK3 β as the most likely kinase to phosphorylate Ser133 (Kuster et al. 2013). As noted above, cMyBP-C may act as a brake on crossbridge attachment. This brake is assumed to be relieved by PKA-mediated phosphorylation (Stelzer et al.

2006; Colson et al. 2008). Moreover, evidence suggests that cMyBP-C contains a number of phosphorylation sites which site-specifically may influence contractile function (Wang et al. 2014).

Implications for systolic/diastolic function

A large number of post translation modifications of regulatory sarcomeric proteins influence myocardial function by causing either an increase or a decrease in calcium sensitivity of tension development. It is still unclear whether in concert these alterations are adaptive or maladaptive during the development of heart failure.

Length dependent activation

Frank-Starling law of the heart

Otto Frank (Frank 1895) and Ernest Starling and colleagues (Patterson et al. 1914) independently provided evidence concerning the relation between end diastolic volume and cardiac function, now known as the Frank–Starling law of the heart. It consists of two components: a relatively slow one, related to alterations in Ca^{2+} -handling (Allen and Kurihara 1982), and a fast component, operating inside the heart on a beat to beat basis. The basis of this fast component resides inside the sarcomere. It reflects two independent myocardial properties: the sarcomere length dependence of T_{max} and of EC_{50} . As discussed earlier, the cardiomyocyte in vivo operates at the ascending limb of the tension–sarcomere length relationship and increased filling results in an increase in sarcomere length and thus in an increase in the tension generating capacity as more myosin heads are able to bind to the thin filament.

The second aspect of the fast component of the Frank–Starling relation, the decrease in EC_{50} with sarcomere length, is much less well understood. An attractive hypothesis that fuelled research over the last 30–40 years is based on the isovolumical behaviour of elastic material such as muscle cells (Matsubara and Elliott 1972). Accordingly, an increase in length directly results in a decrease in muscle width. An increase in sarcomere length thus implies that the distance between the actin/thin and myosin/thick filament decreases. Since the myofilaments are (negatively) charged, such change in distance between them may influence the affinity of binding of (positively) charged Ca^{2+} -ions. Evidence in favour of this hypothesis was obtained through experiments in skinned muscle cells, e.g. (Godt and Maughan 1981; Stienen et al. 1985; Wang and Fuchs 1995). Osmotic compression of skinned muscle cells by inert high molecular weight polymers, such as PVP or Dextrans that did not penetrate the myofilament lattice

indeed resulted in a decrease in EC_{50} , similar to that observed when the cells were stretched. However, X-ray diffraction studies by the Tombe and coworkers (Konhilas et al. 2002) have revealed that there is not a unique relation between myofilament lattice spacing and EC_{50} in isometrically compressed and stretched permeabilised cardiac trabeculae. In addition, evidence has been provided that modification of a single residue on cTnI (threonine 144; Thr-> Pro) modulated the effect of sarcomere length on EC_{50} (Tachampa et al. 2007). Apparently other mechanisms are involved in or contribute to length dependent activation.

Implications for systolic/diastolic function

Recent studies in human cardiomyocytes and in animal models showed that the relation between EC_{50} and sarcomere length was enhanced by PKA-mediated phosphorylation or pseudo phosphorylation of the Ser 22/24 on cTnI (Konhilas et al. 2003; Hanft et al. 2013; Wijnker et al. 2014a). In addition, it was observed in tissue from a hypertrophic cardiomyopathy patient carrying a mutation in cTnT that the decrease in EC_{50} upon stretch from a sarcomere length of approximately 1.8–2.2 μm was blunted (Sequeira et al. 2013). These and related findings in the literature described above indicate that the length dependent activation in healthy and myocardium and the modulation thereof in diseased myocardium are important for systolic function but its molecular basis still remains elusive.

Stretch activation

Stretch activation, the increase in tension in a muscle after stretch, is an important and well characterised process in insect flight muscle (Bullard and Pastore 2011). Stretch activation has also been shown to be present in cardiac muscle (Steiger 1977) and in skeletal muscle (Galler et al. 1997) albeit that the increase in tension is not maintained and slowly decays towards the initial isometric level. The importance of stretch activation for cardiac function in vivo and the involvement of myosin binding protein C are well established in murine myocardium (Tong et al. 2008). A recent study (van Dijk et al. 2014) revealed that both the amplitude as well as the rate constants involved in the stretch activation response in human myocardium are modulated by the free Ca^{2+} concentration and PKA-mediated phosphorylation in a similar way as observed in murine myocardium. Moreover, van Dijk et al. showed that in patients carrying a (heterozygous) truncation mutation in cMyBP-C, apparently sufficient cMyBP-C remained to preserve the main characteristics of crossbridge kinetics.

Conclusions

- A wealth of information has become available on the relation between contractile function and heart failure. This provides an entirely new class of treatment options directly targeted to sarcomeric proteins.
- The reduction in contractile performance during systole can be attributed predominantly to a loss of cardiomyocytes (necrosis), myocyte disarray, a decrease in myofibrillar density, a reduction in the tension generating capacity at the molecular level and limited energy supply. This leads to a decline in systolic function and in the ejection fraction of the heart. Diastolic dysfunction can be attributed mainly to fibrosis and an increase in titin stiffness which result in an increase in stiffness of the ventricular wall. This leads to a loss in stroke volume but the ejection fraction of the heart is preserved.
- A large number of post translation modifications of regulatory sarcomeric proteins influence myocardial function by altering calcium sensitivity of tension development and myocardial stiffness. It is still unclear whether in concert these effects are adaptive or maladaptive during the disease process.
- Recent studies indicate that the Frank–Starling mechanism is blunted when cTnI Ser22/24 phosphorylation is reduced. This may limit the power output of the failing heart during exercise and stress, but also offers treatment opportunities.

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