Cardiac function and modulation of sarcomeric function by length

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The Frank–Starling relationship provides beat-to-beat regulation of ventricular function by matching ventricular input and output. This review addresses the subcellular mechanisms by which the ventricle adjusts its output (i.e. stroke volume) by changes in end-diastolic volume. The subcellular processes are placed in the context of the four phases of the cardiac cycle with emphasis on the sarcomeric properties that mediate the number of force-generating cross-bridges recruited during pressure development. Additional mechanistic insight is provided regarding the factors that regulate myocyte loaded shortening speeds, which are paramount for dictating ejection volume. Emphasis is placed on the interplay between cross-bridge-induced cooperative activation of the thin filament and cooperative deactivation of the thin filament induced by muscle shortening. The balance of these two properties seems to determine systolic haemodynamics, and how this balance is modulated by sarcomere length, in part, underlies the Frank–Starling relationship.

1. Introduction

Human cardiac output is remarkably fine-tuned by ventricular filling volume, whereby an increase in end-diastolic volume leads to an appropriate increase in stroke volume.1 This control feature of the cardiac pump provides beat-to-beat tuning of systemic blood supply to the demands of the peripheral tissues and is important for equilibrating the output between right and left ventricles over time. Any malfunction of this control system will invariably lead to mismatches between supply and demand of peripheral energy, which could severely compromise organ system function and over time could result in fluid buildup such as pulmonary oedema (which occurs with congestive heart failure). The relationship between ventricular filling and ventricular output was first described in the early 20th century by Otto Frank2 in frog ventricles and Ernest Starling in mammalian heart–lung preparations,3 and has become known as the Frank–Starling relationship. The Frank–Starling relationship has been central in the development of ideas about the control of cardiac output over the last half century. The Frank–Starling relationship (i.e. ventricular function curves) has been experimentally determined to be modulated by a number of factors including autonomic innervation, humoral factors, exercise, coronary ischaemia, anaemia, shunts, toxic shock, and anaesthesia (Figure 1).

This has allowed for translation of the Frank–Starling relationship to the clinic as a reference indicator of the physiological and pathophysiological features of the ventricle. Importantly, the Frank–Starling relationship may become greatly depressed (in some cases nearly 10-fold) in late-stage cardiac failure.4–6 So just how do the ventricles appropriately adjust their output to end-diastolic volume and what leads to a diminishment of this control system compared with a normal, healthy heart? This review will address this question by examining the left ventricular cardiac cycle and defining the sub-cellular processes thought to mediate each phase. This is to provide helpful context for how filling and its consequent changes in myocyte length alter ventricular output and what processes may fall into disarray in failing myocardium.

2. Myofibrillar regulation of the cardiac cycle

The first phase of the cardiac cycle is ventricular filling whereby pressure in the left atrium exceeds pressure in the left ventricle and blood enters the ventricle through the atrioventricular valve. During ventricular filling the myoplasmic [Ca2+] is low (~10⁻⁷ M) and force generating interactions are minimal between myosin on the thick filament and actin on the thin filaments. Force generating transitions are inhibited in the absence of Ca²⁺ due to the relative position of tropomyosin, which tends to be trapped by troponin in a position that covers the...
myosin-binding domains on actin monomers. Since thin filaments are confined to the ‘blocked’ state in the absence of Ca\(^{2+}\), individual myocytes are considered ‘relaxed’ and are passively strained during filling to an end-diastolic length. Passive lengthening of myocytes over the working sarcomere length range is modulated primarily by titin with contributions from extracellular matrix proteins and the pericardium. Titin is a 3–4 MDa protein that spans the full half sarcomere from Z-line to M-line [for review of titin see Granzier and Labelit (Figure 2)]. Titin molecules contain extensible and non-extensible regions, where the non-extensible regions provide a scaffold for associated proteins and serve as a filamentous anchor for attachment to the Z-lines and M-lines within the sarcomere. The extensible regions span the I-band region of the sarcomere and play a prominent role in passive resistance over the working range of cardiac sarcomeres (i.e. ~1.8–2.3 μm). The extensible regions of titin contain a PEVK region (rich in proline, glutamate, valine, and lysine), tandem immunoglobulin (Ig) repeats, and an adjustable N2B region.

These three regions form the ‘molecular spring’ region whereby their straightening contributes to passive force. Together these titin regions, in response to volume filling, help establish the sarcomere length of healthy cardiac myocytes just prior to electrical excitation of the ventricles.

The second phase of the cardiac cycle commences after electrical excitation of the myocytes, whereby myoplasmic Ca\(^{2+}\) increases from ~10^{-7} to ~10^{-5} M. The increase in myoplasmic Ca\(^{2+}\) results in its binding to the low affinity Ca\(^{2+}\) binding site on the amino terminus of cardiac troponin C. This binding triggers an allosteric signalling cascade that releases cardiac troponin I from actin and ‘untraps’ tropomyosin such that it translates towards the groove between actin filaments, which exposes myosin binding sites on actin. This defines the transition of the thin filament from the ‘blocked’ state to the ‘closed’ state. In the ‘closed’ state, the position of tropomyosin is thought to allow for increased numbers of weakly bound cross-bridges to interact with actin as well as provision of a population of actin monomers that are made available for strongly bound (high stiffness), non-force generating cross-bridges. Strong cross-bridge binding initiates the thin filament transition from the ‘closed’ state to the ‘open’ state by translating tropomyosin further into the groove between actin filaments. It is only in the ‘open’ state that strongly bound cross-bridges are thought to make the transition from strongly bound non-force generating to strongly bound force-generating. Thus, in this model, full activation of thin filament regions only occurs in the presence of both Ca\(^{2+}\) and strongly bound cross-bridges, wherein Ca\(^{2+}\) first allows cross-bridges to bind and these in turn promote stereospecific binding. Importantly, strongly bound cross-bridges likely influence additional cross-bridge binding by cooperative spread of the tropomyosin positional shift to neighbouring tropomyosins and the propensity of strongly bound cross-bridges to maintain tropomyosin position in the ‘open’ state. Structural data implies that strongly bound cross-bridges influence tropomyosin molecules a minimum of three regulatory units (where one regulatory unity spans ~38 nm and is composed of seven actins, one troponin complex, and one tropomyosin) for a total distance of ~115 nm centred from the individual strongly bound cross-bridge. These structural studies are consistent with skinned muscle fibre experiments that also suggested the cooperative spread of activation by a strongly bound cross-bridge to be ~3 regulatory units (i.e. ~115 nm). Interestingly, a calculation of the number of cross-bridges that interact with thin filaments during activation yields ~1 cross-bridge bound per regulatory unit (for detailed calculation see Gordon et al. ). This calculation assumed 300 myosins per thick filament, 0.75 μm of each thin filament is in the overlap region, and 30% of all cross-bridges interact during a maximal isometric activation. Interestingly, since the fraction of interacting cross-bridges would fall during submaximal Ca\(^{2+}\) activation and further during sarcomere shortening as cross-bridge detachment is accelerated, this would again yield approximately one strongly bound cross-bridge per three tropomyosins (i.e. a total of ~115 nm along the thin filament). While this calculation is applicable for both cardiac and skeletal mammalian myofibrils, the single fibre experimental analysis was performed using fast-twitch skeletal muscle fibres; moreover, the structural analysis was performed with skeletal muscle proteins in the presence of rigor cross-bridges. Similar structural studies have not been performed with cardiac muscle proteins or in the context of cycling cross-bridges. However, experiments investigating the cooperative spread of thin filament activation have been examined using skinned cardiac muscle preparations. It appears that Ca\(^{2+}\) binding to cardiac troponin C is insufficient to completely activate one regulatory unit. However, strong binding cross-bridges appear to yield a greater degree of cooperative activation in cardiac muscle compared with skeletal muscle. Along these lines, cooperative activation of steady-state force has been found to be greater in cardiac and fast-twitch skeletal muscle compared with slow-twitch skeletal muscle. Nonetheless, the exact distance of lateral spread of thin filament activation induced by a cycling cross-bridge in cardiac muscle remains to be elucidated. Certainly, both Ca\(^{2+}\) and cross-bridge-induced cooperative activation of the thin filament are important determinants of the number of

Figure 1 Ventricular function curves showing the Frank–Starling relationship. In normal hearts (blue line) a change in end-diastolic volume leads to a change in stroke volume appropriate to meet the metabolic requirements of the tissue. The Frank–Starling relationship may be modulated (leftward and upward (green line) to yield more stroke volume for a given end-diastolic volume and a greater change in stroke volume with a change in end diastolic volume (e.g. β-adrenergic stimulation). Conversely, the relationship may be shifted downward and rightward (yellow and red lines), which has been defined as heart failure. With heart failure an increase in end-diastolic volume results in a smaller increase in stroke volume, one that is inadequate to meet the demands of the tissues.
force-generating cross-bridges formed in response to the Ca\(^{2+}\)-bound troponin C signal. Another important determinant of the number of force generating cross-bridges appears to be the lateral spacing between thick and thin filaments, which dictates the proximity of cross bridges to actin monomers (i.e. it determines the effective concentration of cross-bridges likely to undergo force generating transitions). Intact cardiac myocytes exhibit constant volume behaviour, thus, increased myocyte length will reduce interfilament lattice spacing, which will increase the effective concentration of myosin cross-bridges and yield increased Ca\(^{2+}\) sensitivity of myofibrillar force at least over an optimal range of spacing (for review see Fuchs and Martyn). Ca\(^{2+}\) sensitivity of force is thought to be paramount in determining the number of force generating cross-bridges during a heartbeat as decreases in length clearly decrease twitch force without alterations in the intracellular Ca\(^{2+}\) transient in intact mammalian ventricular preparations. The changes in lattice spacing with length are thought to be mediated by titin, which contains a spring-like region composed of a PEVK region, multiple Ig domains, and an adjustable N2B region, all that when extended physically pull the thick and thin filaments closer together. TnC, cardiac troponin C; TnI, cardiac troponin I; TnT, cardiac troponin T; MyBP-C, myosin binding protein-C. (Figure modified from).

Figure 2  Schematic of cardiac myofilament proteins at long (2.3 \(\mu\)m) and short (1.8 \(\mu\)m) sarcomere length. Increased myocyte length will exhibit reduced interfilament lattice spacing bringing cross-bridges in closer proximity to actin monomers and thus influencing the number of force generating cross-bridges. The changes in lattice spacing with length are thought to be mediated by titin, which contains a spring-like region composed of a PEVK region, multiple Ig domains, and an adjustable N2B region, all that when extended physically pull the thick and thin filaments closer together. TnC, cardiac troponin C; TnI, cardiac troponin I; TnT, cardiac troponin T; MyBP-C, myosin binding protein-C. (Figure modified from).

The ejection phase of the cardiac cycle begins once left ventricular pressure rises sufficiently to open the aortic valve. The volume of blood ejected is largely determined by the extent of myocyte shortening, which depends on two main factors (i) the number of force generating cross-bridges and (ii) the intrinsic rate of cross-bridge cycling. As mentioned earlier, the number of force-generating cross-bridges is determined by the myoplasmic [Ca\(^{2+}\)] and the cooperative activation of the thin filament by strong binding cross-bridges, which is mediated, in part, by the spacing between myofilaments. The number of strongly bound cross-bridges will dictate where on the force–velocity curve the ensemble of cross-bridges will perform. More force-generating cross-bridges yield less force per cross-bridge allowing faster cycling per cross-bridge that results in more shortening and, ultimately, more stroke volume. It is important to understand that thin filament activation levels are highly dynamic and change as a function of time and in response to sarcomere shortening. Once myocyte shortening commences, in fact, there appears to be a stimulus to deactivate the thin filament. This results from the actuality that as the thin filament slides toward the middle of the sarcomere there is less strain per cross-bridge...
and, thus, less force per cross-bridge. In addition, reduced strain per cross-bridge results in faster cross-bridge detachment so there are fewer strong binding cross-bridges to maintain the ‘open’ state via cross-bridge induced cooperative activation of the thin filament (Figure 4). Furthermore, sarcomere shortening increases interfilament spacing, providing an additional stimulus to reduce the number of strongly bound cross-bridges by decreasing the probability of a detached cross-bridge from re-attaching to the thin filament as it slides past the thick filament. Overall, these processes would act to progressively decrease the number of force-generating cross-bridges, resulting in shortening-induced cooperative deactivation of the thin filament. Shortening-induced cooperative deactivation has been observed consistently during shortening of isolated striated muscle preparations. This mechanism may, in fact, provide an important myofibrillar brake system, which could be important in preventing excessive pressure development in the face of diminishing chamber size during ejection. In addition, shortening-induced cooperative deactivation is likely a major factor for termination of phase three and, thus, control of ventricular relaxation.

When pressure in the aorta exceeds pressure in the left ventricle the semilunar valve will close and phase four (isovolumic relaxation) begins. The factors that determine myocyte relaxation are the underpinnings for the onset and duration of phase four. Certainly, the removal of sarcoplasmic Ca\(^{2+}\) is an important determinant of myofibrillar relaxation, however, its role may be more permissive, at least in healthy myocytes. This idea is derived from the findings that the fall in thin filament bound Ca\(^{2+}\) seems to precede the fall in force in a cardiac muscle isometric twitch\(^{32-34}\) and ventricular models suggest that the transient of Ca\(^{2+}\) bound to thin filaments is virtually complete while the ventricle is still in the ejection phase.\(^{35,36}\) In addition, recent studies have demonstrated that myofibrillar relaxation rates are considerably slower than rates of Ca\(^{2+}\) dissociation from cardiac troponin C in human cardiac myofibrils.\(^{37,38}\) These findings implicate that myofibrillar processes elicited by shortening-induced cooperative deactivation of the thin filament are most important in governing ventricular relaxation in the healthy heart. It is, however, not out of the question that some Ca\(^{2+}\) remains bound to cardiac troponin C for the duration of ejection and, thus, contributes to the rate of relaxation. For instance, in intact myocardium a relatively small amount of Ca\(^{2+}\) is still being removed from the sarcoplasm even during the latter phases of relaxation,\(^{34}\) which would tend to prolong relaxation.

3. Effect of length on sarcomeric function during the cardiac cycle

Within the context of the sub-cellular mechanisms that underlie the four phases of the cardiac cycle, it becomes more apparent how changes in filling volume alter ventricular output. An increase in filling volume (i.e. preload) during phase one will stretch the myocytes causing an increase in sarcomere length within the myofibrils (Figure 2). As sarcomere length is increased from ~1.8 to 2.3 \(\mu\)m there is an increase in force generating capacity of striated muscle due, in part, to more optimal overlap of thick and thin filaments. This is referred to as the ascending limb of the length-tension relationship, first defined by Ramsey and Street in 1940\(^{59}\) and further defined in 1966 in the classic study by Gordon et al.\(^{60}\) using frog fast-twitch skeletal muscle fibers. It is important to point out that cardiac myocytes exhibit a similar (maybe even shallower) ascending limb of the length-tension relationship when maximal Ca\(^{2+}\) activations in permeabilized cardiac myocytes are compared with tetanic forces in fast-twitch skeletal muscle fibres.\(^{61-63}\) The ascending limb of the length-tension relationship, however, becomes much steeper for twitch contractions (in both skeletal muscle\(^{64}\) and cardiac muscle\(^{65}\)) and twitch contractions better simulate the conditions during a cardiac cycle. The sub-cellular basis for the steep length-twitch-tension relationship over the ascending limb is the increased myofibrillar Ca\(^{2+}\) sensitivity of force with increased sarcomere length.\(^{35,63,65}\) The sub-cellular basis for the changes in Ca\(^{2+}\) sensitivity of force with length has been an area of considerable research over the last 25 years (for review see Fuchs and Martin\(^ {34}\)). A plausible biophysical mechanism for greater Ca\(^{2+}\) sensitivity at longer lengths is that stretching of myocytes yields longitudinal and radial forces that reduce the interfilament lattice spacing to increase the effective

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**Figure 3** Sarcomere length-dependent effects on loaded shortening of cardiac muscle. The number of force generating cross-bridges dictates the point on the force-velocity relationship where the ensemble of cross-bridges will function. As sarcomere length increases from 1.8 to 2.3 \(\mu\)m, the number of strongly bound force generating cross-bridges increases so that for a given afterload (or absolute force) (A) the relative load is reduced (B), which will increase the rate of cross-bridge cycling and, ultimately, the extent of myocyte shortening during systolic ejection. (This figure is modified from and depicts theoretical curves based upon data from rat skinned cardiac myocyte preparations\(^ {40}\)).

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**Figure 4** (A) the relative load is reduced (B), which will increase the rate of cross-bridge cycling and, ultimately, the extent of myocyte shortening during systolic ejection. (This figure is modified from and depicts theoretical curves based upon data from rat skinned cardiac myocyte preparations\(^ {40}\)).
Figure 4  Interplay between cooperative thin filament activation and deactivation during the cardiac cycle. Systolic ejection is determined by the balance between cross-bridge induced cooperative activation (left) and shortening induced cooperative deactivation (right). Cross-bridge-induced cooperative activation occurs as strongly bound cross-bridges activate the thin filament, which, in turn, leads to additional cross-bridge binding by the spread of the tropomyosin positional shift. Strong binding cross-bridges are thought to influence tropomyosin molecules a minimum of three regulatory units (i.e. one regulatory unit equals 7 actins/1 troponin/1 tropomyosin) or a span totalling 115 nm centred from the strongly bound cross-bridge, whereas Ca\(^{2+}\) binding to cardiac troponin C appears insufficient to completely activate one full regulatory unit. The transition between phase two and phase three of the cardiac cycle commences with the pendulum swinging from cross-bridge-induced cooperative activation to cooperative deactivation induced by sarcomere shortening, which causes faster detachment rates and a reduction in the number of strongly bound cross-bridges. This process, ultimately, deactivates the thin filament to terminate ejection and begin relaxation (phase four).

concentration of myosin cross-bridges available for binding to Ca\(^{2+}\)-activated thin filaments\(^\text{36,37,66–71}\) (Figure 2). Here the sarcomeres sense an increase in length by physical changes in interfilament lattice spacing induced, in part, by titin strain. However, while there is consistent evidence that Ca\(^{2+}\)-sensitivity of force increases with compression of the interfilament lattice\(^\text{33,37,67,68,71}\) direct examination of interfilament lattice spacing by X-ray diffraction has not always correlated well with length-dependent changes in force production.\(^\text{70,72}\) This may arise from interfilament spacings in experimental myocardial preparations that deviate from the interfilament lattice spacings that are optimal for thin filament responsiveness as suggested by Fuchs and Martyn.\(^\text{34}\) Alternatively, it may indicate a separate, non-mutually exclusive mechanism by which the sarcomeres sense length changes. One possibility is that the strain on titin may more directly correlate with the position of cross-bridges so as to influence probability of binding.\(^\text{33}\) For instance, if increased length yields more cross-bridges in proximity that favours weak binding this would tend to shift both the thin filament and cross-bridge state equilibriums towards positions that favour more strongly bound cross-bridges, which, in turn, would further activate the thin filaments. This is consistent with the X-ray diffraction measurements that showed temperature reduction increased proximity of myosin cross-bridges towards the thin filament (as indicated by the increased \(l_{1,1}/l_{1,0}\) intensity ratio), which resulted in greater Ca\(^{2+}\) sensitivity of force and reduced length dependence of Ca\(^{2+}\) sensitivity of force.\(^\text{73,74}\) The length change may also be sensed by thin filament proteins such as cardiac troponin I since transgenic replacement of cardiac troponin I with slow skeletal troponin I resulted in reduced length dependence of Ca\(^{2+}\) sensitivity of force.\(^\text{72}\) Additionally, Ca\(^{2+}\) binding to cardiac troponin C appears to be increased at longer sarcomere lengths,\(^\text{71,75,76}\) which would contribute to increased levels of thin filament activation and force-generating cross-bridges.\(^\text{77}\) Increased Ca\(^{2+}\)-binding with increased length appears to be more responsive to the presence of more strongly bound force generating cross-bridges rather than changes in sarcomere length per se since the effect was eliminated when force was prevented by vanadate.\(^\text{75,78}\) In fact, Ca\(^{2+}\)-induced force generation has been shown to alter the linear dichroism of cardiac troponin C, labelled near its N-terminus by rhodamine, which suggests cycling cross-bridges alter the N-terminal structure of cardiac troponin C perhaps changing its Ca\(^{2+}\)-binding affinity. In summary, increased sarcomere length (in response to greater filling volume) results in greater Ca\(^{2+}\)-sensitivity of myofibrillar force, which likely is induced by altered interfilament lattice spacing and/or other length-induced allosteric changes along the myofilaments that increase the probability of thin filament activation. As mentioned earlier, the duration of phase two is another factor besides recruitment of force generating cross-bridges that will have an impact on stroke volume. Increased sarcomere length does not appear to speed the rate of force development in cardiac muscle at least at a given activator [Ca\(^{2+}\)]. In fact, when Ca\(^{2+}\)-activation is matched to yield the same relative force at long and short sarcomere length, force development rates are actually faster at short sarcomere length in both \(a\)-MyHC and \(\beta\)-MyHC cardiac preparations.\(^\text{42,79,80}\) This appears to differ from skeletal muscle fibre preparations where rates of force development seem to be slower at short sarcomere length even when Ca\(^{2+}\) activated force levels are matched.\(^\text{18}\) The sub-cellular mechanism for faster rates of force development at short sarcomere length in cardiac myocytes is unknown but physiologically may serve as a
compensatory mechanism to prevent too much of a fall in output due to the relatively large drop in number of force generating cross-bridges that occurs at short sarcomere length.

Once phase 3 (i.e. ejection) begins the amount of blood expelled will be determined by the rate of loaded shortening. Sarcomere length has a major influence on this rate by first determining the number of force generating cross-bridges, which dictates the relative load that the sarcomeres will work against (i.e. afterload). As mentioned earlier, with an increase in preload each given afterload becomes a lower relative load due to greater isometric force producing capabilities (i.e. the force–velocity curves must shift upward) (Figure 3). Thus, loaded shortening velocity is increased as there are more cross-bridges to work against a load, yielding less load per cross-bridge and therefore faster loaded cross-bridge cycling. This is observed consistently in isolated muscle preparations regardless of whether the measurements are made during afterloaded contractions in intact cardiac muscle preparations, during imposition of load clamps in intact cardiac muscle preparations, or during load clamps in permeabilized single myocyte preparations. It also appears that maximum velocity of shortening is sarcomere length-dependent in cardiac muscle preparations, at least during submaximal Ca\(^{2+}\) activations. For comparative purposes, in mammalian fast-twitch skeletal muscle the maximum velocity of shortening was found to be independent of sarcomere length at least over the sarcomere length range of 1.8–2.7 \(\mu m\) based upon slack test analysis. At sarcomere lengths <1.8 \(\mu m\) maximum velocity of shortening decreased linearly with further decreases in sarcomere length, supposedly due to increased internal loads associated with thin filament overlapping with cross-bridges on the opposite side of the sarcomere and thick filaments abutting against Z-discs. As an aside, it is interesting that fibre shortening speed still remains ~50% of maximum at sarcomere lengths of ~1.5 \(\mu m\), implicating considerable thin filament sliding capacity even at very short sarcomere length (perhaps allowed for by thick filament buckling).

The maximum velocity of shortening shows a similar sarcomere length dependence in intact rat cardiac muscle preparations during near maximal Ca\(^{2+}\) activations. However, during submaximal Ca\(^{2+}\) activations maximum shortening velocity was highly dependent on sarcomere length over the sarcomere length range of 1.7–2.2 \(\mu m\) as measured directly by calibrated laser light diffraction. A similar finding was observed in ferret papillary muscle preparations using an electronic feedback system to track muscle segment length. The exact mechanism for length dependence of unloaded shortening remains unclear but may arise from a fixed internal load that cycling cross-bridges must work against. The source of the internal load has been suggested to arise from titin molecules undergoing compression and buckling and/or extracellular components such as collagen. One observation that persists in these experiments is the increased curvature of most length traces (whether from sarcomere length, segment length, or muscle length recordings) during lightly loaded contractions. This is also routinely observed in permeabilized cardiac and skeletal muscle cell preparations regardless of whether preparation length or sarcomere length are monitored. This result implicates either a passive internal load that resides in the sarcomeres and/or loss of cross-bridges during shortening due to shortening-induced cooperative deactivation. Evidence for the latter comes from the experiments in skeletal muscle fibres, whereby reduction of strong binding cross-bridges by a repetitive isotonic shortening protocol eliminated the initial fast component of shortening, and the finding that fibre stiffness (which is thought to reflect the number of strong binding cross-bridges) fell coincident with the changes in curvature of the fibre length trace. Similar experiments have not been performed in cardiac myocyte preparations. Regarding shortening at greater relative loads (i.e. at ~30% of isometric force) where power output is optimal and striated muscle is most efficient and thought to work in vivo, there is considerable sarcomere length dependence of loaded shortening at a given absolute load and this remains even when force velocity curves are normalized for differences in isometric force, i.e. loaded shortening velocity was slower at short sarcomere length. A possible mechanism underlying the decreased loaded shortening and power output at short sarcomere length could be the associated decrease in thin filament activation levels perhaps due to increased inter-filament lattice spacing, which reduces the probability of cross-bridge binding. In fact, when Ca\(^{2+}\)-activated force and presumably thin filament activation levels were matched at short sarcomere length to those at long sarcomere length (by increasing the activator [Ca\(^{2+}\)]) short sarcomere length actually yielded faster loaded shortening velocities and greater peak normalized power output, at least in adult rat myocyte preparations that contained mostly \(\alpha\)-MyHC. This suggests a myofibrillar mechanism that tends to speed loaded cross-bridge cycling to minimize the fall of power at short sarcomere length. Interestingly, the presence of 2% dextran also resulted in faster loaded shortening than at long sarcomere length, again implicating a myofibrillar mechanism that leads to faster loaded cross-bridge cycling at short sarcomere length. A potential mechanism for the speeding of loaded shortening at short sarcomere length (when force is matched) is that as sarcomere length is shortened, titin becomes less taut. This may reduce the impedance on the cross-bridge (which may be mediated by titin’s interaction with myosin-binding protein C (MyBP-C) on the thick filament (Figure 2), which in turn increases myosin head radial and azimuthal mobility. Ultimately, this may lead to faster cross-bridge cycling directly or indirectly by creating more flexible cross-bridges that are more likely to maintain thin filament activation. However, this mechanism alone cannot overcome the decrease in the number of cross-bridges induced by increased lattice spacing at short sarcomere length, which is why loaded shortening is slower at short sarcomere length when activator [Ca\(^{2+}\)] is the same between long and short sarcomere length. These mechanistic ideas are consistent with findings in cardiac myocyte preparations from MyBP-C ablated mice, where loaded shortening was elevated for any given load but especially at high loads. This response (i.e. faster loaded shortening at short sarcomere length), however, may be \(\beta\)-MyHC-dependent as loaded shortening remained slower even when Ca\(^{2+}\)-activated force levels were matched in \(\beta\)-MyHC myocyte preparations. Interestingly though, compression of the lattice caused faster loaded shortening in \(\beta\)-MyHC myocytes.
(similar to that observed in α-MyHC myocytes).\textsuperscript{42} This effect implicates lattice spacing as a key determinant in transducing the sarcomere length response of loaded shortening in cardiac myocytes. The mechanisms underlying the MyHC dependence of sarcomere length on loaded shortening is unclear but may be due to less length-responsive flexibility of the myosin cross-bridges in response to length changes in β-MyHC myocytes but more responsiveness to changes in lattice spacing. These ideas will require careful examination by X-ray diffraction studies. The greater sarcomere length dependence of loaded shortening in β-MyHC cardiac myocytes implicates a steeper Frank–Starling relationship in left ventricles that contain β-MyHC, an observation that has been borne out experimentally.\textsuperscript{40,86} The sarcomere length dependence of loaded shortening in intact cardiac muscle preparations, and the extent of cross-bridge induced cooperative deactivation that occurs in intact preparations remain to be determined. In addition, the effect of sarcomere length on rates of relaxation remains an understudied area.

4. Changes in length-dependent sarcomeric function with heart failure

Heart failure by definition is a downward and rightward shift of the Frank–Starling relationship (i.e. depressed ventricular function curves) compared with the normal, healthy heart\textsuperscript{69} (Figure 1). There are a number of changes to phase one of the cardiac cycle that occur in heart failure. A relatively common occurrence, especially in hypertensive heart disease, is an increase in fibrosis of cardiac tissue.\textsuperscript{90} This will increase the stiffness of the myocardial wall, which means higher diastolic filling pressures are required to fill the ventricle. Increased stiffness also was seen in the spontaneous hypertensive rat model, which was attributed to a decrease in the N2BA (less stiff) isoforms of titin.\textsuperscript{91} Similarly, increases in passive stiffness were seen in human papillary muscle from failing hearts,\textsuperscript{92} in trabeculae from human patients exhibiting dilated cardiomyopathy,\textsuperscript{93} and in diastolic heart failure patients where the increased stiffness was attributed to decreased phosphorylation of titin.\textsuperscript{94} If increased stiffness compromises ventricular filling then end-diastolic sarcomere length may be shorter causing the heart to work lower on the ventricular function curve. In contrast, there is evidence for reduced stiffness (i.e. increased compliance) in human patients with ischaemia-induced left-sided heart failure, which was attributed to an increase in the N2BA (more compliant) isoform of titin.\textsuperscript{45} A more compliant isoform of titin in face of greater filling pressure (that arise with pulmonary congestion) may result in a markedly diminished left ventricular reserve capacity (i.e. inability for sarcomere length to be further elongated or interfilament spacings that are compacted below optimal such that cross-bridge function is impaired\textsuperscript{84}). Regardless of whether ventricular stiffness rises or falls in disease states, any independent modulation of stiffness between the right and left ventricles could undermine a key mission of the Frank–Starling mechanism, which is to match right and left ventricular output, whereby a mismatch may predispose the system to oedema and congestion.

In the context of phase two of the cardiac cycle, myofilaments from failing human hearts have been reported to exhibit an increased Ca\textsuperscript{2+} sensitivity of force\textsuperscript{4,96,97} which was linked to reduced levels of PKA-induced phosphorylation of myofilament proteins.\textsuperscript{96} This is consistent with down-regulated β-adrenergic responsiveness associated with failing hearts.\textsuperscript{98} Increased Ca\textsuperscript{2+} sensitivity of force, in and of itself, may yield reduced length-dependent response of isometric force development as has often been observed.\textsuperscript{68,80,99} This was directly observed in human papillary muscle strips from heart failure patients, which exhibited decreased length-dependent Ca\textsuperscript{2+} sensitivity of force.\textsuperscript{4,100} This, however, is not a consistent finding as intact human myocardium, stretched from 90–100% of muscle length (that yields maximum isometric force), had similar force responses between failing and non-failing myocardium.\textsuperscript{5} Interestingly, though, in this same study, the left ventricular end-diastolic-volume-end-systolic-pressure relationship was depressed considerably in dilated cardiomyopathic hearts compared with donor hearts.\textsuperscript{5} This may implicate changes to elements in parallel with the sarcomeres or changes in the kinetics of cross-bridge interactions. For instance, PKA-induced phosphorylation of myofilaments has been reported to increase rates of force development\textsuperscript{101} and stretch activation,\textsuperscript{102} which would speed pressure development and thus phase two in normal hearts compared with diseased hearts that have reduced PKA phosphorylation of myofibrils. In addition, heart failure has been correlated with increased PKC activity\textsuperscript{103} and PKC-induced phosphorylation has been shown to depress twitch force and kinetics of force development.\textsuperscript{104,105} The effect of heart failure on myocyte-loaded shortening remains unclear. However, here again, PKA-induced phosphorylation has been found to speed-loaded shortening in cardiac myocytes,\textsuperscript{106} while PKC-induced phosphorylation reduced loaded shortening and attenuated the PKA-induced augmentation of power output.\textsuperscript{107} In addition human heart failure is associated with a small shift in MyHC content (~85% β-MyHC in donor hearts to 100% β-MyHC in failing hearts)\textsuperscript{108} and this shift in MyHC is known to result in significantly slower rates of force development,\textsuperscript{39,41} slower loaded shortening, and decreased power output.\textsuperscript{109,110} The effects of heart failure on myofibrillar relaxation rates have not been directly studied but there are a number of studies indicating that Ca\textsuperscript{2+} handling kinetics are slowed with heart failure\textsuperscript{111} and a prolonged Ca\textsuperscript{2+} transient has been shown to circumvent the sarcomeric role in determining relaxation\textsuperscript{52} and inevitably yields defects in relaxation.

5. Summary

The sub-cellular processes that underlie the cardiac cycle involve, to large extent, myofilibrar responses to changes in sarcoplasmic [Ca\textsuperscript{2+}]. The beat-to-beat regulation of ejection is largely determined by the number of strongly bound cross-bridges that interact during systole, which is determined by cooperative activation of the thin filament during phase two (isovolumetric contraction) of the cardiac cycle. Importantly, the degree of cooperative activation is greatly pre-determined by the degree of sarcomeric stretch attained during phase one (ventricular filling). During ejection, the number of strongly bound cross-bridges dictates the degree of shortening but is controlled by the balance of cooperative activation (by strong binding
cross-bridges) and cooperative deactivation induced by shortening (which lowers the number of strongly bound cross-bridges). It remains to be clarified just how sarcomere length directly modulates shortening under physiological loads and how it affects the extent of cooperative deactivation. These questions are at the ‘heart’ of the matter by which chronic adaptations (such as exercise and heart failure) lead to hyper- or hypo-effective Frank-Starling relationships.

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