The Journal of Experimental Biology 211, 2005-2013
Published by The Company of Biologists 2008
doi:10.1242/jeb.003145

# Commentary

# The Frank–Starling mechanism in vertebrate cardiac myocytes

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Accepted 10 April 2008

## Summary

**The Frank–Starling law of the heart applies to all classes of vertebrates. It describes how stretch of cardiac muscle, up to an optimum length, increases contractility thereby linking cardiac ejection to cardiac filling. The cellular mechanisms underlying the Frank–Starling response include an increase in myofilament sensitivity for $Ca^{2+}$, decreased myofilament lattice spacing and increased thin filament cooperativity. Stretching of mammalian, amphibian and fish cardiac myocytes reveal that the functional peak of the sarcomere length (SL)–tension relationship occurs at longer SL in the non-mammalian classes. These findings correlate with *in vivo* cardiac function as non-mammalian vertebrates, such as fish, vary stroke volume to a relatively larger extent than mammals. Thus, it seems the length-dependent properties of individual myocytes are modified to accommodate differences in organ function, and the high extensibility of certain hearts is matched by the extensibility of their myocytes. Reasons for the differences between classes are still to be elucidated, however, the structure of mammalian ventricular myocytes, with larger widths and higher levels of passive stiffness than those from other vertebrate classes may be implicated.**

Key words: sarcomere length–tension relationship, mammals, birds, reptiles, amphibians, fish.

## Introduction

The Frank–Starling mechanism is an intrinsic property of all vertebrate cardiac tissue. It is found in both adult and embryonic myocardium (Asnes et al., 2006) and describes how an increase in muscle length (stretch) increases contractility (Allen and Kentish, 1985). This mechanism is vital for linking changes in venous return (blood returning to the heart), with changes in stroke volume [the volume of blood pumped per beat by the ventricle(s)]. An increase in venous return dilates the ventricle(s), stretching the myocardium thereby increasing the contractility of the muscle and increasing the stroke volume. In this way, the Frank–Starling mechanism is central to the regulation of cardiac output.

Cardiac output (the product of heart rate and stroke volume) is altered to meet the changing demands of the organism [e.g. cardiac output increases with increased activity or temperature (e.g. Burggren et al., 1997)]. This increase can come about through increases in heart rate and/or stroke volume and in most cases both are used to some degree. However, the relative balance of frequency *versus* volume as a modulatory strategy for adjusting cardiac output varies amongst vertebrates. Thus, one might expect the hearts of animals that operate at the extremes of each strategy (i.e. fish and birds/mammals) to show differences in cardiac design across a number of levels of biological organization. The cellular factors associated with the evolution of high heart rates has been reviewed (Lillywhite et al., 1999). Here, we discuss the Frank–Starling mechanism at the cellular level (i.e. the cellular length–tension relationship) across vertebrate classes. We begin by discussing the cellular length–tension relationship as it is currently understood for mammals, before discussing the differences between vertebrate classes. We then focus on cellular adaptations that might enable a myocardium to eject large volumes of blood whilst retaining forceful contractions and thus regulate cardiac output through stroke volume. For an expansion of the themes raised in this *Commentary* and a discussion of the effects of myocardial stretch on the electrical activity of vertebrate hearts see Shiels and White (Shiels and White, 2007). For a comprehensive review of vertebrate cardiac function see Burggren et al. (Burggren et al., 1997).

## The cellular basis for the Frank–Starling response

### Myofilament overlap

Classic experiments by (Gordon et al., 1966) showed the sarcomere length (SL)–tension relationship in skeletal muscle could be explained in terms of thick and thin myofilament overlap (Fig. 1A). They demonstrated that the degree of myofilament overlap determines the potential availability of cross-bridges. As muscle is stretched from resting SL, there is a progressive decrease in double thin filament overlap until an optimum overlap of thick and thin filaments is reached at SLs between 2.0–2.2 μm. At longer lengths, tension falls as myofilament overlap declines with less opportunity for cross-bridge formation (Fig. 1A). In cardiac muscle, the ascending limb of the SL–tension relationship is steeper (red line in Fig. 1A) and a significant increase in active tension occurs over the region of SLs (2.0–2.2 μm in mammals) where the potential availability of cross-bridges remains constant (i.e. when the central cross-bridge head-free zone of the myosin filament is progressively uncovered) (Fabiato and Fabiato, 1975; Allen and Kentish, 1985). Hence, myofilament overlap cannot fully account for the SL–tension relationship in cardiac muscle (see Allen and Kentish, 1985) and much of the remainder of this section will discuss potential additional contributory mechanisms.

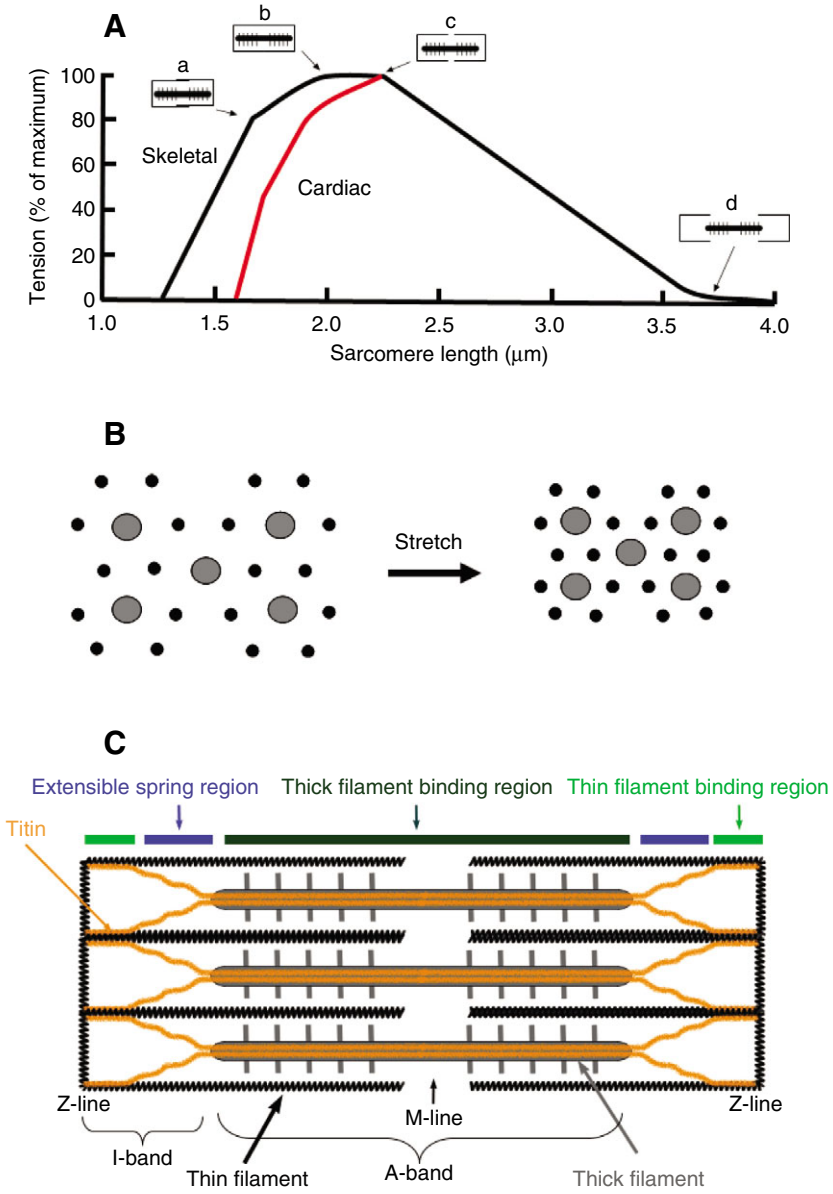


Fig. 1. (A) The sarcomere length (SL)–tension relationship for striated muscle, showing how the degree of thick (myosin) and thin (actin) filament overlap determines the potential availability of cross-bridges and thus tension. Details of the cardiac sarcomeric filaments are given in C and are drawn schematically here in the insets a–d, where the thick line with cross hatching is myosin and the thin line is actin. (a) The position of actin and myosin at short SLs, when myosin comes in contact with the Z-line. There is a rapid decline in tension as SLs decreases (to the left of the arrow). The region between b and c is the range of SLs where the potential availability of cross-bridges remains constant during sarcomere stretch because the central cross-bridge head-free zone of the myosin filament (M-line) is progressively uncovered. SLs to the left of b show how tension decreases when the thin filaments from opposite ends of the sarcomere overlap at the M-line. (d) Tension declines toward zero when the sarcomere is stretched such that there is no overlap between thick and thin filaments. A key feature of the cardiac SL–tension ascending limb is that it is shifted in relation to the skeletal curve, and that force increases over the SL range where myofilament overlap remains constant. This means mechanisms other than overlap play an important role in cardiac muscle tension (see text). The figure is adapted from Gordon et al. (Gordon et al., 1966) and Bers (Bers, 2002). (B) Illustration of a cross section through striated muscle showing the effect of stretch on myofilament lattice spacing. Light grey circles represent thick (myosin) filaments and black circles represent thin (actin) filaments. (C) Schematic diagram of a cardiac sarcomere. The sarcomere is the fundamental unit of contraction and is defined as the region between two Z-lines. Each sarcomere consists of a central A-band (thick filaments) and two halves of the I-band (thin filaments). The I-band from two adjacent sarcomeres meet at the Z-line. The central portion of the A-band is the M-line, which does not contain actin. Also shown are the positioning of titin, actin (thin) and myosin (thick) filaments. The coloured bars at the top indicate key segments of the titin molecule and show the regions bound to the contractile filaments and the extensible region. Figure is adapted from Granzier and Labeit (Granzier and Labeit, 2004).

## Myofilament $Ca^{2+}$ sensitivity

When cardiac muscle is activated by an action potential there is a release of $Ca^{2+}$ from intracellular stores and a transient rise and fall in the cytoplasmic free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$). This $[Ca^{2+}]_i$ transient provokes a twitch contraction, the amplitude of which is typically dependent upon the amplitude of the $[Ca^{2+}]_i$ transient. When striated (both cardiac and skeletal) muscle is stretched there is an immediate increase in the size of the twitch contraction (or active tension). However, Allen and colleagues have shown that in amphibian (Allen and Blinks, 1978) and mammalian (Allen and Kurihara, 1982) cardiac muscle this rapid effect is not associated with an increase in the magnitude of the $[Ca^{2+}]_i$ transient (see Fig. 2A). The rise in tension, in the absence of a rise in $[Ca^{2+}]_i$, indicates that the myofilament sensitivity for $Ca^{2+}$ has increased. These findings have been reproduced at the level of the single cardiac myocyte in mammals (e.g. Calaghan and White, 2004) and rainbow trout (Fig. 2B) (Shiels et al., 2006). In studies using skinned (chemically perforated membranes) cardiac muscle the increase in $Ca^{2+}$ sensitivity is exemplified by the leftward shift in the tension–pCa ($-\log_{10}[Ca^{2+}]$) curve seen with stretch of the sarcomere. This stretch-induced increase in myofilament $Ca^{2+}$ sensitivity is quantified by the change in the $[Ca^{2+}]$ required for half-maximal activation ($\delta pCa_{50}$) as shown for frog in Fig. 2C.

## Thin filament cooperativity

Although it is now widely accepted that the length-dependent increase in the contractility of cardiac muscle is a result of a length-dependent increase in myofibrillar $Ca^{2+}$ sensitivity, the molecular mechanisms have yet to be fully elucidated. Evidence suggests that stretch-induced increases in myofilament $Ca^{2+}$ sensitivity are regulated, in part, by cross-bridge formation. This has been explained by thin filament activation or cooperativity. In the absence of $Ca^{2+}$, the thin filament protein tropomyosin prevents the binding of actin and myosin. Tropomyosin is bound to the troponin complex which contains troponin T (TnT; tropomyosin binding), troponin C (TnC; $Ca^{2+}$ binding) and troponin I (TnI; inhibitory). When $Ca^{2+}$ binds to TnC, its greater interaction with TnI allows tropomyosin to partially move into a groove along the actin thin filament allowing the myosin heads to form weak and strong cross-bridges with actin (Gordon et al.,

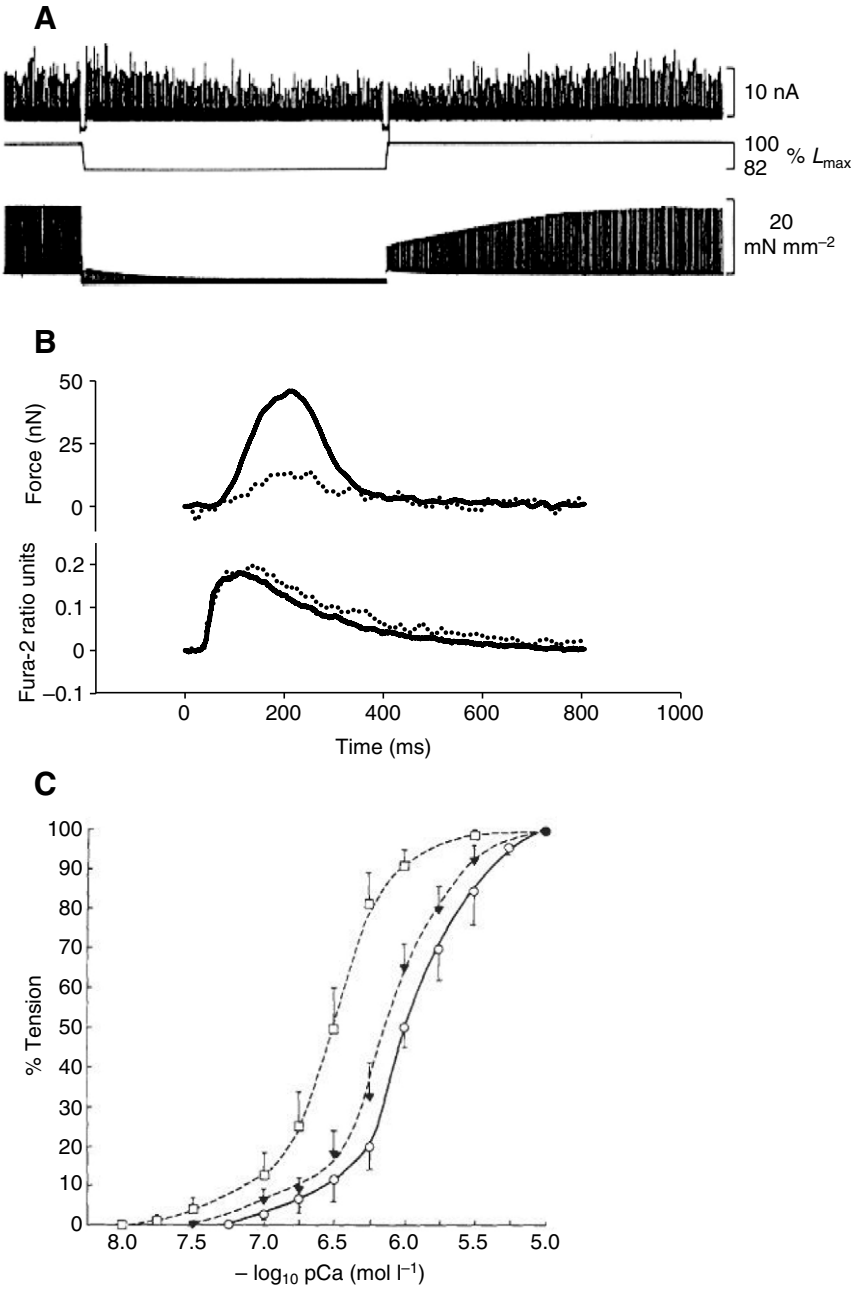


Fig. 2. (A) Stretch increases force in the absence of an increase in the amplitude of the $[Ca^{2+}]_i$ transient. $[Ca^{2+}]_i$ (upper traces, measured by aqueorin, expressed in nA) and tension (lower traces, expressed in mN mm$^{-2}$) in an intact cat trabeculae at 100% (peak of length–tension relationship) and 81% (on ascending limb of length–tension relationship) of $L_{max}$ (length at which force is maximal). Figure is from Allen and Kurihara (Allen and Kurihara, 1982), with permission. (B) Simultaneous measurement of changes in tension (upper panel) and $[Ca^{2+}]_i$ (lower panel) in a ventricular myocyte from the rainbow trout at resting length (dotted line) and after a stretch (solid line), showing the increase in force without an increase in $[Ca^{2+}]_i$. Figure is from Shiels et al. (Shiels et al., 2006), with permission. (C) Force [pCa (–log$_{10}$ Ca mol$^{-1}$)] curves in skinned frog ventricular myocytes. As sarcomere length (SL) is increased there is a leftward shift in the curve indicating an increase in myofilament $Ca^{2+}$ sensitivity. SL: circles, 2.2–2.3 μm; triangles, 2.6–2.7 μm; squares, 3.0–3.1 μm) Figure is from Fabiato and Fabiato (Fabiato and Fabiato, 1978b), with permission.

2001). When a strong binding cross-bridge forms it generates tension but also shifts the tropomyosin molecule further into the actin groove, which increases the probability of a neighbouring myosin head forming a cross-bridge (Fitzsimons and Moss, 1998; Konhilas et al., 2002a). Strong binding cross-bridges also induce cooperative activation of actin by increasing the apparent affinity of TnC for $Ca^{2+}$ (Gordon and Ridgway, 1993) indicating coupling between the $Ca^{2+}$ regulatory sites on TnC and cross-bridge interactions in the thin filament (Fukuda and Granzier, 2005). This mechanism of propagated activation may be particularly important in cardiac muscle (compared with skeletal muscle) where relatively small changes in the proportion of strongly bound cross-bridges have significant effects on the $Ca^{2+}$ sensitivity of force (Fitzsimons and Moss, 1998). Indeed, recent work has shown cardiac muscle activation is controlled locally, within a regulatory unit (made up of seven actin monomers, 1 tropomyosin and 1 troponin complex) and has greater reliance on cross-bridge attachment for promoting cooperative activation than skeletal muscle (Gillis et al., 2007).

### Myofilament lattice spacing

The question now arises as to how stretch might provoke this increase in cross-bridge formation and in the number of strongly bound cross-bridges forming. When muscle is stretched, in addition to an increase in length, there is a decrease in cross-sectional area (Fig. 1B). This latter effect results in compression of the lattice spacing of the muscle (the ordered structure of thick and thin filaments) and the closer proximity of thick and thin filaments is thought to increase the probability of (strong) cross-bridge formation. Evidence to support this explanation comes from studies where changes in $Ca^{2+}$ sensitivity induced by altered SL can be prevented by maintaining a constant preparation width using

osmotic agents such as dextran (Fuchs and Wang, 1996). However, other studies have questioned these interpretations as Konhilas et al. (Konhilas et al., 2002b) showed changes in lattice spacing that were equivalent to the spacing changes caused by increased SL did not increase $Ca^{2+}$ sensitivity.

### Titin and passive tension

Passive tension of the myocardium plays a key role in the Frank–Starling response as it is a critical determinant of ventricular filling. This is because passive tension offers resistance to ventricular filling. Thus, for a given ventricle size, greater passive tension means the myocardium is less distensible and thus harder to fill. The main determinant of passive tension at SLs on the ascending limb of the SL–tension relationship (1.8–2.2 μm in mammals) is the giant elastic protein titin (Wu et al., 2000). There are six molecules of titin in each half sarcomere, meeting in the middle, binding to myosin through the A-band and to actin at the Z-line (Fig. 1C). The extensible portion of the titin molecule is located in the I-band region of the sarcomere and is composed of multiple segments with different extensible properties. Titin develops passive forces that act to draw the Z-lines together as each extensible segment successively unfolds during stretch. With contraction of the sarcomere below resting length (i.e. when the thick filaments are in close proximity to the Z-lines) (see Fig. 1A, inset a), this extensible region is stretched in the opposite direction, imparting restoring force to the contracted sarcomere that pushes the Z-lines apart [see Granzier and Labeit (Granzier and Labeit, 2002) for schematic explanation of restoring forces]. The restoring forces generated by titin contribute to the steepness of the SL–tension relationship at very short SLs (Fig. 1A).

In addition to these longitudinal forces, titin can also produce radial force during stretch as it pulls the thin filament closer to the thick filament, which results in reduced myofilament lattice spacing (Fig. 1B,C). This may help to explain early observations that passive tension was linked to the length-sensitivity of the preparation (Cazorla et al., 1997). Removal of titin in skinned mouse trabeculae increases lattice spacing by around 3 nm throughout the range of SLs on the ascending limb of the length–tension curve, reducing passive tension and the $\delta pCa_{50}$ caused by stretch (Cazorla et al., 2001). This suggests titin modulation of lattice spacing plays a large role in the length-dependent activation of active force in the heart. However, this change in spacing was not confirmed by Konhilas et al. (Konhilas et al., 2002b; Konhilas et al., 2003). Titin strain may also increase the likelihood of actomyosin interaction by increasing the disorder of the myosin heads (Cazorla et al., 2001; Fukuda et al., 2001). In skeletal muscle, stretch can cause a slight extension of the myosin molecule which increases myosin cross-bridge head disorder thereby increasing the chance of cross-bridge formation (Wakabayashi et al., 1994).

Passive tension in the titin spring can be modulated through isoform variation, phosphorylation and $Ca^{2+}$ binding (Fig. 3). Passive tension decreases with phosphorylation (Fukuda et al., 2005b) and increases with $Ca^{2+}$ binding (Fujita et al., 2004) and these responses are isoform-specific as shown in Fig. 3. Two titin isoforms exist in the mammalian heart: the shorter stiffer N2B and longer more compliant N2BA. Their expression profiles are species-, tissue- and developmental stage-dependent and result in differing levels of passive tension (Cazorla et al., 2000b; Fukuda et al., 2005a). In mammal, the expression of N2BA increases with heart size; rat<rabbit<bovine, with adult rodents expressing the short form almost exclusively (Granzier and Labeit, 2004).

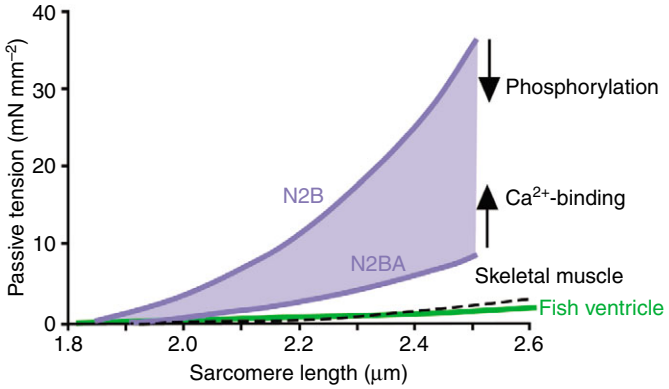


Fig. 3. Illustration of passive tension in striated muscle showing the effect of titin isoform expression (N2A, N2BA and/or combination thereof as indicated by shaded area between lines) and modulation of titin by $Ca^{2+}$ binding or phosphorylation on passive tension in mammalian cardiac muscle. Redrawn from Granzier and Labeit (Granzier and Labeit, 2004). Passive tension data measured from isolated rainbow trout ventricular myocytes [replotted from Shiels et al. (Shiels et al., 2006)] showing the low level of passive force generated by fish heart, even at long sarcomere lengths.

A transmural gradient of long:short isoforms has also been reported in the pig with more long isoform in endocardial than epicardial myocytes (Cazorla et al., 2000a; Cazorla et al., 2000b). This observation suggests that endocardial myocytes would be more compliant than epicardial myocytes. However, titin isoform ratio cannot be the only mechanism that regulates passive tension and stretch sensitivity because rat endocardial myocytes have greater resting tension and display greater stretch-induced increases in myofilament $Ca^{2+}$ sensitivity ($\delta pCa_{50}$) than epicardial myocytes, without there being a transmural difference in titin isoform ratios. The transmural differences in rat heart may be due to greater phosphorylation of myosin light chain 2b by myosin light chain kinase (Cazorla et al., 2000b; Cazorla et al., 2005). This may alter the position of the myosin heads with respect to the backbone of the myosin thick filament and affect the interaction of myosin and actin (Sweeney et al., 1993). Additionally, titin binding to myosin-binding protein C may modulate passive stiffness (Palmer et al., 2004) and be involved in allowing the cross-bridge to sense SL (Fukuda and Granzier, 2005). Thus, the titin isoform ratio, its level of phosphorylation, its binding to $Ca^{2+}$ and sarcomeric proteins, and possibly its quantity, may all modulate passive tension and *via* this, stretch-dependent changes in myofilament $Ca^{2+}$ sensitivity.

## The Frank–Starling response in non-mammalian vertebrates

### Fish

Fish differ from other vertebrate classes in that they modulate cardiac output *via* larger changes in stroke volume than changes in heart rate, the exceptions being very active species such as tuna (Farrell, 1991) and some Antarctic fishes (Axelsson et al., 1992). For example, tuna have limited capacity to increase stroke volume during exercise, and often actually decrease stroke volume coincident with an increase in heart rate (Korsmeyer et al., 1997). Rainbow trout increase heart rate by 50% during exercise but are also able to increase stroke volume threefold (Jones and Randall, 1978; Farrell, 1991). Using an *in situ* heart preparation Farrell and colleagues (e.g. Farrell et al., 1986) have reproduced *in vivo* filling pressures and output pressures and shown that small increases in filling pressure (0.2–0.3 kPa) can give up to threefold increases in stroke volume. Larger increases in filling

pressure are needed to give smaller increases in stroke volume in mammalian preparations.

We hypothesized that the predicted distension of the fish ventricle caused by a threefold increase in stroke volume (Franklin and Davie, 1992) would result in an elongation of individual fish myocytes beyond SLs reported as optimal for active tension development in mammals (i.e. on the descending portion of the mammalian cellular length–tension relationship in Fig. 1A). However, because fish maintain high ejection fractions (close to 1) this suggested that the individual fish myocytes must still contract strongly at such lengths. We investigated the relationship between contractile strength and SL by attaching single trout ventricular myocytes to carbon fibres of known compliance and stretching cells along their longitudinal axis (Fig. 4A) while recording passive and active tension (Shiels et al., 2006). We found that resting SL in isolated trout myocytes was similar to that of mammals (about 1.85 μm), as was thin filament length (about 0.95 μm), but we observed a twofold extension of the functional ascending limb of the length–tension relationship compared to what had previously been demonstrated for mammalian myocytes (Fig. 4B) (Cazorla et al., 2000a) or small multicellular preparations (Kentish et al., 1986). Thus active tension continues to increase in fish myocytes at longer SL when compared with mammalian myocytes, indicating that in fish, active tension increases beyond the length for optimum overlap of myofilaments (Fig. 4B) (Shiels et al., 2006). We concluded that because fish myocytes are able to increase their contractility over a greater range of lengths than mammalian myocytes, a fish ventricle is able to pump a greater range of volumes than a mammalian ventricle.

### Amphibians

Investigation into amphibian myocardium has also demonstrated an extended SL–tension relationship when compared with mammalian myocardium. The ascending limb of the SL–tension relationship of amphibian cardiac muscle extends up to 3 μm (Allen and Blinks, 1978) with similar SLs reported in skinned (Fabiato and Fabiato, 1978a) and intact (Tarr et al., 1981) frog myocytes. The resting SL of amphibian myocytes is consistently reported to be 2–2.3 μm (Fabiato and Fabiato, 1978a; Tarr et al., 1981) close to the optimal SL for mammals. This is associated with longer (Page, 1974) and more variable thin myofilaments (in frog atria 0.8–1.3 μm compared with 0.9–1.1 μm in rat ventricle) (Robinson and Winegrad, 1979). In frog (*Rana pipiens*) trabecular preparations performing work loops, optimal SLs for work were 2.5–2.6 μm, and the optimal SL for isometric tension were even longer (Syme and Josephson, 1995). Thus, like trout, the frog heart is able to develop force and perform work at SL longer than those expected to yield optimum myofilament overlap.

The sensitivity of the amphibian heart to stretch is species dependent with the slope of the Frank–Starling response in the

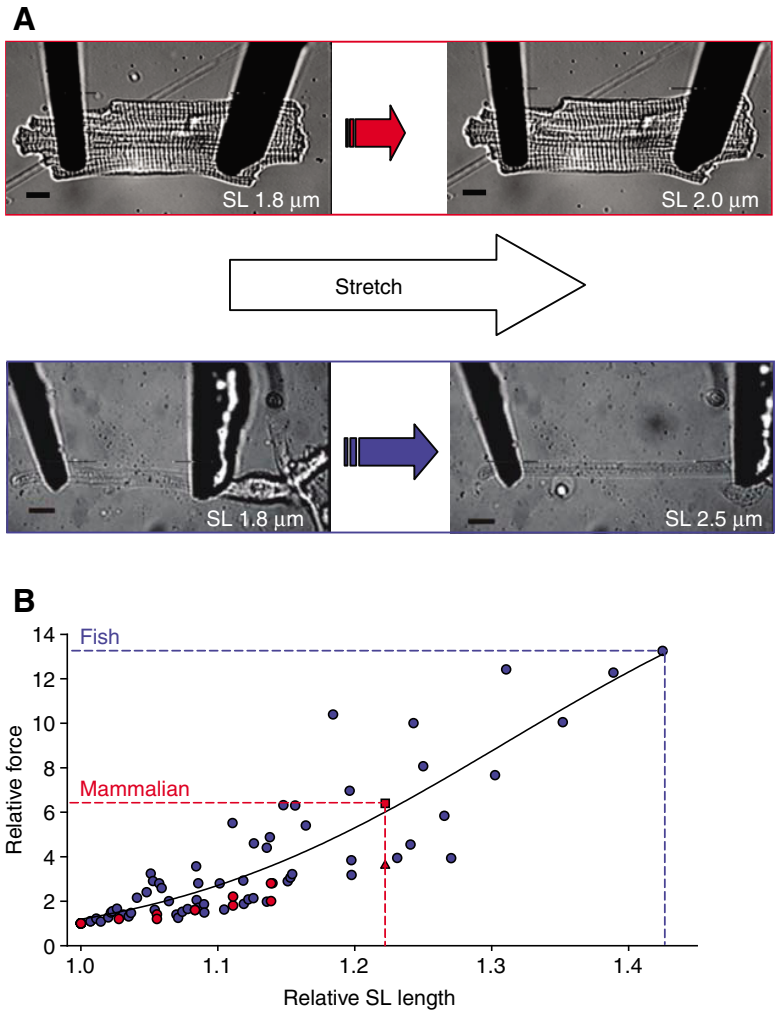


Fig. 4. (A) Stretch of single rat (top) and single trout (bottom) ventricular myocytes held between carbon fibres with resting and stretched sarcomere length (SL) shown on the image. Scale bars, 10 μm. (B) Mean SL– active tension relationships for trout (blue) and mammalian (red) ventricular myocytes where 1.0=1.84±0.02 μm for *x*-axis and 1.0=0.19±0.03 nN μm$^{-2}$ for *y*-axis. The red square (ferret) and red triangle (rat) are data extrapolated to the peak of the mammalian length–tension relationship (2.2 μm). Boxes show the operating range of SLs over which each species act and the corresponding changes in force [adapted from Shiels et al. (Shiels et al., 2006)]. Mammalian data (rat and ferret) are replotted from Cazorla et al. (Cazorla et al., 2000a).

salamander, *Ambystoma tigrinum* (McKean et al., 2002) being less sensitive to filling pressure than in the toad, *Bufo marinus* (McKean et al., 1997) under similar experimental conditions. However, although, amphibians have the capacity to support large increases in stroke volume, they regulate cardiac output through changes in both heart rate and stroke volume. The marine toad (*B. marinus*) increased stroke volume by 50–90% and heart rate by 100–115% during exercise, depending on experimental temperature (Hedrick et al., 1999; Gleeson et al., 1980). Amphibians are prone to desiccation and this may influence the prevalence of frequency- over volume-modulation of cardiac output (Burggren et al., 1997).

### Reptiles and birds

Data are unavailable for single reptilian myocytes but whole hearts studies in turtles [*Chrysemys scripta*, red-eared slider (Farrell et al., 1994); *Emydura signata*, Brisbane short-necked turtle (Franklin, 1994)], snakes [*Python molurus*, Burmese python (Wang et al., 2002)], varanid lizards [*Varanus exanthematicus* (Gleeson et al., 1980)] and crocodiles [*Crocodylus porosus*, saltwater crocodile (Franklin and Axelsson, 1994)] show that the reptilian myocardium is sensitive to the Frank–Starling mechanism. However, reptiles predominantly control cardiac output during increased activity through increased heart rate (Butler et al., 2002). In the active savannah monitor lizard (*V. exanthematicus*), exercise caused stroke volume to increase by approximately 30% while heart rate doubled (Gleeson et al., 1980), whereas only heart rate increased to elevate cardiac output in the semi-aquatic varanid [*V. mertensi* (Frappell et al., 2002)], and the iguanid lizard [*Iguana iguana* (Gleeson et al., 1980)]. Intriguingly, to offset diving bradycardia, stroke volume can increase more than fourfold during breath-hold diving in the red-eared slider turtle [*C. scripta* (Burggren et al., 1997)].

Limited information exists on the role of the cellular Frank–Starling response in bird hearts. Based on work from whole bird hearts and histological sections (Wu et al., 2004), pressure–volume relationships and SLs at end-diastolic and end-systolic volumes in birds are closer to values in mammals than amphibians or fish. Reports exist of increased cardiac output based solely on changes in heart rate in ducks (fourfold) (Grubb, 1982) and pigeons (sixfold) (Peters et al., 2005). However, in the developing chick, both heart rate and stroke volume increase to elevate cardiac output (Burggren et al., 2004).

## Potential reasons for the differences between vertebrate classes

### *In vivo* sarcomere lengths

We have discussed how an increase in SL over certain ranges leads to an increase in contractility (e.g. Fig. 1A). Work has been carried out on mammalian hearts which characterize the changes in SL that actually occur during the *in vivo* cardiac cycle (Stevens and Hunter, 2003). This entailed detailed structural mapping because strain is influenced by fibre orientation (Gilbert et al., 2007). The general conclusion is that end systolic SL is around 1.9–2.0 μm and end diastolic SL is around 2.2–2.4 μm, thus mammalian hearts work on the upper half of the ascending length–tension curve. Unfortunately, similar cellular information is not available for non-mammalian vertebrates. However, in contrast to the mammalian species that have been studied, Farrell (Farrell, 1991) has clearly illustrated how most fish hearts operate over the entire range of their Frank–Starling curve, with the exception being tuna hearts, which appear to operate on the upper portion (Farrell 1991; Blank et al., 2002). The large ejection fractions coupled with the large range of stroke volumes in fish hearts means a greater range of SLs are experienced during the ejection phase of the cardiac cycle. This could result in greater 'shortening deactivation' (as SLs shorten the $Ca^{2+}$ sensitivity of the myofilaments and the tension developed decreases in a manner opposite to the increase in myofilament $Ca^{2+}$ and tension that occurs when SLs increase; see Fig. 2) of the fish myocardium and reduced myocardial efficiency because of low contractility at the end of systole. A strategy to overcome this situation would be to extend the ascending limb of the SL–tension relationship and operate across the peak. That is, rather than just shortening down the ascending limb, the muscle shortens up the descending limb initially, such that cross-bridge formation and active tension would rise during initial ejection. It is not known whether fish use this strategy, however, it could explain how fish are able to maintain high ejection fractions and high stroke volumes. Such a strategy in mammals would lead to high levels of passive tension due to the sharp increase in mammalian passive tension from both titin and connective tissue that occurs above SLs of 2.2 μm (Wu et al., 2000) but this might not be the case in the low pressure systems of fish. In support of this suggestion, a recent study on the flatfish heart reports a very steep Frank–Starling relationship and the highest stroke volumes yet recorded for a teleost, coincident with very low systemic pressures and a very compliant myocardium (compared with mammals) (Mendonça et al., 2007).

The major difference between the SL–tension relationship of mammals, amphibians and fish is the extended ascending limb of the non-mammalian myocytes. Even accounting for the slightly longer thin filament length of amphibians, if the ascending limb of the SL–tension relationship in fish and amphibians extends to 3 μm, active force in amphibians and fish is increasing beyond optimal myofilament overlap which occurs at 2.0–2.3 μm (see Fig. 1A). Thus, force is increasing while the maximum number of potential cross bridges is falling. This is likely related to a sustained increase in myofilament $Ca^{2+}$ sensitivity. This is demonstrated for frog myocardium in Fig. 2C by the leftward shift in the tension–pCa curve ($\delta pCa_{50}$) of about 0.55 pCa units over the SL range 2.2–3.1 μm at 15°C (Fabiato and Fabiato, 1978b). For comparison, in rat myocytes at 22°C, a $\delta pCa_{50}$ of 0.1 pCa unit from 5.41 was seen when SL increased from 1.9 to 2.25 μm (Fitzsimons and Moss, 1998), and a $\delta pCa_{50}$ of 0.13 to 0.19 units from 5.7 when SL increased from 1.9 to 2.3 μm (Cazorla et al., 2005). $Ca^{2+}$ sensitivity falls with falling temperature (Harrison and Bers, 1990) but if this is ignored, these figures roughly equate to a threefold fall in the $Ca^{2+}$ required for half maximal activation ($[Ca^{2+}]_{50}$) per 1 μm increase in SL in both amphibians and mammals (with frog having a lower $[Ca^{2+}]_{50}$ at resting length; 1 μmol $l^{-1}$ frog *versus* 2–4 μmol $l^{-1}$ rat). Thus, consistent with data from intact myocytes, the range of SL to which the myofilaments can be stretched, rather than the slope of the SL–$\delta pCa_{50}$ (i.e. the stretch-induced increase in myofilament $Ca^{2+}$ sensitivity for a given increase in SL) appear to differ in frog and rat. We are unaware of similar measurement in fish myocardium although the $Ca^{2+}$-affinity of fish TnC has been measured (see below).

From these studies it seems the extensibility of amphibian and fish myocytes, coupled to a maintained increase in myofilament $Ca^{2+}$ sensitivity over a large range of SLs, are key to their extended SL–tension relationship. The difficult and still unanswered question is: by what mechanisms do these properties occur?

### Myocyte morphology

Non-mammalian vertebrate myocytes from both the atria and the ventricles have an extended length to width ratio (length:width) when compared with mammals. Typically mammalian ventricular

myocytes are two to three times wider than other cell types (see Table 1 and Fig. 4A) and only mammalian ventricular myocytes possess a well-defined t-tubular system. This t-tubular network allows the myocytes to have a greater cross-sectional area and still produce a uniform $Ca^{2+}$ transient (e.g. Shiels and White, 2005). Amphibian and fish myocytes are longer and thinner than mammalian myocytes, and their myofibrils are located at the periphery of the cell (Vornanen, 1998). Furthermore, irregularities in the lattice structure of frog myocardium have been noted (Robinson and Winegrad, 1979). Thinner cells, fewer parallel sarcomeres, and because of this, fewer parallel titin molecules, in a less ordered lattice structure will affect the passive properties of the myocytes.

## Passive tension

The capacity for greater sarcomere extension in fish and amphibian myocardium may be linked to the low passive tension developed during stretch. The passive tension curve of trout myocytes remains relatively shallow and essentially linear (see Fig. 3) compared with active tension at SL extensions of up to 40% beyond resting length (Shiels et al., 2006) whereas in both intact mammalian tissue (e.g. Kentish et al., 1986) and in skinned single mammalian myocytes (e.g. Cazorla et al., 2000a; Wu et al., 2000), steep increases in passive tension are typical for stretches of 20%. Passive tension is important for cardiac function as it affects diastolic wall tension and is thus a key determinant in cardiac filling. Low passive tension would allow greater end-diastolic volume in the heart, increasing the ability to adjust output *via* changes in stroke volume.

The contribution of titin to passive tension was discussed earlier and is summarized in Fig. 3. At present, the titin isomers in each adult vertebrate class are unknown. However, based on the extensibility of trout myocytes (Shiels et al., 2006) and the information available for mammals, one would expect non-mammalian vertebrates to express a high proportion of a titin isoform analogous to the compliant N2BA found in the mammalian heart. In mammalian hearts, the stiffer titin isoform (N2B) has been implicated in early rapid diastolic filling by exerting restoring forces which aids sarcomeric re-lengthening. This is thought to allow higher intrinsic heart rates with short diastolic filling times in small rodents (Granzier and Labeit, 2002). Because restoring forces are reduced as compliance is increased (Wu et al., 2000), it is plausible that elevation of cardiac output in fish, amphibians and reptiles may be equally, but oppositely, aided in volume regulation by expressing a more extensible titin isoform. This is because less restoring force will decrease end-systolic volume. Indeed, fish hearts are known to have a much higher ejection fraction than mammals with end-systolic volume close to zero over a physiological range of afterloads (Farrell and Jones, 1992; Franklin and Davie, 1992). Thus, the extensible nature of the fish (and amphibian) myocyte with low passive tension may facilitate increased stroke volume both by increasing end-diastolic volume and decreasing end-systolic volume.

Recently, two genes have been reported in the zebrafish (*ttna* and *ttnb*) that are orthologues of human titin, and show mRNA expression of compliant and stiff isoforms in the developing heart (Seeley et al., 2007). Co-expression of these isoforms in the adult zebrafish heart has not been investigated nor has isoform expression in other fish species. The frog ventricle was found to express only one isoform of titin, the identity of which remains to be determined (Neagoe et al., 2003). Turkey ventricular myocardium appears to express only the shorter, stiffer isoform of titin (Wu et al., 2004). This may suggest that working environment (i.e. temperature, heart rate) may also be important in setting passive tension. The titin isoform(s), their level of stretch-induced phosphorylation and their quantity (both per sarcomere and per unit cell cross-sectional area), are all factors that may underlie the passive properties of non-mammalian vertebrate hearts and awaits discovery.

## Troponins

The affinity of TnC for $Ca^{2+}$ increases with stretch. Early studies revealed myofibrils from fish (Churcott et al., 1994) and frog (Harrison and Bers, 1990) heart have a greater $Ca^{2+}$ sensitivity than those from the mammalian heart. This response, at least in fish, involves greater $Ca^{2+}$ sensitivity of the fish isoform of TnC (Gillis et al., 2003). Unfortunately the length-dependent change in myofilament $Ca^{2+}$ sensitivity has not been investigated in fish. The role of TnI in myofilament length-dependent activation has also not been investigated in lower vertebrates, although it is known to play a significant role in mammals (Tachampa et al., 2007). Information on the relationship between TnC $Ca^{2+}$ sensitivity, TnI regulation of length-dependent activation and cross-bridge cooperativity could provide insight into whether the inherently greater $Ca^{2+}$ sensitivity of fish and frog myofibrils affects the ability of stretch to modulate $Ca^{2+}$ sensitivity.

## Environment

Fish, amphibians and reptiles are ectotherms and therefore their hearts are often required to function across a range of thermal environments. Adjusting cardiac output *via* changes in stroke volume may be crucial for ectotherms, since environmental factors such as temperature and oxygen have a direct effect on cardiac frequency. Indeed, pacemaker firing rate can be significantly reduced at cold temperatures in ectotherms, particularly aquatic ectotherms such as fish, whose hearts rapidly equilibrate with ambient temperature because the coronary blood supply comes

Table 1. Comparative morphometric data for vertebrate ventricular myocytes

| | Trout | Frog | Turtle‡ | Lizard§ | Turkey¶ | Rat* |
|---|---|---|---|---|---|---|
| Cell length (μm) | 159.8‡ | 300** | 189.1 | 151.2 | 136 | 141.9 |
| Cell width (μm) | 9.9‡ | 5** | 7.2 | 5.9 | 8.7 | 32.0 |
| Cell depth (μm) | 5.7‡ | n/a | 5.4 | 5.6 | n/a | 13.3 |
| Cell capacitance (pF) | 46† | 75†† | 42.4 | 41.2 | 25.9 | 289.2 |
| Cell volume (pl) | 2.5† | 2.9‡‡ | 2.3 | 2.3 | n/a | 34.4 |
| SA/V ratio (pF $pl^{-1}$) | 18.2† | 25.8‡‡ | 18.3 | 18.2 | n/a | 8.44 |
| t-tubular system | No§§ | No†† | No‡ | No§ | No¶¶ | Yes§§ |

SA/V is surface area/volume. Data are means, but s.e.m. (when known) has been left out for clarity. *(Satoh et al., 1996); †(Vornanen 1998); ‡(Galli et al., 2006); §(G. L. Galli, personal communication); ¶(Kim et al., 2000); **(Goaillard et al., 2001); ††(Bean et al., 1984); ‡‡[derived from Goaillard et al. (Goaillard et al., 2001), assuming an elliptical cross sectional area]; §§(Shiels and White, 2005); ¶¶(Jewett et al., 1971) for finch and humming bird (not turkey).

directly off the gill at the water interface (Farrell and Jones, 1992). Similarly, hypoxia (low oxygen) results in a profound bradycardia in most fish species, which could significantly reduce cardiac output in the absence of concomitant increases in stroke volume (for a review, see Farrell, 2007). Fortunately, because diastolic filling time increases as heart rate decreases with cold temperatures or hypoxia, end-diastolic volume increases and causes myocardial stretch and, thus, through the Frank–Starling mechanism, increased stroke volume.

Cold temperatures also decrease myofilament $Ca^{2+}$ sensitivity (Gillis et al., 2000) which could reduce the ability of the heart to eject the larger volume of blood. The greater inherent $Ca^{2+}$ sensitivity of TnC in fish hearts has been suggested to offset this direct and negative effect of cold temperature on myofibril $Ca^{2+}$ sensitivity (Harrison and Bers, 1990; Churcott et al., 1994). We hypothesise that this feature is augmented by maintenance of the SL-dependent increase in myofilament $Ca^{2+}$ sensitivity at the long SL necessary for volume regulation. The corollary to this scenario is that warm temperatures may pose a problem for volume modulation of cardiac output as (1) the inherently high $Ca^{2+}$ sensitivity in ectotherms limit off-loading of $Ca^{2+}$ from the myofibrils at warm temperatures (Tibbits et al., 1991) and (2) the high heart rates at warm temperatures will limit diastolic filling times. This may help to explain why the upper limit of heart rate is 2.0 Hz (120 beats $min^{-1}$) for a large number of fish species (Farrell, 1991).

## Conclusions

Although there are many gaps in our knowledge it seems that variations in cardiac mechanosensitivity exist within a framework of basic physiological parameters whereby mechanical stimulation increases contractility. The major difference between mammalian and amphibian/fish myocytes appear to be the extended ascending limb of the cellular SL–tension relationship. Additional work is needed to characterise the cellular length–tension relationship in reptiles and birds. Because of the significance of these features in the evolution of vertebrates, such studies are vital to truly grasping the changing role of volume-modulated cardiac output across vertebrate classes. Key questions that remain to be answered are: (1) what is the mechanism(s) by which active force continues to increase beyond optimal myofilament overlap in amphibian and fish myocardium, and (2) how do the changes in SL in the intact heart of non-mammalian vertebrates compare with those seen in mammals? If the differences in the emphasis of mechanical modulation are the result of the cardiac environment, species that span the normal distinctions between vertebrate classes – lizards with high body temperatures, tuna with high heart rates, and mammals that undergo torpor – may prove particularly interesting.

## Glossary

| | |
|---|---|
| Active tension | increase in muscle tension during activation (during systole) |
| $[Ca^{2+}]_i$ | free intracellular calcium concentration |
| $[Ca^{2+}]_i$ | transient: the rise and fall in $[Ca^{2+}]_i$ within cardiac myocytes that accompanies each cardiac cycle/twitch |
| Cardiac output | the volume of blood pumped by the heart per unit time; the product of heart rate and stroke volume |
| Ejection fraction | the end-diastolic volume minus the end-systolic volume as a fraction of the end-systolic volume, i.e. the fraction of blood at the end of ventricular filling that is pumped out during the subsequent contraction. A value of 1 means complete emptying |
| Endocardium | the myocardium on the inner side of the ventricle(s) |
| Epicardium | the myocardium on the outer side of the ventricle(s) |
| Myocyte | an individual cardiac muscle cell |
| Myofilament | either a thick filament made up principally of myosin molecules or a thin filament made up of actin molecules and associated troponin and tropomyosin complexes |
| Passive stiffness | the resistance of the resting muscle to deformation by an applied force; the inverse of compliance |
| Passive tension | muscle tension at rest (during diastole) |
| $pCa_{50}$ | $-\log_{10}$ $Ca^{2+}$ concentration that activates 50% of maximal force |
| Stroke volume | the volume of blood ejected into the aorta by a single contraction of the ventricle(s) |
| t-tubular system | invaginations of the myocyte sarcolemma that carry electrical impulse to the interior of the cell and house a high level of proteins associated with excitation–contraction coupling |
| Venous return | the return of blood to the heart *via* the venous system |
| $\delta pCa_{50}$ | change in $pCa_{50}$, used as an index of changes in myofilament $Ca^{2+}$ sensitivity in response to, e.g. a change in sarcomere length |

The authors thank A. P. Farrell, T. E. Gillis and O. Cazorla for insightful discussions and the anonymous reviewers for their helpful comments. The University of Manchester and the University of Leeds are acknowledged for support.

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