Troponin and Titin Coordinately Regulate Length-dependent Activation in Skinned Porcine Ventricular Muscle

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We investigated the molecular mechanism by which troponin (Tn) regulates the Frank-Starling mechanism of the heart. Quasi-complete reconstitution of thin filaments with rabbit fast skeletal Tn (sTn) attenuated length-dependent activation in skinned porcine left ventricular muscle, to a magnitude similar to that observed in rabbit fast skeletal muscle. The rate of force redevelopment increased upon sTn reconstitution at submaximal levels, coupled with an increase in Ca2+ sensitivity of force, suggesting the acceleration of cross-bridge formation and, accordingly, a reduction in the fraction of resting cross-bridges that can potentially produce additional active force. An increase in titin-based passive force, induced by manipulating the prehistory of stretch, enhanced length-dependent activation, in both control and sTn-reconstituted muscles. Furthermore, reconstitution of rabbit fast skeletal muscle with porcine left ventricular Tn enhanced length-dependent activation, accompanied by a decrease in Ca2+ sensitivity of force. These findings demonstrate that Tn plays an important role in the Frank-Starling mechanism of the heart via on–off switching of the thin filament state, in concert with titin-based regulation.

INTRODUCTION

Cardiac pump function is enhanced as ventricular filling is increased (i.e., the Frank-Starling law of the heart) (Katz, 2002). The “Law of the Heart” is a manifestation of the sarcomere length (SL) dependence of myocardial activation, in which active force is a function of the resting SL (Fukuda and Granzier, 2005). The molecular mechanism of length-dependent activation is still not fully understood.

In striated muscle, the formation of strongly bound cross-bridges, i.e., attachment of myosin to actin, is a stochastic process (Huxley, 1957). It has therefore been proposed that the attachment probability increases as interfilament lattice spacing is reduced, resulting in an increase in Ca2+ sensitivity of force (Fukuda and Granzier, 2005, and references therein). Recent studies using synchrotron x-ray diffraction have revealed that titin (originally known as connectin)-based passive force acts as an interfilament lattice spacing modulator within the physiological SL range in cardiac muscle (Cazorla et al., 2001; Fukuda et al., 2003). It has also been pointed out that titin-based passive force may strain the thick filament, allowing the shift of myosin heads from the thick filament backbone to the thin filament (Fukuda et al., 2001b).

It has however been reported that changes in the lattice spacing are not the only factor modulating length-dependent activation (Konhilas et al., 2002a,b; 2003).

Indeed, we previously varied the thin filament activation level, by changing the concentration of MgADP (Fukuda et al., 2000) or inorganic phosphate (Fukuda et al., 2001a), and demonstrated that the length dependence is a function of the fraction of recruitable (i.e., resting) cross-bridges that can potentially produce additional active force upon attachment to actin (Fukuda and Granzier, 2005).

It is widely known that length-dependent activation is more pronounced in cardiac muscle than in skeletal muscle, within the physiological SL range (e.g., Allen et al., 1974; Allen and Kentish, 1985; Babu et al., 1988; Gulati et al., 1991). This difference likely results in part from the differential expression of titin in cardiac vs. skeletal muscle (e.g., Granzier and Labeit, 2004); a higher passive force due to the shorter extensible I-band region of cardiac titin(s) may more effectively modulate the lattice spacing and/or the thick filament structure.

It is also possible that the differential expression of troponin (Tn) in cardiac vs. skeletal muscle underlies the differing magnitude of length-dependent activation. Indeed, it was proposed that cardiac troponin C (TnC) may act as a unique “length sensor” that somehow senses changes in SL (Babu et al., 1988; Gulati et al., 1991). However, this proposal was later challenged by the Moss group with a series of careful experiments (Moss et al., 1991; McDonald et al., 1995) and, currently, is in dispute.

Abbreviations used in this paper: BDM, 2,3-butanedione monoxime; PLV, porcine left ventricular muscle; SL, sarcomere length; Tn, troponin.
from the papillary muscle of the left ventricle in Ca2+ free Tyrode's mal, HCO3− 95% O2 at 30 °C) containing 30 mM 2,3-butanedione monoxime activation, focusing on how this phenomenon is regulated via on-off switching of the thin filament state in association with titin-based regulation.

The present study was accordingly undertaken to integrate the molecular mechanisms of length-dependent activation, focusing on how this phenomenon is regulated via on-off switching of the thin filament state in association with titin-based regulation.

MATERIALS AND METHODS

All experiments performed in the present study conform to the Guide for the Care and Use of Laboratory Animals (1996, National Academy of Sciences, Washington D.C.).

Preparation of Skinned Muscle and Measurement of Isometric Force

Active and passive forces were measured with skinned porcine left ventricular muscle (PLV), based on our previously published procedure (Fukuda et al., 2000, 2001a,b, 2003). Porcine hearts (animals, ~1.0 yr) were obtained from a local slaughterhouse. Muscle strips (~1–2 mm in diameter and ~10 mm in length) were dissected from the papillary muscle of the left ventricle in Ca2+-free Tyrode's solution (135 mM Na+, 5 mM K+, 1 mM Mg2+, 98 mM Cl−, 20 mM HCO3−, 1 mM HPO42−, 1 mM SO42−, 20 mM acetate, 10 mM glucose, 5 IU/liter insulin, pH 7.35, when equilibrated with 5% CO2−95% O2 at 30 °C) containing 30 mM 2,3-butanedione monoxime (BDM). Psoas muscles were obtained from white rabbits (2–3 kg).

Both cardiac and skeletal muscles were skinned in relaxing solution (5 mM MgATP, 40 mM Na2,N,N,N-tetraethyl-2-aminoethanesulfonic acid [BES], 1 mM Mg2+, 10 mM EGTA, 1 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0) containing 1% (wt/vol) Triton X-100 and 10 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0) containing 1% (wt/vol) Triton X-100 and 10 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0) containing 1% (wt/vol) Triton X-100 and 10 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0) containing 1% (wt/vol) Triton X-100 and 10 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0) containing 1% (wt/vol) Triton X-100 and 10 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0) containing 1% (wt/vol) Triton X-100 and 10 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0) containing 1% (wt/vol) Triton X-100 and 10 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0) containing 1% (wt/vol) Triton X-100 and 10 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0) containing 1% (wt/vol) Triton X-100 and 10 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0) containing 1% (wt/vol) Triton X-100 and 10 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0) containing 1% (wt/vol) Triton X-100 and 10 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0) containing 1% (wt/vol) Triton X-100 and 10 mM dithiothreitol [DTT], 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 100 mM ionic strength [adjusted by K-propionate], pH 7.0).

Small thin preparations (~100 μm in diameter and ~2 mm in length) were carefully dissected from the above strips for isometric force measurement (Fukuda et al., 2003). Single fibers were used for experiments with rabbit psoas muscle. SL was measured during relaxation with a spatial autocorrelation function (model 901A HVSL, Aurora Scientific) through an inverted microscope (model Diaphot 300, Nikon) and a CCD camera (model IPX-VGA210L, IMPERX), and recorded by a 16-bit A/D and 16-bit D/A converter (model 802B, Aurora Scientific). Force and length signals were digitized, controlled, and recorded by a 16-bit A/D and 16-bit D/A converter (model 600A, Aurora Scientific). In brief, the preparation was bathed in an activating solution and steady isometric force was allowed to develop. Subsequently, the muscle was rapidly slackened by 20% of its original length in 1 ms, followed by unloaded shortening for 20 ms after which it was stretched back to its original length in 1 ms. Force redevelopment following the slack-restretch maneuver and force recovery to the original steady-state value reflect the rate of cross-bridge cycling (Brenner and Eisenberg, 1986; Metzger et al., 1989). A kp–pCa curve was obtained by first maximally activating the muscle at pCa 4.5, and then in a series of submaximally activating solutions at pCa 6.0, 5.75, 5.5, and 5.0 (in this order; pCa 6.0 was only used for skeletal Tn (sTn)–reconstituted muscles due to increased Ca2+ sensitivity of force), and lastly at pCa 4.5. kp was obtained by fitting the force redevelopment process with a single exponential function: F(t) = F∞ × (1 − exp(−ktpCa) + Fres, where F(t) is the force at any time t after restretch, F∞ is the steady-state force, and Fres is the residual force obtained immediately after the slack–restretch maneuver (regression coefficient R > 0.7 for all data). Isometric force was measured just before the slack–restretch maneuver, and kp was normalized at pCa 4.5 obtained at the end of experiment. Measurements were performed at 15°C.

Tn Exchange

The Tn exchange was performed based on our previously published procedure (Shiraishi et al., 1995), with modifications. The sTn complex was purified from rabbit fast skeletal muscle and stored at −80°C. In brief, after measuring active force (and kp) at pCa 4.5 at SL 1.9 μm, the cardiac muscle preparation was
bathed in rigor solution (10 mM BES, 150 mM K-propionate, 2.5 mM EGTA, 5 mM MgCl\textsubscript{2}, pH 7.0) containing 2 mg/ml sTn and 80 mM BDM for 1 h at 25°C. The presence of BDM was found to be essential to avoid contraction upon Tn exchange and the ensuing damage to the sarcomere structure, allowing us to obtain two force–pCa curves at short and long SLs, with little or no rundown in active or passive force. Then, the preparation was washed with normal relaxing solution at 15°C for 10 min with gentle agitation to remove excess sTn, resulting in only a small (\(\sim\)10%) increase in the band intensity of each Tn subunit upon sTn reconstitution.

Similarly, reconstitution was performed on single fibers of rabbit psoas muscle under the same condition for 1 h in the presence of the Tn complex purified from porcine left ventricular muscle (cTn; 6 mg/ml). cTn was stored at -80°C.

We also conducted a set of experiments where cardiac or skeletal muscle was treated with the Tn complex identical to the endogenous one. In these experiments, cardiac muscle was treated with 2 mg/ml cTn for 1 h, and skeletal muscle with 2 mg/ml sTn for 1 h (for both force measurements and gel electrophoreses).

Gel Electrophoresis
SDS-PAGE was conducted based on the previous study (Fukuda et al., 2003; Udaka et al., 2008). Muscle preparations were solubilized and electrophoresed on Laemmli gels (acrylamide, 3.5% in stack and 15% resolving gel; pH 9.3). Cardiac (N2B and N2BA isoforms) and skeletal titins were checked by using 2–7% acrylamide gradient gels. Gels were scanned and digitized (ES-2200, EPSON), and densitometry was performed using One-D-Scan (v. 2.03, Scanalytics Corporation). Tn isoforms were identified by commigration with known standards. We quantified the reconstitution ratio by comparing the ratio of troponin T (TnT) or TnI to actin with that obtained for the original muscle (i.e., either rabbit psoas or porcine left ventricular muscle).

Statistics
Significant differences were assigned using the appropriate paired or unpaired Student’s t test. Regression analysis was performed using the least-square method (Fukuda et al., 2001b). Data are expressed as mean ± SEM, with \(n\) representing the number of muscles. Statistical significance was assumed to be \(P < 0.05\). NS indicates \(P > 0.05\).
TABLE I
Summary of the Values of Passive Force, Maximal Active Force, pCa50, ΔpCa50, and nH in Control PLV, sTn-reconstituted PLV, and Control Rabbit Psoas Muscles

| SL  | Passive force | Maximal force | pCa50 | ΔpCa50 | nH |
|-----|---------------|---------------|-------|--------|----|
| μm  | mN/mm²        | mN/mm²        |       |        |    |
| PLV | 1.9           | 54.89 ± 2.24  | 5.56 ± 0.01 | 3.83 ± 0.20 |
|     | 2.3           | 70.25 ± 3.87  | 5.81 ± 0.02 | 0.24 ± 0.01 | 3.11 ± 0.29 |
| PLV (+sTn) | 1.9   | 51.43 ± 2.07  | 5.79 ± 0.01 | 0.14 ± 0.02 | 3.20 ± 0.20 |
|     | 2.3           | 58.26 ± 3.58  | 5.93 ± 0.02 | 0.14 ± 0.02 | 3.01 ± 0.10 |
| Rabbit psoas (+sTn) | 1.9 | 161.72 ± 8.91  | 5.89 ± 0.03 | 0.12 ± 0.01 | 3.40 ± 0.27 |
|     | 2.3           | 178.68 ± 8.74  | 6.01 ± 0.02 | 0.12 ± 0.01 | 3.40 ± 0.27 |

Control experiments

| PLV (+cTn) | 1.9 | 50.70 ± 3.15  | 5.60 ± 0.01 | 3.28 ± 0.11 |
|            | 2.3 | 67.73 ± 3.70  | 5.82 ± 0.05 | 0.23 ± 0.04 | 2.60 ± 0.11 |
| Rabbit psoas (+sTn) | 1.9 | 129.40 ± 14.02 | 5.86 ± 0.03 | 4.21 ± 0.31 |
|            | 2.3 | 132.58 ± 17.78 | 5.97 ± 0.04 | 0.11 ± 0.01 | 4.42 ± 0.55 |

Passive force was obtained at the steady state. PLV (+sTn), sTn-reconstituted PLV. Control experiments, muscles treated with the Tn complex identical to the endogenous one (see text). PLV (+cTn), cTn-reconstituted PLV (n = 5); Rabbit psoas (+sTn), sTn-reconstituted rabbit psoas muscle (n = 6). (P > 0.05 compared with corresponding values for original muscles, except for nH at SL 1.9 μm in PLV (+cTn)). Maximal force was obtained by activating muscle at pCa 4.5 prior to construction of the force–pCa curve at each SL (passive force was measured just prior to activation at pCa 4.5). Numbers in parentheses indicate maximal force values obtained prior to reconstitution (P > 0.05 compared with corresponding values obtained after reconstitution).

*P < 0.05 compared with corresponding values for control PLV.

**P < 0.05 compared with corresponding values for sTn-reconstituted PLV.

RESULTS

Effect of sTn Reconstitution on Length-dependent Activation in Cardiac Muscle

A representative 15% gel (Fig. 1 A, left) demonstrates that endogenous Tn subunits were effectively replaced by their rabbit fast skeletal counterparts in PLV. Subsequent treatment with cTn incorporated cardiac Tn subunits into the sTn-treated muscle by ~40% (see legend of Fig. 1 A for quantitative analysis). Both N2BA and N2B titins were not degraded during Tn exchange (Fig. 1 A, left; see passive force in B and C).

Chart recordings of active force (Fig. 1 B) indicate that upon sTn reconstitution, maximal force decreased only slightly (Table I). At submaximal levels active force increased upon sTn reconstitution to an extent greater at SL 1.9 μm than at 2.3 μm, resulting in a decrease in the stretch-induced gain (i.e., from 52% to 21% at pCa 5.75).

Force–pCa curves revealed that sTn reconstitution increased Ca2+ sensitivity of force and diminished ΔpCa50 (Fig. 1 C, left). ΔpCa50 became similar to that obtained with rabbit fast skeletal (psoas) muscle (Fig. 1 C, right). Similarly, the SL-dependent increase in maximal Ca2+-activated force diminished upon sTn reconstitution and became similar to that obtained in rabbit psoas muscle (Fig. 1 D).

We confirmed that the treatment with the Tn complex identical to the endogenous one did not significantly change active and passive properties of cardiac and skeletal muscles at either SL (Table I; see also Fig. 1 A and Fig. 2 A), suggesting that our Tn exchange protocol creates reconstituted preparations that are interchangeable with the original muscles.

Effect of cTn Reconstitution on Length-dependent Activation in Skeletal Muscle

We then investigated whether reconstitution of skeletal thin filaments with cTn enhances length-dependent activation. A representative 15% gel shows that endogenous skeletal Tn subunits were decreased by ~50% with a concomitant appearance of cardiac Tn subunits (Fig. 2 A). The incorporated cardiac Tn subunits were effectively replaced by skeletal isoforms upon subsequent sTn treatment (see legend of Fig. 2 A for quantitative analysis). Titin was unaffected by the reconstitution procedure (Fig. 2 A, right).

Chart recordings (Fig. 2 B) indicate that upon cTn reconstitution maximal force decreased slightly (Table II). Submaximal active force decreased upon cTn reconstitution to an extent greater at SL 1.9 μm than at 2.3 μm, and resulted in an increase in the stretch-induced gain (i.e., from 27% to 43% at pCa 6.0). Force–pCa curves revealed that Ca2+ sensitivity of force decreased and, importantly, ΔpCa50 increased (Fig. 2 C). ΔpCa50 was similar to that obtained in PLV (see Fig. 1 C). Similarly, the SL-dependent increase in maximal Ca2+-activated force increased upon cTn reconstitution, and became similar to that obtained in PLV (Fig. 2 D).

Effect of sTn Reconstitution on Cross-Bridge Kinetics in Cardiac Muscle

The increase in Ca2+ sensitivity of force upon sTn reconstitution in cardiac muscle may result from the accelerated myosin binding to actin. Therefore, we tested the effect of sTn reconstitution on ktr. At maximal activation, ktr did not significantly change upon sTn reconstitution (see legend of Fig. 3). However, it increased significantly at...
In the present study, we demonstrated that quasi-complete reconstitution of cardiac thin filaments with sTn diminished length-dependent activation, to a similar level as that observed in skeletal muscle. sTn accelerated the cross-bridge formation, and length-dependent activation strongly correlated with titin-based passive force, irrespective of the Tn isoform. Furthermore, reconstitution of skeletal thin filaments with cTn enhanced length-dependent activation, accompanied by a decrease in Ca$^{2+}$ sensitivity of force.

Several groups reported an inverse relationship between the cooperative thin filament activation and length-dependent activation. Using a strong-binding, non-force-generating analogue of myosin subfragment-1 (NEM-S1), Fitzsimons and Moss (1998) first demonstrated that length-dependent activation is attenuated at high cooperative activation, consistent with our previous observation with MgADP (Fukuda et al., 2000). Conversely, length-dependent activation is reportedly enhanced at low cooperative activation, regardless of the activator, i.e., either Ca$^{2+}$ (Fukuda et al., 2001a) or rigor cross-bridges (Smith and Fuchs, 1999). These findings are consistent with the notion that the magnitude of length-dependent activation depends on the fraction of recruitable cross-bridges.

DISCUSSION

In the present study, we demonstrated that quasi-complete reconstitution of cardiac thin filaments with Stn diminished length-dependent activation, to a similar level as that observed in skeletal muscle. Stn accelerated the cross-bridge formation, and length-dependent activation strongly correlated with titin-based passive force, irrespective of the Tn isoform. Furthermore, reconstitution of skeletal thin filaments with cTn enhanced length-dependent activation, accompanied by a decrease in Ca$^{2+}$ sensitivity of force.

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conclude that sTn shifts the equilibrium of the thin filament state toward the “on” state and subsequently reduces the fraction of recruitable cross-bridges, resulting in blunted length-dependent activation, with a magnitude similar to that in skeletal muscle. This view is supported by the result of Fig. 2 that cTn decreased Ca$^{2+}$ sensitivity of force and enhanced length-dependent activation in skeletal muscle.

The reconstitution ratio was lower in rabbit psoas fibers with cTn (50% with 6 mg/ml; Fig. 2 A), as compared with that in PLV with sTn (100% with 2 mg/ml; Fig. 1 A). Moreover, 40% of the skeletal Tn subunits reconstituted in PLV were exchanged for cardiac isoforms upon cTn.

sTn increased Ca$^{2+}$ sensitivity of force (Fig. 1) and submaximal $k_t$ (Fig. 3) in cardiac muscle. Upon sTn reconstitution, $k_t$ became more sensitive to Ca$^{2+}$ than force, and the active force–$k_t$ relationship was shifted upward. These results indicate that sTn accelerates cross-bridge cycling in cardiac muscle. $k_t$ is the sum of the apparent rate of cross-bridge attachment ($f_{app}$) and detachment ($g_{app}$) (Brenner, 1988). Therefore, it can increase due to an increase in either $f_{app}$ or $g_{app}$, or both. Considering, however, that sTn increased Ca$^{2+}$ sensitivity of force, the increase in $k_t$ is likely related to the relative increase in $f_{app}$, resulting in an increase in the fraction of strongly bound cross-bridges. We therefore conclude that sTn shifts the equilibrium of the thin filament state toward the “on” state and subsequently reduces the fraction of recruitable cross-bridges, resulting in blunted length-dependent activation, with a magnitude similar to that in skeletal muscle. This view is supported by the result of Fig. 2 that cTn decreased Ca$^{2+}$ sensitivity of force and enhanced length-dependent activation in skeletal muscle.

The reconstitution ratio was lower in rabbit psoas fibers with cTn (~50% with 6 mg/ml; Fig. 2 A), as compared with that in PLV with sTn (~100% with 2 mg/ml; Fig. 1 A). Moreover, ~40% of the skeletal Tn subunits reconstituted in PLV were exchanged for cardiac isoforms upon cTn.

Table II

Summary of the Values of Passive Force, Maximal Active Force, pCa$^{50}$, ΔpCa$^{50}$, and nH in cTn-reconstituted Rabbit Psoas Muscle

| SL    | Passive force | Maximal force | pCa$^{50}$ | ΔpCa$^{50}$ | nH   |
|-------|---------------|---------------|------------|-------------|------|
| μm    | mN/mm$^2$     | mN/mm$^2$     |            |             |      |
| Fig. 2 C |
| Rabbit psoas (+cTn) | 1.9 | ~0 | 137.61 ± 8.67$^b$ (167.24 ± 13.15$^b$) | 5.66 ± 0.04$^a$ | 2.97 ± 0.34$^a$ |
| 2.3 | 4.72 ± 0.82$^c$ | 183.43 ± 10.81$^b$ | 5.89 ± 0.05$^c$ | 0.24 ± 0.02$^c$ | 2.23 ± 0.13$^b$ |

Passive force was obtained at the steady state. Numbers in parentheses indicate maximal force values obtained prior to cTn reconstitution (P < 0.05 compared with corresponding values obtained after cTn reconstitution). nH decreased upon cTn reconstitution (see Table I), consistent with previous studies (Piroddi et al., 2003; de Tombe et al., 2007).

$^a$P < 0.05 compared with corresponding values for control rabbit psoas muscle (Table I).

$^b$P < 0.05 compared with corresponding values for control PLV (Table I).
treatment (Fig. 1 A), whereas the cardiac Tn subunits reconstituted in rabbit psoas muscle were almost completely replaced by skeletal isoforms upon sTn treatment (Fig. 2 A). These results indicate that cTn has a lower binding affinity to both cardiac and skeletal thin filaments than sTn. By using rabbit psoas myofibrils, Piroddi et al. (2003) and de Tombe et al. (2007) achieved higher levels of reconstitution with cTn (~100% in Piroddi et al., 2003, and ~90% in de Tombe et al., 2007), and reported that Ca\(^{2+}\) sensitivity of force increased, which, as the authors acknowledge, contradicts the intrinsic characteristics of cardiac vs. skeletal muscle. The effect of cTn reconstitution on Ca\(^{2+}\) sensitivity of force in skeletal muscle likely varies with the protocol. In the present study, we treated single fibers in Ca\(^{2+}\)-free rigor solution containing BDM for a relatively short period (i.e., 1 h) to achieve partial reconstitution (~50%), and, therefore, the abnormal on-off switching of the thin filament state that occurs possibly due to the damage upon reconstitution may have been avoided.

TnI directly modulates the thin filament state, in association with TnT and Tm (Solaro and Rarick, 1998). Indeed, it has been reported that Ca\(^{2+}\) sensitivity of force is increased and length-dependent activation is blunted in hearts of transgenic mice expressing the slow skeletal TnI isoform (Arteaga et al., 2000; Konhilas et al., 2003). More recently, Tachampa et al. (2007) reported that a substitution of threonine for proline at position 144 in the inhibitory region of cTnI blunted length-dependent activation, via an unsolved mechanism. On the other hand, our results indicate that cardiac Tn reconstituted in rabbit psoas muscle was almost completely replaced by skeletal isoforms upon sTn treatment. These findings suggest that the protocol used in our study may be more effective in achieving partial reconstitution with cTn, which could be useful in future studies.

**Table III**

| SL (μm) | Passive force (mN/mm\(^2\)) | Maximal force (mN/mm\(^2\)) | pCa\(_{50}\) | pCa\(_{50}\) change (ΔpCa\(_{50}\)) | nH |
|---------|-----------------|-----------------|----------|-----------------|-----|
| 1.9     | Control PLV     | 57.52 ± 1.92    | 5.56 ± 0.01 | 4.33 ± 0.19    |     |
| 2.3 (high PF) | 14.50 ± 1.04\(^a\) | 82.23 ± 3.40\(^a\) | 5.89 ± 0.01\(^a\) | 0.33 ± 0.01\(^a\) | 3.77 ± 0.19 |
| PLV (+sTn) | 56.33 ± 1.23 (59.21 ± 3.36) | 5.80 ± 0.03\(^b\) | 3.76 ± 0.08 |
| 2.3 (high PF) | 15.45 ± 0.83\(^c\) | 68.53 ± 2.34\(^c\) | 6.01 ± 0.02\(^c\) | 0.21 ± 0.01\(^c\) | 3.84 ± 0.41 |

Passive force was obtained ~10 s after increasing SL to 2.3 μm. Numbers in parentheses indicate maximal force values obtained prior to sTn reconstitution (P > 0.05 compared with corresponding values obtained after sTn reconstitution).\(^a\)P < 0.05 compared with corresponding values obtained after sTn reconstitution. \(^b\)P < 0.05 compared with corresponding values obtained in experiments under steady-state passive force (Table I). \(^c\)P < 0.05 compared with corresponding values for control PLV at high passive force (high PF).
hand, isoform switching of either TnC (Moss et al., 1991; McDonald et al., 1995) or TnT (Chandra et al., 2006) reportedly does not alter length-dependent activation. Therefore, it is reasonable to conclude that the attenuation of length-dependent activation by sTn in PLV may primarily result from the replacement of TnI from cardiac to skeletal isoform.

Consistent with the previous reports (Cazorla et al., 2001; Fukuda et al., 2001b, 2003), length-dependent activation was enhanced with an increase in passive force in both control and sTn-reconstituted muscles, and the length dependence linearly correlated with titin-based passive force (Fig. 4). Currently, the role of interfilament lattice spacing in length-dependent activation is under controversy; some groups have published papers that support the lattice spacing hypothesis (e.g., McDonald and Moss, 1995; Fuchs and Wang, 1996; Fukuda et al., 2000), but the de Tombe group has reported that Ca\(^{2+}\) sensitivity of force and the lattice spacing are not well correlated (e.g., Konhilas et al., 2002a, b, 2003). More recently, Pearson et al. (2007) successfully showed in vivo that the lattice spacing plays a pivotal role in the regulation of ventricular pressure (see also Solaro, 2007). The aim of the present study was not to test the lattice spacing hypothesis; however, the result of Fig. 4 is consistent with the notion that titin-based passive force promotes actomyosin interaction, such as by regulating the lattice spacing (Cazorla et al., 2001; Fukuda et al., 2003) and/or by straining the thick filament (Fukuda et al., 2001b), regardless of the thin filament state. Hence, we consider that titin-based passive force operates as a triggering factor in length-dependent activation.

It has been reported that length-dependent activation is attenuated in myocardium from the failing human heart (Morano et al., 1994; Schwingler et al., 1994). Therefore, the importance of the present study is further highlighted by the reports on Tn mutations (e.g., Harada and Morimoto, 2004), as well as by those on changes in titin isoform expression (e.g., Granzier and Labeit, 2004), in heart failure, since our findings indicate that the attenuation of length-dependent activation in failing myocardium may be due, at least in part, to the changes in Tn and/or titin. Future studies should be directed to elucidating the relations between length-dependent activation and sarcomere protein expression changes in various modes of heart failure.

In conclusion, Tn and titin regulate the Frank-Starling mechanism of the heart in a coordinated fashion, such that the fraction of cross-bridges formed via titin-based passive force varies in a Tn isoform-dependent manner.

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