Sarcomere length-dependent Ca$^{2+}$ activation in skinned rabbit psoas muscle fibers: coordinated regulation of thin filament cooperative activation and passive force

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Abstract In skeletal muscle, active force production varies as a function of sarcomere length (SL). It has been considered that this SL dependence results simply from a change in the overlap length between the thick and thin filaments. The purpose of this study was to provide a systematic understanding of the SL-dependent increase in Ca$^{2+}$ sensitivity in skeletal muscle, by investigating how thin filament “on–off” switching and passive force are involved in the regulation. Rabbit psoas muscles were skinned, and active force measurements were taken at various Ca$^{2+}$ concentrations with single fibers, in the short (2.0 and 2.4 μm) and long (2.4 and 2.8 μm) SL ranges. Despite the same magnitude of SL elongation, the SL-dependent increase in Ca$^{2+}$ sensitivity was more pronounced in the long SL range. MgADP (3 mM) increased the rate of rise of active force and attenuated SL-dependent Ca$^{2+}$ activation in both SL ranges. Conversely, inorganic phosphate (Pi, 20 mM) decreased the rate of rise of active force and enhanced SL-dependent Ca$^{2+}$ activation in both SL ranges. Our analyses revealed that, in the absence and presence of MgADP or Pi, the magnitude of SL-dependent Ca$^{2+}$ activation was (1) inversely correlated with the rate of rise of active force, and (2) in proportion to passive force. These findings suggest that the SL dependence of active force in skeletal muscle is regulated via thin filament “on–off” switching and titin (connectin)-based interfila-
ment lattice spacing modulation in a coordinated fashion, in addition to the regulation via the filament overlap.

Keywords Muscle mechanics · Ca$^{2+}$ sensitivity · Troponin · Titin

Abbreviations
SL Sarcomere length
PLV Porcine left ventricular muscle
cTn Porcine left ventricular troponin
sTn Rabbit fast skeletal troponin

Introduction

Striated muscle, either skeletal or cardiac, exhibits sarco-
mere length (SL) dependence of active force generation. It is well established that this SL dependence involves a mechanism by which Ca$^{2+}$ sensitivity of active force increases upon SL elongation in skeletal and cardiac muscles (e.g., [1, 2]). It is generally thought that skeletal muscle can operate over a broad SL range (i.e., ~1.6 to ~3.6 μm) and indeed displays both ascending and descending limbs of the SL-active force curve, in contrast to cardiac muscle that normally exhibits only the ascending limb (e.g., [2, 3]).

A well-known study by Gordon et al. [3] demonstrated that the SL dependence of active force generation in skeletal muscle is a simple, linear function of the overlap length between the thick and thin filaments. However, the findings of later studies have suggested that a change in the thick–thin filament overlap is not the sole factor underlying the SL dependence, and additional mechanisms are
likewise involved. Allen and Moss [4] reported that a reduction in interfilament lattice spacing plays a pivotal role in SL-dependent Ca\(^{2+}\) activation in the short SL range (≈1.6 to ≈2.5 μm; i.e., on the ascending limb). More recently, Shimamoto et al. [5] extended this finding by using single myofibrils of rabbit psoas muscle and confirmed that a change in the lattice spacing of only ≈1.0 nm causes a dramatic change in active force over a broad range of SL (≈2.2 to ≈3.8 μm), under conditions where active force is elicited not only by Ca\(^{2+}\) but also by MgADP (and subsequent formation of strongly bound, actomyosin–ADP cross-bridges via thin filament cooperative activation).

Unlike in cardiac muscle where SL-dependent Ca\(^{2+}\) activation forms the basis for the Frank–Starling law of the heart (e.g., [6] and references therein), the physiological significance of the length dependence in skeletal muscle is still not fully understood. Recently, however, Llewellyn et al. [7] reported that SL dramatically varies in skeletal muscle (gastrocnemius muscle in mice) in vivo upon electric stimulation, in the relatively long SL range of ≈2.6 to ≈3.0 μm. Given the significance of this previous work, we consider that it is of importance to systematically investigate how active force production varies in response to SL elongation in both the short and long SL ranges, at various thin filament activation levels, because the operating SL range may vary depending on various factors, such as the type of muscle or the animal species.

In the present study, by using skinned single fibers of rabbit psoas muscle, we investigated SL-dependent Ca\(^{2+}\) activation in two different SL ranges (short range, 2.0–2.4 μm, and long range, 2.4–2.8 μm) under conditions where thin filament cooperative activation is varied by the application of MgADP or Pi [8].

Materials and methods

All experiments performed in this study conformed to the Guide for the Care and Use of Laboratory Animals (1996, National Academy of Sciences, Washington, DC, USA). Also, all experiments were performed in accordance with the Guidelines on Animal Experimentation of The Jikei University School of Medicine (Tokyo, Japan).

Preparation of skinned muscle and muscle mechanics

Muscle strips (1–2 mm in diameter and ≈10 mm in length) were dissected from psoas muscle of white rabbits (2–3 kg) in Ca\(^{2+}\)-free Tyrode’s solution containing 30 mM 2,3-butanedione monoxime (BDM) (as in [8–11]). Rabbit psoas muscles were skinned in relaxing solution [5 mM MgATP, 40 mM BES, 1 mM Mg\(^{2+}\), 10 mM EGTA, 1 mM dithiothreitol, 15 mM phosphocreatine, 15 U/ml creatine phosphokinase, 180 mM ionic strength (adjusted by K-propionate), pH 7.0] containing 1% (w/v) Triton X-100 and 10 mM BDM overnight at ≈3°C. Muscles were stored for up to 3 weeks at −20°C in relaxing solution containing 50% (v/v) glycerol. All solutions contained protease inhibitors [0.5 mM phenylmethylsulfonyl fluoride (PMSF), 0.04 mM leupeptin, 0.01 mM E64].

Isometric active and passive forces were measured at 15°C, according to the method in our previous studies with single fibers at SL 2.0 and 2.4 μm (or at SL 2.4 and 2.8 μm) [9–11]. Briefly, the single fiber was first immersed in relaxing solution, and SL was set at 2.0 μm. SL was measured by laser diffraction during relaxation. Active and passive forces were measured at SL 2.0 μm, and 20 min after elongation of SL to 2.4 μm (at which time passive force reached the quasi-plateau level), force measurements were repeated, as described in our previous studies [8, 10]: rundown <10% for active and passive forces in all cases. In some experiments, active and passive forces were measured at SL 2.4 and then at 2.8 μm (rundown <10% for active and passive forces in all cases). MgADP or inorganic phosphate (Pi) was added to the individual activating solutions in accordance with our previous studies [8, 12, 13]. When active force was measured, pCa was adjusted by Ca/EGTA [8–14].

Because titin-based passive force reportedly increases from ≈0 nN/mm\(^2\) at SL ≈2.0 μm in an exponential manner in rabbit psoas muscle [10, 15], we used SL 2.0 μm as the control SL in the present study. Also, considering our previous finding that maximal Ca\(^{2+}\)-activated force was greatest at SL ≈2.4 μm in rabbit psoas muscle under the same experimental condition [9], we investigated the effects of MgADP or Pi on SL-dependent Ca\(^{2+}\) activation at SL 2.0–2.4 μm (i.e., on the ascending limb) and 2.4–2.8 μm (i.e., on the descending limb). The difference between the values of the mid-point of the force–pCa curve (i.e., pCa\(_{50}\)) at SL 2.0 (or 2.4) and 2.4 (or 2.8) μm was used as an index of the SL dependence of Ca\(^{2+}\) sensitivity (expressed as ΔpCa\(_{50}\); [8, 10, 12–14]). The steepness of the force–pCa curve was expressed as n_H. At the end of the active and passive force measurement, single fibers were treated with KCl/KI (e.g., [14, 15]), and the ratio of titin-based passive force to total passive force was obtained. Because titin-based (KCl/KI-sensitive) passive force was ≈100% at SL 2.4 and 2.8 μm in all cases (as in [15]), we disregarded the contribution of non-titin-based passive force in the present analysis.

For the measurement of the rate of rise of active force, SL was set at 2.0 μm in relaxing solution. Then, the preparation was immersed in low-EGTA (0.5 mM) relaxing solution for 1 min, and transferred to the control activating solution (pCa 4.5) without MgADP or Pi, followed...
by relaxation. Then, the procedure was repeated in the presence of MgADP or Pi, and the time to half-maximal activation was compared with that obtained in the preceding contraction in the same preparation, hence minimizing the effect of diffusion that is dependent upon the muscle thickness (see [8]). The ratio of the time to half-maximal activation, defined as $t_{1/2}$, was used as an index of cooperative activation [8].

Troponin treatment

Troponin (Tn) treatment was performed based on our previously published procedure (see [8, 10, 11, 16] and references therein). Briefly, after performing the force–pCa protocol without any treatment, the skinned fiber was bathed in rigor solution (10 mM BES, 150 mM K-propionate, 2.5 mM EGTA, 5 mM MgCl$_2$, pH 7.0), containing the whole cardiac troponin complex (cTn, 6 mg/ml; extracted from bovine left ventricular muscle) or the whole skeletal troponin complex (sTn, 2 mg/ml; extracted from rabbit fast skeletal muscle), and 80 mM BDM for 60 min at 25°C. As noted in our previous report [10], the presence of BDM was essential to avoid contraction upon treatment and the ensuing damage to the sarcomere structure. Then, the preparation was washed with normal relaxing solution at 15°C for 10 min with gentle agitation to remove excess Tn molecules. The results of our previous studies demonstrated that the exchange ratio for cTn (or the Tn complex with bovine left ventricular TnT) and sTn (or the Tn complex with rabbit fast skeletal TnT) was $\sim 50$ and $\sim 100\%$, respectively, in skinned rabbit psoas muscle fibers [10, 16].

Statistics

Significant differences were assigned using the paired or unpaired Student’s $t$ test as appropriate. Data are expressed as mean ± SEM, with $n$ representing the number of muscles. Linear regression analyses were performed in accordance with the method used in previous studies [10, 17]. Statistical significance was assumed to be $P < 0.05$. NS indicates $P > 0.05$.

Results

Effect of MgADP or Pi on the rate of rise of active force

First, we investigated the effect of various concentrations of MgADP (0–10 mM) or Pi (0–20 mM) on the rate of rise of active force. We found that MgADP significantly decreased $t_{1/2}$ at low concentrations (1 and 3 mM) but increased $t_{1/2}$ at a high concentration (10 mM), and Pi exerted similar effects at low (3 and 5 mM) and high (20 mM) concentrations (Fig. 1a, b). The accelerating effect of MgADP was greatest at 3 mM, and the decelerating effect of Pi became significant at 20 mM. Therefore, in the following experiments, we used 3 mM MgADP to enhance thin filament cooperative activation and 20 mM Pi to reduce it, based on our previous study on porcine left ventricular muscle (PLV) (cf. [8]).
Effects of MgADP or Pi on SL-dependent Ca$^{2+}$ activation in the short SL range

Next, we investigated the effect of MgADP or Pi on SL-dependent Ca$^{2+}$ activation in the short SL range, i.e., 2.0–2.4 μm. Under the control condition without MgADP or Pi, an increase in SL from 2.0 to 2.4 μm increased maximal Ca$^{2+}$-activated force (as in [9]) and increased Ca$^{2+}$ sensitivity of force by ~0.15 pCa units (i.e., ΔpCa$_{50}$; see Fig. 2a, b; Table 1).

We found that 3 mM MgADP shifted the force–pCa curve leftward to a greater magnitude at SL 2.0 μm than at 2.4 μm, and consequently decreased ΔpCa$_{50}$ (Fig. 2a). Conversely, 20 mM Pi shifted the force–pCa curve rightward to a greater magnitude at SL 2.0 μm than at 2.4 μm, and consequently increased ΔpCa$_{50}$ (Fig. 2b). The effects of 3 mM MgADP or 20 mM Pi on $t_{1/2}$ are summarized in Fig. 2c (the preparations used for Fig. 2a, b). The values of pCa$_{50}$, nH and maximal force at SL 2.0 and 2.4 μm under various conditions are summarized in Table 1.

Consistent with the finding of our previous study [10], cTn treatment shifted the force–pCa curve rightward to a greater magnitude at SL 2.0 μm than at 2.4 μm, and consequently increased ΔpCa$_{50}$ (Fig. 3a). However, sTn treatment did not affect Ca$^{2+}$ sensitivity at either SL. Accordingly, ΔpCa$_{50}$ was not significantly altered by sTn treatment. cTn treatment increased $t_{1/2}$ (as in [10]); however, sTn treatment did not significantly alter it. The values of pCa$_{50}$, nH and maximal force at SL 2.0 and 2.4 μm with and without cTn or sTn treatment are summarized in Table 2.

In Fig. 4, we summarize the relationship between $t_{1/2}$, pCa$_{50}$ and ΔpCa$_{50}$ obtained under various conditions, i.e., in the presence of MgADP or Pi, and cTn or sTn treatment. As found in PLV [8], a significant linear relationship was obtained in each graph, with the slope for rabbit psoas muscle being lower than that for PLV in a relationship of $t_{1/2}$ versus pCa$_{50}$ (Fig. 4b) and that of $t_{1/2}$ versus ΔpCa$_{50}$ (Fig. 4c).

Effects of MgADP or Pi on SL-dependent Ca$^{2+}$ activation in the long SL range

We then investigated the effect of MgADP or Pi on SL-dependent Ca$^{2+}$ activation in the long SL range, i.e., 2.4–2.8 μm. Under the control condition without MgADP or Pi, an increase in SL from 2.4 to 2.8 μm lowered maximal Ca$^{2+}$-activated force (as in [9]) but it increased Ca$^{2+}$ sensitivity of force by ~0.20 pCa units (i.e., ΔpCa$_{50}$; see Fig. 5a; Table 3). This magnitude of increase was significantly greater than that observed when SL was varied between 2.0 and 2.4 μm (Fig. 5a).

As observed in a study in the short SL range, MgADP at 3 mM shifted the force–pCa curve leftward, to a magnitude greater at SL 2.4 μm than at 2.8 μm, and consequently decreased ΔpCa$_{50}$ (Fig. 5b). Conversely, Pi at 20 mM shifted the force–pCa curve rightward, to a magnitude

![Fig. 2](https://example.com/fig2.png)
greater at SL 2.4 μm than at 2.8 μm, thereby increasing ΔpCa50 (Fig. 5c). The relative impact on SL-dependent Ca2+ activation by MgADP or Pi (∼40 and ∼75% for MgADP and Pi, respectively) was similar to that observed in experiments where SL was varied between 2.0 and 2.4 μm. The values of pCa50, nH and maximal force obtained in the absence or presence of MgADP or Pi are summarized in Table 3.

Figure 6 shows the relationship between passive force and the magnitude of SL-dependent Ca2+ activation in the absence and presence of MgADP or Pi, obtained in the short and long SL ranges. ΔpCa50 increased linearly as a function of passive force in the absence and the presence of MgADP or Pi (P < 0.05 in all cases), with the position of the regression line moved downward and upward, respectively, upon application of MgADP and Pi (slope was significantly increased by Pi but it was not significantly changed by MgADP).

Discussion

We demonstrated in the present study that the magnitude of SL-dependent Ca2+ activation in rabbit psoas muscle fibers depends on the level of thin filament cooperative activation in both short and long SL ranges (Figs. 2 and 5). Despite the same magnitude of elongation, SL-dependent Ca2+ activation was more pronounced in the long SL range, with the magnitude in linear proportion to passive force under varying degrees of thin filament cooperative activation (Fig. 6). We discuss these findings focusing on the role of thin filament cooperative activation in the regulation of SL-dependent Ca2+ activation in skeletal muscle.

As reported in our recent work on PLV [8], in the present study, MgADP accelerated the rate of rise of active

Table 1 Summary of the values of passive force, maximal active force, pCa50 and Hill coefficient (nH) in rabbit psoas muscle fibers under various conditions (see Fig. 2)

| SL (μm) | Passive force (mN/mm²) | Maximal force (mN/mm²) | pCa50 | ΔpCa50 | nH |
|---------|------------------------|------------------------|-------|--------|----|
| Control |                        |                        |       |        |    |
| 2.0     | ~0                     | 168.50 ± 7.74          | 5.86 ± 0.01 |        |    |
| 2.4     | 3.58 ± 0.49            | 191.83 ± 8.98          | 6.00 ± 0.01 | 0.15 ± 0.01 | 3.31 ± 0.16 |
| +ADP    |                        |                        |       |        |    |
| 2.0     | ~0                     | 164.07 ± 20.64* (135.53 ± 18.46) | 5.97 ± 0.02<sup>a</sup> |        |    |
| 2.4     | 3.47 ± 0.20            | 184.79 ± 21.68         | 6.06 ± 0.01<sup>a</sup> | 0.09 ± 0.01<sup>a</sup> | 3.30 ± 0.16 |
| +Pi     |                        |                        |       |        |    |
| 2.0     | ~0                     | 85.14 ± 6.69<sup>b</sup> (165.49 ± 15.15) | 5.39 ± 0.01<sup>a</sup> |        |    |
| 2.4     | 3.81 ± 0.27            | 144.23 ± 10.92<sup>b</sup> | 5.66 ± 0.04<sup>a</sup> | 0.27 ± 0.03<sup>a</sup> | 3.93 ± 0.17<sup>b</sup> |

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 ADP or Pi application (* P < 0.05 compared with the prior value). Upon SL elongation from 2.0 to 2.4 μm, maximal force was significantly (P < 0.05) increased in all groups, due presumably to a change in the filament overlap (e.g., [5, 9]).

<sup>a</sup> P < 0.05 compared with the corresponding values in the control group (no ADP or Pi)
force at low concentrations (1 and 3 mM), but MgADP decelerated it at a high concentration (10 mM) in rabbit psoas muscle fibers (Fig. 1). We consider that, at low MgADP concentrations, the actomyosin-ADP complex promotes cross-bridge attachment via enhanced thin filament cooperative activation (similar to the effect of N-ethylmaleimide-subfragment 1; e.g., [18, 19]), while at high MgADP concentrations, large fractions of the complex cause slowing of contraction operating as a dragging force (see [8] and references therein). On the other hand, Pi at low concentrations has been reported to decrease the fraction of the slowly cycling cross-bridges, resulting in acceleration of the rate of rise of active force (e.g., [8, 20]). However, as demonstrated previously by us in PLV, the application of high concentrations of Pi (e.g., 20 mM) is likely to decrease the fraction of strongly bound cross-bridges below a certain threshold value, resulting in slowing of contraction via reduced thin filament cooperative activation (Fig. 1). Therefore, under the present experimental settings, we consider that 3 mM MgADP enhances thin filament cooperative activation while 20 mM Pi reduces it in rabbit psoas muscle fibers. Here, one may point out that an alteration of thin filament cooperative activation is likely to change the \( n_H \) of the force–pCa curve, but no significant correlation existed between the \( n_H \) and \( \Delta pC_{a50} \) in the present study (Supplementary Fig. 1; see also [8, 21] for cardiac muscle). However, it is still unknown whether or to what extent the \( n_H \) reflects thin filament cooperative activation (as discussed in detail in [8]). We therefore consider that, in skeletal muscle, as well as in cardiac muscle [8], \( t_{1/2} \) more precisely reflects a sensitive change in thin filament cooperative activation than the \( n_H \) of the force–pCa curve.

It has been considered that a change in the overlap between the thick and thin filaments (or a change in the double-overlap between these filaments in the short SL.

### Table 2

Summary of the values of passive force, maximal active force, pCa50 and Hill coefficient (\( n_H \)) in rabbit psoas muscle fibers under various conditions (see Supplementary Fig. 1)

| SL (\( \mu m \)) | Passive force (mN/mm²) | Maximal force (mN/mm²) | pCa50 | \( \Delta pC_{a50} \) | \( n_H \) |
|-----------------|------------------------|------------------------|-------|----------------------|--------|
| Control 2.0     | ~0                     | 168.50 ± 7.74          | 5.86 ± 0.01 | 4.12 ± 0.12        |
| 2.4             | 3.58 ± 0.49            | 191.83 ± 8.98          | 6.00 ± 0.01 | 0.15 ± 0.01        | 3.31 ± 0.16 |
| +cTn 2.0        | ~0                     | 145.24 ± 5.88* (173.66 ± 8.49) | 5.64 ± 0.04^a | 3.02 ± 0.30^a    |
| 2.4             | 3.19 ± 0.91            | 186.12 ± 7.63          | 5.86 ± 0.04^a | 0.22 ± 0.02^a     | 2.19 ± 0.10^a |
| +sTn 2.0        | ~0                     | 171.04 ± 6.44 (178.06 ± 7.59) | 5.88 ± 0.01 | 0.15 ± 0.01        | 3.41 ± 0.18  |
| 2.4             | 3.78 ± 0.15            | 192.40 ± 7.50          | 6.03 ± 0.01 | 0.15 ± 0.01        | 3.41 ± 0.18  |

Values in the control group are the same as in those in Table 1. 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 cTn or sTn treatment (* \( P < 0.05 \) compared with the prior value). Upon SL elongation from 2.0 to 2.4 \( \mu m \), maximal force was significantly \( (P < 0.05) \) increased in both cTn and sTn groups, due presumably to a change in the filament overlap (e.g., [5, 9]).

\( ^a P < 0.05 \) compared with the corresponding values in the control group.

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Fig. 4 Linear regression analyses between \( t_{1/2} \), pCa50 and \( \Delta pC_{a50} \).

- **A** Relationship between pCa50 and \( \Delta pC_{a50} \) \((R = 0.98, P < 0.0005)\).
- **B** Relationship between \( t_{1/2} \) and pCa50 \((R = 0.95, P < 0.05)\).
- **C** Relationship between \( t_{1/2} \) and \( \Delta pC_{a50} \) \((R = 0.97, P < 0.01)\). In (a–c), plots were constructed using the data in Figs. 2 and 3. pCa50 obtained at SL 2.0 \( \mu m \). Dashed lines in (a–c) data obtained with PLV in our previous study [8] under the same experimental condition with SL varied between 1.9 and 2.3 \( \mu m \). The values of pCa50 and \( t_{1/2} \) were obtained at the slack SL (1.9 \( \mu m \)) for PLV.
range) underlies the basis for SL-dependent Ca\(^{2+}\) activation in skeletal muscle (e.g., [3]). We found in the present study that, in addition to the regulation via the filament overlap (as indicated by SL-dependent changes in maximal force; see Tables 1, 2 and 3), SL-dependent Ca\(^{2+}\) activation in skeletal muscle is influenced by thin filament cooperative activation (Fig. 4) and passive force (Fig. 6). Previously, by using PLV, we provided experimental as well as mathematical evidence that at high thin filament cooperative activation states, cross-bridge recruitment upon lattice spacing reduction due to titin extension becomes less pronounced due to a decrease in the fraction of ''recruitable'' cross-bridges (that have ATP and therefore can potentially produce active force), resulting in the attenuation of SL-dependent Ca\(^{2+}\) activation [8]. Therefore, the inverse correlation between the rate of rise of active force and \(D_{pCa50}\) (Fig. 4c) obtained by varying the level of thin filament cooperative activation, directly by Tn treatment or indirectly by application of MgADP or Pi, suggests that SL-dependent Ca\(^{2+}\) activation is regulated via "on–off" switching of the thin filament state in rabbit psoas muscle fibers, as in PLV [8].

The present findings (Figs. 2, 5) are consistent with the data obtained in PLV, suggesting that SL-dependent Ca\(^{2+}\) activation is modulated via thin filament cooperative activation in skeletal muscle, in coordination with titin-based lattice spacing modulation. The greater dependence of passive force in the presence of Pi (i.e., greater slope value in Fig. 6) is likely to suggest that cross-bridge recruitment via titin-based lattice spacing reduction is enhanced at low cooperative activation states, due to an increase in the fraction of "recruitable" cross-bridges (as demonstrated previously in cardiac muscle; see [8, 13]). Given the recent findings that the working SL range of skeletal muscle is relatively long in vivo (i.e., \(\sim 2.6\) to \(\sim 3.0\) \(\mu m\) in gastrocnemius muscle of mice, and \(\sim 3.0\) to \(\sim 3.4\) \(\mu m\) in extensor digitorum muscle of humans) [7], the greater SL-dependent Ca\(^{2+}\) activation in the longer SL range (Fig. 5; Table 3) may be a compensatory mechanism for the decrease in the filament overlap and subsequent reduction in active force. This possible compensatory effect may be of particular physiological significance under low force-
because the enhanced SL-dependent Ca$^{2+}$ reported that passive force of several mN/mm$^2$ reduces the concentration increases; see [11] and references therein), generating states during fatigue (where the myoplasmic Pi activation (Fig. 6). Relationship between passive force and SL-dependent Ca$^{2+}$ just prior to activation at pCa 4.5). Numbers in parentheses indicate maximal force values obtained prior to ADP or Pi application (*P < 0.05 compared with the prior value). Upon SL elongation from 2.4 to 2.8 μm, maximal force was significantly (P < 0.05) decreased in all groups, due presumably to a change in the filament overlap (e.g., [5, 9]).

$^a$ P < 0.05 compared with the corresponding values in the control group (no ADP or Pi)

generating states during fatigue (where the myoplasmic Pi concentration increases; see [11] and references therein), because the enhanced SL-dependent Ca$^{2+}$ activation may have an effect to minimize the reduction in active force upon stretch at Ca$^{2+}$ concentrations below the saturating level.

Konhilas et al. [22] demonstrated by using X-ray diffraction that an increase in SL from ~2.0 to ~4.0 μm linearly reduces the lattice spacing in both fast and slow skeletal muscles of the rat. More recently, Irving et al. [15] reported that passive force of several mN/mm$^2$ reduces the lattice spacing linearly with no significant effect on myosin mass transfer from the thick filament backbone to thin filaments in rabbit psoas muscle fibers, over the range of SL from 2.0 to 3.4 μm. In the present study, we found that the magnitude of SL-dependent Ca$^{2+}$ activation was more pronounced in the long SL range than in the short SL range in the absence and the presence of MgADP or Pi (Figs. 2 and 5). We therefore consider that, in addition to the regulation via the filament overlap, titin-based passive force and subsequent lattice spacing reduction plays a pivotal role in regulating SL-dependent Ca$^{2+}$ activation in skeletal muscle, with the magnitude depending on the state of the thin filaments (Fig. 6).

It was found that the slope of the linear regression line in the relationship of $t_{1/2}$ versus ΔpCa$_{50}$ was ~40% less in skeletal muscle than in cardiac muscle obtained under the same experimental condition (Fig. 4c; cf. [8]). This suggests that the dependence on thin filament cooperative activation in setting the magnitude of SL-dependent Ca$^{2+}$ activation is ~40% less in skeletal muscle than in cardiac muscle. Likewise, the lesser slope value for skeletal muscle in the relationship of $t_{1/2}$ versus pCa$_{50}$ (~30%; Fig. 4b) compared to that for cardiac muscle suggests that the positive feedback effect on Ca$^{2+}$-binding to TnC via cross-bridge formation due to enhanced thin filament cooperative activation is ~30% less in skeletal muscle than in cardiac muscle. Therefore, these findings may in part account for the well-known phenomenon that SL-dependent Ca$^{2+}$ activation is less in skeletal muscle than in cardiac muscle (cf. [6, 8, 10]).

In conclusion, our findings suggest that, in addition to the regulation via the filament overlap, SL-dependent Ca$^{2+}$ activation in skeletal muscle is regulated via thin filament cooperative activation and titin-based lattice spacing modulation in a coordinated fashion. This is an area of future research to investigate whether and how the regulation is altered in sarcomeric disease, such as in various forms of skeletal muscle atrophy (e.g., [9, 11] and references therein).

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| SL (μm) | Passive force (mN/mm$^2$) | Maximal force (mN/mm$^2$) | pCa$_{50}$ | ΔpCa$_{50}$ | n$_H$
|--------|----------------|----------------|----------|------------|---
| Control | 2.4 3.68 ± 0.36 | 189.33 ± 12.16 | 5.98 ± 0.01 | 3.57 ± 0.33 |
| 2.8 8.65 ± 0.47 | 172.17 ± 9.82 | 6.18 ± 0.01 | 0.20 ± 0.01 | 4.64 ± 0.10 |
| +ADP | 2.4 3.68 ± 0.41 | 198.87 ± 7.68* (180.38 ± 9.45) | 6.17 ± 0.02$^a$ | 3.45 ± 0.09 |
| 2.8 9.72 ± 0.63 | 183.87 ± 9.13 | 6.29 ± 0.02$^a$ | 0.12 ± 0.01$^a$ | 4.30 ± 0.14 |
| +Pi | 2.4 3.42 ± 0.30 | 133.17 ± 9.35* (196.67 ± 4.59) | 5.61 ± 0.02$^a$ | 2.89 ± 0.26 |
| 2.8 8.68 ± 0.69 | 118.50 ± 8.99$^a$ | 5.99 ± 0.03$^a$ | 0.34 ± 0.02$^a$ | 3.65 ± 0.18$^a$ |

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 ADP or Pi application ($^*P < 0.05$ compared with the prior value). Upon SL elongation from 2.4 to 2.8 μm, maximal force was significantly ($P < 0.05$) decreased in all groups, due presumably to a change in the filament overlap (e.g., [5, 9]).

$^a$ P < 0.05 compared with the corresponding values in the control group (no ADP or Pi)
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