Mechanisms of enhanced force production in lengthening (eccentric) muscle contractions

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Herzog W. Mechanisms of enhanced force production in lengthening (eccentric) muscle contractions. J Appl Physiol 116: 1407–1417, 2014. First published February 21, 2013; doi:10.1152/japplphysiol.00069.2013.—In contrast to isometric and shortening contractions, many observations made on actively lengthening muscles cannot be readily explained with the sliding filament and cross-bridge theory. Specifically, residual force enhancement, the persistent increase in force following active muscle lengthening, beyond what one would expect based on muscle length, has not been explained satisfactorily. Here, we summarize the experimental evidence on residual force enhancement, critically evaluate proposed mechanisms for the residual force enhancement, and propose a mechanism for residual force enhancement that explains all currently agreed upon experimental observations. The proposed mechanism is based on the engagement of the structural protein titin upon muscle activation and an increase in titin’s resistance to active compared with passive stretching. This change in resistance from the passive to the active state is suggested to be based on 1) calcium binding by titin upon activation, 2) binding of titin to actin upon activation, and 3) as a consequence of titin-actin binding—a shift toward stiffer titin segments that are used in active compared with passive muscle elongation. Although there is some experimental evidence for the proposed mechanism, it must be stressed that much of the details proposed here remain unclear and should provide ample research opportunities for scientists in the future. Nevertheless, the proposed mechanism for residual force enhancement explains all basic findings in this area of research.

residual force enhancement; titin; cross-bridge theory; eccentric contractions

MUSCLE CONTRACTIONS ARE DIVIDED into constant length (isometric), shortening (concentric), and lengthening (eccentric) contractions. Whereas the mechanics of isometric and shortening contractions are well described by the currently accepted molecular mechanisms of muscle contraction—the sliding filament (51, 53, 55) and cross-bridge theories (49, 52, 54, 109)—the mechanics of lengthening contractions are not (49, 50, 121). A. F. Huxley (49) realized this in his first description of the cross-bridge theory, when realizing that the maximal force during lengthening contractions was 5.33 times the maximal isometric force, F0, which was much greater than that typically observed experimentally (1.8 × F0). Also, the heat liberation in lengthening muscles was predicted to be much bigger than that observed experimentally (45, 49). However, Huxley made suggestions on how these discrepancies between the cross-bridge model and experimental observation could be eliminated, at least qualitatively, by assuming, for example, for the excess heat generation, that the cross-bridge bonds in lengthening contractions were not detached by chemical reactions but by mechanical “breaking” (49).

Another shortcoming of the cross-bridge theory that has not been rectified to date is the inability to predict the persistent increase in force of an actively lengthened muscle compared with the force under the same conditions but in the absence of active lengthening (121): the so-called “residual force enhancement” (22). Residual force enhancement had been observed well before the development of the initial cross-bridge theory (1), and thus its absence from cross-bridge thinking is an oversight. In his book, “Reflections on Muscle,” Huxley (50) remarks on the residual force enhancement and lengthening muscle by saying, “I imagine that special features have been evolved which allow this elongation (of muscles) to take place without damaging the muscle,” and “I suspect that many of the unexplained phenomena, such as those I have just described here (associated with muscle lengthening) are related to these special features, and have little relation to the processes that take place during shortening”. In this review, I will focus on these unexplained phenomena during muscle lengthening, with emphasis on residual force enhancement, and the features that have evolved in muscles to help explain the molecular mechanisms of force production in actively lengthening muscles.
MECHANISMS OF CONTRACTION

To understand why the sliding filament-based cross-bridge theory cannot explain residual force enhancement, we need to briefly review the current thinking on the molecular mechanisms of contraction. In 1953, Hanson and Hugh Huxley (31) suggested that contraction of muscles occurred by the sliding of two sets of filaments: the thick or myosin-based and the thin or actin-based filaments. This idea was supported in a set of twin papers in *Nature* by Andrew Huxley and Niedergerke (51) and Hugh Huxley and Hanson (55), providing overwhelming evidence that muscle contraction did not occur by a shortening of the thick filaments, as had been assumed up to that time (101). Andrew Huxley then provided a first mathematical description of how this sliding of myofilaments was powered (49). He proposed that the thick myosin filament contained side pieces—cross bridges—that cyclically attached to binding sites on the thin actin filaments and that the cross bridges pulled the actin past the myosin filaments, thereby producing shortening and force. Each cross-bridge attachment/detachment cycle was powered by the hydrolysis of one ATP. One of the consequences of this theory is that the maximal isometric force of a muscle only depends on muscle (sarcomere) length—greatest when actin and myosin filaments overlap completely and when a maximal number of cross-bridge attachments are possible and becoming zero at sarcomere lengths when myofilament overlap ceases to exist (25). However, experimental observations over the past half-century show consistently that the steady-state isometric force of a muscle not only depends on muscle length (for a given level of activation) but also depends on its history of contraction—greater if a muscle is actively stretched and smaller if a muscle is actively shortened prior to the isometric contraction (4, 10, 12, 14, 16, 17, 19, 20, 22, 23, 27, 30, 35, 36, 36a, 36b, 42–44, 47, 59, 60, 60a, 61, 62, 65, 66, 69–73, 75–77, 82–84, 89, 95, 97, 98, 102–104, 106, 107, 110, 111, 113, 115, 117, 119, 120). This history-dependent behavior of muscle contraction cannot be predicted with the cross-bridge theory, primarily because of the assumption that different cross-bridge states are connected by rate constants that are exclusively dependent on the relative position of a cross bridge’s equilibrium location and its corresponding, nearest feasible attachment site (121). Therefore, we are left with two conclusions: either the cross-bridge theory is not correct and needs replacement, or the cross-bridge theory is generally correct but needs amendment to account for the mechanical properties of muscles during and after active lengthening. We favor the second of these possibilities and will argue so below by demonstrating that forces during and following active lengthening are not only governed by actin-myosin-based cross-bridge forces but also by forces arising from structural proteins, whose resistance to stretch changes with activation and force production.

**ENHANCED FORCE DURING AND FOLLOWING LENGTHENING CONTRACTIONS**

Residual force enhancement refers to the observation that the steady-state isometric force of a muscle is greater following active muscle lengthening compared with the corresponding force following a purely isometric reference contraction (Fig. 1). Residual force enhancement was first described systematically in 1952 (1) and has been observed consistently in muscle (e.g., refs. (1, 47, 91)], single-fiber (e.g., refs. (21, 22, 70, 108, 115), myofibril (e.g., refs. (60–62), and single-sarcomere preparations (76). Force enhancement increases with increasing lengthening magnitude (e.g., refs. (1, 12, 22)), at least up to a certain amount of stretch (47), is independent (or nearly so) of the speed of lengthening (e.g., refs. (1, 22)), is reduced when active shortening of muscles is preceded by active shortening (37, 75), is greater on the descending than the ascending limb of the force-length relationship (91, 102), is associated with a substantial decrease in metabolic energy requirement per unit of force (58), is highly correlated with the transient force at the end of active lengthening (12), and is comprised, at least in part, of a passive component—the so-called passive force enhancement (36a)—which is not eliminated instantaneously.

**Fig. 1.** Force enhancement (FE) and passive FE (PFE) in skeletal muscles following active elongation. A: cat soleus muscle stretched by 3, 6, and 9 mm, showing increased peak forces after stretch, increased FE, and increased PFE with increasing magnitudes of stretch. ∆ Muscle Length, change in muscle length. B: single-myofibril preparation from rabbit psoas, showing PFE. C: isolated single sarcomere from rabbit psoas muscle showing FE and forces in the enhanced state that clearly exceed the isometric force at optimal sarcomere length (O-FE; 2.4 μm) preceding active lengthening O-FE [Borrowed with permission from Ref. (34)].
when muscles are deactivated (39, 44). The passive force enhancement also increases with increasing magnitudes of muscle lengthening and for increasing muscle lengths and can be abolished instantaneously by shortening the muscle from its stretched to its original length (39, 44).

Despite this abundance of consistent observations on residual force enhancement and the general agreement on the properties associated with force enhancement, there is great debate on the molecular mechanisms responsible for residual force enhancement (19, 35, 36c, 89).

**MECHANISMS UNDERLYING FORCE ENHANCEMENT**

A number of mechanisms have been proposed to explain the molecular workings of residual force enhancement following active muscle lengthening. Some of these are based on the framework of the cross-bridge theory. For example, it has been suggested that force enhancement might be caused by a stress-dependent decrease in the rate of cross-bridge detachment from actin, thereby increasing the proportion of attached cross bridges following active muscle lengthening compared with isometric reference contractions. This increase in the proportion of attached cross bridges would then be associated with the increased force observed in the enhanced state (83). Other mechanisms are not based on the cross-bridge theory. For example, the idea that force enhancement is caused by the engagement of a passive structural element upon activation involves a mechanism of force production during active lengthening that does not rely on actin-myosin-based cross-bridge force [e.g., ref. (96)]. Below, we will discuss the major mechanisms associated with force enhancement and will attempt to explain the advantages and limitations of each of the proposals.

**Cross-Bridge Theory-Based Mechanisms of Force Enhancement**

Nonuniformity based on instability. Arguably, the most frequent explanation for residual force enhancement has been the so-called “sarcomere-length nonuniformity theory.” This theory is based on the idea that muscle segments and sarcomeres are mechanically unstable on the descending limb of the force-length relationship, an idea first promoted by A. V. Hill in 1953 (46). Therefore, a muscle stretched on the descending limb of the force-length relationship would produce great elongations in some parts of the muscle and little elongation in other parts (Fig. 2). The parts with little elongation would contain sarcomeres with greater myofilament overlap than an average sarcomere during a purely isometric reference contraction (where sarcomeres are assumed to remain relatively uniform in length) and thus would be able to produce more active force. The parts of the muscle subjected to great lengthening were thought to compensate for the lack of active force by increased passive forces (Fig. 2). Therefore, the enhanced forces following active muscle lengthening would be achieved with increased active force in some parts of the muscle and increased passive force in the overstretched parts of the muscle (63, 86, 87, 89, 91). The advantage of this theory is that it can explain, at least theoretically, force-enhanced states of muscles within the framework of the cross-bridge theory. However, its disadvantage is that ~60% of the working range of a muscle (the entire descending limb of the force-length relationship) would be unstable, thus producing unpredictable results and causing sarcomeres to be overstretched to a degree where they would be considered damaged (90, 116), and one might ask the question: why would the most basic contractile machinery of animal movement evolve in a way that 60% of its working range was unstable and prone to injury by overstretching sarcomeres?

The sarcomere-length nonuniformity theory relies on the idea that serially arranged sarcomeres are unstable on the descending limb of the force-length relationship (46) and based on its mathematical representation, predicts that at steady state, force enhancement cannot occur on the ascending limb of the force-length relationship, and force in the enhanced state cannot exceed the maximal isometric force obtained at optimal sarcomere length (22, 23, 87). Below, we will review the evidence for these assumptions and predictions of the sarcomere-length nonuniformity theory.

**Sarcomere instability.** Initial testing of the idea of sarcomere instability on the descending limb of the force-length relationship involved active lengthening of muscle and fiber preparations, fixing them “instantaneously” in the stretched state, and comparing the amount of overstretched sarcomeres following active lengthening with the amount observed following purely isometric or shortening contractions (5, 9, 88, 100). Results of such experiments typically demonstrated an increased proportion of overstretched (popped) sarcomeres compared with reference contractions not involving muscle lengthening. An advantage of these experiments is that sarcomeres are tested in their native environment, with sarcomere structures fully intact and structural proteins providing support (19). A limitation of these experiments is that individual sarcomere dynamics could not be observed directly, and damage to overstretched regions of muscles and fibers could not be uniquely associated with sarcomere instability, rather than, for example, a structural
weakness of the muscle/fiber at the location of sarcomere disruption.

In contrast to single-fiber and muscle preparations, isolated myofibrils represent serially arranged sarcomeres, stripped of all supporting features, except those imbedded in the sarcomeres themselves. Furthermore, individual sarcomere lengths can be measured continuously during lengthening, and the forces measured at the end of the myofibril represent the instantaneous, dynamic forces transmitted by each sarcomere [e.g., ref. (6)], neglecting the small, inertial forces of a sarcomere compared with the much greater contractile forces. When stretching activated myofibrils on the descending limb of the force-length relationship, individual sarcomeres (107) and half-sarcomeres all elongate but to different degrees (60). On the surface, this might be interpreted as support of sarcomere-length instability. However, force in all myofibrils increased with stretch on the descending limb of the force-length relationship, thereby providing a positive restoring (stabilizing) force in all sarcomeres and half-sarcomeres (60). Furthermore, when myofibrils were held in the lengthened position to observe sarcomere dynamics following lengthening, sarcomeres remained at steady lengths and were never stretched quickly beyond actin-myosin filament overlap, as predicted by the nonuniformity theory (60, 107). Finally, careful measurement of sarcomere and half-sarcomere dynamics in selected myofibril preparations reveals that stretching on the descending limb appears to produce more consistent sarcomere and half-sarcomere lengths compared with purely isometric contractions, because of increased stiffness in the long half-sarcomeres compared with the corresponding short half-sarcomeres (60). This result is also supported by early works on half-sarcomere dynamics in skinned fibers, where half-sarcomere nonuniformities decreased with increasing sarcomere lengths, presumably because of the stabilizing action of titin (48), and work on intact fibers, where segment lengths were found to be more stable after stretch compared with the corresponding isometric reference contractions (22).

In summary, in preparations where the dynamics of sarcomeres and half-sarcomeres can be measured directly, (half-) sarcomeres appear perfectly stable, indicated by the positive restoring force during lengthening and the stable (half-) sarcomere lengths following lengthening (34, 35, 60, 107). This interpretation is also consistent with decreased A-band shifts for increasing sarcomere lengths in fiber preparations and the decreased variation in segment (22), sarcomere (99), and half-sarcomere lengths (60) following active lengthening compared with the corresponding isometric reference contractions, suggesting a stabilizing effect of muscle stretching and a decrease in differences of segmental and (half-) sarcomere lengths.

**Force enhancement on the ascending limb of the force-length relationship.** A crucial prediction of the sarcomere-length nonuniformity theory is that force enhancement cannot occur on the positively sloped, ascending part of the force-length relationship [e.g., refs. (86, 87, 91)]. The positive slope provides stabilizing, restoring forces and thus precludes sarcomere-length instabilities and associated sarcomere-length nonuniformities (34, 35). Nevertheless, even the earliest reports of force enhancement in whole muscle preparations found force enhancement on the “stable,” ascending limb of the force-length relationship (1). This early result on whole muscles was supported by more recent work on cat soleus (91, 111). Whereas the authors of that latest work argued that there was no force enhancement on the ascending limb of the force-length relationship, their data show the opposite result: force enhancement in 14 out of 15 data points shown and a maximum enhancement of ~15% (91). Similarly, a very small but consistent force enhancement on the ascending limb of the force-length relationship has been observed in single-fiber preparations (70, 102), sarcomeres (105), and mechanically isolated half-sarcomeres (60), and although consistently observed in entire muscle and myofibril preparations, this result is not uniquely supported in some intact fiber preparations, where force enhancement on the ascending limb was not observed (21, 22).

In summary, work on whole muscle and myofibril preparations seems to show force enhancement on the ascending limb of the force-length relationship consistently. However, this force enhancement is small compared with that observed on the descending limb of the force-length relationship. Results on single-fiber preparations are mixed, some showing small but consistent force enhancement (70, 102), whereas others do not (21, 22); therefore, the final judgment on this issue cannot be made. Ultimately, the existence of force enhancement on the ascending limb might depend on structural variations between muscles, for example, where on the force-length relationship passive forces become engaged.

**Enhanced forces that exceed the isometric force at optimal sarcomere length.** Probably, the most important prediction of the sarcomere-length nonuniformity theory is that at steady state, forces in the enhanced state cannot exceed the isometric forces at optimal sarcomere length, although theoretical works have shown that very small enhancements above the plateau forces are possible if sarcomere lengths have not reached steady-state conditions [e.g., refs. (13, 116)]. Edman and colleagues (21) were the first to systematically check whether forces in the enhanced state (after active muscle lengthening) exceed the maximal isometric force at optimal length. They initially concluded that “yes,” they did but then reverted their decision with experiments in which the steady-state isometric forces were followed for a longer period of time after completion of muscle lengthening (22). However, in the classic work by Abbott and Aubert (1), the authors suggest that forces in the enhanced state of entire muscles exceed the maximal isometric forces at optimal muscle length. These results are supported by more recent evidence in whole muscle (91, 111), single-fiber (70, 102, 108), myofibril (60), and single-sarcomere preparations (76). Also, results of 12 half-sarcomeres in a single myofibril showed an average force enhancement of 44% above the maximal isometric force at optimal length (60), and findings from 10 isolated sarcomere preparations stretched on the descending limb of the force-length relationship resulted in an average of 37% greater forces in the enhanced state than those obtained for corresponding, purely isometric contractions performed at optimal sarcomere length (76).

In summary, there is strong evidence that given the right lengthening conditions, forces in the enhanced state of muscles, fibers, myofibrils, sarcomeres, and half-sarcomeres can easily exceed the isometric forces obtained at optimal length. The lone dissenting paper, where this issue was analyzed carefully (22), also showed forces after active fiber lengthening in excess of the isometric plateau forces but only by a few percent, and that was interpreted as within the margin of...
show greater sarcomere disruptions than nonstretched control specimens, evidence in single fibers and myofibrils suggests that lengthening reduces variations in segmental (21), sarcomere, and half-sarcomere lengths (48, 60).

Noncross-Bridge Theory-Based Mechanisms of Force Enhancement

With the use of sarcomere-length nonuniformity, force enhancement has been explained within the cross-bridge theory framework for over three decades (e.g., refs. (19, 23, 86, 87)). However, the finding of substantial (37% on average and >50% in isolated cases) force enhancement above the isometric plateau forces in single-sarcomere preparations (76) cannot be explained with increased overlap of myofilaments and associated increase in force in sarcomeres or half-sarcomeres. Similarly, results of 300% increased forces in calcium-activated myofibrils compared with nonactivated myofibrils, pulled beyond actin-myosin filament overlap (Fig. 3), are incompatible with an explanation based on actin-myosin-based cross-bridge forces (78). Therefore, it appears that there are other mechanisms causing the vast majority of the enhanced forces observed following active muscle lengthening.

**Passive force enhancement.** The idea that force enhancement might be associated with the engagement of a passive structural element upon activation has been proposed more than three decades ago (21), and corresponding muscle models of such a mechanism have been described (24). The idea of engagement of a passive element has intuitive appeal, because force enhancement is known to increase with increasing magnitudes of stretch (e.g., refs. (1, 11, 22, 47)) to cause an increase in the subsequent shortening velocity (22) and to be independent of the speed of muscle lengthening (e.g., ref. (21)). However, empirical evidence for such a mechanism was lacking until 2002, when it was discovered in cat soleus muscles that force enhancement had a distinct, passive component [hereafter, called “passive force enhancement” (36a)], which was not abolished upon deactivation (Fig. 1). Subsequent research demonstrated that this passive force enhancement was also contained in single-myofibril preparations (61, 62), suggesting that it was a sarcomeric property. Elimination of titin from sarcomeres abolished the passive force enhancement, indicating that titin might be an important player in (passive) force enhancement (62, 78).

Edman et al. (22) argued that if force enhancement was indeed based on the engagement of a passive structural element upon muscle activation, then shortening a muscle prior to lengthening should eliminate the force enhancement associated with such a passive component. When performing shortening-stretch experiments, force enhancement was the same as that observed for muscle lengthening alone, suggesting that either engagement of a passive element did not exist or that this passive element had to re-engage after the shortening contraction. When repeating shortening-stretch experiments in whole muscle (37, 74) and single-fiber preparations (106), we observed a substantial shortening, magnitude-dependent decrease in force enhancement, in contrast to published results (22). Our experiments had been performed with no break between the shortening and the lengthening contractions, whereas previous experiments contained such a break. When repeating shortening-stretch experiments with breaks of 500 ms and 1,000 ms,
we observed that the effect of shortening on the subsequent lengthening-induced force enhancement diminished (106), indicating that there appears to be an engagement of a passive structural component upon activation, and when shortening precedes lengthening, this component will deduct from the observed force enhancement, except if the break between shortening and lengthening exceeds a critical threshold (~1 s, in our case), and force enhancement becomes identical to that observed without previous shortening. This last result suggests that the passive structural element is disrupted by shortening but given sufficient time, can re-engage and produce normal force enhancement.

In summary, there is evidence from a variety of different mechanical tests on different structural levels that force enhancement is associated with the engagement of a passive structural element. Since passive force enhancement is observed in single myofibrils, and the passive forces in myofibrils are known to originate primarily from the structural protein titin (Fig. 4A) and since elimination of titin function from sarcomeres abolishes all passive force enhancement, it is reasonable to speculate that titin is, at least in part, responsible for the passive and possibly the total force enhancement observed in skeletal muscles (34).

MECHANISM OF FORCE ENHANCEMENT IN SKELETAL MUSCLE

In this last segment, I will propose a possible mechanism for residual force enhancement, based on the engagement of a structural passive component—the giant molecular spring titin. Many of the details of this proposed mechanism need further elucidation, and there is no claim of completeness or exclusiveness of action. However, in view of the fact that substantial (in excess of 50%) force enhancement above the plateau of the force-length relationship has been observed in single sarcomeres (76) and half-sarcomeres (60) and that forces, three times those observed in passive elongations of myofibrils, are obtained by active lengthening of myofibrils to lengths beyond actin-myosin overlap (78), it appears that some passive structural framework is required to explain these results.

Although segmental, sarcomere, and half-sarcomere-length nonuniformities are clearly part of everyday muscle contraction, current evidence suggests that if anything, stretching reduces such nonuniformities on the fiber segment (22), sarcomere (48, 60), and half-sarcomere level (60) compared with corresponding, purely isometric reference contractions. Thus there is no denying that sarcomere-length nonuniformities play a big part in muscle contraction, but they are by no means exclusive to stretches on the descending limb of the force-length relationship and thus may possibly be an associate rather than a cause of residual force enhancement.

If titin is indeed responsible for part of the force enhancement and is “engaged” upon activation, as first suggested more than 30 years ago (21), then this engagement could occur in principally two ways: 1) by increasing titin’s spring stiffness through an increase in its inherent stiffness or 2) by increasing titin’s spring stiffness through a reduction in its free spring length (Fig. 4B). An increase in titin’s stiffness upon activation would then result in greater forces from titin when a muscle is stretched actively compared with when it is lengthened passively (Fig. 4C). Intuitively, this is an appealing mechanism, as it provides for low-resistance elongation of passive muscles, which is what one wants, and for high-resistance elongation of active muscle, becoming stronger when actin-myosin overlap and associated cross-bridge forces decrease and when in the absence of another mechanism, muscles would be unstable and vulnerable to injury. Most intriguingly, such a mechanism would allow for high-force production in lengthening muscle for little metabolic cost.

Increases in the Inherent Stiffness of Titin

The idea that titin’s contribution to force production in muscles changes with contractile conditions is, by now, well accepted (28, 34, 35, 95). Aside from long-term adaptations of titin to functional demands by differential splicing (26, 28, 29, 93, 94), instantaneous changes to titin’s stiffness are possible by phosphorylation and calcium binding. Phosphorylation experiments have been conducted primarily in cardiac muscle and in general, have resulted in decreased force with increasing calcium concentrations (26, 125) and thus are not directly relevant in the current context. However, calcium binding to glutamate-rich motifs of the Pro-Glu-Val-Lys (PEVK) region of titin has been shown to increase passive force in mouse soleus muscle fibers (68)—an observation that was supported by experiments in single myofibrils from rabbit psoas muscle, whose active-force capacity was abolished by deletion of troponin C from the thin myofilament (62).

Fig. 4. Proposed mechanism for the residual FE observed in skeletal muscles following active lengthening. A: micrograph of myofibril (top), micrograph of an isolated sarcomere (middle), and schematic illustration of a sarcomere with Z-bands, thick and thin filaments, and location of titin (bottom). B: schematic illustration of a partial (part of the left side) sarcomere. Top: 2 partial sarcomeres at rest: the left sarcomere is at a relatively short length; the right sarcomere at a relatively long length. When passively stretched to the same length from their initial position (middle), the 2 sarcomeres will achieve the same internal structural organization and thus will exert the same passive force. However, when stretched actively (bottom) from the 2 initial lengths, the 2 sarcomeres will achieve different internal structural arrangements, which will result in different force contributions through titin. In the initially short sarcomere (left initial position), titin binding to actin upon activation (proposed here to occur near the PEVK segment) will occur close to the Z-band; thus when stretched, the distal portion of titin (the free segment that can still act as a spring) is stretched to a great degree and will produce a relatively large force. In contrast, in the sarcomere with the initially long length (right initial position), titin will bind to actin upon activation relatively far away from the Z-band; thus upon active lengthening, the free segment of titin is not as much stretched as for the sarcomere with the short initial length, and thus the force will be smaller (compared with the initially short sarcomere) after the active lengthening. Note also that we propose that there is calcium binding upon activation to the distal Ig domain segments (indicated by the coloring), thereby increasing their stability, and thus the force required to unfold the distal Ig domain segments in active compared with passive lengthening. Finally, in this scenario, only the distal Ig domain segments of titin are available for elongation, and these segments—if we assume they must unfold upon elongation, even for physiologically relevant muscle-stretch magnitudes—are stiffer than the average stiffness for the whole titin protein, thereby contributing further to the increased resistance of titin in active compared with passive stretching. C: schematic illustration of the shift in passive forces (associated with titin) to shorter lengths and to increased stiffness upon activation. Such a shift allows for a straightforward explanation for all general observations that have been made in active and passive elongations of skeletal muscles.

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Recently, we examined the ability of Ig domains of titin to bind calcium and change their mechanical properties. Fluorescence spectroscopy revealed a change in the microenvironment of the isolated I27 Ig domain of titin with calcium in a reversible and dose-dependent manner (18). Further investigation of the unfolding characteristics of eight linked Ig domains demonstrated an increase in unfolding force of ~40 pN (20% increase) and an increase in stiffness, suggesting force and stiffness regulation in the presence of calcium (muscle activation). Since normal forces on titin are estimated to be between 0 and 10 pN (26, 122), the increase in force for Ig domain unfolding in the presence of calcium is substantial. It has been
argued that Ig domain unfolding is likely not relevant for physiological sarcomere lengths (26). For example, for rabbit psoas fibers, unfolding of Ig domains is estimated to occur at sarcomere lengths ranging between 3.0 and 3.5 μm (33, 64), whereas the physiologic range of sarcomere function (2.0–2.6 μm) is thought to be substantially smaller (33). However, in the next sections, we will argue that Ig domain unfolding might be relevant for physiologic sarcomere excursions in the active state, even though it likely plays only a minor or no role at all when muscles are lengthened passively.

Increases in Titin Stiffness by Reduction of the Free Spring Length

Although changes in titin’s stiffness by calcium-modulated, titin-based forces, when muscles are actively stretched, are well acknowledged, these effects seem to be of minor magnitude compared with the >100% of residual force enhancements observed for optimized stretch conditions and the >40% higher forces in the enhanced state compared with isometric forces at optimal muscle length (60, 76, 78). Another way of adjusting a molecular spring’s stiffness is by altering its free spring length. For titin, the free spring length can be changed effectively if specific segments were bound to the rigid actin filament. Experimental evidence suggests that this is indeed the case (2, 8, 15, 56, 67, 79–81, 92, 118, 124). However, alternative interactions are possible—for example, a winding of titin onto a rotating actin filament in lengthening and shortening contractions (85, 95). Nevertheless, titin binding to actin offers a simple way of adjusting titin’s stiffness and force in the active compared with the passive state. Solid-phase binding assays suggest that the most promising area for titin-actin interactions is the PEVK segment (124). If indeed the PEVK segment were to interact with the actin myofilament upon muscle activation, then this could explain many of the observations on actively lengthening muscles that have defied consistent explanation and would in no way diminish the role of the cross-bridge theory. It merely would add titin as a force regulator to the existing, cross-bridge-based force regulation in skeletal muscle. Below, we propose how titin might work as a force regulator in active muscle, in parallel with actin-myosin-based cross-bridge forces.

PROPOSAL FOR TITIN’S ROLE IN FORCE REGULATION IN ACTIVELY LENGTHENING MUSCLE

Titin extends from the M-band of the sarcomere to the Z-band, with the I-band region of titin acting as a molecular spring with serially aligned spring segments (Fig. 4A). Imagine titin in two initial positions, as shown in Fig. 4B: one representing a short and the other a long sarcomere. If a muscle is now stretched passively from either of the two initial positions to the same final position, the passive force will be the same, independent of the initial sarcomere length (Fig. 4B). However, if a muscle is lengthened while activated from the two initial positions, titin will bind to the actin filament at the PEVK segment, and calcium will bind to titin. Titin binding to actin would naturally occur closer to the Z-band for the initially short sarcomere and farther away from the Z-band for the more elongated sarcomere, thereby increasing the distance from the titin binding site to the edge of the A-band for the short compared with the long (initial) sarcomere, thus causing more stretch (and thus more force in titin) for the initially short compared with the initially long sarcomere (Fig. 4B).

Also, binding of the PEVK segment to the actin filament upon activation would only leave the distal Ig domain segment capable of acting as a free spring, thereby causing Ig domains to unfold within the physiological range of muscle excursion for active lengthening, whereas this would likely not be the case for passive muscle lengthening (33). Since Ig domain unfolding is known to require high forces (7, 32, 112, 114, 123) and since Ig domains have been shown to bind calcium and become harder to unfold by doing so (18), the free spring length available in active lengthening muscle is much stiffer than in passively lengthening muscle. Therefore, titin’s force regulations in actively compared with passively lengthened muscle include several force-regulating mechanisms: 1) calcium binding, 2) titin binding to the actin filament, and 3) use of the stiff, distal Ig domain segment.

In turn, titin binding to actin depends on the initial sarcomere length, as titin binds closer to the Z-band for short compared with long initial sarcomere lengths. Furthermore, titin binding to actin reduces the free spring length of titin, and the free spring length (distal Ig domain) is, on average, much stiffer than the average stiffness of the unbound (passive) titin and might cause Ig domain unfolding at much shorter lengths, possibly physiologically relevant sarcomere lengths, in active lengthening compared with passive lengthening of muscles (33, 64).

The mechanism of titin force regulation in actively lengthened muscle would explain a series of currently unexplained phenomena, including: 1) force enhancement above the isometric forces observed at optimal muscle length (Fig. 4C) (1, 60, 70, 76, 91, 102, 108); 2) the passive force enhancement observed in actively stretched muscles (3, 23, 27, 39, 44, 61, 62); 3) the increasing force enhancement with increasing stretch magnitudes (1, 11, 21, 22, 47); and 4) the reduction of the energetic cost (per unit of force) for isometric contractions in the enhanced state compared with the corresponding, purely isometric reference contractions (58).

In 1980, Andrew Huxley (50) summarized the unexplained observations of actively lengthening muscles and the fact that these observations could not be explained with the cross-bridge theory. He predicted that special features would be discovered and that these special features would explain many of the unexplained phenomena of actively lengthening muscle. Here, we propose that one of these special features is force regulation through titin, as described above and illustrated in Fig. 4. We admit that many of the features described in our proposal need careful and detailed exploration in the future. However, the proposed mechanism of force enhancement, if nothing else, is consistent with experimental observations made in actively lengthened muscles that could not be explained previously. It is my hope that the mechanism proposed above will undergo critical testing using a variety of approaches on different structural levels of skeletal muscle.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: W.H. conception and design of research; W.H. analyzed data; W.H. interpreted results of experiments; W.H. prepared figures; W.H. edited and revised manuscript; W.H. approved final version of manuscript.

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