Eccentric muscle contraction

Skeletal muscles are exquisitely designed machines for producing force and work. Positive work is produced when muscles are shortening, because the force of the muscle and the displacement that it produces are in the same direction. The active shortening of muscles is referred to as shortening (20) or **concentric** contraction. When a muscle is activated and its length does not change, no external work is produced. This is referred to as an **isometric** contraction and occurs, for example when holding an object while maintaining a constant body configuration. Muscles are also well designed to absorb work, for example when the active knee extensor muscles elongate when walking down a set of stairs or a steep hill. In these situations, muscles are activated to such a degree that the force they produce is smaller than the external force acting on the muscles, thus the muscles are stretched or elongated despite the force they produce. In these situations, the force of the muscles (tending to shorten the muscles) is in the opposite direction to the displacement of the muscles’ insertion sites, thus the work “performed” is negative, or work is absorbed. Contractions in which active muscles are stretched or elongated are referred to as lengthening (20) or **eccentric** contractions. It is these eccentric contractions that we are concerned with here for a variety of reasons, primarily because they have been ill explained traditionally, and the properties of eccentrically working muscles do not agree well with predictions made by the currently accepted mechanism of muscle contraction, the cross-bridge theory.

Below, we study the effects of eccentric contractions within the framework of the generally accepted theory for muscle contraction, the sliding filament and cross-bridge theories (37; 40; 44). During eccentric contractions of moderate stretch speed, a muscle exerts more force for a given amount of activation than it would for an isometric or a concentric contraction. This property of muscle has been observed for a good century, and was systematically described by A.V. Hill (34), without an explicit explanation of the underlying mechanism. In his formulation of the first cross-bridge model, Huxley (1957) adjusted the rate functions of cross-bridge attachment and detachment in such a manner that his predictions of force as a function of muscle shortening and stretch speed (the so-called force-velocity properties of muscle) matched Hill’s (34) data well. In the cross-bridge model, force during eccentric contractions is greater than for a corresponding isometric or concentric contraction because the attached cross-bridges are more strained, and thus produce more force, and depending on the selection of the rate constants, the number of cross-bridges that is attached at any given time can also be greater than it would be for a corresponding isometric or concentric contraction.
Skeletal muscles are also known to have so-called history-dependent properties. Most importantly in the context of his review, muscles often show substantial amounts of residual force enhancement. Residual force enhancement refers an increase in the long-lasting, steady-state isometric force following an eccentric contraction, compared to the corresponding isometric force not preceded by an eccentric contraction (1; 17; 32). Classic cross-bridge models cannot predict the residual force enhancement property (83), but residual force enhancement is a well-acknowledged and generally accepted property of skeletal muscles (1; 15-17; 66; 70; 73; 80). In order to account for residual force enhancement specifically, or history-dependent properties of skeletal muscles in general, the predictions of cross-bridge type models need to be carefully analyzed, and modifications to existing cross-bridge models are required if we ever want to make accurate predictions of muscle forces during and following eccentric contractions.

This review is focussed on the events following eccentric contractions, the history-dependent effects, rather than eccentric contractions per se. This approach has the advantage that it must challenge our current thinking on the molecular events underlying muscle contraction, since history-dependent properties are not captured within the frame work of the cross-bridge theory (37; 83). Furthermore, towards the end of this review, I will propose a potential mechanism that explains the history-dependent properties of muscles with just a small addition to the existing cross-bridge theory, thereby retaining all the predictive abilities of the existing muscle contraction paradigm, while aiding in the predictions of the mechanics and energetics of eccentric contractions and associated history-dependent properties. While many of the experimental evidence laid out below is obtained from reduced preparations, primarily myofibrils and single sarcomeres, and while experiments are often performed outside the physiological length range of in situ muscles in order to isolate active from passive force contributions, there is no reason to believe that the observed phenomena do not occur within the physiologically relevant lengths of in situ muscle contraction. Furthermore, the physiological relevance of the findings presented below has not been fully established, but the focus here is not on the practical applications, but rather on the basic mechanisms of how muscles contract and regulate force not only in isometric and concentric, but also during and after eccentric contractions, a field that has remained unexplored for too long (26; 38).

**Sliding filament and cross-bridge theory**

In the first part of the 20th century, muscle contraction was thought to occur through the shortening of long filamentous proteins in the centre of sarcomeres: the thick or myosin filaments(39). However, the discovery that the thick filaments of isolated myofibrils(42; 44) and intact fibres(40) do not appreciably shorten for a variety of contractile conditions, led to the idea that there are two sets of filaments (thin - actin and thick - myosin) that slide relative to one another, thereby accounting for the large length changes observed in muscle contraction. This has become known as the “sliding filament theory”. The relative sliding of these two sets of filaments has been associated with projections (cross-bridges) originating on the thick filaments that cyclically interact with the thin filaments, pulling the thin past the thick filaments using
energy from the hydrolysis of adenosine triphosphate (ATP) (37). This theory of muscle contraction is known as the cross-bridge theory, and although details of the original theory developed over half a century ago have been changed (41; 43; 77), the basic premises underlying the cross-bridge theory have been retained.

The cross-bridge theory explains well observations made in isometrically and concentrically contracting muscles, but fares less well for observations in eccentrically contracting muscles. The isometric force-length relationship, particularly the so-called plateau and descending limb region, is well explained by the overlap of the thick and thin filaments, the arrangement of cross-bridges on the thick filaments, and the assumptions governing the cross-bridge theory (22) (Figure 1). Similarly, the concentric part of the force-velocity relationship (34) is well explained with the changing cross-bridge attachment distribution and the asymmetric properties of the rate constants of attachment and detachment of the cross-bridge theory (37). Specifically, force decreases during concentric contractions with increasing speeds of muscle shortening because the proportion of attached cross-bridges and the force per cross-bridge decrease. This behaviour is well predicted by traditional cross-bridge models.

However, the cross-bridge theory has failed in explaining some basic properties of skeletal muscles observed during eccentric contractions. For example, in the original cross-bridge model, eccentric force and energy requirements were vastly overestimated, and cross-bridge models are inherently unable to account for the residual force enhancement (17) observed in skeletal muscles following eccentric muscle contraction (83). Residual force enhancement is defined as the increase in isometric steady-state force following an eccentric contraction compared to the isometric steady-state force obtained during a purely isometric contraction at the corresponding length and activation. Andrew Huxley, who developed the first mathematical framework for the cross-bridge theory, acknowledged the shortcomings of his theory for eccentric contractions, and remarked that “there is a wide, and probably difficult, field for investigation here (referring to eccentric contractions), and I expect that it holds a number of surprises”. Somewhat prophetically, he continued to state regarding eccentric contractions that: “I imagine that special features have evolved which allow elongation to take place without damaging the muscle” (38).

In the following, we would like to share some findings made on eccentrically contracting muscles, specifically the residual force enhancement, and offer a possible explanation for these findings. The proposed molecular model of muscle contraction arising from these findings includes, aside from the contractile filaments actin and myosin, the structural protein “titin” as the third sarcomeric myofilament contributing to active force production during and following eccentric contractions (Figure 2). By including titin into the molecular mechanism of skeletal muscle contraction, experimental results of eccentric contractions can be explained readily while retaining the predictive power of the cross-bridge theory for isometric and concentric contractions. However, it must be emphasized that this newly proposed mechanism of muscle
contraction is by no means fully proven, but it provides feasible answers to hitherto unexplained phenomena in a simple manner, without affecting the role of actin and myosin in the cross-bridge theory.

**Properties of eccentric muscle contractions**

A muscle that is stretched while activated (eccentric contraction) produces substantially more force than a muscle that is contracting isometrically or a muscle shortening at the same speed (13; 18; 34; 71). Much of this increased force in eccentric contraction can be explained with the cross-bridge theory by realizing that the proportion of attached cross-bridges increases and the cross-bridge distribution distances (Huxley’s 1957 “x”-distance) increase in eccentric compared to isometric and concentric contractions (37). However, a muscle stretched while activated at a relatively slow speed (so that a steady-state cross-bridge distribution is achieved; that is a cross-bridge distribution in which the proportion of attached cross-bridges and the average force per cross-bridge is constant) should always reach the same peak force at the end of a stretch independent of the stretch magnitude (37; 41; 83). However, this is not the case, as peak forces at the end of a stretch increase with increasing stretch magnitudes (7; 32) (Figure 3A). Furthermore, following active stretching, a muscle’s steady-state isometric force remains greater than the corresponding force (same length and same activation) obtained during a purely isometric contraction. This observation is referred to as residual force enhancement; a property consistently observed across all structural levels of muscle (Figure 3A)(17; 26; 27; 80).

In 2002, our group discovered that this residual force enhancement observed in the activated muscle often persisted following deactivation (i.e. in the passive muscle), and this has become known as the passive force enhancement (32) (Figure 3A). Although not specifically identified as such, others have observed passive force enhancement following eccentric contractions as well (47; 67). Since residual force enhancement has been shown to be proportional to the force at the end of an eccentric contraction, it has been assumed that the residual force enhancement manifests itself during the eccentric phase of muscle contraction and is not a phenomenon that only emerges following the stretch of an active muscle (7). Similarly, passive force enhancement has been shown to be proportional to residual force enhancement (7) (Figure 3B); therefore residual force enhancement has been thought to be at least in part associated with a passive structural element of muscle (17; 30; 32; 70). Once it had been demonstrated that passive force enhancement is a property of isolated myofibrils and sarcomeres, and that passive force enhancement disappears after removal of the structural protein titin, titin became associated with the passive and the residual force enhancement in skeletal muscle contraction (26-28; 31; 64; 69).

In order to test the hypothesis that residual force enhancement establishes itself during the active stretching phase, and that it is independent of actin-myosin based cross-bridge forces, we stretched activated and passive myofibrils beyond actin myosin filament overlap, thereby eliminating any possibility of cross-bridge contributions to force. We found in rabbit psoas and mouse psoas myofibrils that forces in actively stretched myofibrils were 2-4 times greater than...
those measured in passively stretched myofibrils at lengths where all sarcomeres had been
cnostified to have lost actin-myosin filament overlap (55) (Figure 4). These findings support the
idea that activation of muscle produces substantial force that is independent of the actin-myosin
based cross-bridge forces. Since elimination of titin in myofibril preparations abolishes these
effects (Figure 4), it seems safe to assume that titin is implicated in the additional force observed
in actively stretched muscles.

Residual force enhancement has been associated for a long time with the development of
sarcomere length non-uniformities when a muscle is stretched on the descending limb of the
force-length relationship (65; 66). This theory, which originated in Hill’s (35) instability
theorem, was laid to rest when we demonstrated that force enhancement could be observed in
single sarcomere preparations, and that forces in the enhanced state could vastly exceed (in
excess of 50%) the purely isometric forces obtained at optimal sarcomere lengths (Figure 5A, B).
These observations cannot be explained with increased sarcomere length non-uniformities, but
are well explained with an increase in force in a passive structural sarcomeric protein, such as
titin.

The three filament sarcomere model

In the cross-bridge theory, sarcomeres are represented with the two contractile filaments actin
(also referred to as the thin filament) and myosin (or the thick filament). The three filament
sarcomere model, in addition, contains the filament titin (Figure 2). Titin is the largest known
protein in the human body with a molecular weight ranging from approximately 3-4MDa. Titin
spans the half sarcomere from the Z-bands, which border the sarcomeres, to the M-line in the
middle of the sarcomere. In the A-band region of the sarcomere (Figure 2), titin is thought to be
tightly bound to the thick filaments. In the I-band region (Figure 2), titin acts as a molecular
spring centering the thick filaments in the sarcomere and providing passive resistance to muscle
stretch. I-band titin in skeletal muscles consists of a proximal immunoglobulin (Ig) domain, an
N2A region, the PEVK domain so named for its abundance of proline, glutamate, valine and
lysine, and a distal Ig domain. Upon stretching of a muscle, the randomly arranged Ig domains
are straightened, thereby producing small passive forces (23). Further elongation causes a
stretching of the PEVK domain. These two “spring” elements are thought to be working within
the functional range of muscle contraction. Finally, at long sarcomere lengths, Ig domains start to
unfold. While alignment of the Ig domains and stretching of the PEVK domain are associated
with an essentially elastic behaviour of titin, unfolding of the Ig domains is associated with a
great loss of energy, and thus a highly visco-elastic properties (24; 25; 50; 63).

In summary, titin’s traditional functional role is associated with centering the thick filaments in
the sarcomere and providing low level passive force when sarcomeres become over-stretched.

New mechanism of muscle force production
As outlined above, actively stretched myofibrils have vastly increased forces compared to passively stretched myofibrils in the absence of actin-myosin filament overlap, and thus in the absence of cross-bridge based forces. It has been demonstrated that at least part of this increased force is associated with a passive structural element of muscle, and furthermore, that elimination of titin in single myofibrils abolishes all force transmission across sarcomeres (55) and all residual and passive force enhancement (49). These observations led to the idea that titin is a molecular spring that increases its stiffness and thus its force, in active compared to passive muscle contraction. There are two basic ways in which a molecular spring, like titin, might increase its stiffness: (i) by increasing its inherent spring stiffness and/or (ii) by shortening its spring length. In the following, we will consider these two possibilities.

**Increasing titin’s inherent spring stiffness:** When a muscle is activated, calcium is released from the sarcoplasmic reticulum into the interior of muscle fibres, interacting with the regulatory protein troponin C, which in turn allows for cross-bridge binding and force production. It has been demonstrated that titin also binds calcium upon activation, and in doing so, becomes stiffer, and when stretched, produces more force in the “activated” compared to the “passive” state. Specifically, calcium has been found to bind to the glutamate-rich region of titin’s PEVK domain (53) and to the I27 immunoglobulin domain of cardiac titin (14) and increase titin’s stiffness in both cases. Other calcium binding sites on titin might exist and they might change titin’s inherent spring stiffness, but systematic investigations are required to test this idea. Nevertheless, there is good evidence that titin binds calcium upon muscle activation and in doing so, increases its stiffness and force when active muscles are stretched.

**Shortening titin’s spring length:** When a spring is stretched by a given amount, its stiffness and resistance to stretch depend on the spring’s free length. It has been suggested that titin’s free spring length is changed upon muscle activation by binding of titin’s proximal region to the stiff actin myofilaments (27; 28; 64; 69). Specifically, there is evidence that the N2A region of titin binds to actin in vivo, and that the PEVK domain of titin binds to actin in vitro (6; 9; 51; 62). The experimental evidence and theoretical support for these mechanical properties of titin are examined in the following.

**Experimental Evidence**

Figure (4) demonstrates that titin-based forces are significantly increased in actively compared to passively stretched myofibrils. At sarcomere lengths in excess of 4.0µm, actin-myosin based active forces cannot contribute to the total force measured in rabbit psoas myofibril preparations because of the loss of overlap between the contractile filaments (29; 60; 84). Even if one assumes that there might be sarcomere (or half-sarcomere) length non-uniformities, there would need to be some (half-) sarcomeres whose force generation exclusively relies on titin.

In order to identify the effects of calcium activation on titin-based forces in the absence of cross-bridge forces, myofibrils were stretched from an average sarcomere length of 2.4 to an average
length of 3.4µm at low [pCa=8.0] and high concentrations [pCa=3.5] of calcium. Cross-bridge forces in these preparations were inhibited either by the deletion of the regulatory protein troponin C (49), or by the cross-bridge inhibitor (20mM) 2,3 butanedione monoxime (BDM)(55). Calcium activation caused an increase in passive force of approximately 25% (from 30 to 40nN/µm²) (Figure 6A), a result consistent with the findings obtained by others (Figure 6B)(53). However, this increase in calcium activated titin force of 25% is much smaller than the increase in force observed in activated myofibrils stretched beyond actin-myosin filament overlap (Figure 4). Therefore it appears that calcium activation alone only produces a small increase in titin-based force. The reminder of the increase in force observed in Figure (4) might be associated with a force-dependent binding of the titin spring to actin.

In order to test if the proximal parts of titin might bind to actin upon force production, we labelled titin near its PEVK domain using monoclonal primary antibodies F146.9B9 (Enzo Life Sciences Inc. NY, USA) for titin PEVK and anti-myomesin (Developmental Studies Hybridoma Bank, IA, USA) conjugated with Alexa Fluor 488 (Invitrogen, NY, USA). Stretching these labelled myofibrils passively showed the expected increase in titin lengths for the proximal and the distal portions of I-band titin (Figure 7A). However, stretching activated myofibrils resulted in a partial elongation of the proximal portion of titin for the initial part of the stretch, while this portion stopped elongating for the final part of the stretch, suggesting that it might have been fixed in length, for example by attaching to the rigid actin filament (Figure 7B). The distal portion of titin elongated throughout the entire activated myofibril stretch protocol, and took on the entire elongation of half-sarcomeres once the proximal portion stopped elongating, suggesting that the distal portion remained a free spring in the activated condition, elongating much more than in the passive stretch conditions.

In summary, these experimental results suggest that titin binds calcium upon activation, thereby increasing its spring stiffness, and that some proximal part of titin (the exact location of which needs to be determined), may bind to actin (or another rigid structural component within the sarcomere), thereby potentially decreasing titin’s free spring length in the I-band region, thus possibly increasing titin’s stiffness and its force.

**Theoretical Support**

We built a classical coarse grain cross-bridge model of half sarcomeres calculating the active forces based on cross-bridge interactions and the passive forces based on elongations of titin. Active forces were calculated using a five state model(72). The model of force-elongation properties of single titin filaments accounts for the complex structure of titin, as well as for the stochastic nature of its unfolding. This approach leads to a stochastic model with the length of the half sarcomere and its rate of change in length as input parameters(57; 78). Here, we assumed a stretching velocity of 0.1µm/sarcomere/s. Furthermore, we based our calculations on the well-characterized rabbit psoas titin 3,400-kD isoform, which contains 50 proximal and 22 distal Ig domains, as well as approximately 800 PEVK residues(21)
Since classical two filament cross-bridge models have the limitations outlined above, we expanded the cross-bridge theory by including titin as a third sarcomeric myofilament with activation-dependent properties. We assumed that titin can increase its stiffness by binding calcium upon activation and by titin binding to actin upon active force production by the cross-bridges.

In order to account for the effects of calcium binding to titin, we expanded the classical cross-bridge model by altering titin’s elastic properties and the energy barriers for the unfolding of titin’s Ig domains in the presence of activation (or equivalently high calcium concentration in the sarcoplasm reflecting muscle activation). Implementing the above described experimental findings into the three filament model resulted in experimentally-observed enhanced passive forces in activated myofibrils. The predicted passive stress-elongation curves also coincided well with experimental results in which myofibrils were activated with calcium but cross-bridge interactions were inhibited by 2,3 butanedione monoxime (BDM) and troponin C depletion. However, these passive forces were only marginally greater than those obtained for myofibrils that were passively stretched beyond actin-myosin filament overlap (Figure 8). There was no statistically significant residual force enhancement under these conditions, as the effect of calcium binding to titin for purely isometric contractions and for isometric contractions following active muscle stretching was similar.

In a refinement of the model, we expanded the calcium binding model by introducing the possibility of titin binding to actin upon muscle activation, as has been suggested based on experimental evidence. By allowing titin to bind to the rigid actin filament upon activation, titin’s free spring length is reduced, leading to increased passive forces upon active muscle stretching. In a first approximation, we assumed that titin binds to the nearest actin binding site. Therefore, the main determinant of passive force increase due to titin-to-actin binding is the position of the binding site on titin relative to actin prior to activation and stretch. This model approach allows for simulating and analysing all experimental conditions, provided that titin is at or above its slack length at the time of activation.

We then compared predictions of the three filament model with and without titin-to-actin binding to experimental results of purely passive stretches, and active stretches with and without the possibility of titin to bind to actin upon activation (Figure 8). For these predictions, we assumed that the titin binding site is located at the N2A region, leaving the PEVK and distal Ig domain as titin's free spring, while the entire proximal Ig domain was eliminated from elongation. Predictions of the three filament model without titin-to-actin binding (but binding of calcium to titin) agreed almost perfectly with the experimental results of activated myofibrils with inhibited cross-bridges. Similarly, predictions of the three filament model with titin-to-actin binding predicted well the experimental results of active myofibril stretching with normal cross-bridge interactions.
The three filament model with titin-to-actin binding also predicts residual force enhancement and its associated properties. For example, residual force enhancement is predicted to be independent of the speed of active muscle stretching, but depends directly on the magnitude of stretching (e.g. (17)). This model also naturally accounts for the passive force enhancement observed following deactivation of an actively stretched muscle (e.g. (32)), and further predicts the observed dependence of passive force enhancement on the magnitude of stretch. The exact amount of force enhancement (for given stretch magnitudes and muscle lengths) depends on titin’s binding site to actin. A best approximation of our model predictions with experimental data indicates that titin binding to actin occurs near the middle of titin’s PEVK segment(79).

A New Model of Muscle Contraction

Muscle activation and force production have been exclusively associated with the contractile proteins actin and myosin and their interaction as described by the cross-bridge theory(37; 41; 43; 77). Passive structures, such as the collagen fibril network associated with muscle connective tissue, and structural sarcomeric proteins, such as titin, nebulin, desmin and dystrophin, were assumed to contribute to passive and sarcomere internal structural forces as given by their viscoelastic properties. Here, based on experimental observations and theoretical support, we propose that at least one of these structural proteins, titin, also contributes to “active” force production by proposing that titin changes its stiffness and force by binding calcium at specific sites, and by attaching to actin upon muscle activation and force production (Figure 9).

Specifically, it has been shown that calcium binds to the glutamate-rich region of titin’s PEVK domain(53) and to selected immunoglobulin domains(14), and by doing so, increases its spring stiffness and thus its force when stretched in the activated compared to the passive state. Similarly, there is evidence from isolated protein experiments, in vitro motility assays, and intact sarcomeres and myofibrils that titin’s proximal immunoglobulin domain, and possibly part of the N2A and PEVK region attach prominently to actin (6; 9; 51; 56; 58; 61; 62; 68; 68; 82; 86), thereby shortening titin’s free spring length in the I-band region, thus increasing titin’s force and stiffness at a given sarcomere length. It is this latter phenomenon that we think produces the vast increases in force observed in actively (compared with passively) stretched myofibrils and single, mechanically isolated sarcomeres(54; 55; 74).

The molecular details of the proposed three filament model of sarcomere force production (actin, myosin, titin) need careful elucidation, although preliminary results and theoretical support provide good evidence for an active role of titin in muscle contraction. If shown to be correct, this new model would have substantial advantages over the classic two-filament (actin and myosin only) cross-bridge model. For example, it would provide a ready explanation for the stability of the thick filaments in the centre of sarcomeres(36), and the stability of sarcomeres on the descending limb of the force-length relationship(2; 35; 87).
More importantly though, the three filament model of muscle contraction would naturally explain the universally observed residual force enhancement and passive force enhancement properties of muscles (1; 17; 32; 33), and the increased metabolic efficiency of eccentric contractions (19) and force production in the enhanced state (48). Furthermore, it would be an appealing solution to the problem of actin-myosin based force loss on the descending limb of the force-length relationship (22), while retaining all properties successfully predicted by the cross-bridge model for isometric and concentric muscle contraction.

Although many of the experiments supporting the role of titin in active force production were performed at non-physiological lengths, beyond actin-myosin filament overlap, to determine the passive properties of an activated (calcium saturated) muscle, the proposed new model of muscle contraction, if proven correct, would be of great physiological relevance. First and foremost, an increase in titin’s stiffness and decrease in its zero strain length would ensure the stability of myosin filaments in the centre of sarcomeres. The cross-bridge theory is devoid of such stability, as has been pointed out decades ago (45; 46). Similarly, titin’s proposed role would also provide a simple explanation for the observed stability of sarcomeres on the descending limb of the force-length relationship (e.g. (75)), when the cross-bridge model predicts instability that causes sarcomeres to tear themselves apart (35; 65; 67; 87). Furthermore, titin’s proposed action would also elegantly account for the residual force enhancement (1; 17), the passive force enhancement (32; 33), and static tension properties described in the literature (3; 4; 10; 76). It would also account for the energetic efficiency of eccentric muscle contraction (5; 12; 52; 81), and the observed decrease in metabolic cost per unit of force for muscles in the force enhanced compared to muscles in purely isometric reference states (48). In summary, the proposed action of titin in the new three filament model of muscle contraction, in conjunction with the actin-myosin based cross-bridge theory, could predict many basic properties of skeletal muscle contraction in a simple and intuitively obvious manner, thereby expanding the predictive power of models of muscle contraction to a substantial degree.

It has been pointed out that stretching of sarcomeres to very long lengths might cause a dislodging of titin from its A-band attachments (85), and if so, could produce irreversible damage to the structure and force production of sarcomeres. However, as pointed out by Wang et al. (1991), the point where sarcomeres start to “yield” is not necessarily associated with the dislodging of titin, but rather, is associated with the start of unfolding of titin’s Ig domains (25; 50). This unfolding of Ig domains is associated with a great hysteresis in the force elongation/shortening curves for titin, and thus is also associated with a great loss of the proteins potential energy. However, given the right “recovery” conditions, Ig domains have been shown to refold and full recovery of the mechanical properties of titin are possible (24), indicating that even when sarcomeres are stretched to extreme lengths (>6µm), there is no irreversible damage to titin’s integrity and structure. This is of particular relevance, as it has been suggested that sarcomere lengths well beyond 3.4µm are easily achieved by many in situ muscles during animal movements, including humans (e.g. (8; 11; 59)). Recently, Dr. Lieber presented results of
average sarcomere lengths in spastic muscles of patients with cerebral palsy that were way beyond actin-myosin filament overlap (4.2µm) and sometimes averaged in excess of 6µm (unpublished results presented at the World Congress of Biomechanics, Boston, 2014). It is good to know that although such sarcomere lengths would render them incapable of producing active, actin-myosin based force, these sarcomeres would not necessarily be damaged structurally and thus might recover following appropriate treatment intervention.

Andrew Huxley prophetically predicted that eccentric contractions were very different from concentric and isometric contractions, that investigating eccentric contractions would reveal surprising results, and that special features might be identified that make eccentric contractions possible (even within the frame work of the cross-bridge theory)(38). Here, we suggest that the special feature that functionally helps eccentric contractions and explains all unexplained observations for actively lengthening muscles is the structural spring protein titin whose stiffness and force is adjusted upon activation and force production. The most basic result of this new model of muscle contraction is that muscle can be stretched passively against little resistance, while upon activation and force production, titin-based force becomes dominant and contributes to eccentric force at virtually no metabolic cost. Albeit not proven to be correct, this three filament model including titin as a variable stiffness molecular spring, would solve the problem of eccentric muscle contraction and history-dependent properties immediately, would provide stability to muscles at long length, would prevent damage to muscles in eccentric contractions, but most importantly, is intuitively appealing for its simplicity and beauty in design.

References


Figure 1: Sarcomere force-length relationship for rabbit skeletal muscles showing the ascending (green), plateau (blue) and descending limb (red) regions of the relationship with schematic representations of “sarcomeres” and their overlap between actin and myosin filaments for crucial lengths. For a full explanation of this relationship, please consult (22).
Figure 2: Schematic figure of a sarcomere with the contractile proteins actin (thin filament) and myosin (thick filament), and the molecular spring titin which stabilizes myosin in the centre of sarcomeres, provides most of the passive force in isolated sarcomeres and myofibrils, and is thought to change its stiffness in an activation (calcium) and force (cross-bridge attachment) dependent manner. Titin spans the half sarcomere from the Z-band to the M-line. Its I-band segment consists of a proximal (close to the Z-band) and a distal (close to the A-I band junction) immunoglobulin (Ig) domains, the N2A region, and the PEVK segment. All I-band segments of titin are extensible giving titin its spring-like properties and function. Bottom: Domain composition for a skeletal muscle titin isoform and the approximate location of the F146 label attached to titin in experiments described below.
Figure 3: (A) Force-time and length-time histories of a passive (p), an isometric reference (f), and three isometric-stretch-isometric muscle contractions of 3, 6, and 9mm stretch magnitude (3, 6, 9), starting at optimal muscle length of 107mm indicated here as 0 length. Note that the peak forces at the end of the stretch (occurring at about 5s) increase with increasing stretch magnitudes, despite identical stretch speed and final length. Note furthermore that the three stretch contractions of the active muscle show increased steady-state isometric forces after stretching (9-10s) compared to the isometric reference contraction. This is called residual force enhancement (FE). Finally, note that after deactivation of the muscle (stimulation to the muscles via the nerve was stopped, this occurs in the figure at about 10s), the passive force of the active stretch contractions is greater than the passive forces of the isometric contraction and the passive muscle stretch. This has become known as the passive force enhancement (PFE). (B) Relationship between passive force enhancement and total force enhancement. Cat soleus, 35°C, activated for 8s (shaded area in A) through maximal tibial nerve stimulation at a frequency of 30Hz and square wave pulse duration of 0.1ms.
Figure 4: Stress (force/cross-sectional area) as a function of sarcomere length for slow stretching (0.1µm/s/sarcomere) of active and passive myofibrils. Slow continuous stretching. The area of myofilament overlap is shaded in grey and goes to sarcomere lengths of approximately 4.0µm for rabbit psoas myofibrils; the area of no myofilament overlap is in white and contains all sarcomere lengths >4.0µm. Note that for the continuous stretching, the passive myofibrils always have substantially less force than the activated myofibrils in the region where actin-myosin based cross-bridge forces are zero. When titin is eliminated from the myofibril preparations, no force transmission occurs in actively or passively stretched myofibrils.
Figure 5: (A) Stress as a function of time in a single, isolated sarcomere, and (B) normalized force as a function of sarcomere length for isometric contractions and for isometric contractions following active stretching of single sarcomeres. Forces in single sarcomeres are substantially greater after active stretching compared to the purely isometric reference contractions [grey diamonds and black square indicating the mean value for the reference contractions], thereby demonstrating force enhancement (FE). Forces after stretching of active sarcomeres [green triangles with the mean indicated by the black circle] are also substantially greater than the isometric forces prior to stretch at a sarcomere length of 2.4µm [brown circle] (i.e. optimal length for rabbit psoas sarcomeres, that is the length at which the isometric force reaches peak force/stress values), therefore exhibiting forces in the enhanced state that are greater than the maximal isometric forces at optimal sarcomere length in these single sarcomere preparations (OFE). OFE indicated in (A) shows the difference between the enhanced force at a sarcomere length of 3.4µm compared to the corresponding maximal isometric force of a single sarcomere at optimal length (2.4µm), while OFE shown in (B) shows the mean increase in force in the enhanced state (at 3.4µm) compared to the mean of the maximal isometric force at optimal sarcomere length (2.4µm) across all sarcomeres tested (n=8). The observation shown as the raw data in (A) was observed in all eight single sarcomeres tested in this manner. Rabbit psoas myofibrils at 21°C.
Figure 6: Stress (force/cross-sectional area) in a single myofibril (A) and skinned fibres (B) as a function of calcium concentration. In (A) stretching of a single myofibril whose cross-bridge based force was eliminated by the deletion of troponin C from actin. Stretching was performed from an average sarcomere length of 2.4 to 3.4µm and caused a greater peak stress (~11s) and steady-state stress (50-60s) in the presence of high calcium [pCa=3.5] compared to low calcium concentrations [pCa=8.0]. In (B) higher stresses are also observed when skinned fibres are pulled from an average sarcomere length of 2.0 to 3.0µm in a high [pCa=4] compared to a low [pCa=9] calcium concentration and in the presence of a cross-bridge inhibitor (2,3 butanedione monoxime) that eliminates all cross-bridge based active forces(53).
Figure 7: Passive (A) and active (B) stretching of two representative myofibrils each while measuring the half sarcomere length (open circles) and the length of the proximal segment of titin from the Z-band to the F146 antibody label at the distal end of titin’s PEVK segment (open diamonds). Note that for the passive stretching (A), the half sarcomere and proximal titin segment length increase approximately linearly, as expected, while for the active stretching (B), the proximal titin segment is stretched partially, until it does not seem to elongate further at a sarcomere length of approximately 2.3µm for one myofibril (blue diamonds) and approximately 3.0µm for the other myofibril (brown diamonds). It is assumed that when the proximal titin segment elongation stops, titin might have bound to actin (or another structural component of the sarcomere), thus the entire stretch of the half sarcomere has to be taken up by the distal titin segment whose elongation is indicated as the difference between the corresponding open diamonds and open circles in (A) and (B). While the distal segment elongation is linear in passive stretching (A), it increases dramatically for active stretching (B) when the proximal segment does not elongate any further. Note that when the proximal segment elongates initially in the active myofibrils (A), its length and rate of elongation is approximately the same as shown for the passive myofibril stretching (B), suggesting that initially, prior to the proposed binding of titin to actin, passive and active elongations of titin are virtually identical within the error of measurement.
Figure 8: Theoretical predictions of myofibril stresses (forces/cross-sectional areas) as a function of sarcomere lengths for simulated stretching of myofibrils at a speed of 0.1µm/sarcomere/s using the three filament sarcomere model described in the text. From the bottom to the top, the solid lines represent purely passive stretch conditions (green), stretching of activated \[pCa=3.5\] myofibrils not accounting for titin-actin binding (light blue), and stretching of activated myofibrils incorporating titin-actin binding (dark blue). The green and blue dots represent the average passive (±1SD) and active stresses measured in psoas myofibrils stretched at a speed of 0.1µm/sarcomere/s at 21°C. The passive predictions fit well with experimental observations indicating that titin may indeed be the primary passive force in isolated myofibrils. The active predictions underestimate the average stresses measured experimentally, but this can be adjusted readily, for example, if the location of titin-actin interaction is changed to a more distal location on titin.
Figure 9: Schematic illustration of the proposed new mechanism of muscle contraction involving a three-filament (involving titin) rather than just the classic two-filament sarcomere model (actin and myosin). In (A) a passively stretched half-sarcomere, in which titin is elongated in its entirety in accordance with the stiffness properties of its serially arranged springs (i.e. Ig domain, PEVK domain and N2A domain springs), is shown with its corresponding low passive force. In (B) the half-sarcomere is stretched in the presence of calcium (activation), calcium is bound to specific segments on titin and increases the stiffness of these segments. However, titin does not bind to actin (a scenario that happens when actin-myosin based cross-bridge forces are inhibited, as we have done for example, by deleting the regulatory protein troponin C from actin or by inhibiting strong cross-bridge attachments through 2,3 butanedione monoxime). In this situation, the passive force is higher (yellow trace) compared to the purely passive stretch shown in (A) (blue trace). Finally, in (C) the half-sarcomere is stretched in the activated myofibril and cross-bridge forces are naturally present. In this “normal” eccentric contraction scenario, calcium is thought to bind to titin, as in (B), and in addition, titin is assumed to bind to actin in a hitherto unknown manner, thereby shortening its free spring length, thus increasing its stiffness and thus force when muscles are stretched. In this situation, the force produced by titin (purple trace) would be even higher than the titin force in (B) (yellow trace), because the “free” spring length of titin is reduced, thus titin is stiffer and produces more resistance (force) at a given sarcomere length.