 MCB, vol.6, no.4, pp.229-241, 2009

Molecular Basis of Force Development by Skeletal Muscles During and After Stretch

Dilson E. Rassier*

Abstract: When activated skeletal muscles are stretched at slow velocities, force increases in two phases: (i) a fast increase, and (ii) a slow increase. The transition between these phases is commonly associated with the mechanical detachment of cross-bridges from actin. This phenomenon is referred to as *force enhancement during stretch*. After the stretch, force decreases and reaches steady-state at levels that are higher than the force produced at the corresponding length during purely isometric contractions. This phenomenon is referred to as *residual force enhancement.* The mechanisms behind the increase in force during and after stretch are still a matter of debate, and have physiological implications as human muscles perform stretch contractions continuously during daily activity. This paper briefly reviews the potential mechanisms to explain stretch forces, including an increased number of cross-bridges attached to actin, an increased strain in cross-bridges upon stretch, the influence of passive elements upon activation and sarcomere length non-uniformities.

Keyword: force enhancement, cross-bridge kinetics, pre-powerstroke, titin, sarcomere

1 Introduction

Muscle contraction is driven by cyclical interactions between myosin cross-bridges and actin filaments, a mechanical process coupled with ATP hydrolysis (Figure 1). The cooperative action of many myosin molecules will shorten the sarcomeres and produce force; when length changes are not imposed to the muscles during activation they produce an isometric contraction. However, if muscles are stretched while activated, they produce a substantial increase in force (5, 16, 17, 21, 22, 36, 37, 43, 47, 48, 56) while the rate of ATP hydrolysis is decreased (2, 35). This increase in force has been referred to as *force enhancement during stretch.* After stretch, force decays and reaches a steady-state, which is higher than the force obtained at the corresponding length during purely isometric contractions. This increase in force has been referred to as *residual force enhancement* (1, 18, 19, 26, 29, 48, 49, 51, 52, 58).

Although there has been much research in the field, the molecular bases for the force enhancement during and after stretch are still unknown, and different mechanisms have been proposed without conclusive arguments, which generates heated debate in the literature (24, 25, 39, 40, 60). Understanding the mechanisms behind the stretch-induced force enhancement will lead to a better comprehension of the basic mechanisms of contraction. Furthermore, stretch forces are important for everyday muscle actions. Muscles work continuously in shortening-stretch cycles and play a key role in storing and releasing energy. Muscles can act as dynamic stabilizers of joints during stretch, protecting ligaments and tendons from injury during lengthening actions (e.g. walking downstairs, landing from jumping) and securing the musculoskeletal system from sudden length changes. This paper will review the potential mechanisms of force enhancement produced during and after stretch, with emphasis to studies performed with isolated muscle fibres and myofibrils, preparations that allow measurements of sarcomere/fibre length, providing direct insight into the molecular mechanisms of contraction.

* Department of Kinesiology and Physical Education, Faculty of Education, McGill University, 475 Pine Avenue West, Montreal (PQ), Canada - H4W 1S4. Email: dilson.rassier@mcgill.ca; phone: 514-2984184, ext 0558

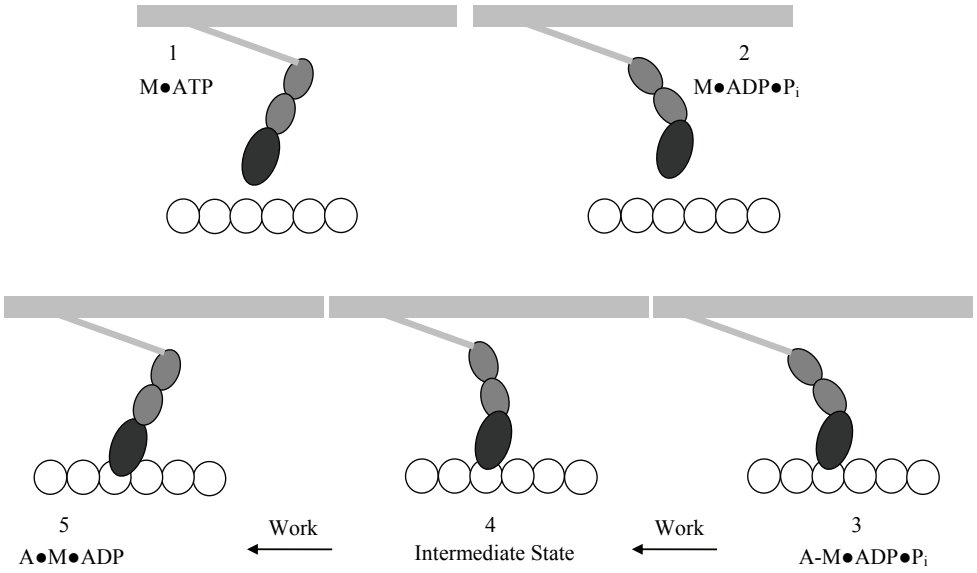

Figure 1: Simplified representation of the actomyosin interaction, based on biochemical (20, 41) and structural (54) studies. In state 1, myosin is detached from actin, and ATP is bound to the active site, forming the M•ATP complex. During the transition to stage 2, hydrolysis of the nucleotide is associated with a reorientation of the light chains of myosin from the post powerstroke to the pre powerstroke position. At this point the cross-bridge may or may not attach to actin, entering stage 3. In stage 3, the M•ADP•P_i complex attaches weakly to actin, forming the complex A-M•ADP•P_i, in which little (or no) force is generated. Following, the light chain domain assumes a new orientation (stage 4), representing the powerstroke generated with ADP and P_i bound to myosin. Release of P_i leads to a strongly bound state (A•M•ADP•P_i), and additional work is performed as the light chain region changes orientation to the post powerstroke conformation (state 5). As a result of the reorientation of the light chains, the powerstroke is performed and force is generated. At the end of the powerstroke, ATP binds to myosin, causing detachment of myosin from actin, returning to stage 1 (M•ATP) [Adapted from Karatzaferi et al. (30)].

2 Characteristics of the force enhancement during stretch and residual force enhancement

A typical experiment investigating the characteristics of force enhancement during and after stretch is shown in Figure 2. When the stretch is performed at slow velocities (<2 L_o•s^{-1}), the *force enhancement during stretch* has two components, (i) a steep phase (called phase I hereafter), in which force increases significantly during stretch of a few nanometers per half-sarcomere, and (ii) a slow phase (called phase II hereafter), in which force increases less steeply than during the first phase or remains unchanged (16, 17, 22, 46-48, 52). A few studies have observed an additional break-point just after the beginning of stretch (21, 46) - this review will not discuss this early break as it is not always detected and has not been thoroughly investigated.

The transition between phases I and II is typically associated with the mechanical detachment of cross-bridges after they reach a critical extension (17, 22, 47, 56). Studies that investigate the critical sarcomere extension (SL_c) needed to achieve this transition show average values between 14 nm and 20 nm when sarcomere length is not controlled (17, 56) and between 8 nm and 10 nm when sarcomere length is controlled (22, 36) or when individual sarcomere lengths are directly measured (47). The force obtained at the transition point increases as a function of stretch velocity to reach a maximum of $\sim$2.0 the maximal isometric force (P_o) at 1.0 μm•sec^{-1}•half-sarcomere (half-saturation at

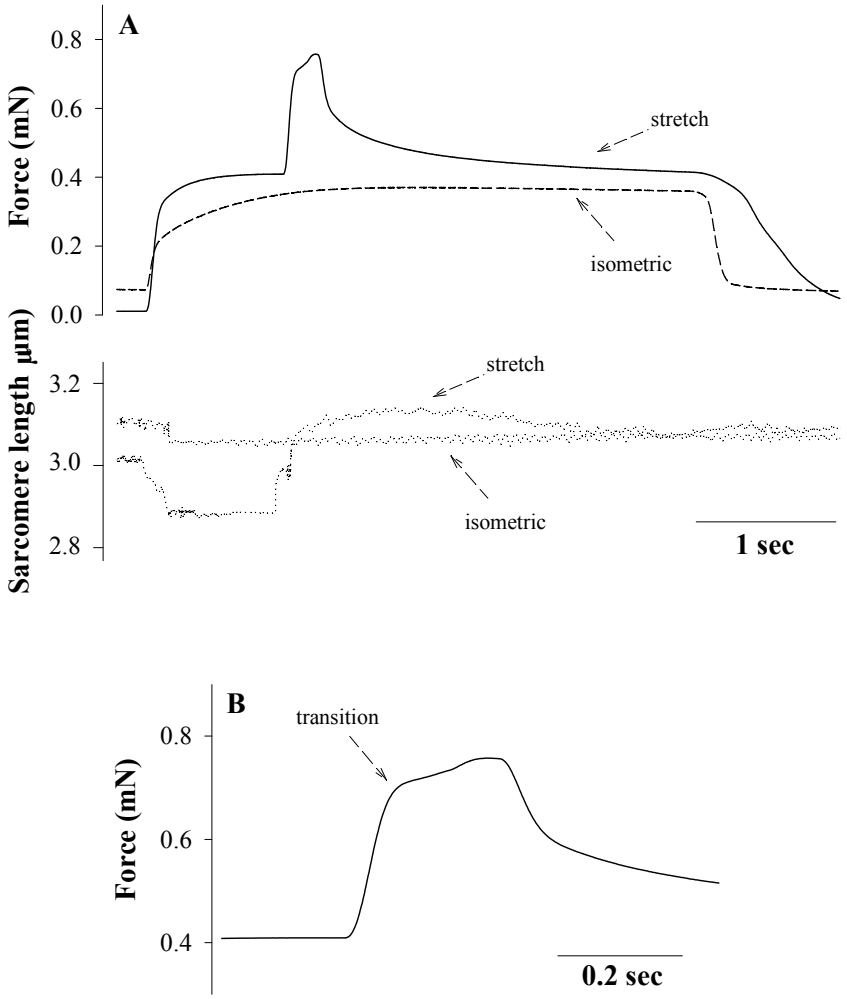

Figure 2: Force response of a single muscle fibre to an active stretch performed from an average sarcomere length of 2.88μm to 3.10μm. (A) Top panel show forces and bottom panel shows the sarcomere length tracings. Force increases substantially during stretch. After stretch, force decays in two phases: a fast decay and a slow decay that leads to a steady-state situation. The force at steady-state is higher than the isometric force produced at the corresponding sarcomere length, i.e. residual force enhancement is observed. These contractions were performed with the same fibre during one experiment, and were tested such that the final sarcomere length at the end of the contraction would be similar, taking into account the amount of shortening observed during activation. (B) Detail of the force enhancement produced during stretch. Force rises in two phases: a fast phase, in which force increases significantly over a few magnitude of stretch, and a slow phase, in which force increases less steeply than during the first phase. The transition between these two phases occurs at a critical stretch magnitude, supposedly caused by the mechanical detachment of cross-bridges after they reach a critical extension during stretch.

$\sim$0.5 μm•sec^{-1}•half-sarcomere) (16, 17, 21, 36, 44, 46, 47). After the transition between phases I and II, force continues to increase or remains unchanged; results differ according to the preparation used and experimental protocol (16, 17, 21, 36, 44, 46, 47). Although the reason for such difference is not clear, it may be related to fibre type composition, as studies commonly observe an absence of force increase in phase II in soleus fibres but not in psoas fibres (46, 56).

After the stretch, the force decay can be usually fitted by a bi-exponential function. Initially, the force drops to $\sim$1.4-1.5 times the maximal isometric force at a rate of 50•sec^{-1} - 250•sec^{-1}. Then, the force decays slowly (2•sec^{-1}-10•sec^{-1}) (16, 46) before reaching a steady-state (phase III). The new steady-state force is higher than that produced during isometric contractions at the corresponding length, i.e. *residual force enhancement* (called phase III hereafter). The residual force enhancement is long lasting (>6 s), increases with increasing amplitudes of stretch (1, 18, 52), and increasing sarcomere length to an optimal of $\sim$2.6 μm (18). Opposite to the force enhancement in phases I and II, the residual force enhancement is independent of the speed of stretch (18, 46, 52).

3 Mechanisms

The mechanisms behind the different phases of force enhancement with stretch are under investigation without conclusive results. Although several mechanisms have been proposed, they can be generally classified into sarcomeric mechanisms, including (i) cross-bridges kinetics and (ii) the involvement of passive elements; or structural mechanisms, associated primarily with (iii) sarcomere non-uniformity and instability.

3.1 Cross-bridges involvement during the stretch–induced force enhancement

The involvement of cross-bridges in the force enhancement produced during stretch has been broadly investigated, but the results are controversial. There is general agreement that stretch-induced changes in cross-bridges properties will influence the force during phase I and possibly during phase II, while cross-bridge involvement in phase III is less clear.

Phases I and II. Force enhancement in phase I has been attributed primarily to (i) an increased in the mean cross-bridge force (11-13, 22, 36, 44), (ii) an increase in the number of cross-bridges attached to actin (9, 34), or a combination of both. Because the force transient in phase I occurs very rapidly, studies have measured muscle fibre stiffness in phase II as an indicator of cross-bridge attachment in phase I. The results invariably show an increase in stiffness during stretch (9, 14, 22, 34, 36), but authors disagree on the contribution of the increased stiffness to the total force enhancement.

Investigators that observed an increase in stiffness between 10%-20% calculated that it is not large enough to explain the force enhancement during stretch (22). Instead, they suggest that the force enhancement is caused largely by an increased mean force produced by the cross-bridges. Evidence for such hypothesis comes from studies in which the transition in force, presumably associated with the critical cross-bridge extension before it detaches from actin, is calculated using the amount of sarcomere extension - the "critical sarcomere stretch" (17, 22, 47). These studies have shown that situations that increase the force produced by strong-bound cross-bridges, including increasing experimental temperatures (12), lowering the ionic strength (11) or stretching slowly the fibers (13) decrease the critical sarcomere stretch, suggesting that cross-bridges that are already strained would resist to lower strains before detaching from actin. Conversely, studies that induce a decrease in force produced by cross-bridges before stretch increase the critical sarcomere stretch (10, 22, 47, 48).

An increased in cross-bridge force without an increased in the number of cross-bridges could be caused by different mechanisms. It has been suggested that the increased force during stretch phase I is caused by cross-bridges working in a pre-powerstroke state that precedes phosphate release (10, 22, 46, 47, 55)(stage 3, Figure 1). These cross-bridges would not produce substantial force during isometric contractions, but large forces

when stretched. Studies that manipulated cross-bridges into pre-powerstroke states using different interventions, including 2,3-Butanedione monoxime (BDM) (47, 48), N-benzyl-p-toluene sulphonamide (BTS) (46), high concentrations of phosphate (56), vanadate (V_i) together with aluminum floride (AlF_4) (10, 22), or increasing ionic strength (22), show a substantial decrease in isometric force without a concomitant decrease in stretch forces during phase I, increasing the stretch force/isometric force ratios. Conversely, increasing temperature, which conceptually shifts cross-bridges towards strongly-bound states, show opposite results and the stretch force/isometric force ratios are decreased (45, 63). This hypothesis is tempting specially because pre-powerstroke cross-bridges could resist to the applied stretch while producing force without large energy requirements, consistent with previous studies showing low ATP consumption during stretch (2, 35).

Although the above-mentioned studies suggest that an increased mean cross-bridge force can explain (at least) partly the increase in stretch forces, the idea is not without controversy. Increases in muscle fiber stiffness in values ranging between 22% and 60% without concomitant changes in the cross-bridge mean force have also been observed (9, 34), suggesting that the force enhancement is caused by an increase in the number of cross-bridges attached to actin. Linari et al. (34) observed that the myosin meridian X-ray reflection (I_{M3}), which arises from the 14.5 nm axial repeat of the myosin heads in the thick filament, is depressed during stretching of the active muscle fibers. The authors suggested that the decrease in the I_{M3} signal is caused by an increased number of cross-bridges attached to actin, which could be associated with the engagement of the second cross-bridge in the myosin S2 segment. The attachment of a second cross-bridge to an actin binding site situated next to the actin already bound would be favored by the change in strain or conformation caused by the first cross-bridge, which could start a cooperativity mechanism that would increase force. Recently, Brunello et al. (9) were able to show that a stretch of only 5 nm is sufficient to double the number of myosin motors attached to action in a few millisecond, which strengthens such possibility.

Phase III. Since cross-bridges are implicated in force enhancement during phase I and possibly in phase II, it is appealing to consider that stretch-induced changes in cross-bridges would also enhance force in phase III. A major limitation with this possibility is that changes in mean cross-bridge forces and/or cross-bridge number caused by stretch would have to be maintained for many attachment/detachment cycles after the stretch, an unlikely scenario. While Linari et al. (34) observed that stiffness was still enhanced by 7% at 300 ms after the end of stretch of intact frog fibres, which leads the possibility that cross-bridge number may be maintained elevated, Getz et al. (22) observed that stiffness returned to isometric levels within 10 ms after stretch of permeabilized rabbit psoas fibres.

Studies that evaluated muscle fibre stiffness in phase III are few and inconclusive. Sugi and Tsuchiya (58) showed that stiffness decreased after stretch reaching the same level as that observed during isometric contractions at the corresponding length, while Julian and Morgan (29) showed that stiffness increased during stretch and remained virtually constant after stretch. Rassier and Herzog (49) observed an increase in stiffness in phase IIII when compared to isometric contractions at corresponding lengths, but they did not measured the sarcomere length, which limits their interpretation, although giving indirect support for this hypothesis. More research is needed to directly evaluate this possibility, but even if this mechanism works, it would explain only part of force enhancement in phase III. Force increases at levels that are higher than those observed during isometric contractions (33, 52), and thus in a region where the number of cross-bridges is not optimized.

Summary. The involvement of cross-bridges in the stretch-induced force enhancement in phase I and possibly phase II is well accepted, but there is controversy as the mechanisms by which cross-bridges increase the force. While experimental evidence suggests that an increased strain which

leads to an increased mean cross-bridge force would be responsible for most of the force enhancement, recent studies show that a significant increase in stiffness may be obtained by the engagement of a second myosin head during stretch, which would ultimately increase the number of cross-bridges attached to actin. The mechanism remains a matter of debate, but most likely a combination of both factors will contribute to the final increase in force during phases I and II, which will depend heavily on the preparation and conditions investigated. Changes in cross-bridges may be carried out after stretch and explain part of the residual force enhancement (phase III), although more evidence is needed to support (or deny) such hypothesis as results are limited and inconclusive.

3.2 Passive elements involvement in stretch–induced force enhancement

Although cross-bridges are the main active force generators inside the sarcomeres, it has been known for many years that significant passive forces are produced at long muscle lengths. A distinct possibility suggested in the literature is that the force increase during phases II and III may be caused by non cross-bridge, viscoelastic structures (19), and more specifically titin molecules. Titin spans the half-sarcomere attaching to the Z-lines, thick and thin filaments, and provides much of the passive force in muscle fibres (23, 32, 62, 64). It is now recognized that the PEVK segment of titin binds Ca^{2+} with high affinity (31, 65), changing the structure and decreasing the persistence length of the molecule (31). Since a decrease in persistence length increases the stiffness, the force produced by titin is enhanced when the PEVK segment binds to Ca^{2+}. Accordingly, Labeit et al. (31) have shown that single muscle fibers in which actin filaments were depleted (and therefore myosin-actin interaction was inhibited), show an upwards shift in the sarcomere length-passive force relationship with increasing Ca^{2+} concentrations.

The hypothesis that "activation" of titin is responsible for the stretch-induced force enhancement would be consistent with studies showing that when muscle fibers are activated in the presence of cross-bridge inhibitors and are stretched, a non-cross-bridge dependent stiffness is observed, referred to as "static stiffness" (6-8). If confirmed, this mechanism would explain the differences in force enhancement during phase II observed in muscles with different fibre types, as titin isoforms are different in muscles with predominantly fast or slow fibre types.

If titin is responsible for the force enhancement in phase II, and the potential changes on its properties with stretch would be maintained while activation persists, it could also influence force produced during phase III. Studies evaluating the effects of cross-bridge inhibitors on the force enhancement in phase III strengthen this hypothesis. Administration of BDM (48, 49) and BTS (46), or decreasing temperature (57), interventions that should decrease the proportion of strongly-bound cross-bridges attached to actin, increase significantly the residual force enhancement in phase III, suggesting that passive elements are implicated. Furthermore, studies with single muscle fibres (48, 53) and myofibrils (27, 28) show that after an active stretch the force remains elevated even after deactivation of the muscle - a phenomenon referred to as "passive force enhancement" (Figure 3). The passive force enhancement is directly associated with phase III (28, 53), and it is also higher than the passive forces produced after purely passive stretches at similar sarcomere lengths. Although the passive force enhancement is taken from forces measured after the contraction, it is indicative that activation changes the passive forces. Recently, it has been shown that passive force enhancement is present in myofibrils that were activated while cross-bridge interactions with actin was inhibited by depletion of Troponin C (TnC), but it was not observed in myofibrils depleted of titin (27). The passive force enhancement shares striking similarities with the stretch-induced increase in static stiffness (6-8).

Summary. There is evidence that force enhancement in phases II and III is associated with the involvement of passive elements inside the sarcomeres, and most specifically with the properties of titin that may change as a result of Ca^{2+} activation. Studies show that when cross-bridge

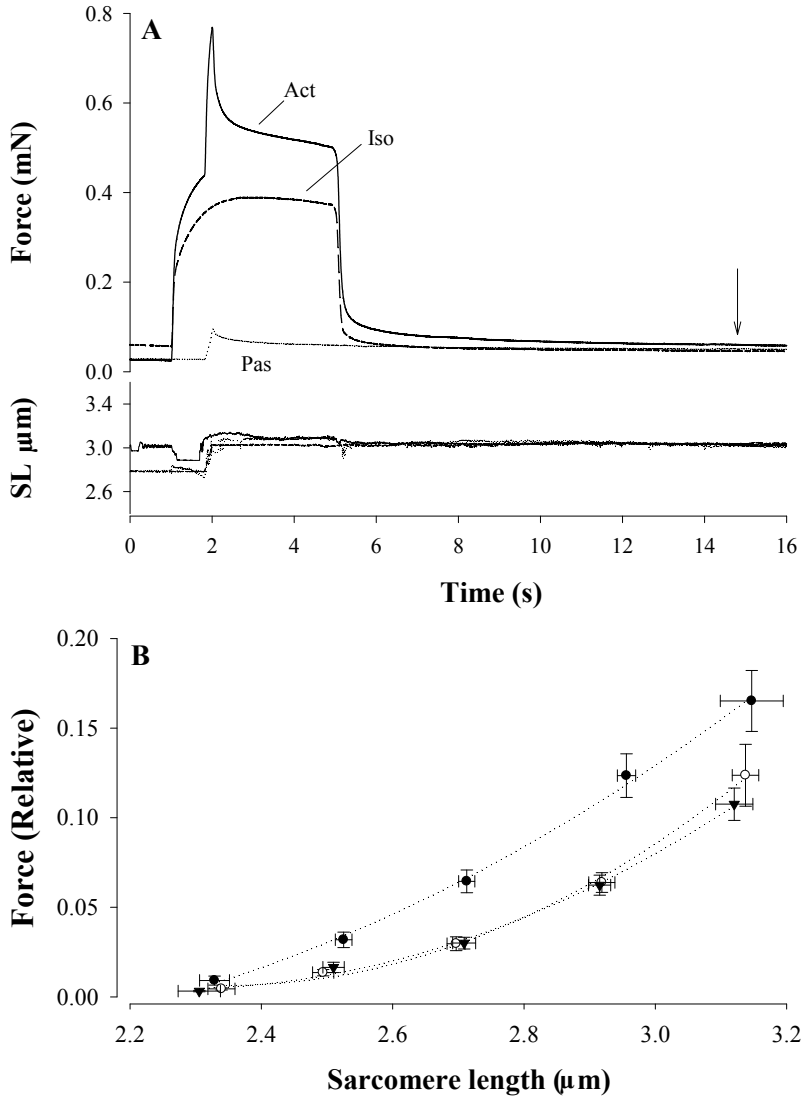

Figure 3: (A) Force response of a single muscle fibre to an active stretch (from 2.87μm to 3.13μm), a passive stretch (2.78μm to 3.08μm) and an isometric contraction (3.10μm). Force enhancement is observed during and after stretch, and also following deactivation of the muscle fibre. The actively stretched muscle has a higher passive force than the passively stretched muscle and the isometric reference contraction at the corresponding length (time of measurements shown with an arrow). (B) Mean ($\pm$ standard error of the mean) values are shown from experiments similar to the one performed in panel A. Closed circles indicate passive forces following a stretch produced during muscle activation, open circles indicate passive forces following a stretch without activation, and triangles indicate passive forces following an isometric contraction. Data were grouped into intervals of 0.2μm (range: 2.2μ–3.2μm). With the exception of sarcomere lengths of 2.2μm and 2.4μm, there is a significant increase ($p<0.05$) in the passive force following active stretch compared with the passive force following isometric contraction and passive stretch [Adapted from Rassier et al. (53)].

inhibitors are used during experiments, muscle fibres and myofibrils still present a large increase in force when stretched. A fraction of the force enhancement persists during phase III, and even after deactivation of muscles, suggesting that the changes in the properties of titin are long lasting and may explain part of the force enhancement.

3.3 Sarcomere non-uniformity involvement in stretch–induced force enhancement

It has been proposed that stretch forces are associated with structural changes in sarcomeres, most specifically sarcomere length non-uniformity and instability (29, 38). The descending limb of the isometric force-length relationship has been proposed to be unstable due to its negative slope. Such argument has not been accepted without argument (3, 4, 61, 66), as the negative slope of the force-length relationship is commonly derived trough static, isometric tests, not necessarily correct to evaluate system instability. Nonetheless, it has been suggested that, upon activation and stretch along this "unstable" region of muscle functioning, small differences in the yield tensions of the sarcomeres (as a result of their different lengths) will lead to an increase in the sarcomere length non-uniformity. The sarcomeres will continue changing lengths at varying velocities - the weakest sarcomeres will lengthen very rapidly at the expense of sarcomeres that will elongate slowly (if at all). At one point the sarcomeres that are elongating rapidly become unable to hold the tension and will be stretched to the point where they will lose filament overlap ("popping sarcomere"), and will be limited only by passive forces. The process will then be repeated with the next weakest sarcomere. The sarcomeres that are stretched beyond filament overlap stabilize high force with strong sarcomeres that would hardly stretch.

Since force enhancement in phase I is observed in conditions that maintain sarcomere length uniformity and is commonly observed along the plateau of the force-length relationship [e.g. (22, 36)], sarcomere length non-uniformity and popping sarcomeres have been associated with phases II and especially III. Theoretical models incorporating sarcomere instabilities (15, 38) can produce the residual force enhancement, and studies using electron microscopy show significant non-uniformities following stretch of isolated muscles (59).

The sarcomere non-uniformity theory has not been accepted without debate (24, 25, 39, 40, 60) and direct evidence for such hypothesis is lacking. Experiments with isolated fibres show that force enhancement can be obtained on the "stable", ascending limb of the force-length relationship (42) and above the plateau of the force-length relationship when stretch conditions are optimized (33, 52), which weakened the hypothesis that sarcomere instability is responsible for the residual force enhancement. Force enhancement has also been observed without large sarcomere length non-uniformity (46) or when muscle fibre segments are clamped during and after stretch through a feedback system (18, 58).

Recently, experiments conducted with isolated myofibrils, a preparation in each individual sarcomere can be tracked during experiments, failed to confirm the predictions of the sarcomere length non-uniformity theory. These studies have shown that the tension rise during and after stretch is accomplished with the development of substantial sarcomere non-uniformity but without sarcomere popping, i.e. sarcomeres never stretched to the point where sarcomeres would lose filament overlap (Figure 4) (26, 50, 51, 60). Furthermore, in several occasions sarcomeres are observed to be stable, without an increase in the degree of length non-uniformity after stretch (Figure 4). Telley et al. (60) suggested that sarcomere-length non-uniformity could still produce force enhancement in phase III without popping sarcomeres, but the authors never evaluated sarcomere behaviour when force achieved a steady-state level, and therefore could not evaluate directly the characteristics of the residual force enhancement.

Summary. When muscle fibres and myofibrils are stretched along the descending limb of the force length relationship, a substantial degree of sarcomere length non-uniformity is observed. However, such non-uniformity does not necessarily translate into large instabilities, and although

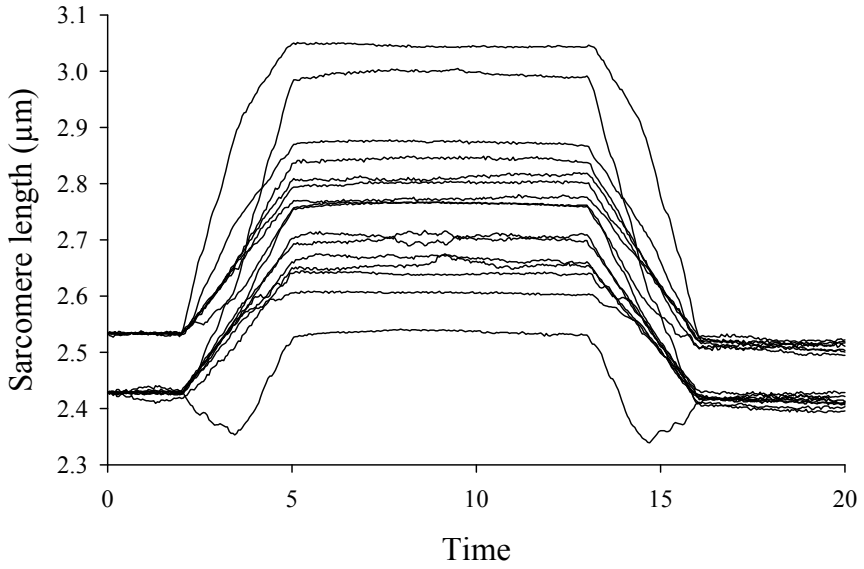

Figure 4: Behaviour of individual sarcomeres during stretch in an activated myofibril. The initial and final average sarcomere lengths were 2.47μm and 2.75μm, respectively. Note that all sarcomeres stretch during the stretch of the myofibril, except by one that initially shortens and then stretches. Sarcomere length non-uniformity increases during stretch, but after stretch sarcomeres are maintained isometric until the myofibril is shortened back to the initial length. At this point all sarcomeres shorten to their initial length. Note that sarcomeres do not achieve a length in which they would lose completely filament overlap.

it can play a role in some experimental conditions, force enhancement is observed when sarcomere is clamped isometric during contractions. Most evidence with single fibres and recent experiments performed with myofibrils suggest that sarcomere length non-uniformity cannot explain uniquely force enhancement in phases II and III.

4 Conclusion

The mechanisms of force enhancement induced by stretch has generated much debate in the field of muscle physiology. The research will continue, but a single explanation will not likely emerge easily, as different experimental conditions seem to lead to opposing results. Several mechanisms are associated with the force enhancement, which are complex and likely complementary to each other; while some mechanisms are associated mainly with phases I and II, others are long lasting and associated with phase III. Stretch likely induces both an increase in the number of cross-bridges attached to actin, and a higher force produced by individual cross-bridges. Besides, stretch utilizes the energy stored in passive elements, especially titin that changes its properties with Ca^{2+}-induced muscle activation. Stretch has also been associated with an increase in sarcomere length non-uniformity and popping sarcomeres, but recent evidence fail to confirm such hypothesis, as popping sarcomeres are not observed during and after stretch of isolated myofibrils. The role of sarcomere length non-uniformity on force enhancement needs further, detailed investigation.

References

1. Abbot BC and Aubert X. The force exerted by active striated muscle during and after change of length. *J Physiol* 117: 77-86, 1952.

2. Abbott BC, Aubert XM and Hill AV. The absorption of work by a muscle stretched during a single twitch or a short tetanus. *Proc R Soc Lond B Biol Sci* 139: 86-104, 1951.

3. Allinger TL, Epstein M and Herzog W. Stability of muscle fibers on the descending limb of the force-length relation. A theoretical consideration. *J Biomech* 29: 627-633, 1996.

4. Allinger TL, Herzog W and Epstein M. Force-length properties in stable skeletal muscle fibers–theoretical considerations. *J Biomech* 29: 1235-1240, 1996.

5. Bagni MA, Cecchi G and Colombini B. Crossbridge properties investigated by fast ramp stretching of activated frog muscle fibres. *J Physiol* 565: 261-268, 2005.

6. Bagni MA, Cecchi G, Colombini B and Colomo F. A non-cross-bridge stiffness in activated frog muscle fibers. *Biophys J* 82: 3118-3127, 2002.

7. Bagni MA, Cecchi G, Colomo F and Garzella P. Development of stiffness precedes cross-bridge attachment during the early tension rise in single frog muscle fibres. *J Physiol* 481: 273-278, 1994.

8. Bagni MA, Colombini B, Geiger P, Berlinguer PR and Cecchi G. Non-cross-bridge calcium-dependent stiffness in frog muscle fibers. *Am J Physiol Cell Physiol* 286: C1353-C1357, 2004.

9. Brunello E, Reconditi M, Elangovan R, Linari M, Sun YB, Narayanan T, Panine P, Piazzesi G, Irving M and Lombardi V. Skeletal muscle resists stretch by rapid binding of the second motor domain of myosin to actin. *Proc Natl Acad Sci U S A* 104: 20114-20119, 2007.

10. Chinn M, Getz EB, Cooke R and Lehman SL. Force enhancement by PEG during ramp stretches of skeletal muscle. *J Muscle Res Cell Motil* 24: 571-578, 2003.

11. Colombini B, Bagni MA, Cecchi G and Griffiths PJ. Effects of solution tonicity on cross-bridge properties and myosin lever arm disposition in intact frog muscle fibres. *J Physiol* 578: 337-346, 2007.

12. Colombini B, Nocella M, Benelli G, Cecchi G and Bagni MA. Effect of temperature on cross-bridge properties in intact frog muscle fibers. *Am J Physiol Cell Physiol* 294: C1113-C1117, 2008.

13. Colombini B, Nocella M, Benelli G, Cecchi G and Bagni MA. Crossbridge properties during force enhancement by slow stretching in single intact frog muscle fibres. *J Physiol* 585: 607-615, 2007.

14. Colomo F, Lombardi V and Piazzesi G. The recovery of tension in transients during steady lengthening of frog muscle fibres. *Pflugers Arch* 414: 245-247,

15. Denoth J, Stussi E, Csucs G and Danuser G. Single muscle fiber contraction is dictated by inter-sarcomere dynamics. *J Theor Biol* 216: 101-122, 2002.

16. Edman KA, Elzinga G and Noble MI. Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *J Physiol* 281: 139-155, 1978.

17. Edman KA, Elzinga G and Noble MI. Critical sarcomere extension required to recruit a decaying component of extra force during stretch in tetanic contractions of frog skeletal muscle fibers. *J Gen Physiol* 78: 365-382, 1981.

18. Edman KA, Elzinga G and Noble MI. Residual force enhancement after stretch of contracting frog single muscle fibers. *J Gen Physiol* 80: 769-784, 1982.

19. Edman KA and Tsuchiya T. Strain of passive elements during force enhancement by stretch in frog muscle fibres. *J Physiol* 490: 191-205, 1996.

20. Eisenberg E, Hill TL and Chen Y. Cross-bridge model of muscle contraction. Quantitative analysis. *Biophys J* 29: 195-227, 1980.

21. Flitney FW and Hirst DG. Cross-bridge detachment and sarcomere 'give' during stretch of active frog's muscle. *J Physiol* 276: 449-465, 1978.

22. Getz EB, Cooke R and Lehman SL. Phase transition in force during ramp stretches of skeletal muscle. *Biophys J* 75: 2971-2983, 1998.

23. Granzier H, Labeit D, Wu Y and Labeit S. Titin as a modular spring: emerging mechanisms for elasticity control by titin in cardiac physiology and pathophysiology. *J Muscle Res Cell Motil* 23: 457-471, 2002.

24. Herzog W, Lee EJ and Rassier DE. Residual force enhancement in skeletal muscle. *J Physiol* 574: 635-642, 2006.

25. Herzog W and Leonard TR. Response to the (Morgan and Proske) Letter to the Editor by Walter Herzog (on behalf of the authors) and Tim Leonard. *J Physiol* 2006.

26. Joumaa V, Leonard TR and Herzog W. Residual force enhancement in myofibrils and sarcomeres. *Proc Biol Sci* 275: 1411-1419, 2008.

27. Joumaa V, Rassier DE, Leonard TR and Herzog W. The origin of passive force enhancement in skeletal muscle. *Am J Physiol Cell Physiol* 294: C74-C78, 2008.

28. Joumaa V, Rassier DE, Leonard TR and Herzog W. Passive force enhancement in single myofibrils. *Pflugers Arch* 2007.

29. Julian FJ and Morgan DL. The effect on tension of non-uniform distribution of length changes applied to frog muscle fibres. *J Physiol* 293: 379-392,

30. Karatzaferi C, Chinn MK and Cooke R. The force exerted by a muscle cross-bridge depends directly on the strength of the actomyosin bond. *Biophys J* 87: 2532-2544, 2004.

31. Labeit D, Watanabe K, Witt C, Fujita H, Wu Y, Lahmers S, Funck T, Labeit S and Granzier H. Calcium-dependent molecular spring elements in the giant protein titin. *Proc Natl Acad Sci U S A* 100: 13716-13721, 2003.

32. Labeit S and Kolmerer B. Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science* 270: 293-296, 1995.

33. Lee EJ and Herzog W. Residual force enhancement exceeds the isometric force at optimal sarcomere length for optimized stretch conditions. *J Appl Physiol* 105: 457-462, 2008.

34. Linari M, Lucii L, Reconditi M, Casoni ME, Amenitsch H, Bernstorff S, Piazzesi G and Lombardi V. A combined mechanical and X-ray diffraction study of stretch potentiation in single frog muscle fibres. *J Physiol* 526 Pt 3: 589-596, 2000.

35. Linari M, Woledge RC and Curtin NA. Energy storage during stretch of active single fibres from frog skeletal muscle. *J Physiol* 548: 461-474, 2003.

36. Lombardi V and Piazzesi G. The contractile response during steady lengthening of stimulated frog muscle fibres. *J Physiol* 431: 141-171, 1990.

37. Mansson A. The tension response to stretch of intact skeletal muscle fibres of the frog at varied tonicity of the extracellular medium. *J Muscle Res Cell Motil* 15: 145-157, 1994.

38. Morgan DL. An explanation for residual increased tension in striated muscle after stretch during contraction. *Exp Physiol* 79: 831-838, 1994.

39. Morgan DL and Proske U. Sarcomere popping requires stretch over a range where total tension decreases with length. *J Physiol* 574: 627-628, 2006.

40. Morgan DL and Proske U. Can all residual force enhancement be explained by sarcom-

ere non-uniformities? *J Physiol* 578: 613-615, 2007.

41. Pate E and Cooke R. A model of crossbridge action: the effects of ATP, ADP and Pi. *J Muscle Res Cell Motil* 10: 181-196, 1989.

42. Peterson DR, Rassier DE and Herzog W. Force enhancement in single skeletal muscle fibres on the ascending limb of the force-length relationship. *J Exp Biol* 207: 2787-2791, 2004.

43. Piazzesi G, Francini F, Linari M and Lombardi V. Tension transients during steady lengthening of tetanized muscle fibres of the frog. *J Physiol* 445: 659-711, 1992.

44. Piazzesi G, Linari M, Reconditi M, Vanzi F and Lombardi V. Cross-bridge detachment and attachment following a step stretch imposed on active single frog muscle fibres. *J Physiol* 498: 3-15, 1997.

45. Piazzesi G, Reconditi M, Koubassova N, Decostre V, Linari M, Lucii L and Lombardi V. Temperature dependence of the force-generating process in single fibres from frog skeletal muscle. *J Physiol* 549: 93-106, 2003.

46. Pinniger GJ, Ranatunga KW and Offer GW. Crossbridge and non-crossbridge contributions to tension in lengthening rat muscle: force-induced reversal of the power stroke. *J Physiol* 573: 627-643, 2006.

47. Rassier DE. Pre-power stroke cross bridges contribute to force during stretch of skeletal muscle myofibrils. *Proc Biol Sci* 275: 2577-2586, 2008.

48. Rassier DE and Herzog W. Active force inhibition and stretch-induced force enhancement in frog muscle treated with BDM. *J Appl Physiol* 97: 1400, 2004.

49. Rassier DE and Herzog W. Relationship between force and stiffness in muscle fibers after stretch. *J Appl Physiol* 99: 1769-1775, 2005.

50. Rassier DE, Herzog W and Pollack GH. Dynamics of individual sarcomeres during and after stretch in activated single myofibrils. *Proc Biol Sci* 270: 1740, 2003.

51. Rassier DE, Herzog W and Pollack GH. Stretch-induced force enhancement and stability of skeletal muscle myofibrils. *Adv Exp Med Biol* 538: 501-515, 2003.

52. Rassier DE, Herzog W, Wakeling J and Syme DA. Stretch-induced, steady-state force enhancement in single skeletal muscle fibers exceeds the isometric force at optimum fiber length. *J Biomech* 36: 1309-1316, 2003.

53. Rassier DE, Lee EJ and Herzog W. Modulation of passive force in single skeletal muscle fibres. *Biol Lett* 1: 342-345, 2005.

54. Rayment I, Holden HM, Whittaker M, Yohn CB, Lorenz M, Holmes KC and Milligan RA. Structure of the actin-myosin complex and its implications for muscle contraction. *Science* 261: 58-65, 1993.

55. Roots H, Offer GW and Ranatunga KW. Comparison of the tension responses to ramp shortening and lengthening in intact mammalian muscle fibres: crossbridge and non-crossbridge contributions. *J Muscle Res Cell Motil* 28: 123-139, 2007.

56. Stienen GJ, Versteeg PG, Papp Z and Elzinga G. Mechanical properties of skinned rabbit psoas and soleus muscle fibres during lengthening: effects of phosphate and Ca2+. *J Physiol* 451: 503-523, 1992.

57. Sugi H. Tension changes during and after stretch in frog muscle fibres. *J Physiol* 225: 237-253, 1972.

58. Sugi H and Tsuchiya T. Stiffness changes during enhancement and deficit of isometric force by slow length changes in frog skeletal muscle fibres. *J Physiol* 407: 215-229, 1988.

59. Talbot JA and Morgan DL. Quantitative analysis of sarcomere non-uniformities in active

muscle following a stretch. *J Muscle Res Cell Motil* 17: 261-268, 1996.

60. Telley IA, Stehle R, Ranatunga KW, Pfitzer G, Stussi E and Denoth J. Dynamic behaviour of half-sarcomeres during and after stretch in activated rabbit psoas myofibrils: sarcomere asymmetry but no 'sarcomere popping'. *J Physiol* 573: 173-185, 2006.

61. ter Keurs HE, Iwazumi T and Pollack GH. The sarcomere length-tension relation in skeletal muscle. *J Gen Physiol* 72: 565-592, 1978.

62. Trombitas K, Greaser M, Labeit S, Jin JP, Kellermayer M, Helmes M and Granzier H. Titin extensibility in situ: entropic elasticity of permanently folded and permanently unfolded molecular segments. *J Cell Biol* 140: 853-859, 1998.

63. Wang G and Kawai M. Effect of temperature on elementary steps of the cross-bridge cycle in rabbit soleus slow-twitch muscle fibres. *J Physiol* 531: 219-234, 2001.

64. Watanabe K, Nair P, Labeit D, Kellermayer MS, Greaser M, Labeit S and Granzier H. Molecular mechanics of cardiac titin's PEVK and N2B spring elements. *J Biol Chem* 277: 11549-11558, 2002.

65. Yamasaki R, Berri M, Wu Y, Trombitas K, McNabb M, Kellermayer MS, Witt C, Labeit D, Labeit S, Greaser M and Granzier H. Titin-actin interaction in mouse myocardium: passive tension modulation and its regulation by calcium/S100A1. *Biophys J* 81: 2297-2313, 2001.

66. Zahalak GI. Can muscle fibers be stable on the descending limbs of their sarcomere length-tension relations? *J Biomech* 30: 1179-1182, 1997.