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Characterization of the passive component of force enhancement following active stretching of skeletal muscle

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Summary

The mechanisms causing the steady-state force enhancement following active skeletal muscle stretching are not well understood. Recently, we found direct evidence that part of the force enhancement is associated with the engagement of a passive component. In this study, we reproduced the conditions that give consistent passive force enhancement and evaluated the mechanical properties of this passive force enhancement so as to gain insight into its source. The three primary results were that (1) the passive force enhancement is long lasting (>25 s), (2) passive force enhancement was reduced in a dose-dependent manner by the amount of shortening preceding active muscle stretching, and (3) passive force enhancement could be abolished 'instantaneously' by shortening–stretching the passive muscle by an amount equivalent to the active stretch magnitude. Together with the remaining results, we conclude that the source of the passive force enhancement must be arranged in parallel with the contractile force, it must consist of a viscoelastic molecular spring whose stiffness characteristic can be reset by shortening, and it must have a characteristic length that is governed by the length of the contractile components, possibly the sarcomeres. Based on these results, the molecular spring titin emerges as a possible candidate for the passive component of the steady-state force enhancement observed in this and previous studies.

Key words: passive force, skeletal muscle, stretching, titin, cross-bridge theory, sarcomere length, stability, molecular spring, calcium.

Introduction

It is well accepted that force increases when an active muscle is stretched (e.g. Fenn, 1924; Katz, 1939; Hill, 1938). This increase in force has been associated with an increased strain in the cross-bridges, and an increased proportion of attached cross-bridges (Huxley, 1957; Huxley and Simmons, 1971). Similarly, it has been observed that the steady-state force reached approximately 3–5 s following active muscle stretch is also substantially greater than the corresponding isometric force (e.g. Abbott and Aubert, 1952; Edman et al., 1978, 1982). In contrast to the increase in force during stretch, the force enhancement following stretch is independent of the stretch speed, but increases with stretch magnitude (Edman et al., 1978, 1982). Force enhancement following stretch is long-lasting (>20 s) (Abbott and Aubert, 1952), is associated with a similar or an increased stiffness at steady-state compared to that during a purely isometric contraction at the same length (Sugi and Tsuchiya, 1988; Linari et al., 2000; Herzog and Leonard, 2002), does not exceed the isometric force at optimal length (Edman et al., 1982), and has primarily been found on the descending limb of the force–length relationship (Edman et al., 1978, 1982; Morgan et al., 2000). Although the mechanisms underlying the steady-state force enhancement following muscle stretch remain unknown, the sarcomere length non-uniformity theory has frequently been used to explain this phenomenon (Julian and Morgan, 1979; Morgan, 1990, 1994; Morgan et al., 2000; Edman and Tsuchiya, 1996; Herzog, 1998; Herzog and Leonard, 2002).

Edman et al. (1978, 1982) were the first to propose that the "*residual force enhancement after stretch was compatible with recruitment of a passive elastic element in parallel with the contractile system*". This notion was further supported by indirect evidence in single fiber and whole muscle preparations (Edman and Tsuchiya, 1996; DeRuiter et al., 2000; Lee and Herzog, 2002), and was used in theoretical considerations (Noble, 1992; Herzog, 1998; Herzog and Leonard, 2002) and mathematical modeling of the force enhancement effect (Forcinito et al., 1998). Recently, we reported direct evidence for passive force enhancement in the cat soleus, in single fibres of frog, and in voluntary contractions of human adductor pollicis (Herzog and Leonard, 2002; Rassier et al., 2003, in press; Lee and Herzog, 2002). The purpose of the present study was to characterize the mechanical properties of this newly detected passive component that contributes to the steady-state force enhancement following active muscle stretch, and to determine, or eliminate, possible candidate structures that may cause this passive force enhancement.

Materials and methods

Experiments were performed using ten cat soleus muscles from five adult outbred animals of mass 3.4 kg (±0.3 kg). The mean soleus mass was 3.1 g (±0.4 g). All procedures were approved by the University's committee on the Care of Animals in Research.

Preparation

The procedures for animal preparation, force and length measurements have been described before (Herzog and Leonard, 1997). Here, only the salient features are repeated. Cats were anaesthetized using a nitrous oxide, halothane (5%), oxygen mixture, and were then intubated and maintained at 0.8–1.0% halothane throughout the remainder of the experiment. Cats were regularly checked for ear, pupil and paw pressure reflexes, and halothane was adjusted accordingly. The soleus, soleus tendon and calcaneus were exposed using a single cut on the posterior, lateral shank. The soleus tendon was isolated from the rest of the Achilles tendon and was cut from the calcaneus with a remnant piece of bone. The muscles surrounding the soleus (plantaris and both heads of the gastrocnemius) were bluntly dissected away from the soleus, and the corresponding tendons were cut leaving the soleus isolated from any other muscle. A second cut was made on the posterior, lateral thigh and the tibial nerve was exposed and implemented with a bipolar cuff-type electrode for soleus stimulation (Herzog and Leonard, 1997). The nerve was left fully intact to ensure a consistent, long-term preparation. The cat was secured in a prone position in a hammock and the pelvis, thigh and shank of the experimental hindlimb were fixed with bilateral bone pins to a stereotaxic frame. The bone piece at the distal end of the soleus tendon was attached with sutures to a muscle puller (MTS, Eden Prairie, MN, USA; natural frequency >10 kHz). When attaching the bone piece, about half of the soleus tendon is wrapped around the attachment clamp on the muscle puller, thus providing excellent fixation of the distal end of the soleus. The free tendon was usually about 10 mm long following fixation and provided little compliance to the preparation because of the great stiffness of the soleus tendon that makes it virtually rigid within the range of muscle forces (Baratta and Solomonow, 1990). The soleus forces and excursions were measured continuously by the muscle puller and were sampled at a frequency of 200 Hz, except for tests in which stiffness was assessed (2000 Hz). Nerve stimulation was performed using a voltage that exceeded the α-motoneuron threshold by a factor of three (3T) to ensure full soleus stimulation (Herzog and Leonard, 1997). Stimulation pulses were monopolar and of 0.1 ms duration. Stimulation frequency was 30 Hz, which produces fused tetanic contractions of the cat soleus, and the duration of stimulation varied as a function of the specific test. The exposed soleus was covered with saline-soaked gauze, and was heated with an infrared lamp to keep the muscle temperature between 30–35°C.

Experimental protocol

At the beginning of each experimental protocol, the isometric force–length relationship of the cat soleus was determined. Peak tetanic forces (30 Hz) were determined from a length near active insufficiency (zero force) until a muscle length that was 12 mm longer than the length at which active force (total force – passive force) was maximal. Length steps were 3 mm, and typically 12–15 length steps were required to cover the target range. The muscle length at the right end of the isometric force plateau (Gordon et al., 1966) was designated 0 mm. Increases in muscle length are defined as positive; i.e. +9 mm refers to a muscle length that is 9 mm longer than the 0 reference length. Note that the 0 mm reference length is typically associated with an active isometric force that is equal or just a little bit smaller (<5%) than the maximal, active isometric force.

Following the determination of the force–length relationship, seven tests were performed. Test 1 was aimed at determining the long-term stress–relaxation rate of the passive component of force enhancement and comparing it to the stress–relaxation rate of the passive component following purely isometric contractions. Passive force enhancement (the force enhancement measured after deactivation of the muscle) was produced by stretching the active muscle from 0 mm to +9 mm (i.e. approx. 9% of the total muscle length or approx. 21% of the optimal fiber length; Herzog and Leonard, 2002) at a speed of 3 mm s^{-1} (i.e. about 7% of optimal fiber length s^{-1}), deactivating the muscle 5 s after the end of stretch, and then measuring the passive force decay for a period of >25 s (Fig. 1). Note that 5 s after deactivation of the muscle, all deactivation force transients have subsided (Huxley, 1957; Huxley and Simmons, 1971). The loss of force of the passive elements at this point in time reflects the viscoelastic properties of the tissues involved in passive force production.

Test 2 was aimed at determining the stiffness of the passive component of force enhancement and comparing it to the corresponding stiffness of the passive force following a purely isometric contraction or a passive stretch. Passive force enhancement was obtained by stretching the muscle as described in test 1, and stiffness of the passive components was assessed by a quick stretch (1 mm at 50 mm s^{-1}) at 5 s following deactivation of the muscle (Fig. 2).

Test 3 was aimed at quantifying the amount of loss of passive force enhancement by a quick shortening of the muscle. Passive force enhancement was obtained by stretching the muscle as described in test 1. 5 s following deactivation, the muscle was shortened by 4.5 or 9 mm (i.e. 50 or 100% of the active stretch amplitude), at a speed of 18 mm s^{-1}, and immediately stretched back to its original length at a speed of 18 mm s^{-1}. The passive force enhancements at 0.2 s prior to and 2 s following this passive shortening–stretch cycle were compared (Fig. 3).

Test 4 was aimed at isolating the passive component of force enhancement from the total force enhancement (active plus passive component). Passive force enhancement was obtained as in test 1. 5 s following deactivation of the muscle, the muscle was activated again isometrically for 5 s, and any

remnant force enhancement was quantified at 4.8 s after reactivation of the muscle (label 3, Fig. 4).

Test 5 was aimed at determining whether shortening the muscle prior to the stretch protocol influenced total and passive force enhancement. All stretches were identical to those described in test 1 (9 mm amplitude at 3 mm s^{-1}). Preceding the stretches, the muscle was shortened by 3, 6 and 9 mm at a speed of 9 mm s^{-1}, and total force enhancement (the force enhancement measured while the muscle was still activated at 4.8 s following the stretch) and passive force enhancement (5 s following deactivation) were compared for the different conditions (Fig. 5). Note that 4.8 s following the active stretch, all force transients associated with the dynamic stretch have mostly subsided (e.g. Huxley, 1957; Huxley and Simmons, 1971; Herzog and Leonard, 2002). Therefore, the force enhancement observed at this point in time may be considered a steady-state value. We have shown previously that the force enhancement at 4.8 s following the active stretch is virtually identical to that at 25–30s following the active stretch (Herzog and Rassier, 2002), as the force–time curves of the test and reference contraction are virtually 'parallel' to each other. Parallelism in this study was assessed by approximating the force–time curves of the test contraction by a best-fitting straight line from 4.3–4.8 s following the active stretch and comparing this slope statistically to that obtained for the corresponding period of time of the isometric reference contraction (Wakeling et al., 2000). To our knowledge, all force enhancements described in the literature on mammalian muscle at or near physiological temperatures were made at times <4.8 s following the active stretch. Even in single fibers at low temperatures, where contractile processes occur at a much reduced rate, force enhancement measurements are rarely made at ≥4.8 s following the stretch (e.g. Edman et al., 1978: 179 ms, 478 ms and about 4.5 s; Edman et al., 1982: 4.5–6.0 s).

In test 6, the stiffness at the time of steady-state force enhancement following stretch was determined and compared to the stiffness of a purely isometric contraction at the same length. Force enhancement was obtained as described in test 1. Stiffness was determined using a quick stretch of 1mm amplitude at a speed of 50 mm s^{-1} at 4.8 s following the end of the stretch phase (Fig. 6).

Finally, test 7 was aimed at determining whether or not the steady-state force-enhanced values could exceed the isometric force values at optimal length. For these tests, the muscles were stretched actively from an initial length of –6 mm to a final length of +3 mm. The activation was maintained for 5 s following the end of the stretch, and comparisons of values from the force-enhanced tests and the isometric reference tests at optimal muscle length were made at 4.8 s following the stretch.

All test contractions, in all seven experimental protocols, were preceded and followed by isometric reference contractions at the corresponding final length. If these two reference contractions were not within 0.1 N (i.e. approx. 0.4% of the maximal isometric force), the test trial was rejected, therefore any damage or fatigue effect could not have produced the observed force enhancement or passive force enhancement.

Data analysis

The force enhancement was determined as the difference between the isometric force following the stretch and the isometric reference force at the corresponding length. Force values were taken 4.8 s after the end of the stretch, when a near steady-state force had been reached (Herzog and Leonard, 2002). Similarly, the passive force enhancement was assessed as the difference between the passive isometric force following the stretch test and the passive isometric force following the corresponding isometric reference contraction. Force values were taken 5 s following deactivation of the muscle, when the transient force decay following deactivation had subsided (Herzog and Leonard, 2002). Non-parametric, repeated measures statistics (Wilcoxon matched-pair; Hinkle et al., 1979) were used to test for force enhancement and passive force enhancement. The level of significance was chosen at α=0.05.

Results

The rate of force-relaxation of the passive force following active muscle stretch was significantly greater (by 164%) than the corresponding rate following isometric contractions (Table 1). Since force-relaxation rates typically depend on the absolute force, it could be argued that the rate of passive force decrease was greater following active stretch contractions than that following isometric contractions, because the passive force was also greater following stretch compared to isometric. In order to test this argument, we performed selected isometric tests at muscle lengths greater than +9 mm to achieve passive forces (but not passive force enhancement) that were greater than the passive forces following the active stretch contractions. However, even in these cases, the rate of passive force relaxation was similar to that of the isometric contractions at the shorter length and was significantly smaller than the rate of passive force decrease following active muscle stretching (Fig. 1). This result is evidence that the viscoelastic nature of the passive force in the force enhanced state is different from the viscoelasticity obtained following isometric contractions at the corresponding lengths.

Stiffness of the passive muscle tendon unit following active stretching was significantly greater (31%) than the stiffness following an isometric contraction at the corresponding muscle length or passive stretching of the muscle (Table 1). An example result of the stiffness assessment is shown in Fig. 2. This result suggests that the passive force enhancement is associated with the recruitment of an additional passive element, or the change in stiffness of a passive element.

When a muscle that has a substantial amount of passive force enhancement was quickly released (and immediately stretched back) by an amount that was equal to the active stretch that was used to produce the passive force enhancement (i.e. 9 mm in our case), all passive force enhancement was

abolished instantaneously. When the same muscle was only released (and immediately stretched back) by 50% of the initial active stretch (i.e. 4.5 mm in our case), the passive force enhancement was almost completely retained (Table 1, Fig. 3). This behaviour is not consistent with that of a viscoelastic material, but could be explained with a material whose stiffness properties can change in a discontinuous way.

Following a 9 mm stretch of the active muscle from its zero reference length, we always observed a substantial amount of force enhancement at 4.8 s following the active stretch, and we always observed a corresponding passive force enhancement (Fig. 4, Table 1). Upon reactivation of the muscle, there remained a certain amount of force enhancement that was smaller than both the initial total force enhancement and the passive force enhancement. Following deactivation after the second stimulation period, passive force enhancement was still present in all muscles (Fig. 4, Table 1).

When shortening the muscle by 3, 6 and 9 mm prior to the 9 mm stretch, it was found that total and passive force enhancement decreased with increasing magnitudes of shortening (Table 1, Fig. 5). When subtracting the passive force enhancement value from the total force enhancement value, the remaining (active) force enhancement was independent of the amount of shortening preceding the stretch (Table 1). This last result suggests that the decrease in total force enhancement may be explained completely by the decrease in passive force enhancement. Therefore, it appears that the amount of shortening preceding the stretch directly affects the passive but not the active component of force enhancement.

Reports in the literature of stiffness measurements following active muscle stretch, when a steady-state force enhancement

Table 1. *The primary values determined in this study*

Test 1. Rate of force relaxation (mN s^{-1})

Test:	
Isometric	11±8
Experimental	29±7

Test 2. Stiffness of passive component (N mm^{-1})

Test:	
Isometric	4.2±1.0
Experimental	5.5±1.1

Test 3. Amount of force enhancement (N) before and after passive shortening–stretch

	Before release	After release	Difference (before – after)
Excursion (mm):			
4.5	3.0±1.2	2.4±1.1	0.6±0.4
9.0	2.7±0.3	0.0±0.7	2.7±0.5

Test 4. Force enhancement (N)

Total during 1st activation (1)*	4.8±0.5
Passive after 1st activation (2)*	3.0±0.4
Passive during 2nd activation (3)*	0.6±0.6
Passive after 2nd activation (4)*	1.4±0.5

Test 5. Total and passive force enhancement (N) when stretch was preceded by shortening

	Total	Passive	Active (Total – passive)
Shortening (mm)			
0	5.0±1.0	3.3±0.7	1.7±0.4
3	4.6±0.7	2.7±0.6	1.9±0.3
6	3.8±0.7	2.1±0.8	1.7±0.3
9	3.6±0.9	2.1±0.8	1.5±0.2

Test 6. Stiffness of the activated muscle (N mm^{-1})

Test:	
Isometric	10.4±1.1
Experimental	11.6±1.0

Test 7. Force enhancement above isometric force at optimum length (%) 5.3±2.7

Values are means ±1 S.D.
For details of tests 1–7, see text.
*Number of force enhancement refers to Fig. 4.

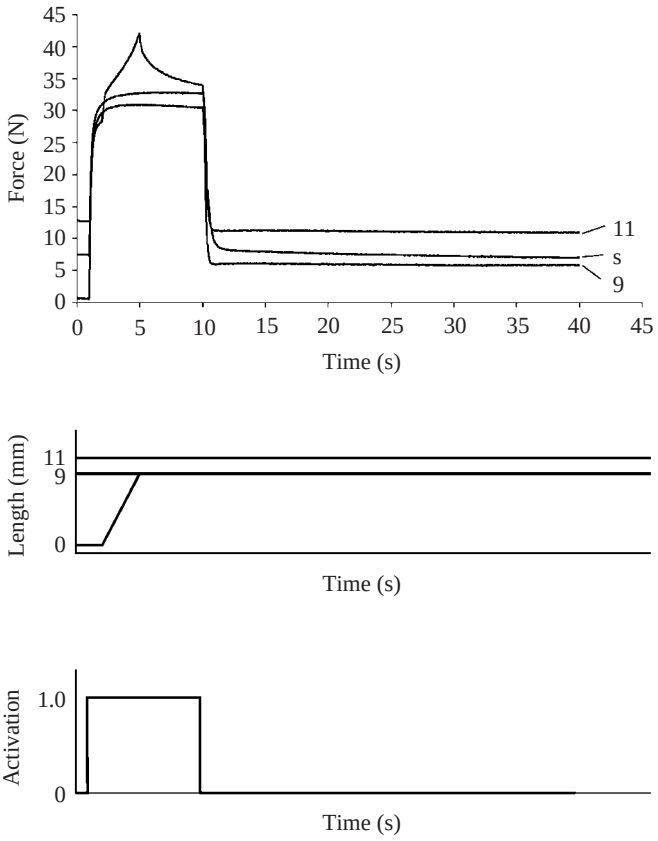

Fig. 1. Representative force–time histories of two isometric and one experimental stretch contraction held for approximately 30 s beyond deactivation. The isometric contractions were performed at lengths of +9 and +11 mm (9, 11, respectively). The stretch test (s) was performed from 0 to +9 mm at a constant speed of 3 mm s^{-1}. Note that the passive force following active stretch is greater than the corresponding passive force following isometric contraction (9), and decays at a greater rate than those of the two isometric contractions (9, 11).

has been achieved, have not been consistent. Sugi and Tsuchiya (1988) reported no increase in stiffness in the force-enhanced state compared to the isometric reference value, whereas Linari et al. (2000) found such an increase in single fibres from frog. Unfortunately, the results by Linari et al. (2000) must be interpreted with caution, as they were obtained very quickly following the stretch (175 ms), and therefore may not be relevant for the steady-state conditions discussed here. Interestingly, the sarcomere length non-uniformity theory of force enhancement predicts a decrease in stiffness in the force-enhanced state compared to the isometric reference state (Morgan et al., 2000). We found a consistent, and statistically significant, increase in muscle stiffness in the steady, force-enhanced state compared to the isometric reference value (Table 1, Fig. 6). The average increase in stiffness (11.5%) was similar in magnitude to the average steady-state force enhancement (15.3%) observed in this study.

Finally, we detected force values in the force-enhanced state that exceeded the active isometric forces at optimal muscle length (Fig. 7). Although these force-enhanced values did not exceed the isometric plateau forces by a great amount (5.3±2.7%), this observation was made consistently in all ten muscles (P<<0.05), and was statistically significant, for all muscles when stretched by 9 mm at a speed of 3 mm s^{-1} to a final length of +3 mm.

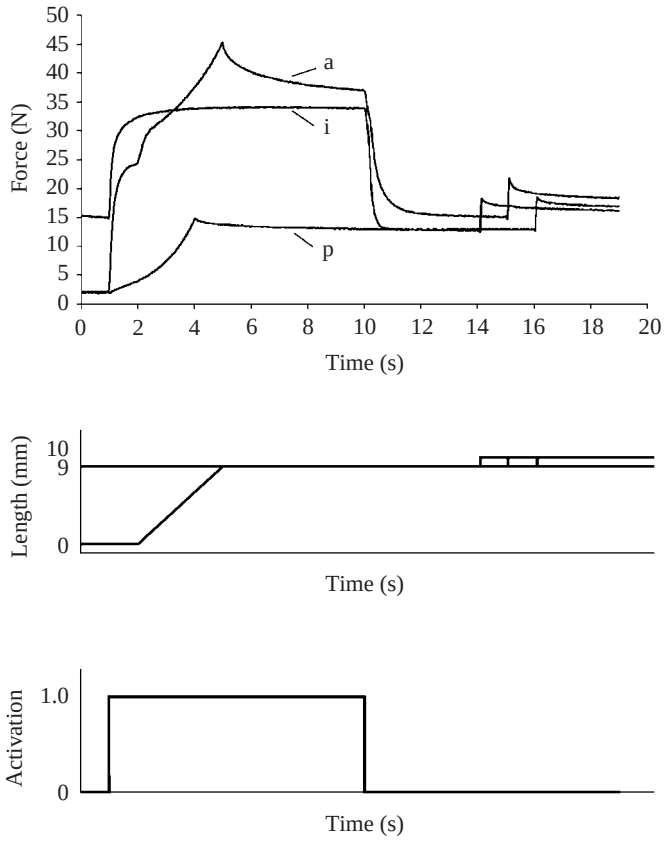

Fig. 2. Representative force–time histories of a passive stretch test (p), the corresponding active stretch test (a, 0 to +9 mm at 3 mm s^{-1}), and the corresponding isometric contraction (i) at a length of +9 mm. Stiffness of the deactivated muscle was determined by a 1 mm stretch at 50 mm s^{-1} at about 5 s following cessation of muscle stimulation. Passive stiffness was significantly greater following the active stretch tests compared to the passive stretch tests and the isometric reference contractions.

Discussion

There are dozens of studies that demonstrate that the steady-state force following active muscle stretching is greater than the corresponding purely isometric force (e.g. Abbott and Aubert, 1952; Edman et al., 1978, 1982; Edman and Tsuchiya, 1996; Linari et al., 2000; Morgan et al., 2000; DeRuiter et al., 2000). However, the mechanisms underlying force enhancement following active stretch are not known. Edman et al. (1978, 1982) speculated more than two decades ago that force enhancement may be associated with the recruitment of an elastic component that is arranged in parallel to the contractile component. They speculated that this parallel elastic component was recruited upon activation and stretch.

Edman and Tsuchiya (1996) found steady-state force

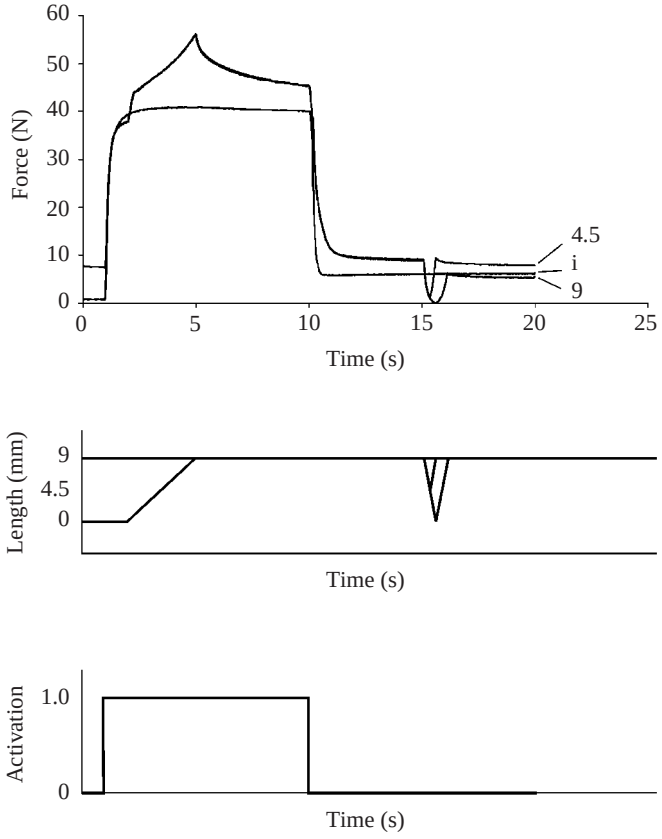

Fig. 3. Representative force–time histories of two identical stretch tests (0 to +9 mm at 3 mm s^{-1}) and the corresponding isometric reference contraction (i) at +9 mm. Approximately 5 s following deactivation, the actively stretched muscles were released and immediately stretched again by 4.5 or 9 mm (4.5 and 9, respectively). When shortened–stretched by 4.5 mm (50% of the active stretch), passive force enhancement was almost completely maintained. When shortened–stretched by 9 mm, passive force enhancement was abolished ‘instantaneously’.

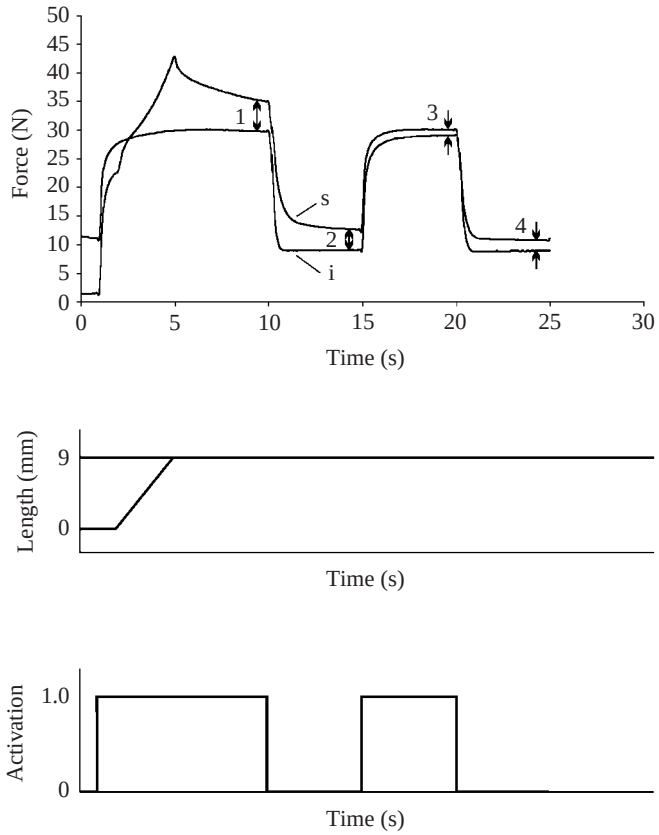

Fig. 4. Representative force–time histories of an isometric reference contraction (i) and an experimental stretch contraction (s, 0 to +9 mm at 3 mm s^{-1}). Following the first activation, the muscle was left deactivated for 5 s before it was activated again at the final length (+9 mm for the isometric reference contraction and the experimental stretch test). Note the passive force enhancement following the first (2) and second (4) deactivation, and the decreased 'passive' force enhancement during the second activation period (3) compared to the passive force enhancement prior to and following the second activation.

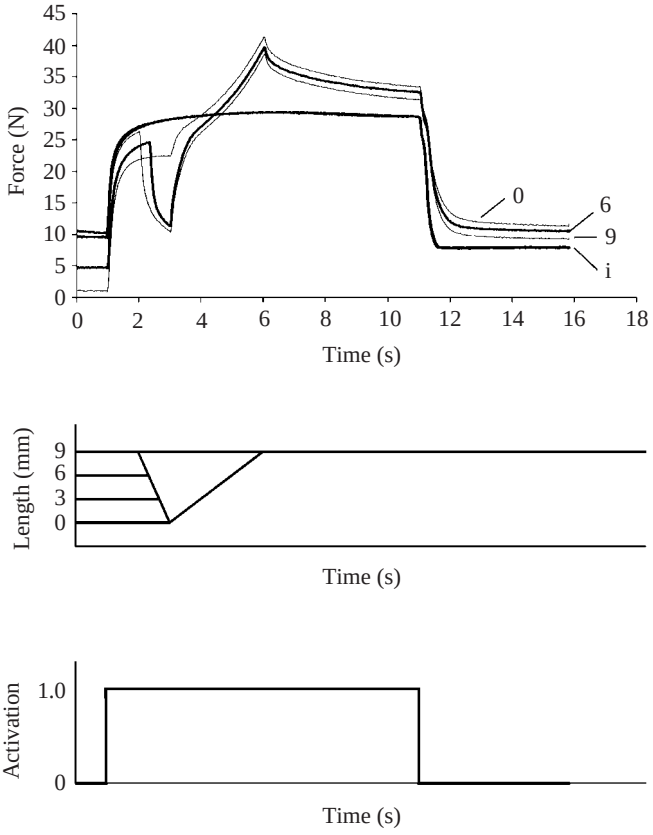

Fig. 5. Representative force–time histories of three experimental stretch contractions (0 to +9 mm at 3 mm s^{-1}) that were preceded by active shortening of 0, 6 and 9 mm (0, 6 and 9, respectively). Also shown is an isometric reference contraction (i, +9 mm). Note how increasing the amount of shortening decreases the total and the passive force enhancement to a similar degree.

enhancement after stretch of frog muscle fibres. This force enhancement was linearly related to the slow component of tension rise during stretch. In addition, when released against a small load, the shortening transients of the previously stretched fibres exhibited a greater and steeper decrease than those obtained from isometric contractions without previous stretching. These results were interpreted as originating from the elongation of a passive, elastic, cytoskeletal protein.

Recently, we found direct evidence for passive force contribution to force enhancement following active stretch in the cat soleus (Herzog and Leonard, 2002). This passive force enhancement was found as a persistent force enhancement following deactivation of an actively stretched muscle. Passive force enhancement was independent of the stretch speed, was directly dependent on stretch magnitude, and contributed as much as 84% to the total force enhancement for the greatest stretch amplitudes tested. Similar results to those obtained for cat soleus have also been obtained for single fibres from frog *Rana pipiens* (Rassier et al., 2003, in press) and for *in vivo* human adductor pollicis (Lee and Herzog, 2002). Although not impossible, it might prove difficult to identify the exact structure(s) responsible for the passive force enhancement. As a first step in this direction, we determined the mechanical properties of the passive force enhancement following active muscle stretch. By doing this, we hoped to gain insight into what structural models might be useful in explaining the passive force enhancement.

Out of the seven tests performed in this study, we felt that the results of three were particularly revealing. The first of these results was the fact that the passive force enhancement was long-lasting and persisted for >25 s in all cases (Fig. 1, Table 1). Edman and Tsuchiya (1996) had proposed a model of passive force enhancement based on strain of elastic elements and variations in filament overlap caused by non-uniform length changes within the fibre volume. They argued that regions with greater filament overlap are likely to generate the steady-state force enhancement following active stretch. The elastic elements that were recruited during stretch were presumed to support regions in which filament overlap had been reduced, thereby providing a force equilibrium. However,

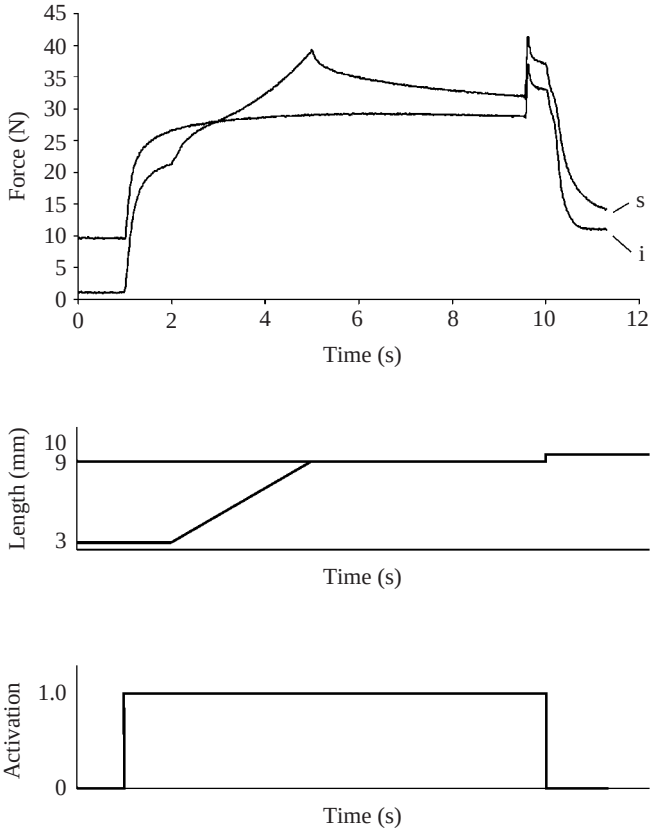

Fig. 6. Representative force–time histories of an experimental stretch test (s, 0 to +9 mm at 3 mm s^{-1}) and the corresponding isometric reference contraction (i) at the final stretch length (+9 mm). At 4.8 s following the end of the active stretch, muscle stiffness was determined by a quick stretch (1 mm at 50 mm s^{-1}). The average stiffness for the experimental stretch contractions was 11.5% greater than the stiffness for the isometric reference contractions.

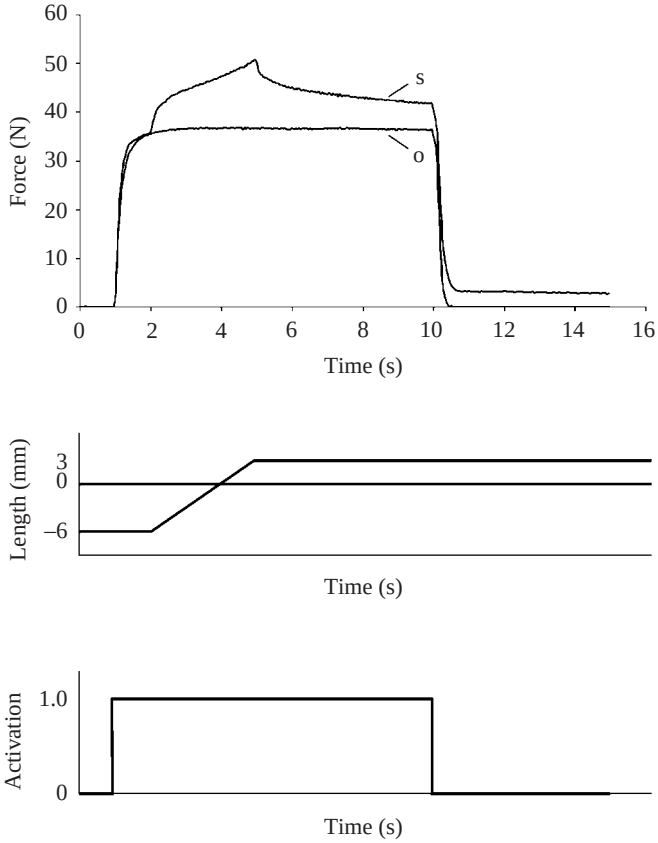

Fig. 7. Representative force–time histories of the active force of an experimental stretch test (s, –6 to +3 mm at 3 mm s^{-1}), and an isometric contraction at the optimum length of the muscle (o). Note that the steady-state isometric force following the active stretch contraction is greater than the purely isometric force at muscle optimum length.

in their model, deactivation of the muscle (fibre), which results in a loss of the active force, would also result in the loss of the passive component of the force enhancement because of the proposed in-series arrangement of the passive and active component (fig. 9B in Edman and Tsuchiya, 1996). However, we found in all cases that passive force enhancement persisted for a long time following deactivation, thereby eliminating the passive force enhancement model by Edman and Tsuchiya (1996), at least for the conditions and the muscle tested here.

The second result that we judged important was that passive force enhancement was reduced in a dose-dependent manner with the magnitude of shortening preceding muscle stretch, whereas the active component of force enhancement was unaffected by shortening (Fig. 5, Table 1). This result suggests that 'engagement' of the passive component of force enhancement occurs at the length at which the muscle is activated. If stretched after activation, the passive component will provide additional force. If shortened, the effect of the passive component of force enhancement decreases in a dose-dependent manner with the magnitude of shortening preceding the stretch. This result is in contrast to the findings by Edman et al. (1982), who reported that force enhancement in single frog fibres was independent of shortening preceding fibre stretching. However, aside from the obvious difference in preparations (cat soleus *vs.* frog tibialis anterior fibres), there was a methodological difference between the two studies that we thought might be crucial. Edman et al. (1982) separated the stretch from the shortening by a 1 s delay, whereas in our experiments, stretch of the soleus followed the shortening instantaneously. It seemed quite possible that the 1 s delay used by Edman et al. (1982) may have reset the 'initial' length of the structures contributing to the passive force enhancement, and may have produced the result that shortening preceding stretch does not influence force enhancement. However, in the meantime we have repeated the shortening–stretch experiments with various delays between the shortening and the stretch phase, as was done by Edman et al. (1982), but we could not reproduce Edman's results, neither in single fibres of frog (N=12) (Herzog and Rassier, 2003) nor in the cat soleus (N=6) (Herzog, 2002).

Probably the most important result of this study was the fact that the passive force enhancement could be eliminated 'instantaneously' by shortening (and stretching back) the

deactivated, passive muscle by 9 mm (i.e. 100% of the stretch amplitude) (Fig. 3, Table 1). In contrast, shortening (and stretching back) by 4.5 mm (50% of the stretch amplitude) left the passive force enhancement virtually unaffected. This result is evidence that the passive force enhancement is not caused by a purely viscoelastic component that is stretched and whose force is slowly decaying following the active stretch. Rather, it appears that a structural protein is either 'engaged' at the initial muscle length, or that the 'stiffness' of a structural protein is changed by active muscle stretch. This event is reversible by shortening the passive muscle to its original length, but is not significantly altered by shortening the muscle by 50% of its active stretch amplitude.

Finally, all of the remaining results support the idea that the passive component of force enhancement must be in parallel with the contractile component. This suggestion is supported by the increased stiffness of the passive and active muscle following active stretch compared to the purely isometric contractions at the corresponding muscle lengths (Figs 2, 6; Table 1). Furthermore, we found steady-state isometric forces that were in excess of the active isometric forces at optimum muscle length (after accounting for the passive forces associated with the increase in muscle length), suggesting that a parallel force component was added to the contractile force (Fig. 7). Finally, the passive force enhancement prior to and following a second isometric contraction (Fig. 4), was significantly greater than the passive force enhancement during the second isometric contraction[1], suggesting that the passive component of force enhancement is in-parallel to the contractile component, and therefore was shorter in the active compared to the passive state. Therefore, passive force enhancement was smaller in the active compared to the passive muscle.

Possible explanation of results

The idea that force enhancement has the properties of a passive 'elastic' element has been proposed, but not directly demonstrated before (Edman et al., 1982; Noble, 1992; Edman and Tsuchiya, 1996; DeRuiter et al., 2000). The molecular spring titin has been suggested to fill this role, and although we do not have direct evidence for a possible contribution of titin to the passive force enhancement observed here, titin's properties and structural arrangement appear consistent with the results found in this study.

First, titin is arranged in parallel with the active force producing cross-bridges, at least if we assume that half-sarocomeres remain uniform in length. If we assumed that half-sarcomere lengths became non-uniform, as did Edman and Tsuchiya (1996), the titin in the elongated half-sarcomere would resist the active forces in the corresponding shortened half-sarcomere, and in that case, titin would be arranged in-series with the active force-producing elements, and the persistent passive force enhancement following deactivation of the stretched muscle observed in this study, would not be possible.

In order for titin to contribute to the force enhancement, as observed here, titin's stiffness, or its characteristic length, would need to change for actively stretching muscle compared to isometrically contracting or passively stretched muscle. There is no direct evidence that titin changes its stiffness or characteristic length upon active stretching. However, titin has been found to change its stiffness under specific conditions. Tatsumi et al. (2001) showed that the secondary structures of the elastic part of titin were changed by the binding of calcium ions. They concluded from this result that the stiffness of titin changes during the contraction–relaxation cycle. Similarly, Yamasaki et al. (2001) found that cardiac titin interacted with actin in a dose-dependent manner based on the concentration of the soluble calcium-binding protein S100A1. These interactions were shown to modulate the passive stiffness, and were hypothesized to provide a mechanism for changing titin-based force prior to active contraction. Summarizing, titin has been found to change its stiffness, and therefore, characteristic force. This change has been associated with calcium concentration. Therefore, it appears feasible to hypothesize that titin's characteristic stiffness may be increased when stretching an active compared to a relaxed muscle. This increased stiffness may be the reason for the observed passive component of force enhancement. This thinking would be consistent with the increased stiffness in the force-enhanced (active and passive) states compared to the isometric states (Fig. 6, Table 1). It would also allow for the possibility to produce steady-state forces following active stretch that exceed the peak active isometric forces at muscle optimum length (Fig. 7). Also, with titin being arranged in parallel to the contractile element (in uniform half-sarcomeres), the result that passive force enhancement is smaller in the active compared to the relaxed muscle (because of contractile element shortening) is also accounted for (Fig. 4). Finally, it is well known that titin's stiffness is associated, in part, with the unfolding of molecular knots in the immunoglobulin region (Rief et al., 1997). It has been shown that refolding of the immunoglobulin domains only occurs when force in titin becomes very low and titin is relaxed to its initial characteristic length. This property of titin would explain why a 4.5 mm shortening of the passive muscle did not abolish the passive force enhancement, but a 9 mm shortening (corresponding to the amount of active stretch) eliminated all passive force enhancement instantaneously.

In summary, we were able to measure some crucial properties of muscle in the passive force-enhanced state. These properties can be used to eliminate possible candidate models, for example, the one proposed by Edman and Tsuchiya (1996), and include others, for example, titin, as long as half sarcomeres remain at uniform length. However, it should be stressed that further experiments must be performed to identify the true source of the passive component of force enhancement.

[1]It has been shown in previous experiments that active force enhancement is abolished following deactivation of the muscle (Abbott and Aubert, 1952; Morgan et al., 2000).

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References

Abbott, B. C. and Aubert, X. M. (1952). The force exerted by active striated muscle during and after change of length. *J. Physiol.* **117**, 77-86.

Baratta, R. and Solomonow, M. (1990). The dynamic response model of nine different skeletal muscles. *IEEE Trans. Biomed. Eng.* **37**, 243-251.

DeRuiter, C. J., Didden, W. J. M., Jones, D. A. and de Haan, A. (2000). The force-velocity relationship of human adductor pollicis muscle during stretch and the effects of fatigue. *J. Physiol.* **526**, 671-681.

Edman, K. A. P., Elzinga, G. and Noble, M. I. M. (1978). Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *J. Physiol.* **281**, 139-155.

Edman, K. A. P., Elzinga, G. and Noble, M. I. M. (1982). Residual force enhancement after stretch of contracting frog single muscle fibers. *J. Gen. Physiol.* **80**, 769-784.

Edman, K. A. P. and Tsuchiya, T. (1996). Strain of passive elements during force enhancement by stretch in frog muscle fibres. *J. Physiol.* **490**, 191-205.

Fenn, W. O. (1924). The relation between the work performed and the energy liberated in muscular contraction. *J. Physiol.* **58**, 373-395.

Forcinito, M., Epstein, M. and Herzog, W. (1998). Can a rheological muscle model predict force depression/enhancement? *J. Biomech.* **31**, 1093-1099.

Gordon, A. M., Huxley, A. F. and Julian, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol.* **184**, 170-192.

Herzog, W. (1998). History dependence of force production in skeletal muscle: a proposal for mechanisms. *J. Electromyogr. Kinesiol.* **8**, 111-117.

Herzog, W. (2002). Force enhancement during and following eccentric muscle contraction. *Proc. IV World Congr. Biomechanics*. Calgary, Canada: The University of Calgary.

Herzog, W. and Leonard, T. R. (1997). Depression of cat soleus forces following isokinetic shortening. *J. Biomech.* **30**, 865-872.

Herzog, W. and Leonard, T. R. (2002). Force enhancement following stretching of skeletal muscle: a new mechanism. *J. Exp. Biol.* **205**, 1275-1283.

Herzog, W. and Rassier, D. E. (2002). History dependence of skeletal muscle force production: a forgotten property. *J. Mech. Med. Biol.* **2**, 347-358.

Herzog, W. and Rassier, D. E. (2003). The effects of shortening on the stretch-induced force enhancement in muscle fibers. *Biophys. J. Book Abstr.* **2739-Pos**, 560a.

Hill, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. Lond.* **126**, 136-195.

Hinkle, D. F., Wiersma, W. and Jurs, S. G. (1979). *Applied Statistics for the Behavioral Sciences*. pp. 332-367. Chicago: Rand McNally College Publishing Co.

Huxley, A. F. (1957). Muscle structure and theories of contraction. *Prog. Biophys. Biophys. Chem.* **7**, 255-318.

Huxley, A. F. and Simmons, R. M. (1971). Proposed mechanism of force generation in striated muscle. *Nature* **233**, 533-538.

Julian, F. J. and Morgan, D. L. (1979). Intersarcomere dynamics during fixed-end tetanic contractions of frog muscle fibres. *J. Physiol.* **293**, 365-378.

Katz, B. (1939). The relation between force and speed in muscular contraction. *J. Physiol.* **96**, 45-64.

Lee, H. D. and Herzog, W. (2002). Force enhancement following muscle stretch of electrically and voluntarily activated human adductor pollicis. *J. Physiol.* **545**, 321-330.

Linari, M., Lucii, L., Reconditi, M., Vannicelli Casoni, M. E., Amenitsch, H., Bernstorff, S. and Piazzesi, G. (2000). A combined mechanical and x-ray diffraction study of stretch potentiation in single frog muscle fibres. *J. Physiol.* **526.3**, 589-596.

Morgan, D. L. (1990). New insights into the behavior of muscle during active lengthening. *Biophys. J.* **57**, 209-221.

Morgan, D. L. (1994). An explanation for residual increased tension in striated muscle after stretch during contraction. *Exp. Physiol.* **79**, 831-838.

Morgan, D. L., Whitehead, N. P., Wise, A. K., Gregory, J. E. and Proske, U. (2000). Tension changes in the cat soleus muscle following slow stretch or shortening of the contracting muscle. *J. Physiol.* **522**, 503-513.

Noble, M. I. M. (1992). Enhancement of mechanical performance of striated muscle by stretch during contraction. *Exp. Physiol.* **77**, 539-552.

Rassier, D. E., Herzog, W. and Pollack, G. H. (in press). Stretch-induced force enhancement and stability of skeletal muscle myofibrils. In *Advances in Experimental Medicine and Biology* (ed. H. Sugi). New York: Kluwer Academic/Plenum Publishers.

Rassier, D. E., Herzog, W., Wakeling, J. and Syme, D. (2003). Stretch-induced, steady-state force enhancement in single skeletal muscle fibers exceeds the isometric force at optimal fibre length. *J. Biomech.* **36**, 1309-1316.

Rief, M., Gautel, M., Oesterhelt, F., Fernandez, J. M. and Gaub, H. E. (1997). Reversible unfolding of individual titin immunoglobulin domains by AFM. *Science* **276**, 1109-1112.

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

Tatsumi, R., Maeda, K., Hattori, A. and Takahashi, K. (2001). Calcium binding to an elastic portion of connectin/titin filaments. *J. Mus. Res. Cell Motil.* **22**, 149-162.

Wakeling, J., Herzog, W. and Syme, D. (2000). Force enhancement and stability in skeletal muscle fibers. *XI Congr. Can. Soc. Biomech.* p. 145. Montreal, Canada: The University of Montreal.

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