The Journal of Experimental Biology 211, 3001-3008
Published by The Company of Biologists 2008
doi:10.1242/jeb.021204

Is a parallel elastic element responsible for the enhancement of steady-state muscle force following active stretch?

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Accepted 17 July 2008

SUMMARY

For over 50 years, it has been recognised that muscles from many different species of animals are able to generate a higher steady-state isometric force after active stretch than during a purely isometric contraction at the same length. This is known as 'residual force enhancement' (rFE). The mechanism underlying this phenomenon remains controversial. One proposal is that an elastic element parallel to the cross-bridges becomes stiffer, or is engaged, when the muscle is activated and generates force when stretched. If this is indeed the sole mechanism, then rFE should be eliminated by subsequently shortening the muscle by a distance equal to or greater than the initial stretch. We tested this hypothesis using six intact single fibres from frog lumbrical muscle. The fibres were activated and stretched to generate rFE and then rapidly shortened by between 25% and 700% of the initial stretch distance. In contrast to previous reports, we found that rapid shortening induced a depression of subsequent isometric force. We used two methods to account for this force depression when calculating rFE, thereby obtaining upper and lower bounds for the true rFE. With both methods of calculation, rFE was significantly greater than zero when shortening distance was equal to stretch distance (P=0.0004 and P=0.03, respectively). Therefore, our hypothesis was not supported. We conclude that rFE is unlikely to be generated solely by a parallel elastic element.

Key words: muscle mechanics, fibre, cell, slack test, eccentric contraction, lengthening.

INTRODUCTION

It is well known that the steady-state isometric force generated by a muscle is higher after active stretch than during a purely isometric contraction at the same muscle length (Abbott and Aubert, 1952). Stretch has also been shown to increase force during subsequent shortening (Edman et al., 1978). This 'residual force enhancement' (rFE) after stretch has been observed in muscles from fish and amphibians (Abbott and Aubert, 1952), as well as in muscles from mammals (Herzog and Leonard, 2002) including humans (Lee and Herzog, 2002). This effect is of particular interest because it is not predicted by the widely accepted 'cross-bridge' model of muscle contraction (Huxley, 1957). Therefore, it is important to understand the mechanism underlying rFE in order to determine whether it can be explained by making minor modifications to our understanding of muscle contraction or whether current models need to be completely revised. Despite extensive study, the mechanism of rFE is still the subject of intense debate (e.g. Herzog et al., 2006; Morgan and Proske, 2007).

One mechanism that has been proposed is that the extra force is provided by an elastic element, which acts in parallel to the cross-bridges. It is proposed that this element stiffens, or is engaged, when the muscle is activated so that it exerts more force when the muscle is stretched while activated than it would if the muscle were stretched while inactive and then activated isometrically (Fig. 1). This mechanism was first proposed in 1978 by Edman et al. and has found recent support as a partial (Herzog and Leonard, 2002) or complete (Pinniger et al., 2006; Telley and Denoth, 2007) explanation for rFE. It has been suggested that the elastic element involved could be titin (Bagni et al., 2002; Herzog and Leonard, 2002), a protein that is an important contributor to passive force in many muscles (Tskhovrebova and Trinick, 2003). There is evidence that the stiffness of titin increases upon muscle activation in response to the rise in intracellular calcium concentration (Joumaa et al., 2008; Labeit et al., 2003).

The above mechanism is appealing because it corresponds well with the known properties of rFE. The force exerted by an elastic element increases with the magnitude of stretch and is independent of the velocity of stretch, and rFE has been shown to have similar characteristics (Edman et al., 1978; Edman et al., 1982; Schachar et al., 2004). Previously, we have shown that when stretch distance is increased, by starting the stretch at progressively shorter initial lengths, the amount of rFE increases with stretch magnitude only up to a certain point and then levels off (Bullimore et al., 2007). This is consistent with an elastic element that has very low stiffness at short lengths, which is typical of biological soft tissues (Fung, 1967). The absolute amount of rFE generated by a given stretch shows only small changes in response to the application of drugs that have a substantial influence on the force produced by the cross-bridges (Bagni et al., 2002; Pinniger et al., 2006; Rassier and Herzog, 2004a) and is maximum at a much longer muscle length than that at which the cross-bridges produce maximum force (Edman et al., 1982; Schachar et al., 2004). This suggests that rFE is generated by structures other than the cross-bridges. rFE has also been shown to be associated with an increase in the passive force remaining after the muscle has been deactivated ('passive force enhancement'), suggesting that it may be associated with modification of a passive element (Herzog and Leonard, 2002).

One way of testing this proposed mechanism is to activate a muscle, allow it to shorten and then to stretch it again by the same amount or, conversely, to stretch it and then allow it to shorten by the same amount. In either case, no rFE should result from the stretch because an elastic element that is shortened or stretched and then returned to its original length generates the same force as it did initially. Several experiments of this type have been performed with conflicting results. Edman et al. found that rapid shortening performed either ~1 s before or immediately before, a stretch had no effect on rFE compared with the stretch alone (Edman et al., 1982; Edman et al., 1984). Herzog and Leonard and Lee et al. found that when stretch was followed by shortening the effects of the stretch were eliminated, leaving a depression of isometric force as would have occurred with shortening alone (Herzog and Leonard, 2000; Lee et al., 2001). When shortening was followed by stretch, the rFE after stretch was either eliminated (Herzog and Leonard, 2000) or substantially reduced (Lee et al., 2001). However, interpretation of these two studies is complicated by the fact that, because they were designed to address a different question, they used low velocities of shortening at which a substantial amount of force was produced. Shortening under these conditions is known to depress the subsequent isometric muscle force, whereas shortening at high or maximal velocities has been found not to cause force depression (Edman et al., 1993; Herzog and Leonard, 1997; Herzog and Leonard, 2007; Marechal and Plaghki, 1979). This factor makes it difficult to determine whether the measured reduction in rFE was due to the superimposed effects of this force depression or to a direct reduction in the rFE itself. Rassier and Herzog also found that prior shortening reduced the rFE induced by stretch but again used sub-maximal shortening velocities (Rassier and Herzog, 2004b). In general, a difficulty with interpreting all studies where shortening is performed before stretch is that, as we do not know the identity of the elastic element or the mechanism of stiffening, we do not know whether the resting length of the element would be re-established after shortening, allowing it to still generate rFE during a subsequent stretch. For example, titin is known to form bonds with actin (Bianco et al., 2007) and it is possible that such bonds could reform during and after shortening so that titin would still be loaded during a subsequent stretch.

The present study was designed to overcome these limitations. Shortening was performed after stretch, to avoid the possibility of the elastic element being re-established at the shorter length, and was at maximal shortening velocity to avoid inducing force depression. It was hypothesised that rFE would be eliminated by shortening a distance equal to or greater than the initial stretch.

MATERIALS AND METHODS

Muscle fibres and apparatus

We used six intact, single fibres dissected from the lumbrical muscles of the frog, *Xenopus laevis* (Daudin). All procedures were approved by an Animal Care Committee at the University of Calgary, Alberta, Canada. The fibres were measured using an optical microscope fitted with an eyepiece reticle. Fibre length, measured with the fibre just pulled taut in the dissection bath, was 1.37–2.15 mm. Cross-sectional area, determined by measuring the maximum and minimum diameters at the midpoint of the fibre and assuming an elliptical cross-section, was 1940–11,570 μm^2.

The experiments were performed using a mechanical testing apparatus (model 801A; Aurora Scientific, Ontario, Canada). The fibre was suspended between a force transducer (model 400A; step response time, 0.3 ms) and a length controller (model 322C-I; step response time, 0.7 ms) in a bath containing Ringer's solution (composition: NaCl, 115 mmol l^{-1}; KCl, 2.5 mmol l^{-1}; $CaCl_2$, 1.8 mmol l^{-1}; Na_2HPO_4, 2.15 mmol l^{-1}; NaH_2PO_4, 0.85 mmol l^{-1}; pH 7.0). The solution was stored in a 500 ml reservoir adjacent to the apparatus and was circulated through the bath at a rate greater than 0.45 ml min^{-1} during the experiment. Bath temperature was measured using a thermocouple probe placed within a few millimetres of the fibre and was controlled using Peltier modules and a feedback control loop. The mean temperature of individual experiments was varied between 7.7 and 13°C depending on the temperature at which the fibre produced a good force response; however, temperature did not vary by more than 0.6°C during any individual experiment or by more than 0.3°C (usually less than 0.1°C) between contractions that were compared in order to calculate rFE. Electrical stimulation was delivered using two platinum plate electrodes situated alongside the fibre. Stimulus pulses were 0.5 ms square waves, and current was adjusted to the lowest value that gave maximal twitch force at room temperature (55–90 mA).

The fibres were attached to the force transducer and length controller *via* thin wire hooks. They were attached to these hooks using aluminium foil clips, which were folded around the tendons at the ends of the fibres. The clips were modified from those described by Ford et al. so that they had pieces that could be wrapped around the shaft of the hook to prevent movement of the clip with respect to the hook (Ford et al., 1977).

Experimental protocol

During the initial stages of the experiment, 1 s contractions with 2 or 3 min rest periods were used. Stimulation frequency was gradually increased in sequential contractions until a fused contraction was obtained. Ten 'conditioning' contractions were performed at this frequency to bring the fibre into a state where peak force was approximately constant in successive tetani. Stimulation frequency was then adjusted to give maximal isometric force, and a force–length relationship was obtained at this frequency by performing seven isometric contractions at intervals of 5% fibre length. The relationship between peak force and fibre length was fitted with a second-order polynomial and the length at which force was maximal (optimal length, L_{opt}) was identified to the nearest 2.5% fibre length. Passive forces were typically low over the range used (1.2–3.2% of maximal isometric force at 1.2 L_{opt}) and subtracting passive force did not affect the choice of L_{opt}. The maximum isometric stress generated by the fibres was 270 kPa to 360 kPa, which is consistent with previous measurements on *Xenopus laevis* twitch fibres over a similar temperature range (Lannergren et al., 1982).

For the remainder of the experiment, 3 s contractions with 6 min rest periods were used. The stimulation frequency was adjusted to give contractions that were fused, or almost fused, and could be sustained for 3 s (23 Hz to 46 Hz). The fact that the stimulation frequency was slightly less than maximal was not a concern because it has been shown that halving the stimulation rate relative to that required for a fused contraction does not significantly affect the rFE expressed as a percentage of isometric force (Rassier and Herzog, 2005).

rFE was elicited by applying stretches of 5% fibre length, with a final length of 1.2 L_{opt} and a speed of 0.1 fibre lengths s^{-1}. The stretch was followed by a rapid shortening with a distance equal to 0, 25, 50, 75, 100, 150, 200, 300 or 400% of stretch distance. In some fibres, shortening distances of 500, 600 and 700% stretch distance were also applied. To allow the fibres to shorten at their maximum velocity, the lever arm of the length controller was

repositioned as quickly as possible. The speed of the lever arm was always at least 2.9 times the maximal velocity of the fibre (as measured using the 'slack test', see below).

Three contractions were performed for each shortening distance in the following order: (1) 'isometric-shortening' – the fibre was activated at $1.2\,L_{opt}$, held isometric for 1.5 s, then rapidly shortened by the specified distance and held isometric for the remainder of the activation period; (2) 'purely isometric' – the fibre was activated and held isometric at the final length of the corresponding isometric-shortening contraction; and (3) 'stretch-shortening' the fibre was activated at $1.15\,L_{opt}$, stretched to $1.2\,L_{opt}$, held isometric for 600 ms, and then rapidly shortened by the specified distance and held isometric for the remainder of the activation period. The isometric-shortening and purely isometric contractions provided control conditions relative to which rFE in the stretch-shortening contractions was calculated. The isometric-shortening contraction was compared with the purely isometric contraction to determine whether shortening caused any force depression. Contractions were performed in the above order so that a higher force in the stretch-shortening compared with the other two contractions or a lower force in the isometric-shortening contraction compared with the purely isometric contraction could not be attributed to fatigue. Shortening was performed 600 ms after stretch as a compromise between allowing some of the stretch-induced transients to subside and avoiding fatigue due to excessively long periods of activation. We did not expect these transients to influence the final force however because they are eliminated by rapid shortening (Edman et al., 1984). To determine the longitudinal stiffness of the fibre, a short rapid stretch (0.25% fibre length in 1 ms) was performed immediately before the end of activation. An example of raw data from one set of three contractions with a shortening distance of 100% of stretch distance is shown in Fig. 2.

Throughout the experiment, force immediately before shortening in each isometric-shortening contraction was recorded to monitor fibre condition. This force varied by less than 4.5% during the testing of each of the fibres.

As force depression was unexpectedly observed after rapid shortening, several tests were performed on two fibres to investigate this further. In all cases, the fibres were allowed to shorten at their maximum velocity, as in the rest of the experiment. Fibre 1 was always shortened from $1.2\,L_{opt}$ to $1.1\,L_{opt}$ and fibre 2 was always shortened from $1.1\,L_{opt}$ to $1.05\,L_{opt}$ but the stimulation rate and number of periods of force redevelopment were varied. The reasoning behind the investigations performed is explained in the Discussion. Force depression was calculated as described below by comparing force in the shortening contractions with force in a purely isometric contraction at the final length (which was performed immediately after the shortening contraction in question). In the control condition, the shortening was performed at the same time as in the isometric-shortening contractions used in the rest of the study (1.5 s into activation). To investigate the effect of increasing stimulation frequency, the control condition was repeated but with a 10 Hz increase in stimulation frequency in both the shortening and purely isometric contractions. The number of periods of force development occurring during a contraction was varied by: (1) performing the shortening 20 ms into activation so that there was only one period of force development (as opposed to two periods in the isometric-shortening contractions: initial force development and force redevelopment after shortening); and (2) breaking the shortening into two or (in one fibre) three equal steps at intervals of 1 s or 0.5 s, respectively, to give three or four periods of force development. When shortening was broken into multiple steps, the final step was always performed at the same time as the shortening in the control contraction.

Data recording and analysis

Force and length data were recorded at 10 kHz. To reduce file size and processing time, the data points were then averaged in groups of 10 to produce smoothed data at 1 kHz, except in the regions of the rapid force changes during shortening and the stiffness test, which were left at 10 kHz. These smaller files were analysed in order to obtain the data and figures presented here.

The force at the end of each contraction was calculated by taking the mean force over a $100\,ms^{-1}$ period immediately before the stiffness test, i.e. beginning 1.39 s after shortening (and at the equivalent time during the purely isometric contraction). rFE was calculated in two ways: (1) by subtracting force in the isometric-shortening contraction from force in the stretch-shortening contraction and expressing the result as a percentage of force in the isometric-shortening contraction; and (2) by subtracting force in the purely isometric contraction from force in the stretch-shortening contraction and expressing the result as a percentage of force in the purely isometric contraction. Force depression was calculated by subtracting force in the isometric-shortening contraction from force in the purely isometric contraction and expressing the result as a percentage of force in the purely isometric contraction.

The slopes of the force–time records were determined by linear regression over a 500 ms period immediately before the stiffness test. They were compared between the three different types of contraction and used to determine whether the force traces were converging or diverging at the time when rFE and force depression were calculated. Stiffness was calculated by dividing the change in force during the stiffness test by the change in length.

The protocol used in the present study, with a series of rapid shortening steps of different distances, is similar to the 'slack test' used to measure maximal shortening velocity (Edman, 1979). We took advantage of this to determine whether maximal shortening velocity was influenced by prior stretch. Shortening steps that ended at lengths shorter than $0.95\,L_{opt}$ (i.e. those greater than 500%) were excluded because maximal shortening velocity is known to decrease at short muscle lengths (Edman, 1979), probably due to internal resistance within the fibre. Shorter steps, where there was not an appreciable duration of unloaded shortening (those less than 200 or 300% depending on the fibre), were also excluded. In two fibres, shortening steps of 250 and 350% were included in the slack test, although they were excluded from the rest of the analysis because they were not performed by the other fibres. This gave three to five data points for each slack test. The 10 kHz force data were smoothed by applying a 21 point moving average. Visual examination confirmed that the smoothed curve remained centred within the range of the variation caused by noise in the raw data. For each contraction, the time after the start of shortening at which force began to redevelop was calculated. This was determined by finding the time at which force crossed a threshold of 5 μN above baseline (approximately 0.25% maximal isometric force). This was typically about 10 ms after the force first began to rise but was used instead of the initial rise because it was easier to detect accurately and objectively. This overestimate of the time for force development will not influence the calculated velocity as long as the initial rate of force rise is independent of the size of the shortening step. This seems a reasonable assumption because the initial rate of force rise is expected to depend primarily upon the properties of the series elasticity (which should be independent of step size) and the force–velocity relationship (which should vary little with step size

because all final lengths were close to the plateau of the force–length relationship). The relationship between time of force redevelopment and step size was analysed separately for the isometric-shortening and stretch-shortening contractions using linear regression, and maximal shortening velocity was determined by taking the inverse of the calculated slope. Time for force redevelopment was treated as the dependent variable because it was expected to include more error than step size.

Statistical analysis

Significant differences of rFE and force depression from zero were detected by calculating 95% confidence intervals for each shortening distance using the *t*-distribution for N–1 degrees of freedom, where N is the number of fibres. For example, for shortening steps that were performed by all fibres (N=6), the confidence interval was calculated as 2.571 (± s.e.m.). If the confidence interval did not include zero, the data point was taken to be significantly different from zero. The data for stiffness and for force–time slope were analysed using analysis of variance (anovan.m, Matlab release 13, The Mathworks Inc., Natick, MA, USA) with fibre, shortening distance and contraction type (isometric-shortening, stretch-shortening or purely isometric) as factors and two-factor interactions included. This analysis indicated, however, that there were significant interactions between shortening distance and contraction type, so the data were reanalysed for each shortening distance separately with fibre and contraction type as factors. Because there were significant differences between fibres, only shortening distances that were performed by all fibres were included. *Post hoc* comparisons for the effect of contraction type were made using a Bonferroni adjustment for multiple comparisons (multcompare.m, Matlab). Maximal shortening velocities in the isometric-shortening and stretch-shortening contractions were compared using a paired *t*-test (Microsoft Excel). Throughout, P<0.05 was considered significant.

RESULTS

Our aim was to determine the effect of subsequent shortening on the rFE induced by a stretch. We allowed the fibres to shorten rapidly in order to avoid shortening-induced force depression. However, despite this, we did observe some force depression, i.e. force at the end of the isometric-shortening contractions was consistently lower than force at the end of the purely isometric contractions. This force depression was maximal for shortening distances between 150 and 300% stretch distance and was absent for shortening distances equal to or greater than 600% stretch distance (Fig. 3).

The occurrence of force depression meant that rFE calculated relative to the isometric-shortening and purely isometric contractions was not the same (Fig. 4). These two methods of calculation provide maximum and minimum bounds, respectively, for the true rFE (see Discussion). rFE calculated relative to the isometric-shortening contraction decreased as shortening distance increased but when shortening distance was 100% of the stretch distance rFE was still significantly above zero [7.6±2.3% (mean ± s.d.); P=0.0004]. It remained significantly above zero until a shortening distance of 500% stretch distance was reached. rFE calculated relative to the purely isometric contraction also initially decreased with shortening distance and was significantly above zero when shortening distance was 100% of stretch distance [1.4±1.2% (mean ± s.d.); P=0.03]. However, rFE calculated using this method was significantly less than zero for shortening distances between 200 and 400% of stretch distance suggesting that it was an underestimate of the true rFE.

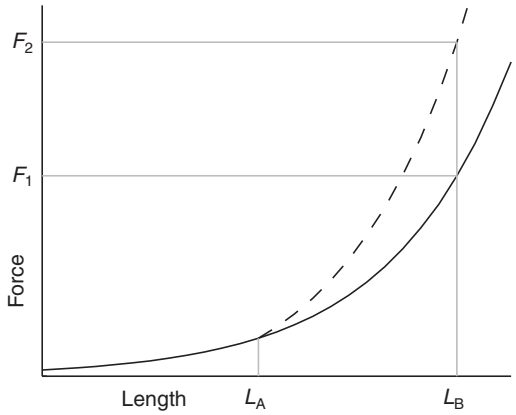

Fig. 1. Schematic illustrating how residual force enhancement (rFE) could be generated by a parallel elastic component (PEC) that increases in stiffness when the muscle is activated. The solid line is the force–length relationship of the PEC in a relaxed muscle. The broken line is the force-length relationship of the PEC when the muscle is activated with the PEC at the length L_A. The stiffness of the PEC, but not the force, increases upon activation. If an isometric contraction is performed when the PEC is at L_B, it exerts a force F_1. However, if the muscle is activated when the PEC is at L_A and is then stretched until the PEC reaches L_B, the PEC force will be F_2. The rFE is F_2–F_1.

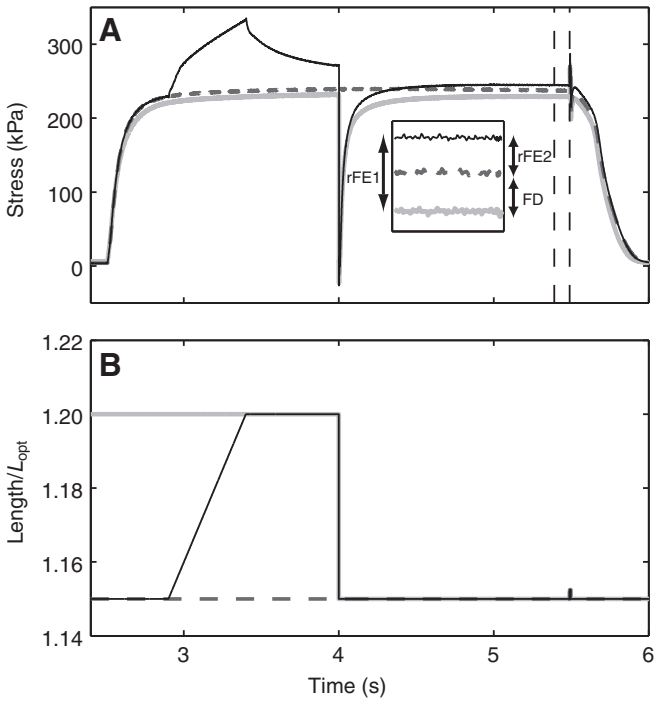

Fig. 2. Example of raw data for stress against time (A) and length against time (B) for one set of three contractions with a shortening distance equal to 100% of stretch distance. L_{opt}=optimal length. Isometric-shortening contraction–thick, solid, light-grey line; purely isometric contraction–thick, broken, dark-grey line; stretch-shortening contraction–thin, solid, black line. Force is transiently negative after shortening because of damped oscillations, which may have been caused by vibrations in the wire hook attached to the force transducer. Vertical broken lines indicate the period over which mean force was calculated before the stiffness test. Inset shows expansion of force records between broken lines, with double-headed arrows indicating the force enhancement calculated by two different methods (rFE1,rFE2) and the force depression (FD).

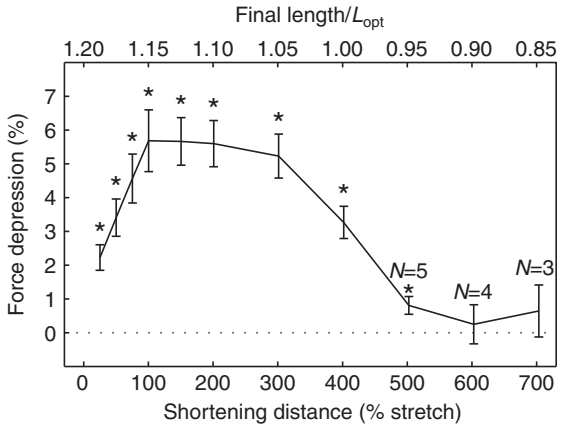

Fig. 3. Force depression (% isometric force) induced by rapid shortening through various distances (shortening distance expressed as a percentage of stretch distance in the corresponding stretch-shortening contraction for consistency with the other figures). Top axis gives corresponding final fibre length relative to optimal length (L_{opt}). Error bars indicate means ± s.e.m. Labels give number of fibres (*N*) when this was less than six. *Different from zero, $P<0.05$.

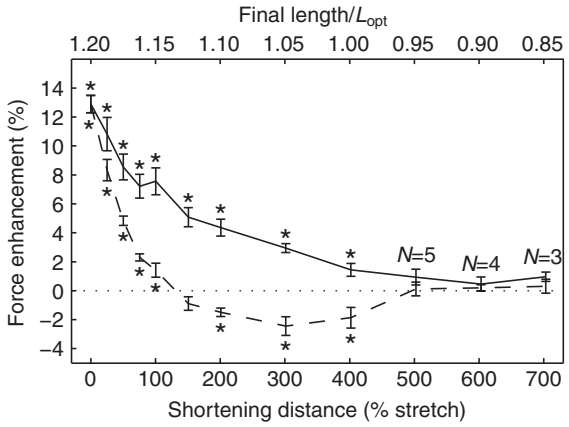

Fig. 4. Residual force enhancement remaining after rapid shortening by various distances (shortening distance expressed as a percentage of stretch distance). Force enhancement is calculated in two different ways: (A) relative to a contraction without stretch but with shortening (solid line); and (B) relative to a purely isometric contraction (broken line). These two methods represent maximum and minimum bounds, respectively, for the true residual force enhancement. In both cases, force enhancement was significantly greater than zero when shortening distance was 100% of stretch distance. The first data point shows the residual force enhancement without shortening. Top axis gives corresponding final fibre length relative to optimal length (L_{opt}). Error bars indicate means ± s.e.m. Labels give number of fibres (*N*) when this was less than six. *Different from zero, $P<0.05$.

The observed force depression was further investigated in two fibres by varying the stimulation frequency and number of periods of force development during a contraction. In both fibres, force depression increased when stimulation frequency was increased by 10 Hz and increased as the number of periods of force development increased (Fig. 5).

For all three types of contraction, the mean force–time slopes were negative when force was measured. For shortening distances up to and including 150%, the slopes of the stretch-shortening and isometric-shortening contractions were significantly different, such

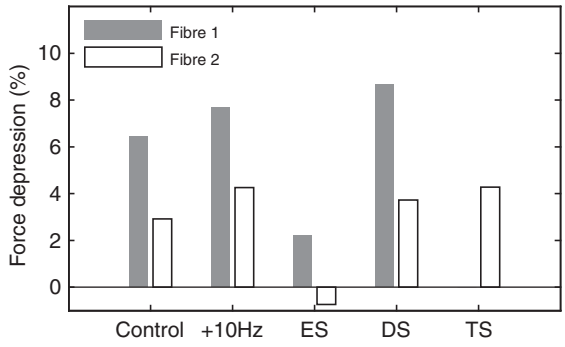

Fig. 5. Effect of altering stimulation rate and number of periods of force redevelopment on the force depression in two muscle fibres. 'Control', same conditions as in the rest of the study with shortening 1500 ms into activation so that there were two periods of force development; '+10Hz', same as 'Control' except for a 10 Hz increase in stimulation frequency; 'ES' (early-shortening), shortening performed 20 ms into activation so that there was only one period of force development; 'DS' (double-shortening), shortening broken into two equal steps with a 1000 ms gap so that there were three periods of force development; 'TS' (triple-shortening), shortening broken into three equal steps with 500 ms gaps so that there were four periods of force development (only performed on fibre 2). For all trials performed on each fibre, total shortening distance and final length were the same. However, shortening distance and final length were different in the two fibres (see Materials and methods), which may explain the different magnitudes of force depression observed. Force depression increased when stimulation frequency was increased, in contrast to what would be expected for the 'movement effect' (Edman, 1975; Edman, 1980). Force depression increased as the number of periods of force development was increased but the relationship was not linear.

that they were converging. This means that if rFE had been measured later it would have been smaller. However, the rate of convergence was low. For example, for a shortening distance of 100%, the mean rate of convergence was 0.008±0.005 mN s^{-1} (mean ± s.d.). If this rate had been maintained, the mean time taken to eliminate the rFE would have been 16.2±7.6 s (mean ± s.d.). Therefore, the rFE that we observed at this shortening distance can be considered long-lasting. For shortening distances between 200 and 400%, the force–time slopes were not significantly different in the stretch-shortening and isometric-shortening contractions. This is important because it shows that, over a range of shortening distances for which we had hypothesised that rFE would be absent, the observed rFE was not, on average, changing significantly over time.

For all shortening distances, the force–time slopes for the stretch-shortening and purely isometric contractions were significantly different such that they were diverging, i.e. rFE calculated relative to the purely isometric contractions was increasing with time.

For all shortening distances, the force–time slopes for the isometric-shortening and purely isometric contractions were significantly different such that they were converging, i.e. force depression was decreasing with time. For a shortening distance of 100%, the rate of convergence was 0.024±0.006 mN s^{-1} (mean ± s.d.). If this rate of convergence had been maintained, the force depression would have been eliminated after a further 3.3±0.6 s (mean ± s.d.).

When the stiffness data were analysed using an ANOVA that combined all shortening distances and all fibres, there was a significant interaction between contraction type and shortening distance. When the data were analysed for each shortening distance separately, stiffness in the isometric-shortening contractions was

significantly lower than in the purely isometric contractions for shortening distances of 25, 75, 150 and 300% and significantly lower than in the stretch-shortening contractions for shortening distances of 25, 50, 75 and 300%.

Maximal shortening velocity was not significantly affected by whether shortening was preceded by active stretch or by a purely isometric contraction (isometric-shortening, 3.76±0.96 fibre lengths s^{-1}; stretch-shortening, 3.75±0.60 fibre lengths s^{-1} (mean ± s.d.); P=0.97).

DISCUSSION

Implications for the mechanism of residual force enhancement

To determine whether a parallel elastic element is responsible for rFE, we tested the hypothesis that rFE is eliminated by shortening the muscle by a distance equal to or greater than the initial stretch. We used rapid shortening in order to avoid shortening-induced force depression. However, despite this, we did observe force depression after shortening. This observation was consistent across all fibres and is interesting as it differs from what has been observed previously (Edman et al., 1993; Herzog and Leonard, 1997; Herzog and Leonard, 2007; Marechal and Plaghki, 1979). This observation has potentially important implications for understanding the mechanism underlying force depression. Possible reasons for the difference between the results of the present study and those of previous studies are discussed below.

The occurrence of shortening-induced force depression meant that the shortening could have had a dual effect on the force after stretch, i.e. a direct depression of force, as well as a reduction in rFE. Calculating the rFE relative to the isometric-shortening contraction implicitly assumed that the direct force depression was the same regardless of whether shortening was preceded by stretch. This is supported by studies that have found that prior stretch did not influence the amount of force depression induced by subsequent shortening (Herzog and Leonard, 2000; Lee et al., 2001). However, these studies used slower shortening than the present study and a different preparation so the findings may not be directly applicable. It is possible that, under the conditions used in the present study, prior stretch protected the fibres from the effects of shortening to some extent, so that less force depression occurred after stretch. In this case, rFE calculated relative to the isometric-shortening contraction would be an overestimate.

The opposite extreme is to assume that prior stretch completely protects the fibres from shortening-induced force depression. If this is true then rFE should be calculated relative to the purely isometric contraction. However, rFE calculated in this way was significantly below zero for shortening distances of between 200 and 400% of stretch distance (Fig. 4), i.e. force in the stretch-shortening contractions was consistently lower than in the purely isometric contractions over this range. It is unlikely that this depression of force was due to the stretch because stretch is consistently found to enhance subsequent force production (Herzog et al., 2006). Therefore, this decrease in force can be attributed to the effects of shortening and implies that the stretch did not completely protect the fibres from force depression. This indicates that rFE calculated by this method is probably an underestimate.

The true rFE is therefore expected to lie between the two curves shown in Fig. 4. For both curves the rFE for a shortening distance equal to 100% of stretch distance was significantly above zero. Therefore, we can reject the hypothesis that rapid shortening by a distance equal to the initial stretch eliminates rFE. We conclude that, although stiffening of a parallel elastic element may play a role in rFE, it is unlikely to provide a complete explanation.

Our findings also do not support the idea that rFE is generated solely by cross-bridges that become ‘stuck’ or detach very slowly after stretch because a period of rapid shortening would force these cross-bridges to detach. An alternative idea is that the forced detachment of cross-bridges during stretch puts them into an alternative biochemical state, which enables them to generate more force than usual (Herzog, 1998) and, which persists despite rapid shortening. In this case, the results of the present study suggest that the number of cross-bridges remaining in this state decreases as the period of shortening increases. We found no difference in maximal shortening velocity following a stretch or an isometric contraction, which concurs with previous findings based on the slack test (Edman and Tsuchiya, 1996) and extrapolation of the hyperbolic force-velocity relationship (Edman et al., 1978; Sugi and Tsuchiya, 1981). Therefore, any change in the cross-bridges that is induced by stretch does not produce a detectable change in maximal shortening velocity.

The finding that stiffness was significantly lower in the isometric-shortening contractions compared with the other two conditions suggests that stretch may have caused an increase in stiffness whereas shortening caused a decrease. In this case, the lack of a difference in stiffness in the stretch-shortening and purely isometric contractions could be explained by the effects of stretch and shortening cancelling out whereas the lower stiffness in the isometric-shortening conditions could be explained by the effect of stretch alone. This explanation is also compatible with the observation that significant differences in stiffness tended to occur at smaller shortening distances where force depression was high (Fig. 3), although the difference in stiffness was not always significant over this range.

Titin is known to bind to actin (Jin, 1995; Soteriou et al., 1993) and to modify *in vitro* motility of actin moving on myosin (Li et al., 1995; Niederländer et al., 2004). However it is not known whether these properties have an influence on active muscle mechanics *in vivo*. Bianco et al. used optical tweezers to measure the rupture forces of titin–actin bonds and concluded that these bonds could act to stabilise resting muscle structure and to modulate the viscous properties of active muscle (Bianco et al., 2007). If these interactions are also able to influence steady-state active muscle forces then this raises the possibility that differences in titin–actin bonding might play a role in the higher forces observed at the end of the stretch-shortening contractions in the present study.

Possible explanations for the force depression induced by rapid shortening

In contrast to the present findings, previous studies have not found any force depression after rapid shortening (Edman et al., 1993; Herzog and Leonard, 1997; Herzog and Leonard, 2007; Marechal and Plaghki, 1979). This difference may be due to differences in methodology. Edman et al., shortened muscle fibres at the beginning of activation before significant force had been developed [see figs 2 and 3 in Edman et al. (Edman et al., 1993)] whereas we shortened muscle fibres from the isometric plateau. When we shortened early in activation in two fibres we found a small force depression in one fibre and a small increase in force in the other fibre (Fig. 5). Herzog and Leonard measured force depression using a final muscle length on the ascending limb of the force–length relationship (Herzog and Leonard, 2007) whereas, for shortening distances up to 400% stretch distance, we used final lengths on the descending limb. Herzog and Leonard did not determine the force–length relationship but may also have used final lengths on the ascending limb (Herzog and Leonard,

1997). However, Maréchal and Plaghki used a very similar protocol to that used in the present study (shortening by 7% fibre length from the tension plateau on the descending limb of the force–length relationship) but did not find any force depression after shortening at the highest velocity that they used (Maréchal and Plaghki, 1979).

In addition to the methodological differences discussed above, two other possible explanations for the observed force depression were considered. The first explanation was that we observed the shortening induced force depression or 'movement effect' described by Edman (Edman, 1975; Edman, 1980). This is different from the permanent depression of isometric force described by Maréchal and Plaghki (Maréchal and Plaghki, 1979) and Herzog and Leonard (Herzog and Leonard, 1997) in that it is transient, is not influenced by the velocity of shortening and decreases as activation level increases. Edman reported that the movement effect lasts up to 800–900 ms after shortening (Edman, 1975; Edman, 1980). By contrast, we found a depression of force almost 1.5 s after shortening and calculated that this would have remained for at least another 3.3 s if stimulation had continued. When we increased stimulation frequency by 10 Hz in two fibres, percentage force depression also increased (Fig. 5) whereas the movement effect would be expected to decrease. Therefore, the force depression that we observed does not appear to be the same phenomenon as described by Edman.

The second explanation for the observed force depression that was considered was that it was the same phenomenon as described by Maréchal and Plaghki (Maréchal and Plaghki, 1979), and that it did not occur during the period of rapid unloaded shortening but during the time when force was redeveloping and the sarcomeres were shortening and doing work against the tendons at the end of the fibre. The muscle fibres that we used were relatively short so that a given extension of the tendons would allow more shortening per sarcomere than in a longer fibre. The purely isometric contraction with which the isometric-shortening contraction was compared also included a period of force development when the muscle was initially activated. Therefore, this explanation only makes sense if some of the effects of the initial period of force development in the isometric-shortening contraction persisted despite the rapid shortening and the fact that force remained at zero for an appreciable time for the longer shortening distances. This is consistent with the findings that rapid shortening, during which force dropped to zero, did not completely eliminate the force depression induced by a previous period of slow shortening (Herzog and Leonard, 2007).

To investigate this idea further, we performed a series of tests in two fibres in which the distance of shortening was kept constant but the number of periods of force development was varied (Fig. 5). In both fibres we found that the amount of force depression increased with the number of periods of force development, as would be expected if the force depression arose during force development. Lee et al. also determined the effect of using multiple shortening steps (Lee et al., 2001). They found that the number of steps did not affect the amount of force depression. However, because they used slow speeds of shortening, which would be expected to induce a large amount of force depression, it is likely that the force depression during force redevelopment was insignificant relative to that during shortening, explaining the difference from the results observed in the present study.

The preliminary investigations that we performed support the possibility that the force depression arose during force development rather than during the period of unloaded shortening but further experiments with a greater number of fibres are needed to confirm this. It is important to establish whether force depression does occur after unloaded shortening because this has implications for the mechanism of force depression. For example, Maréchal and Plaghki proposed that force depression arises from a stress-induced inhibition of cross-bridge attachment in the region of new overlap that forms between actin and myosin filaments during shortening (Maréchal and Plaghki, 1979). This implies that unloaded shortening, when stress is zero, should not induce force depression.

Consideration of possible interactions between force enhancement and force depression raises the question of whether they have the same underlying mechanism. These two effects exhibit a number of differences in their characteristics suggesting that they may have different mechanisms. For example, force depression is very sensitive to shortening velocity (Edman et al., 1993; Herzog and Leonard, 1997; Marechal and Plaghki, 1979) whereas force enhancement is insensitive to stretch velocity (Edman et al., 1978); force depression continues to increase as shortening distance increases but force enhancement increases with stretch distance only up to a point and then levels out (Bullimore et al., 2007), and force depression and force enhancement have different relationships to muscle length (Schachar et al., 2004). These differences suggest that the underlying mechanisms are not the same but this does not address how force depression and enhancement might be connected in stretch-shortening or shortening-stretch experiments.

Role of rFE *in vivo*

In life, muscles rarely perform ramp stretches followed by prolonged isometric contractions. Common tasks, such as locomotion, typically involve short periods of activation during which muscle length and velocity vary continuously; however, this does not mean that rFE cannot play a role in such tasks. It has been shown that rFE is present almost immediately after the end of stretch (Edman et al., 1984), that isotonic as well as isometric force is enhanced (Edman et al., 1978), and that rFE occurs in muscles that are voluntarily activated to submaximal levels (Oskouei and Herzog, 2005). Therefore, it seems likely that rFE influences muscle force during everyday activities. This effect is expected to be beneficial. Muscle stretch will occur whenever the load on a muscle exceeds the isometric force at the current level of activation. While such stretch is a desirable component of many normal activities, it will also occur when the force on a muscle is unexpectedly high, e.g. when an animal stumbles or slips, steps in a hole or is carrying a load. By enhancing the force-producing capacity of a muscle after stretch, rFE would help the muscle to support loads that are higher than expected until activation level can be increased.

Conclusions

We have shown that rFE is unlikely to arise solely from a parallel elastic element that increases in stiffness when the muscle is activated. Other mechanisms that have been proposed include non-uniformity of half-sarcomere properties and behaviour (Edman and Tsuchiya, 1996; Morgan, 1990; Telley et al., 2003) and an increase in the force generated by the cross-bridges (Herzog, 1998; Linari et al., 2000). It is also quite possible that rFE is generated by more than one mechanism, e.g. the combined effect of a passive and an active component (Herzog and Leonard, 2002). This would not be surprising because, as discussed above, rFE is expected to play a beneficial role during everyday activities. From an evolutionary viewpoint, this implies that any feature that results in rFE would be favoured by natural selection. Therefore, it is quite possible that muscle has developed several features that cause rFE. If this is the case, then it presents a significant challenge to experimenters who must develop techniques for partitioning the observed rFE between different causes.

We thank Elias Tomaras for his help in designing the clips for attaching the fibres to the apparatus and the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canada Research Chairs programme for funding. Equipment and facilities used for this research were made available by funding from the Canadian Foundation for Innovation and the Alberta Innovation Research Program.

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