Force enhancement in single skeletal muscle fibres on the ascending limb of the force–length relationship

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Summary

It has been assumed that force enhancement in single fibres of skeletal muscles only occurs on the descending, and not the ascending or plateau region, of the force–length relationship. This assumption has been based, however, on theoretical considerations or isolated experiments, in which neither stretch conditions nor fibre lengths were optimized for force enhancement. Therefore, the purpose of this study was to investigate the residual, steady-state force enhancement following active stretch in single muscle fibres of frog on the ascending limb of the force–length relationship. Twenty-nine stretch experiments on ten single fibres from the lumbrical muscle of the frog *Rana pipiens* were carried out on the ascending limb of the force–length relationship. Force enhancement was observed in 28 out of the 29 tests. Moreover, the force produced for stretch experiments finishing at optimal fibre length always exceeded the force obtained for an isometric contraction at optimal length. We conclude from these results that steady-state force enhancement occurs systematically on the ascending limb of the force–length relationship, and that the steady-state force in the enhanced state can easily exceed the maximal isometric force of the fibre.

Key words: force enhancement, muscle mechanics, single fibre, striated muscle, cross-bridge theory, force production, frog, *Rana pipiens.*

Introduction

It is well accepted that the residual, steady-state isometric force following active stretch of a muscle or fibre on the descending limb of the force–length relationship is greater than the purely isometric force at the corresponding length (Abbott and Aubert, 1952; Edman et al., 1978, 1982; Julian and Morgan, 1979b; Herzog and Leonard, 2000; Lee and Herzog, 2002). This phenomenon is referred to as force enhancement following stretch (Herzog, 1998), and its origin remains unknown.

Force enhancement is known to increase with increasing magnitudes of stretch (Abbott and Aubert, 1952; Edman et al., 1978, 1982), but is independent, or at least very insensitive, to the speed of stretch (Edman et al., 1978, 1982; Sugi and Tsuchiya, 1988). One of the crucial ideas regarding the mechanisms associated with residual force enhancement following stretch has been the suggestion that force enhancement does not occur in regions where the active isometric force increases with increasing muscle/fibre length; i.e. the so-called ascending limb of the force–length relationship (Edman et al., 1978, 1982; Julian and Morgan, 1979b). Rather, force enhancement has been thought to occur only on the so-called descending limb of the force–length relationship, a region in which the active isometric force decreases with increasing muscle/fibre length (Abbott and Aubert, 1952; Edman et al., 1978, 1982; Julian and Morgan,

1979b; Herzog and Leonard, 2000), and in which sarcomere lengths are assumed to be unstable (Hill, 1953) and non-uniform in length (Julian and Morgan, 1979b; Morgan, 1990, 1994; Allinger et al., 1996). Therefore, force enhancement has been associated frequently with the occurrence of instability and the associated non-uniformities in sarcomere lengths (Julian and Morgan, 1979a,b; Edman et al., 1982; Morgan, 1990, 1994; Edman and Tsuchiya, 1996; Morgan et al., 2000).

According to the sarcomere length non-uniformity theory, force enhancement can only occur on the descending, but not the ascending limb of the force–length relationship for two reasons: (i) the ascending limb has a positive force–length slope, therefore sarcomere lengths are stable and non-uniformities cannot occur, and (ii) even if sarcomere length non-uniformities occurred on the ascending limb (i.e. some sarcomeres would be shorter and some longer than the average length), the sarcomeres that would be shorter than average would have a decreased force potential compared to the average sarcomere length (Gordon et al., 1966). Therefore, only a decrease, but not an increase, in force could result from sarcomere length non-uniformities on the ascending limb of the force–length relationship.

Recently, Herzog and Leonard (2002) showed a small, but consistent force enhancement on the ascending portion of the force–length relationship in the cat soleus. However, their

result may have been caused by a few fibres of the whole muscle that might have been on the descending limb of the force–length relationship, while the whole muscle exhibited properties associated with the ascending limb of the force–length relationship. In order to answer the question of whether force enhancement can occur on the ascending portion of the force–length relationship, experiments on the single fibre level were needed. Thus, the purpose of this study was to investigate the residual, steady-state force enhancement following active stretch in single fibres of frog on the ascending limb of the force–length relationship. Based on results obtained with whole muscle, we hypothesized that there would be a small but consistent force enhancement on the ascending, positive slope, of the force–length relationship, provided that stretch conditions were optimized. If so, the steady-state forces following stretch should exceed the purely isometric forces at optimal fibre length, if the fibre was stretched from some initial length on the ascending limb of the force–length relationship to the optimal fibre length.

Materials and methods
Muscle preparation
Single fibres (N=10) were dissected from the lumbrical muscle of the frog, *Rana pipiens* Schreber. Care was taken to ensure that all fibres were free of any debris from dead adjacent fibres. Treatment of the animals, and all experimental procedures, were approved by the University of Calgary's committee for the ethical use of animals in research.

Experimental setup
The tendons of the dissected fibres were gripped with small T-shaped pieces of aluminum foil as close to the fibre as possible. The fibres were mounted in an experimental chamber between a force transducer (Sensonor AE801, SensorOne Technologies Corp., Sausalito, CA, USA) and a servomotor length controller (Aurora Scientific, Aurora, ON, Canada). The chamber was filled with Ringer's solution (NaCl 115 mmol, KCl 3 mmol, $CaCl_2$ 3 mmol, NaH_2PO_4 2 mmol, $NaHCO_3$ 20 mmol, pH 7.5). The temperature was controlled at ~8°C (range: 6–10°C) during all experiments.

Stimulation (Grass S88, Grass Instruments, West Warwick, RI, USA) was given through two parallel platinum wire electrodes on either side of the fibre mounted in the experimental chamber. Square pulses (0.4 ms duration) were delivered with an amplitude 25% above the voltage that produced maximal force (range: 50–90 V). Each fibre was tested individually to induce a completely fused tetanic contraction with the lowest stimulation frequency possible (range: 23–26 Hz).

Initial procedures
After determining the optimal voltage for stimulation, the fibre was left to twitch every 90 s for at least 30 min. The fibre was then visually inspected for signs of damage. The magnitudes of the twitches after the conditioning period were compared to the initial twitch values to examine the quality and viability of the fibre. If any decrease in force was observed, the fibre was discarded at this point.

Experimental procedures
An experimental force–length curve was determined for each fibre using 2 s contractions to define the ascending limb, the optimal length, and the descending limb of the force–length relationship (Gordon et al., 1966; Morgan et al., 2000). The length at which the greatest active force was obtained was defined as 0% (optimal length), and was associated with an average sarcomere length of 2.1 μm in accordance with results of length-clamped (Gordon et al., 1966) and fixed-end single fibre preparations (Lutz and Rome, 1994). Lengths below 0% were defined as the ascending limb, and were designated with negative length values (i.e. a length of –10% refers to a fibre length that is 10% smaller than optimal length), and the corresponding average sarcomere lengths were assumed to change proportionally with fibre length. A rest period of 5–6 min was given between contractions to avoid fatigue of the fibre. For the remainder of the experiment, isometric reference contractions at the optimal length were systematically used to check if force remained constant throughout testing. If the force dropped more than 2% between adjacent reference contractions, or if the reference force decreased by a total of 10% or more of the initial maximum isometric force at any point during testing,

Fig. 1. Representation of the time course of stimulation for the experimental stretches. Here, 0% length refers to the length at which active maximal isometric force was obtained (average sarcomere length about 2.1 μm; Lutz and Rome, 1994). The fibres were activated at an initial length for 1 s (sufficient for the force to reach the maximal isometric force at that length), then stretched 10% of fibre length to the final length in 0.25 s (40% s^{-1}). The fibre was then held at this final length for another 4.75 s (sufficient time for steady-state isometric force to be established; Edman et al., 1982).
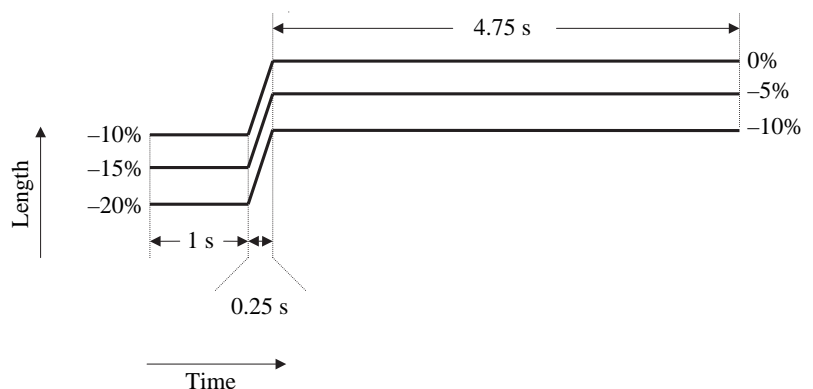
the fibre was immediately discarded, and the results were eliminated from analysis.

After determining the force–length relationship and the optimal length, isometric contractions (square pulses, 0.4 ms duration, 6 s stimulation train), were performed at lengths of 0%, –5%, –10%, –15% and –20%; i.e. on the ascending limb of the force–length relationship (average sarcomere length of approximately 2.1, 2.0, 1.9, 1.8 and 1.7 μm, respectively). Then, three active stretches were performed along the ascending limb of the force–length relationship from lengths of –10% to 0% (optimal length), –15% to –5%, and –20% to –10% (Fig. 1). For the stretch tests, fibres were shortened
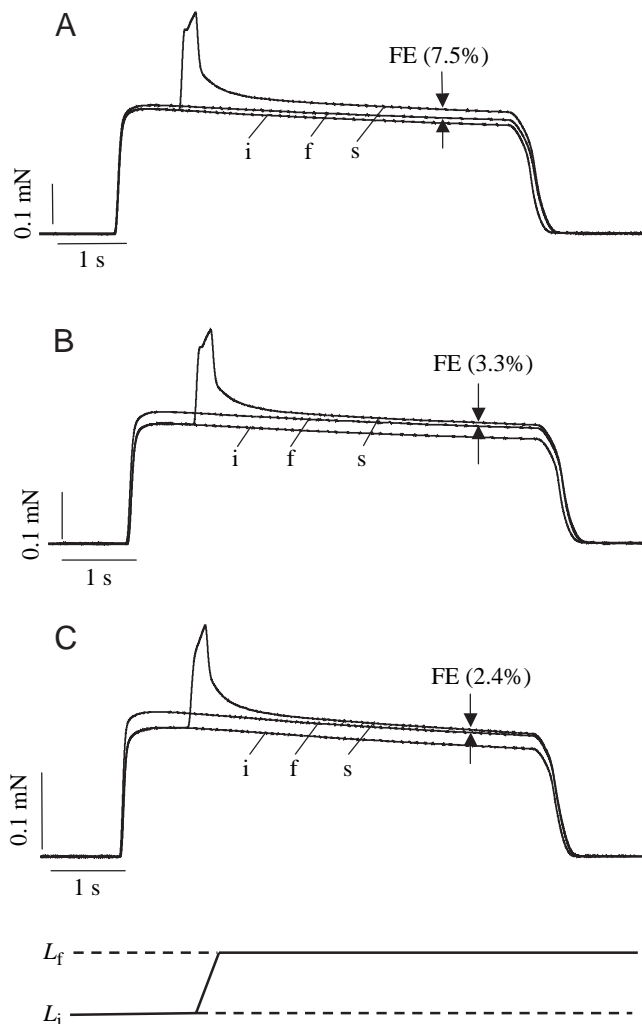
Fig. 2. Typical experiment, conducted with one fibre. (A–C) Two isometric force traces with the fibre at the initial length (i, bottom trace), and the final length (f, middle trace), as well as the stretch contraction from the initial to the final fibre length (s, top trace). In A, the final length corresponds to the optimal length for force development; i.e. at this length, the purely isometric force was maximal. Note that the steady-state force enhancement is greater than any of the isometric forces, although only by a small amount in C. Initial lengths: –10%, –15% and –20% for A, B and C, respectively. Stretch speed was 40% of fibre length s^{-1}, and stretch amplitude was 10% of fibre length in all cases.

passively to the initial length (L_i), where they were stimulated for 1 s, which was sufficient time for the force to reach maximal isometric force (e.g. Edman et al., 1978, 1982; Julian and Morgan, 1979b; Edman and Tsuchiya, 1996). Fibres were then stretched to the final length (L_f) at a speed of 40% of fibre length s^{-1} (i.e. in 0.25 s), where they were held activated for another 4.75 s (Fig. 1).

Data analysis and statistics

The active forces recorded at 3.75 s after the stretch were used for statistical analysis, when force had reached a steady-state level (i.e. the stretch test force–time traces were parallel to the isometric reference force–time traces, e.g. Edman et al., 1982). Parallelism was evaluated by fitting a linear regression line through the data points for the 0.5 s period preceding the instant at which force enhancement was evaluated. The slopes for both the isometric reference and the stretch contractions were determined and evaluated for differences using a repeated-measures analysis of variance (RM-ANOVA) ($P<0.05$). Comparisons of forces at 3.75 s after stretch with the isometric reference forces at the corresponding lengths were made by one-way RM-ANOVA, followed by contrasts chosen *a priori* when indicated. A level of significance of $P<0.05$ was used for all analyses. Results are shown as means ± S.E.M.

Results

Ten fibres were tested using stretches beginning at initial lengths of –10% and –15%, and nine fibres were tested beginning at an initial length of –20%. For all ten fibres tested beginning at lengths of –10% and –15%, and eight out of the nine fibres tested beginning at –20%, the steady-state forces following active stretch were greater than the purely isometric reference contractions at the corresponding length, L_f (Fig. 2A–C). There was no evidence of any passive forces in any of the reference or stretch contractions. Furthermore, there was no evidence of passive force enhancement as reported previously when single fibres were stretched while activated on the descending limb of the force–length relationship (Herzog and Leonard, 2002; Rassier et al., 2003).

In many cases, the steady-state forces following active stretch were greater than the isometric reference forces at optimal fibre length (Fig. 3). For the tests beginning at a length of –10% and finishing at 0% (optimal) length, all ten fibres showed force enhancement that exceeded the isometric force at optimal length by 4.9±0.9%, with a range of 0.8–10.9%. For the tests beginning at a length of –15%, eight out of the ten fibres showed force enhancement exceeding the force at optimal length. The mean force enhancement for these tests was 4.5±0.9%, and they exceeded the maximal isometric force by 2.4±1.0%. For the tests beginning at a length of –20%, the mean force enhancement was 2.0±0.6%, and the steady-state forces following active stretching never exceeded the purely isometric reference force at optimal length.

Fig. 3. Mean ascending limb portion of the force–length relationship (gray circles, solid line) and steady-state forces (mean ±1 S.D.) following stretch tests (black squares, connected to initial lengths by broken lines) in lumbrical fibres. Forces were normalized with respect to the maximal isometric force (dotted line) for comparison across fibres. Fibre lengths were normalized relative to the optimal fibre length (0%), i.e. the length at which the isometric force was greatest. Note that steady-state force enhancement for stretches beginning at lengths –10 and –15% produced average forces greater than the isometric force at optimal length (0%). Optimal average sarcomere length was based on Lutz and Rome (1994), who demonstrated that maximal force in single frog fibres for fixed-end contractions (as produced here) correspond to an average sarcomere length of 2.1 μm. The remaining average sarcomere lengths were calculated proportionally to the fibre length change.
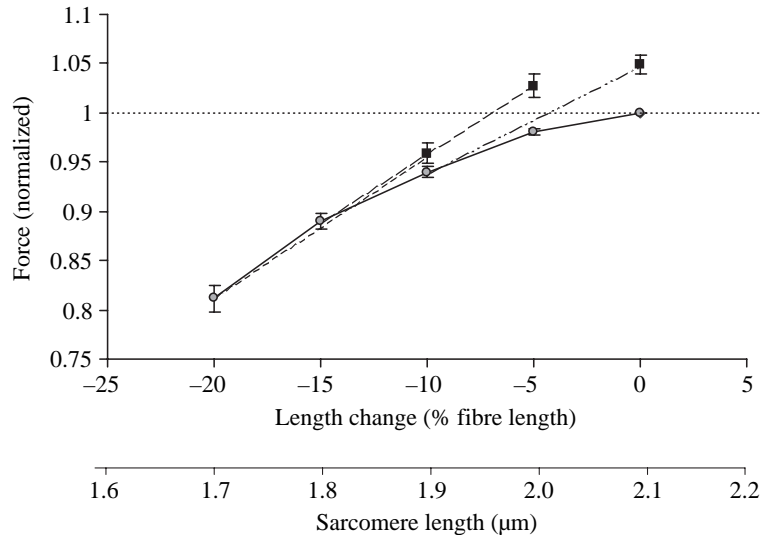
Discussion

In the search for mechanism(s) of force enhancement, two ideas have played a crucial role in researchers' thinking. The first of these ideas was that force enhancement was said to not occur on the ascending limb of the force–length relationship; the second, that the forces associated with force enhancement could not exceed the purely isometric forces at a muscle or fibre length at which the maximal active isometric force was observed. These two ideas were used in support of the so-called sarcomere length non-uniformity theory. This theory states that force enhancement following active muscle/fibre stretching is associated with the development of non-uniformities in sarcomere lengths (e.g. Julian and Morgan, 1979b; Zahalak, 1997; Morgan et al., 2000), and that these non-uniformities are caused by sarcomere length instability (Hill, 1953; Gordon et al., 1966) that occurs on the negative slope of the force–length relationship, the descending limb, but not the positive slope, the ascending limb of the force–length relationship (e.g., Hill, 1953; Allinger et al., 1996).

However, it has been shown that stretch-induced, steady-state force in single skeletal muscle fibres can exceed the isometric force at optimal length. Herzog and Leonard (2002) and Rassier et al. (2003) found evidence for the recruitment of a passive elastic element that contributed to the force enhancement. They suggested that possibly this 'passive force enhancement' may account for the steady-state forces above the isometric reference force at optimal fibre lengths. In the present study, no passive forces were observed in any test, and no passive force enhancement either. However, this does not preclude that passive force enhancement may cause the steady-state isometric forces following stretch to exceed the purely isometric forces at optimal length. In contrast to long muscle/fibre lengths, the passive force enhancement at short lengths would have to disappear in this scenario, and thus, would not be directly observable.

Edman et al. (1978) showed force enhancement above the forces obtained at optimal length for stretch magnitudes of 0.2 μm per sarcomere (about 10% of fibre length) in their single fibres from frog (*Rana temporaria*) semitendinosus (their figure 4A). However, they argued that this force enhancement was transient and decayed rapidly, and was not visible if the fibre was held for 4.5 s following the stretch (their figure 6A). However, the evidence for this latter statement was based on a stretch of 0.08 μm per sarcomere, which corresponds to only about 4% of fibre length. Therefore, it seems quite possible that Edman et al. (1978) might have found similar results as shown here, had they performed systematic stretch experiments with greater stretch amplitudes on the ascending limb of the force–length relationship.

There are a number of studies in which residual force enhancement has been observed following stretch of whole muscles (Abbott and Aubert, 1952; Herzog and Leonard, 2000, 2002; Herzog et al., 2003). However, the observed force enhancement may have been caused by some fibres that were already on the descending portion of the force–length relationship, while the majority of fibres were still on the ascending limb. This scenario would be associated with ascending limb behaviour of the whole muscle, and simultaneously explain why force enhancement was relatively small in these studies. Here, we observed small, but consistent, force enhancement on the ascending limb of the force–length relationship in single fibres. This result implies that sarcomere length non-uniformities may develop on the ascending portion of the force–length relationship, in contrast to what has been found experimentally (Julian and Morgan, 1979b; Morgan, 1990, 1994), and has been predicted theoretically (Allinger et al., 1996).

The mean force enhancement (about 3–5%, with peak values reaching about 10%) for the stretch protocols used here are much smaller than what has typically been observed on the descending limb of the force–length relationship (Abbott and Aubert, 1952; Edman et al., 1978, 1982; Julian and Morgan, 1979b; Herzog and Leonard, 2000). Thus, it might be argued that force enhancement on the ascending limb is not important

for practical applications. However, from a mechanistic point of view, the results of this study are significant, because they show that systematic force enhancement is obtained at fibre lengths at which sarcomere length non-uniformity cannot contribute to the total force enhancement. Therefore, force enhancement may be associated with yet another mechanism that has not been considered in the past, or might be caused by passive elements whose effect disappears with deactivation in short, but not long, muscle fibres.

Conclusions

There is a small, but systematic, steady-state force enhancement on the ascending limb of single fibres from frog that can exceed (for specific stretch conditions) the maximal isometric forces obtained at optimal fibre length. This force enhancement is difficult to reconcile within the framework of the sarcomere length non-uniformity theory, and it was not associated with an increase in the passive force following stretch of activated fibres. Therefore, it is not related in an obvious way to the passive force enhancement, as observed at long muscle and fibre lengths. Nevertheless, sarcomere length non-uniformities and/or passive force enhancement cannot be completely excluded as factors contributing to the total force enhancement observed here. However, there exists the possibility that force enhancement on the ascending limb of the force–length relationship may be caused by a mechanism other than those associated with force enhancement on the descending limb.

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