Residual force enhancement exceeds the isometric force at optimal sarcomere length for optimized stretch conditions

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Submitted 3 October 2006; accepted in final form 16 May 2008

Lee E-J, Herzog W. Residual force enhancement exceeds the isometric force at optimal sarcomere length for optimized stretch conditions. J Appl Physiol 105: 457–462, 2008. First published May 22, 2008; doi:10.1152/japplphysiol.01109.2006.—Residual force enhancement (FE) following stretch of an activated muscle is a well accepted property of skeletal muscle contraction. However, the mechanism underlying FE remains unknown. A crucial assumption on which some proposed mechanisms are based is the idea that forces in the enhanced state cannot exceed the steady-state isometric force at a sarcomere length associated with optimal myofilament overlap. Although there are a number of studies in which forces in the enhanced state were compared with the corresponding isometric forces on the plateau of the force-length relationship, these studies either did not show enhanced forces above the plateau or, if they did, they lacked measurements of sarcomere lengths confirming the plateau region. Here, we revisited this question by optimizing stretch conditions and measuring the average sarcomere lengths in isolated fibers, and we found that FE exceeded the maximal isometric reference force obtained at the plateau of the force-length relationship consistently (mean ± SD: 4.8 ± 2.1%) and by up to 10%. When subtracting the passive component of FE from the total FE, the enhanced forces remained greater than the isometric plateau force (mean ± SD: 4.3 ± 2.0%). Calcium-induced increases in passive forces, known to be present in single fibers and myofibrils, are too small to account for the FE observed here. We conclude that FE cannot be explained exclusively with a stretch-induced development of sarcomere length nonuniformities, that FE in single fibers may be associated with the recruitment of additional contractile force, and that isometric steady-state forces in the enhanced state are not uniquely determined by sarcomere lengths.

skeletal muscle fibers; mechanisms of contraction; cross-bridge theory; sliding filament theory

THE STEADY-STATE ISOMETRIC forces following stretching of an active fiber or muscle exceed the steady-state forces obtained for purely isometric contractions at the corresponding length (1, 4, 9, 14). This property of skeletal muscle has been called “steady-state” or “residual” force enhancement and has first been described systematically by Abbott and Aubert (1). It is distinctly different from the increase in force during stretch, which has been described by Hill (11) and is explained by the actin-myosin cross-bridge kinetics (13).

Residual force enhancement has been found to increase with increasing magnitudes of stretch (1, 2, 4, 5, 29), is thought to be independent of the speed of stretch (4, 5, 30), and has been observed consistently on the descending limb of the force-length relationship (4, 5, 25). However, there is some controversy as to whether force enhancement exists on the ascending limb and plateau of the force-length relationship. Force enhancement has been observed on the ascending limb/plateau in some whole muscle preparations (1–3, 9), but not in others (e.g., Ref. 22). However, the results on whole muscle preparations have been criticized because it is not known whether some of the fibers might be operating on the descending part of the force-length relationship, thereby causing the observed force enhancement, whereas the majority of the fibers operate on the ascending part and so produce increasing force with increasing muscle length (i.e., ascending limb behavior). Similarly, force enhancement has been observed on the ascending limb/plateau in some single-fiber preparations (e.g., Ref. 23) but not in others (e.g., Ref. 5). The ascending limb in Peterson et al. (23) was identified by an increase in isometric force with increasing fiber length, whereas the actual sarcomere lengths were not measured. Therefore, it could not be determined whether the ascending limb/plateau corresponded to sarcomere lengths shorter/equal, respectively, than those known to give optimal overlap between actin and myosin filaments [i.e., 2.0–2.2 μm for frog fibers; Gordon et al. (8)].

Whether or not force enhancement exists on the plateau of the force-length relationship is crucial in terms of evaluating possible mechanisms for this phenomenon. The most accepted mechanism for force enhancement is associated with the idea that some weak sarcomeres are pulled beyond myofilament overlap by active stretching on the “unstable” (12) descending limb of the force-length relationship and that these sarcomeres are supported exclusively by passive forces, whereas the remaining sarcomeres are stretched only by a small amount, if at all (20–22). Therefore, force in the enhanced state is given by the active force of the short, strong sarcomeres, and it is greater than the corresponding purely isometric force for which sarcomere lengths are assumed to be relatively uniform (19, 22). According to this theory, hereafter referred to as the sarcomere length nonuniformity theory, force enhancement cannot exceed the purely isometric forces on the plateau of the force-length relationship (21, 22). However, there are no systematic studies in which residual force enhancement on the plateau of the force-length relationship has been investigated while optimizing stretch conditions and simultaneously measuring sarcomere length.

The purpose of this study was to test whether there is residual force enhancement that exceeds the steady-state isometric force obtained at average optimal sarcomere length, i.e., on the plateau of the force-length relationship. The plateau was identified in single-fiber experiments by finding the sarcomere length of maximal active isometric force production and was

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confirmed by average sarcomere length measurements across a midsection of the fiber using a laser diffraction approach. Stretch magnitudes and sarcomere lengths were carefully chosen based on pilot work to maximize the probability of finding residual force enhancement exceeding the isometric forces at the plateau of the force-length relationship.

METHODS

Muscle fiber preparation. Twelve single fibers of the lumbrical muscles (2–3 mm in length and ~80 μm in diameter) from frogs, Rana pipiens, were used for all experiments. Frogs were killed by decapitation, and single fibers were isolated by mechanical dissection. Treatment of the frogs and all experimental procedures were approved by the University of Calgary committee for the ethical use of animals in research.

Force and fiber length measurements. After isolating a single fiber in a dissecting bath, the tendons at either end of the fibers were gripped with small T-shaped pieces of aluminum clips as close as possible to the fibers to avoid tendon compliance as much as possible. Fibers were then attached to a force transducer (Sensonor) at one end and a servomotor length controller (Aurora Scientific) at the other end. The experimental chamber containing the fiber was placed on an inverted microscope (Eclipse TE300, Nikon). The chamber was filled with physiological Ringer solution (in mM: 115 NaCl, 3 KCl, 3 CaCl2, 2 NaH2PO4, and 20 NaHCO3, pH = 7.5), and the temperature of the Ringer solution was kept constant at 9°C by a controller (VWR Scientific Products).

Stimulation (Grass S88, Grass Instruments) of fibers was achieved through two platinum wire electrodes that were placed inside the chamber parallel to the muscle fibers. Square wave pulses (0.4-ms duration) were delivered at an amplitude of 25% above the voltage (50–80 V) that elicited maximal force. The frequency of stimulation was chosen individually for each fiber to induce a fused tetanic contraction at physiologically relevant frequencies (30–40 Hz). Fiber lengths were measured before testing with a calibrated eyepiece (error <0.02 mm).

Sarcomere length measurement. Average sarcomere lengths were measured with a laser diffraction technique (7, 27, 32) using a He-Ne laser beam (633-nm wavelength, ~0.5-mm diameter; Meredith Instruments). The laser beam was projected vertically onto a midsection of the fibers, and the diffraction pattern was recorded by a fast single-array charge-coupled device camera (Line scan PL-2048EP, Pulnix) with 2048 pixels. Average sarcomere lengths were determined in real time based on the first-order diffraction angle using a custom-designed detector (28).

Before each testing session, the sarcomere length detector was calibrated using two optical gratings (92 and 110 lines/mm, Edmund Optics) and higher order diffraction patterns. The accuracy of the laser diffraction system was <2% of sarcomere length within the range of 1.82 to 3.03 μm.

Experimental procedures. At the beginning of the experiment, stimulation parameters were determined with 1-s tetanic contractions, and fibers were then paced for 40 min with twitch contractions every 90 s.

After pacing, fibers were inspected visually for damage, and they were evaluated for any decrease in force with 1-s tetanic isometric contractions. If there was any visible damage or a decrease in isometric force, the fiber was discarded. The force-sarcomere length relationship was determined with 2-s tetanic isometric contractions (3-min intervals) to identify the plateau and descending limb of the force-sarcomere length relationship. The length that gave the maximal active isometric force was then taken as the optimal fiber length.

One set of experimental tests consisted of five individual contractions: the first two contractions were isometric reference contractions at the final length (the length to which the fiber was stretched to) and the optimal length. The third contraction was the test contraction in which the activated fiber was stretched from some initial to the final length. The last two contractions were a repeat of the two isometric reference contractions at the optimal and final length. If the isometric reference force at the optimal length differed by 0.2% within a set, or decreased by >10% from its initial value at any time during testing, the fiber was discarded and the results were not included in the analysis.

Five sets of experimental tests were attempted at different points along the force-length relationship as shown schematically in Fig. 1. The first set started at the optimal sarcomere length (hereafter referred to as 0% length). The second and third set started at 3.3% longer and shorter, and the forth and fifth set at 6.7% longer and shorter, than optimal length. All contractions lasted for 5 s and were separated by a 6 min rest interval. Stretch magnitudes were 10% of the nominal optimal fiber length, and were performed at a nominal speed of 50% fiber length/s. These stretch conditions had been identified previously to produce forces in the enhanced state that exceeded the isometric reference forces at optimal fiber length and did not cause damage during repeat measurements as required here. Fibers were activated at the initial length for 800 ms before stretch, and they were held isometrically at the final length for 4 s (Fig. 1). Force, fiber length, and sarcomere length were recorded at a frequency of 1,000 Hz.

Data analysis. To obtain a mean ± SE force-sarcomere length relationship, data from all fibers (n = 12) were grouped according to sarcomere lengths into bins of 0.1 μm ranging from 1.85 to 2.75 μm.

Steady-state forces were approximated by measurements made at 4.5 s following the onset of activation. Force enhancement was defined as the increase in the steady-state isometric force following active fiber stretch compared with the steady-state isometric reference force at the corresponding final length obtained immediately before the test contraction (ΔFEfinal in Fig. 1). Furthermore, the steady-state isometric force following active fiber stretch was also compared with the maximal isometric force at optimal length obtained immediately before the corresponding test contraction (ΔFEopt in Fig. 1). This definition for force enhancement and force enhancement above the plateau was adopted so that any stretch-induced damage to the fibers or any fatigue accumulation during a given set of tests would decrease the observed force enhancement, and thus values obtained here, if anything at all, would underestimate the true force enhancement and...
also would underestimate the true force enhancement above the isometric plateau forces. To make comparisons across fibers, force enhancement was normalized with respect to the corresponding isometric reference force. Similarly, force enhancement above the isometric force at optimal sarcomere length was determined across fibers by normalizing all values relative to the corresponding isometric reference forces at optimal sarcomere length.

Passive forces were measured at 1 and 5 s after deactivation, and the passive force enhancement was defined as the increase in passive force following active stretch compared with the passive force following the corresponding isometric reference contraction (ΔPFE in Fig. 1) (9). The passive force enhancement was normalized with respect to the maximal isometric force at the corresponding final length for comparison across fibers. The amount of active force enhancement was obtained by subtracting the passive force enhancement from the total force enhancement. To determine the mean total force enhancement and passive force enhancement, data from all fibers \( n = 12 \) were grouped into bins of 0.1 μm of sarcomere length ranging from 2.0 to 2.4 μm.

RESULTS

Maximal active isometric forces were found to occur consistently at average sarcomere lengths ranging from 2.0 to 2.2 μm, thereby confirming that maximal forces were obtained near sarcomere lengths associated with optimal actin-myosin filament overlap in frog skeletal muscle fibers (8). The isometric forces were perfectly stable [that is, there was no creep as is sometimes observed in segment or sarcomere clamped preparations (8)], and they were associated with a constant average sarcomere length throughout the isometric steady-state phases of the contractions (Fig. 2).

The steady-state isometric force following active fiber stretch was greater than the purely isometric reference force at the corresponding final length for all fibers and each test contraction (Figs. 2A and 3A). The mean ± SD force enhancement across all fibers and all sarcomere lengths was 10.4 ± 3.9%, reaching a peak value of 16.1% at the longest sarcomere length (Fig. 3A). In contrast to the force obtained for the purely isometric reference contractions, there was no distinct decrease of the peak isometric forces following active fiber stretching beyond sarcomere lengths of 2.2 μm (Fig. 3).

Although force enhancement has been observed previously in single fiber preparations (4–6, 25, 30), novel to the literature is the result that forces in the enhanced state clearly exceeded the isometric reference forces obtained at optimal sarcomere lengths \( (P < 0.001) \), i.e., the plateau of the force-length relationship (Figs. 2 and 3). This result was obtained for all fibers and each stretch condition and for comparison with the isometric reference forces preceding the test contractions; therefore, any fatigue or damage to the fiber would have decreased the amount of force enhancement that was measured here. The mean ± SD force enhancement above the isometric plateau forces was 4.8 ± 2.1% with peak values reaching 10%.

There was a consistent, albeit small passive force enhancement averaging 0.7% (at 1 s after deactivation) or 0.4% (at 5 s after deactivation) of the active isometric reference forces. When subtracting this passive contribution from the total force enhancement, the remaining active component of the force enhancement was virtually unaffected, and more importantly, forces in the active enhanced state still clearly exceeded the peak isometric reference force obtained on the plateau of the force-length relationship (Fig. 3B).

DISCUSSION

Residual force enhancement has been observed for more than half a century (e.g., Ref. 1) in a variety of muscles and structural preparations ranging from single fibers (e.g., Refs. 4–6, 14, 30) to in vivo human muscles (e.g., Refs. 2, 3, 17). However, one crucial question that has eluded satisfactory explanation is how much force can there be in the enhanced state? More specifically, can force in the enhanced state (i.e., after active stretch) exceed purely isometric reference forces at sarcomere lengths associated with optimal actin-myosin filament overlap? According to the most accepted mechanism for force enhancement, the sarcomere length nonuniformity theory, forces in the enhanced state cannot exceed the purely isometric forces on the plateau of the force-length relationship (19, 20, 22).

Edman et al. (4, 5) examined this question carefully and concluded that forces in the enhanced state did not clearly exceed the isometric reference forces at optimal sarcomere lengths in single fibers of frog skeletal muscle. Based on their results, they discussed that residual force enhancement was not a property of the cross bridges, as had been suggested by Sugi and Tsuchiya (30), and was not associated with the recruitment of additional contractile material. However, others have observed force enhancement above the isometric plateau forces in...
of 0.2% from the original value) and would have exceeded the isometric reference forces at the plateau for each test, thereby lending support to the idea that our results were not an artifact of early evaluation but that they are different from the results obtained by Edman et al. (5) at 6 s.

Average sarcomere lengths, as measured by laser diffraction, stayed constant in the steady-state phase following active fiber stretching, implying that average sarcomere lengths within the target zone remained approximately constant. These results are in agreement with those reported by Edman et al. (5), who found that active stretching had a stabilizing effect on sarcomere lengths and that force enhancement persisted even in fibers that were stretched under conditions of segment length control. Similarly, Tolley et al. (31) demonstrated in single myofibrils of rabbit psoas that stretching provided a stabilizing effect on sarcomere lengths on the descending limb of the force-length relationship, and Herzog et al. (10) also demonstrated that sarcomeres remained perfectly stable and at constant lengths immediately following active stretching of isolated myofibrils on the descending limb of the force-length relationship.

We used a laser diffraction approach to measure average sarcomere lengths, similar to many previously published papers on single fiber mechanics (5, 24, 30, 32). This approach does not allow for the identification of individual sarcomere lengths, nor can it provide more than just a rough estimate of sarcomere length distribution within the target region, and sarcomere behavior outside the target region remains unknown. These shortcomings of the laser diffraction approach have been pointed out in the literature (7, 15, 18, 27), and they present limitations for studies in which individual sarcomere lengths, sarcomere length distributions, sarcomere length control, or information of all sarcomeres of a fiber is required. However, these limitations are not relevant for the purposes of the present study because all we needed to know is that our measurements of force enhancement above the isometric plateau forces were made with some sarcomeres at optimal length, and it can be assumed fairly safely that if the center of the first order diffraction pattern is within 2.0–2.2 μm (as it was in our study), some of the sarcomeres are at those lengths too. Whether or not some sarcomeres were outside those lengths, or if some sarcomeres outside the target region behaved vastly different from those in the target region, does not matter, as the sarcomere length nonuniformity theory does not allow for force enhancement to be greater than those observed for purely isometric contractions at optimal sarcomere lengths (i.e., the plateau of the force-length relationship) for any sarcomere length distribution (19, 20, 22).

In this study, we also observed a small but consistent passive force enhancement, as had been observed previously in whole muscle preparations (9). Therefore, the forces in the enhanced state exceeding the isometric plateau forces could have been caused by this passive force enhancement. However, when subtracting the passive component of the force enhancement, the residual forces remained greater than the isometric reference forces at optimal sarcomere lengths. Even when evaluating passive force enhancement at 1 s after deactivation to maximize the passive contribution to the total force enhancement, the average active force enhancement was still 4.1 ± 1.7% above the plateau of the force-length relationship. The idea that the residual force enhancement might be caused by an whole muscles (e.g., Ref. 1) and single fibers (e.g., Refs. 23, 25), supporting our result presented here, but they were somewhat inconclusive as sarcomere lengths were not measured in these studies.

Here, we found the novel result that forces in the enhanced state consistently and systematically exceeded the purely isometric reference forces obtained at optimal sarcomere lengths in single fibers of frog skeletal muscle. This observation was made in all fibers and for each test contraction, and the forces in the enhanced state exceeded the isometric reference forces at the plateau by 4.8 ± 2.1%, on average, with peak values reaching up to 10%. Although it has been shown that force enhancement is long lasting [≥30 s; Abbott and Aubert (1)], we, in agreement with the published literature, chose a specific instant in time for evaluation of the force enhancement (4.5 s after the onset of activation). At that instant in time, the force-time histories of the test and reference contractions were still converging, and thus, had we evaluated force enhancement at a later instant in time, it would have been smaller. To estimate the force enhancement at 6 s, as did Edman et al. (5), we calculated what the force enhancement would have been at that time, assuming that the convergence of the two curves remained constant. At 6 s, the average force enhancement above the plateau would have been 4.6% (i.e., a mere decrease

![Fig. 3. Mean ± SE force-sarcomere length relationship (●) and mean total force enhancement (A) and active force enhancement (B) (△, mean force enhancement; △, peak force enhancement). Solid lines show linear regression approximations representing the plateau region and the descending limb of the force-sarcomere length relationship. All data were grouped into bins of 0.1 μm of sarcomere length ranging from 1.85 to 2.75 μm for the force-sarcomere length relationship and from 2.0 to 2.4 μm for the force enhancement results.](http://jap.physiology.org/doi/10.1152/jappl.00150.2008)
increase in stiffness of a passive element on activation was strengthened by the work of Labeit et al. (16), who showed a statistically significant increase in passive forces when single fibers were stretched at a high (pCa = 4.0) compared with a low (pCa = 9) calcium concentration. This calcium-induced increase in passive force reached a peak value of ~10 mN/ 

mm² at a sarcomere length of ~3.0 μm, but was only about 1 mN/mm² at sarcomere lengths of 2.0–2.4 μm. This value is in the same range as the passive force enhancement measured in this study, and it is much too small to account for the entire force enhancement above the isometric plateau.

We found clear evidence that forces in the enhanced state exceeded the isometric reference forces at optimal sarcomere lengths (i.e., on the plateau of the force-length relationship). This result persisted when the passive force enhancement was accounted for, thus suggesting that force enhancement might be associated with the recruitment of additional contractile force. Because this additional force does not appear to be associated with a corresponding increase in fiber stiffness (30), we propose that it might be caused by an increase in the average force per cross bridge. This could potentially be explained by a change in the proportion of attached crossbridges in different attached states. For example, we have observed that residual force enhancement is substantially increased, and it may reach values of over 150% in fiber preparations whose cross-bridge kinetics are biased towards the weakly bound states by addition of 10 mM 2,3-butanedione monoxime (BDM). However, and more importantly in the context of this study, not only did BDM cause an increase in the relative force enhancement, it also caused an increase in the absolute force enhancement of >40% in the 5 and 10 mM BDM conditions compared with control (0 mM) and low-level (2 mM) BDM conditions (26). Therefore, it appears that force enhancement may be associated (at least in part) with a stretch-induced facilitation of transition of cross bridges from the weakly to strongly bound states. Such a mechanism could cause force enhancement without a corresponding increase in stiffness, but further research is needed to investigate this proposed mechanism more carefully.

The isometric force-sarcomere length relationship has a distinct change in slope at a sarcomere length of ~2.2 μm (Fig. 3), indicating the change from the plateau to the descending limb region, as observed by Gordon et al. (8). However, following active stretching, there was no change in slope to indicate that the descending limb of the force-length relationship had been reached (Fig. 3). Rather, the isometric steady-state forces after stretching remained approximately constant between 2.1 and 2.4 μm, indicating that not only does active stretching provide for additional steady-state isometric force (i.e., force enhancement) but it also provides for a more extended plateau region than that obtained for purely isometric contractions, thereby offsetting the anticipated loss of force with a decrease in actin-myosin filament overlap. This result has important functional implications, as the “region of maximal force production (i.e., the plateau of the force-length relationship)” appears to cover a vastly greater sarcomere length range following active stretch compared with purely isometric contractions, thereby optimizing fiber and muscle function, force and work potential during everyday movements.

ACKNOWLEDGMENTS

We thank Dr. Dilson Rassier for fruitful discussions.

GRANTS

This study was supported by grants from the Natural Sciences and Engineering Research Council of Canada and The Canada Research Chairs Program. We thank the Faculty of Kinesiology (graduate studies) at the University of Calgary for financial support.

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