Force depression in single myofibrils

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Submitted 1 October 2009; accepted in final form 4 December 2009

Joumaa V, Herzog W. Force depression in single myofibrils. J Appl Physiol 108: 356–362, 2010. First published December 10, 2009; doi:10.1152/japplphysiol.01108.2009.—Force depression after active shortening has been observed in different muscle preparations. It has been assumed that force depression is caused by the development of sarcomere length nonuniformities after shortening. However, this hypothesis has never been investigated in a preparation where individual sarcomere lengths could be directly measured. Here, we investigated force depression in single myofibrils (n = 11) and tracked simultaneously the changes in individual sarcomere lengths (n = 60) before, during, and after shortening and after a purely isometric contraction performed at the final length. Shortening produced force depression in all myofibrils (mean ± SE; 30.9 ± 3.9%). During shortening, all sarcomeres shortened, but not by the same amount. Sarcomere lengths were nonuniform, with the same mean SD before (0.11 ± 0.06 μm) and after shortening (0.11 ± 0.06 μm) and after a purely isometric contraction at the final length (0.10 ± 0.05 μm). Furthermore, greater shortening magnitudes were found for sarcomeres that were long in the initial isometric configuration. Nonuniformities of half-sarcomere lengths were also the same before (SD = 0.13 μm) and after (SD = 0.14 μm) shortening. We conclude from these results that the development of sarcomere (or half-sarcomere) length nonuniformities does not play a major role in force depression. Rather, force depression seems an intrinsic property of individual (half-) sarcomeres and muscle contraction.

history dependence; sarcomere length nonuniformity; sarcomere instability; stiffness; stress-induced inhibition of cross bridges

It is generally accepted that the steady-state isometric force following shortening of an activated muscle is smaller than the corresponding steady-state force obtained for a purely isometric contraction at the corresponding length (1, 4, 9, 11, 12, 23, 24, 27). This phenomenon is referred to as force depression.

It is well known that force depression increases with increasing shortening magnitudes (1, 11, 23) and decreases with increasing shortening speeds (1, 11, 22–24). Force depression is long lasting (>20 s) (1, 12, 20) but can be abolished instantaneously by deactivation for a period long enough for force to drop to zero (1, 11). Force depression has been systematically observed in whole muscle preparations (1, 11, 23, 24), human skeletal muscles activated voluntarily and through electrical nerve stimulation (4, 20, 21), and single-fiber preparations (5, 9, 18, 27).

The mechanisms underlying force depression are still unknown. It has been assumed that force depression might be caused by the development of sarcomere length nonuniformities during shortening (5, 18, 24). According to this hypothesis, if shortening occurs on the “unstable” (13) descending limb of the force-length relationship, sarcomere lengths are assumed to go from a relatively uniform state for isometric contractions to a highly nonuniform state during shortening. Specifically, short sarcomeres are supposed to shorten more than average and move from the descending to the ascending limb of the force-length relationship, while long sarcomeres are assumed to barely shorten at all (24). This redistribution of sarcomeres associated with shortening is thought to lead to reduced forces compared with the corresponding isometric contractions in which sarcomere lengths are assumed to remain relatively uniform (5, 18, 24). However, force depression experiments have never been performed in a preparation where force and individual sarcomere lengths could be measured continuously, thereby testing the nontrivial assumptions underlying this theory.

Indirect attempts have been made to test the sarcomere length nonuniformity theory of force depression using single-fiber preparations in which average sarcomere length of selected fiber segments were measured (5, 9). However, these measurements cannot provide more than a rough qualitative glimpse at this complex issue, as length measurements are averaged across ~500,000 sarcomeres when using laser diffraction in a normal-sized single fiber. Therefore, interpretation of the contribution of single sarcomeres to the fiber force or determination of the development of sarcomere length nonuniformities during shortening is less certain in fiber or muscle preparations. Furthermore, in a muscle or single fiber, sarcomeres are connected to neighboring sarcomeres in a complex manner that leaves them neither strictly in series nor strictly in parallel with each other; thus the system becomes statically redundant and any attribution of sarcomere function to fiber or whole muscle behavior becomes virtually impossible.

Therefore, the aim of this study was to investigate force depression in skeletal muscle in a preparation that allows for unique attribution of this property to the individual sarcomere. This requires a preparation in which individual sarcomere lengths can be measured accurately and continuously and in which sarcomere lengths can be uniquely related to sarcomere force. Isolated myofibrils, a string of sarcomeres that are perfectly in series with each other, offer that possibility and eliminate the idea of force depression through transverse connections across myofibrils and fibers.

MATERIALS AND METHODS

Myofibril preparation. Rabbits were euthanized by an intravenous injection of 1 ml of a pentobarbital solution (240 mg/ml), a protocol approved by the University of Calgary’s Animal Care and Ethics Committee. Strips of psoas muscle were dissected, tied to small wooden sticks, and stored in rigor solution for 12 h at 4°C, then in a rigor-glycerol (50:50) solution at −20°C for 2 wk before testing. On the day of the experiments, a small piece of muscle tissue (2 mm length) was cut and subsequently blended in rigor solution. A small amount of the blended mixture was placed in a chamber positioned on top of a movable stage mounted on an inverted microscope (Zeiss Axiovert 200M). After 5 min of stabilization, the rigor solution was replaced by a relaxing solution, and myofibrils in suspension were washed away leaving those settled at the bottom of the chamber.
A myofibril with a good striation pattern was fixed to a glass needle at one end and to a nanolever at the other end (29), allowing for length change and force measurement, respectively. The striation pattern of the myofibril was projected onto a linear photodiode array (10,680 elements), which generated a signal with light and dark peaks representing the sarcomere striation pattern. The centroids of the A-bands were determined, and sarcomere lengths were calculated as the distance between adjacent A-band centroids by an algorithm that tracks the signal’s peak positions continuously during the experiments. The experiments were simultaneously visualized using a CCD camera and recorded by a video recorder.

Mechanical tests. Tests were performed at room temperature (~22°C). Myofibrils (n = 11) were activated by adding an activating solution of high calcium concentration (pCa = 3.5) at an average nominal sarcomere length of ~2.8 μm and then shortened at a speed of 0.1 μm·s⁻¹·sarcomere⁻¹ to an average nominal sarcomere length of ~2.4 μm. Myofibrils were then held isometrically for 1 min and deactivated by adding a relaxing solution (pCa = 8.0). After a rest period of 3–5 min, myofibrils were reactivated at the final sarcomere length of 2.4 μm.

Data analysis. Forces were normalized by myofibril cross-sectional area and expressed as stress (kN/m²).

Force depression for a myofibril was defined as the difference in the steady-state isometric force following shortening, and the purely isometric reference contraction at 2.4-μm sarcomere length. Passive forces were not measured separately, as they have been shown to be negligible in this range of sarcomere lengths (<2.8 μm) (2, 16, 17), and as all comparisons of reference and shortening test contractions were made at the same length; thus passive forces, if present, would have been the same for the experimental and reference contractions.

Since half-sarcomeres have been shown to form independent units behaving nonuniformly within the same sarcomere (28), we further investigated force depression and nonuniformity at the half-sarcomere level in myofibrils where all the Z-lines and A-bands were visible during the entire experiment.

For comparison of sarcomere and half-sarcomere length nonuniformities from before to after shortening and for force depression between experimental and isometric reference contractions across

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**Fig. 1.** Raw data used for sarcomere length analysis. A: phase-contrast image of a myofibril. B: intensity peaks collected from the linear photodiode array.

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**Fig. 2.** Example of a myofibril response to activation at an average sarcomere length (SL) of ~2.8 μm, then shortened to an average sarcomere length of ~2.4 μm, deactivated, and then activated again. Note that the force after active shortening is smaller than the purely isometric force at the same (final) length and the (initial) length preceding shortening. Arrows from left to right along the time axis indicate the time of activation (initial length = 2.8 μm), deactivation (final length = 2.4 μm), and reactivation at the final length (2.4 μm).
myofibrils, nonparametric Mann-Whitney U-paired tests were used. The level of significance was set at $\alpha = 0.05$.

Solutions. The rigor, relaxing, and activating solutions were identical to those described previously (26).

RESULTS

Figure 1 shows a mounted myofibril between the glass needle and the nanolever and the intensity peaks collected from the linear photodiode array used for sarcomere length measurements.

Shortening produced force depression (Fig. 2) in all 11 myofibrils (mean ± SE; 30.9 ± 3.9%).

Five myofibrils (of 11 myofibrils) in which the striation pattern could be detected during the whole experiment were used for the sarcomere length analysis. A total number of 60 sarcomeres was used for the analysis of individual sarcomere lengths throughout the entire trial. During shortening, all sarcomeres shortened, but not by the same amount (Figs. 3 and 4). Sarcomere lengths before and after shortening were nonuniform (Fig. 4) with the same mean SD (0.11 ± 0.06 μm) before and after shortening. Furthermore, greater shortening magnitudes were found for sarcomeres that were long in the initial isometric configuration (Fig. 5).

Half-sarcomere lengths ($n = 18$) from one myofibril in which Z-lines and A-bands were clearly visible during the entire experiment were determined. As observed for the full sarcomeres, nonuniformities of half-sarcomere lengths were the same before (SD = 0.13 μm) and after (SD = 0.14 μm) shortening (Fig. 6A). Furthermore, half-sarcomere shortening was greater for initially long half-sarcomeres (Fig. 6B).

Sarcomere length nonuniformities were also measured after the purely isometric contractions performed at an average sarcomere length of 2.4 μm. As shown in Fig. 7, nonuniformities after a purely isometric contraction were similar to the nonuniformities found after active shortening with a mean SD of 0.10 ± 0.05 μm.

DISCUSSION

The main finding of this study is that force depression developed by myofibrils was not associated with the develop-
ment of sarcomere length nonuniformities in the shortening phase of testing. Rather, sarcomere length nonuniformities were the same in the isometric contraction preceding and following shortening and after a purely isometric contraction at the final length of the myofibril on the descending limb of the force-length relationship, thereby suggesting that although these nonuniformities are a regular part of myofibril contractions, they are not produced by shortening, nor are they enhanced by shortening of the myofibril.

The sarcomere length nonuniformities observed in the myofibril preparations are of the same magnitude as those observed in single myofibrils (26, 28), and are somewhat

Fig. 5. Shortening magnitude as a function of initial SLs for individual sarcomeres \((n = 60)\) from 5 myofibrils. On average, initially long sarcomeres tend to shorten more than initially short sarcomeres \((r^2 = 0.11, P < 0.05)\). This correlation exists for each myofibril individually. Inset: examples of changes in SLs as a function of time for 2 sarcomeres from 2 myofibrils. The 2 sarcomeres shown are the longest and shortest before shortening. The shortening amplitude is higher for the initially long compared with the initially short sarcomere.

Fig. 6. A: half-sarcomere \((n = 18)\) lengths before (white) and after (black) shortening. Starting at left, each pair of half-sarcomeres belongs to the same sarcomere. B: shortening magnitude as a function of the initial half-sarcomere lengths for individual half-sarcomeres. The initially long half-sarcomeres tend to shorten by a greater amount than the initially short half-sarcomeres, as indicated by the positive slope of the best fitting linear regression line \((r^2 = 0.21, P < 0.05)\).
smaller than those found along the full length of intact frog fibers (15). These results suggest that the structural nonuniformities in myofibrils are similar to those observed in intact muscles and that sarcomere length nonuniformities do not contribute substantially to the force depression property. Differences in sarcomere lengths observed between myofibrils and intact fibers can be explained by the fact that sarcomere lengths in single fibers represent an average length of ~500,000 sarcomeres arranged in series and in parallel and therefore cannot be compared directly to sarcomere length variations between single adjacent sarcomeres, as observed here in myofibrils.

When shortening on the descending limb of the force-length relationship, the sarcomere length nonuniformity theory predicts that sarcomere lengths become more nonuniform, which they did not. More specifically, the theory predicts that sarcomeres that are short in the initial isometric steady-state condition will shorten to a greater extent than sarcomeres that are initially long. In fact, for force depression to occur, the short sarcomeres need to go onto the ascending limb of the force-length relationship to a level where their isometric force is the same as that of the initially long sarcomeres that shorten little or not at all. However, we observed exactly the opposite: that is, long initial sarcomeres tended to shorten more than initially short sarcomeres (Fig. 5). In agreement with the results obtained at the full sarcomere level, half-sarcomere (n = 18) nonuniformity did not increase with shortening and initially long half-sarcomeres tended to shorten more than initially short half-sarcomeres (Fig. 6B).

Nonuniformities observed before and after shortening were similar to nonuniformities observed after the purely isometric

Fig. 7. Individual SLs as a function of time after a purely isometric contraction at a SL of 2.4 μm for the myofibril shown in Fig. 4. SLs are nonuniform with a SD of ±0.10 μm. Inset: SLs after an active shortening as shown in Fig. 4.

Fig. 8. Expected (filled squares) and measured forces (open squares) for all 60 sarcomeres used in this study. Steady-state isometric forces are smaller for all sarcomeres following shortening compared with the expected isometric reference forces. Expected forces were calculated according to Gordon et al. (8) and accounting for the differences in myofilament lengths between frog and rabbit (10, 25) psoas muscles. Since myofibrils are formed of sarcomeres arranged in series, measuring the force at the end of a myofibril gives the instantaneous force in each sarcomere. By measuring individual SLs before, during, and after shortening, we could calculate the amount of force expected for each sarcomere according to its filament overlap.

Fig. 9. Force depression (FD) as a function of shortening magnitude for individual sarcomeres (n = 60). There is a correlation (r² = 0.55, P < 0.05) between FD and shortening magnitude.
contracted performed at the final length (average sarcomere length = 2.4 μm), suggesting that non-uniformities were not related to the presence of sarcomeres on the descending limb of the force-length relationship.

According to the sarcomere length nonuniformity theory, force depression originates from the development of nonuniformities in lengths between sarcomeres. In a myofibril preparation, with sarcomeres perfectly in series with each other, force depression can be quantified directly for an individual sarcomere when its lengths can be measured during experiments. For all 60 sarcomeres where length measurements were made, force depression was observed and varied from a few percent to just over 50% (Fig. 8). These results strongly suggest that force depression is a sarcomeric property and thus can and does occur in the absence of sarcomere length nonuniformities.

Sarcomeric force depression and shortening magnitude were positively correlated (Fig. 9), a result that agrees with findings on the whole muscle and single-fiber level (1, 22, 27).

Force depression in a single sarcomere may occur because of a decrease in the proportion of attached cross bridges or a decrease in the average force per cross bridge. Changes in stiffness can be used to distinguish between these two possibilities, as stiffness can be related to the proportion of attached cross bridges (6). It is well acknowledged that stiffness is also associated with the compliance of actin and myosin, and in myofibrils, the compliance of titin (7, 14, 19). In a first approximation, it can be assumed that actin, myosin, and titin compliance remain constant after shortening; therefore, changes in stiffness are likely related to changes in the proportion of attached cross bridges. Since force depression is associated with a systematic decrease in muscle and fiber stiffness (20, 27), it may be assumed that the decrease in force is associated with a decrease in the number of attached cross bridges.

Marechal and Plaghki (23) suggested that force depression might be caused by an inhibition of cross-bridge attachment following shortening. A possible mechanism for this inhibition is that actin filaments entering the overlap zone during active (in contrast to passive) shortening are stressed (7, 19). This may cause a change in the orientation of cross-bridge attachment sites (3), thereby decreasing the probability of cross-bridge attachment. Marechal and Plaghki (23) suggested that inhibition of cross bridges would only occur in the actin-myosin overlap zone that was newly formed during shortening. If so, forces in the depressed state should not be smaller than the isometric forces preceding shortening on the descending limb of the force-length relationship. However, we found that this condition was typically not met (e.g., Fig. 2), and the average force in the depressed state was 9.5% lower than the average force in the isometric contractions preceding shortening. Similar results have been obtained by others (e.g., Ref. 20, Figs. 3 and 4; Ref. 9, Figs. 2–4). Therefore, it appears that cross-bridge inhibition following shortening might not only occur in the newly formed, but the entire acto-myosin overlap zone.

Conclusion. Force depression is a property of individual sarcomeres and thus not rely on the development of nonuniformities between sarcomeres. Although this finding does not exclude the possibility that sarcomere length nonuniformity is a contributor to force depression, it provides proof that force depression can occur in the absence of such nonuniformities.

ACKNOWLEDGMENTS

We thank Azim Jinha for writing the analysis programs and Tim Leonard for insightful discussions.

REFERENCES