Force depression following skeletal muscle shortening is long lasting

W. Herzog*, T.R. Leonard, J.Z. Wu

Human Performance Laboratory, Faculty of Kinesiology, The University of Calgary, 2500 University Drive N.W., Calgary, Canada AB T2N 1N4

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Abstract

In a recent manuscript, Edman (1996) reported that force depression following shortening was a transient phenomenon. A transient response would not fit into the mechanism of force depression suggested by Herzog and Leonard (1997) who argued that force depression following shortening was associated with a stress-related inhibition of cross-bridge attachments in the actomyosin overlap zone formed during the shortening phase. The purpose of this study was to test whether force depressions were long lasting or transient, and in the process, to quantify the relationship between force depression and the amount of shortening and the shortening force. It was found that force depression in cat soleus (35–37 °C) was long lasting and was linearly related to the amount of shortening and the shortening force. This latter result suggested that force depression might possibly be related to a single scalar variable; the mechanical work performed by the muscle during the shortening phase. Although the present study was not designed to test this hypothesis, pilot results support the idea that force depression following shortening contractions might be explained exclusively by the muscular work during the shortening phase. © 1998 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The fact that the isometric force of skeletal muscles is depressed following shortening compared to a purely isometric reference contraction has been known for almost half a century (Abbott and Aubert, 1952). This phenomenon is generally accepted (Marechal and Plagheki, 1979; Sugi and Tsuchiya, 1988; Edman et al., 1993), and its effect on in vivo muscle function is considered important (Edman, 1996). However, the mechanism causing force depression following shortening is unknown and has been a matter of great debate (Herzog and Leonard, 1997).

Recently, we proposed and tested a “new” mechanism for force depression following shortening. This mechanism is based on the idea that force depression is associated with a stress-related inhibition of cross-bridge attachments in the actomyosin overlap zone that is formed during shortening (Marechal and Plagheki, 1979; Herzog and Leonard, 1997). Although the observed results agreed qualitatively with the predictions made based on this particular mechanism, there remained two unresolved problems: (1) the reason for the inhibition of the cross-bridge attachments in the newly formed overlap zone could not be determined. Specifically, it could not be asserted whether the reason for the cross-bridge inhibition was a transient biochemical phenomenon, as suggested by Edman (1996), or a long-lasting mechanical phenomenon as suggested by Herzog and Leonard, (1997); and (2) the force depression could not be quantified in terms of the two variables implicated in the mechanism of force depression; the shortening distance and the force during the shortening phase.

The purpose of this study was to specifically test whether force depression following shortening was long lasting or transient, and to quantify the relationship between force depression on the one hand and the extent of shortening and the force during shortening on the other hand.

2. Methods

Experiments were performed on in situ cat soleus muscles (n = 5). Cats were anesthetized using a
halothane–nitrous oxide–oxygen mixture, a nerve stimulating electrode was implanted surgically on the tibial nerve distal to the junction with the common peroneal nerve, and the soleus tendon was isolated from the remainder of the Achilles tendon. The tendon was cut at the distal attachment leaving a remnant piece of bone. The animal was fixed in a stereotaxic frame using sharp, bilateral bone pins at the pelvis, the femoral condyles, and the malleoli. The tendon with its remnant bone piece was attached to a muscle puller which measured force and displacement. The muscle puller was an MTS strength testing machine with a 100 N (= 10 V) load cell and a 20 mm (= 10 V) displacement measuring device (Herzog and Leonard, 1997). The reference length of the muscle was set using a pair of sutures which were attached on the soleus tendon and the tibia at an included ankle angle of 80°. This reference length was called 0 mm, and shortening and lengthening of the muscle from its reference length is given in mm, negative for shortening and positive for lengthening. Therefore, −6 mm indicates a muscle length that is 6 mm shorter than the reference length.

Three conceptual tests were performed. In the first test, an isometric reference contraction was performed at the reference length (0 mm) for a minimum of 25 s. Following the reference contraction, the muscle was activated at a length of +8 mm, for about 2 s, shortened to 0 mm at a speed of 4 mm s⁻¹, and left activated at 0 mm for at least another 20 s. Stimulation parameters for this experiment were: stimulation period 25–35 s; level of stimulation – three times the α-motoneuron threshold (3 T, which stimulates all α-motoneurons); frequency of stimulation 10 and 30 Hz; pulse duration – 0.1 ms.

In the second test, an isometric reference contraction was performed for 8 s at a length of −4 mm. Following the reference contraction, the muscle was activated for 2 s at a length of −2, 0, +2, and +4 mm, shortened to −4 mm at a speed of 4 mm s⁻¹, and held isometrically at −4 mm until the total contraction time was 8 s. Shortening experiments from each length were repeated three times, observing rest periods of 1–2 min. Following each set of three shortening contractions from a given length, a repeat isometric reference contraction was performed. The stimulation parameters were: 8 s, 3 T, 30 Hz, 0.1 ms (see above).

In the third test, an isometric reference contraction was performed for 8 s at a length of 4 mm. Following the reference contraction, the muscle was stimulated for 3 s at a length of +4 mm, shortened to −4 mm at a speed of 4 mm s⁻¹, and held isometrically at −4 mm for another 3 s. During the shortening phase, the activation was varied by changing the current supplied to the tibial nerve from no activation to full activation (3T) in 5–7 steps. The mean force during the shortening period was calculated for each activation protocol.

Shortening experiments at each activation level were repeated three times, followed by an isometric reference contraction. Rest periods between trials were 1–2 minutes. Except for the variable activation during the shortening period, the stimulation parameters were: 8 s, 3 T, 30 Hz, 0.1 ms.

Force depression following shortening was quantified as:

\[
1 - \frac{(D_1 + D_2 + D_3)/3}{F} \times 100\%
\]

where \(F\) represents the isometric reference force; \(D_1\), \(D_2\), and \(D_3\) represent the force following shortening for three repeat trials (except for the long lasting tests in which only one experimental trial was performed). Eq. (1) was used at the exact time when shortening of the muscle was finished, and at 0.5 s intervals thereafter until the end of contraction.

![Fig. 1. Representative force–time curves for an isometric reference contraction at 0 mm length and an isometric contraction (0 mm) that is preceded by a shortening contraction from +8 to 0 mm at a speed of 4 mm s⁻¹. The isometric forces following the shortening contraction were always lower than the corresponding isometric reference forces for all muscles (n = 5) and both rates of tibial nerve stimulation (30 Hz, a; 10 Hz b). From these results, it was concluded that force depressions in cat soleus muscle at physiologic temperatures were long lasting rather than transient, as proposed by Edman (1996).](image)
All experiments were performed at a soleus temperature of 35–37°C. All procedures were approved by the animal ethics committee of the University of Calgary.

3. Results

For cat soleus, force depression following shortening was always long lasting (Figs. 1 and 2). This result was observed in all muscles and at both frequencies of stimulation (10 and 30 Hz). Following the shortening phase, there was always a recovery of force over several seconds, i.e. the isometric force trace and the force trace following muscle shortening were approaching each other. However, for the past 10–15 s of these tests, the reference and experimental force–time traces remained virtually parallel, particularly for the fully fused (30 Hz) contractions (Figs. 1 and 2).

As observed previously in the cat soleus (Herzog and Leonard, 1997) and other muscles or isolated fibres, force depression increased with increasing extents of shortening (Fig. 3). When quantifying the force depression following shortening of 2, 4, 6 and 8 mm, it appeared that the relationship was virtually linear for all times analyzed following the end of the shortening contraction (Fig. 4).

When releasing the muscle for a given distance at a given speed at different levels of activation (and therefore different amounts of force during the shortening phase), force depression was increased with increasing forces during the shortening phase (Fig. 5). When plotting force depression as a function of the mean force during shortening, the relationship was negative for the time point at the exact end of the shortening phase (Fig. 6, trace 0), and was positive and reasonably linear for the remaining time points following shortening.

Fig. 2. Percent of force depression calculated using Eq. (1) as a function of time for the two experiments (30 and 10 Hz) shown in Fig. 1. Time = 0 s represents the instant when the shortening phase was finished. Note that the trace for the 30 Hz contraction is virtually parallel to the horizontal axis for the last 10–15 s, indicating a constant value of force depression during that phase. The 10 Hz trace never reaches a perfectly constant value, but the amount of force depression appears to decrease slowly even towards the very end of the experiment.

Fig. 3. Representative force–time curves for isometric reference contractions (i) at −4 mm length and for isometric contractions (−4 mm) that are preceded by shortening contractions of amplitude 2 mm (2), 4 mm (4), 6 mm (6), and 8 mm (8). Note, that the raw unsmoothed data are shown for four isometric reference contractions and for three experimental contractions for each of the shortening distances.

Fig. 4. Representative force depressions (in %) as a function of the shortening distances (in mm) at 0–4.5 s after the end of the shortening contractions. Force depressions are virtually linearly related to the shortening distances at all instants in time, approaching a steady-state force depression at about 2.5 s.
4. Discussion

The origin of force depression following shortening is unknown. Knowing whether force depressions are transient or long-lasting should help eliminate and/or identify possible mechanisms. In a recent manuscript, it had been argued that force depressions in skeletal muscle are transient because the mechanism responsible for force depression following shortening was a reduction in affinity for calcium at the thin filament regulatory sites during the shortening phase. Once the shortening phase was finished, the inhibition was released and normal force was shown to be reestablished within 1.0–1.5 s (Edman, 1996; his Fig. 3). However, for the contraction shown by Edman (1996), force was not fused, therefore the force in the muscle fibre relaxed to a certain degree after each stimulus. Also, shortening of the muscle was done at the beginning of the contraction in a virtually unloaded state. Therefore, the transient nature of the force depression could have been associated with the force release, rather than the reestablishment of normal calcium affinity following the shortening phase or the fact that shortening occurred in the unloaded state. Even in our experiments performed at a frequency of stimulation of 10 Hz, one might argue that there is a transient recovery of the force depression (Fig. 2), although recovery was never complete within the 20–30 s test period. A complete force release during a phase of force depression has been shown to abolish force depressions instantaneously (Abbott and Aubert, 1952; Herzog and Leonard, 1997). Therefore, in order to test if force depression following shortening was transient or long lasting, tests were performed for fused (30 Hz) contractions and loaded shortening conditions.

Abbott and Aubert (1952) already demonstrated long-lasting force depressions (about 20 s) in dogfish muscle. Their work cannot be accepted as proof for long-lasting force depressions, because their experiment was performed at 0°C, a temperature which slows down the contractile process so dramatically that the 20 s force depression may only be equivalent to a couple of seconds in mammalian skeletal muscle at 37°C. The results obtained here on cat soleus at 35–37°C strongly support the idea that force depression is indeed long lasting (Figs. 1 and 2). Combined with the result that force depression can be abolished instantaneously if force is released (Abbott and Aubert, 1952; Herzog and Leonard, 1997), a mechanical inhibition of cross-bridge attachment in the newly formed overlap zone appears attractive. One possible candidate for such a mechanical inhibition (among many others) is thin filament compliance (Goldman and Huxley, 1994; Huxley et al., 1994; Kojima et al., 1994; Wakabayashi et al., 1994), and the corresponding change in orientation between thick filament cross-bridges and thin filament attachment sites (Daniel et al., 1998).
If the above proposed mechanism of force depression is correct, one would expect a linear relationship between force depression and the amount of muscle shortening, always assuming uniform mechanical properties along the thin filament and a similar mean force of shortening. The mean forces in the shortening tests shown in Figs. 3 and 4 were similar, and the expected linear relationship was observed in all cases and at all times.

Furthermore, if the proposed mechanism of force depression is correct, one would also expect a linear relationship between the steady-state force depression and mean force during shortening, at least for the tests performed here. In our tests, submaximal forces were achieved by decreasing the current of stimulation to the motor nerve. Decreasing the current will reduce the number of activated motor units, but the motor units that are activated are activated maximally. Therefore, during the submaximal tests, the activated motor units contract maximally, while the remaining motor units are silent (of course, there might be a small number of motor units just at the motoneuron threshold that are partially activated, but it can be assumed that the number of these motor units is small). For this scenario, one would expect the relationship between mean force of shortening and force depression to be linear, because during the isometric contraction following the shortening phase, all motor units are recruited. Those which were active during the shortening phase should show the full inhibition effect; those which were silent should show no force depression, thus creating a situation where total force depression is linearly related to the number of active fibres, and therefore, the force during shortening.

Thin filament compliance results in increased deformation of the thin filament with increasing force on the filament. Furthermore, thin filament deformations are largest in the non-overlap zone (Forcinito et al., 1997) and they might restrict normal cross-bridge attachment once the “deformed” thin filament enters the overlap zone during shortening. The larger the amount of shortening, the greater the newly formed area of overlap between thick and “deformed” thin filament, and thus, the greater the inhibition to cross-bridge attachment in this zone.

From the results of this study, the proposal that force depression is related to a biochemical inhibition occurring during the shortening phase which disappears once shortening is finished (Edman, 1996) cannot be supported. Rather, it appears that for fused or partially fused tetanic contractions, force depression following shortening is long lasting but can be abolished instantaneously by releasing muscle force to zero levels for a short period of time.

Force depression following shortening appears to be linearly related to the amount of shortening and the mean force during the shortening phase. If these results are generally correct (i.e. for all muscles and shortening conditions), it would be trivial to incorporate force depression into any cross-bridge model of muscle contraction. Such an adaptation of the cross-bridge theory could be done by assuming that the attachment distribution function is calculated differently for two distinct zones of overlap: the zone that existed before shortening is treated using the normal distribution attachment function (Huxley, 1957; Huxley and Simmons, 1971); the zone of overlap formed during the shortening phase is treated with a distribution attachment function similar to that described above, except for an “inhibition” factor which is proportional to the force during shortening.

The fact that force depression appears to be positively related to the amount of shortening and the force during the shortening phase suggested to us that perhaps force depression might be related to a single scalar variable; the mechanical work produced by the muscle during the shortening phase. Although our experiments were not aimed at testing this hypothesis, we correlated the muscular work during the shortening phase with the steady-state force depression for two experimental conditions: the condition in which (a) the amount of shortening was varied (Fig. 3), and (b) the amount of force was varied (Fig. 5). For both situations, we found a reasonable straight line approximation of similar slope, and a virtually zero intercept (Fig. 7). This pilot result suggests that the muscular work during shortening might account for most of the observed force depressions observed in this study and in others. If correct for all situations, this result would imply that the speed of shortening is not associated with force depression, a result that has been taken for granted since the pioneering work in this area of research by Abbott and Aubert (1952). Clearly, the mechanism of force depression proposed here, and its possible explanation by a single scalar variable must be tested rigorously in the future.

4.1. Final comments

Often, force depression following shortening is associated with sarcomere length non-uniformities (Edman et al., 1993; Sugi and Tsuchiya, 1988), particularly as they occur on the descending limb of the force–length relationship. Our experiments were performed on the ascending limb of the force–length relationship (Fig. 3) where sarcomere length non-uniformities are minor (Edman et al., 1993), therefore, sarcomere length non-uniformities are likely not a significant factor in the force depressions observed here.

In order to further test the idea that force depressions might be caused by an inhibition of cross-bridge attachments following shortening, muscle stiffness should be quantified during the isometric reference contractions and the isometric contractions following shortening. We hypothesize that stiffness should be lower in the isometric
contractions following shortening than the isometric reference contractions, and that the decrease in stiffness should be proportional to the force depression. Although such stiffness measurements have been made in single frog muscle fibres (Sugi and Tsuchiya, 1988), we are not aware of any such measurements in either mammalian or whole muscle preparations.

Force depressions can be abolished instantaneously by a complete force release (Abbott and Aubert, 1952; Herzog and Leonard, 1997). However, force release in these experiments was accomplished by deactivation which might give the observed result for reasons different than the force release. Therefore, force releases during states of force depression should be performed in the future mechanically and at full activation (e.g. by quick release). We hypothesize that force depressions are abolished completely and instantaneously following a force release produced by a quick shortening of a fully activated muscle.

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References


