The relationship between force depression following shortening and mechanical work in skeletal muscle

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Abstract

Force depression following muscle shortening was investigated in cat soleus \((n = 6)\) at 37°C for a variety of contractile conditions with the aim to test the hypotheses that force depression was independent of the speed of shortening and was directly related to the mechanical work produced by the muscle during shortening. Force depression was similar for tests in which the mechanical work performed by the muscle was similar, independent of the speed of shortening (range of speeds: 4–256 mm/s). On the other hand, force depression varied significantly at a given speed of shortening but different amounts of mechanical work, supporting the hypothesis that force depression was not speed but work dependent. The variations in the mechanical work produced by the muscle during shortening accounted for 87–96% of the variance observed in the force depression following shortening further supporting the idea that the single scalar variable work accounts for most of the observed loss in isometric force after shortening. The results of the present study are also in agreement with the notion that the mechanism underlying force depression might be associated with an inhibition of cross-bridge attachments in the overlap zone formed during the shortening phase, as proposed previously (Herzog and Leonard, 1997. Journal of Biomechanics 30 (9), 865–872; Maréchal and Plaghki, 1979. Journal of General Physiology 73, 453–467). © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The isometric force in muscle following shortening against resistance is lower (depressed) compared to the isometric force obtained at the same length without prior shortening. This observation has first been described by Abbott and Aubert (1952), and has since been confirmed on many occasions (e.g. Edman et al., 1993; Herzog and Leonard, 1997; Maréchal and Plaghki, 1979; Sugi and Tsuchiya, 1988). The mechanism underlying force depression following shortening is not known, although several mechanisms have been proposed in the past (Edman et al., 1993; Granzier and Pollack, 1989; Herzog and Leonard, 1997; Maréchal and Plaghki, 1979). Because of the lack of a generally accepted mechanism for force depression following muscle shortening, researchers have largely refrained from attempting to describe force depression mathematically. One of the exceptions is the work by Maréchal and Plaghki (1979) who showed that force depression is linearly related to the amount of shortening and that the linearity constant depended on where on the force–length relationship the experiments were performed. They further described that force depression was an inverse exponential function of the speed of shortening. Similar findings have been reported by others (Abbott and Aubert, 1952; De Ruiter et al., 1998).

Recently, based on results in cat soleus, it was proposed that force depression may be independent of the speed of shortening, but rather may depend on the force during shortening (Herzog and Leonard, 1997). This finding led to the suggestion of a mechanism of force depression following shortening based on the mechanical deformation of actin filaments entering the actin-myosin overlap zone during shortening (Maréchal and Plaghki, 1979), and a corresponding change in the relative orientation between myosin cross-bridges and actin-binding sites that might reduce the probability for cross-bridge attachments in the newly formed overlap zone. Since
actin myofilaments are known to be compliant (Goldman and Huxley, 1994; Higuchi and Goldman, 1995; Huxley et al., 1994; Kojima et al., 1994; Wakabayashi et al., 1994), and since this compliance is associated with changes in the three-dimensional orientation of actin and myosin filaments (Daniel et al., 1998), a stress-dependent inhibition of cross-bridges in the overlap zone formed during shortening becomes an attractive (and possible) mechanism (Herzog, 1998). Results on isolated frog fibres show that stiffness decreases in parallel with the amount of force depression following shortening (Sugi and Tsuchiya, 1988), supporting the idea that force depression may be associated with a decrease in the number of attached cross-bridges compared to purely isometric contractions.

If force depression following shortening is caused by a stress-dependent deformation of actin (and possibly myosin) filaments, force depression should be independent of the speed of shortening, but should depend on the amount of shortening and the stress (force) acting on the myofilaments during shortening. Furthermore, force depression should occur continuously during the shortening phase. Therefore, it appears that force depression following shortening might depend primarily on a single scalar variable, the work performed by the muscle during the shortening phase. Although it has been suggested that work may be associated with force depression (De Ruiter et al., 1998; Granzier and Pollack, 1989; Herzog, 1998), no systematic study has been conducted to investigate the possible relationship between work and force depression in skeletal muscle.

The purpose of this study was to test whether or not the single scalar variable work could account for most of the observed force depression following skeletal muscle shortening under a variety of conditions. Specifically, shortening contractions were performed by keeping two of the three determinant variables of shortening (amount of shortening, speed of shortening, and muscle activation during shortening) constant, while the third variable was varied in a systematic way. Furthermore, tests were performed with variable speeds and variable activation of the muscle during the shortening phase.

2. Methods

Force depression following shortening was determined in cat soleus (n = 6) using a setup that has been described earlier (Herzog and Leonard, 1997; Herzog, 1998). Briefly, cats were anaesthetized using a nitrous oxide, halothane, oxygen mixture. The soleus, soleus tendon and calcaneus were exposed using a single cut on the posterior, lateral shank. The soleus tendon was isolated from the rest of the Achilles tendon and was cut from the calcaneus with a remnant piece of bone after a reference length (corresponding to an 80° included ankle angle) had been determined.

A second cut was made on the posterior, lateral thigh and the tibial nerve was exposed and implemented with a bipolar cuff-type electrode (Herzog et al., 1995) for soleus stimulation. The cat was secured in a prone position in a hammock and the pelvis, thigh, and shank of the experimental hindlimb were fixed with bilateral bone pins to a stereotaxic frame. The bone piece at the distal end of the soleus tendon was attached with sutures to a muscle puller (MTS, Eden Prairie, MN, natural frequency > 10 kHz). The soleus forces (100 N = 10 V) and excursions (20 mm = 10 V) were measured continuously by the muscle puller and were collected at a frequency of 200 Hz, except for tests exceeding a shortening speed of 64 mm/s for which data were collected at 1000 Hz. The muscle length corresponding to the 80° included ankle angle was taken as zero length; shortening from that length was taken as negative, lengthening as positive. For example, a muscle length of −4 mm corresponds to a length 4 mm shorter than the reference length.

Six series of tests were performed. In the first series of tests, an isometric contraction at a given length was followed by a shortening contraction at a given speed and constant activation but varying amount of shortening, followed by a second isometric contraction at the new (shortened) length. The final length was always −4 mm; the starting lengths were (typically) −2, 0, +2, +4, and +6 mm. The speed of shortening was 4 mm/s and the stimulation of the tibialis nerve was three times the x-motoneuron threshold (3 T), 30 Hz, 0.1 ms pulse duration, and 8 s train duration. For the cat soleus (a primarily slow twitch fibred muscle (Ariano et al., 1973)), this stimulation protocol resulted in a fused tetanic contraction of (likely) all motor units.

In the second series of tests, isometric-shortening-isometric contractions were performed with constant activation (3 T, 30 Hz, 0.1 ms, 8 s) and constant amount of shortening (8 mm) but varying speeds (typically, 4, 8, 16, 32, 64, 128 and 256 mm/s).

In the third series of tests, isometric-shortening-isometric contractions were performed at a constant speed (4 mm/s) over a constant shortening range (8 mm) but varying levels of activation during the shortening phase ranging from 0 to 3 T in about 4–8 steps. The exact current provided to the tibial nerve for the varying levels of activation were different from muscle to muscle and changed within a muscle for different tests because of the ever changing threshold level for x-motoneuron stimulation. The activation of the muscle was constant for the initial and final isometric contractions (3 T, 30 Hz, 0.1 ms).

The fourth series of tests was identical to the third, except there was no shortening, and therefore no work performed by the muscle, when the level of activation was changed.

In the fifth series of tests, shortening over a given distance (8 mm) and constant activation (3 T, 30 Hz,
0.1 ms) was performed using a variety of changing speeds. The changes in speed were controlled by the computer that controlled the muscle puller. In our setup, any speed change that can be expressed as a function of time can be implemented. The specific speed changes chosen for this experiment are shown as part of Fig. 5 in the results section. In the last series of tests, the amount of shortening (8 mm) and the speed of shortening (4 mm/s) were kept constant whereas activation during the shortening phase was varied.

In all six series of tests, two identical isometric-shortening-isometric tests were preceded and followed by an isometric reference contraction at the final length. Force depression was determined as the difference in force between the isometric reference contraction and the steady-state isometric force following shortening (i.e., 3 s after the end of any shortening contraction in a given experiment). This difference in force was determined at the same instant in time for the reference and the experimental contractions to avoid any bias caused by fatigue-related loss of force. Work performed by the muscle was calculated as the area under the muscle force-length change graph. Calculating the work for each trial of all six series of tests gave, on average, about 50-60 values per muscle that could be related to the force depression values. These 50-60 values represent 25-30 independent measurements that were repeated once. Since the repeat measurements were nearly identical in all cases, they were not used for deriving the relation between work and force depression. A least-squares fitting power function that was forced through the origin (zero force depression, zero work) was used to describe the relationship between work and force depression. Also, force depression values were plotted as a function of the speed of shortening. All experimental procedures were approved by the animal ethics committee of the University of Calgary.

3. Results

Force depression following muscle shortening was directly related to the amount of shortening (Fig. 1a); similarly, the work performed by the muscle was increased with increasing amounts of shortening (Fig. 1c). Force depression was inversely related to the speed of shortening (Fig. 2a), and, for a given amount of shortening and constant activation, work of the muscle was decreased with increasing speeds of shortening (Fig. 2c). This latter result is caused by a decrease in the force during the shortening phase as the speed of shortening increases. It is merely a reflection of the force-velocity property of skeletal muscle (Hill, 1938).

Force depression was increased for increasing levels of tibial nerve stimulation (Fig. 3a). Increased stimulation caused an increase in the force during the shortening phase, and since the amount and speed of shortening were kept constant, the work performed by the muscle during shortening was also increased for increased tibial nerve stimulations (Fig. 3c). When activation was decreased for a 2 s period without any shortening of the muscle, no work was performed in the process and there was no force depression upon full reactivation (Fig. 4).

Performing isometric-shortening-isometric contractions at variable speeds or variable stimulation of the tibial nerve during the shortening phase resulted in force depressions that were directly related to the different amounts of work performed by the shortening muscle (Figs. 5 and 6, respectively).

The work performed by the muscle during shortening accounted, on average, for 92% (± 3% S.D.) of the force depression for all six muscles with a range from 87 to
96% Figs. 7(a–f). Although, a casual glance at Figs. 7(a–f) might give the impression that the force depression-work relationships vary much across muscles, it should be noted that the raw data for all muscles are remarkably similar. When combining all force depression and work values shown in Figs. 7(a–f) in a single graph, the resulting power relationship is still strong (Fig. 8; $r^2 = 0.85$), i.e. 85% of the variance in force depression across all six muscles and all six tests was explained by the variance in the mechanical work of the muscle.

When plotting force depression as a function of the shortening speed, two observations were made: first, force depression was similar for experiments performed at vastly differing speeds provided that the work during shortening was similar, and second, force depression varied greatly for a given speed of shortening, provided that the work varied as well (Fig. 9).

4. Discussion

Force depression following shortening in skeletal muscles has been associated primarily with the amount of shortening, and the shortening speed (Abbott and Aubert, 1952; Maréchal and Plaghki, 1979). The most common mechanism related to force depression has been sarcomere length non-uniformity (Edman et al., 1993). For a variety of reasons, shortening speed and sarcomere length non-uniformity appear less attractive as candidate mechanisms at present than they might have in the past. For example, it has been demonstrated earlier (Herzog and Leonard, 1997), and in this study, that force depression may vary substantially in tests in which the shortening distance and speed are kept constant and force during

Fig. 2. Representative force–time histories of isometric reference contractions at a muscle length of $-4$ mm, and force–time histories for isometric-shortening-isometric contractions (a) at different speeds of shortening varying from 4 to 128 mm/s (b). Shortening distance (8 mm) and activation (maximal) were kept constant. The corresponding work–time histories for the above contractions (c).

Fig. 3. Representative force–time histories of isometric reference contractions at a muscle length of $-4$ mm, and force–time histories of isometric-shortening-isometric contractions at four levels of activation varying from zero to maximal during the shortening phase (a) Note, that the magnitude of force depression increased with increasing work performed by the muscle; i.e. the amount of force depression increased from trace b to c, to d, to e, as expected. The shortening distance (8 mm) and shortening speed (4 mm/s) were kept constant (b). The corresponding worktime histories for the above conditions (c).
shortening is varied (Fig. 3). Furthermore, we found consistently that force depression was similar when the mechanical work performed by the muscle during shortening was similar, even if the speed of shortening was changed from the low (4 mm/s) to the high extreme (256 mm/s) tested in this study (Fig. 9). These results suggest that it is the work rather than the speed of shortening that is related to the force depression.

Similarly, the sarcomere length non-uniformity mechanism cannot explain the observation by Granzier and Pollack (1989) that force depression is virtually identical for fixed end and sarcomere length controlled contractions in isolated frog skeletal muscle fibres. Also, sarcomere length non-uniformity has been suggested as
Fig. 7. Relationship between the amount of depressed force as a function of the work performed by the muscle during the shortening phase for all six muscles (a–f) and for all six experimental conditions (the different symbols are associated with the different experimental conditions). Also, shown is the best-fitting power function that goes through the origin (0, 0) of the force–work plot and the corresponding coefficient of variation, $R^2$.

a mechanism for force enhancement following eccentric contractions (Edman and Tsuchiya, 1996), and it is hard to reconcile how a single mechanism may explain two opposite phenomena (force depression and force enhancement) simultaneously.

Recently, it has been found that actin myofilaments are compliant (Goldman and Huxley, 1994; Higuchi and Goldman, 1995; Huxley et al., 1994; Kojima et al., 1994; Wakabayashi et al., 1994) and may account for as much as 50–70% of the total sarcomere compliance (Daniel
between actin and myosin one would expect the newly formed overlap zone to be produced. If the amount of shortening is constant, the work performed depends on the amount of shortening. For this situation, the larger the amount of shortening, the greater the newly formed overlap zone (Fig. 11, n) and the larger the force depression, presumably because there is an increased area of overlap between actin and myosin filaments containing deformed actin filaments with a reduced probability for cross-bridge attachments compared to the same zone of overlap in an isometric contraction.

Although it has been mentioned anecdotally that work might be related to force depression (De Ruiter et al., 1998; Granzier and Pollack, 1989; Maréchal and Plaghki, 1979), no systematic study has ever been performed to explore the possible relationship between these two variables. Here, we show that the work performed by the muscle explains virtually the entire variation in force depression for a variety of different tests (Figs. 7 and 8). In addition, the relationship between the absolute force depression and the absolute work across all six muscles was similar (Fig. 8), resulting in a strong relationship across all muscles and all tests ($r^2 = 0.85$). We conclude from these results that work is a good descriptor of force depression, that force depression is likely not related to the speed of shortening but rather to the force or work that is changed with changing speeds (Fig. 9), and that work might not only be a descriptor of force depression but may point directly to an underlying mechanism.

If force depression following muscle shortening is caused by stress-induced inhibition of cross-bridge attachments in the newly formed actin–myosin overlap zone, two so far untested hypotheses should hold: (1) muscle in the force-depressed state should be less stiff than muscle in the normal isometric state. Although, such a correlation between stiffness and depressed force has been demonstrated in an earlier study on frog fibre preparations (Sugi and Tsuchiya, 1988), single fibres might behave distinctly different than whole mammalian skeletal muscle. (2) Force depression should be abolished instantaneously if the stress in the muscle is completely released. Although such stress release experiments have been performed in the past (Abbott and Aubert, 1952; De Ruiter et al., 1998; Herzog and Leonard, 1997), they were accomplished by interrupting the stimulation during the force-depressed phase. Therefore, one might argue that the recovery of force was related to the interruption of stimulation and the muscle’s reactivation after a sufficient period of time, rather than the release of force accompanying the interruption of stimulation. In order to test whether a stress release without a change in activation will abolish force depression, a muscle should...
be shortened first to produce force depression. Then, the muscle should be allowed to shorten a second time at a speed exceeding its maximal speed of shortening, therefore, most of the second shortening contraction is made at zero external force, and so, no stress on the contractile myofilaments. Based on the stress-dependent crossbridge inhibition mechanism, the force depression that was present following the first shortening contraction...
should be abolished completely following the second shortening contraction.

Summarizing, this study provides first systematic evidence that force depression is largely accounted for by the mechanical work performed by the muscle during shortening (Figs. 7 and 8). Furthermore, the results of this study indicate that force depression following shortening at a wide variety of speeds is the same provided the work performed during the shortening phase is about the same (Fig. 9). Finally, the muscle work during the shortening phase may directly point to a mechanism of force depression based on cross-bridge inhibition caused by mechanical deformation of myofilaments. Although the present study cannot rule out the most popular mechanism associated with force depression: sarcomere length non-uniformity; it provides support for a mechanism based on myofilament deformation (Herzog and Leonard, 1997; Herzog, 1998; Maréchal and Plaghki, 1979) and the associated change in three-dimensional geometry of cross-bridge attachment (Daniel et al., 1998).

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References


