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RESEARCH ARTICLE

New insights into force depression in skeletal muscle

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SUMMARY

Force depression observed following active shortening is not well understood. Previous research suggested that force depression might be associated with a stress-induced inhibition of cross-bridges in the newly formed overlap zone following shortening. Our aim was to investigate this theory in skinned fibres and determine whether there was an inhibition of the attachment of cross-bridges or a decrease in the force produced per cross-bridge. The stress-induced inhibition of cross-bridge theory gives testable predictions, including: (1) skinned fibres should show proportional force and stiffness depression, (2) force after shortening should not be lower than force before shortening, (3) stiffness following shortening should not be lower than stiffness before shortening and (4) force depression should decrease when the stress during shortening is decreased. In agreement with these predictions, force and stiffness depression were approximately proportional, and force depression decreased with decreasing stress during shortening. However, in contrast to the predictions of the stress-induced inhibition of cross-bridge theory, force after shortening from sarcomere lengths of 2.8 and 3.0 μm to a sarcomere length of 2.4 μm was smaller than force before shortening, and this was not accompanied by a corresponding decrease in stiffness. We conclude that the stress-induced inhibition of cross-bridge theory, as proposed previously, cannot be the only mechanism for force depression, but that there is an additional, stress-induced inhibition of cross-bridges in the old overlap zone. Furthermore, both mechanisms, inhibition of cross-bridge attachment and reduction of force produced per cross-bridge, contribute to force depression. Inhibition and/or reduction of force depend(s) on the amount of stress imposed on actin during the shortening phase.

Key words: history dependence, stress-induced inhibition, cross-bridge, force, shortening, skinned fibre, stiffness, BDM.

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INTRODUCTION

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 (Abbott and Aubert, 1952; De Ruiter et al., 1998; Granzier and Pollack, 1989; Herzog and Leonard, 1997; Herzog et al., 1998; Marechal and Plaghki, 1979; Morgan et al., 2000; Sugi and Tsuchiya, 1988). This phenomenon, known as force depression, has been systematically observed in whole muscle preparations (Abbott and Aubert, 1952; Herzog and Leonard, 1997; Marechal and Plaghki, 1979; Morgan et al., 2000), human skeletal muscles (De Ruiter et al., 1998; Lee and Herzog, 2003; Lee et al., 1999), single intact fibres (Edman et al., 1993; Granzier and Pollack, 1989; Julian and Morgan, 1979; Sugi and Tsuchiya, 1988) and single myofibrils (Joumaa and Herzog, 2010). Force depression increases with increasing shortening magnitudes (Abbott and Aubert, 1952; Herzog and Leonard, 1997; Marechal and Plaghki, 1979) and decreases with increasing shortening speeds (Abbott and Aubert, 1952; Herzog and Leonard, 1997; Leonard and Herzog, 2005; Marechal and Plaghki, 1979; Morgan et al., 2000). Force depression is long lasting (>20 s) (Abbott and Aubert, 1952; Herzog et al., 1998; Lee and Herzog, 2003), but can be abolished instantaneously when force drops to zero (Abbott and Aubert, 1952; Herzog and Leonard, 1997).

The mechanism underlying force depression is still unclear. It has been suggested that force depression is caused by sarcomere length instabilities on the descending limb of the force–length relationship, which results in the development of sarcomere length non-uniformities during active shortening. These sarcomere length non-uniformities are thought to cause a decrease in isometric forces if they are preceded by active shortening (Morgan et al., 2000). However, there is still debate about whether sarcomere length non-uniformity is the cause of force depression (Joumaa and Herzog, 2010).

More than three decades ago, Marechal and Plaghki (Marechal and Plaghki, 1979) suggested that force depression might be associated with a stress-induced inhibition of cross-bridges in the newly formed overlap zone following shortening. According to Marechal and Plaghki (Marechal and Plaghki, 1979), when a sarcomere is activated isometrically, the thin filament is strained because of its compliant nature. This strain causes distortion of actin and a change in the orientation of the cross-bridge attachment sites (Daniel et al., 1998) that is proportional to the actin deformation, thereby inhibiting cross-bridges either by decreasing the probability of cross-bridge attachments or by preventing attached cross-bridges from producing force. Because actin filaments are anchored to myosin in the A-band region, whereas they are free in the I-band region, deformations of actin would be expected to be big in the I-band region and small in the A-band region. During active shortening, strained and deformed parts of actin filaments previously present in the I-band region enter the overlap zone, forming a ‘new’ overlap zone in which cross-bridges are inhibited.

Marechal and Plaghki (Marechal and Plaghki, 1979) proposed the stress-induced inhibition of cross-bridge theory following force depression experiments performed in whole muscle preparations.

However, experiments at the whole-muscle level cannot provide more than a rough glimpse at a mechanism that is occurring at the sarcomere level. In their experiments, sarcomere lengths could not be measured, and force is a combination of active and passive structures that are arranged in series and in parallel in the muscle, and therefore their force cannot be directly attributed to sarcomeres. Attempts at testing the validity and limitations of the stress-induced inhibition of cross-bridge theory in a preparation where sarcomere lengths could be measured and force could reflect what is occurring at the sarcomere level have never been made. Therefore, the aim of this study was to investigate the stress-induced inhibition of cross-bridge theory in skinned fibres. In skinned fibres, sarcomere lengths can be measured readily, and force measured at the end of the fibre is the force produced only by sarcomeres inside the fibre as the connective tissue surrounding fibres is eliminated. Furthermore, we wanted to determine whether there was an inhibition of the attachment of cross-bridges or a reduction of the force produced per cross-bridge. It has been shown that stiffness can be used as a measure of the percentage of attached cross-bridges (Ford et al., 1981); therefore, stiffness was measured in all reference contractions and the corresponding test contractions.

If the stress-induced inhibition of cross-bridges was the origin of force depression, and if there was an inhibition of the attachment of cross-bridges rather than a reduction of the force produced per cross-bridge, the following predictions should hold: (1) skinned fibres should show proportional force and stiffness depression; (2) because the inhibition of cross-bridges only occurs in the newly formed overlap zone, force after shortening should not be lower than force before shortening; (3) stiffness following shortening should not be lower than stiffness before shortening; and (4) because actin deformation is stress dependent, force depression should decrease when the stress during shortening is decreased.

MATERIALS AND METHODS

Skinned fibre preparation

New Zealand white rabbits [*Oryctolagus cuniculus* (Linnaeus 1758]) were euthanized by an intravenous injection of 1 ml of a pentobarbital solution (240 mg ml^{-1}), 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 a skinning solution for 12 h at 4°C, then in a skinning-glycerol (50:50) solution at –20°C for 2 weeks (Mounier et al., 1989).

On the day of the experiments, a single fibre segment (~1.5–2 mm length) was dissected from the skinned muscle biopsy using a binocular microscope and transferred to an experimental glass chamber containing the relaxing solution. One end of the fibre was glued to the hook of a length controller and the other end to the hook of a force transducer (Aurora Scientific Inc., Model 400A, Aurora, ON, Canada), allowing control of length and force measurements, respectively. Sarcomere lengths were measured using optical diffraction of a He-Ne laser beam. All experiments were performed at ~22°C.

Mechanical tests

Force and stiffness depression in comparison to the purely isometric contraction, and the decrease in force and stiffness in comparison to force and stiffness before shortening, were measured for four shortening magnitudes, from initial average sarcomere lengths of 2.8, 3.0, 3.2 and 3.6 μm to an average sarcomere length of 2.4 μm, performed at a speed of shortening of 0.05 fibre lengths s^{-1}. The initial average sarcomere length before shortening is designed as $L_{S,i}$, which is 2.8, 3.0, 3.2 or 3.6 μm. The final average sarcomere length after shortening is constant (2.4 μm) and the shortening magnitudes are 0.4, 0.6, 0.8 and 1.2 μm, respectively.

Twenty fibres were used in this study. For each shortening magnitude, the following protocol was performed. Fibres were set at an average sarcomere length of 2.4 μm. Fibres were then stretched passively to $L_{S,i}$ (2.8, 3.0, 3.2 or 3.6 μm), held isometrically for 1 min, activated, shortened to an average sarcomere length of 2.4 μm at a speed of shortening of 0.05 fibre lengths s^{-1}, held isometrically for 30 s and then deactivated. A period of 5 min rest was given to the fibres between tests. Fibres were then reactivated at the final average sarcomere length of 2.4 μm in order to measure reference force. Stiffness was calculated during a quick stretch–release cycle of 0.2% fibre length at a speed of 1 fibre length s^{-1} performed before and after activation at $L_{S,i}$, after active shortening to the average sarcomere length of 2.4 μm and after reference contractions at 2.4 μm.

Stiffness (instantaneous stiffness) was measured as the difference between the peak force reached after the quick stretch and the force before stretch divided by the amplitude of the stretch. Force and stiffness depression were defined as the difference between the steady-state isometric force and stiffness following active shortening and the purely isometric force and stiffness at 2.4 μm sarcomere length. Force and stiffness decrease compared with the force and stiffness before shortening were defined as the difference between the steady-state isometric force and stiffness following shortening (at the short length, 2.4 μm) and the force and stiffness for the purely isometric contraction before shortening (at the longer length, $L_{S,i}$).

To compare active force and stiffness at different sarcomere lengths (before shortening at sarcomere lengths ranging from 2.8 to 3.6 μm and after shortening at a sarcomere length of 2.4 μm), passive force and stiffness measured before activation were subtracted from the total force and stiffness measured after activation.

Experiments with 2,3-butanedione monoxime

Force depression tests were performed on an additional 10 fibres exposed to 2,3-butanedione monoxime (BDM). BDM is thought to decrease active force by inhibiting the release of the inorganic phosphate in the cross-bridge cycle and therefore keeping cross-bridges in pre-power stroke states (Herrmann et al., 1992). Because BDM decreases the active isometric force and the stress on fibres, these experiments were performed to test the effect of decreases in stress before and during shortening on force depression.

Solutions

We used the following three solutions to keep the fibres passive (relaxing solution), remove old fluid during fluid exchanges (washing solution) and activate fibres and induce contractions (activating solution): (1) skinning or relaxing solution (in mmol l^{-1}): potassium propionate (170), magnesium acetate (2.5), MOPS (20), K_2EGTA (5) and ATP (2.5), pH 7.0; (2) washing solution (in mmol l^{-1}): potassium propionate (185), magnesium acetate (2.5), MOPS (10) and ATP (2.5), pH 7.0; and (3) activating solution (in mmol l^{-1}): potassium propionate (170), magnesium acetate (2.5), MOPS (10), ATP (2.5) and free Ca^{2+} buffered with EGTA (CaEGTA and K_2EGTA were mixed to obtain the pCa value of 4.2), pH 7.0.

RESULTS

Shortening produced force depression (Figs 1, 2, Tables 1, 2) in all fibres for all shortening magnitudes. The mean (±s.e.m.) force depression was 27.7±3.3, 40.5±3.7, 50.7±4.0 and 70.1±3.7% after shortening from sarcomere lengths of 2.8, 3.0, 3.2 and 3.6 μm, respectively, to an average sarcomere length of 2.4 μm.

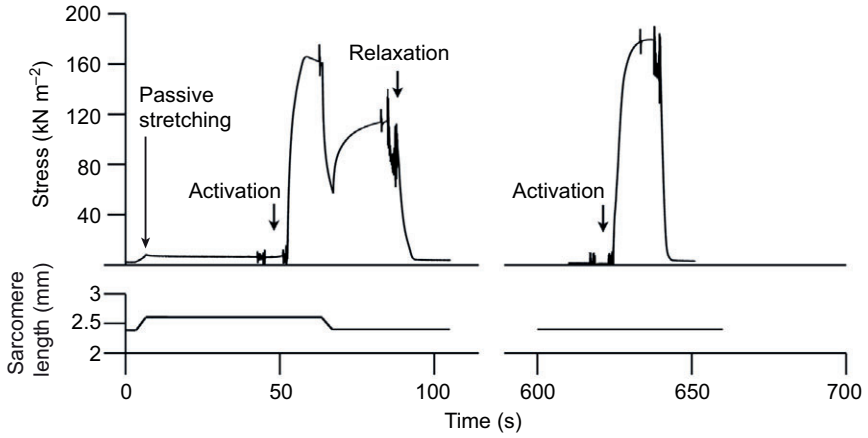

Fig. 1. Typical fibre response when stretched passively from an average sarcomere length of 2.4 μm to an average sarcomere length of 2.8 μm, activated, then shortened to an average sarcomere length of 2.4 μm, deactivated and then activated again. Note that the force after active shortening is smaller 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 passive stretching, activation (initial length=2.8 μm), deactivation (relaxation; final length=2.4 μm) and re-activation at the final length (2.4 μm). A fibre was activated by adding first a washing solution (free of EGTA and calcium) and then an activating solution (with high concentration of calcium). The noise on the graph indicates the time when the solution was changed. The sudden change in force observed after passive stretching (barely visible), activation at 2.8 μm, shortening to 2.4 μm and activation at 2.4 μm indicates the stretch–shortening cycle performed to measure stiffness.

Stiffness depression was observed in all fibres (Table 2, Fig. 2) and was correlated with the amount of force depression (Fig. 3).

In contrast to the predictions of the stress-induced inhibition of cross-bridge theory, force after shortening from average sarcomere lengths of 2.8 and 3.0 μm to an average sarcomere length of 2.4 μm was smaller than force before shortening (Fig. 1, Tables 1, 2). However, shortening from sarcomere lengths of 3.2 and 3.6 μm resulted in forces that were greater than the forces for the purely isometric contractions at the initial length.

Stiffness following shortening was not changed compared with stiffness before shortening when the initial sarcomere lengths were 2.8 and 3.0 μm, and was increased when the initial sarcomere lengths were 3.2 and 3.6 μm (Tables 1, 2).

The application of BDM led to an expected decrease in isometric reference force (at a sarcomere length of 2.4 μm) of 32.4±3.2%. Force depression for the BDM conditions existed for all shortening magnitudes but was consistently less than that for the shortening trials without BDM (Fig. 4). In the presence of BDM, force and stiffness decrease compared with force and stiffness before shortening were less than the decreases observed without BDM (Fig. 5).

DISCUSSION

The aim of this study was to investigate specific predictions on the mechanisms of force depression based on the stress-induced inhibition of cross-bridge theory (Marechal and Plaghki, 1979) in skinned fibres. Furthermore, we wanted to determine whether there was an inhibition of the attachment of cross-bridges or a reduction in the force produced per cross-bridge following active shortening in skinned fibres. The stress-induced inhibition of cross-bridge theory is based on the idea that when a sarcomere is activated, there is a stress-induced distortion of the thin filament in the I-band region of the sarcomere and possibly a change in the orientation of the cross-bridge attachment sites (Daniel et al., 1998; Marechal and Plaghki, 1979). During active shortening, strained and deformed parts of the actin filament enter the overlap zone, forming a new overlap zone in which the cross-bridges are inhibited either by decreasing the probability of cross-bridge attachments or by preventing attached cross-bridges from producing normal force. The stress-induced inhibition of cross-bridge theory gives testable predictions, many of which were supported by our results; however,

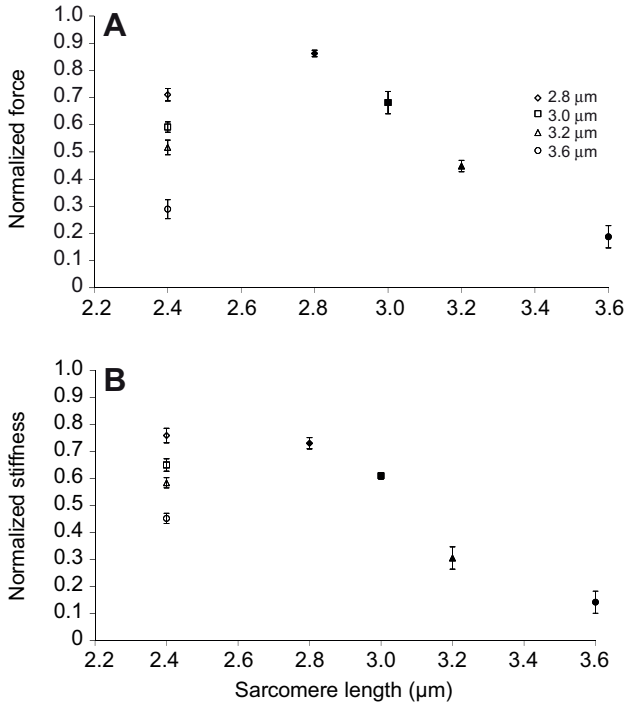

Fig. 2. Force (A) and stiffness (B) normalized to force and stiffness measured for the purely isometric contraction performed at a sarcomere length of 2.4 μm (values are means ± s.e.m.). Diamonds, squares, triangles and circles represent force and stiffness before (closed symbols) and after shortening (open symbols, depressed state at the sarcomere length of 2.4 μm) from sarcomere lengths of 2.8, 3.0, 3.2 and 3.6 μm, respectively.

Table 1. Stress and stiffness before and after shortening and after reference contraction

	Before shortening		After shortening to 2.4 μm		Reference contraction at 2.4 μm	
Initial sarcomere length (μm)	Stress ($kN\,m^{-2}$)	Stiffness ($mN\,mm^{-1}$)	Stress ($kN\,m^{-2}$)	Stiffness ($mN\,mm^{-1}$)	Stress ($kN\,m^{-2}$)	Stiffness ($mN\,mm^{-1}$)
2.8	161±4*	14.6±1.1	134±7	15.2±1.2	188±5*	20.0±2.0*
3.0	124±5*	11.0±1.0	110±6	12.2±1.1	180±4*	18.3±2.1*
3.2	90±2*	6.7±0.6*	102±5	10.6±0.9	191±6*	19.3±1.4*
3.6	46±3*	3.2±0.3*	64±4	8.0±0.9	189±4*	18.6±2.5*

Values are means ± s.e.m. Asterisks indicate a significant difference in stress or stiffness before shortening and in reference contractions compared with after shortening (Wilcoxon test, $P<0.05$).

the prediction that force after shortening should not be lower than force before shortening was rejected. This implies that the inhibition of cross-bridges in the newly formed overlap zone alone cannot explain all of the observed force depression.

All skinned fibres showed force depression, which increased with increasing shortening magnitudes, thereby confirming results in human muscle (De Ruiter et al., 1998; Lee and Herzog, 2003; Rousanoglou et al., 2007), whole isolated muscle (Abbott and Aubert, 1952; Herzog and Leonard, 1997; Marechal and Plaghki, 1979; Meijer et al., 1998; Morgan et al., 2000) and single fibre preparations (Granzier and Pollack, 1989; Sugi and Tsuchiya, 1988). As skinned fibres are formed of myofibrils arranged in parallel and in series, we suggest that mechanisms occurring within myofibrils and in particular within sarcomeres likely contribute to force depression. It has been assumed that stiffness is related to the number of attached cross-bridges (Ford et al., 1981). However, stiffness is also associated with the compliance of actin, myosin and titin (Goldman and Huxley, 1994; Horowits et al., 1986; Kojima et al., 1994). If we assume that actin, myosin and titin compliance is approximately similar for the experimental and reference conditions (at a given length), we can infer that the decrease in stiffness observed in this study at the sarcomere length of 2.4 μm is likely associated with a reduction in the proportion of attached cross-bridges. Therefore, it appears that force depression is likely associated with an inhibition of cross-bridge attachments. Similar decreases in stiffness following active muscle shortening were observed by Sugi and Tsuchiya (Sugi and Tsuchiya, 1988) in single fibres, and by Lee and Herzog (Lee and Herzog, 2003) in human adductor pollicis.

Shortening from average sarcomere lengths of 2.8 and 3.0 μm to 2.4 μm produced a decrease in force compared with the isometric forces before shortening. This decrease in force was not in accordance with the predictions of the stress-induced inhibition of cross-bridge theory, which requires that force after shortening is equal to or greater than the force before shortening. However, we may speculate that inhibition of cross-bridges occurs in the entire actin–myosin overlap zone and not only in the newly formed overlap zone as proposed by Marechal and Plagkhi (Marechal and Plagkhi, 1979). Because this force decrease was not accompanied by a corresponding decrease in stiffness, and because strongly and weakly bound cross-bridges are assumed to contribute equally to stiffness (Forcinito et al., 1997; Forcinito et al., 1998; Huxley, 1957; Huxley and Simmons, 1971), we can infer that a shortening-induced transformation of cross-bridges from strongly to weakly bound states contributed to the observed force depression. Furthermore, forces following shortening from initial sarcomere lengths of 3.2 and 3.6 μm were slightly greater than the isometric forces prior to shortening whereas the corresponding stiffness values were substantially greater (Table 1), suggesting that the proportion of force-producing (strongly bound) *versus* non-force-producing (weakly bound) cross-bridges was decreased following fibre shortening.

We may speculate that inhibition of cross-bridge attachment, or confinement to the weakly bound state, depends on the amount of stress in the sarcomere and the associated deformation of actin. It seems that inhibition of cross-bridges following active shortening is associated with high stresses on actin and great deformation, whereas transformation to weakly bound states is caused by small stresses. The difference in forces following shortening compared with the isometric forces before shortening for short and long initial sarcomere lengths could be explained according to this hypothesis. Force following shortening was significantly lower than force before shortening for initial sarcomere lengths of 2.8 and 3.0 μm, but not for initial sarcomere lengths of 3.2 and 3.6 μm. In fact, the shorter the initial sarcomere length, the higher the force decrease compared with force before shortening (Tables 1, 2). Activation at a sarcomere length of 2.8 μm is associated with a large force (approximately 80% of the force produced at the plateau region of the force–length relationship) and a large stress; therefore, it is thought that actin

Table 2. Percentage of force and stiffness depression and decrease

Initial sarcomere length (μm)	Force depression (%)	Stiffness depression (%)	Force decrease (%)	Stiffness decrease (%)
2.8	27.7±3.3*	25.4±3.9*	17.7±2.1*	–3.6±4.26#
3.0	40.5±3.6*	34±4.3*	12.9±4.7*	–10.1±10.9#
3.2	50.7±4.0*	43.4±4.1*	–16.5±13.4*	–91.1±26.4*,#
3.6	70.8±3.7*	60.0±2.6*	–44.7±16.0*	–167.8±35.7*,#

Force and stiffness depression percentages were defined as the difference between the steady-state isometric force and stiffness following active shortening and the purely isometric force and stiffness at 2.4 μm sarcomere length, divided by the purely isometric force and stiffness at 2.4 μm sarcomere length. Force and stiffness decreases were defined as the difference between the steady-state isometric force and stiffness before active shortening and the force and stiffness obtained after shortening to 2.4 μm sarcomere length, divided by the force and stiffness before shortening.

*, A significant change in force or stiffness relative to the force and stiffness, respectively, for the purely isometric contractions at the final length (second and third columns) and the initial length (last two columns); #, a significant difference between force decrease and stiffness decrease (Wilcoxon test, $P<0.05$ length).

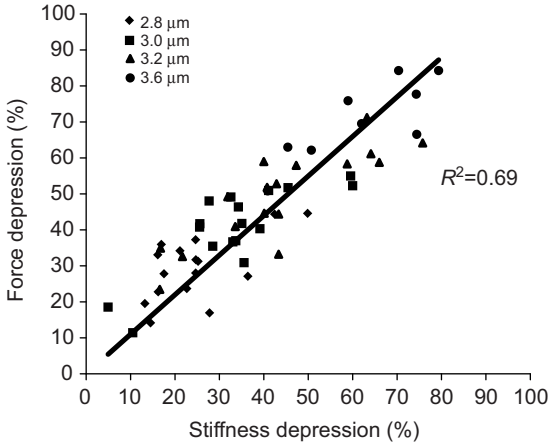

Fig. 3. Force depression as a function of stiffness depression after shortening from sarcomere lengths of 2.8, 3.0, 3.2 and 3.6 μm to a sarcomere length of 2.4 μm. There is a significant correlation (P<0.05) between force and stiffness depression across all shortening magnitudes (slope=1.1). Diamonds, squares, triangles and circles represent shortenings from sarcomere lengths of 2.8, 3.0, 3.2 and 3.6 μm, respectively.

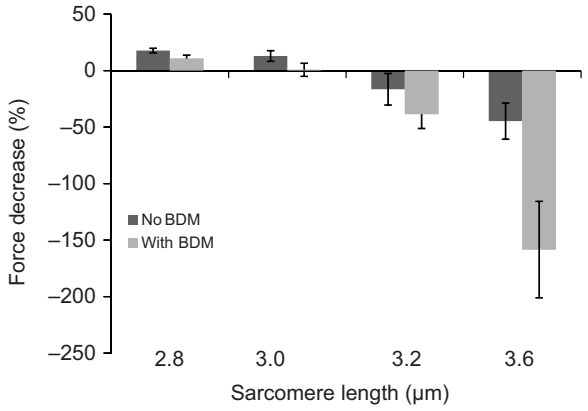

Fig. 5. Mean (±s.e.m.) force decrease after shortening compared with force before shortening in the absence (black) and presence (grey) of BDM. The change in force observed in the presence of BDM is significant at all sarcomere lengths (Wilcoxon test, P<0.05). Sarcomere lengths (2.8, 3.0, 3.2 and 3.6 μm) represent the initial sarcomere length before active shortening to the final sarcomere length of 2.4 μm.

filaments are deformed to a great extent in the I-band region and little in the A-band region (Daniel et al., 1998). Following shortening, most cross-bridge attachments would be expected to be inhibited in the newly formed overlap zone, and some cross-bridges would be expected to be transformed into weakly bound states in the old overlap zone, resulting in smaller forces after shortening than force before shortening, while stiffness remains essentially unchanged. In contrast, activation at 3.6 μm is associated with a small force (approximately 18% of the force produced at the plateau of the force–length relationship) and a low stress. Therefore, actin deformations would be expected to be small, thereby causing small inhibitions of cross-bridge attachments, and thus the loss of force owing to inhibition would be more than offset by the gain of force owing to increasing myofilament overlap. Consequently, force would be higher after shortening than the isometric force at the long initial length.

Experiments with the cross-bridge inhibitor BDM confirmed that force depression is stress dependent. BDM decreased active force and stress during shortening and, as a result, force depression

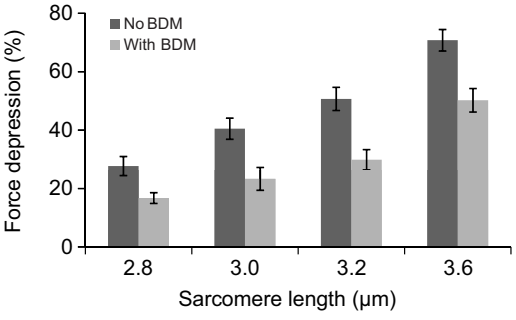

Fig. 4. Mean (±s.e.m.) force depression in the absence (black) and presence (grey) of 2,3-butanedione monoxime (BDM). Force depression is significantly smaller in the presence of BDM compared with the normal conditions at all sarcomere lengths (Wilcoxon test, P<0.05). Sarcomere lengths (2.8, 3.0, 3.2 and 3.6 μm) represent the initial sarcomere length before active shortening to the final sarcomere length of 2.4 μm.

and the decrease in force relative to the isometric force at the initial length were decreased. In contrast, as it has been shown that titin's stiffness might increase in various experimental conditions (Labeit et al., 2003; Joumaa et al., 2008), we can speculate that titin's stiffness increases after active shortening and may thus explain the discrepancy between the decrease in force and stiffness observed after active shortening. However, because it is known that titin-based forces in rabbit psoas fibres only start coming into effect at average sarcomere lengths of approximately 2.8 μm (Bartoo et al., 1997; Joumaa et al., 2008) and because fibres here were shortened to an average sarcomere length of 2.4 μm, it is safe to assume that titin-based stiffness does not play a role at the fibre lengths tested in this study. Furthermore, although the increase in titin stiffness reported by Labeit et al. (Labeit et al., 2003) and Joumaa et al. (Joumaa et al., 2008) was small, enormous increases in titin stiffness would be required to compensate for the decrease in active stiffness observed in our study.

Another hypothesis that has been proposed to explain force depression has been the development of sarcomere length non-uniformities during active shortening on the descending limb of the force–length relationship (Edman et al., 1993; Julian and Morgan, 1979; Morgan et al., 2000). According to this hypothesis, sarcomeres are assumed to shorten by different amounts because of instability of sarcomere length and force on the descending limb of the force–length relationship (Hill, 1953). This non-uniform behaviour leads to a situation in which sarcomeres, after attaining force equilibrium, produce tension that is smaller than that produced at the corresponding length obtained during an isometric contraction, in which sarcomeres are assumed to be at relatively uniform lengths. However, there is still debate about whether sarcomere length non-uniformity is the cause of force depression (Joumaa and Herzog, 2010).

Conclusions

We conclude that the stress-induced inhibition of cross-bridge theory, as proposed by Marechal and Plaghki (Marechal and Plaghki, 1979), explains most but not all of the force depression

observed here. We propose that in addition to this inhibition, there is also a stress-induced inhibition of cross-bridges in the old overlap zone and a reduction in the average force per cross-bridge that contributes a small but measurable amount to force depression. Inhibition of cross-bridge attachment, or the reduction of average cross-bridge force, seems to depend crucially on the amount of stress imposed on actin; high stress favouring the former mechanism and low stress the latter.

FUNDING

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