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RESEARCH ARTICLE

Energy cost of isometric force production after active shortening in skinned muscle fibres

Venus Joumaa*, Alex Fitzowich and Walter Herzog

ABSTRACT

The steady-state isometric force after active shortening of a skeletal muscle is lower than the purely isometric force at the corresponding length. This property of skeletal muscle is known as force depression. The purpose of this study was to investigate whether the energy cost of force production at the steady state after active shortening was reduced compared with the energy cost of force production for a purely isometric contraction performed at the corresponding length (same length, same activation). Experiments were performed in skinned fibres isolated from rabbit psoas muscle. Skinned fibres were actively shortened from an average sarcomere length of 3.0 µm to an average sarcomere length of 2.4 µm. Purely isometric reference contractions were performed at an average sarcomere length of 2.4 µm. Simultaneously with the force measurements, the ATP cost was measured during the last 30 s of isometric contractions using an enzyme-coupled assay. Stiffness was calculated during a quick stretch–release cycle of 0.2% fibre length performed once the steady state had been reached after active shortening and during the purely isometric reference contractions. Force and stiffness following active shortening were decreased by 10.0±1.8% and 11.0±2.2%, respectively, compared with the isometric reference contractions. Similarly, ATPase activity per second (not normalized to the force) showed a decrease of 15.6±3.0% in the force-depressed state compared with the purely isometric reference state. However, ATPase activity per second per unit of force was similar for the isometric contractions following active shortening (28.7±2.4 mmol l^{-1} mN^{-1} s mm^3) and the corresponding purely isometric reference contraction (30.9±2.8 mmol l^{-1} mN^{-1} s mm^3). Furthermore, the reduction in absolute ATPase activity per second was significantly correlated with force depression and stiffness depression. These results are in accordance with the idea that force depression following active shortening is primarily caused by a decrease in the proportion of attached cross-bridges. Furthermore, these findings, along with previously reported results showing a decrease in ATP consumption per unit of force after active muscle stretching, suggest that the mechanisms involved in the steady-state force after active muscle shortening and active muscle lengthening are of distinctly different origin.

KEY WORDS: ATPase activity, Force depression, Cross-bridge cycling, Stiffness, Efficiency, Residual force enhancement, Concentric muscle contraction, Eccentric muscle contraction, Isometric muscle contraction

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INTRODUCTION

Force at the steady state following active shortening of a skeletal muscle is reduced compared with the force produced after a purely isometric contraction performed at the same final length (Abbott and Aubert, 1952). This force depression (FD) has been observed in all muscle preparations, ranging from whole muscles (Abbott and Aubert, 1952; Herzog and Leonard, 1997; Maréchal and Plaghki, 1979; Meijer et al., 1998; Morgan et al., 2000) to human skeletal muscles (De Ruiter et al., 1998; Lee and Herzog, 2003; Power et al., 2014), single fibres (Edman et al., 1993; Granzier and Pollack, 1989; Joumaa et al., 2012; Julian and Morgan, 1979; Minozzo and Rassier, 2013; Sugi and Tsuchiya, 1988) and myofibrils (Joumaa and Herzog, 2010). Force depression increases with increasing shortening magnitudes (Abbott and Aubert, 1952; Herzog and Leonard, 1997; Maréchal and Plaghki, 1979), and decreases with increasing shortening speeds (Abbott and Aubert, 1952; Herzog and Leonard, 1997; Leonard and Herzog, 2005; Maréchal and Plaghki, 1979; Morgan et al., 2000).

The mechanisms underlying FD remain unclear. Because sarcomeres are thought to be unstable on the descending limb of the force–length relationship, it has been suggested that sarcomeres shorten by different amounts during active shortening. This non-uniform shortening of sarcomeres results in the force being reduced compared with a situation in which force is produced by sarcomeres of relatively uniform lengths (Morgan et al., 2000). Another mechanism for force depression was proposed by Maréchal and Plaghki in 1979. Maréchal and Plaghki (1979) suggested that force is reduced after active shortening because of a stress-induced inhibition of cross-bridge attachment in the myofilament overlap region formed after shortening. It has been shown that the actin and myosin filaments are compliant (Goldman and Huxley, 1994; Kojima et al., 1994) and thus when muscle is activated, stress might cause changes in the conformation of actin monomers, possibly resulting in an angular distortion of the myosin binding sites on actin (Daniel et al., 1998). This distortion may cause inhibition of cross-bridge formation in the newly formed overlap zone when muscle is then shortened and the stress in actin filaments is maintained (Leonard and Herzog, 2005). Another mechanism proposed for FD has been the accumulation of fatigue products [H^+ and inorganic phosphate (P_i)] during shortening (Granzier and Pollack, 1989). It has been shown that ATP consumption increases steeply with the amount of work performed by the muscle and, consequently, increasing H^+ and P_i concentrations (Woledge et al., 1985). Therefore, one might predict a great increase in H^+ and P_i concentrations when mechanical work is performed during shortening compared with an isometric contraction, leading to a decrease in force after shortening (Granzier and Pollack, 1989).

Despite this abundance of information regarding the properties and mechanisms of force depression, there has been no investigation of the cost of force production during the isometric state after active shortening compared with purely isometric contractions. Recently, it was found that the ATP cost per unit of force was reduced by 17%

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in the isometric state after active stretching compared with the purely isometric contraction at the corresponding length (Joumaa and Herzog, 2013), suggesting that skeletal muscle becomes more economical after active stretch and that its metabolic and energetic properties depend on the history of contraction. Therefore, the purpose of this study was to investigate whether the energy cost of force production at the steady state after active shortening was reduced compared with the energy cost of force production for a purely isometric contraction performed at the corresponding length. Experiments were performed in skinned muscle fibres and the energy cost of force production was determined using an enzyme-coupled assay (Glyn and Sleep, 1985) as previously described (Joumaa and Herzog, 2013).

MATERIALS AND METHODS

Skinned fibre preparation

Six-month-old female New Zealand White rabbits were euthanized by an intravenous injection of 1 ml of pentobarbital (240 mg ml^{-1}), a protocol approved by the University of Calgary's Life and Environmental Sciences Animal Care and Ethics Committee. Strips of psoas muscle were then dissected, tied to small wooden sticks and stored in a skinning solution (see solutions below) 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 was dissected from the skinned muscle biopsy and transferred to an experimental glass chamber containing a 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, Model 400A, Ontario, Canada), allowing for control of fibre length and measurement of force, respectively. Sarcomere lengths were measured using optical diffraction of a He-Ne laser beam. Before experimentation, all fibres were bathed for 5 min in a relaxing solution containing 0.5% (v:v) of Triton X-100. Fibre volume was calculated assuming the fibre has a cylindrical shape. All experiments were performed at ∼15°C.

Mechanical tests

Active shortening contraction

Skinned fibres (n=16) were set at an average sarcomere length of 2.4 µm in the relaxing solution and then passively stretched to an average sarcomere length of 3.0 µm, held for 20 s and activated by changing the relaxing solution to a high calcium activating solution. Fibres were then actively shortened to an average sarcomere length of 2.4 µm at a speed of 0.1 fibre length s^{-1}. After steady state forces had been reached, fibres were quickly transferred to another bath of activating solution for 30 s and then relaxed (Fig. 1).

Reference contraction

After a rest period of 5 min, the isometric reference contraction test was performed. Fibres were activated at an average sarcomere length of 2.4 µm and then quickly transferred to another bath of activating solution for 30 s, then to a relaxing solution.

To minimize the effect of fatigue on force depression, and to have similar contraction conditions between the isometric reference and the active shortening tests, we performed the active shortening tests prior to the reference tests and fibres were activated for the same duration during the active shortening and the isometric reference tests.

Stiffness measurements

Stiffness was calculated during a quick stretch–release cycle of 0.2% fibre length at a speed of 1 fibre length s^{-1} performed once the steady state had been reached after active shortening and during the purely isometric reference contraction.

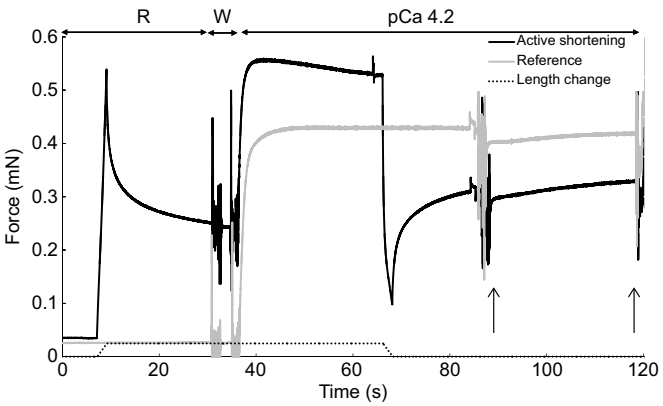

Fig. 1. Active shortening and purely isometric reference contractions. Reference: the fibre was activated at a sarcomere length of 2.4 µm then transferred to another bath of activating solution for 30 s (between the two vertical arrows). Active shortening: the fibre was passively stretched to a sarcomere length of 3.0 µm, activated, actively shortened to a sarcomere length of 2.4 µm and then transferred to another bath of activating solution for 30 s (between the two vertical arrows). The fibre was activated by adding first a washing solution (W; free of EGTA and calcium) and then an activating solution of pCa 4.2. R, W and pCa 4.2 indicate the solution in which the fibre is bathed. R, relaxing solution. The noise in the graphs indicates the time when the fibre was transferred between solutions. The dotted line along the time axis indicates the change in length of the fibre during the active shortening test. The sudden change in force observed before transfer to the activating solution for 30 s indicates the stretch–release cycle performed to measure stiffness. The average force produced by the fibre shown in this figure (diameter=80 µm, length=1.14 mm) during the last 30 s of activation was 0.32 and 0.41 mN for the active shortening and the reference contractions, respectively.

Metabolic cost

Metabolic cost was quantified by measuring the ATPase activity using an enzyme-coupled assay (de Tombe and Stienen, 1995; Glyn and Sleep, 1985; Locher et al., 2009; Mateja et al., 2013; Ottenheijm et al., 2011). The activating solution in which the fibres were bathed for 30 s before deactivation after the purely isometric reference contraction and the active shortening contraction was kept and used to quantify the amount of ADP produced (Joumaa and Herzog, 2013). ADP was coupled first to the synthesis of pyruvate and ATP from phosphoenolpyruvate, a reaction that is catalyzed by the enzyme pyruvate kinase, and subsequently to the synthesis of lactate, a reaction that is catalyzed by the enzyme lactate dehydrogenase and during which NADH is oxidized to NAD^+. The breakdown of NADH was determined photometrically by measuring the absorbance of 340 nm UV light (Chandra et al., 2009; de Tombe et al., 2007; Glyn and Sleep, 1985).

The absorbance signal was calibrated using known amounts of ADP and monitoring the NADH absorbance. ADP produced by the fibres was converted to the amount of ATP used during contraction by assuming that the ATP used during contraction is equal to the ADP produced.

Data analysis

Force depression

Force depression was determined as the difference in the steady-state isometric force following active shortening and the purely isometric force at 2.4 µm sarcomere length. The percentage of force depression was expressed as a function of the isometric reference force.

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Stiffness (instantaneous stiffness)

Stiffness was measured as the difference between the peak force reached after the quick stretch and the force immediately before the stretch divided by the amplitude of the stretch. Stiffness depression was defined as the difference in stiffness measured at the steady state following active shortening and the stiffness measured for the purely isometric reference contractions performed at an average sarcomere length of 2.4 µm. The percentage of stiffness depression was expressed as a function of the stiffness for the purely isometric reference contraction. Fibres that did not show stiffness and force depression were discarded from analysis, as force and stiffness depression was a required outcome for comparison with isometric reference contractions (Joumaa et al., 2012; Lee and Herzog, 2003; Sugi and Tsuchiya, 1988).

Metabolic cost

The absolute amount of ATP (not normalized to force) used during the last 30 s of isometric contraction before deactivation was compared between the active shortening and the reference contractions. In order to obtain the ATPase activity per unit of force, the absolute amount of ATP used during the last 30 s before deactivations was divided by the corresponding force and compared between the reference contraction and the corresponding active shortening contraction.

Statistical analysis

The non-parametric Wilcoxon test ($P<0.05$) was used to compare the amount of absolute ATP per second and the amount of ATP per second per unit of force used during the purely isometric reference contractions and the corresponding isometric contractions after active shortening.

Solutions

The solutions used were as follows. 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. Washing solution (in mmol l^{-1}): potassium propionate (185), magnesium acetate (2.5), MOPS (20) and ATP (2.5), pH 7.0. 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 mixed in order to obtain the pCa 4.2 value), pH 7.0. To every 100 µl of activating solution in which the fibres bathed for 30 s, we added phosphoenolpyruvate (15 mmol l^{-1}), pyruvate kinase (400 U ml^{-1}), lactate dehydrogenase (450 U ml^{-1}) and NADH (20 mmol l^{-1}).

RESULTS

Fig. 1 shows the force–time history of a typical experiment. As shown in the reference contraction test, force did not decrease over the duration of activation, suggesting that the effect of P_i accumulation and fatigue was negligible on force production.

The average amount of ATP used per second per unit of force for the purely isometric reference contractions normalized to fibre volume was 30.9±2.8 mmol l^{-1} mN^{-1} s mm^3. ATPase activity per second per unit of force for the isometric contractions following active shortening (force-depressed state; 28.7±2.4 mmol l^{-1} mN^{-1} s mm^3) was similar to that for the purely isometric reference contractions. The resolution of the technique used to measure the ATPase activity was 0.05 mmol l^{-1} (Fig. 2). In contrast, absolute ATPase activity per second (not normalized to force) was decreased by 15.6±3.0% in the force-depressed state compared with the purely isometric reference state. Furthermore, the reduction in absolute ATPase activity per second was significantly correlated with force depression (Fig. 3).

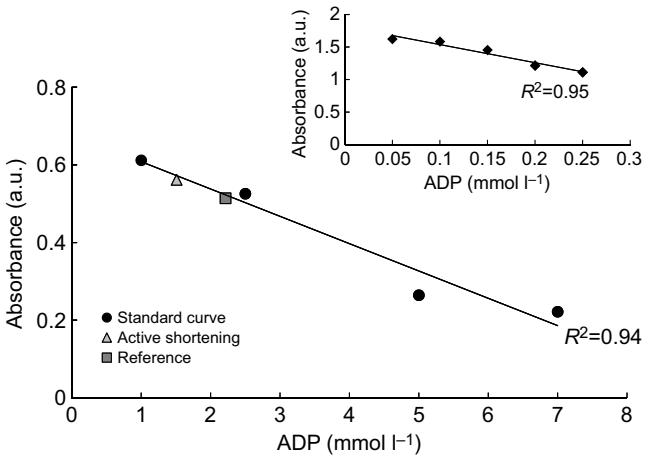

Fig. 2. NADH absorbance as a function of ADP concentration. The standard curve was established by adding known amounts of ADP. When the concentration of ADP increases, the amount of NADH transformed into NAD^+ increases and therefore the absorbance is reduced. The activating solution in which the fibre was bathed for the last 30 s of active shortening (active shortening) and purely isometric (reference) contractions was used to measure the ATPase activity using an enzyme-coupled assay, leading to the transformation of NADH into NAD^+. The absorbance of NADH in the reference and active shortening states was used to calculate the amount of ADP produced. The amount of ADP produced by the fibre shown in Fig. 1 was 1.51 and 2.21 mmol l^{-1} for the active shortening and reference contractions, respectively. The inset shows that the enzyme-coupled assay method used in this study was able to detect small differences in the concentration of ADP (in the order of 0.05 mmol l^{-1}).

Force and stiffness were decreased following active shortening by 10.0±1.8% and 11.0±2.2%, respectively, compared with the isometric reference contractions. Stiffness depression was significantly correlated with the amount of force depression (Fig. 4) and the reduction in the absolute ATP used per second (Fig. 5).

DISCUSSION

The aim of this study was to investigate whether the energy cost of force production at the steady state after active shortening was reduced compared with the energy cost of force production for a purely isometric contraction performed at the corresponding length. Our main findings are that the absolute amount of ATP use in the force depressed state is reduced compared with isometric reference

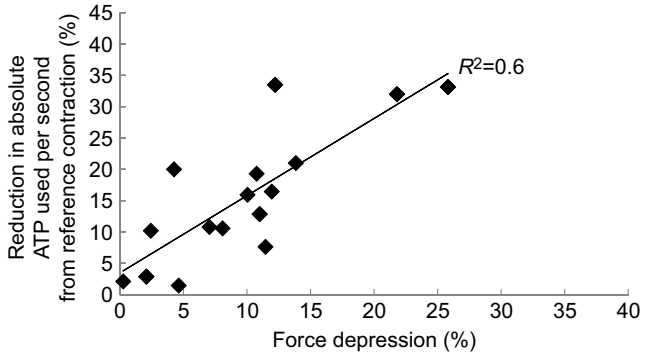

Fig. 3. Percent reduction in absolute ATP consumption per second as a function of force depression. Fibres (n=16) were actively shortened from an average sarcomere length of 3.0 µm to an average sarcomere length of 2.4 µm.

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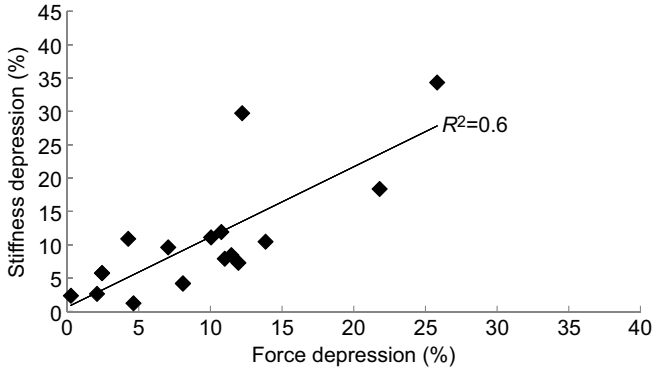

Fig. 4. Relationship between stiffness depression and force depression. Fibres (n=16) were actively shortened from an average sarcomere length of 3.0 µm to an average sarcomere length of 2.4 µm.

levels, but that once the ATP use is normalized to the amount of force, the differences in metabolic cost are abolished.

Metabolic cost in skeletal muscle is measured by ATP consumption. It is well known that force is produced by a skeletal muscle when myosin heads cyclically attach to actin filaments. One molecule of ATP is used during each attachment–detachment cycle (Huxley, 1957). Many organelles in the muscle fibres hydrolyze ATP to power their functions. Skinned fibres exposed to low concentrations (0.5%) of detergents (Triton or Brij) have been shown to be extremely well suited for assessing ATP consumption by the contractile filaments and cross-bridges (Chandra et al., 2009; de Tombe et al., 2007; Stephenson et al., 1989). Treatment of skinned fibres with Triton or Brij disrupts the intracellular membranous compartments and eliminates ATP-dependent membrane pumps such as the sodium-potassium pump and the sarcoplasmic reticulum calcium pump (Fink et al., 1986; Stephenson et al., 1981, 1989). Therefore, ATP use can be related exclusively to the cross-bridges in skinned fibres (Fink et al., 1986; Stephenson et al., 1981, 1989). In the present study, absolute ATP cost per second was reduced for steady-state isometric force following active shortening, suggesting that less ATP is used by the cross-bridges compared with the corresponding purely isometric reference contractions. According to the cross-bridge theory (Huxley, 1957), a reduction in ATP consumption per second might be due to (1) a decrease in the number of cycling cross-bridges or (2) an increase in the time of the attachment–detachment cycle of a cross-bridge.

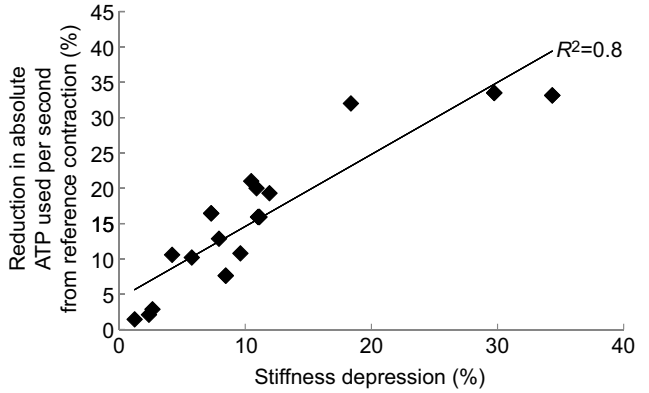

Fig. 5. Percentage reduction in absolute ATP consumption per second as a function of stiffness depression. Fibres (n=16) were actively shortened from an average sarcomere length of 3.0 µm to an average sarcomere length of 2.4 µm.

When ATP consumption was normalized to the amount of force produced during the steady state after active shortening, it showed no difference compared with the purely isometric contraction, suggesting that the decrease in ATP consumption was accompanied by a similar decrease in force. The first and second suggestions stated above could potentially explain this result. During cross-bridge cycling, ATP is used to power the attachment–detachment cycles and simultaneously produce force. How can the decrease in the number of cycling cross-bridges explain the reduction in force along with the decrease in ATP consumption? The amount of force developed by a muscle can vary by changes in the proportion of attached cross-bridges and the duty ratio of cross-bridge cycling (the time a cross-bridge spends generating force during its cycle relative to the duration of the cycle) (Huxley, 1957). Assuming that the duty ratio remains constant after active shortening, a reduction in the number of attached cross-bridges as proposed above would lead to a decrease in force along with the decrease in ATP use. For this argument to hold, one needs to assume that the duty ratio is essentially unaffected by the active shortening, otherwise force would not be depressed in parallel with the reduction in ATP consumption.

Regarding our second suggestion, if the total cross-bridge cycle time was indeed extended, the time a cross-bridge spends generating force and the duty ratio cannot increase; otherwise the reduced ATP consumption would not be associated with a corresponding decrease in force. Therefore, the two most plausible explanations for the decrease in energy consumption along with the parallel reduction in force are: (1) a decrease in the number of cycling cross-bridges while maintaining the duty ratio of the cross-bridges, or (2) an increase in the cross-bridge cycle time and a corresponding decrease in the duty ratio of the cross-bridges, that is, the cross-bridge cycle time is primarily increased because of increases in times of cross-bridge states not associated with force production.

These two possibilities can be interpreted in terms of a two-state cross-bridge model with one attached cross-bridge state or force-generating state and one detached cross-bridge state or non-force-generating state (Huxley, 1957), and rate constants f and g for attachment and detachment of the cross-bridges, respectively. The proportion of force generating cross-bridges at steady state is described by α, where α is the ratio $f/(f+g)$. Steady state force (F) is described by: $F=n\times F_{CB}\times\alpha$, where n is the number of cycling cross-bridges and F_{CB} is the average force produced per cross-bridge. In order to generate a decrease in the number of cycling cross-bridges while maintaining the duty ratio of a cross-bridge, n must be reduced without affecting α. For this case, active shortening would decrease the number of cross-bridges involved in active cycling but not their cycling kinetics. In contrast, our results could also be explained by maintaining n, while decreasing α, either by decreasing f or increasing g. For this case, active shortening would change the cycling kinetics of the cross-bridges.

It has been shown in this study (Fig. 3) and by others (Joumaa et al., 2012; Lee and Herzog, 2003; Sugi and Tsuchiya, 1988) that force depression after active shortening is correlated with stiffness depression. It is well accepted that stiffness is associated with the number of attached cross-bridges as well as the compliance of actin, myosin and titin (Ford et al., 1981; Goldman and Huxley, 1994; Granzier et al., 2000; Kojima et al., 1994). However, it has been assumed that actin, myosin and titin compliance remains constant after active shortening compared with the purely isometric reference

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contractions; therefore, changes in stiffness are thought to be primarily caused by changes in the number of attached cross-bridges (Joumaa et al., 2012; Lee and Herzog, 2003; Sugi and Tsuchiya, 1988). According to the two-state cross-bridge model, this reduction in the number of attached cross-bridges is consistent with our findings that force depression is associated with either a decrease in n while α is maintained or a decrease in α while n is maintained. Therefore, our results suggest that force depression is caused by a decrease in the steady state number of attached cross-bridges, and that this reduction in the number of attached cross-bridges is caused by either a decrease in actively participating cross-bridges or a change in the cross-bridge kinetics, resulting in a decreased duty ratio of the cross-bridges.

Our results are consistent with the stress-induced inhibition of cross-bridge attachment theory proposed by Maréchal and Plaghki (1979). According to this theory, actin filaments in the I-band region are strained upon force production and this strain might produce a rotational distortion of the cross-bridge attachment sites, and so might inhibit cross-bridge attachments in regions of actin that enter the actin–myosin filament overlap zone when a muscle is actively shortening. Our results are also consistent with a titin-based mechanism recently proposed to explain the reduction in stiffness and the number of attached cross-bridges after active shortening. In a theoretical model, Rode et al. (2009) suggested that titin could play a role in force depression by interfering with cross-bridge formation during active shortening. They proposed that the PEVK region of titin binds to actin during isometric contraction, and when sarcomeres actively shorten and myosin enters the range of actin that contains the attached PEVK region, the number of binding sites on actin available for myosin is reduced and thus active force is decreased compared with a purely isomeric force performed at the final length. It has been shown that some small amount of titin (approximately 20%) might be degraded by the skinning process (Joumaa et al., 2008b); therefore, if titin was involved in the reduction of the number of attached cross-bridges during active shortening, titin degradation might prevent the titin-based decrease in the number of attached cross-bridges, and therefore the amount of force depression observed in our study might be underestimated. However, the idea of passive structural elements and titin playing a role in force depression is merely a theoretical proposal (Forcinito et al., 1997; Rode et al., 2009) with no experimental support to date, and thus needs to be considered with caution.

The findings of the present study could also be interpreted in terms of the sarcomere length non-uniformity theory proposed to explain force depression (Morgan et al., 2000). According to this theory, if active shortening is occurring from the descending limb to the plateau region of the force–length relationship, some sarcomeres shorten very little so that their overlap would not increase and stay similar to that of the starting length and others would shorten a great deal, beyond the plateau and on to the ascending limb to balance out force (Morgan et al., 2000). Based on this view, Morgan et al. (2000) predicted that the amount of overlap between thin and thick filaments and the number of attached cross-bridges would slightly vary after active shortening from the initial starting length before shortening. Given that the amount of overlap and the number of attached cross-bridges are greater for purely isometric contractions performed at the plateau region than at the descending limb (Gordon et al., 1966), this means that the number of cross-bridges after active shortening would be reduced in the force-depressed state compared with a purely isomeric contraction performed at the plateau. This reduction in overlap and the number of attached cross-bridges in the force-depressed state would involve a decrease in the absolute amount of ATP used after active shortening and little change in the ATPase consumption per unit of force. The findings of the present study therefore support the ideas of the sarcomere length non-uniformity theory. However, previously reported observations contradict the predictions of the non-uniformity theory, making it an unlikely mechanism for force depression. For example, it has been shown by Granzier and Pollack (1989) that force depression is virtually identical for fixed-end and sarcomere-length controlled contractions in isolated frog muscle fibres. Furthermore, the development of sarcomere length non-uniformities was studied in single myofibrils and it was found that sarcomere length dispersion did not increase after active shortening compared with the purely isometric contraction or the initial state before shortening (Joumaa and Herzog, 2010).

It has been well documented that the steady-state isometric force after active stretching is enhanced compared with the corresponding purely isometric reference contractions (Abbott and Aubert, 1952; Edman et al., 1978; Hahn et al., 2007; Herzog and Leonard, 2002; Joumaa et al., 2008a; Minozzo et al., 2013; Pinniger et al., 2006). This phenomenon is called residual force enhancement. The mechanisms associated with active muscle lengthening and shortening, and whether residual force enhancement mirrors force depression, remain a matter of debate (Edman et al., 1978; Herzog and Leonard, 2000; Morgan et al., 2000; Rassier and Herzog, 2004). The results of the present study strongly support the idea that distinctly different mechanisms are involved in force enhancement and force depression. Recently, we found that the ATPase activity per unit of force was reduced in the force-enhanced compared with the purely isometric reference state (Joumaa and Herzog, 2013). It was suggested that skeletal muscle becomes more efficient when actively stretched, either by increasing the amount of force produced per cross-bridge or by engaging a passive element, such as the molecular spring titin; mechanisms that increase force without an appreciable increase in metabolic cost (Joumaa and Herzog, 2013). In contrast, active muscle shortening does not change the ATP cost per unit of force, and thus does not influence the metabolic efficiency of muscle contraction. Furthermore, our results, and those from others, agree with the idea that force depression is associated with a decrease in the number of attached cross-bridges rather than changes in the force produced per cross-bridge (Joumaa et al., 2012; Lee and Herzog, 2003; Minozzo and Rassier, 2013; Sugi and Tsuchiya, 1988). Therefore, mechanism(s) of force depression should focus on how the proportion of attached cross-bridges might be decreased, while the relative cost of force production remains unchanged after active shortening.

Fibres in our experiments were activated for more than 80 s. Long activation periods were required for the active shortening test, in order to allow the fibres to produce their maximal active force, perform the active shortening, then for the force after shortening to reach a steady state and finally for the ATPase measurement. These prolonged activation durations might result in P_i accumulation, fatigue and force reduction. However, the amount of P_i in resting fibres has been shown to vary between 1 and 6 mmol l^{-1} (Kushmerick et al., 1992). In our experiments, the amount of ADP produced by the fibres during the 30 s of activation did not exceed 5 mmol l^{-1} because of the vast volume available in these experiments compared with the volume of an intact fibre. Knowing that the amounts of ADP and P_i produced during activation are similar, we can safely assume that the amount of P_i produced in our experiments is less than 5 mmol l^{-1} and therefore well within the

range observed in fibres at rest, and would not induce fatigue. This is consistent with the negligible reduction in force observed in Fig. 1 for extended periods of activation. Although the prolonged contractions did not seem to produce enough P_i to reduce force, future experiments aimed at investigating the effect of P_i accumulation and muscle fatigue on the kinetics of cross-bridge cycling in the context of force depression are required.

ATPase activity could not be measured in real time with force production in this study. The ADP accumulated in the last 30 s of contraction was measured after the experiments using an enzyme-coupled assay (Joumaa and Herzog, 2013). ATP hydrolysis rates could vary over long periods of activation and between conditions; therefore, future investigations aimed at measuring force and ATPase activity in real time are required.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
V.J. contributed to the conception, design, execution and interpretation of the findings, and drafting and revising the article. A.F. contributed to the execution and interpretation of the findings, and drafting and revising the article. W.H. contributed to the conception, design, and interpretation of the findings, and drafting and revising the article.

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References
Abbott, B. C. and Aubert, X. M. (1952). The force exerted by active striated muscle during and after change of length. *J. Physiol.* **117**, 77-86.
Chandra, M., Mamidi, R., Ford, S., Hidalgo, C., Witt, C., Ottenheijm, C., Labeit, S. and Granzier, H. (2009). Nebulin alters cross-bridge cycling kinetics and increases thin filament activation: a novel mechanism for increasing tension and reducing tension cost. *J. Biol. Chem.* **284**, 30889-30896.
Daniel, T. L., Trimble, A. C. and Chase, P. B. (1998). Compliant realignment of binding sites in muscle: transient behavior and mechanical tuning. *Biophys. J.* **74**, 1611-1621.
De Ruiter, C. J., De Haan, A., Jones, D. A. and Sargeant, A. J. (1998). Shortening-induced force depression in human adductor pollicis muscle. *J. Physiol.* **507**, 583-591.
de Tombe, P. P. and Stienen, G. J. M. (1995). Protein kinase A does not alter economy of force maintenance in skinned rat cardiac trabeculae. *Circ. Res.* **76**, 734-741.
de Tombe, P. P., Belus, A., Piroddi, N., Scellini, B., Walker, J. S., Martin, A. F., Tesi, C. and Poggesi, C. (2007). Myofilament calcium sensitivity does not affect cross-bridge activation-relaxation kinetics. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R1129-R1136.
Edman, K. A., Elzinga, G. and Noble, M. I. (1978). Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *J. Physiol.* **281**, 139-155.
Edman, K. A., Caputo, C. and Lou, F. (1993). Depression of tetanic force induced by loaded shortening of frog muscle fibres. *J. Physiol.* **466**, 535-552.
Fink, R. H., Stephenson, D. G. and Williams, D. A. (1986). Potassium and ionic strength effects on the isometric force of skinned twitch muscle fibres of the rat and toad. *J. Physiol.* **370**, 317-337.
Forcinito, M., Epstein, M. and Herzog, W. (1997). Theoretical considerations on myofibril stiffness. *Biophys. J.* **72**, 1278-1286.
Ford, L. E., Huxley, A. F. and Simmons, R. M. (1981). The relation between stiffness and filament overlap in stimulated frog muscle fibres. *J. Physiol.* **311**, 219-249.
Glyn, H. and Sleep, J. (1985). Dependence of adenosine triphosphatase activity of rabbit psoas muscle fibres and myofibrils on substrate concentration. *J. Physiol.* **365**, 259-276.
Goldman, Y. E. and Huxley, A. F. (1994). Actin compliance: are you pulling my chain? *Biophys. J.* **67**, 2131-2133.
Gordon, A. M., Huxley, A. F. and Julian, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol.* **184**, 170-192.
Granzier, H. L. and Pollack, G. H. (1989). Effect of active pre-shortening on isometric and isotonic performance of single frog muscle fibres. *J. Physiol.* **415**, 299-327.
Granzier, H., Helmes, M., Cazorla, O., McNabb, M., Labeit, D., Wu, Y., Yamasaki, R., Redkar, A., Kellermayer, M., Labeit, S. et al. (2000). Mechanical properties of titin isoforms. *Adv. Exp. Med. Biol.* **481**, 283-300; discussion 300-4.
Hahn, D., Seiberl, W. and Schwirtz, A. (2007). Force enhancement during and following muscle stretch of maximal voluntarily activated human quadriceps femoris. *Eur. J. Appl. Physiol.* **100**, 701-709.
Herzog, W. and Leonard, T. R. (1997). Depression of cat soleus forces following isokinetic shortening. *J. Biomech.* **30**, 865-872.
Herzog, W. and Leonard, T. R. (2000). The history dependence of force production in mammalian skeletal muscle following stretch-shortening and shortening-stretch cycles. *J. Biomech.* **33**, 531-542.
Herzog, W. and Leonard, T. R. (2002). Force enhancement following stretching of skeletal muscle: a new mechanism. *J. Exp. Biol.* **205**, 1275-1283.
Huxley, A. F. (1957). Muscle structure and theories of contraction. *Prog. Biophys. Biophys. Chem.* **7**, 255-318.
Joumaa, V. and Herzog, W. (2010). Force depression in single myofibrils. *J. Appl. Physiol.* **108**, 356-362.
Joumaa, V. and Herzog, W. (2013). Energy cost of force production is reduced after active stretch in skinned muscle fibres. *J. Biomech.* **46**, 1135-1139.
Joumaa, V., Leonard, T. R. and Herzog, W. (2008a). Residual force enhancement in myofibrils and sarcomeres. *Proc. Biol. Sci.* **275**, 1411-1419.
Joumaa, V., Rassier, D. E., Leonard, T. R. and Herzog, W. (2008b). The origin of passive force enhancement in skeletal muscle. *Am. J. Physiol. Cell Physiol.* **294**, C74-C78.
Joumaa, V., Macintosh, B. R. and Herzog, W. (2012). New insights into force depression in skeletal muscle. *J. Exp. Biol.* **215**, 2135-2140.
Julian, F. J. and Morgan, D. L. (1979). The effect on tension of non-uniform distribution of length changes applied to frog muscle fibres. *J. Physiol.* **293**, 379-392.
Kojima, H., Ishijima, A. and Yanagida, T. (1994). Direct measurement of stiffness of single actin filaments with and without tropomyosin by in vitro nanomanipulation. *Proc. Natl. Acad. Sci. USA* **91**, 12962-12966.
Kushmerick, M. J., Moerland, T. S. and Wiseman, R. W. (1992). Mammalian skeletal muscle fibers distinguished by contents of phosphocreatine, ATP, and Pi. *Proc. Natl. Acad. Sci. USA* **89**, 7521-7525.
Lee, H.-D. and Herzog, W. (2003). Force depression following muscle shortening of voluntarily activated and electrically stimulated human adductor pollicis. *J. Physiol.* **551**, 993-1003.
Leonard, T. R. and Herzog, W. (2005). Does the speed of shortening affect steady-state force depression in cat soleus muscle? *J. Biomech.* **38**, 2190-2197.
Locher, M. R., Razumova, M. V., Stelzer, J. E., Norman, H. S., Patel, J. R. and Moss, R. L. (2009). Determination of rate constants for turnover of myosin isoforms in rat myocardium: implications for in vivo contractile kinetics. *Am. J. Physiol. Heart Circ. Physiol.* **297**, H247-H256.
Maréchal, G. and Plaghki, L. (1979). The deficit of the isometric tetanic tension redeveloped after a release of frog muscle at a constant velocity. *J. Gen. Physiol.* **73**, 453-467.
Mateja, R. D., Greaser, M. L. and de Tombe, P. P. (2013). Impact of titin isoform on length dependent activation and cross-bridge cycling kinetics in rat skeletal muscle. *Biochim. Biophys. Acta* **1833**, 804-811.
Meijer, K., Grootenboer, H. J., Koopman, H. F. J. M., van der Linden, B. J. J. J. and Huijing, P. A. (1998). A Hill type model of rat medial gastrocnemius muscle that accounts for shortening history effects. *J. Biomech.* **31**, 555-563.
Minozzo, F. C. and Rassier, D. E. (2013). The effects of Ca^{2+} and MgADP on force development during and after muscle length changes. *PLoS ONE* **8**, e68866.
Minozzo, F. C., Baroni, B. M., Correa, J. A., Vaz, M. A. and Rassier, D. E. (2013). Force produced after stretch in sarcomeres and half-sarcomeres isolated from skeletal muscles. *Sci. Rep.* **3**, 2320.
Morgan, D. L., Whitehead, N. P., Wise, A. K., Gregory, J. E. and Proske, U. (2000). Tension changes in the cat soleus muscle following slow stretch or shortening of the contracting muscle. *J. Physiol.* **522**, 503-513.
Mounier, Y., Holy, X. and Stevens, L. (1989). Compared properties of the contractile system of skinned slow and fast rat muscle fibres. *Pflugers Arch.* **415**, 136-141.
Ottenheijm, C. A. C., Lawlor, M. W., Stienen, G. J. M., Granzier, H. and Beggs, A. H. (2011). Changes in cross-bridge cycling underlie muscle weakness in patients with tropomyosin 3-based myopathy. *Hum. Mol. Genet.* **20**, 2015-2025.
Pinniger, G. J., Ranatunga, K. W. and Offer, G. W. (2006). Crossbridge and non-crossbridge contributions to tension in lengthening rat muscle: force-induced reversal of the power stroke. *J. Physiol.* **573**, 627-643.
Power, G. A., Makrakos, D. P., Stevens, D. E., Herzog, W., Rice, C. L. and Vandervoort, A. A. (2014). Shortening-induced torque depression in old men: Implications for age-related power loss. *Exp. Gerontol.* **57**, 75-80.

Journal of Experimental Biology (2017) 220, 1509-1515 doi:10.1242/jeb.117622

Rassier, D. E. and Herzog, W. (2004). Considerations on the history dependence of muscle contraction. *J. Appl. Physiol.* **96**, 419-427.

Rode, C., Siebert, T. and Blickhan, R. (2009). Titin-induced force enhancement and force depression: a 'sticky-spring' mechanism in muscle contractions? *J. Theor. Biol.* **259**, 350-360.

Stephenson, D. G., Wendt, I. R. and Forrest, Q. G. (1981). Non-uniform ion distributions and electrical potentials in sarcoplasmic regions of skeletal muscle fibres. *Nature* **289**, 690-692.

Stephenson, D. G., Stewart, A. W. and Wilson, G. J. (1989). Dissociation of force from myofibrillar MgATPase and stiffness at short sarcomere lengths in rat and toad skeletal muscle. *J. Physiol.* **410**, 351-366.

Sugi, H. and Tsuchiya, T. (1988). Stiffness changes during enhancement and deficit of isometric force by slow length changes in frog skeletal muscle fibres. *J. Physiol.* **407**, 215-229.

Woledge, R. C., Curtin, N. A. and Homsher, E. (1985). Energetic aspects of muscle contraction. *Monogr. Physiol. Soc.* **41**, 1-357.