Sarcomere length dependence of the rate of tension redevelopment and submaximal tension in rat and rabbit skinned skeletal muscle fibres

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1. We examined the hypothesis that in skeletal muscle the steep relationship between twitch tension and sarcomere length (SL) within the range 2·30 to 1·85 μm involves SL-dependent alterations in the rate of tension development.

2. In skinned preparations of both rat slow-twitch and rabbit fast-twitch skeletal muscle fibres the rate of tension redevelopment (k_{tr}) at 15 °C was reduced at short SL (~2·00 μm) compared with a longer SL (~2·30 μm). In submaximally activated fibres, the decrease in k_{tr} over this range of lengths was greater in fast-twitch fibres (38% reduction) than in slow-twitch fibres (14% reduction).

3. Ca^{2+} sensitivity of tension, as assessed as the pCa ($-\log[Ca^{2+}]$) for half-maximal activation, or pCa_{50}, decreased to a greater extent in rabbit fast-twitch skeletal muscle fibres than in slow-twitch fibres from both rabbit and rat when SL was reduced from ~2·30 to ~1·85 μm. The ΔpCa_{50} over this SL range was 0·24 ± 0·07 pCa units in fast-twitch fibres from rabbit psoas muscle. The ΔpCa_{50} for slow-twitch fibres from rabbit and rat soleus muscle was 0·08 ± 0·02 and 0·10 ± 0·04 pCa units, respectively.

4. Osmotic compression of both slow-twitch and fast-twitch fibres at a SL of 2·00 μm increased k_{tr} to values similar to those obtained at a SL of 2·30 μm in the absence of dextran. This result indicates that the slower rate of tension redevelopment at short SL is due in large part to the increase in interfilament lattice spacing associated with shorter SL.

5. Taken together, these results suggest that length dependence of twitch tension is, in part, due to length dependence of isometric cross-bridge interaction kinetics, an effect that is mediated by length-dependent changes in interfilament lattice spacing.

During twitch contractions of skeletal muscle, Ca^{2+} is released from the sarcoplasmic reticulum and binds to sites on the thin filament protein troponin C, thereby allowing interactions of myosin with actin. The increase in myoplasmic Ca^{2+} concentration (Blinks, Rüdel & Taylor, 1978), the binding of Ca^{2+} to the low affinity sites of troponin C (Robertson, Johnson & Potter, 1981), and the Ca^{2+}-induced conformational changes in thin filament proteins (Kress, Huxley & Faruqi, 1986) are presumably all too rapid to limit the rate of tension development. Thus, the rate of rise of twitch tension and consequently the amount of tension developed during a twitch, is primarily modulated by the rate of cross-bridge transition(s) to strongly bound force-generating states.

Isometric twitch tension changes dramatically as a function of length in striated muscle (Rack & Westbury, 1969; Close, 1972; Gordon & Pollack, 1980; ter Keurs, Risnsburger, van Heuningen & Nagelsmit, 1980; Roszek, Baan & Huijing, 1994). In frog sartorius muscle for example, peak isometric twitch tension falls to near zero when sarcomere length (SL) is reduced from ~2·1 to 1·7 μm (Close, 1972). A number of factors may contribute to the rapid fall-off of twitch tension as muscle length is reduced (for review see Allen & Kentish, 1985). One probable factor is the change in the extent of thick and thin filament overlap that is known to occur as SL is shortened. However, according to the SL–tension relationship in tetanically stimulated muscle fibres from frog (Gordon, Huxley & Julian, 1966), such changes in filament overlap can only account for ~20% of the near-complete loss of twitch tension over a SL range of 2·20 to 1·80 μm. This suggests that length dependence of twitch tension also involves changes in the activation state of the myofilaments. For example, twitch tension could decrease at short SL because of a reduction in sensitivity of myofilaments to Ca^{2+}. Consistent with this idea, between sarcomere lengths of 2·20 and 1·60 μm, steady-state tension decreases to a greater extent during submaximal Ca^{2+} activations than it does during maximal Ca^{2+} activations (Allen & Moss, 1987).

Length dependence of twitch tension might also involve changes in the rate of cross-bridge transitions from weakly bound to strongly bound, force-generating states. Such transitions may be slower at shorter SL, thereby reducing the population of force-generating cross-bridges and, thus, tension during a twitch. In the present study we tested this idea by measuring length dependence of the rate of tension redevelopment in Ca^{2+} activated skinned slow-twitch and fast-twitch skeletal muscle fibres.

This work has been described in part at the 1995 and 1997 Biophysical Society Meetings and by McDonald & Moss, 1995.

METHODS

Muscle preparation

Adult male New Zealand rabbits were killed by rapid injection of an overdose of sodium pentobarbitone (50–60 mg (kg body weight)$^{-1}$) via the lateral ear vein. Psoas and soleus muscles were isolated and bundles of approximately fifty muscle fibres were prepared and stored as described previously (Allen & Moss, 1987). Sprague–Dawley rats (225–250 g) were anaesthetized with sodium pentobarbitone (30 mg (kg body weight)$^{-1}$ I.P.) and the soleus muscle was removed for preparation of muscle bundles. Fibre bundles were skinned by exposure to glycerol-containing relaxing solution overnight at 4 °C. Fibre bundles were stored for up to 3 weeks in relaxing solution containing 50% glycerol (v/v) at −20 °C. Since soleus muscle contains ~15% fast-twitch fibres, all soleus fibres used for mechanical measurements were analysed by SDS–PAGE and silver staining to assess protein content. The results include data only from soleus fibres that were characterized as slow-twitch fibres based upon expression of type I myosin heavy chains.

Experimental apparatus

For mechanical measurements, single skinned fibres were pulled free from the ends of the bundles using finely shaped forceps. Each fibre was mounted between a force transducer (model 403, sensitivity of 20 V g^{-1} and resonant frequency 600 Hz, or model 400, sensitivity of 2 V g^{-1}, resonant frequency of 2 kHz; Cambridge Technology, Inc., Cambridge, MA, USA) and a DC torque motor (model 300, Cambridge Technology, Inc.) in an experimental apparatus similar to that used previously in our laboratory (Moss, 1979). Using a stereomicroscope at ×50 magnification, the ends of the fibre were placed in stainless steel troughs (25 gauge hypodermic needle tubing) glued to styluses extending from the active elements of the force transducer and the DC torque motor. The fibre ends were secured by overlaying a 0·5 mm length of 4/0 monofilament nylon suture, which were tied into the troughs with two loops of 10/0 monofilament suture. This attachment minimized end compliance of the fibre preparation.

Prior to mechanical measurements the experimental apparatus was mounted on the stage of an inverted microscope (model IMT-2, Olympus Instrument Co., Japan). Fibre length, width, and striation spacing were measured, monitored, and recorded using a video camera mounted on the microscope. Muscle fibre force and length signals were digitized at 1000 Hz using a 12-bit A/D converter (AT-MIO-16F-5, National Instruments Corp., Austin, TX, USA) and each was displayed and stored on a personal computer using custom software (LabView for Windows, National Instruments Corp.). Muscle length changes were driven by computer-generated voltage commands to the torque motor via a 12 bit D/A converter (AT-MIO-16F-5, National Instruments Corp.) using the custom software.

SL measurement

SL was measured by laser diffraction using a system similar to the one previously described in detail by de Tombe & ter Keurs (1990). Fibre segments were illuminated by a helium–neon laser beam (model 05-LHP151, 5 mW output, 632·8 nm wavelength, Melles-Groit, Carlsbad, CA, USA) perpendicular to the long axis of the segment, and the position of the first-order diffraction line was monitored with a 512-element photodiode array (model RC 105, Reticon, Sunnyvale, CA, USA) that was scanned electronically every 0·5 ms. A glass coverslip was placed over the chamber containing the fibre to eliminate distortion of the first-order diffraction line by the fluid meniscus of the activating solutions. Median position of the first-order intensity distribution was converted into a voltage proportional to SL using a non-linear amplifier (Biomedical Technical Support Centre, University of Calgary, Calgary, Canada). The transfer function of the amplifier was adjusted using glass diffraction gratings of known spacing. SL was also measured visually on the video monitor at the beginning of each experiment to confirm the calibration of the system.

Experimental protocol

Length dependence of Ca^{2+} sensitivity of tension. All mechanical experiments were performed at 15 °C. Two tension–pCa relationships were characterized for each fibre: a tension–pCa relationship was first characterized at short SL (~1·85 μm) after which the fibre was lengthened to a longer SL (~2·25 μm) and a second tension–pCa relationship was obtained. This sequence for obtaining tension–pCa relationships was optimal for resolving the striation pattern throughout the protocol since the quality of the pattern at short lengths was sometimes reduced once the fibre was activated at the longer length. Tension–pCa relationships were characterized by first maximally activating the fibre and subsequently transferring the fibre into a series of solutions of submaximal pCa. At each pCa, a steady tension was allowed to develop and the fibre was then rapidly slackened to determine total tension (Fig. 2). The amount of active tension generated at each pCa was calculated as the difference between total tension and passive tension. Passive tension was assessed by slackening the fibre while in the relaxed state. In order to determine any decline in tension-generating capability, the fibre was maximally activated at the beginning and end of the protocol used to obtain each tension–pCa relationship. For a given curve, all fibres maintained at least 80% of initial maximal tension. Tensions in submaximally activating solutions were expressed as fractions of peak tension (P_0) measured in solution of pCa 4·5 at the same SL. The P_0 value used to normalize submaximal tensions was obtained by linear interpolation between successive maximal activations. In this protocol, SL was monitored during each activation by video microscopy.

SL dependence of rate of tension redevelopment (k_{tr}). The kinetics of tension development were assessed using a modification of the procedure originally described by Brenner & Eisenberg (1986). Fibres were transferred from relaxing to activating solution and tension was allowed to develop to a plateau. Subsequently, slack equivalent to 15–20% of original muscle length was rapidly introduced at one end of the muscle via the torque motor and this was followed by a brief period (25–100 ms) of unloaded shortening. Unloaded shortening has been shown to reduce dynamic stiffness (which was used as an index of the proportion of attached cross-bridges) in psoas fibres to ~20% of that measured during isometric

contraction (Brenner & Eisenberg, 1986). Dissociation of some of the remaining cross-bridges was accomplished by rapidly restretching the muscle to the original length. Tension redevelopment following the slack–restretch manoeuvre results from reattachment of cross-bridges to the thin filament and redistribution of cross-bridges into force-generating states. In most cases, a small residual tension was present following the slack–restretch manoeuvre just prior to tension redevelopment (Fig. 1). Residual tensions were quantified and expressed as percentage of peak tension for each particular activation. For slow-twitch fibres, residual tensions were greater during maximal activations ($19 \pm 7\%$, $n = 30$) than during submaximal activations ($10 \pm 4\%$, $n = 36$). Also, residual tensions during maximal activations were less at long SL ($16 \pm 7\%$, $n = 15$) than at short SL ($21 \pm 6\%$, $n = 15$). Similarly, during submaximal activations, residual tensions were significantly lower at long SL ($6 \pm 4\%$, $n = 12$) than either short SL ($12 \pm 3\%$, $n = 12$) or short SL + dextran ($12 \pm 5\%$, $n = 12$). For fast-twitch fibres, residual tensions were also lower at long SL ($1 \pm 6\%$, $n = 9$) than at short SL (8 ± 4, $n = 9$).

It is necessary to maintain constant SL during tension redevelopment, since sarcomere shortening in the central portion of the muscle as a result of end compliance leads to an underestimation of k_{tr}. We utilized an adaptive feedback system to control SL as originally described by Wolff, McDonald & Moss (1995). A proportional error and proportional error-squared feedback control algorithm was implemented with custom software (LabView for Windows, National Instruments Corp.), employing a 25 Hz finite, impulse-response, low-pass digital filter on the SL signal (corrected for a linear phase delay). SL was clamped during tension redevelopment to ± 5 nm (half-sarcomere)$^{-1}$ within three to four iterations as illustrated in Fig. 1. Rates of tension development were measured during maximal and submaximal activations of slow-twitch fibres and submaximal activations of fast-twitch fibres. We were unable to measure k_{tr} adequately in maximally activated fast-twitch psoas fibres. Psoas fibres exhibited somewhat more end compliance than slow-twitch fibres and, during the repeated prolonged activations necessary for these experiments the striation pattern deteriorated to such an extent that sarcomere length control was not feasible.

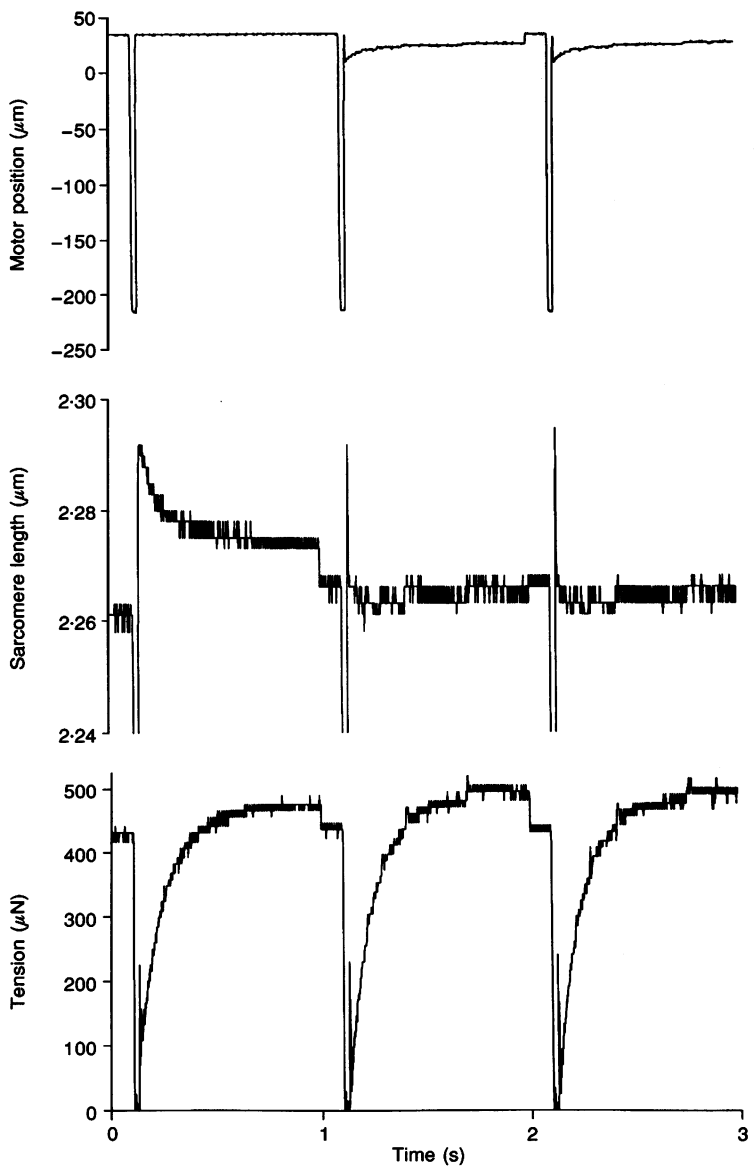

Figure 1. Records of motor position, sarcomere length (SL) and tension illustrating the method of SL control

In this example, SL was clamped to ± 2 nm (half-sarcomere)$^{-1}$ during tension redevelopment following the length release–restretch procedure using an adaptive feedback control system. SL control, in most cases, decreased the residual tension present immediately after the release–restretch protocol and increased the rate of tension redevelopment. In this case, a psoas fibre was submaximally activated (pCa 5·9) and the k_{tr} was 7·94 s^{-1} in the absence of control and increased to 8·30 s^{-1} in the final controlled iteration. Following the slack–restretch manoeuvre, tension redeveloped to a value somewhat greater than obtained prior to the length change (also see Figs 7 and 8). This overshoot was observed in both fast- and slow-twitch fibres and was most prevalent during submaximal activations at long SL. The mechanism(s) underlying this phenomenon is unknown.

The protocol for determining SL dependence of k_{tr} and the effects of myofilament lattice spacing on k_{tr} consisted of measuring k_{tr} at long SL, short SL, and short SL following osmotic compression using high molecular weight dextran polymers. The order of these measurements was random. For each condition (i.e. long SL, short SL, and short SL + dextran), the muscle fibre was activated to a steady-state tension, tension redevelopment was measured using the slack–restretch manoeuvre and then the fibre was transferred back to relaxing solution. Fibre width was measured by video-microscopy during steady-state activation before the slack–restretch manoeuvre. For osmotic compression experiments, the concentration of dextran in activating solutions was adjusted by 2–4% to obtain a fibre width similar to that at long SL. For some experiments SL was measured by videomicroscopy both before and after the slack–restretch manoeuvre to confirm the SL determined by laser diffraction.

Solutions

The compositions of the relaxing and activating solutions used in mechanical measurements were (mM): EGTA, 7; free Mg^{2+}, 1; imidazole, 20; MgATP, 4; creatine phosphate, 14·5; at pH 7·0. Various Ca^{2+} concentrations between 10^{-9} M (relaxing solution) and $10^{-4·5}$ M (maximal Ca^{2+} activating solution) were used with sufficient KCl to adjust ionic strength to 180 mM. The final concentrations of each metal, ligand, and metal–ligand complex at 15 °C were determined with the computer program of Fabiato (1988). The total $[K^+]$, $[Na^+]$, and $[Cl^-]$ in pCa 9·0 solution were 110, 39, and 90 mM, respectively, and for pCa 4·5 solution these concentrations were 110, 39 and 89 mM, respectively. Glycerol-relaxing solution for fibre bundle storage and relaxing solution in which the dissections and fibre isolations were performed contained (mM): EGTA, 2; $MgCl_2$, 1; MgATP, 4; imidazole, 10; and KCl, 100; held at pH 7·0.

Fibres were osmotically compressed by adding dextran (503 000 MW, Sigma Chemical Co.) to relaxing and activating solutions to a final concentration of 2–4 g dextran $(100\ ml)^{-1}$. The dextran concentration was varied between 2 and 4% in order to achieve the best match fibre width between conditions of long SL and short SL + dextran.

Data and statistical analyses

The form and mid-point (pCa_{50}) of the tension–pCa relationship were determined by Hill plot analysis of the data (Shiner & Solaro, 1984). Two separate straight lines were fitted to tension data above and below 0·5 P_0 by the least-squares analysis using the following equation:

$$\log[P_r/(1-P_r)] = N(\log[Ca^{2+}] + pCa_{50}),$$

where P_r is tension as a fraction of P_0 and pCa_{50} is the Ca^{2+} concentration for half-maximal activation. The slopes of the two phases of the Hill plot, above and below pCa_{50}, were N_1 and N_2, respectively.

For purposes of displaying the data (Fig. 4), mean tension–pCa data were best fitted using the following equation:

$$P_r = [Ca^{2+}]^N/(([Ca^{2+}]_{50})^N + [Ca^{2+}]^N),$$

where P_r is tension as a fraction of P_0, $[Ca^{2+}]_{50}$ is the Ca^{2+} concentration for half-maximal activation, and N is the Hill coefficient.

Tension redevelopment following the length release–restretch manoeuvre was well fitted by a single exponential function:

$$F = F_{max}(1 - \exp(-k_{tr}t)) + F_{res},$$

where F is tension at time t, F_{max} is maximal tension, and k_{tr} is the rate constant of tension redevelopment. F_{res} represents the residual tension present immediately after the length release–restretch manoeuver. Curve fitting and statistical analyses were performed using SigmaPlot software (Jandel, Corte Madera, CA, USA).

To determine whether there were significant differences in fibre dimensions, pCa_{50}, or k_{tr} as a function of SL, paired t tests were used. A one-way repeated-measures ANOVA was used to compare fibre dimensions, k_{tr}, and relative tensions between long SL, short SL, and short SL + dextran groups. A Student–Newman–Keuls test was used as a *post hoc* test to assess the differences among means. All values are means ± S.D. and $P < 0·05$ was chosen as indicating significance.

RESULTS

Length dependence of Ca^{2+} sensitivity of tension

To determine the effect of SL on Ca^{2+} sensitivity of tension, tension–pCa relationships were characterized in slow-twitch and fast-twitch skeletal muscle fibres activated at two different sarcomere lengths. Original data showing the effects of SL on steady-state tension are presented in Figs 2 and 3. Panels *A–D* in Fig. 2 show photomicrographs and corresponding tension records from a rat soleus fibre activated in solutions of various free Ca^{2+} concentrations. The photomicrographs demonstrate that SL was reliably measured during steady-state contractions. When activated at pCa 5·8, the fibre generated 49% of peak tension (P_0) at SL of ~2·30 μm, which decreased to 32% P_0 when SL was reduced to ~1·85 μm. Figure 3 shows qualitatively similar results from a fast-twitch fibre from rabbit psoas muscle. At pCa 5·9 the fibre generated a tension of 73% P_0 at a SL of ~2·25 μm and tension decreased to 37% P_0 when SL was reduced to ~1·85 μm. These examples illustrate that length-dependent variations in submaximal tension are greater in a rabbit fast-twitch fibre than in a rat slow-twitch fibre over a similar range of SL, i.e. ~2·30 to 1·85 μm. Mean tension–pCa relationships are shown for rat soleus and rabbit psoas fibres at long and short SL in Fig. 4, and fibre dimensions and Ca^{2+} sensitivities of tension are summarized in Table 1. For rat soleus fibres, reducing SL from 2·31 ± 0·05 to 1·88 ± 0·02 μm increased the $[Ca^{2+}]$ for half-maximal activation (i.e. decreased pCa_{50}) by ~0·10 pCa units, i.e. the Ca^{2+} sensitivity of tension decreased. For psoas fibres, reducing SL from 2·28 ± 0·05 μm to 1·86 ± 0·03 μm decreased pCa_{50} to a greater extent (i.e. ~0·24 pCa units).

Additional experiments showed that the greater length dependence of Ca^{2+} sensitivity in rabbit fast-twitch fibres *versus* rat slow-twitch fibres was due exclusively to fibre-type differences rather than inter-species variation. Slow-twitch fibres from rabbit soleus muscle exhibited length-dependent changes in Ca^{2+} sensitivity of tension that were virtually identical to those in rat slow-twitch fibres: increasing SL from ~1·85 to ~2·30 μm increased pCa_{50} by 0·08 ± 0·02 pCa units (results not shown).

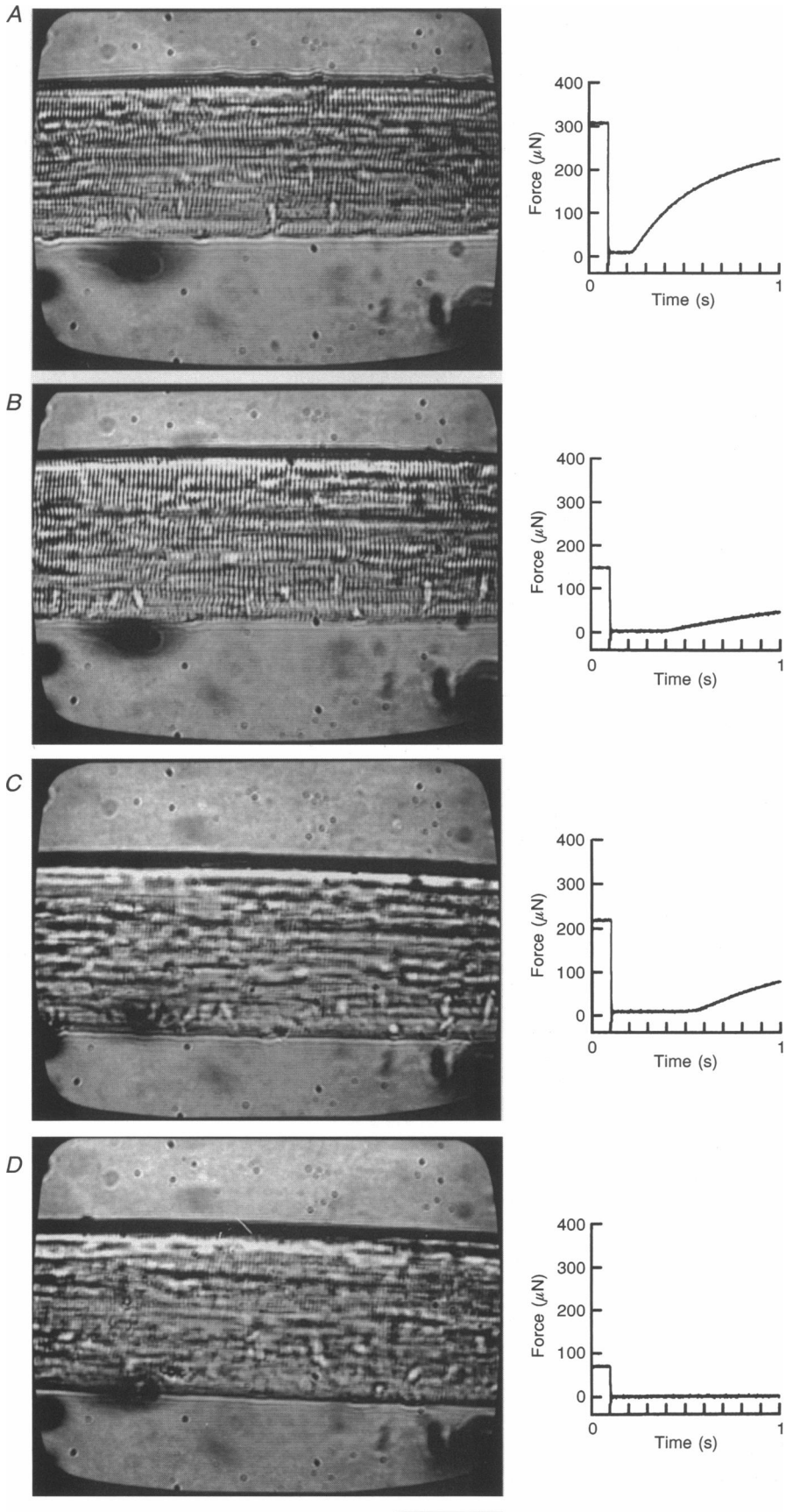

Figure 2. Photomicrographs and tension records from a rat slow-twitch soleus fibre at two different SL

Total tension developed at a given pCa was determined by first allowing the fibre to attain steady-state tension and then rapidly slackening the fibre. Following the rapid change in fibre length (i.e. ~20% of the fibre segment length) tension fell to zero and this initial drop in tension was used to determine total tension. The amount of active tension developed at each pCa was calculated as the difference between total tension and passive tension, which was assessed by slackening the fibre while in the relaxed state (data not shown). In panels *A* and *B* the slow-twitch fibre was maintained at a SL of ~2·30 μm, while in panels *C* and *D* the fibre was shortened to a SL of ~1·85 μm. When activated at pCa 5·8 at long SL (panel *B*) the fibre generated 49% of the peak tension (P_0) of 308 μN (pCa 4·5, panel *A*). When activated in solution of pCa 5·8 at short SL (panel *D*) the fibre generated 32% of the P_0 of 220 μN (pCa 4·5, panel *C*). Calibration bar, 50 μm.

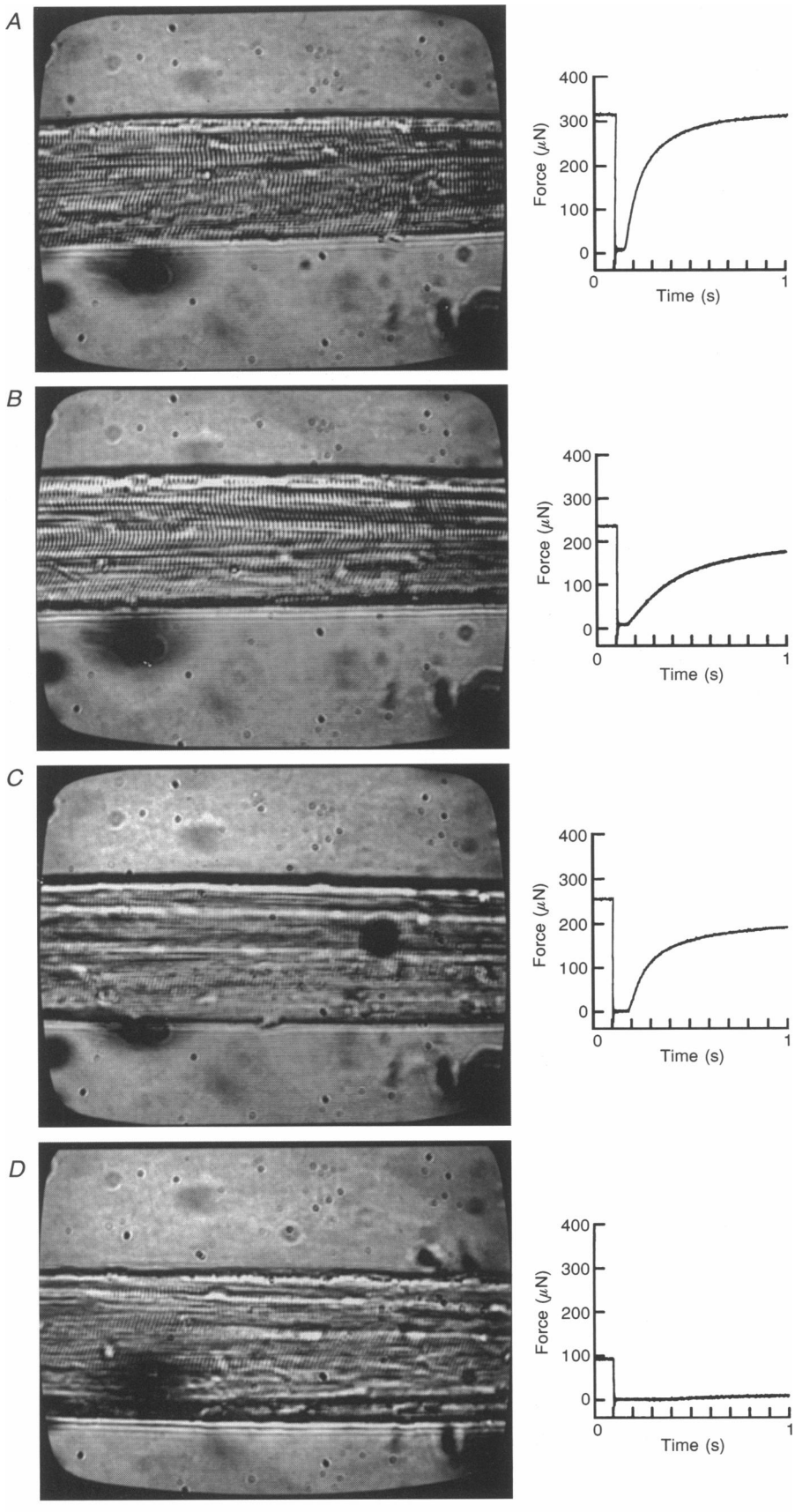

Figure 3. Photomicrographs and tension records of a rabbit fast-twitch psoas fibre activated at long and short SL

In solution of pCa 5·9 at long SL (~2·25 μm; panel *B*), the fibre generated 73% of the peak tension (P_0) of 317 μN (pCa 4·5; panel *A*). When SL was reduced to ~1·85 μm, tension in pCa 5·9 solution (panel *D*) decreased to 37% of the P_0 of 258 μN (pCa 4·5, panel *C*). Calibration bar, 50 μm.

Table 1. Dimensional characteristics, Hill coefficients and pCa_{50} values from rat slow-twitch and rabbit fast-twitch fibres

	Fibre segment length (mm)	Segment width (μm)	Sarcomere length (μm) pCa 9·0	Sarcomere length (μm) pCa 4·5	Maximal tension (μN)	N_2	N_1	pCa_{50}
Slow-twitch fibres								
Long	1·94 ± 0·17	85·0 ± 13·8	2·39 ± 0·04	2·31 ± 0·05	267 ± 124	2·42 ± 0·77	1·33 ± 0·24	5·94 ± 0·14
Short	1·58 ± 0·14*	92·6 ± 13·2*	—	1·87 ± 0·03*	254 ± 82*	2·96 ± 0·52	1·62 ± 0·33*	5·84 ± 0·13*
Fast-twitch fibres								
Long	1·73 ± 0·35	74·7 ± 15·1	2·40 ± 0·05	2·28 ± 0·05	268 ± 129	12·0 ± 3·4	2·30 ± 0·52	5·99 ± 0·07
Short	1·40 ± 0·30*	81·9 ± 16·7*	—	1·86 ± 0·03*	223 ± 140*	7·66 ± 1·87*	1·92 ± 0·52*	5·75 ± 0·14*

Values are means ± S.D. For slow twitch fibres, $n = 11$. For fast twitch fibres, $n = 6$. At short length, fibres were slackened during relaxation and actively shortened to the desired sarcomere length (~1·85 μm). *$P < 0·05$ *vs.* long SL.

Length dependence of rate of tension redevelopment (k_{tr})

Since the rate of rise of twitch tension is most probably limited by the rate of cross-bridge transitions from weakly bound to strongly bound states, changes in this rate with changes in SL could play a significant role in determining length–twitch tension relationships. In a previous report, Stephenson & Williams (1982) observed a tendency for tension development to be slower at short SL than at long SL when Ca^{2+} was rapidly raised to submaximal levels. In the present study, we examined SL dependence on the rate of tension redevelopment following a rapid length release and restretch manoeuvre in tonically activated rat slow-twitch and rabbit fast-twitch skinned muscle fibres. An adaptive feedback control system was used to maintain constant SL during tension redevelopment since shortening of the central portion of the muscle fibre against the compliant ends is known to reduce artifactually the rate of tension development.

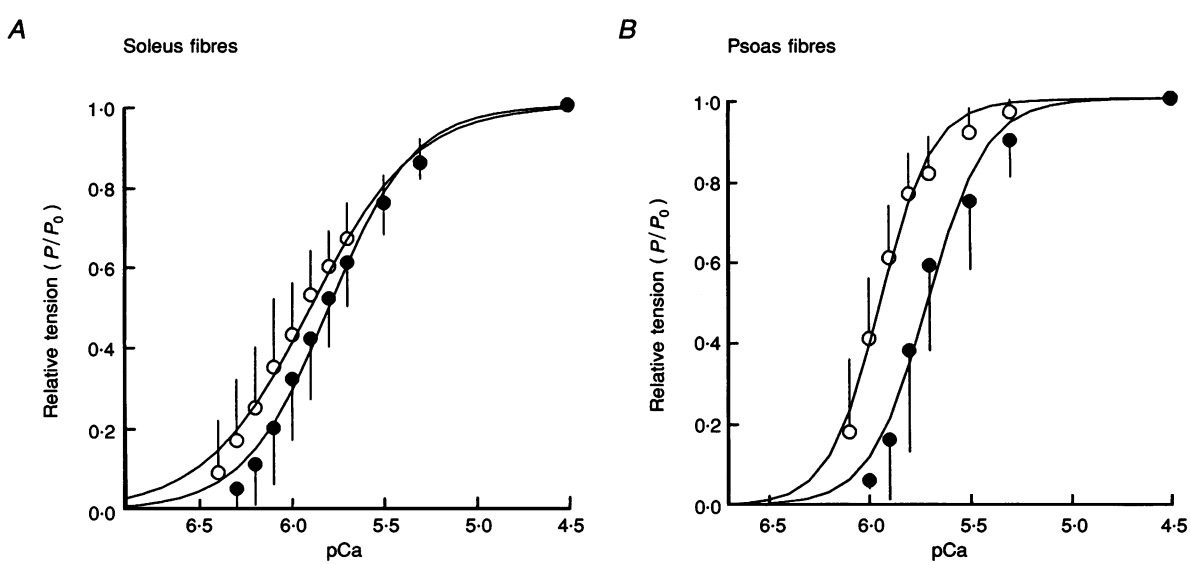

Figure 4. Mean tension–pCa relationships from rat slow-twitch soleus fibres and rabbit fast-twitch psoas fibres at long and short SL

Mean fibre dimensions and Ca^{2+} sensitivities of tension values are presented in Table 1. Data points are means ± S.D. Mean tension–pCa data were fitted using the following equation:

$$P_r = [Ca^{2+}]^N/(([Ca^{2+}]_{50})^N + [Ca^{2+}]^N),$$

where P_r is tension as a fraction of peak tension, $[Ca^{2+}]_{50}$ is the Ca^{2+} concentration for half-maximal activation, and N is the Hill coefficient. ●, short SL; ○, long SL.

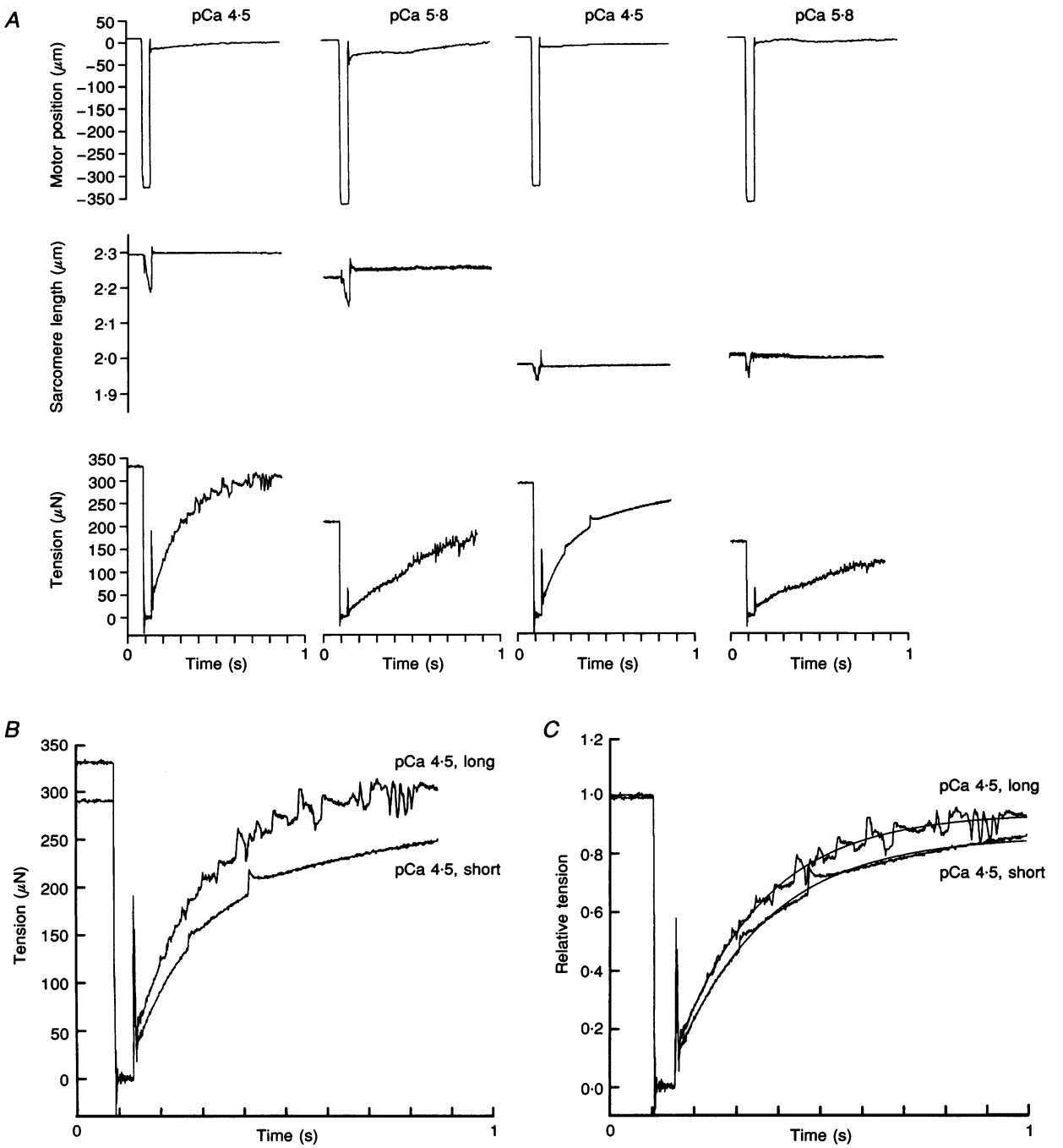

Figure 5. Representative records showing the effect of SL on steady-state tension and the rate of tension redevelopment in a rat slow-twitch soleus fibre

Panel *A* shows motor position, SL, and tension traces at two Ca^{2+} concentrations and at both long and short SL. Following the rapid release of the fibre, the fibre shortened under zero load. During unloaded shortening the sarcomere length pattern was undetected due to random scatter of the laser light by the slackened fibre. Sarcomere length pattern was again recorded after restretch of the fibre. Panel *B* (raw data) and Panel *C* (normalized data) show tension records during a slack–restretch manoeuvre applied to the fibre at both long and short SL during maximal activations (pCa 4·5). The rate constants of tension redevelopment (k_{tr}) are 4·85 s^{-1} and 4·53 s^{-1} at long and short lengths, respectively. In panel *C*, single exponential curves were best fitted to the tension redevelopment records, as described in the Methods using the following equation:

$$F = F_{max}(1 - \exp(-k_{tr}t)) + F_{res},$$

where F is tension at time t, F_{max} is maximal tension and F_{res} is the residual tension immediately following the length release–restretch manoeuvre .

Table 2. Length dependence of k_{tr} in rat slow-twitch and rabbit fast-twitch fibres

	SL (μm)	Relative tension P/P_0	k_{tr} (s^{-1})
Slow-twitch fibres			
Maximal activation			
($n = 15$)	2·28 ± 0·02	1·0	5·15 ± 0·85
($n = 15$)	1·99 ± 0·04*	0·90 ± 0·04*	4·12 ± 0·70*
Submaximal activation			
($n = 12$)	2·29 ± 0·02	0·53 ± 0·09	1·95 ± 0·48
($n = 12$)	1·99 ± 0·03*	0·41 ± 0·07*	1·67 ± 0·45*
Fast-twitch fibres			
Submaximal activation			
($n = 6$)	2·27 ± 0·01	0·72 ± 0·08	6·57 ± 1·62
($n = 6$)	2·01 ± 0·03*	0·38 ± 0·17*	4·07 ± 0·86*

Values are means ± S.D.; SL, sarcomere length. Relative tensions are expressed as a fraction of tension obtained during maximal activation (pCa 4·5) at long SL. * $P < 0·05$ *vs.* long SL.

Table 3. Effect of osmotic compression on k_{tr} in rat slow-twitch and rabbit fast-twitch fibres

	Dextran	SL (μm)	Width (μm)	Relative tension P/P_0	k_{tr} (s^{-1})
Slow-twitch fibres					
Maximal activation					
($n = 6$)	—	2·28 ± 0·02	71 ± 16	1·0	5·43 ± 0·82
($n = 6$)	—	1·99 ± 0·04*	76 ± 18*†	0·91 ± 0·04*†	4·57 ± 0·45*†
($n = 6$)	+	1·99 ± 0·04*	70 ± 15	0·97 ± 0·04*	5·29 ± 1·07
Submaximal activation					
($n = 12$)	—	2·29 ± 0·02	78 ± 10	0·53 ± 0·09	1·95 ± 0·48
($n = 12$)	—	1·99 ± 0·03*	84 ± 12*†	0·41 ± 0·07*†	1·67 ± 0·45*†
($n = 12$)	+	1·99 ± 0·03*	80 ± 11	0·59 ± 0·12	2·01 ± 0·50
Fast-twitch fibres					
Submaximal activation					
($n = 3$)	—	2·25 ± 0·03	86 ± 2	0·65 ± 0·05	7·84 ± 1·08
($n = 3$)	—	1·96 ± 0·05*	92 ± 1*†	0·32 ± 0·08*†	5·34 ± 0·13*†
($n = 3$)	+	1·93 ± 0·05*	84 ± 1	0·76 ± 0·06	7·90 ± 1·64

Values are means ± S.D.; SL, sarcomere length. Relative tensions are expressed as a fraction of tension obtained during maximal activation (pCa 4·5) at long SL. * $P < 0·05$ *vs.* long SL. † $P < 0·05$ *vs.* short SL plus dextran.

Figure 5*A* shows examples of fibre segment length, SL, and tension records in response to slack–restretch manoeuvres applied to a rat slow-twitch fibre during maximal and submaximal activations at long and short SL. The time courses of tension redevelopment at pCa 4·5 are enlarged and superimposed in Fig. 5*B* (raw data) and 5*C* (normalized data). At long SL, steady-state tension was greater than at short SL and the rate of tension redevelopment was slightly faster (long SL, k_{tr} was 4·85 s^{-1}; short SL, k_{tr} was 4·53 s^{-1}). The length dependence of k_{tr} is summarized in Table 2 for both rat slow-twitch fibres and rabbit fast-twitch fibres. For maximally activated slow-twitch fibres, reducing SL by ~0·3 μm from ~2·28 μm resulted in a 20% reduction in k_{tr} (5·15 ± 0·85 s^{-1} *versus* 4·12 ± 0·70 s^{-1}, $P < 0·001$). For submaximally activated slow-twitch fibres, a similar reduction in SL resulted in a 14% decrease in k_{tr} (Fig. 5*A*, Table 2). On the other hand, steady-isometric tension decreased by a relatively greater extent (23%) during submaximal activations than during maximal activations (10%) over this same SL range, i.e. ~2·29 to ~2·00 μm.

One possible mechanism to explain the length dependence of the rate of tension redevelopment is that the rate of cross-bridge binding is reduced at short SL due to an increase in lateral spacing between thick and thin filaments (Rome, 1968). To test this idea, we studied the effects on k_{tr} due to osmotic compression of interfilament lattice spacing at short SL. These results are summarized in Table 3. In

submaximally activated slow-twitch fibres, osmotic compression at short SL increased k_{tr} to values similar to those obtained at long SL in the absence of dextran. In the example shown in Fig. 6, osmotic compression with 2% dextran increased tension at short SL from 78 to 147 μN and increased k_{tr} from 1·02 to 1·85 s^{-1}, a value essentially identical to that obtained in the same fibre at long SL in the absence of dextran, i.e. 1·86 s^{-1}.

In an attempt to distinguish whether the effect of dextran on k_{tr} was due to myofilament compression *per se* or a dextran-induced change in the level of activation of the thin filaments, osmotic compression experiments were repeated on fibres during maximal activation, in which the thin filaments should be fully activated (Lehrer, 1994). Osmotic compression of maximally activated fibres at short SL was found to increase k_{tr} to values similar to those at long SL (see Table 3), suggesting that myofilament spacing *per se* is an important determinant of k_{tr}. Of course, such a result does not exclude the possibility that numbers of cross-bridges bound to the thin filament might contribute to length-dependent changes in k_{tr} at low levels of Ca^{2+} activation.

We also assessed k_{tr} as a function of SL in fast-twitch fibres from rabbit psoas muscle and obtained qualitatively similar findings; these are summarized in Table 2. Figure 7 shows representative records of tension redevelopment in a submaximally activated fibre at long (2·28 μm) and short (1·99 μm) SL. In this case, isometric tension in the untreated control fibre was 421 μN at long SL and 147 μN at short SL, while k_{tr} was 6·87 s^{-1} at long SL and 3·59 s^{-1} at short SL. Overall, in submaximally activated psoas fibres, reducing SL from 2·27 ± 0·01 to 2·01 ± 0·03 μm resulted in a 38% decrease in mean k_{tr} (6·57 ± 1·62 to 4·07 ± 0·86 s^{-1}) while tension decreased from 0·72 ± 0·08 to 0·38 ± 0·17 P_0. Thus, similar changes in SL of slow-twitch fibres and fast-twitch fibres resulted in greater changes in both k_{tr} and Ca^{2+} sensitivity of tension in the fast-twitch fibres.

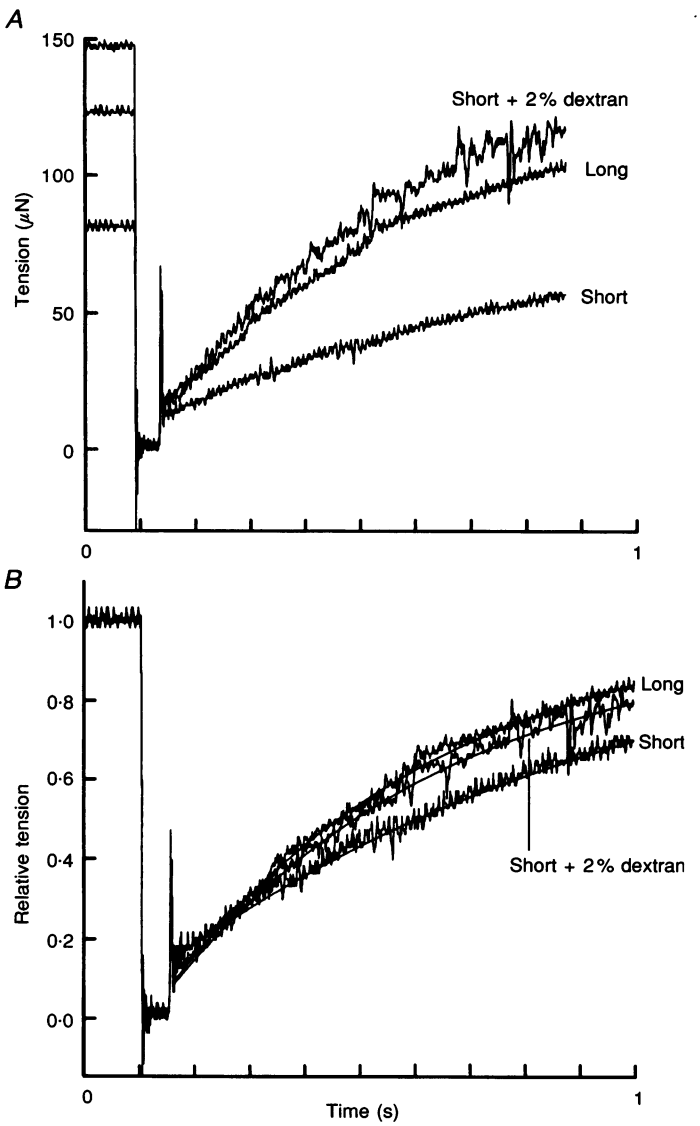

Figure 6. The effects of 2% dextran on steady-state tension and k_{tr} due to osmotic compression of a submaximally activated (pCa 5·8) slow-twitch fibre from rat soleus muscle

Panel *A* shows raw records of tension redevelopment and panel *B* shows these same records normalized to steady-isometric tension with the fitted exponential curves. In this experiment osmotic compression of the fibre at short SL (1·97 μm) increased tension from 78 to 147 μN and increased k_{tr} from 1·02 to 1·85 s^{-1}, which was similar to the k_{tr} of 1·86 s^{-1} measured in the same fibre at long length in the absence of dextran.

If the length dependence of cross-bridge kinetics in fast-twitch fibres is due primarily to changes in myofilament spacing, osmotic compression of fast-twitch muscle fibres at short length should increase k_{tr} to values near those measured in the same fibres at long SL. As summarized in Table 3, this was found to be the case. As a specific example, Fig. 8 shows records of tension redevelopment in a submaximally activated fast-twitch muscle fibre at short SL, long SL, and at short SL in the presence of 3% dextran: k_{tr} was 8·69 s^{-1} at long length, decreased to 5·22 s^{-1} at short length, and recovered to 9·46 s^{-1} when osmotically compressed at short SL. These results also show that osmotic compression of fast-twitch fibres had a greater effect on k_{tr} than in slow-twitch fibres, which is consistent with the greater length dependence of k_{tr} in fast-twitch fibres. However, in both slow-twitch and fast-twitch fibres, the rates of tension redevelopment at long and short lengths were similar when the myofilament spacing at short lengths was experimentally manipulated to closely match the spacing at long lengths.

DISCUSSION

Possible mechanisms for length dependence of twitch tension in skeletal muscle

The fall-off of isometric-twitch tension following reductions in SL is an important functional characteristic of striated muscle. Several factors may contribute to this rather steep relationship. First, the amount of Ca^{2+} released to the myoplasm may change as a function of SL. To date there is no evidence to support this idea since the level of myoplasmic free Ca^{2+} during a twitch differed only slightly

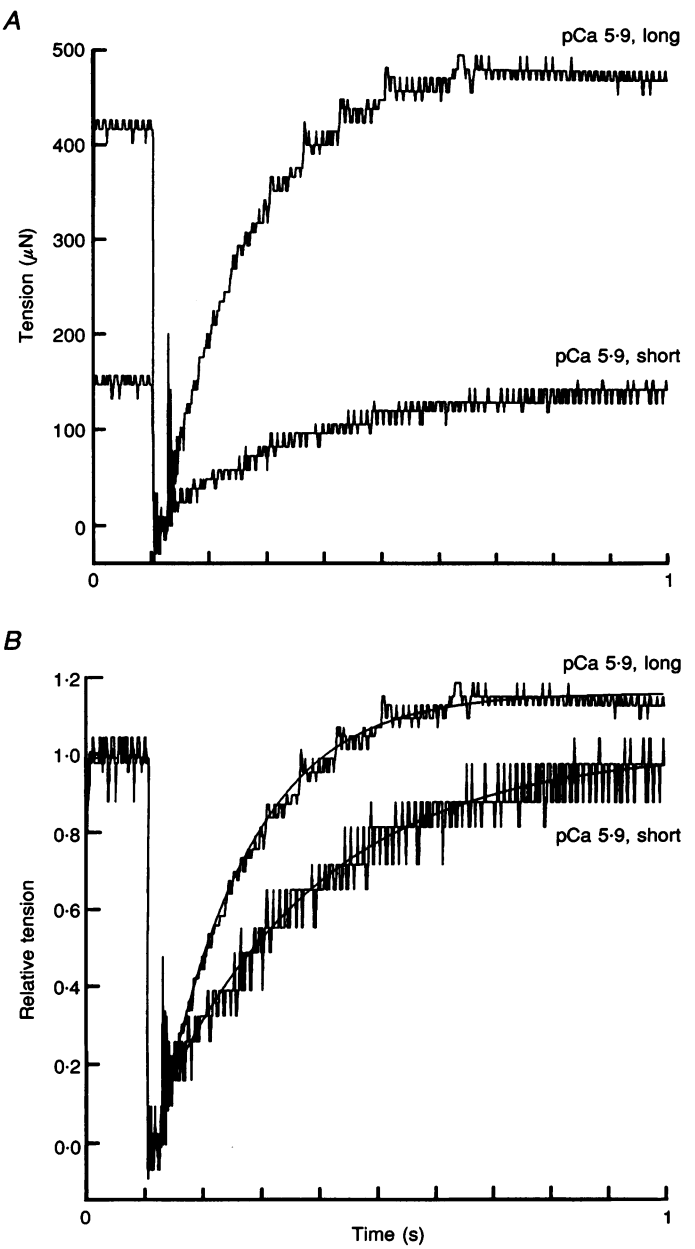

Figure 7. The effect of SL on steady-state tension and k_{tr} in a submaximally activated (pCa 5·9) fast-twitch fibre from rabbit psoas muscle

In this example, isometric tensions at long and short SL were 421 and 147 μN, respectively. Rates of tension redevelopment (k_{tr}) were 6·87 s^{-1} at long SL and 3·59 s^{-1} at short SL.

when SL was reduced from 2·4 to 1·4 μm (Blinks, Rüdel & Taylor, 1978). These experiments were, however, performed on amphibian fibres and the results might not be generally applicable to mammalian fibres. Second, there would be small but significant decreases in twitch tension as length is reduced from optimum due to changes in overlap of thick and thin filaments (Gordon, Huxley & Julian, 1966). Third, since the increase in myoplasmic Ca^{2+} during a twitch is transient and therefore submaximal for much of the time course of a twitch, the decrease in Ca^{2+} sensitivity of tension observed previously at short SL in skinned skeletal muscle fibres (Endo, 1973; Allen & Moss, 1987; Fuchs & Wang, 1991; this study) probably contributes significantly to the decline of twitch tension. Fourth, since isolated skeletal muscles at rest do not ordinarily assume a SL of much less than 1·90–2·00 μm (i.e. slack length), experimental measurements of twitch tension at SL less than slack length require that the muscle be slackened while at rest and then actively shortened to the isometric SL during the subsequent twitch. In these cases, the muscle will shorten to the desired length during part of the myoplasmic Ca^{2+} transient, leaving only the remaining portion of the Ca^{2+} transient for isometric tension development. Also, as the slackened muscle shortens, dissociation of Ca^{2+} from troponin C (Gordon & Ridgeway, 1987; Allen & Kentish, 1988) would further reduce the level of activation of the thin filaments once the isometric phase of contraction began.

Our finding that the rate of isometric tension redevelopment decreases at short SL provides evidence for an additional mechanism involved in length dependence of twitch tension. The slowing of the rate at which cross-bridges proceed from detached or weakly bound states to strongly bound, force-generating states would reduce the number of force-generating cross-bridges and, thus, twitch tension in response to a transient increase in myoplasmic $[Ca^{2+}]$.

Length dependence of Ca^{2+} sensitivity of tension

Since myoplasmic $[Ca^{2+}]$ is submaximal for much of a twitch, the reduced Ca^{2+} sensitivity of tension at short SL would contribute to decreased twitch tension. In the present study, we characterized the effect of SL on Ca^{2+} sensitivity of tension in skinned preparations of both slow-twitch and fast-twitch muscle fibres. When SL was reduced from ~2·25 to ~1·85 μm, Ca^{2+} sensitivity of tension decreased significantly in both fibre types, the decrease being greater in fast-twitch fibres than in slow-twitch fibres. Stephenson & Williams (1982) reported a similar change in Ca^{2+} sensitivity of tension in slow-twitch fibres as found in this study but, in contrast, length-dependent changes in Ca^{2+} sensitivity were greater in slow-twitch fibres than in fast-twitch fibres. However, their study examined Ca^{2+} sensitivity of tension over a SL range of 2·2 to 3·6 μm, which is much greater than the one examined in this study. Gulati, Sonnenblick & Babu (1990) reported that Ca^{2+} sensitivity of

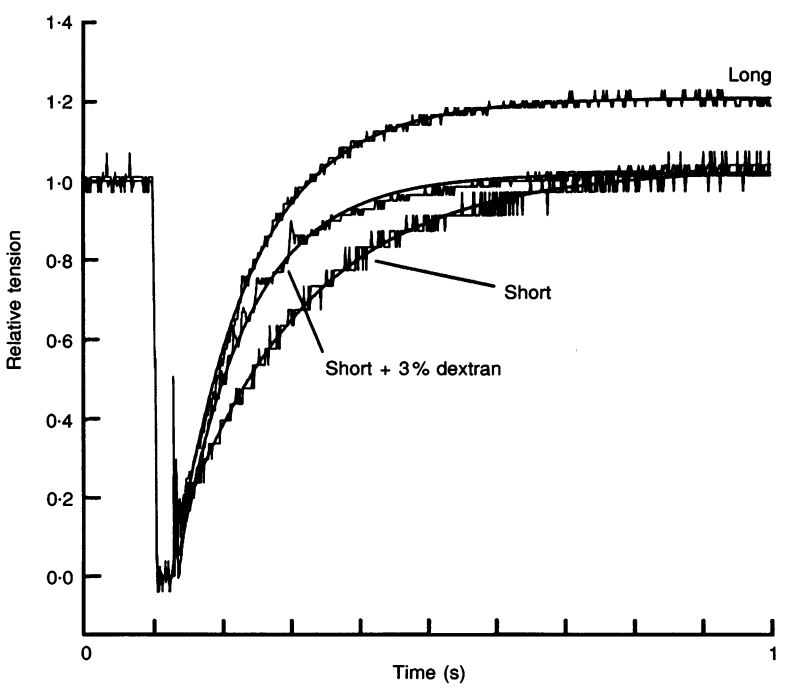

Figure 8. The effect of osmotic compression with 3% dextran on k_{tr} in a rabbit fast-twitch psoas fibre during submaximal activation

Records shown are normalized to the steady isometric tension for each condition, i.e. long SL, short SL, or short SL + 3% dextran. Osmotic compression of the fibre at short SL increased k_{tr} from 5·22 to 9·46 s^{-1}, a value similar to the k_{tr} of 8·69 s^{-1} obtained at long SL in the absence of dextran.

tension decreased to a greater extent ($\Delta pCa_{50} \approx 0{\cdot}13$ pCa units) in slow-twitch fibres than in fast-twitch fibres ($\Delta pCa_{50} \approx 0{\cdot}03 \pm 0{\cdot}01$ pCa units) when SL was reduced from 2·40 to 1·90 μm. Thus, these results differ quantitatively from those of the present study, but the basis for this difference is not clear.

A number of mechanisms might be involved in conferring greater length dependence of Ca^{2+} sensitivity of tension in fast-twitch fibres. Fibre-type-dependent variations in protein isoforms is one possibility, in particular troponin C which differs structurally and functionally between slow-twitch and fast-twitch fibres (reviewed by Parmacek & Leiden, 1991). Slow-twitch fibres contain the cardiac isoform of troponin C (cTnC) which has one regulating Ca^{2+} binding site while fast-twitch fibres contain the skeletal isoform of troponin C (sTnC) which has two regulatory Ca^{2+} binding sites. So far, experimental evidence does not support the idea that sTnC confers greater length dependence of Ca^{2+} sensitivity of tension. Replacement of sTnC with cTnC in fast-twitch fibres has been reported to either increase (Gulati, Sonnenblick & Babu, 1990) or have no effect (Moss, Nyowe & Greaser, 1991) on length dependence of Ca^{2+} sensitivity of tension. Also, incorporation of sTnC in place of cTnC in thin filaments of cardiac myocytes in transgenic mice did not alter the length dependence of Ca^{2+} sensitivity of tension (McDonald, Field, Parmacek, Soonpaa, Leiden & Moss, 1995). The possibility that other thin or thick filament protein isoforms mediate fibre-type-specific differences in length dependence of Ca^{2+} sensitivity of tension remains to be studied.

A more likely basis for the greater length dependence of Ca^{2+} sensitivity of tension in fast-twitch fibres is the greater apparent co-operativity of Ca^{2+} activation of tension in this fibre type. Several studies have suggested that the Ca^{2+} sensitivity of tension is modulated by the number of strong-binding cross-bridges bound to the thin filament (Hofmann & Fuchs, 1987; Allen & Moss, 1987; Sweitzer & Moss, 1990; Swartz & Moss, 1992). Since the steepness of the tension–pCa relationship is significantly greater in fast-twitch fibres (N_2 at $12{\cdot}0 \pm 3{\cdot}4$ at long SL) than in slow-twitch fibres (N_2 at $2{\cdot}42 \pm 0{\cdot}77$ at long SL), it is reasonable to conclude that the thin filament of fast-twitch muscle is more highly co-operative. As a result, its activation state would be expected to vary more dramatically as a result of length-induced variations in cross-bridge number. At short lengths, there may be a reduction in strongly bound bridges due to increased filament lattice spacing and a consequent decrease in the probability of cross-bridge binding, and this in turn would reduce the degree of co-operative activation of the thin filament and disproportionately reduce isometric tension. On the other hand, activation of slow-twitch fibres is considerably less co-operative and more continuously graded with Ca^{2+} concentration. In this case the increase in filament lattice spacing at short lengths would reduce the number of strongly bound cross-bridges and more nearly proportionately reduce tension. Thus, the reduction in tension would be less than in fast-twitch muscle fibres.

Another possible mechanism contributing to greater length dependence of Ca^{2+} sensitivity of tension in fast-twitch fibres is the greater length and lattice-spacing dependence of the rate of tension redevelopment observed in the present study. To interpret tension redevelopment data, cross-bridge cycling has been characterized in terms of a two-state model consisting of a distribution between weakly bound, non-force-generating states and strongly bound, force-generating states (Brenner, 1988). In this scheme, force-generating transitions are governed by an apparent rate constant f_{app} and the detachment transitions are governed by an apparent rate constant g_{app}. According to this model (Brenner, 1988), both the position and shape of the tension–pCa relationship are determined by Ca^{2+}-dependent modulation of the proportion of cycling cross-bridges in the force-generating state, i.e. $f_{app}/(f_{app} + g_{app}) \times$ (total number of cross-bridges available), where an increase in f_{app} relative to g_{app} yields a leftward shift in the tension–pCa relationship. The observations of greater rates of tension redevelopment (k_{tr}) and greater Ca^{2+} sensitivity of tension at long SL are consistent with this model, assuming that the changes in k_{tr} are primarily determined by changes in f_{app}. Also consistent with this model, were the greater length-dependent changes in steady tension in submaximally activated fast-twitch fibres that were associated with greater changes in k_{tr}. Therefore, the greater length dependence of k_{tr} in fast-twitch fibres may contribute to the greater change in Ca^{2+} sensitivity of tension. Alternatively, Ca^{2+} sensitivity of tension and k_{tr} may vary independently in response to changes in SL, or the SL-induced change in k_{tr} could depend upon the force generated at a given Ca^{2+} concentration as suggested by Hofmann & Fuchs (1987).

Mechanisms for length dependence of k_{tr}

The most likely explanation for the reduction in k_{tr} at short SL is the increased lateral spacing between thick and thin filaments as SL is reduced, since a greater distance between thick and thin filaments should decrease the probability of cross-bridge attachment to actin. We tested this hypothesis by examining the effects on k_{tr} due to the reduction in spacing between the thick and thin filaments achieved by osmotic compression of the myofilament lattice. Our findings that compression of the filament lattice at short SL increased k_{tr} in both slow-twitch and fast-twitch fibres to values similar to those obtained at long SL supports the notion that length dependence of k_{tr} is mediated, at least in part, by changes in myofilament spacing. The identity of the cross-bridge transitions that are influenced by SL and interfilament spacing is unknown. Zhao & Kawai (1993) used sinusoidal analyses of steady-state activated skeletal muscle fibres to assess the effects of osmotic compression on elementary steps in the cross-bridge cycle. They concluded that osmotic compression caused cross-bridges to accumulate in force-generating states by reducing the rate of reversal of

the power stroke as well as the rate constants of an isomerization step predicted by their model to follow the power stroke. However, these inferred changes in rate constants may be model specific since rate constants describing the power stroke (k_4 and k_{-4}) and the isomerization step (k_6) in their model are approximately an order of magnitude greater than the rate constant of tension redevelopment (Brenner & Eisenberg, 1986 and this study) and the isometric ATPase activity observed in psoas fibres (Brenner & Eisenberg, 1986).

ALLEN, D. G. & KENTISH, J. C. (1985). The cellular basis of the length–tension relation in cardiac muscle. *Journal of Molecular & Cellular Cardiology* **17**, 821–840.

ALLEN, D. G. & KENTISH, J. C. (1988). Ca^{2+} concentration in the myoplasm of skinned ferret ventricular muscle following changes in muscle length. *Journal of Physiology* **407**, 489–503.

ALLEN, D. G. & KURIHARA, S. (1982). The effects of muscle length on intracellular Ca^{2+} transients in mammalian cardiac muscle. *Journal of Physiology* **327**, 79–94.

ALLEN, J. D. & MOSS, R. L. (1987). Factors influencing the ascending limb of the sarcomere length tension relationship in rabbit skinned muscle fibres. *Journal of Physiology* **390**, 119–136.

ALLEN, D. G., NICHOLS, C. G. & SMITH, G. L. (1988). The effects of changes in muscle length during diastole on the Ca^{2+} transient in ferret ventricular muscle. *Journal of Physiology* **406**, 359–370.

BLINKS, J. R., RÜDEL, R. & TAYLOR, S. R. (1978). Ca^{2+} transients in isolated amphibian skeletal muscle fibres: detection with aequorin. *Journal of Physiology* **277**, 291–323.

BRENNER, B. (1988). Effect of Ca^{2+} on cross-bridge turnover kinetics in skinned single rabbit psoas fibers; implications for regulation of muscle contraction. *Proceedings of the National Academy of Sciences of the USA* **85**, 3265–3269.

BRENNER, B. & EISENBERG, E. (1986). Rate of force generation in muscle: correlation with actomyosin ATPase activity in solution. *Proceedings of the National Academy of Sciences of the USA* **83**, 3542–3546.

CLAFLIN, D. R., MORGAN, D. L., STEPHENSON, D. G. & JULIAN, F. J. (1994). The intracellular Ca^{2+} transient and tension in frog skeletal muscle fibres measured with high temporal resolution. *Journal of Physiology* **475**, 319–325.

CLOSE, R. I. (1972). The relations between sarcomere length and characteristics of isometric twitch contractions of frog sartorius muscle. *Journal of Physiology* **220**, 745–762.

DE TOMBE, P. P. & TER KEURS, H. E. D. J. (1990). Force and velocity of sarcomere shortening in trabeculae from rat heart: effects of temperature. *Journal of Physiology* **66**, 1239–1254.

ENDO, M. (1973). Length-dependence of activation of skinned muscle fibres by calcium. *Cold Spring Harbor Symposium on Quantatative Biology* **37**, 505–510.

FABIATO A. (1988). Computer programs for calculating total from specified free or free specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. *Methods in Enzymology* **157**, 378–417.

FUCHS, F. & WANG, Y.-P. (1991). Force, length, and Ca^{2+}-troponin C affinity in skeletal muscle. *American Journal of Physiology* **261**, C787–792.

GORDON, A. M., HUXLEY, A. F. & JULIAN, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *Journal of Physiology* **184**, 170–192.

GORDON, A. M. & POLLACK, G. H. (1980). Effects of Ca^{2+} on the sarcomere length–tension relation in rat cardiac muscle. *Circulation Research* **47**, 610–619.

GORDON, A. M. & RIDGEWAY, E. B. (1987). Extra Ca^{2+} on shortening in barnacle muscle. *Journal of General Physiology* **90**, 321–340.

GULATI, J., SONNENBLICK, E. & BABU, A. (1990). The role of troponin C in the length dependence of Ca^{2+}-sensitive force of mammalian skeletal and cardiac muscles. *Journal of Physiology* **441**, 305–324.

HIBBERD, M. G. & JEWELL, B. R. (1982). Ca^{2+} and length-dependent force production in rat ventricular muscle. *Journal of Physiology* **329**, 527–540.

HOFMANN, P. A. & FUCHS, F. (1987). Effect of length and cross-bridge attachment on Ca^{2+} binding to cardiac troponin C. *American Journal of Physiology* **253**, C541–546.

KRESS, M., HUXLEY, H. E. & FARUQI, A. R. (1986). Structural changes during activation of frog muscle studied by time-resolved x-ray diffraction. *Journal of Molecular Biology* **188**, 325–342.

LEHRER, S. (1994). The regulatory switch of the muscle thin filament: Ca^{2+} or myosin heads? *Journal of Muscle Research and Cell Motility* **15**, 232–236.

MCDONALD, K. S., FIELD, L. J., PARMACEK, M. S., SOONPAA, M., LEIDEN, J. M. & MOSS, R. L. (1995). Length dependence of Ca^{2+} sensitivity of tension in mouse cardiac myocytes expressing skeletal troponin C. *Journal of Physiology* **483**, 131–139.

MCDONALD, K. S. & MOSS, R. L. (1995). Osmotic compression of single cardiac myocytes eliminates the reduction in Ca^{2+} sensitivity of tension at short sarcomere length. *Circulation Research* **77**, 199–205.

METZGER, J. M. & MOSS, R. L. (1990). Ca^{2+}-sensitive cross-bridge transitions in mammalian fast and slow skeletal muscle fibres. *Science* **247**, 1088–1090.

MOSS, R. L. (1979). Sarcomere length–tension relations of frog skinned muscle fibres during Ca^{2+} activation at short lengths. *Journal of Physiology* **292**, 177–202.

MOSS, R. L., NWOYE, L. O. & GREASER, M. L. (1991). Substitution of cardiac troponin C into rabbit muscle does not alter the length dependence of Ca^{2+} sensitivity of tension. *Journal of Physiology* **440**, 273–289.

MOSS, R. L., SWINFORD, A. E. & GREASER M. L. (1983). Alterations in the Ca^{2+} sensitivity of tension development by single skeletal muscle fibres at stretched lengths. *Biophysical Journal* **43**, 115–119.

PARMACEK, M. S. & LEIDEN, J. M. (1991). Structure, function, and regulation of troponin C. *Circulation* **84**, 991–1003.

RACK, P. M. H. & WESTBURY, D. R. (1969). The effects of length and stimulus rate on tension in the isometric cat soleus muscle. *Journal of Physiology* **204**, 443–460.

ROBERTSON, S. P., JOHNSON, J. D. & POTTER, J. D. (1981). The time-course of Ca^{2+} exchange with calmodulin, troponin, parvalbumin, and myosin in response to transient increases in Ca^{2+}. *Biophysical Journal* **34**, 559–569.

ROME, E. (1968). X-ray diffraction studies of the filament lattice of striated muscle in various bathing media. *Journal of Molecular Biology* **37**, 331–344.

ROSZEK, B., BAAN, G. C. & HUIJING, P. A. (1994). Decreasing stimulation frequency-dependent length-force characteristics of rat muscle. *Journal of Applied Physiology* **77**, 2115–2124.

SHINER, J. S. & SOLARO, R. J. (1984). The Hill coefficient for the Ca^{2+}-activation of striated muscle contraction. *Biophysical Journal* **46**, 541–543.

STEPHENSON, D. G. & WILLIAMS, D. A. (1982). Effects of sarcomere length on the force–pCa relation in fast- and slow-twitch skinned muscle fibres from the rat. *Journal of Physiology* **333**, 637–653.

SWARTZ, D. R. & Moss, R. L. (1992). Influence of a strong-binding myosin analog on Ca^{2+} sensitive mechanical properties of skinned skeletal muscle fibres. *Journal of Biological Chemistry* **267**, 20497–20506.

SWEITZER, N. K. & Moss, R. L. (1990). The effect of altered temperature on Ca^{2+}-sensitive force in permeabilized myocardium and skeletal muscle. *Journal of General Physiology* **96**, 1221–1245.

TER KEURS, H. E. D. J., RISNSBURGER, W. H., VAN HEUNINGEN, P. & NAGELSMIT, M. J. (1980). Tension development and sarcomere length in rat cardiac trabelucae. *Circulation Research* **46**, 703–714.

WOLFF, M. R., MCDONALD, K. S. & Moss, R. L. (1995). Rate of tension development in cardiac muscle varies with level of activator Ca^{2+}. *Circulation Research* **76**, 154–160.

ZHAO, Y. & KAWAI, M. (1993). The effect of the lattice spacing change on cross-bridge kinetics in chemically skinned rabbit psoas muscle fibres. *Biophysical Journal* **64**, 197–210.

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