RESEARCH ARTICLE

Additional in-series compliance reduces muscle force summation and alters the time course of force relaxation during fixed-end contractions

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ABSTRACT

There are high mechanical demands placed on skeletal muscles in movements requiring rapid acceleration of the body or its limbs. Tendons are responsible for transmitting muscle forces, but, because of their elasticity, can manipulate the mechanics of the internal contractile apparatus. Shortening of the contractile apparatus against the stretch of tendon affects force generation according to known mechanical properties; however, the extent to which differences in tendon compliance alter force development in response to a burst of electrical impulses is unclear. To establish the influence of series compliance on force summation, we studied electrically evoked doublet contractions in the cane toad peroneus muscle in the presence and absence of a compliant artificial tendon. Additional series compliance reduced tetanic force by two-thirds, a finding predicted based on the force–length property of skeletal muscle. Doublet force and force–time integral expressed relative to the twitch were also reduced by additional series compliance. Active shortening over a larger range of the ascending limb of the force–length curve and at a higher velocity, leading to a progressive reduction in force-generating potential, could be responsible. Muscle–tendon interaction may also explain the accelerated time course of force relaxation in the presence of additional compliance. Our findings suggest that a compliant tendon limits force summation under constant-length conditions. However, high series compliance can be mechanically advantageous when a muscle–tendon unit is actively stretched, permitting muscle fibres to generate force almost isometrically, as shown during stretch–shorten cycles in locomotor activities. Restricting active shortening would likely favour rapid force development.

KEY WORDS: Series elastic compliance, Tendon stiffness, Muscle–tendon interaction, Active shortening, Cane toad

INTRODUCTION

Animals must be capable of producing rapid movements to interact successfully with their physical environment. Locomotor activities employed during prey capture and predator evasion place especially high mechanical demands on skeletal muscle performance. For instance, the ankle extensors of the kangaroo rat and cat generate peak forces during jumping that exceed those measured *in situ* under maximal isometric conditions (Walmsley et al., 1978; Biewener et al., 1988). High mechanical outputs are not confined to accelerations, which necessitate that muscles generate mechanical energy. Skeletal muscles must also rapidly absorb mechanical energy to quickly arrest the body's motion (Gabaldón et al., 2004). Small animals in particular must have neuromuscular systems adept at generating forces at very high rates because of the comparatively shorter time that their limbs spend in contact with the ground during locomotion (Kram and Taylor, 1990).

Muscle fibre force production is largely constrained by the force–length (Gordon et al., 1966) and force–velocity (Fenn and Marsh, 1935) properties of the contractile apparatus. For a given activation level, contractile force is a function of myofilament overlap and cross-bridge cycling dynamics. Contractile speed, including the maximum velocity of fibre shortening and maximum rates of force development and relaxation, is governed by the kinetics of actin–myosin interaction and sarcoplasmic reticulum Ca^{2+} release and reuptake (Briggs et al., 1977; Edman et al., 1988; Metzger and Moss, 1990; Gordon et al., 2000). Fibre type-specific expressions of the motor protein myosin (Edman et al., 1988; Bottinelli et al., 1991), and fibre type-related differences in Ca^{2+} dynamics (Baylor and Hollingworth, 2003; Barclay, 2012) and contractile apparatus Ca^{2+} sensitivity (Stephenson and Williams, 1981) mean that the mechanical output of whole muscle is also a function of fibre type composition (Harridge et al., 1996). The performance of skeletal muscle in functional terms, as either a powerful motor or brake, is ultimately dependent on both high contractile force and speed being effectively transferred to the skeletal system.

To exert influence over the skeletal system, internally generated forces must be transmitted to the skeletal system via elastic tendinous structures. As the passive structures of the series elastic element (SEE) elongate under muscle tension according to their structural and material properties (Lieber et al., 1991), series elastic compliance presents as a mechanical obstacle to force transmission. When the length of the muscle–tendon unit (MTU) is held constant during activation, active muscle fibres are permitted to shorten against the stretch of the SEE (Griffiths, 1991; Buchanan and Marsh, 2001). The amount of internal shortening afforded by the SEE, expressed as a percentage of resting fibre length or the fibre length at which force is maximal (i.e. fixed-end compliance), is largely a function of muscle design. Fixed-end compliance increases as the relative difference in length between the SEE and muscle fibre increases (Zajac, 1989). Animal and human skeletal muscles cover a broad range of tendon length-to-fibre length ratios (Zajac, 1989; Biewener and Roberts, 2000). As a consequence, the fixed-end compliance of skeletal muscle varies considerably (Biewener and Roberts, 2000): horse superficial digital flexor 81% (Brown et al., 2003; Swanstrom et al., 2005), human medial gastrocnemius 35% (Narici et al., 1996) cat medial gastrocnemius

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List of symbols and abbreviations	
FTI	force–time integral
ISI	interstimulus interval
L_0	muscle fibre bundle length at which twitch force is maximal
MTU	muscle–tendon unit
SEE	series elastic element

28% (Griffiths, 1991), guinea fowl lateral gastrocnemius 13% (Buchanan and Marsh, 2001), frog semitendinosus 11% (Lieber et al., 1991, 1992), hopping mouse gastrocnemius 6% (Ettema, 1996) and rat gastrocnemius 5% (Ettema, 1996). Experimental findings support distinct mechanical and functional roles for tendons of low and high series compliance (Biewener et al., 1981; Biewener and Blickhan, 1988; Ettema, 1996). Some studies have concluded that the structural design of the SEE appears to be ecologically driven (e.g. Biewener and Blickhan, 1988).

As the SEE influences both force transmission and internal shortening, and is diverse in morphology, series elastic compliance is an important mechanical determinant of the rate of force development and the force-generating capacity of muscle. Much of what we know about the effects of series elastic compliance on contractile behaviour is owing to the work of A. V. Hill. Hill (1951) established that adding compliance in-series with muscle reduced twitch force and prolonged the time to peak tension. Hill (1949) also demonstrated that when the effect of series elastic compliance on internal contractile dynamics was effectively abolished, by applying a rapid, yet brief stretch to the MTU at stimulation onset, twitch force and the rate of force rise increased substantially. A similar response was observed when active shortening against the stretch of the SEE was largely prevented by a feedback control signal that adjusted the length of an isolated bullfrog plantaris MTU (Sawicki and Roberts, 2009). We recently demonstrated for the human triceps surae, which has high fixed-end compliance, that the mechanical properties of the twitch could be dramatically increased by only a modest reduction in fascicle shortening amplitude and velocity induced by a rapid stretch of relatively low amplitude (Mayfield et al., 2016). Stretch of the MTU early in the twitch was shown to also influence the force generated by a second stimulating pulse delivered at the post-stretch MTU length, suggesting that the depressant effect of active shortening on contractile force may influence force summation under conditions where internal shortening is permitted. Findings of this nature highlight the necessity to consider muscle–tendon interaction when interpreting muscle contractile properties under constant-length conditions. Whether differences in series elastic compliance over the range experienced by animals influence the relative summation of force owing to a burst of electrical impulses is less clear.

Applying a rapid stretch to the MTU, as a means for simulating lower series elastic compliance, is limited in that the effective stiffness of the SEE is modified only for the duration of the stretch. Further to this, the degree to which the effective stiffness is modified by a given amplitude of stretch is dependent on the active state of the muscle during the stretch. There is less relative resistance to active shortening when activation is achieved with two stimuli, particularly at a high frequency of stimulation, owing to higher rates of shortening and force rise (Mayfield et al., 2016). A more suitable model for studying the direct influence of series compliance on force summation would permit the compliance of the SEE to be manipulated to align with different physiological compliances for comparative purposes, but also be maintained for the duration of the

contraction. Serial attachment of a thin latex strip to an isolated muscle preparation has been demonstrated to be an effective means for simulating in vivo tendon dynamics (Lichtwark and Barclay, 2010).

The purpose of the present study was to examine the influence of series elastic compliance on force summation owing to doublet stimulation [brief interstimulus interval (ISI)]. We attached a compliant artificial tendon, as described above, in-series with a fibre bundle isolated from the peroneus muscle of a cane toad (Rhinella marina). Twitch and doublet force were measured during fixed-end contractions at a range of fibre bundle lengths in the absence and presence of a compliant tendon to characterize the effects of series compliance on muscle contractile properties and force summation. On the basis that additional series compliance would permit an increase in active shortening of the internal contractile apparatus and, therefore, bring about a reduction in force-generating capacity, we hypothesized that additional series compliance would reduce relative force summation.

MATERIALS AND METHODS
Animals
Cane toads [Rhinella marina (Linnaeus 1758)] were captured in local watercourses and housed in an enclosure at The University of Queensland until use. Toads were placed in a refrigerator (4°C) for approximately 1 h prior to euthanasia to ease stress on the animal. Toads were euthanized by double pithing. All animal handling procedures were carried out in accordance with the guidelines of The University of Queensland's Animal Ethics Committee.

In vitro preparation
The peroneus muscle and tendons were dissected from both hind limbs, blotted on filter paper (Whatman Grade 1) and placed in Ringer's solution at room temperature. Bundles of fibres (N=15) were then dissected from the isolated muscle under a microscope. Aluminium foil clips were attached to the proximal and distal tendinous regions of each fibre bundle for mounting of the preparation. The distal clip was designed to permit the inclusion of a thin strip of latex in-series with the fibre bundle (Lichtwark and Barclay, 2010). The dimensions of the latex strip determined the series elastic compliance of the preparation. The latex strip, subsequently referred to as a compliant artificial tendon, was of dimensions that permitted the fibre bundle to actively shorten by 20–25% of its resting length during a fixed-end tetanic contraction (based on the approximate force-generating capacity of the muscle bundles).

The muscle preparation was mounted between a force transducer and micrometre, and lay submerged in Ringer's solution in a temperature-controlled bath (custom built). The Ringer's solution was of the following composition (mmol l^{-1}): NaCl 112, $CaCl_2$ 2.5, $MgCl_2$ 1, HEPES 20, KCl 3.3 and glucose 5, adjusted to pH 7.4 at room temperature using NaOH. Force development and relaxation in vertebrate skeletal muscle exhibit strong thermal dependencies (Bennett, 1984). Measurements in the present study were made at 30°C to maximize relative doublet summation in amphibian muscle (Ranatunga, 1977) and increase the force-limiting potential of the compliant artificial tendon.

In vitro data collection
An electrical stimulator (SD9 Stimulator, Grass Technologies, Quincy, MA, USA) delivered supramaximal square-wave pulses to fibres via two fine platinum wire electrodes that ran parallel to the length of the fibre bundle in close proximity. Pulse width was set at

500 µs and stimulus strength set 10% above that eliciting maximum twitch force (field strength of approximately 30–40 V cm^{-1}). With the muscle preparation mounted at the aluminium foil clips at either end, fibre bundle length was adjusted to maximize twitch force (optimal length, L_0). Force output was sampled and displayed at 2000 Hz with a 16-bit Micro1401 and Spike2 data collection system (Cambridge Electronics Design, Cambridge, UK).

In the absence of the compliant artificial tendon, twitch, doublet and tetanic force were measured at L_0. Additional measurements of twitch and doublet force were subsequently made at fibre bundle lengths of L_0-2 mm ($L_{0-2\text{ mm}}$), L_0-1 mm ($L_{0-1\text{ mm}}$) and L_0+1 mm ($L_{0+1\text{ mm}}$) to examine the effect of series compliance on the known length dependence of force summation. Passive force was recorded at each fibre bundle length and subsequently used to set fibre bundle length with the compliant artificial tendon serially attached (see below). An interval of at least 1 min was provided between successive twitch and doublet contractions to minimize the effects of muscle fatigue and oxidative stress. A final measurement of twitch force was obtained at L_0 to assess muscle performance. Once force measurements had been completed without the artificial tendon serially attached, the aluminium foil clip at the distal end of the preparation was dismounted from the micrometre and the free end of the artificial tendon was mounted. To obtain the desired fibre bundle length with the artificial tendon mounted, the length of the now more compliant MTU was adjusted until the passive force was equal to that previously measured at the corresponding fibre bundle length without the artificial tendon mounted. Twitch and doublet force were subsequently measured at each of the four fibre bundle lengths, and tetanic force was measured at L_0. A final measurement of twitch force was obtained at L_0 to assess muscular fatigue. Doublet stimulation was performed with ISIs of 5, 20 and 40 ms. The dynamic behaviour of fibre bundles serially attached to a complaint artificial tendon was recorded by a high-speed (240 Hz) compact digital camera fixed directly above the bath for estimation of muscle strain.

Data analysis
Force signals were imported into MATLAB (R2014a, MathWorks, Natick, MA, USA) for processing and analysis. To remove undesired high-frequency components from the imported data, a custom-written script was implemented to apply a second-order, 80 Hz low-pass Butterworth filter (dual pass). Twitch and doublet contractions were analyzed to determine contraction time, half-relaxation time, peak active force and the force–time integral (FTI). The contraction time of doublet responses was defined as the time interval between the second stimulus pulse and peak force. Peak active force in the presence of a compliant artificial tendon was calculated after estimating the reduction in passive force owing to shortening of the fibre bundle against the stretch of the artificial tendon. Traditional approaches to calculating active force assume passive force to remain constant during active force development, subtracting passive force at rest from peak total force to determine active force. Though this may be an appropriate method when internal shortening is restricted by a stiff SEE or by the removal of tendinous tissues, and thus when the length of the parallel elastic element remains relatively constant, it underestimates the active force developed by a muscle in-series with a compliant SEE (MacIntosh and MacNaughton, 2005; Hoffman et al., 2012). To approximate the reduction in passive force during contraction owing to active shortening in the presence of a compliant artificial tendon, fibre bundle length during twitch and doublet contractions at L_0 was measured. A quadratic function was then applied to values of

passive force obtained at each resting fibre bundle length, and the reduction in passive force during active force development owing to active shortening was determined from the interpolated passive force–length data. Peak active force was calculated by subtracting the corrected passive force from peak total force. This process was performed only for trials with the artificial tendon attached. Active shortening was assumed to be negligible in the absence of an artificial tendon. Accordingly, passive force at rest was used for the calculation of peak active force for twitch and doublet contractions without an artificial tendon. Relative force summation was calculated by normalizing doublet force to twitch force at each fibre bundle initial length. Measurements of fibre bundle strain (Δfibre bundle length/L_0) at L_0 were calculated from digitized 2D images.

Statistical analysis
To determine whether there were effects of series elastic compliance and fibre bundle initial length on each parameter of muscle performance, a two-way repeated measures ANOVA (tendon compliance×fibre bundle initial length) was performed in statistical software (Prism 6, GraphPad Software, La Jolla, CA, USA). A post hoc analysis was performed using Tukey's method of controlling significance level for multiple pair-wise comparisons. Statistical significance was set at $P \leq 0.05$. Data are reported in the text as means±s.d., and presented in the figures as means±s.e.m.

RESULTS
Additional series elastic compliance in the form of a compliant latex strip led to an increase in the amplitude and rate of active shortening and a reduction in peak force during tetanic, doublet and twitch fixed-end contractions. An example of the time courses of fibre bundle force and shortening during each contraction type with and without a compliant artificial tendon attached is shown in Fig. 1. Waveform-averaged tetanic, doublet and twitch force for fixed-end contractions performed at L_0 with and without serial attachment of a compliant artificial tendon are illustrated in Fig. 2. The addition of a compliant artificial tendon reduced tetanic force at L_0 by 66±9% ($P<0.01$; Fig. 1A). Additional series compliance also reduced the tetanic rate of force development and relaxation by 70% (5.52±2.2 versus 1.57±0.49 mN ms^{-1}, $P<0.01$) and 59% (5.36±1.75 versus 2.06±0.5 mN ms^{-1}, $P<0.01$), respectively. Muscle fibre bundle strain against the stretch of the compliant artificial tendon during a tetanic contraction at L_0 was 22±4%. Additional series compliance reduced twitch and doublet force by 65±8% and 68±6%, respectively.

Fig. 3 illustrates passive and active forces, and the FTI, as a function of fibre bundle initial length for twitch and doublet contractions performed with and without a compliant artificial tendon attached. Passive tension increased as a function of fibre bundle length in the presence and absence of additional series compliance ($P<0.01$), but the change in passive tension owing to step increases in fibre bundle length did not differ between compliant and stiff conditions ($P=0.55$; Fig. 3A,B). This indicates that the length of the fibre bundle prior to contraction at each length step was similar with and without the compliant tendon attached. Additional series compliance reduced the active force and FTI of twitch and doublet contractions ($P<0.01$) at all fibre bundle initial lengths. Twitch active force and FTI were reduced by 54–63% and 53–73%, respectively, depending on the initial length of the fibre bundle (Fig. 3A,C). Doublet active force and FTI were reduced by 60–68% and 58–75%, respectively, depending on the initial length of the fibre bundle (Fig. 3B,D).
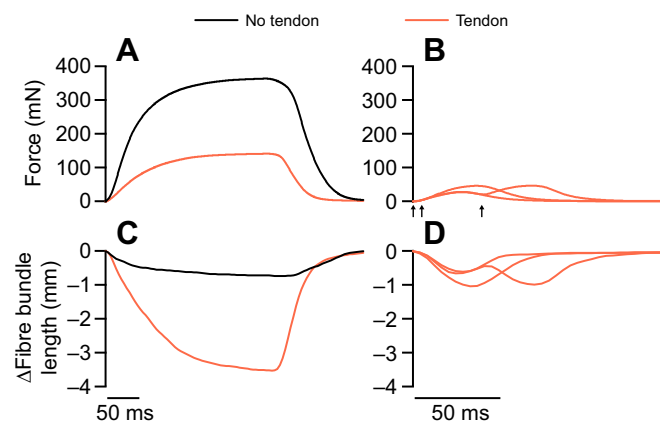
Fig. 1. Typical time course of force production and shortening during fixed-end contractions of a cane toad peroneus muscle fibre bundle with and without a compliant artificial tendon attached in-series. Additional series compliance led to a reduction in tetanic force (A) and a substantial increase in the amplitude and rate of active shortening (C). Active shortening during force generation owing to twitch and doublet stimulation (B) in the presence of additional series compliance was also of a considerable amplitude (D). Active shortening during a doublet was in fact of a greater amplitude than that permitted under maximal conditions in the absence of additional series compliance. Fibre bundle length changes during twitch and doublet contractions in the absence of additional compliance were not measurable and, therefore, were assumed to be negligible. Arrows in B denote timing of stimulation pulses. Doublet ISIs are 5 and 40 ms. Data are shown from the same muscle fibre bundle.

Twitch and doublet force responses were normalized to peak twitch force for the purpose of examining relative force summation and the time course of force rise and relaxation as a function of series compliance. Waveform-averaged normalized force responses are illustrated in Fig. 4, where the effect of additional series compliance on the relative magnitude and time course of the summated response is clearly visible. Most notably, the addition of a compliant artificial tendon reduced relative force summation at the shortest fibre bundle initial lengths and accelerated the decay of force at the longest fibre bundle initial lengths (Fig. 4A,C). There were main effects of series compliance and fibre bundle initial length on both relative force summation and the relative FTI (Fig. 5). The addition of a compliant tendon reduced both relative force summation (5 and 40 ms, $P<0.01$) and the relative FTI (5 and 40 ms, $P<0.01$). However, the effect of initial length on force summation and FTI changed as a function of ISI. With respect to an ISI of 5 ms, both force summation ($P<0.01$) and FTI ($P<0.01$) decreased as a function of increasing fibre bundle initial length (Fig. 5A,C). Conversely, force summation increased as a function of initial
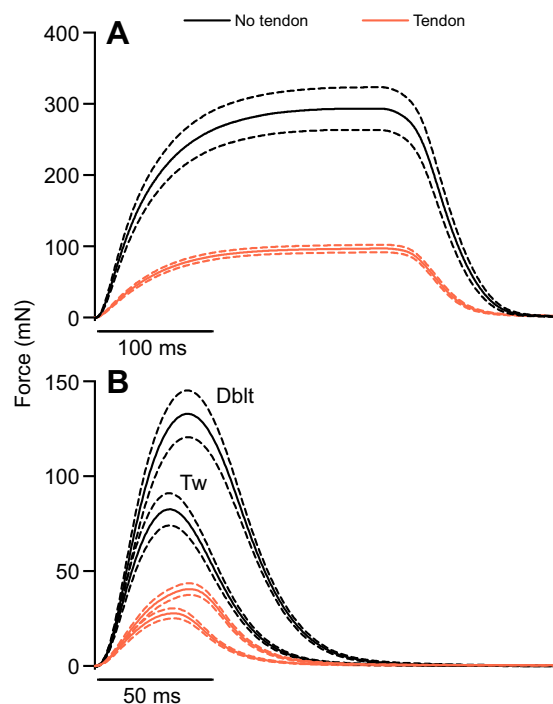

Fig. 2. Tetanic, doublet and twitch force during fixed-end contractions with and without a compliant artificial tendon attached in-series. Additional series compliance considerably reduced tetanic (A), doublet (Dblt; B) and twitch (Tw; B) force. Data are waveform averages (solid) and the s.e.m. (dashed) of all fibre bundle data (N=15). Tetanic stimulation was for 240 ms at 100 Hz. Doublet stimulation ISI is 5 ms.
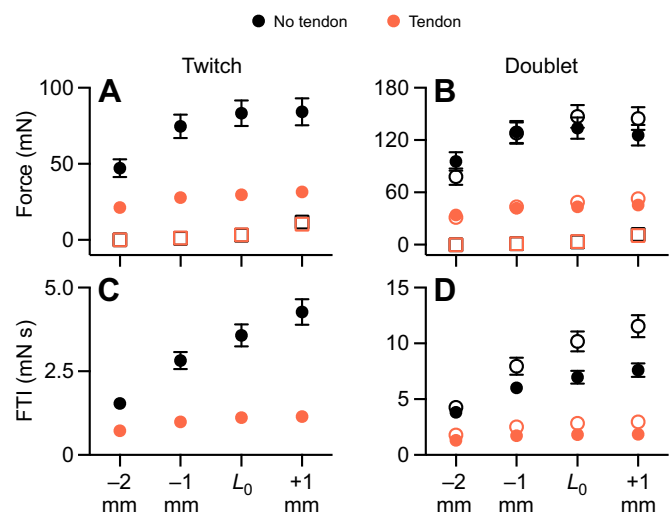

Fig. 3. Twitch and doublet active force and force–time integral (FTI) as a function of fibre bundle initial length for fixed-end contractions with and without a compliant artificial tendon attached in-series. Passive tension at rest (open squares) did not differ between conditions at each length step ($P=0.55$), indicating similar fibre bundle lengths prior to contraction. Additional series compliance considerably reduced twitch and doublet active force (A,B) and FTI (C,D) at all fibre bundle initial lengths ($P<0.01$). Doublet data are for ISIs of 5 ms (solid circles) and 40 ms (open circles). Data are means±s.e.m., N=15. A two-way repeated-measures ANOVA (Tukey post hoc test) was used for determination of statistical significance.
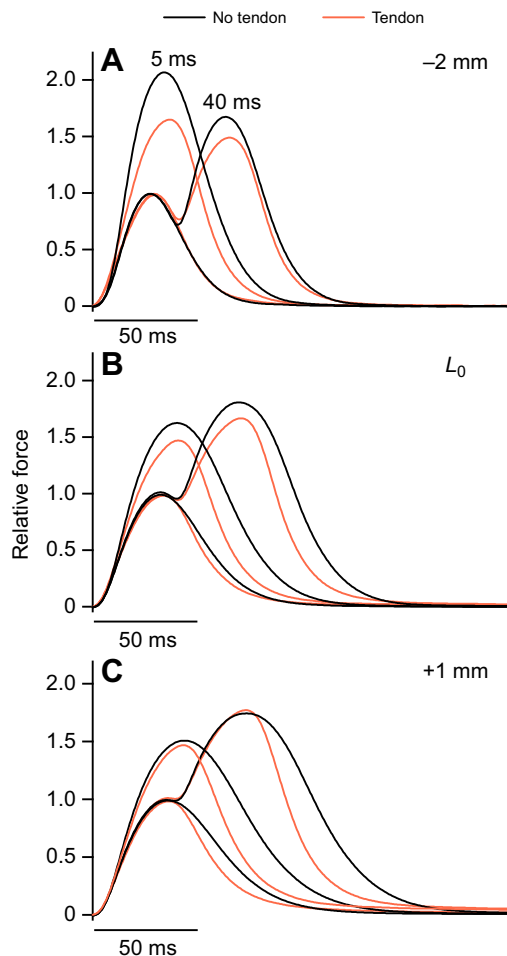
Fig. 4. Relative force summation for fixed-end contractions with and without a compliant artificial tendon attached in-series. Twitch and doublet force at $L_{0-2\,mm}$ (A), L_0 (B) and $L_{0+1\,mm}$ (C) are expressed relative to peak twitch force at the same fibre bundle initial length for compliant and stiff conditions. Additional series compliance reduced relative force summation at fibre bundle initial lengths between $L_{0-2\,mm}$ and L_0. Additional series compliance also tended to prolong the rise time of force and accelerate the time course of force relaxation. Data are waveform averages of all fibre bundle data (N=15).
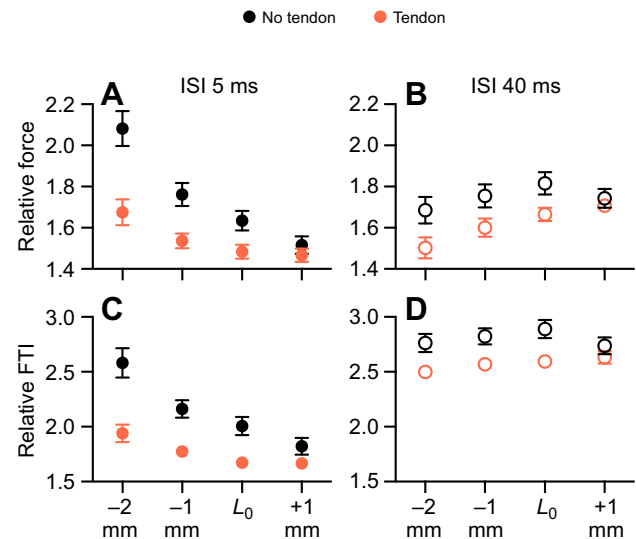

Fig. 5. Relative force summation and relative FTI as a function of fibre bundle initial length for fixed-end contractions with and without a compliant artificial tendon attached in-series. Additional series compliance reduced relative force summation (A,B) and relative FTI (C,D) (P<0.01) in a length-dependent manner. There was a main effect of fibre bundle initial length for both measures (P<0.01). The direction of the length-dependent response was a function of ISI. Peak doublet force and FTI were normalized to corresponding values of the twitch response at the same fibre bundle initial length. Data are means±s.e.m., N=15. A two-way repeated-measures ANOVA (Tukey *post hoc* test) was used for determination of statistical significance.

length for twitch and doublet (5 ms) contractions (tendon compliance×fibre bundle initial length interaction, P<0.01), and was almost absent at a $L_{0+1\,mm}$ (Fig. 6A,B).

length for an ISI of 40 ms (P<0.01; Fig. 5B). There was no main effect of initial length on relative FTI for an ISI of 40 ms (P=0.07; Fig. 5D). The force-limiting effect of additional series compliance was greatest at short fibre bundle initial lengths for both relative force summation (tendon compliance×fibre bundle initial length interaction: 5 and 40 ms, P<0.01) and relative FTI (tendon compliance×fibre bundle initial length interaction: 5 ms, P<0.01; 40 ms, P<0.05).

Additional series compliance altered the time course of force development and relaxation for twitch and doublet contractions (Fig. 6). The size of the effect was dependent on fibre bundle initial length. There were main effects of both series compliance and fibre bundle initial length on contraction time and half-relaxation time. The addition of a compliant tendon increased twitch and doublet contraction time by 3–9% (P<0.01) and 1–12% (5 and 40 ms, P<0.01), respectively, depending on the initial length of the fibre bundle (Fig. 6A,B). Contraction time also increased as a function of increasing initial length for twitch (P<0.01) and doublet contractions (5 and 40 ms, P<0.01; Fig. 6A,B). The size of the series compliance effect decreased as a function of increasing initial
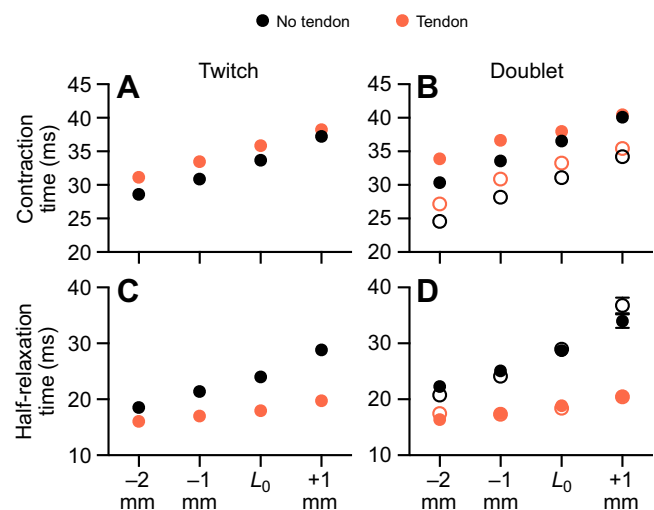

Fig. 6. Contraction time and half-relaxation time of fixed-end contractions with and without a compliant artificial tendon attached in-series. Additional series compliance prolonged the contraction time (A,B) and reduced the half-relaxation time (C,D) of twitch and doublet contractions (P<0.01) in a length-dependent manner. The effect of additional series compliance was considerably larger for the half-relaxation time. Both measures increased as a function of fibre bundle initial length (P<0.01); however, they were less sensitive to changes in fibre bundle initial length in the presence of additional series compliance. Doublet data are for ISIs of 5 ms (solid symbols) and 40 ms (open symbols). Data are means±s.e.m., N=15. A two-way repeated-measures ANOVA (Tukey *post hoc* test) was used for determination of statistical significance.

The effect of additional series compliance on the time course of force rise was modest with respect to the accelerative effect of compliance on force relaxation. The addition of a compliant tendon reduced twitch and doublet half-relaxation time by 13–32% ($P<0.01$) and 16–44% (5 and 40 ms, $P<0.01$), respectively, depending on the initial length of the fibre bundle (Fig. 6C,D). Half-relaxation time increased as a function of increasing length for twitch ($P<0.01$) and doublet contractions (5 and 40 ms, $P<0.01$; Fig. 6C,D). The size of the series compliance effect increased as a function of fibre bundle initial length for twitch and doublet (5 and 40 ms) contractions (tendon compliance×fibre bundle initial length interaction, $P<0.01$). However, in contrast to the effect of additional series compliance on contraction time, half-relaxation time was reduced by a considerable amount at all fibre bundle initial lengths.

DISCUSSION

In this study, a compliant artificial tendon was attached in-series to an isolated muscle preparation as a means of investigating the effects of series elastic compliance on force summation. In addition to substantially constraining muscle force generation, active shortening of the internal contractile apparatus afforded by additional series compliance reduced doublet force summation and altered the time course of force rise and relaxation. Doublet force and doublet FTI expressed relative to the twitch were lower when a muscle fibre bundle was serially attached to a compliant artificial tendon. Additional series compliance also brought about an increase in the rise time of twitch and doublet force, and accelerated the time course of force decay. The reduced contractile output of a muscle fibre bundle in-series with a compliant SEE can be understood in terms of the dynamic factors that influence force generation in skeletal muscle. The same factors that constrain contractile apparatus force output likely contribute to limiting force summation under conditions where active shortening against stretch of the SEE is substantial.

A compliant artificial tendon reduced the peak force and FTI of all contraction types to one-third of that generated under comparatively stiff conditions. A loss in force-generating potential of this magnitude is predictable when the effects of series elastic compliance on muscle fibre dynamics are considered. Muscle fibre bundle shortening against the extension of the compliant artificial tendon was measured to be 22% of resting bundle length during a fixed-end tetanic contraction at L_0 in the present study. Thus, the addition of a compliant tendon allowed for fibre bundles to shorten onto the ascending limb of the force–length curve, where force generation is reduced (Gordon et al., 1966). The absence of an appreciable increase in twitch and doublet force in response to step increases in initial length suggests that the artificial tendon was of a sufficient compliance to permit fibre bundles to shorten onto a similar low-force region of the ascending limb. This assertion is in agreement with models of muscle–tendon interaction, which predict that the force–length relationship should shift rightward and become skewed toward initial sarcomere lengths on the ascending limb as series elastic compliance increases (Lieber et al., 1992). The plantaris muscle of the bullfrog has a high fixed-end compliance (~30%; Sawicki et al., 2015b), but avoids operating at fibre lengths on the ascending limb during the take-off phase of jumping by beginning at lengths on the descending limb. Force attenuation in this muscle is mitigated by actively shortening from lengths beyond optimum length onto the plateau region prior to take-off (Azizi and Roberts, 2010).

The addition of a compliant tendon reduced the relative amplitude and relative FTI of the doublet response for both ISIs employed in

the present study. Altered force summation in the presence of additional series compliance is likely to be due to, at least in part, considerable muscle fibre shortening during the rise of force. Though shortening velocity was not measured in the present study, a higher velocity of shortening can be inferred from the larger amplitude of shortening permitted in the presence of additional series compliance. High velocities of shortening under constant-length conditions, and in response to high-frequency doublet stimulation, have previously been demonstrated in vivo for a muscle group with a compliant tendon (Mayfield et al., 2015). A significant reduction in the rate of force development under the compliant conditions imposed in the present study suggests that the fibre bundles did in fact operate at velocities at which force production is considerably limited (Hill, 1938). When a second pulse is delivered during the early rise in twitch force, before or immediately after the peak in fibre shortening velocity, a higher peak velocity of shortening is achieved. For instance, individual muscles of the human triceps surae achieve peak velocities during a high-frequency doublet that are 50% higher than during a twitch (Mayfield et al., 2015). We argue that the higher velocity of active shortening afforded by the compliant tendon was sufficient to cause a considerable reduction in the force-generating capacity of the contractile apparatus during force development, such that the relative force contribution of a second stimulating pulse was reduced with respect to stiffer conditions. Twitch force has been shown to increase significantly in response to a modest reduction in the velocity of shortening induced by an increase in SEE effective stiffness (Mayfield et al., 2016). Sandercock (2000) also concluded that a higher velocity of active shortening during force development could likely explain the reduction in force summation demonstrated when individual parts of whole muscle were activated simultaneously rather than individually, arguing that a common SEE permitted greater internal shortening during synchronous activity.

Additional series elastic compliance also affected the operating lengths of muscle fibre bundles during activation owing to the first and second stimulating pulses. Greater amplitudes of active shortening meant that muscle fibre bundle lengths at the onset of the second pulse were shorter and less favourable for force production. Additional shortening that occurs as force rises in response to a second pulse is also considerable under compliant conditions (Mayfield et al., 2015). Therefore, fibres in the compliant condition were allowed to continue to move down the ascending limb of the force–length curve, further limiting the force-generating potential of the second stimulus. We believe that the reduction in relative force summation in the compliant condition likely reflects a shortening-induced reduction in force-generating capacity, as explained by the force–length and force–velocity relationships. History-dependent contractile properties, specifically those concerned with actin–myosin interaction subsequent to active shortening (Edman, 1975; Herzog and Leonard, 1997), may also contribute to a progressive loss in force-generating potential.

When values of force summation reported in the literature for animal (Ranatunga, 1977; Wallinga-de Jonge et al., 1980; Parmiggiani and Stein, 1981) and, specifically, human muscles (Duchateau and Hainaut, 1986; Baudry et al., 2005) are considered, the reduction in relative force summation owing to additional series elastic compliance was small. This finding may be due, in part, to the animal model selected. In agreement with previous work on frog muscle at 22°C (Ranatunga, 1977), relative force summation in response to high-frequency doublet stimulation in the absence of additional series compliance was low relative to mammalian muscle (Parmiggiani and

Stein, 1981; Duchateau and Hainaut, 1986) and did not exceed that predicted from linear summation of the twitch, except at a short fibre bundle length. Therefore, the potential for internal shortening afforded by additional compliance to reduce relative force summation was likely low. Considerably larger twitch-to-tetanus ratios have also been reported for amphibian muscle (see Close, 1965), even at temperatures exceeding physiological norms (Ranatunga, 1977). This suggests that, relative to a twitch, there is a low capacity for a second stimulus to bring about a significant increase in mechanical activation in amphibian muscle. By contrast, the relative force contribution of a second stimulus in mammalian muscle can be two to three times larger in amplitude than that of the twitch and be of an augmented speed and duration (Parmiggiani and Stein, 1981; Duchateau and Hainaut, 1986; Baudry et al., 2005), giving rise to a summated response three to four times larger than the twitch.

Mammalian muscle may have been more suitable for this investigation on the basis that there are elements of excitation–contraction coupling that clearly operate in a nonlinear manner to promote force generation when excitation occurs in quick succession. High series compliance may have additional consequences for force summation in mammalian muscle if shortening of the internal contractile apparatus were to interfere with one or more of these processes (e.g. thin filament activation; Edman, 1975; Gordon et al., 2000). Differences in excitation–contraction coupling may reflect the different lifestyles of various taxa. Ca^{2+} release and reuptake kinetics, and Ca^{2+} regulation of thin filament activation likely play crucial roles in determining force summation. Inactivation of Ca^{2+} release, whereby less Ca^{2+} is released from the sarcoplasmic reticulum in response to a second action potential when it immediately follows an initial action potential, is indeed a property that differs between amphibian and mammalian muscles (Caputo et al., 2004). Amphibian and mammalian muscle also have distinct sarcoplasmic reticulum ryanodine receptor (Ca^{2+} release channel) isoforms, Ca^{2+} release channel structural configuration at the site of Ca^{2+} release, and arrangement of the junctional membranes (Jayasinghe and Launikonis, 2013), which position the ryanodine receptors relative to the contractile apparatus (see Hollingworth and Baylor, 2013).

Additional series compliance induced a modest increase in the rise time of twitch and doublet force. The time to peak twitch tension has been shown previously to become systematically delayed as the compliance of an external spring is increased (Hill, 1951; Bawa et al., 1976). It has been suggested that increasing the series elastic compliance increases the length and duration for which the contractile apparatus must shorten before force equilibrium is reached (Hill, 1951). The delay in peak force induced by additional series compliance in the present study was small with respect to that observed for frog sartorius at 0°C (Hill, 1951), but similar to that observed for cat plantaris at a physiological temperature and when additional series compliance reduced twitch force by a similar extent (∼70%; Bawa et al., 1976). Some of this variation may be explained by the thermal (Bennett, 1984; Stephenson and Williams, 1985), fibre-type (Stephenson and Williams, 1981, 1985) and length dependence (Stephenson and Williams, 1982) of numerous processes in the series of events leading to contraction and relaxation. It also remains unclear what influence active shortening has on the Ca^{2+} sensitivity of the contractile apparatus (Gordon et al., 2000), which increases as a function of sarcomere length (Stephenson and Williams, 1982) when examined under purely isometric conditions.

Half-relaxation time was reduced considerably in the presence of additional series compliance, especially at longer initial fibre bundle lengths. Relative to the rise time of force in the presence of additional series elastic compliance, half-relaxation time was also less sensitive to step increases in initial fibre bundle length. The decay of twitch force has been shown previously to be more sensitive to initial muscle length than that of the rise time of force (Close, 1964; Wallinga-de Jonge et al., 1980). We suggest that the amplitude of active shortening permitted under the compliant conditions in the present study may have limited the influence of initial length on relaxation kinetics. Assuming on the basis of the force–length data obtained in the compliant condition that fibre bundles shortened onto a similar region of the ascending limb, differences in active fibre bundle length were likely less than the differences in initial length caused by the lengthening steps. Similar active lengths could explain the depressed effect of initial length on half-relaxation time. This conclusion is supported by evidence demonstrating the sensitivity of half-relaxation time to initial length to be lower at lengths below L_0 (Jewell and Wilkie, 1960; Close, 1964) and our own finding that the time course of force decay was abbreviated more for doublet stimulation, which induced additional shortening. In agreement with the literature, half-relaxation time was more sensitive to initial fibre bundle length (52–56% increase) than was force rise time (30–32% increase) when the compliant tendon was absent. Bawa and colleagues (1976) reported a decrease in twitch half-relaxation time in response to an increase in spring compliance for cat soleus, but found half-relaxation time to increase for plantaris under similar conditions. Fibre type composition and the length range over which the muscle is tested could potentially modify the effect that series compliance has on force rise and relaxation by influencing the Ca^{2+} sensitivity of the contractile apparatus.

A greater relative rate of force relaxation in the presence of additional series elastic compliance may also reflect the reduced ability of strongly bound cross-bridges to resist the lengthening imposed upon them by the recoil of the elastic artificial tendon at the onset of relaxation. It has been argued that as sarcomere length non-uniformities arise at the onset of contractile apparatus deactivation (Edman and Flitney, 1982), the capacity for strongly attached cross-bridges to avoid further slippage or 'give' rapidly declines (Caputo et al., 1994). Length perturbations imposed during the early phase of force relaxation have been demonstrated to accelerate the transition between slow and fast relaxation phases and abbreviate the decay of active tension (Caputo et al., 1994; Tesi et al., 2002). The strain dependence of myosin detachment kinetics has recently been simulated in a cross-bridge model (Campbell, 2016). The same model showed that actin–myosin movement during relaxation permitted by series elastic compliance accelerated the time course of force decay (Campbell, 2016). A transient increase in the myoplasmic Ca^{2+} concentration subsequent to a brief stretch delivered at relaxation onset, relative to an isometric control (Caputo et al., 1994; Wang and Kerrick, 2002), suggests that the dissociation of force-generating cross-bridges may also mobilize Ca^{2+} from the thin filament, which could act to further accelerate the decay of force (Caputo et al., 1994).

Our findings indicate that high series elastic compliance limits force summation and accelerates relaxation when MTU length is held constant. On this basis it might be argued that a compliant tendon would be detrimental to muscular performance during maximal efforts, for example, during jumping in anuran amphibians, given that this form of locomotion requires the mechanical behaviour of several lower limb muscles to be maximal to rapidly extend the hind limb (Peplowski and Marsh, 1997; Roberts and Marsh, 2003). However, by allowing contractile element length changes to be decoupled from MTU length changes,

series elastic compliance, in fact, plays an important role in amplifying mechanical power, especially during jumping in frogs (Astley and Roberts, 2012, 2014). Series compliance provides a means for a catapult mechanism of power production, that is, strain energy can be stored in the SEE via muscle shortening during the loading phase and released at a rapid rate when the SEE recoils during forward propulsion (Astley and Roberts, 2012; Sawicki et al., 2015b). An elastic mechanism of this form enables MTU power generated during jumping to be amplified with respect to the capacity of the muscle (Peplowski and Marsh, 1997; Navas et al., 1999). Power amplification is reliant on dynamic processes generating moments that oppose joint excursion attempted by the muscle as it generates force and actively stretches the SEE, storing elastic energy (Astley and Roberts, 2014). Though an elastic tendon is imperative for power amplification, large-amplitude active shortening similar to that permitted by the compliant artificial tendon may render the muscle incapable of generating forces sufficient for jumping. The tendons of some anuran hind limb extensors are in fact quite stiff (Lieber et al., 1991; Trestik and Lieber, 1993) with respect to a number of distal muscles in humans and animals that participate in stretch–shorten cycles during locomotor tasks (Morgan et al., 1978; Griffiths, 1991; Narici et al., 1996). However, force loss associated with high series compliance can be compensated for by operating initially at fibre lengths on the descending limb, as has been shown for the bullfrog plantaris, which is likely permitted to achieve long starting lengths by possessing relatively compliant passive properties (Azizi and Roberts, 2010).

High series elastic compliance may be more desirable in species in which the MTU is actively stretched before shortening. A compliant tendon can permit near-isometric muscle fibre dynamics during stretch of the MTU, allowing muscle to function as a rigid force-producing strut (Biewener et al., 1981; Roberts et al., 1997). Energy stored in the stretched tendon can then be returned to the skeletal system when the tendon recoils during limb propulsion (Alexander and Vernon, 1975; Roberts et al., 1997). Strut-like muscle behaviour and cycling of elastic energy have been demonstrated clearly in the gastrocnemius and plantaris of tammar wallabies during hopping (Biewener et al., 1998), the gastrocnemius of turkeys during running (Roberts et al., 1997), and the gastrocnemius (Lichtwark et al., 2007; Farris and Sawicki, 2012) and soleus (Lai et al., 2015) of humans during walking and running. However, muscles with high series compliance are not confined to economic force production and elastic energy savings. *In vitro* studies of cyclical MTU behaviour indicate that the timing of activation can be modulated to achieve brake- or motor-like muscle performance (Sawicki et al., 2015a). Naturally occurring bursts of high-frequency motor unit activity, which can resemble doublet stimulation, are thought to be crucial to rapid force development (Duchateau and Baudry, 2014). High-frequency motor unit doublet discharges have been recorded at the onset of ballistic efforts (Van Cutsem et al., 1998) and fast corrective movements (Grimby, 1984), and during locomotor activity (Hennig and Lømo, 1985). Force summation in a compliant MTU owing to doublet stimulation or a burst of pulses may be more pronounced and bring about higher rates of force development if activation were to be coupled with stretch of the MTU, which would act to restrict active shortening relative to constant-length conditions.

In conclusion, force summation during a fixed-end contraction is reduced in the presence of a compliant artificial tendon designed to permit significant internal shortening. Shorter operating lengths and higher velocities of shortening relative to comparatively stiff conditions are believed to lead to a progressive reduction in relative force-generating capacity, as predicted by the force–length and force–velocity properties of the contractile apparatus. Active shortening afforded by a compliant tendon might also accelerate the decay of force. Though these findings demonstrate that high series compliance limits rapid force development under constant-length conditions, muscles with compliant tendons generate large forces economically during locomotor tasks owing to stretch–shorten cycles that allow for minimal internal shortening.

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