

RESEARCH ARTICLE

Effects of series elastic compliance on muscle force summation and the rate of force rise

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ABSTRACT

Compliant tendons permit mechanically unfavourable fascicle dynamics during fixed-end contractions. The purpose of this study was to reduce the effective compliance of tendon and investigate how small reductions in active shortening affect twitch kinetics and contractile performance in response to a second stimulus. The series elastic element (SEE) of the human triceps surae ($N=15$) was effectively stiffened by applying a 55 ms rotation to the ankle, through a range of 5 deg, at the onset of twitch and doublet [interstimulus interval (ISI) of 80 ms] stimulation. Ultrasonography was employed to quantify lateral gastrocnemius and soleus fascicle lengths. Rotation increased twitch torque (40–75%), rate of torque development (RTD; 124–154%) and torque-time integral (TTI; 70–110%) relative to constant-length contractions at the initial and final joint positions, yet caused only modest reductions in shortening amplitude and velocity. The torque contribution of the second pulse increased when stimulation was preceded by rotation, a finding unable to be explained on the basis of fascicle length or SEE stiffness during contraction post-rotation. A further increase in torque contribution was not demonstrated, nor was an increase in doublet TTI, when the second pulse was delivered during rotation and shortly after the initial pulse (ISI of 10 ms). The depressant effect of active shortening on subsequent torque generation suggests that compliant tendons, by affording large length changes, may limit torque summation. Our findings indicate that changes in tendon compliance shown to occur in response to resistance training or unloading are likely sufficient to considerably alter contractile performance, particularly maximal RTD.

KEY WORDS: Twitch, Triceps surae, Active shortening, History-dependent properties, Rate of force development, Tendon stiffness

INTRODUCTION

Contractile force generated by skeletal muscle in response to two closely spaced electrical impulses often exceeds that which is predicted from the linear summation of twitch force (Ranatunga, 1977; Duchateau and Hainaut, 1986a). Relative to the twitch, the force contribution owing to the second stimulus often demonstrates an augmented rise time, peak force, force–time integral and rate of force rise (Parmiggiani and Stein, 1981; Duchateau and Hainaut, 1986a). From a mechanical perspective, this mode of activation is an effective means of rapidly increasing muscle output. High-frequency single motor unit discharges resembling doublet or burst stimulation

have been shown to be present prior to or at the onset of force rise during voluntary efforts involving a ballistic contraction (Desmedt and Godaux, 1978; Van Cutsem et al., 1998) or fast corrective movement (Grimby, 1984; Hennig and Lomo, 1985). In some instances, initial discharge frequencies are higher than that necessary to achieve maximum tetanic force (Marsh et al., 1981; Van Cutsem et al., 1998), suggesting that a maximal rate of force development may be desired (Buller and Lewis, 1965; Van Cutsem et al., 1998). If indeed high-frequency neural activity stems from the effects of strong excitatory inputs necessary during ballistic contractions (Duchateau and Baudry, 2014), rapid force summation may play a crucial role in such movements.

Both physiological and mechanical mechanisms may contribute to non-linear force summation. Upon excitation of skeletal muscle, the contractile apparatus is brought into an active state through the binding of Ca^{2+} ions to regulatory proteins on the thin filament. The activation dynamics of muscle, especially the kinetics of sarcoplasmic Ca^{2+} release and reuptake (Duchateau and Hainaut, 1986b; Barclay, 2012), are likely important determinants of force generation in response to consecutive stimuli. Indeed, doublet force summation is influenced by factors that alter the Ca^{2+} sensitivity of the contractile apparatus (Stephenson and Williams, 1982, 1985; Sweeney and Stull, 1990), including muscle length (Wallinga-de Jonge et al., 1980; Mela et al., 2002), muscle temperature (Ranatunga, 1977) and post-activation potentiation (Baudry et al., 2005). These same factors have been shown to also influence the twitch:tetanus ratio (Ranatunga, 1977; Stein and Parmiggiani, 1981; Moore and Stull, 1984), which appears to be inversely related to doublet force summation (Duchateau and Hainaut, 1986a). Differences in doublet force summation between fast and slow twitch muscle (Ranatunga, 1977; Wallinga-de Jonge et al., 1980) may be well explained by fibre-type-specific sarcoplasmic reticulum Ca^{2+} kinetics (Baylor and Hollingworth, 2003; Barclay, 2012) and contractile apparatus Ca^{2+} sensitivity (Stephenson and Williams, 1985).

The nonlinear load-extension property of the series elastic element (SEE) and the requirement to take up slack in the SEE prior to the transmission of active tension (Parmiggiani and Stein, 1981) have been cited as mechanical factors that likely influence doublet force summation. Muscle length may also be of consequence to force summation for reasons that extend beyond myofibrillar overlap and contractile apparatus Ca^{2+} sensitivity. Recent evidence suggests that the dependence of the force–length relationship on muscle activation level is partly due to differences in the amount of internal work that must be performed on passive elements for effective force transmission at different muscle lengths (Holt and Azizi, 2014).

We recently conducted work in a muscle group that does not demonstrate greater than linear force summation under constant-length conditions and suggested that muscle–tendon interaction may have additional implications for force summation (Mayfield

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List of symbols and abbreviations

C ₂	torque contribution of second stimulation pulse
ISI	interstimulus interval
LG	lateral gastrocnemius
L _o	optimal fascicle length for force generation
MTU	muscle–tendon unit
PF	plantar flexion
ROT	rotation
RTD	rate of torque development
SEE	series elastic element
SOL	soleus
TTI	torque–time integral
US	ultrasound

et al., 2015). Force summation in response to doublet stimulation was shown to be comparatively low for the human triceps surae, a group of muscles arranged in-series with a long, elastic tendinous element (i.e. Achilles tendon). Active shortening of both the lateral gastrocnemius (LG) and the soleus (SOL) against the extension of the SEE was substantial during a twitch and increased considerably with doublet stimulation, irrespective of the interval between stimuli (5–100 ms). We concluded that force summation was limited, at least in part, by high series elastic compliance affording significant internal shortening and, therefore, a progressive loss in force-generating capacity according to the known force–length (Gordon et al., 1966) and force–velocity (Fenn and Marsh, 1935) properties of muscle. A similar reduction in force summation has been demonstrated during tetanic contractions when individual parts of whole muscle are stimulated simultaneously, rather than individually, and is likely owing to the common in-series elastic compliance allowing a higher amplitude and velocity of shortening (Sandercock, 2000; Perreault et al., 2003). Despite numerous examples of human and animal lower limb muscles experiencing significant internal shortening under constant-length conditions (see Roberts, 2002), the role the history dependence of force generation (Abbott and Aubert, 1952) plays in reduced force summation has received relatively little attention. Active shortening has a depressant effect on force generation (Edman, 1975; Maréchal and Plaghki, 1979; Herzog and Leonard, 1997) that may contribute to limiting the force-generating potential of stimuli within a burst.

Although the activation dynamics of a muscle are expected to largely explain force summation, the relative importance of prior shortening and instantaneous fibre length and velocity for force development remains unclear. If high-frequency motor unit discharges are assumed to be driving the rapid rise of force underpinning ballistic contractions, high series elastic compliance may limit performance on the basis that larger and more rapid changes in fibre length will be permitted. A more definitive means of determining the influence of active shortening on force summation would be to experimentally alter the compliance of the SEE.

While it is very difficult to non-invasively change the compliance of the tendinous structures that make up the SEE, one can simulate a SEE of lower compliance by applying a stretch to the muscle–tendon unit (MTU) during activation to restrict active shortening against the extension of the SEE. A. V. Hill (1949) demonstrated that the series elastic compliance of frog sartorius could be effectively abolished during a twitch by imposing a very brief, rapid stretch immediately after stimulation. Hill reduced the influence of in-series compliance to such an extent that twitch force increased very abruptly to a near-tetanic level. In these

experiments, the fibres of frog sartorius were likely contracting relatively isometrically, or potentially with some degree of active stretch, as the stretch amplitude imposed (10–15% of muscle length) likely exceeded the fixed-end compliance of the parallel fibred muscle (Jewell and Wilkie, 1958). By comparison, the ankle extensors of humans (Fukunaga et al., 2001; Lichtwark and Wilson, 2005), and of some other animals (Griffiths, 1991; Roberts, 2002), have a long external tendon that acts to buffer muscle fibre length changes when the whole muscle is stretched. Stretch of such muscles is expected to impose much more subtle reductions in compliance, and therefore fibre shortening, which may not have a significant impact on the force generated in response to a twitch or successive stimuli.

The present study set out to simulate a state of reduced SEE compliance according to an *in vivo* implementation of the paradigm employed by Hill (1949), but in a muscle with high series compliance such that the increase in effective stiffness induced by stretch would be more modest. We aimed first to determine the extent to which the rate of force development during a twitch is influenced by subtle changes in active shortening induced by an increase in SEE effective stiffness. Second, we aimed to determine whether force summation is sensitive to changes in active shortening that occur during the preceding twitch as a result of a transient increase in SEE effective stiffness. A brief stretch was applied to the MTUs of the human triceps surae at the onset of a twitch that induced only modest reductions in active fascicle behaviour relative to constant-length conditions. A second stimulus was applied immediately after the stretch. We hypothesized that small changes in active shortening resulting from an increase in SEE effective stiffness would substantially increase twitch force because of reductions in fibre shortening velocity. We also hypothesized that a reduction in active shortening during the twitch would increase the absolute force contribution of the second stimulus owing to the history dependence of force generation.

MATERIALS AND METHODS**Ethical approval**

The experimental protocols were approved in accordance with the ethical review guidelines and processes of the Human Ethics Committee of The University of Queensland. The ethical guidelines set out by the Declaration of Helsinki were adhered to and written informed consent was obtained from all subjects.

Subjects

Fifteen male subjects (mean±s.d.; age: 26±3 years; body mass: 84±14 kg; height: 183±7 cm) free of neuromuscular injury or impairment participated in this investigation.

Experimental protocol

Participants lay prone on a padded bench with knees completely extended. The right foot was secured to the footplate of a custom-built microprocessor-controlled torque motor and the ankle joint was aligned with the axis of rotation of the dynamometer. Supramaximal electrical stimuli were delivered percutaneously to the tibial nerve over the popliteal fossa and the resulting plantar flexion torque was measured. The change in active length of LG and SOL fascicles was determined from ultrasound (US) imaging. Constant-length reference contractions, whereby MTU length remained fixed, were performed with the ankle in 5 deg of plantar flexion (5 deg PF) relative to a neutral position (0 deg; tibia 90 deg to sole of foot). The effective stiffness of the SEE was increased by imposing a rapid, brief, 5 deg dorsiflexion rotation at the onset of

electrical stimulation. Ankle rotation functioned to stretch the active MTU and, therefore, performed mechanical work on the SEE, which in turn provided additional resistance to active shortening. The fascicle behaviour permitted during rotation reflected that expected for a muscle in-series with a less compliant tendon, where active shortening at the expense of SEE lengthening is less pronounced.

The imposed rotation was 55 ms in duration and typically achieved a peak velocity of 250 deg s⁻¹. The delay between stimulation and footplate rotation was designed such that stretch of the MTU resisted active fascicle shortening, but did not cause active fascicle lengthening. Specifically, rotation was programmed to begin immediately after the onset of active fascicle shortening. An appropriate delay, as confirmed by a reduction in fascicle shortening soon after stimulation and an absence of any fascicle stretch, was determined from US data collected during pilot experiments. LabVIEW software (National Instruments, Austin, TX, USA) and a custom-written control program were implemented for precise control of footplate rotation with respect to the timing of electrical stimulation (Fig. 1). Ankle position for the rotation and constant-length conditions is illustrated in Fig. 1C.

By increasing the effective stiffness of the SEE and, therefore, reducing active fascicle shortening, LG and SOL fascicles operated at longer lengths during rotation contractions than during the constant-length reference contractions performed at the initial joint position (5 deg PF). On this basis it was assumed that a component of the plantar flexion torque produced during rotation contractions would be due to a rightward shift of fascicle length on the force-length curve, and thus, an improvement in myofilament overlap. To approximate the contribution of the force-length property alone, a constant-length reference contraction was performed at 0 deg, the post-rotation joint position (see Fig. 1). This measure was

particularly important for assessing the torque contribution owing to the second pulse, which was delivered at 0 deg, and thus at a longer MTU length (see below). As the rotation was designed to limit active shortening without causing active lengthening, the actual increase in active fascicle length associated with stiffening of the SEE was considerably less than the difference in active length between the two constant-length conditions. Therefore, this method quantifies the upper limit of contribution from the force-length relationship.

A second stimulation pulse was delivered after the rotation had been completed (i.e. in the absence of altered compliance), and during the constant-length contractions at 5 deg PF and 0 deg after the same time interval, to ascertain whether the fascicle mechanics preceding a stimulus influence contractile performance. An interstimulus interval (ISI) of 80 ms was implemented for this purpose. Activating the muscle after rotation allowed for any changes in the torque contribution and fascicle behaviour in response to the second pulse, relative to the constant-length conditions, to be attributed to differences in the mechanics of the initial contraction. The torque contributed by the second pulse to the total response (C_2) was quantified by subtracting twitch torque from doublet torque, and compared between constant-length and rotation conditions. We presumed that SEE stiffness post-rotation would return to a level similar to that evident at the same joint position without prior rotation. An estimate of SEE stiffness post-rotation was used to verify this presumption (see Signal processing and analysis).

For a small subset of participants ($N=5$), doublet stimulation was also performed with an ISI of 10 ms to maximize fascicle shortening velocity. In these experiments, the second pulse was delivered during the stretch, with the aim of furthering our understanding of the effects of fascicle velocity on force summation.

Twitch and doublet contractions were performed twice for each of the three contraction conditions: constant-length contraction at 5 deg PF, constant-length contraction at 0 deg and rotation contraction. The ordering of conditions was randomized. The stimulation protocol was repeated for a second US transducer position (see Ultrasound measurements).

Peripheral nerve stimulation

A constant current electrical stimulator (DS7AH, Digitimer, Hertfordshire, UK) delivered a 500 μ s square pulse transcutaneously to the tibial nerve to evoke supramaximal twitches from the triceps surae. An anode (sensor area 80 mm²; Kendall, Mansfield, MA, USA) was positioned in the midline of the leg, 2 cm proximal to the horizontal skin crease overlying the popliteal fossa in the knee. A cathode of similar size and type was placed on the skin crease after locating the exact position of the tibial nerve using successive stimulations elicited via a custom probe. The stimulator current was increased in 5 mA increments until there was no further increase in twitch torque. The stimulator current was increased by a further 25% to ensure maximal activation throughout the duration of the investigation.

Ultrasound measurements

B-mode images were collected with a research US system (SonixMDP, Ultrasonix, Analogic, Peabody, MA, USA). Images of the LG and SOL were acquired separately at rates of 240 and 207 Hz, and at depths of 25 and 30 mm, respectively. A 60 mm linear transducer (L14-5W/60, Ultrasonix, Analogic) was positioned over the mid-belly of the LG and the orientation optimized according to the pennation of the muscle being imaged.

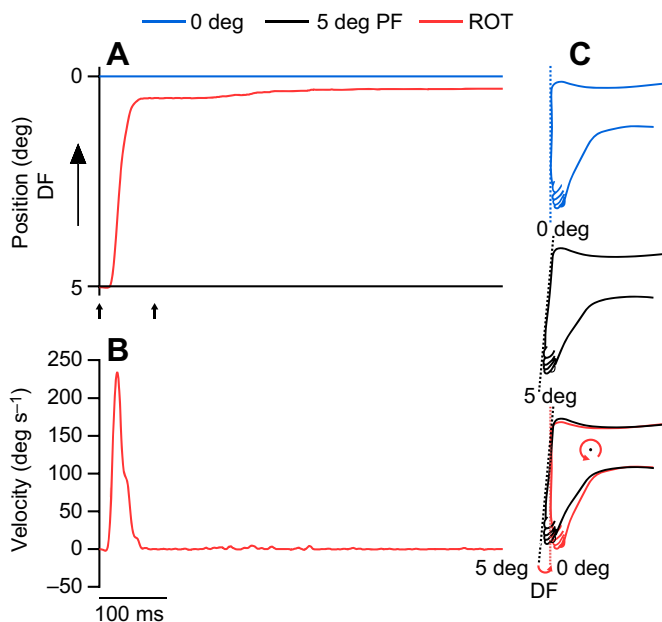


Fig. 1. Experimental protocol. The ankle was rapidly rotated from 5 deg PF to 0 deg at the onset of a twitch. A second pulse was delivered 80 ms after the initial pulse, once the dorsiflexion (DF) rotation was complete. Footplate position (A) and velocity (B) with respect to the timing of stimulation (vertical arrows) are shown for rotation contractions (ROT) and the constant-length contractions performed at the initial (5 deg PF) and final (0 deg) joint positions. (C) Schematic representation of joint position for each condition.

Because of the variable pennation of SOL fibres, an appropriate image of the SOL was obtained with the transducer positioned more distally or overlying the mid-belly of the medial gastrocnemius.

Instantaneous fascicle length was determined for each frame of US data through implementation of a custom-written, automatic tracking algorithm in MATLAB (MathWorks, Natick, MA, USA). The optical flow algorithm used to compute changes in fascicle length has been described in detail elsewhere (Cronin et al., 2011). Briefly, the tracking software allows the user to manually define a region of interest and fascicle endpoints. A fascicle that intersected the central area of the region of the interest was selected for analysis. If an endpoint(s) extended beyond the image border, the endpoint position was extrapolated according to the pennation of the fascicle and orientation of the aponeurosis. This process is performed for the initial frame only. For subsequent frames, a least squares fit of an affine transformation is applied to the optical flow within the designated region of interest, which is in turn used to compute fascicle endpoint displacements in a frame-by-frame fashion. Instantaneous fascicle velocity was obtained by differentiating the automatically tracked fascicle length data.

Signal processing and analysis

The torque signal was amplified (BKI-5, Nobel Elektronik, Karlskoga, Sweden) and then sampled and displayed at 2000 Hz using 12-bit Micro3 and Spike2 software (Cambridge Electronic Design, Cambridge, UK). Torque and fascicle data were imported into MATLAB to undergo further processing and for analysis. To correct for torque artefacts during the acceleration and deceleration phases of the rotation owing to the inertial properties of the footplate and lever arm of the dynamometer, and the participant's foot, unloaded rotations were performed with a model foot. External torque measured during the acceleration and deceleration phases of the simulated condition was subtracted from the net torque measured during experimental recordings to calculate the plantar flexion torque that is due to active and passive muscle forces. Undesired high-frequency components were removed from torque and fascicle data by a low-pass (30 Hz), second-order Butterworth filter (dual-pass to correct any time shift) implemented in a custom-written script.

Peak torque, torque–time integral (TTI) and peak rate of torque development (RTD) were determined for twitch and doublet contractions, and for the torque contribution of the second stimulation pulse. Torque was zeroed at contraction onset for measurements of active torque. TTI was defined as the area under the active torque–time trace from the onset of stimulation to the time when torque had decreased by 75% from its peak (75% relaxation). TTI was not quantified for the entire contractile response (i.e. to complete relaxation) because of the increase in passive tension at rest subsequent to stretch of the MTU. Similarly, the TTI of constant-length contractions performed at 0 deg was quantified from the active torque–time trace (i.e. zeroed torque) to limit the contribution of passive tension.

The degree to which stretch of the MTU induced changes in SEE stiffness was determined from differences in estimates of mean SEE stiffness during torque development. Mean SEE stiffness was calculated from the change in torque and the corresponding change in fascicle length. The latter was assumed to be proportional to the amplitude of lengthening experienced by the SEE. The estimate of mean stiffness for the rotation condition was based on the assumption that the MTU length was constant and, therefore, represents a measure of mean effective stiffness. Stretch of the MTU

simulated a state of higher SEE stiffness rather than modify the SEE at a structural or material level. Mean SEE stiffness during torque development owing to the second stimulus was also estimated to ascertain whether stiffness post-rotation had returned to a level similar to that evident during a constant-length contraction at the same joint position. This measure was calculated after quantifying the contribution of the second stimulation pulse to both total torque and total fascicle length change. Although a nonlinear toe region has been demonstrated for the load–extension curve of tendon at low forces (Ker, 1981; Maganaris and Paul, 1999), the tendon is expected to operate through this nonlinear region in each condition. A measure of mean SEE stiffness during torque development should be sufficient for discerning the effect of MTU stretch on the mechanical state of the SEE.

Statistical analyses

Statistics pertaining to torque and fascicle data were grouped according to experimental condition (i.e. 5 deg PF, 0 deg and rotation), stimulus type (i.e. twitch, doublet and C₂) and ISI (i.e. 10 and 80 ms), and are reported as means±s.d. One-way repeated-measures ANOVAs were performed for comparisons between experimental conditions for a given stimulus type. A *post hoc* analysis was performed using Tukey's method of controlling significance level for multiple pair-wise comparisons. Paired Student's *t*-tests were performed for comparisons between ISIs for the rotation condition only. Statistical significance was set at $P \leq 0.05$ for all comparisons. Data displayed in figures represent means±s.e.m. Statistical tests were performed using Prism 6 software (GraphPad, La Jolla, CA, USA).

RESULTS

Plantar flexion torque and fascicle length changes in response to twitch and doublet stimulation are illustrated in Fig. 2. A brief, rapid rotation of the ankle at the onset of a twitch caused a modest reduction in the extent of active shortening in both the LG and SOL with respect to the constant-length contraction at the initial joint position (5 deg PF). The restriction of active shortening against the extension of the SEE was associated with a concomitant increase in the rate of torque development and an increase in twitch amplitude. Active lengthening was absent during rotation in both muscles. Together, these findings indicate that stretching the MTU at contraction onset functioned to increase the effective stiffness of the SEE, and is therefore, a suitable model for investigating the effects of series elastic compliance on contractile performance.

Twitch and doublet torque were largest in the rotation condition (Fig. 2A), exceeding the torque generated during constant-length contractions at both the initial and final (0 deg) joint positions. Active shortening of LG (Fig. 2B) and SOL (Fig. 2C) fascicles during a twitch was considerable in all conditions, including contractions in which the ankle was rapidly rotated. The amplitude of additional shortening upon stimulation a second time, just prior to force relaxation, was also substantial. Relative to the constant-length contractions at the initial and final joint position, LG and SOL fascicles operated at an intermediate length during torque development for the rotation contractions.

Rotation was estimated to have increased the mean effective stiffness of the LG and SOL SEE relative to the constant-length contraction at the initial joint position by 109 and 141%, respectively ($P < 0.01$; Fig. 3). Mean SEE stiffness increased as a function of MTU length for the constant-length contractions (i.e. mean SEE stiffness was greater during a constant-length contractions at the final joint position), but was still substantially

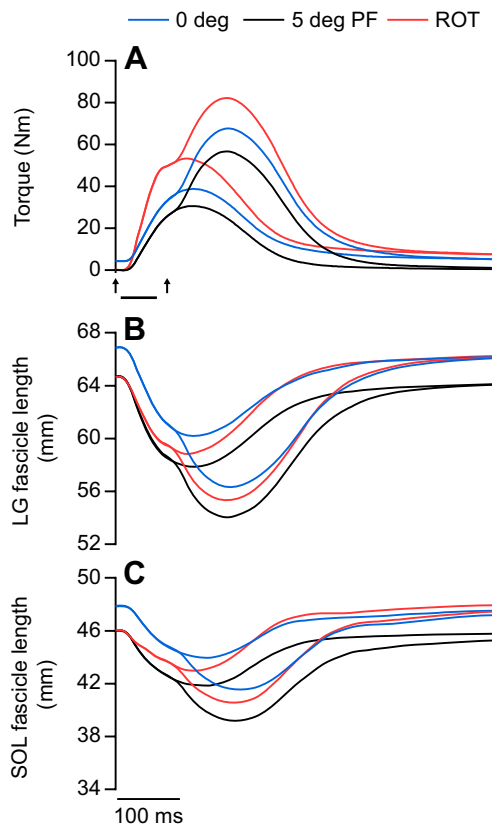


Fig. 2. Contractile behaviour of the triceps surae in response to twitch and doublet stimulation performed with and without a rotation imposed at stimulation onset to increase the effective stiffness of the series elastic element (SEE). The ankle was rapidly rotated through 5 deg of dorsiflexion (ROT) at the onset of stimulation to stretch the muscle–tendon unit and to restrict active shortening. Waveform averaged torque (A) and fascicle length [B, lateral gastrocnemius (LG); C, soleus (SOL)] for rotation contractions are compared with measurements made during constant-length contractions at the initial (5 deg PF) and final (0 deg) joint positions. Vertical arrows and horizontal bar denote the timing of stimulation and rotation, respectively. Rotation dramatically increased twitch and doublet torque, yet only caused a modest reduction in active shortening of LG and SOL fascicles. Torque and LG data, $N=15$. SOL data, $N=11$.

higher during the rotation condition (84–104%, $P<0.01$). No difference in mean SEE stiffness during torque development owing to the second stimulation pulse was detected between the rotation contraction and the constant-length contraction at the final joint position, for either LG (6% increase, $P=0.28$; Fig. 3A) or SOL (4% decrease, $P=0.1$; Fig. 3B). This indicated that any differences in contractile output in response to the second pulse between the two conditions could be attributed to the reduction in active shortening induced by the preceding rotation.

Torque measurements

Twitch and doublet peak torque, TTI and peak RTD for each condition are illustrated in Fig. 4. Rotation increased twitch torque, TTI and peak RTD relative to the constant-length contractions performed at the initial (74, 110 and 154%, respectively; $P<0.01$) and final (37, 81 and 124%, respectively; $P<0.01$) joint positions. Rotation also increased doublet peak torque and TTI relative to the constant-length conditions ($P<0.01$). Peak RTD owing to the second stimulation pulse during the doublet was augmented by rotation, but only with respect to the constant-length contraction at the initial joint position ($P<0.01$).

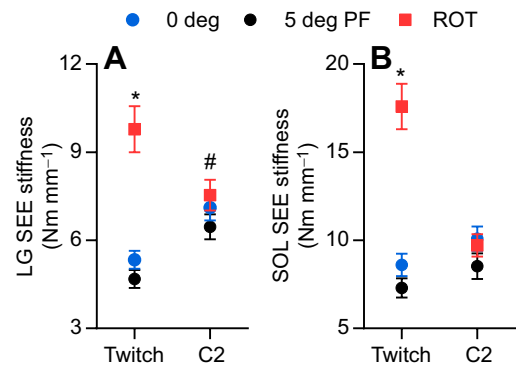


Fig. 3. Mean SEE stiffness of the LG and SOL during twitch and doublet contractions performed with and without an imposed rotation. Rotation (ROT) during the twitch increased the mean effective stiffness of the SEE by a factor of two for both LG (A) and SOL (B). The mean effective stiffness of the SEE during torque development owing to the second stimulation pulse (C_2) was similar after rotation and for a constant-length contraction at the final joint position (0 deg). Data are means \pm s.e.m. (LG, $N=15$; SOL, $N=11$). A one-way repeated-measures ANOVA (Tukey's *post hoc* test) was used for determination of statistical significance. *ROT>5 deg PF, 0 deg, $P<0.01$; #ROT>5 deg PF, $P<0.01$.

Twitch torque was subtracted from doublet torque to quantify the torque contribution of the second stimulation pulse (C_2 ; Fig. 4). Rotation during the twitch increased the amplitude, TTI and peak RTD of the torque response contributed by the second pulse relative to the constant-length contractions at both joint positions ($P<0.01$). The effect of rotation on subsequent contractile performance is more clearly illustrated in Fig. 5, where the kinetics of the second torque contribution for each condition have been normalised to corresponding values at the initial joint position.

Fascicle dynamics

Rotation reduced active shortening of LG and SOL fascicles during twitch and doublet contractions relative to the constant-length contractions at the initial and final joint positions (both $P<0.01$; Fig. 6). Accordingly, LG and SOL fascicles were at a longer mean length during the rise in active tension in rotation contractions

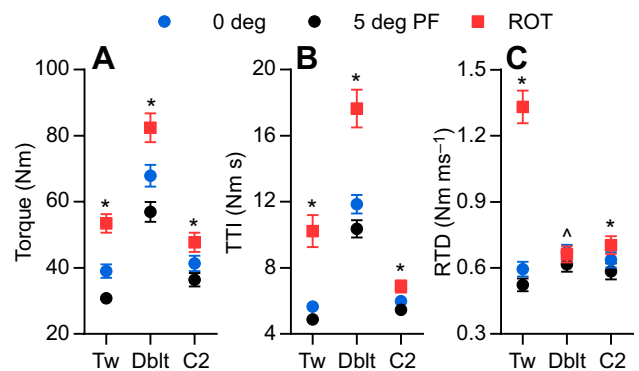


Fig. 4. Mechanical properties of twitch and doublet contractions performed with and without an imposed rotation. Peak torque (A), torque–time interval (TTI) (B) and peak rate of torque development (RTD) (C) of twitch (Tw) and doublet (Dbt) contractions, and of the torque contribution owing to the second stimulation pulse (C_2). Rotation (ROT) increased the peak torque, TTI and peak RTD of the twitch and second torque contribution relative to the constant-length contractions at the initial (5 deg PF) and final (0 deg) joint positions. Data are means \pm s.e.m. ($N=15$). A one-way repeated-measures ANOVA (Tukey's *post hoc* test) was used for determination of statistical significance. *ROT>5 deg PF, 0 deg, $P<0.01$; ^ROT>5 deg PF, $P<0.01$.

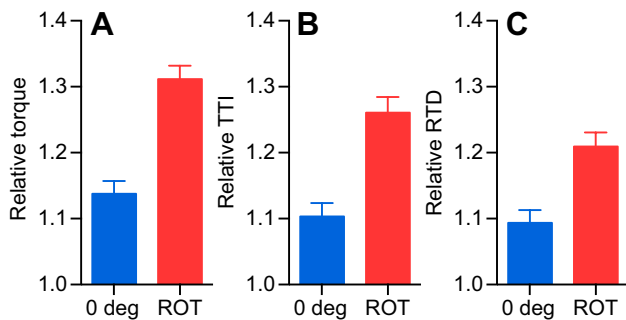


Fig. 5. Torque contribution owing to second stimulation pulse for doublet contractions with and without an imposed rotation. Peak torque (A), TTI (B) and peak RTD (C) have been normalized to corresponding values obtained during the constant-length contraction at the initial joint position (5 deg PF). The torque contribution owing to the second pulse was greater when the second pulse was delivered post-rotation than when delivered during a constant-length contraction at the initial or final (0 deg) joint position. Data are means \pm s.e.m. ($N=15$).

relative to the constant-length contractions performed at the initial joint position ($P<0.01$; see Fig. 2D,E). The reduction in active shortening caused by the increase in effective stiffness of the SEE (1–1.2 mm reduction) was less than the difference in initial fascicle length between the initial and final joint positions (1.9–2.2 mm difference). Therefore, with respect to the constant-length contractions at the final joint position, LG and SOL fascicles were at a shorter mean length during rotation contractions ($P<0.01$; see Fig. 2D,E).

Instantaneous fascicle velocity during twitch and doublet contractions is illustrated in Fig. 7A,B. The effect of rotation on velocity was most pronounced during the period of rotation. Rotation reduced the peak velocity during the active shortening phase of the twitch for both LG ($P=0.01$; Fig. 7C) and SOL ($P<0.01$; Fig. 7D) fascicles relative to the constant-length contraction at the initial joint position. With respect to the twitch contraction at the final joint position, rotation reduced the peak velocity of SOL ($P=0.03$) but not LG fascicles ($P=0.77$). Rotation reduced the peak in shortening velocity brought about by a second pulse relative to the constant-length contraction at the initial

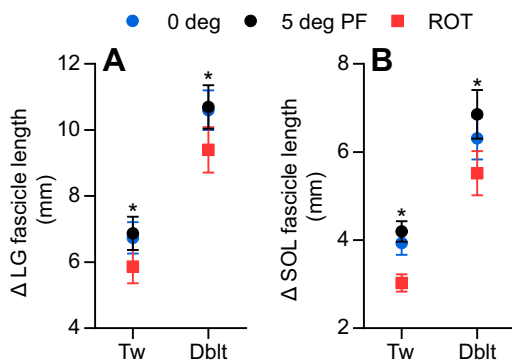


Fig. 6. Active shortening of LG and SOL fascicles in response to twitch and doublet stimulation performed with and without an imposed rotation. Rotation (ROT) induced a modest reduction in the amplitude of active shortening of both LG (A) and SOL (B) fascicles in response to twitch (Tw) and doublet (Dbtl) stimulation relative to the constant-length contractions at the initial (5 deg PF) and final (0 deg) joint positions. Data are means \pm s.e.m. (LG, $N=15$; SOL, $N=11$). A one-way repeated-measures ANOVA (Tukey's *post hoc* test) was used for determination of statistical significance. *ROT<5 deg PF, 0 deg, $P<0.01$.

($P=0.01$) and final ($P<0.01$) joint positions, but for LG fascicles only (SOL: $P=0.74$ and $P=0.99$, respectively). Rotation reduced the mean velocity of fascicle shortening during the doublet relative to the constant-length contraction at the initial joint position (LG: 57 ± 16 versus 64 ± 15 mm s $^{-1}$, $P<0.01$; SOL: 29 ± 9 versus 35 ± 10 mm s $^{-1}$, $P<0.01$), but had no effect on mean velocity relative to a constant-length contraction at the final joint position (LG: $P=0.46$; SOL: $P=0.61$).

Doublet contractile dynamics as a function of ISI

Reducing the ISI of doublet stimulation in the rotation condition from 80 to 10 ms (Fig. 8), such that the second stimulation pulse was delivered during rotation, led to only a modest increase in peak torque (6%, 97.2 ± 22.2 versus 92.0 ± 21.0 Nm, $P<0.01$) and peak RTD (28%, 0.95 ± 0.24 versus 0.75 ± 0.2 Nm ms $^{-1}$, $P<0.01$), and a decrease in doublet TTI (5%, 17.1 ± 4.0 versus 17.9 ± 4.4 Nm s $^{-1}$,

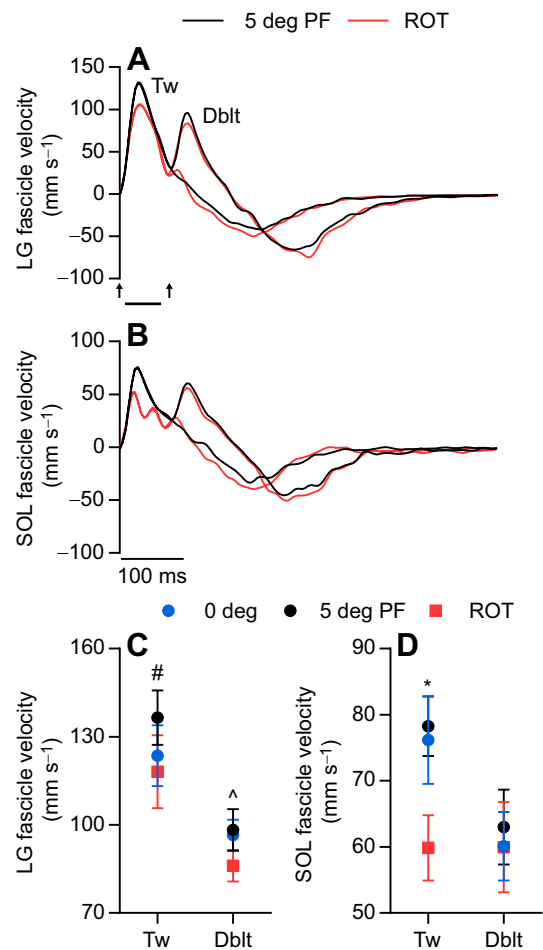


Fig. 7. Velocity of LG and SOL fascicles in response to twitch and doublet stimulation performed with and without an imposed rotation. (A,B) Waveform averaged velocity of LG (A) and SOL (B) fascicles during rotation contractions (ROT) and constant-length contractions at the initial joint position (5 deg PF). The peaks in the velocity response associated with the first and second pulses are denoted Tw and Dbtl, respectively. Vertical arrows and horizontal bar denote the timing of stimulation and rotation, respectively. (C,D) Peak velocities in response to the first and second pulses for LG (C) and SOL (D) fascicles. Rotation reduced the velocity of shortening relative to the constant-length contractions at the initial and final (0 deg) joint positions. Data are means \pm s.e.m. (LG, $N=15$; SOL, $N=11$). A one-way repeated-measures ANOVA (Tukey's *post hoc* test) was used for determination of statistical significance. #ROT<5 deg PF, $P<0.05$; ^ROT<5 deg PF, 0 deg, $P<0.05$; *ROT<5 deg PF, 0 deg, $P<0.01$.

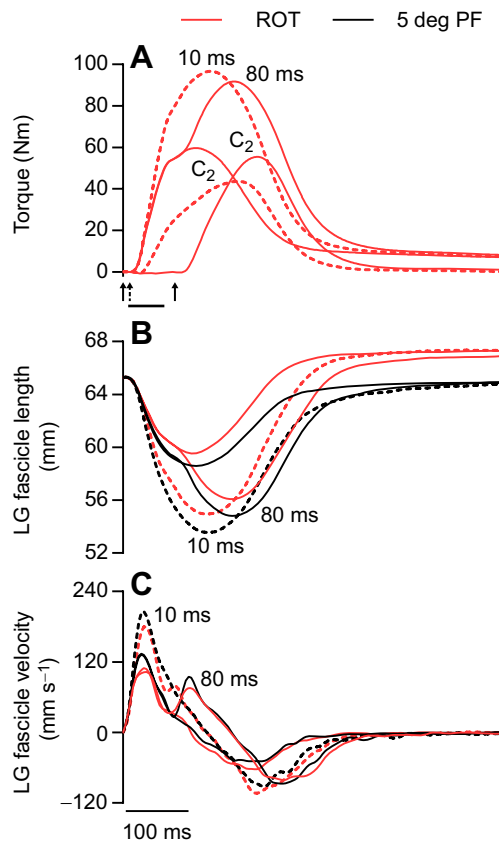


Fig. 8. Effect of doublet interstimulus interval (ISI) on torque and LG fascicle dynamics during contractions performed with an imposed rotation. For a doublet ISI of 10 ms (dashed lines), the second pulse was delivered during rotation (ROT). Relative to a second pulse delivered post-rotation (solid lines), doublet stimulation during rotation brought about a small increase in doublet peak torque (A), but did not increase the torque contribution of the second pulse (C_2). Vertical arrows and horizontal bar denote the timing of stimulation and rotation, respectively. Reducing the ISI to 10 ms increased the amplitude (B) and velocity (C) of active shortening. Fascicle measurements from the constant-length contractions at the initial joint position (5 deg PF) are shown to illustrate the effect of rotation on fascicle dynamics. Fascicle velocity remains high in response to an ISI of 10 ms when stimulation is performed with rotation. Data are waveform averages, $N=5$.

$P=0.05$). Rotation brought about greater changes in the torque contribution of a second stimulation pulse delivered post-rotation (ISI 80 ms) than it did a second pulse delivered during rotation (ISI 10 ms; Fig. 9). The torque contribution owing to a second pulse delivered 10 ms after the initial pulse was 20% smaller in amplitude (44.5 ± 9.9 versus 55.7 ± 11.6 Nm, $P < 0.01$) and demonstrated an 18% lower peak RTD (0.31 ± 0.08 versus 0.37 ± 0.11 Nm ms $^{-1}$, $P = 0.01$). The TTI of the torque contribution also decreased when the ISI was reduced, but the effect was smaller (3%, 7.2 ± 1.8 versus 7.4 ± 1.9 Nm s $^{-1}$, $P = 0.05$).

Reducing the ISI to 10 ms for the rotation condition increased active fascicle shortening for LG (10.4 ± 1.2 versus 9.2 ± 1.5 , $P < 0.01$; see Fig. 8B) and SOL (7.3 ± 2.1 versus 6.4 ± 2 mm, $P = 0.06$); however, a statistically significant reduction was only observed for LG. An ISI of 10 ms also increased peak shortening velocity (LG: 187 ± 24 versus 112 ± 28 mm s $^{-1}$, $P < 0.01$; SOL: 115 ± 35 versus 64 ± 19 mm s $^{-1}$, $P = 0.01$) and mean shortening velocity (LG: 79 ± 10 versus 53 ± 10 mm s $^{-1}$, $P < 0.01$; SOL: 47 ± 12 versus 34 ± 10 mm s $^{-1}$, $P < 0.01$; see Fig. 8C).

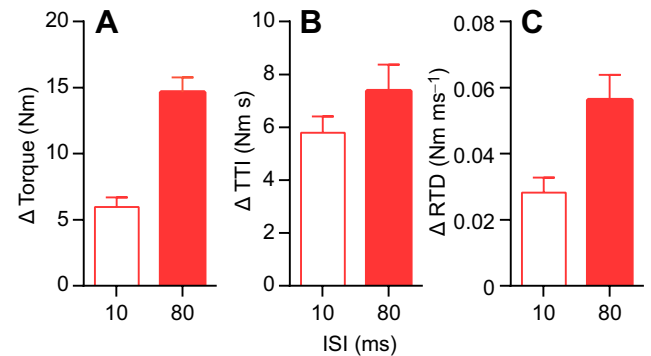


Fig. 9. Rotation-induced changes in the torque contribution of a second stimulation pulse delivered 10 or 80 ms after the twitch. The change in peak torque (A), TTI (B) and peak RTD (C) relative to a corresponding value measured during the constant-length contraction at the initial joint position. Rotation had a greater effect on the torque contribution of a second pulse delivered after rotation (ISI 80 ms) than it did the torque contribution of a second pulse delivered during rotation, shortly after the initial pulse (ISI 10 ms). Data are means \pm s.e.m. ($N=5$).

DISCUSSION

The purpose of the present study was to further our understanding of the role series elastic compliance plays in manipulating force generation and explore the possibility that the extent of active shortening afforded by series compliance may affect force summation. We have shown that modest reductions in the amplitude and velocity of active shortening owing to an increase in effective SEE stiffness were sufficient to substantially augment the mechanical properties of the twitch. Twitch torque, TTI and peak RTD were considerably greater for contractions where active fascicle shortening was restricted by a rapid, small-amplitude stretch than for constant-length contractions performed at the initial and final MTU lengths of the rotation contraction. Our results also show that the torque contribution of a second stimulus was sensitive to the degree of active shortening permitted during the preceding period of contractile activity. The torque contribution increased when prior active shortening was restricted by a transient increase in effective SEE stiffness. As active fascicle lengths post-rotation were shorter than fascicle lengths measured during the constant-length contraction at the final MTU length, our findings suggest that history-dependent properties may influence the force-generating potential of stimuli within a burst.

Stretch of the MTU caused only a modest reduction in the extent to which LG and SOL fascicles actively shortened against the extension of the SEE. The fascicles of both muscles still shortened to lengths that were only 1 mm (1.5–3% of resting length) longer than minimum active lengths achieved during the constant-length contraction at the initial MTU length. By comparison, increasing the effective stiffness of the SEE had a dramatic effect on mechanical output. MTU stretch increased twitch torque and rate of torque development by 74 and 154%, respectively, relative to the constant-length contraction at the initial MTU length. According to the constant-length contractions performed at the final MTU length, an increase in active fascicle length of 2.5 mm would account for 36 and 9% of the increase in twitch torque and RTD, respectively. However, as MTU stretch induced only a 1 mm increase in active fascicle lengths relative to the constant-length contraction at the initial MTU length, we propose that the effects of shifts in position on the force–length curve were likely minor.

Increasing the effective stiffness of the SEE also achieved reductions in peak and mean shortening velocities. Peak velocity

during the twitch was reduced by 18 mm s^{-1} for fascicles of both muscles. Assuming an optimal fascicle length for force generation (L_o) of approximately 70 and 50 mm for LG and SOL, respectively, this equates to an approximate $0.25 L_o \text{ s}^{-1}$ reduction in peak shortening velocity for both muscles. The effect of compliance on velocity in the present study is apparently small when considered relative to a maximum velocity of shortening, which has been estimated to be 10 to $13 L_o \text{ s}^{-1}$ (Zajac, 1989). However, the force–velocity relationship is described by a hyperbolic function, and we estimate that a peak velocity of 1.5 to $2 L_o \text{ s}^{-1}$ would have positioned both muscles on the steepest region of the curve such that a small reduction would likely have a considerable influence on force generation. Accordingly, we propose that more favourable shortening velocities were primarily responsible for the improvement in twitch performance. Our assertions on the relative contribution of the force–length and force–velocity properties are of course based on the premise that fascicle behaviour measured at the level of the mid-belly is representative of behaviour in more proximal and distal muscle regions (Lichtwark et al., 2007). Regional differences in fascicle strain (Azizi and Deslauriers, 2014) might have played some part in the present study, although it is difficult to determine whether this would necessarily have a positive or negative effect.

The role of series elastic compliance in manipulating force summation was explored by determining whether prior active shortening influences force generation under constant-length conditions. We found that restricting the amplitude and velocity of active shortening during the twitch increased the torque contribution of a second pulse. The improvement in contractile performance could not be explained on the basis of the force–length relationship, as both the LG and SOL operated at slightly less favourable fascicle lengths after stretch than during the constant-length contraction at the final MTU length. Prior stretch of the MTU did induce a small reduction in peak shortening velocity in response to the second pulse for LG fascicles, which would be expected to favour force production. However, as there was no detectable difference in mean SEE stiffness during torque development owing to the second pulse between the two conditions, the increase in torque contribution and slowing of shortening velocity are likely due to another mechanism. We interpret these findings as indicating that the greater contractile output post-MTU stretch was owing to altered contraction dynamics during the preceding twitch as a result of a transient increase in SEE effective stiffness. We propose that the known dependence of muscle force generation on prior contractile history might explain the increase in contractile performance observed when prior active shortening was reduced.

A muscle that is allowed to actively shorten to a new length prior to constant-length contractile activity generates less steady-state force than if contracting at the same final muscle length in a purely constant-length manner (Maréchal and Plaghki, 1979; Herzog and Leonard, 1997). Shortening-induced force depression may arise from a stress-induced inhibition of cross-bridge attachment in newly formed (Maréchal and Plaghki, 1979; Herzog and Leonard, 1997) and old overlap zones (Joumaa et al., 2012). If this mechanism operates when significant internal shortening is permitted under constant-length conditions, it is possible that increasing the effective stiffness of the SEE reduces any inhibition of cross-bridge attachment. The cooperative effects of cross-bridge binding on thin filament activation (Gordon et al., 2000; Moss and Fitzsimons, 2010) may have also contributed to the increase in force output when prior active shortening was reduced. Active shortening afforded by a shortening step during the initial period of a contraction depresses the force-generating capacity of the contractile

apparatus during the ensuing period of activation (Edman, 1975, 1980). Deactivation of the contractile apparatus is likely due to a decline in cooperative activation stemming from shortening-induced detachment of strongly bound cross-bridges (Gordon et al., 2000). By affording more favourable fibre dynamics and, therefore, increasing the number of force-generating cross-bridges, stiffening of the SEE might have led to an increase in cross-bridge-mediated activation of the thin filament.

The arrangement of passive elastic structures with respect to the contractile apparatus is complex and unlikely to be of a strictly ‘in-series’ nature (Epstein et al., 2006). Accordingly, our estimate of mean SEE stiffness may not have encapsulated the stiffening and rearrangement of all passive force-transmitting structures. For example, muscle aponeurosis can experience strain in the transverse direction (Azizi and Roberts, 2009; Arellano et al., 2016) that likely contributes to increasing aponeurosis stiffness in the direction of the muscle’s line of action (Azizi and Roberts, 2009). The variable stiffness of aponeurosis may modify the compliance of elastic elements arranged in-series with the contractile apparatus in a muscle-region-dependent manner (Azizi and Roberts, 2009). Subtle force-driven differences in the compliance of some passive force-transmitting pathways may have gone undetected in the present study, and could potentially explain the difference in the contractile output when the muscles were stimulated a second time.

We anticipated that a higher level of muscle activation during the period for which the SEE was effectively stiffened would favour torque production with respect to a contraction in which the second pulse is delivered after this period. Instead, we found that the torque contribution of the second pulse was of a smaller amplitude and TTI, and demonstrated a lower peak RTD when delivered during stretch. The higher doublet stimulation frequency also provided no advantage to the doublet TTI and increased peak doublet torque only modestly. Although a large period of the rise in torque occurred concurrently with stretch of the MTU, a high velocity of shortening was still permitted (2.3 – $2.7 L_o \text{ s}^{-1}$) and can likely explain the absence of any further augmentation. We found peak and mean shortening velocities to increase by 66 – 81% and 38 – 48% when the ISI was reduced to 10 ms, respectively. This suggests that larger stretch amplitudes, by providing greater resistance to active shortening, may be necessary for the contractile apparatus to take full advantage of very high frequencies of activation. From an energetic perspective, these findings may have relevance to the neural control of motor unit behaviour during contractions in which total muscle work or force maintenance is of greater importance than the rate of force rise. Minimizing the activation level or frequency of activation required to achieve a target force is energetically desirable (Barclay et al., 1993, 2008), as the metabolic cost of the contraction will be reduced accordingly.

Our results bear significance to our understanding of how tendon compliance constrains muscle force generation, and of the mechanical implications of physiological changes in tendon compliance. Tendons undergo significant remodelling in response to an increase or decrease in mechanical loading (Magnusson et al., 2008), demonstrating mechanical behaviour indicative of structural and material changes (Reeves et al., 2003, 2005). Stretch of the MTU at twitch onset in the present study was estimated to have increased the effective stiffness of the SEE by 84 – 141% . Similar magnitudes of change in stiffness have been demonstrated for human lower limb tendons in response to resistance training and bed rest. Twelve to 14 weeks of resistance training has been shown to facilitate a 50 – 84% increase in tendon stiffness and elastic modulus in young (Kubo et al., 2001; Malliaras et al., 2013) and older

(Reeves et al., 2003; Onambele-Pearson and Pearson, 2012) adults. Conversely, 21 and 90 days of bed rest may reduce tendon stiffness (and elastic modulus) by 29% (De Boer et al., 2007) and 58% (Reeves et al., 2005), respectively. Moderate reductions in tendon stiffness have been shown to also occur with natural ageing (Onambele et al., 2006; Stenroth et al., 2012). In the present study, the change in effective stiffness was more modest (59–74%) when stretch of the MTU occurred during the short ISI doublet, presumably because of greater internal forces, yet doublet RTD still increased by 75%. This suggests that even relatively small changes in tendon compliance may have an appreciable effect on contractile performance. Studies showing concomitant changes in tendon mechanical properties and peak RTD in response to loading (Kubo et al., 2001; Reeves et al., 2003) and unloading (Kubo et al., 2000) interventions support a strong link between the rise in tension and internal mechanics.

Although our findings highlight the force-limiting behaviour of the compliant Achilles tendon during constant-length conditions, the triceps surae is more accustomed to participating in dynamic contractions where the MTU is subjected to large stretch amplitudes during stretch–shorten cycles (Hof et al., 1983, 2002; Fukunaga et al., 2001). Under the high gravitational and inertial loads experienced during walking, running or hopping, the Achilles tendon lengthens considerably as the whole MTU is stretched, allowing active muscle fibres to operate almost isometrically (Lichtwark and Wilson, 2005, 2006; Lichtwark et al., 2007). Near-isometric behaviour not only permits fibres to operate at velocities that favour force generation, but also allows contractile force to be generated in an economic manner (Roberts, 2002). Doublet or burst discharges during a stretch of considerable amplitude may be capable of promoting rapid force development, provided that the frequency and number of motor units activated are sufficient to minimize fascicle length changes during stretch of the compliant MTU.

Conclusions

The results of this study demonstrate that series elastic compliance has a major impact on the rate of force rise and doublet force summation. We have shown that modest reductions in fascicle shortening amplitude and velocity owing to a reduction in effective series compliance were sufficient to substantially improve contractile performance. We have also provided evidence that suggests that the degree of active shortening afforded by a compliant tendon may be capable of depressing the force-generating potential of consecutive stimuli in a history-dependent manner. On the basis of our findings we propose that the changes in tendon stiffness subsequent to chronic mechanical loading and unloading are likely large enough to considerably augment and depress the maximum rate of force development, respectively. Finally, our results suggest that in a compliant muscle, lower stimulation frequencies may be equally effective as higher frequencies in achieving a desired total force output when activation and MTU stretch are coordinated. A high stimulation frequency may be less favourable in this regard because of very high velocities of shortening.

Acknowledgements

We sincerely thank the subjects for their participation in the study.

Competing interests

The authors declare no competing or financial interests.

Author contributions

D.L.M., A.G.C. and G.A.L. were involved in the conception and design of research. D.L.M. performed experiments and analyzed data. D.L.M., A.G.C. and G.A.L.

interpreted results of experiments. D.L.M. and G.A.L. prepared the figures. D.L.M., A.G.C. and G.A.L. drafted, edited and revised the manuscript, and approved the final version of manuscript.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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