Doublet potentiation in the triceps surae is limited by series compliance and dynamic fascicle behavior

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Mayfield DL, Lichtwark GA, Cronin NJ, Avela J, Cresswell AG. Doublet potentiation in the triceps surae is limited by series compliance and dynamic fascicle behavior. J Appl Physiol 119: 807–816, 2015. First published August 6, 2015; doi:10.1152/japplphysiol.00403.2015.—Activation of skeletal muscle twice in quick succession results in nonlinear force summation (i.e., doublet potentiation). The force contributed by a second activation is typically of augmented amplitude, longer in duration, and generated at a greater rate. The purpose of this study was to examine force summation in a muscle attached to a compliant tendon, where considerable internal shortening occurs during a fixed-end contraction. The triceps surae of 21 (Experiment 1) and 9 (Experiment 2) young adults were maximally activated with doublet stimulation of different interstimulus intervals (ISIs) (5-100 ms) at several muscle lengths. Ultrasound images acquired from lateral gastrocnemius and soleus muscles allowed quantification of dynamic fascicle behavior. Force summation was muscle length dependent. Force augmentation was limited to a short muscle length. Lateral gastrocnemius and soleus fascicles underwent large amounts of active shortening and achieved high velocities in response to doublet stimulation, dynamics unfavorable for force production. Summation amplitude and the sensitivity of summation to ISI were dramatically depressed in the triceps surae after comparison to muscles with less fixed-end compliance. We propose that the internal shortening permitted by high series compliance limited force augmentation by offsetting and/or interfering with activation and cross-bridge processes driving augmentation. High series compliance may also reduce the sensitivity of the summated response to ISI, an assertion supported by predictions from a Hill-type muscle model. These muscles may exhibit greater force augmentation during more accustomed stretch-shorten tasks (i.e., hopping), where the compliance of the Achilles tendon actually enables near-isometric fascicle behavior.

muscle mechanics; muscle force; force summation; muscle-tendon interaction

WHEN SKELETAL MUSCLE IS ACTIVATED twice in quick succession, the summated force response exceeds that expected from the linear addition of two individual twitches (8, 11, 60). Augmentation of the amplitude and duration of force contributed by the second mechanical transient promotes nonlinear force summation, whereby the amplitude of the resultant response can be three to four times larger than the amplitude of the twitch (3, 17, 62). Force augmentation in whole skeletal muscle and single motor units resulting from doublet electrical stimulation, also referred to as doublet potentiation, can enhance contractile performance during subsequent low-frequency stimulation (8, 22, 63), a phenomenon known as the catchlike property (7). During voluntary contractions, skeletal motor units can be activated to discharge briefly at very high frequencies (interstimulus interval, ISI < 20 ms) (5, 14, 67). The presence of doublet discharges at the onset of ballistic contractions suggests that doublet potentiation may in fact be utilized to facilitate rapid force generation during volitional contractions (16, 46).

The contractile processes contributing to force augmentation are not well understood. Consequently, force summation remains a difficult facet of muscle behavior to predict (68). Enhanced Ca2+ dynamics owing to augmented Ca2+ release (18) and greater intracellular Ca2+ summation (6) and enhanced cross-bridge dynamics following cooperative activation of the actin filament consequent to binding of force-generating cross bridges (1, 10, 25) are contractile processes postulated to contribute to doublet potentiation. More effective force transmission subsequent to the initial activation, mediated by muscle fiber shortening and an increase in series stiffness (i.e., removal of slack from within the tendon and aponeurosis), provides a mechanical explanation for the augmented force contributed by a second stimulus (60). This investigation focused on the role of muscle mechanical factors, such as tendon compliance, in regulating force summation from consecutive stimuli.

It has been well demonstrated that the compliance of the series elastic elements (SEE), tendon and aponeurosis, permit muscle contractile elements (CE) to actively shorten during constant-length contractions (27, 40, 42, 59). Hill (35, 36) established that the inclusion of additional series compliance acts to, not only depress the amplitude and rate of tension development, but also delay the development of maximum tension during a twitch. Conversely, abolishing the series compliance altogether by means of a brief, rapid stretch at the onset of activation enhances tension development by limiting the extension of the SEE and thus by limiting the internal shortening.

To date, there has been little consideration for SEE compliance and the magnitude of internal shortening in response to doublet stimulation and how muscle fiber mechanics may influence force augmentation. This is surprising given that the force-generating capacity of the CE is governed by instantaneous length (26) and velocity (21) and exhibits history-dependent properties (2). If the compliance of the SEE is sufficient to permit considerable internal shortening, force-length- and force-velocity-related reductions in muscle fiber force-producing capacity may offset the augmentation of force derived from enhanced Ca2+ and cross-bridge dynamics. A significant leftward shift onto or further down the ascending limb of the force-length curve and high velocity of shortening may also directly interfere with these processes, given that the Ca2+ sensitivity of the CE is length dependent (20, 65) and rapid shortening accelerates cross-bridge detachment (39) and may also reduce the Ca2+ affinity of troponin C (9, 69).
Although the mechanical implications of muscular internal shortening have been well described, it has yet to be established whether an interaction exists between the intrinsic mechanics and the contractile process contributing to force augmentation. Accordingly, the purpose of this investigation was to examine doublet potentiation in a muscle with high series compliance where significant internal shortening is expected. The fascicles of human gastrocnemius and soleus experience large changes in active length during a fixed-end contraction (42, 53) because of their serial attachment to the long, compliant Achilles tendon (54). The relatively high series compliance of this muscle group compared with other human and animal skeletal muscles makes it suitable for this investigation. We hypothesized that the extent of force augmentation in the triceps surae (TS) in response to doublet stimulation would be depressed compared with similar data from muscles with less series compliance.

**METHODS**

**Ethical approval.** The experimental protocols were approved in accordance with the ethical review guidelines and processes of the two institutional review committees where the experiments were conducted. Written, informed consent was obtained from all subjects, and ethical guidelines set out by the Declaration of Helsinki were followed.

Participants. An initial investigation (Experiment 1) was conducted on 21 healthy subjects (mean ± SD; age: 26 ± 3 yr; body mass: 70 ± 11 kg; height: 174 ± 8 cm). A supplementary investigation (Experiment 2) was conducted on a different group of nine subjects (age: 26 ± 5 yr; body mass: 80 ± 16 kg; height: 180 ± 9 cm). Subjects from both groups were free of neuromuscular injury or impairment.

Experimental protocols. In both experiments, TS torque summation and fascicle dynamics were investigated as a function of ISI and knee-ankle joint configuration. Knee and ankle joint positions were manipulated to adjust the length of the gastrocnemius and soleus muscles and allow the length-dependent property of summation to be explored. Fascicle dynamics were quantified by means of two-dimensional ultrasound (US) imaging. In Experiment 1, the ISI range was 5–40 ms (a range typically used for doublet stimulation), and ankle angles of −10° (dorsiflexion), 0° (tibia perpendicular to the sole of the foot), and 20° (plantar flexion) were imaged at an extended (180°) and flexed (90°) knee angle (i.e., 6 joint configurations in total). After completion of Experiment 1, a supplementary investigation was performed with an US imaging system capable of greater temporal resolutions to improve measures of dynamic fascicle behavior, in particular, instantaneous fascicle velocity. A greater ISI range (5–100 ms) was also implemented as part of Experiment 2 to allow for a more complete analysis of the sensitivity of the summated response to stimulation frequency. Doublet stimuli were delivered at an ankle angle of 0° and in the same extended and flexed knee positions as in Experiment 1.

Experimental setup. In Experiment 1, subjects sat on an adjustable chassis with back support. The adjustable framework enabled the hip angle to be kept constant (110°) and allowed manipulation of the knee joint angle. The right foot was securely fixed to a noncompliant, rotational footplate and aligned with the rotational axis. The position of the seat and backrest was adjusted to achieve the desired knee angle. An inelastic strap fixed the thigh to the seat. Two custom-built strain gauges positioned directly under the footplate measured plantar flexion forces generated by the TS during the electrical stimulation protocol. In Experiment 2 (performed in a different laboratory from Experiment 1), participants lay prone on a padded bench top with their knees extended or adopted a passive kneeling position, such that the knee was at 90°, by leaning forward onto a timber box positioned on the bench top. The right ankle was securely fixed to the footplate of a specially constructed microprocessor-controlled torque motor that held the ankle in a 0° neutral position. The ankle was aligned with the rotational axis of the dynamometer.

**Peripheral nerve stimulation.** A constant current electrical stimulator (DST7AH; Digitimer, Hertfordshire, UK) was used to evoke maximal twitches from the TS by means of a 500-μs square pulse delivered transcutaneously to the tibial nerve. An anode (sensor area 79 mm²; Ambu, Copenhagen, Denmark) was positioned 2 cm proximal to the horizontal skin crease overlying the popliteal fossa, and a cathode (sensor area 79 mm²; Ambu) was placed on the skin crease. Before placement of the cathode, a smaller ball-type electrode mounted into a wand was used to locate the position of the tibial nerve. A maximal twitch response was achieved by increasing the stimulator current until there was no further increase in M-wave peak-to-peak amplitude or twitch torque. The stimulator current was increased by a further 25% to ensure that maximal activation was achieved throughout the duration of the investigation. The above process was performed separately for the extended and flexed knee positions to account for any movement between the tibial nerve and stimulating electrodes that may occur as a result of moving the knee.

**Electrical stimulation protocol.** In Experiment 1, doublet stimulation was performed for ISIs of 5, 10, 15, 20, 25, 30, 35, and 40 ms. Single stimulation pulses were also delivered. Each ISI was delivered twice during a randomized stimulation pattern (minimum 20 ms between each stimulus). After randomization of the initial knee angle, the stimulation pattern was delivered at each ankle position in a random order. The knee was then rotated to the remaining angle, and the stimulation protocol was repeated. In Experiment 2, doublet stimulation was performed for ISIs of 5, 20, and 40 ms to align with the initial investigation. Doublet stimulation also included ISIs of 60 and 100 ms to allow the frequency sensitivity of doublet potentiation to be further explored. The stimulation protocol was repeated for each knee position and two US transducer positions (see **US measurements**). In both experiments, five brief, submaximal voluntary contractions (~75% of maximal) were performed before each stimulation battery to minimize thixotropic effects (61).

**Electromyographic recordings.** Bipolar electromyographic (EMG) signals were recorded from lateral gastrocnemius and the lateral aspect of soleus. Before the placement of self-adhesive Ag/AgCl surface electrodes (sensor area 15 mm²; Ambu), the skin was shaved, abraded with sandpaper, and cleaned with ethanol. Recorded surface signals were amplified 100 times and high pass filtered (10-Hz cutoff frequency, NL824, Digitimer).

**US measurements.** In Experiment 1, a PC-based US system (Echo- blaster 128; UAB Telemed, Vilnius, Lithuania) was used to visualize and acquire longitudinal images of soleus and lateral gastrocnemius. A flat, 96-element, linear, multi-frequency probe was positioned over the muscle belly of lateral gastrocnemius, allowing both lateral gastrocnemius and soleus muscles to be visualized simultaneously in the one imaging plane. B-mode (6 MHz) images were acquired at a rate of 80 Hz with a field of view of 50 mm and a focus depth of 18–26 mm.

In Experiment 2, B-mode images were collected with a research US system (SonixMDP; Ultrasonix, Analogic, Peabody, MA) that allowed individual frames to be acquired at a rate of 195 Hz, yielding data of superior temporal resolution. This system was employed to improve the capacity for accurate quantification of instantaneous fascicle velocity. Lateral gastrocnemius and soleus fascicles were imaged at separate locations and at depths of 25 and 30 mm, respectively, to allow for high frame rates. In contrast to Experiment 1, the soleus was imaged more distally, where it is more superficial. Images were acquired with a 60-mm linear probe (L14-5W/60; Ultrasonix, Analogic) with a central frequency of 10 MHz.

Through implementation of a custom-written automatic tracking algorithm in MATLAB (MathWorks, Natick, MA), instantaneous
fascicle length was determined for each frame of US. Fascicle length was defined as the linear distance between fascicle insertion into the deep aponeurosis and fascicle insertion into the superficial aponeurosis. A fascicle that intersected the middle region of the image was selected for analysis. The optical flow estimation algorithm used to quantify fascicle length has been described in detail elsewhere (12) and has been shown to track fascicle length changes with submillimeter resolution during tasks like postural sway (13). Instantaneous fascicle velocity was obtained following differentiation of the automatically tracked fascicle length data.

Signal processing. Force and EMG signals collected during Experiment 1 were simultaneously sampled at 2,000 Hz and displayed using a 16-bit Power 1401 and Spike2 software (Cambridge Electronic Design, Cambridge, UK). The force signal was amplified by a custom-built amplifier before acquisition and later converted to torque using the perpendicular distance from the footplate axis of rotation to the head of the fifth metatarsal. Torque data from Experiment 2 was sampled and displayed at 2,000 Hz using a 12-bit Micro3 1401 and Spike2 software (Cambridge Electronic Design). The signal was amplified before acquisition (BKI-5; Nobel Elektronik, Stockholm, Sweden) and low-pass filtered at 25 Hz by a custom-built analog filter.

Twitch and doublet responses were evaluated to determine peak torque and peak rate of torque development (RTD). To quantify the torque contributed by a second stimulus (C2) in a doublet contraction, the torque trace of a twitch was superimposed and subtracted from the torque trace of the doublet response. To assess the influence of joint configuration, or muscle length, on the nature of torque summation, within-configuration normalization was performed for doublet peak torque and C2 peak torque (doublet torque and C2 torque normalized to peak torque of the twitch response of the same configuration). Normalized doublet peak torque is subsequently referred to as torque summation.

Hill-type muscle model. A Hill muscle model was developed to examine the influence of SEE compliance on force summation and the sensitivity of the summated response to ISI in the absence of history-dependent processes known to affect force production. Details of the muscle model are outlined by Lichtwardt and Wilson (49); however, only the mechanical aspects were implemented here. For this investigation, the two time constants of the stimulation model controlling the time course of the activator were optimized to reach agreement between the force-time predictions of the model and the force-time characteristics of the experimental data.

Two compliance conditions were modeled: 1) stiff, where the SEE permitted a negative CE strain of 7% under complete activation (low fixed-end compliance) to resemble a muscle with a short tendon (51), and 2) compliant, where the SEE permitted the CE to experience a negative strain of 49% under complete activation (high fixed-end compliance), similar to that of the gastrocnemius muscle (53). Doublet stimulation was performed for ISIs between 5 and 120 ms. Doublet force was normalized to peak twitch force for each SEE condition. To determine the relative sensitivity of force summation to ISI for each SEE condition, the smallest doublet response was normalized to the largest doublet response.

Data processing and statistical analysis. All data sets were imported into MATLAB to undergo further processing and analysis. To remove undesired high-frequency components from each of the imported signals, custom-written scripts were implemented to filter the data using a low-pass (30 Hz) second-order Butterworth filter (dual pass to correct any time shift). Statistics pertaining to contractile characteristics and fascicle length data were grouped according to condition and reported as means ± SD. Data displayed in figures represent the means ± SE. A two-way repeated-measures ANOVA (configuration × ISI) with a Tukey’s multiple-comparison correction was performed to identify significant differences in the magnitude of torque summation and measures of fascicle behavior in relation to ISI and joint configuration, treating data from the two experiments separately. The level of statistical significance was set at P < 0.05 for all comparisons. Statistical tests were performed using Prism 6 software (GraphPad, La Jolla, CA).

RESULTS

Torque summation as a function of ISI. Waveform-averaged torque and fascicle-length responses from Experiment 2 are illustrated in Fig. 1. All measures deviate rapidly from the twitch in response to a second activation. Peak torque was greatest in response to an ISI of 5 ms and decreased slightly as the interval duration was extended to 100 ms. The maximum amount of active shortening for both lateral gastrocnemius and soleus fascicles was achieved with an ISI between 5 and 40 ms. Beyond an interval duration of 40 ms, the amplitude of shortening also decreased as a function of ISI. Detailed contractile characteristics of the TS in response to twitch and doublet
stimulation for **Experiment 1** and **Experiment 2** are presented in Tables 1 and 2, respectively.

The plantar flexion torques produced in the two different experimental setups were comparable after correcting for body mass (90°, \( P = 0.79 \); 180°, \( P = 0.88 \)). Mean values of body mass-normalized torque for the two common knee-ankle configurations are shown in Fig. 2 as a function of ISI. Doublet torque decreased significantly as a function of increasing ISI in both experiments (\( P < 0.01 \)). However, the effect was small. In **Experiment 1**, peak torque was most sensitive to stimulation frequency in the extended knee-plantar flexed ankle configuration, in which a 6.3% reduction was observed (significant ISI-by-configuration interaction, \( P < 0.05 \)). The reduction in peak torque as a function of ISI was 1.3–2.2% for all other configurations (Fig. 2). A comparable fall in torque amplitude (3.3–4.6%) was observed for the same ISI range in **Experiment 2** (Fig. 2). Doublet torque continued to decrease in a linear fashion as the interval duration was extended to 60 ms (5.6%) and 100 ms (11.9%). A significant effect of ISI on \( C_2 \) amplitude (\( P < 0.01 \)) was evident in **Experiment 2**. However, in contrast to summation amplitude, \( C_2 \) amplitude increased by 7–10% as the ISI was increased from 5 to 100 ms.

**Fascicle dynamics in response to doublet stimulation.** Lateral gastrocnemius and soleus fascicles shortened substantially in response to twitch (6.3–7.2 mm; 3.9–4.3 mm) and high-frequency doublet stimulation (10.8–11.3 mm; 6.2–7.2 mm) (Fig. 1, B and C). In **Experiment 1**, the magnitude of active shortening of lateral gastrocnemius and soleus fascicles increased significantly as a function of ISI (\( P < 0.01 \)); however, the effect was small and was not demonstrated across the same range in **Experiment 2**. The mean increase in absolute shortening as a function of ISI, collapsed across all configurations of **Experiment 1**, was 0.4 ± 0.4 mm and 0.4 ± 0.3 mm for lateral gastrocnemius and soleus fascicles, respectively. A difference in shortening amplitude of <0.1 mm was observed for the same ISI range in **Experiment 2**. A significant reduction (8–9%, \( P < 0.01 \)) in active shortening relative to the 5-ms ISI was evident only when the ISI was extended to 100 ms (lateral gastrocnemius, −0.9 ± 0.8 mm; soleus, −0.6 ± 0.6 mm), which is consistent with the reduced torque measured at this ISI.

The RTD and velocity of fascicle shortening were temporally coupled and displayed similar sensitivities to ISI (Fig. 3). The amplitudes of both properties were maximized with an ISI of 5 ms and decreased as a function of ISI and as the summated response became less fused. Soleus and lateral gastrocnemius fascicles achieved peak velocities of ~2–3 lengths/s. As the ISI was extended to 40 ms, peak shortening velocity fell by 23% for both muscles. The second peak in active shortening velocity associated with ISIs >40 ms did not surpass that of the initial peak, and thus the maximum velocity of long duration ISIs was of equal amplitude to that of the twitch response. The total reduction in peak shortening velocity beyond an ISI of 40 ms was 30% and 29% for soleus and lateral gastrocnemius, respectively. By contrast, the second peak in RTD remained larger than the initial peak for an ISI of 60 ms.

**Torque summation as a function of joint position.** Torque summation in the TS was length dependent (significant ISI-by-configuration interaction, \( P < 0.01 \)) and characterized by both torque augmentation and depression, depending on the joint configuration (Fig. 4). Doublet potentiation, or greater than linear torque summation (2.04–2.46), was observed only when the ankle was in a position of plantar flexion (Fig. 4A). Torque augmentation, as measured by the amplitude of the torque response contributed by the second stimulus (\( C_2 \)), was greatest in this ankle position (\( C_2 = 1.17–1.49 \)) (Fig. 4C). At neutral and dorsiflexed ankle positions, doublet potentiation was absent and torque summation was in a less than linear fashion (1.77–

**Table 1. Experiment 1: contractile characteristics of triceps surae in response to twitch and doublet stimulation**

<table>
<thead>
<tr>
<th>Ankle Angle</th>
<th>Twitch</th>
<th>5-ms ISI</th>
<th>40-ms ISI</th>
<th>Knee flexed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torque, Nm</td>
<td>14.8 ± 5.6</td>
<td>29.4 ± 9.8</td>
<td>28.7 ± 9.6</td>
<td>30.1 ± 6.3</td>
</tr>
<tr>
<td>Summation</td>
<td>2.04 ± 0.3</td>
<td>1.99 ± 0.3</td>
<td>31.0 ± 10.1</td>
<td>1.80 ± 0.12</td>
</tr>
<tr>
<td>( C_2 )</td>
<td>1.15 ± 0.29</td>
<td>1.19 ± 0.24</td>
<td>1.80 ± 0.12</td>
<td>1.77 ± 0.09</td>
</tr>
<tr>
<td>RTD&lt;sub&gt;peak&lt;/sub&gt;, Nm/ms</td>
<td>0.23 ± 0.09</td>
<td>0.44 ± 0.16</td>
<td>0.37 ± 0.12</td>
<td>0.29 ± 0.12</td>
</tr>
<tr>
<td>Passive FL, mm</td>
<td>51.3 ± 14.8</td>
<td>51.3 ± 14.8</td>
<td>61.7 ± 15.4</td>
<td>61.7 ± 15.4</td>
</tr>
<tr>
<td>( \Delta F_L ), mm</td>
<td>3.3 ± 1.7</td>
<td>4.4 ± 2.2</td>
<td>4.9 ± 2.4</td>
<td>3.6 ± 1.5</td>
</tr>
<tr>
<td>Lateral gastrocnemius</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive FL, mm</td>
<td>58.9 ± 12.9</td>
<td>5.9 ± 1.7</td>
<td>6.5 ± 1.9</td>
<td>5.9 ± 1.7</td>
</tr>
<tr>
<td>( \Delta F_L ), mm</td>
<td>4.8 ± 1.4</td>
<td>6.0 ± 1.4</td>
<td>6.3 ± 1.0</td>
<td>6.3 ± 1.0</td>
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</table>

<table>
<thead>
<tr>
<th>Ankle Angle</th>
<th>Twitch</th>
<th>5-ms ISI</th>
<th>40-ms ISI</th>
<th>Knee extended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torque, Nm</td>
<td>15.8 ± 5.4</td>
<td>33.5 ± 9.9</td>
<td>31.2 ± 9.4</td>
<td>36.0 ± 7.6</td>
</tr>
<tr>
<td>Summation</td>
<td>2.46 ± 0.30</td>
<td>2.29 ± 0.22</td>
<td>60.2 ± 11.8</td>
<td>60.2 ± 11.8</td>
</tr>
<tr>
<td>( C_2 )</td>
<td>1.53 ± 0.31</td>
<td>1.43 ± 0.25</td>
<td>1.85 ± 0.12</td>
<td>1.85 ± 0.12</td>
</tr>
<tr>
<td>RTD&lt;sub&gt;peak&lt;/sub&gt;, Nm/ms</td>
<td>0.20 ± 0.06</td>
<td>0.46 ± 0.14</td>
<td>0.41 ± 0.13</td>
<td>0.45 ± 0.08</td>
</tr>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive FL, mm</td>
<td>44.6 ± 13.4</td>
<td>3.8 ± 1.2</td>
<td>4.3 ± 1.5</td>
<td>3.5 ± 1.4</td>
</tr>
<tr>
<td>( \Delta F_L ), mm</td>
<td>3.2 ± 1.2</td>
<td>6.0 ± 1.4</td>
<td>6.3 ± 1.0</td>
<td>6.3 ± 1.0</td>
</tr>
<tr>
<td>Lateral gastrocnemius</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive FL, mm</td>
<td>64.8 ± 6.5</td>
<td>6.2 ± 2.2</td>
<td>6.9 ± 2.6</td>
<td>4.6 ± 1.2</td>
</tr>
<tr>
<td>( \Delta F_L ), mm</td>
<td>4.4 ± 1.5</td>
<td>6.3 ± 2.2</td>
<td>6.9 ± 2.6</td>
<td>4.6 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. ISI, interstimulus interval; \( C_2 \), peak normalized torque contributed by second stimulus; RTD, peak rate of torque development; FL, fascicle length.

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1.85) (Fig. 4B), independent of knee position. The peak torque contributed by $C_2$ was depressed relative to the twitch for these configurations ($C_2 = 0.92$–0.98) (Fig. 4D). Torque summation in Experiment 2 [flexed knee: 1.99; 95% CI (1.87, 2.1); extended knee: 2.04; 95% CI (1.96, 2.13)] was slightly greater than that observed during Experiment 1 for the two common joint configurations [flexed knee: 1.78; 95% CI (1.74, 1.82); extended knee: 1.83; 95% CI (1.78, 1.89)]. However, doublet potentiation remained absent in Experiment 2, and summation was generally within a similar range. Hypothetical linear summation (i.e., linear addition of the twitch response with a delay equal to the ISI) is illustrated in Figs. 4A, B, and 4C, for the plantar flexed and dorsiflexed ankle positions of Experiment 1 to highlight the length-dependent augmentation and depression of torque.

**Hill-type muscle model.** Results from the Hill-type muscle model support the current experimental observations and are shown in Fig. 5. Relative to a stiff SEE, the inclusion of a SEE with high series compliance (compliant SEE condition) capable of permitting a large degree of internal shortening acted to reduce the magnitude of force summation and depress the sensitivity of the response to ISI (Fig. 5A). Force summation in the compliant condition (1.88) (Fig. 5C) was less than that of the stiff condition (2.15) (Fig. 5B) and less sensitive to ISI (8 vs. 20%).

### DISCUSSION

In the current experiments, doublet potentiation was examined in a muscle with high series compliance, whereby considerable internal shortening is permitted during a fixed-end contraction. Force augmentation was absent in the TS over a range of muscle-tendon unit (MTU) lengths. Doublet potentiation was observed only when the ankle was in plantar flexion, a position at which the length and force-generating capacity of the soleus and lateral gastrocnemius are considerably reduced. At longer TS MTU lengths, where muscle fiber lengths were close to optimal for force production and force transmission is expected to be more effective, torque summed in a less-than-linear fashion or near-linear fashion. Lateral gastrocnemius and soleus fascicles experienced large changes in active length and achieved high velocities of shortening in response to doublet stimulation. These findings support our hypothesis and indicate that mechanically unfavorable fiber dynamics brought about by high series compliance can limit doublet potentiation. The underlying excitation-contraction coupling processes that have been cited to contribute to force augmentation likely remain active in the TS during doublet stimulation [i.e., enhanced $Ca^{2+}$ (18) and cross-bridge dynamics (1, 10)]. However, considerable internal shortening is expected to offset the augmentation caused by these processes and potentially limit summation by directly interfering with certain activa-
tion and cross-bridge-related processes [e.g., length-dependent Ca\(^{2+}\) sensitivity (20)].

A graphical comparison of the current data to force summation data obtained from similar experiments performed on human and animal muscles with less fixed-end compliance supports the current interpretation (see Fig. 6). The human tibialis anterior (3, 4) and cat flexor digitorum longus (62), which both possess considerably lower tendon-to-fiber length ratios than gastrocnemius and soleus (71), achieve a level of summation close to four at high stimulation frequencies. Similarly, the human adductor pollicis has been evidenced to generate a second mechanical response that contributes close to three times as much force as a twitch (17).

Muscle fiber dynamics are rarely discussed in the context of force summation. Any internal shortening should limit force generation by directly influencing cross-bridge dynamics (39). Our results clearly demonstrate that the compliance of the Achilles tendon permits considerable internal shortening in response to twitch and doublet stimulation. Active fascicle lengths were significantly shorter in response to doublet stimulation. A large degree of additional shortening was apparent in both muscles even for doublet contractions where the second stimulus was delivered close to peak twitch tension. Soleus and both gastrocnemii operate almost exclusively on the ascending limb of the length-tension curve (32, 55). Consequently, a significant amount of active shortening would shift both muscles further down the ascending limb and be expected to substantially limit force generation (26).

Active shortening during the development of isometric tension can also facilitate the deactivation of contractile machinery according to the mechanical work performed (i.e., shortening amplitude and force) (30, 31). Some of the loss in isometric tension attributable to additional series compliance has been attributed to extra internal shortening and the mechanical deactivation of muscle (19). Shortening-induced force depression has been explained by stress-induced inhibition of cross-bridge attachment in the newly formed (31, 56) and old filament overlap zones (41). Given the large amount of internal shortening experienced by both muscles, it is likely that force augmentation was in part limited by a reduction in the number of force-generating cross bridges according to both the length-tension relationship and shortening-induced force depression.

However, it appears that a compliant SEE may exert its greatest influence on force summation by permitting high velocities of active shortening. Lateral gastrocnemius and soleus fascicles reached peak velocities equivalent to 2.5–3.0 and 2.0–2.5 lengths/s at the highest stimulation frequencies, re-

![Fig. 3. Waveform-averaged rate of torque development (RTD) (A) and velocity profiles of lateral gastrocnemius (B) and soleus (C) fascicles in response to twitch and doublet stimulation. Doublet responses are for ISIs of 5, 20, 40, 60, and 100 ms. Data are from the extended knee configuration of Experiment 2 (n = 9).](image)

![Fig. 4. Waveform-averaged active twitch and doublet torque (A and B) and normalized torque contribution of second stimulus (C) and for the extended knee-plantar flexed (left) and dorsiflexed (right) configurations of Experiment 1 (n = 21). The plantar flexed and dorsiflexed ankle positions represent short and long muscle-tendon unit lengths, respectively. Doublet responses are for an ISI of 5 ms. Dashed lines (A and B) represent linear summation of the twitch response for an ISI of 5 ms.](image)
and/or facilitating an intensification of Ca\textsuperscript{2+} maximising the summation of myoplasmic Ca\textsuperscript{2+} stimulation frequencies will achieve greater CE activation by shortening velocity. Although it is expected that the highest Fig. 6

second activation at an ISI of 100 ms compared with 5 ms (see with an ISI of 5 ms. The greater peak torque contributed by a first stimulus, which was 30% less than the peak associated substantially smaller than the peak velocity associated with the 70). For an ISI of 100 ms, the velocity of the second peak was reduced by 40–50% at a velocity 20–30% of maximum (37, 70). The Ca\textsuperscript{2+} sensitivity of the CE would also be expected to be sufficiently low that the CE would benefit from augmented Ca\textsuperscript{2+} release and greater Ca\textsuperscript{2+} summation. The absence of dramatic doublet potentiation suggests that the reduction in force production owing to the force-length-velocity relationship partially offset the augmentation stemming from length-dependent processes. However, shorter TS MTU lengths than investigated in the current study (i.e., at or below slack length) may have achieved more dramatic augmentation.

Torque summation in the TS displayed a weak sensitivity to ISI, falling only 12%. A 30% reduction in force summation and 27% reduction in C\textsubscript{2} amplitude has been observed in the human

respectively. These velocities are quite remarkable considering the isometric conditions of the MTU. The maximum velocity of contractile shortening has been estimated to be \sim10–13 lengths/s (71). Force-velocity curves derived from experimental data and the Hill force-velocity relation (34) indicate that the force-generating capacity of both plantar flexors would be reduced by 40–50% at a velocity 20–30% of maximum (37, 70). For an ISI of 100 ms, the velocity of the second peak was substantially smaller than the peak velocity associated with the first stimulus, which was 30% less than the peak associated with an ISI of 5 ms. The greater peak torque contributed by a second activation at an ISI of 100 ms compared with 5 ms (see Fig. 6B) may be partly explained by a significant reduction in shortening velocity. Although it is expected that the highest stimulation frequencies will achieve greater CE activation by maximizing the summation of myoplasmic Ca\textsuperscript{2+} transients (6) and/or facilitating an intensification of Ca\textsuperscript{2+} release (18), internal shortening in the TS may limit force production to the extent that the advantages of high-frequency activation are obscured.

The amount of internal shortening permitted by series compliance during a fixed-end contraction can be largely attributed to the relative difference in length of the SEE and CE (43, 71). Gastrocnemius and soleus have high fixed-end compliances attributable to large tendon-to-muscle fiber length ratios of 9:1 and 11:1, respectively (71). By comparison, the tibialis anterior has a tendon-to-fiber ratio of 3:1 and the muscles of the lower limb in the cat a ratio 2–6:1 (71). SEE stiffness, which may be exaggerated in isolated muscle preparations through removal or reduction of the external tendon, may contribute to the discrepancy observed between the current data and the literature. The relative MTU length at which slack is introduced may also play a role. For example, the human tibialis anterior appears to be slack over a greater length range than the medial gastrocnemius (38, 45). More pronounced force augmentation at short muscle lengths has in fact been attributed to the presence of slack within the SEE, limiting the initial transmission of force (57, 64, 66) and the length-dependent sensitivity of the CE to Ca\textsuperscript{2+} (20, 65). Doublet potentiation was limited at a relatively short MTU length in the current study, where absolute torque was also small. Some degree of slack is likely introduced into the system at this length (29). The Ca\textsuperscript{2+} sensitivity of the CE would also be expected to be sufficiently low that the CE would benefit from augmented Ca\textsuperscript{2+} release and greater Ca\textsuperscript{2+} summation. The absence of dramatic doublet potentiation suggests that the reduction in force production owing to the force-length-velocity relationship partially offset the augmentation stemming from length-dependent processes. However, shorter TS MTU lengths than investigated in the current study (i.e., at or below slack length) may have achieved more dramatic augmentation.

Fig. 6. Normalized torque summation (A) and C\textsubscript{2} amplitude (normalized torque contributed by second stimulus) (B) data from the current study compared with data from human and animal muscles with less fixed-end compliance. Summation data are presented as a function of ISI normalized to contraction time. Data from the extended knee-neutral ankle configuration of Experiment 1 (□) and Experiment 2 (○) have been included and compared with summation data from similar studies (closed shapes). Note the small, but contrasting, increase in C\textsubscript{2} amplitude as a function of ISI observed for triceps surae in this study. ISI\textsubscript{norm}, interstimulus interval normalized to the contraction time of the twitch of the respective muscle. ● and ●, human tibialis anterior (3, 4); ■, cat flexor digitorum longus (62); ▲, cat soleus (62); ■, human adductor pollicis (17). Data from Experiment 1 and 2 and from human tibialis anterior (3, 4) are means. Data from human adductor pollicis (17) and cat flexor digitorum longus and soleus (62) are from a single representative muscle.
tibialis anterior and adductor pollicis, respectively, over a comparable normalized ISI range (see Fig. 6). Given that extra series compliance causes the rise in twitch force to be reduced in amplitude and prolonged in duration (36), the depressed sensitivity to ISI displayed by the TS may well be a function of high series compliance. The Hill-type muscle model implemented in this investigation supported this conclusion. When CE force was modeled as a function of the force-velocity-length relationship and the length-tension properties of the SEE element, a compliant SEE (similar to that possessed by the human gastrocnemius) reduced the sensitivity of the summed response to ISI. We believe that the Achilles tendon reduces the extent to which Ca\textsuperscript{2+} dynamics influence the sensitivity of the summed response to activation frequency by depressing the amplitude and prolonging the development of active tension.

A reduced sensitivity to activation frequency may have important implications for postural control. Experimental evidence suggests that the TS generate ballistic-like torque impulses in an attempt to control center-of-mass motion during quiet standing (52). Given that the amplitude of anterior-posterior sway appears to be modulated by the accuracy of such impulses (52), high series compliance may serve to benefit control by minimizing fluctuations in torque in response to rate coding and motor unit recruitment. Although soleus has been evidenced to generate an ongoing background plantar flexor torque during standing (15, 58), a compliant tendon may still act to limit inaccuracies in torque brought about by variability in motor unit firing rate (33).

In instances where there is a requirement for a larger, more rapid force, the current data suggest that a compliant SEE is unfavorable. At the onset of ballistic voluntary contractions, single motor units can discharge briefly at very high frequencies (14, 44, 67). High-frequency discharges have also been recorded from single motor units during locomotion in the rat (24) and during rapid corrective movements performed during locomotion in humans (28). Doublet discharges at the onset of fast contractions have been suggested to enable rapid force development and thus are probably functionally relevant (16, 46). The addition of a high-frequency doublet, similar to a motor unit doublet, at the onset of slow, constant-frequency electrical stimulation can also augment subsequent force production for up to several seconds (i.e., catchlike property) (22, 63). However, limited augmentation and mechanically unfavorable fascicle dynamics that we have shown here suggest that force enhancement arising from contractile phenomena related to the catchlike response and motor unit doublet potentiation may be less pronounced in the TS during constant-length contractions. We note, however, that the MTU of the TS primarily acts in a stretch-shorten cycle during functional tasks, where the compliance of the Achilles tendon is in fact mechanically advantageous because it enables the muscle fibers of the TS to remain in a near-isometric state relative to the whole MTU (23). During walking (23, 50), running (47) and hopping (48), high series compliance actually promotes fiber dynamics favorable for force production. Therefore, we predict that force augmentation from doublet stimulation may be more pronounced in the TS during such stretch-shorten contractions where internal shortening is limited.

**Conclusion.** Doublet potentiation was absent in the TS of healthy young adults over a range of muscle lengths. Doublet potentiation was evident at a short MTU length; however, the amplitude of augmentation was small relative to values reported for muscles with less fixed-end compliance. We have provided experimental evidence that the series compliance of the Achilles tendon limits force augmentation and depresses the sensitivity of the summed response to ISI in the TS during fixed-end contractions. The fascicles of the TS are permitted to experience substantial shortening in response to twitch and doublet stimulation, behavior that likely offsets and/or interferes with contractile processes driving augmentation. We suggest that the velocity of internal shortening is most critical to limiting augmentation.

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