Length-force characteristics of in vivo human muscle reflected by supersonic shear imaging

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Sasaki K, Toyama S, Ishii N. Length-force characteristics of in vivo human muscle reflected by supersonic shear imaging. J Appl Physiol 117: 153–162, 2014. First published May 29, 2014; doi:10.1152/japplphysiol.01058.2013.—Recently, an ultrasound-based elastography technique has been used to measure stiffness (shear modulus) of an active human muscle along the axis of contraction. Using this technique, we explored 1) whether muscle shear modulus, like muscle force, is length dependent; and 2) whether the length dependence of muscle shear modulus is consistent between electrically elicited and voluntary contractions. From nine healthy participants, ankle joint torque and shear modulus of the tibialis anterior muscle were measured at five different ankle joint angles during tetanic contractions and during maximal voluntary contractions. Fascicle length, pennation angle, and tendon moment arm length of the tetanized tibialis anterior calculated from ultrasound images were used to reveal the length-dependent changes in muscle force and shear modulus. Over the range of joint angles examined, both force and shear modulus of the tetanized muscle increased with increasing fascicle length. Regression analysis of normalized data revealed a significant linear relationship between force and shear modulus ($R^2 = 0.52, n = 45, P < 0.001$). Although the length dependence of shear modulus was consistent, irrespective of contraction mode, the slope of length-shear modulus relationship was steeper during maximal voluntary contractions than during tetanic contractions. These results provide novel evidence that length-force relationship, one of the most fundamental characteristics of muscle, can be inferred from in vivo imaging of shear modulus in the tibialis anterior muscle. Furthermore, the estimation of length-force relationship may be applicable to voluntary contractions in which neural and mechanical interactions of multiple muscles are involved.

Address for reprint requests and other correspondence: K. Sasaki, Laboratory of Exercise and Environmental Physiology, Faculty of Human Sciences and Design, Japan Women’s Univ., Mejirodai 2-8-1, Tokyo 112-8681, Japan (e-mail: sasakik@fc.jwu.ac.jp).

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muscle contractile force would be reflected by muscle shear modulus changes that were consistent between tetanic and maximal voluntary contractions.

METHODS

Participants. Nine healthy volunteers (2 women and 7 men) participated in this study. Their mean (± SD) age, height, and body mass were 28.4 ± 3.9 yr, 170.7 ± 5.3 cm, and 66.3 ± 10.8 kg, respectively. They were recreationally active and accustomed to both strenuous physical activities and measurements of neuromuscular function. All participants were fully informed of the purpose, experimental procedures, and possible risk of this study before giving their written consent to participate. The study protocol was in accordance with the Declaration of Helsinki and was approved by the Ethical Committee for Experimental Research involving Human Subjects, Japan Women’s University.

Experimental design and setup. This study consisted of two experiments performed on different days: the first one (experiment 1) was performed to simultaneously determine ankle joint torque and shear modulus of the tibialis anterior muscle, while the second one (experiment 2) was devoted to the measurement of moment arm length of the tibialis anterior (distal tendon) across a range of ankle joint angles. Experiment 1 was further divided into two sessions, i.e., joint torque and muscle shear modulus were measured during electrically evoked tetanic contractions (TC session) and during maximal voluntary contractions (MVC session). There was a rest of at least 10 min between sessions. The session order was randomly assigned to each participant.

A schematic of the setup common to both experiments is shown in Fig. 1. The participant sat on an experimental chair with the left knee flexed at 90°. The thighs were immobilized with an inelastic strap. The left foot (wearing a thin rubber shoe) was also strapped firmly to a footplate of custom-designed ankle dynamometer (48). The footplate was positioned so that its rotational axis coincided with the anatomical axis of the ankle. An electrical goniometer (XMI10/A, Biometrics, UK) was secured with adhesive tape over the medial aspect of the foot and the lower leg. An ultrasonic probe coated with transmission gel was placed over the tibialis anterior muscle belly (centered at 40% of the length from the popliteal crease to the center of the lateral malleolus). Then the probe position was carefully adjusted to align the longitudinal direction of the probe with that of the muscle. To ensure that both probe position and orientation remained constant throughout the experiment, a self-made probe holder was attached to the skin with adhesive tape.

Experiment 1. In the TC session, electrical stimulation was applied percutaneously to the tibialis anterior using an electric stimulator (SEN-7203, Nihon Koden, Japan) with a stimulus-isolation unit (SS-2185, Nihon Koden). A 5-s tetanic contraction was evoked with an 80-Hz train of 0.25-ms rectangular pulses via two self-adhesive electrodes (HV-BIGPAD, Omron Healthcare, Japan) placed over the proximal and distal portions of the muscle. We stimulated the muscle belly rather than the peroneal nerve because the latter also innervates the peroneus muscles. In fact, studies investigating in vivo angle-torque relationship of dorsiflexor muscles have mostly used muscle belly stimulation (27, 33, 41). The maximal tolerable intensity of electrical stimulation, i.e., the greatest intensity the participant could tolerate without substantial pain or discomfort, was individually determined and was used as the stimulation intensity throughout the session. We did not examine whether this intensity reached a supra-maximal level, because the stimulation of the tibialis anterior muscle belly with excessive current intensity was shown to activate the peroneus muscles and even the triceps surae muscles, resulting in distorted twitch responses (41).

In the MVC session, the participant routinely performed a 5-s submaximal dorsiflexion (perceived exertion level corresponding to 80% of maximal effort) and a subsequent 5-s maximal dorsiflexion for familiarization and warm-up. These contractions would attenuate the influence of history-dependent changes in muscle-tendon mechanical properties (38) on measured variables. Then the data collection was initiated, in which the participant was asked to rapidly increase dorsiflexion torque to a maximum and to maintain maximal effort for 5 s. To assess the degree of voluntary muscle activation, a paired-pulse (doublet) stimulation was delivered to the tibialis anterior muscle during and immediately after each maximal effort. Pulse width, pulse frequency, and stimulus intensity used were the same as those in the TC session.

In both TC and MVC sessions, ankle joint torque and muscle shear modulus were measured at five different ankle joint positions, ranging between −15° (dorsiflexed position) and +25° (plantar flexed position), where 0° denotes neutral position. The order of joint positions was randomized across participants. As measurable joint rotation occurs even in “isometric” contractions in vivo (3, 40, 48), we measured an actual ankle angle during contraction using the electrical goniometer. The measurement was performed twice at each ankle joint position, with a 2-min rest between measurements and a 5-min rest between different joint positions. The torque was measured with a pair of strain gauges bonded symmetrically to the shaft of the footplate. The torque signal was amplified with a signal conditioner (CDV-700A, Kyowa Electronic Instruments, Japan), collected at a

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sampling rate of 10 kHz by using a data acquisition system (PowerLab/16SP, ADInstruments, Australia), and digitally low-pass filtered with a cutoff frequency of 50 Hz for later analysis. The shear modulus along the longitudinal axis of the tibialis anterior muscle was measured with an ultrasonic scanner (Aixplorer version 4.2, Supersonic Imagine, France) and a 4- to 15-MHz linear array probe (SL15–4, Supersonic Imagine). The scanner was used in shear-wave elastography mode with musculoskeletal preset, the measuring principle of which has been described in detail (2, 52). Briefly, the scanner can remotely generate a mechanical shear wave by focusing ultrasound at a given location and image tissues during the wave propagation at a very high-frame rate (up to 20 kHz). A time-of-flight algorithm based on cross-correlation of local displacement-time profiles of two different points at the same depth was used to determine the velocity of shear wave propagation parallel to the probe front face. The spatial and temporal resolutions of the propagation velocity measurement were 1 mm and 1 s, respectively. Given a linearly elastic and transversely isotropic material (43, 46), muscle shear modulus ($\mu$) can be calculated from the shear-wave velocity:

$$\mu = \rho V_s^2$$

where $\rho$ is the density of muscle (generally assumed to be 1,000 kg/m$^3$), and $V_s$ is the shear-wave velocity. The shear modulus was measured from the superficial part of the muscle and visualized as a 15-mm color-coded square superimposed on a B-mode ultrasound image (Fig. 2).

**Experiment 2.** The moment arm length of the tibialis anterior tendon was determined using the in vivo tendon excursion method (25, 35). Briefly, this method involves ultrasound measurement of tendon excursion over a given joint rotation and subsequent calculation of tendon excursion-to-joint rotation ratio. In this experiment, an ultrasonic apparatus (SSD-2000, Aloka, Japan) with a 7.5-MHz linear array probe was used to obtain longitudinal images of the tibialis anterior. The images were obtained during passive joint rotation because the estimation of moment arm length using the tendon excursion method has been validated only at rest (34). However, we later corrected the measured value (see Data analysis), taking into account an increase in moment arm length during contraction (34, 35).

The ankle was cyclically dorsiflexed and plantar flexed through its range of motion by manually rotating the footplate at an angular velocity of $\sim 3°/s$. The participants were asked to completely relax their muscles during the joint rotation, which was monitored by surface electromyographic sensors (SX230, Biometrics) placed on the belly of the tibialis anterior and soleus muscles. A reference electrode (R206, Biometrics) was attached to the right wrist. Ankle joint angle was measured with the goniometer and sampled concurrently with electromyographic signals at 1 kHz using the data acquisition system. The ultrasound images synchronized with the joint angle and electromyographic signals were recorded at 30 Hz using a digital camcorder (NV-GS250, Panasonic, Japan) and LabChart Pro software (ADInstruments).

**Data analysis.** For experiment 1, the ankle joint angle and torque were averaged over 3 s in the middle of muscle contraction where the torque reached a plateau. For the MVC session, the voluntary activation level during contractions was calculated using the following equation (45):

$$VA = 1 - (DT_{sup} \cdot T/T_{max})/DT_{pot}$$

where $VA$ is the voluntary activation level, $DT_{sup}$ the torque induced by the superimposed doublet, $T$ the voluntary torque just before the superimposed doublet, $T_{max}$ the instantaneous maximum of voluntary torque, and $DT_{pot}$ the torque induced by the “potentiated” doublet (i.e., the doublet stimulation immediately after the voluntary contraction). Using bundled software of the ultrasonic scanner, the shear modulus was averaged over a selected circular region of interest (ROI). The diameter of ROI used to determine the muscle elastic properties varies greatly in the literature, from 2 mm (49) to 15 mm (6). A recent study using SSI (29) showed that the ROI size did not affect the averaged value of elastic modulus in resting muscle and tendon. In our pilot experiment, however, we found that muscle tissue deformation associated with forceful contractions occasionally resulted in a relatively defocused image with a large variation of shear modulus. This variation included unnaturally low (or even zero) values of shear modulus, which was rarely found in a clear image and thus considered an artifact. In such cases, the muscle shear modulus could be underestimated if averaged over a large area, so that we decided to use a small-diameter (3 mm) ROI to avoid the underestimation (Fig. 3). One possible problem with the use of small-diameter ROI is that the averaged shear modulus may be variable and not serve as a representative value of a whole muscle, especially when assuming spatial heterogeneity of mechanical response within individual muscles (10). Therefore, the analysis was performed on two different sites in each color-coded square: one near the superficial aponeurosis, and the other near the central aponeurosis. The position of each ROI was adjusted frame by frame such that 1) the average shear modulus was similar to or higher than those of adjacent areas; and 2) the shear modulus variability within each ROI was relatively small (coefficient of variation < 30%). The shear moduli obtained from the two different sites were averaged for further analysis.

Typical time-series data of the joint torque and the muscle shear modulus in the TC session are shown in Fig. 4. As can be seen, both signals showed a similar response to electrical stimulation with a
temporal difference of ~1–2 s. This difference was presumably due both to the phase shift associated with signal processing (i.e., the calculation and visualization of shear-wave propagation velocity) and to the limited time resolution (1 s) in the shear modulus measurement. In this study, the shear modulus of the tibialis anterior muscle was averaged over four data points, where the modulus reached a plateau. The data were not corrected for the resting shear modulus, because 1) the resting shear modulus generally corresponded to only a few percentages of that during contraction and did not change monotonically with joint angle or fascicle length; 2) the strain of parallel elastic component, a major source of the resting muscle stiffness (31), will substantially decrease during contraction (32); and 3) the tendon and soft tissue compliance caused the lateral displacement of the imaged tissues during contraction, so that the sites of shear modulus measurement were not identical between the resting and contracting muscles.

Fascicle length and pennation angle of the tibialis anterior muscle were analyzed from the B-mode ultrasound images taken in the middle of contractions using public domain software (ImageJ, National Institutes of Health, MD). In each ultrasound image, a clear oblique line reflected from interfascicular connective tissue was identified. The fascicle length was defined as the length of oblique line between the superficial and central aponeuroses, whereas the pennation angle was defined as the angle between the fascicle selected for length measurement and the central aponeurosis. When the entire length of the fascicle was not included in the image, the length of the missing portion was estimated using linear extrapolations of the fascicle and aponeuroses (13, 44).

For experiment 2, the excursion of the tibialis anterior central aponeurosis, which can be assumed equal to the tendon excursion when the muscle is at rest (34, 35), was analyzed from the ultrasound images taken during passive joint rotation. As with other researchers (25, 35), we did not take into account the stretch of parallel elastic component because the tendon moment arm length measured with this method has been shown to be comparable with that directly determined from magnetic resonance images (34, 35, 51). Furthermore, we estimated tendon moment arm length during contraction only within the range where passive muscle force was assumed to be small (28).

A reference point, at which a clearly visible fascicle attached to the central aponeurosis, was first identified from an ultrasound image at the beginning of passive joint rotation. The tendon excursion defined as the displacement of the reference point along the longitudinal axis of the muscle was then analyzed using ImageJ software. For each participant, 44 images (4 different images at each of 11 joint angles) captured during full-range, cyclic joint rotations were analyzed. The images were typically obtained every 5° of joint rotation, but near Fig. 4. Typical time-series data of ankle joint torque (A) and shear modulus of the tibialis anterior muscle (B) during tetanic contraction. Note that both the torque and shear modulus showed a similar response to electrical stimulation with a temporal difference of ~1–2 s, which was presumably due both to the phase shift associated with signal processing (i.e., the calculation and visualization of shear-wave propagation velocity) and to the limited time resolution (1 s) in the shear modulus measurement. The joint torque was averaged over 3 s in the middle of muscle contraction, while the shear modulus was averaged over four data points where the modulus reached a plateau.
the end range of motion the interval was adjusted, depending on the flexibility of participants. The moment arm of the tibialis anterior tendon (m) was calculated from:

\[ m = \text{dx/da} \]

where \( \text{dx} \) is the tendon excursion averaged over four different joint rotations with the same amplitude and \( \text{da} \) is the change in joint angle. The relationship between the ankle joint angle and the moment arm of the tibialis anterior was fitted to the following equation (25, 42):

\[ m = R \cdot \sin(a + \Delta) \]

where \( R \) is the theoretical maximum of the moment arm length, \( a \) is the ankle joint angle (90° denotes neutral position), and \( \Delta \) is the difference between the neutral ankle angle and the angle at which the moment arm reaches the maximum. The parameters \( R \) and \( \Delta \) were determined using nonlinear least squares method with a quasi-Newton algorithm.

It has been recognized that the moment arm length substantially increases during forceful muscle contractions (34). We thus estimated moment arm lengths during tetanic contractions from those at rest using the following equation:

\[ M = k \cdot m \]

where \( M \) and \( m \) are the moment arm lengths during tetanic contraction and at rest, respectively, and \( k \) is a scale factor. In this study, the value of \( k \) was determined to be 1.4, from the data of magnetic resonance imaging studies (35–37).

As with earlier studies (12, 33), we assumed negligible contributions of synergists and antagonists to the ankle joint torque during percutaneous electrical stimulation of the tibialis anterior muscle. At each of the five joint angles, the tendon force (i.e., the force transmitted through the tendon) was thus calculated by dividing the electrically evoked ankle joint torque by the estimated moment arm length of the tetanized tibialis anterior. This muscle force, defined as the contractile force acting parallel to the muscle fiber orientation, was then determined by dividing the tendon force by the cosine of pennation angle.

Statistics. Data are expressed as means and SD for all variables because the data distribution was not significantly different from a normal distribution (\( P > 0.14 \), one-sample Kolmogorov-Smirnov test). Repeatability of the two successive measurements was assessed in terms of an intraclass correlation coefficient (ICC), typical error, and a coefficient of variation. Reduced major axis regression (20, 50) was performed to demonstrate a relationship between contractile force and muscle shear modulus, as well as length-dependent changes in both variables. This method is considered statistically more appropriate than ordinary least squares regression when assuming errors in both the \( X \)-axis and \( Y \)-axis variables (50). Mechanical variables obtained in the MVC session were compared with those in the TC session, using two-way (session \( \times \) joint position) repeated-measures ANOVA and post hoc Student’s paired \( t \)-test with the false discovery rate procedure (9) when needed. We treated the joint position as an independent variable in the ANOVA model because the ankle joint angles (measured with the goniometer) during contractions were essentially the same between the TC and MVC sessions (see RESULTS). In addition, the dependence of voluntary activation level on joint position was tested by one-way repeated-measures ANOVA. In all statistical tests, \( P < 0.05 \) was considered significant.

RESULTS

The repeatability of joint torque, fascicle length, pennation angle, shear modulus, and voluntary activation level (MVC session only) between two successive contractions is summarized in Table 1. Except for the voluntary activation level, the ICC values indicated good to excellent repeatability, ranging from 0.76 (pennation angle) to 0.99 (joint torque). The relatively low ICC of voluntary activation level was due mainly to the “ceiling effect” associated with the interpolated twitch technique (45) rather than to the large variability. The values of typical error and coefficient of variation were consistently low. Accordingly, for these variables, the average value of the two successive contractions at the same joint position was used for further statistical analysis.

Table 2 summarizes the measured and calculated variables during tetanic contractions of the tibialis anterior muscle at five different ankle joint positions. As reported earlier (27, 33, 41),

| Table 1. Repeatability of measured variables between two successive contractions |
|---------------------------------|-----------------|-----------------|-----------------|
| ICC                              | Typical Error   | Mean CV, %       |
| Joint torque, N·m               | 0.99 (0.98–0.99) | 1.8 (1.6–2.1)   | 2.6 (2.1–3.2)   |
| Fascicle length, mm             | 0.89 (0.84–0.93) | 2.9 (2.5–3.4)   | 2.6 (2.1–3.1)   |
| Pennation angle, °              | 0.76 (0.66–0.84) | 1.6 (1.4–1.8)   | 6.5 (5.5–7.6)   |
| Shear modulus, kPa              | 0.80 (0.72–0.87) | 18 (15–21)      | 5.9 (4.8–7.0)   |
| Voluntary activation level, %   | 0.51 (0.26–0.70) | 5.4 (4.4–6.8)   | 2.8 (1.7–4.0)   |

Values in parentheses represent 95% confidence intervals. ICC, intraclass correlation coefficient; CV, coefficient of variation.

| Table 2. Variables determined during tetanic contractions |
|---------------------------------|----------------|----------------|----------------|
| Ankle Joint Position            | 1              | 2              | 3              | 4              | 5              |
| Joint angle, °                  | −16.7 ± 5.5    | −5.9 ± 7.8     | 4.2 ± 5.2      | 15.9 ± 6.8     | 25.9 ± 6.3     |
| Joint torque, N·m               | 15.7 ± 9.0     | 22.9 ± 10.9    | 24.4 ± 9.2     | 24.3 ± 11.7    | 21.7 ± 8.2     |
| Fascicle length, mm             | 45.8 ± 8.2     | 49.3 ± 7.4     | 54.8 ± 6.5     | 59.1 ± 5.8     | 63.3 ± 5.4     |
| Pennation angle, °              | 14.5 ± 2.9     | 14.0 ± 1.7     | 12.4 ± 2.2     | 10.8 ± 1.6     | 10.1 ± 2.0     |
| Tendon moment arm length, mm    | 34.0 ± 6.9     | 33.1 ± 6.5     | 31.5 ± 6.5     | 28.0 ± 6.3     | 24.5 ± 6.6     |
| Tendon force, N                 | 502 ± 376      | 739 ± 449      | 811 ± 353      | 915 ± 499      | 938 ± 388      |
| Muscle force, N                 | 518 ± 385      | 763 ± 466      | 832 ± 364      | 932 ± 509      | 954 ± 396      |
| Shear modulus, kPa              | 179 ± 31       | 182 ± 26       | 192 ± 27       | 206 ± 43       | 216 ± 22       |

Values are means ± SD. Positive joint angle denotes plantar flexion. Tendon moment arm length was estimated from tendon excursion during passive joint rotations and literature values.

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the tetanic torque-angle relationship of the tibialis anterior was bell-shaped with a broad peak between the neutral and plantar-flexed positions. The fascicle length increased continuously with plantar flexion, whereas the pennation angle decreased only slightly. The estimated moment arm length of the tibialis anterior tendon gradually decreased with plantar flexion. The tendon and muscle forces, calculated using the above-mentioned variables, increased by ~85% over the range of joint angles examined (from dorsiflexion to plantar flexion). The muscle shear modulus during contraction also increased as the ankle was plantar flexed.

As illustrated with group data of normalized length-force relationship (Fig. 5A), the muscle force measured during tetanic contraction appeared to increase with increasing muscle fascicle length. Regression analysis revealed a significant positive association of normalized muscle force with normalized fascicle length ($R^2 = 0.51, n = 45, P < 0.001$). The muscle shear modulus also depended on fascicle length (Fig. 5B). Regression analysis revealed a significant positive association of normalized muscle shear modulus with normalized fascicle length ($R^2 = 0.42, n = 45, P < 0.001$). As both muscle force and shear modulus showed similar length-dependent changes, we explored the association of these variables. The regression analysis on individual data revealed a significant association of muscle force and shear modulus in only three of nine participants. This was due to a small sample size and relatively low variations of muscle force and shear modulus within each participant. As for normalized group data (Fig. 6), there was a significant linear relationship between muscle shear modulus and muscle force ($R^2 = 0.52, n = 45, P < 0.001$).

Table 3 summarizes the measured variables during maximal voluntary contractions of dorsiflexor muscles at five different ankle joint positions. The two-way repeated-measures ANOVA revealed a significant interaction of “session” (TC and MVC sessions) and “joint position” on the joint torque ($P < 0.001$) and muscle shear modulus ($P = 0.045$), but not on the joint angle ($P = 0.61$), fascicle length ($P = 0.20$), or pennation angle ($P = 0.90$). The voluntary activation level did not differ across joint positions ($P = 0.50$, one-way repeated-measures ANOVA). As shown in Fig. 7, there was a difference in fascicle length-shear modulus relationship between the TC and MVC sessions. The post hoc multiple comparisons showed that, at dorsiflexed positions, the shear modulus measured in the TC session was significantly greater than that in the MVC session ($P = 0.005$ and 0.012 for positions 1 and 2, respectively).

**DISCUSSION**

To our knowledge, the present study is the first of its kind to determine muscle shear modulus in vivo during both tetanic and voluntary isometric contractions using a relatively new imaging technique (SSI) and, further, to explore the association of shear modulus with muscle fascicle length and contractile force. In connection with the length-force relationship of muscle, the length-shear modulus relationship would provide not only valuable information on the capacity of SSI to estimate individual muscle force, but also a clue to the understanding of structures and mechanisms responsible for the stiffness of an active human muscle. The results demonstrated the length dependence of muscle shear modulus, which was similar to that of contractile force (Fig. 5). The significant positive association between muscle force and shear modulus (Fig. 6) supports our hypothesis that length-dependent changes in the muscle contractile force would be reflected by the changes in muscle shear modulus. Nonetheless, the slope of length-shear modulus relationship was steeper in the MVC session than in the TC session (Fig. 7).

Several authors (5–7, 43, 49, 54) have recently used SSI to visualize and quantify the elastic properties of human muscle during contractions, but mostly in a limited range of intensities. This is mainly because, even during submaximal contractions, the muscle shear modulus often reaches the upper limit of measurement range of the SSI scanner. In this study, however, the shear modulus of the tibialis anterior muscle determined in both the TC and MVC sessions fell within the measurement range (0–267 kPa), so that the length-dependent changes can be detected (Fig. 7). This discrepancy may be due, at least in
part, to a large intermuscle difference in the shear modulus during contraction. In fact, Bouillard et al. (7) found that the contraction intensities achieved without reaching the upper limit (267 kPa) of shear modulus measurement were different between muscles: 39.1 ± 12.6% and 25.3 ± 4.2% of MVC for the first dorsal interosseous and the abductor digiti minimi, respectively. In a later study (5), the same group of authors reported a much higher value (58.3 ± 14.2% MVC) for the abductor digiti minimi, implying an interindividual difference in the muscle shear modulus. Also, a recent study on the biceps brachii muscle (54) showed that the shear modulus increased linearly with the contraction intensity (15–60% MVC) at a rate of 3.10 kPa/%MVC. These results suggest that, during voluntary contractions, such muscles become much stiffer than does the tibialis anterior muscle. On the other hand, Chernak et al. (8) reported that the average shear wave velocity of the human medial gastrocnemius muscle in the maximally contracted condition was 8.3 m/s. This value corresponds to a shear modulus of 69 kPa, being much lower than those of the tibialis anterior muscle. On the one hand, the results indicated that the fascicle length and fascicle length of the tetanized tibialis anterior muscle at five different joint angles.

The results indicated that both the force and fascicle length gradually increased with increasing of the degree of plantar flexion (Table 2 and Fig. 5A). Although we did not directly measure the moment arm length during contraction, but estimated it from the tendon excursion during passive joint rotations (25, 35) and literature values (35–37), a similar observation was made by Maganaris (33), who concluded that the tibialis anterior muscle in vivo operates in the “ascending limb” and “plateau region” of the length-force curve. The plateau region was not observed in our data, presumably because of the limited range of ankle joint angles examined. On average, 26° of plantar flexion was the most plantar-flexed position in this study, while the plateau region found by Maganaris (33) corresponded to 30–45° of plantar flexion.

A limitation of this study is that we used the stimulation with maximal tolerable intensity to induce tetanic contractions. In other words, the supramaximality, i.e., whether further increase in stimulation intensity resulted in further torque development, was not assessed. We considered the use of supramaximal muscle stimulation impractical because it would cause both coactivation of adjacent muscles (41) and intolerable pain or discomfort. Although the supramaximal nerve stimulation can be readily achieved, the stimulation of the common peroneal nerve will activate not only the tibialis anterior but also the peroneus longus and peroneus brevis muscles (27, 41). The muscle stimulation with maximal tolerable intensity would minimize the risk of current spread to adjacent muscles, as well as the pain.

Notwithstanding, our results (e.g., the absolute tetanic torque and the torque-angle relationship) are in line with those of earlier studies employing supramaximal stimulation of the common peroneal nerve (41, 44) and stimulation of tibialis anterior muscle belly with maximized torque output (12, 27, 33, 41). Isolated activation of the tibialis anterior muscle is suggested by the difference in dorsiflexion torque between electrically elicited and voluntary contractions (see Tables 2 and 3), given the contribution of the tibialis anterior to the total volume of dorsiflexor muscles (15). Therefore, we can assume that the length-force relationship of the tibialis anterior muscle determined in this study represents an intrinsic contractile property of muscle rather than the artifact of length-dependent change in muscle activation.

Length-dependent change in muscle shear modulus. The shear modulus of the tetanized tibialis anterior muscle increased with increasing fascicle length over the range of joint angles examined (Fig. 5B). As it has been suggested that the muscle shear-wave velocity or shear modulus is influenced by

Table 3. Variables determined during maximal voluntary contractions

<table>
<thead>
<tr>
<th>Ankle Joint Position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Joint angle, °</strong></td>
<td>-16.8 ± 3.9</td>
<td>-6.7 ± 5.9</td>
<td>3.0 ± 5.2</td>
<td>14.1 ± 7.2</td>
<td>25.8 ± 6.6</td>
</tr>
<tr>
<td><strong>Joint torque, N·m</strong></td>
<td>26.1 ± 9.5</td>
<td>38.2 ± 13.9</td>
<td>48.4 ± 12.6</td>
<td>52.9 ± 12.2</td>
<td>51.1 ± 10.0</td>
</tr>
<tr>
<td><strong>Fascicle length, mm</strong></td>
<td>48.7 ± 4.2</td>
<td>52.5 ± 7.4</td>
<td>55.2 ± 5.9</td>
<td>59.9 ± 5.8</td>
<td>61.6 ± 6.0</td>
</tr>
<tr>
<td><strong>Pennation angle, °</strong></td>
<td>15.4 ± 3.3</td>
<td>14.2 ± 2.8</td>
<td>13.3 ± 2.1</td>
<td>11.3 ± 1.7</td>
<td>11.0 ± 2.4</td>
</tr>
<tr>
<td><strong>Shear modulus, kPa</strong></td>
<td>132 ± 24</td>
<td>143 ± 29</td>
<td>169 ± 27</td>
<td>189 ± 24</td>
<td>203 ± 30</td>
</tr>
<tr>
<td><strong>Voluntary activation level, %</strong></td>
<td>93.4 ± 7.6</td>
<td>94.9 ± 4.2</td>
<td>91.3 ± 7.5</td>
<td>93.6 ± 8.1</td>
<td>91.4 ± 5.4</td>
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Values are means ± SD. Positive joint angle denotes plantar flexion.
the angle between fascicles and the probe face (16, 31), this length-shear modulus relationship may result from the change in pennation angle. However, we believe that the effect of pennation angle plays a minor role, because the change in pennation angle with fascicle length was quite small (a difference of 4.4° ± 2.3° between the most dorsiflexed and plantarflexed positions) and not correlated with the change in the absolute shear modulus (r = −0.06, n = 9, P = 0.87). Furthermore, the slope of length-shear modulus relationship differed between the TC and MVC sessions (Fig. 7), despite similar pennation angles.

It is well recognized that, at the cellular level, there is a close link between muscle contractile force and longitudinal stiffness (14). During muscle contraction in vivo, however, it remains unclear as to how the longitudinal stiffness is determined and associated with the contractile force. We found that the shear modulus was linearly related to the muscle force calculated from the results of mechanical and ultrasonographic measurements (Fig. 6). Importantly, this relationship was determined during electrically elicited contractions, where the muscle activation remained almost constant. Therefore, the result showed an association of force and shear modulus more directly than those in previous studies (5–7, 43, 54) using submaximal voluntary contractions, where both the number and average firing rate of muscle fibers activated vary with contraction intensity. Despite the differences in muscles examined and contraction types used, our results are generally consistent with those of Bouillard et al. (5, 7) and extend their findings that SSI can provide an indirect measure of individual muscle force.

The structures responsible for the muscle shear modulus along the longitudinal axis and the mechanisms underlying the increased shear modulus during contraction in vivo remain poorly understood. It has been shown that the muscle fiber stiffness is proportional to the number of attached cross bridges (14) and thus changes linearly with the force developed by the fiber (18). However, Julian and Morgan (26) showed that the single-fiber stiffness did not change in proportion with the force over the shallow ascending limb of the length-force curve, probably because of the compliance of the filament structure. Considering that we measured the shear modulus of the tibialis anterior muscle operating in the ascending limb, the linear force-shear modulus relationship found in this study may not be fully explained by the changes in the number of attached cross bridges. Another possible explanation for the increased shear modulus is the nonlinear elastic properties of connective tissue in series with muscle fibers, as the shear modulus also changes with passive muscle force (8, 28, 31). For instance, De Zee and Voigt (12) showed that the series elastic stiffness of human tibialis anterior muscle progressively increased with increasing force up to a maximum. A similar conclusion was drawn by examining load-elongation characteristics of the tibialis anterior tendon and central aponeurosis (39). However, these findings cannot be generalized to other muscles, because the stiffness of series elasticity typically reaches a constant value at a given level of voluntary force production (19, 30).

Difference in shear modulus between tetanic and maximal voluntary contractions. Unlike tetanic contractions induced by percutaneous muscle stimulation, voluntary contractions involve recruitment of multiple muscles that have different activation strategies and mechanical characteristics and thus contribute to joint torque in varying degrees. Under such natural conditions, it is extremely difficult to evaluate contractile properties of each individual muscle by measuring joint torque and muscle-tendon architecture. Moreover, recent studies (4, 21) have found evidence of in vivo intermuscular force transmission that may affect internal load distribution within and between muscles, although others have claimed that the effect is negligibly small in physiological conditions (47, 53). Therefore, an important question is whether the length-shear modulus characteristics determined using SSI are consistent between artificial (i.e., electrical stimulation of an individual muscle) and natural (i.e., voluntary contraction involving multiple muscles) conditions.

In the MVC session, the muscle shear modulus increased with fascicle length in a manner similar to that observed in the TC session (Fig. 7). This result suggests that SSI can assess the muscle shear modulus and its length dependence, irrespective of contraction mode. It should be noted, however, that the shear modulus measured at short fascicle lengths (i.e., at dorsiflexed positions) was significantly lower in the MVC session than in the TC session. This observation may not impair the validity of SSI to estimate individual muscle force during voluntary contractions, but rather reflect the actual difference in the contractile force between tetanic and voluntary contractions. Although one simple explanation for this difference could be the failure of voluntary drive, this is unlikely because the voluntary activation level was high and independent of ankle joint angles (Table 3). A more plausible explanation is associated with the difference in motor unit firing rates between conditions. In fact, De Luca and Hostage (11) indicated that the average motor unit firing rates in the tibialis anterior muscle during voluntary contractions fell within 5–30 Hz, while we used a pulse frequency of 80 Hz to maximize tetanic torque. The difference in firing rates has been shown to have a greater impact on force production at shorter
muscle length (1, 41) and thus may account for the differences in both muscle force and shear modulus at the short fascicle lengths. Future studies confirming the above explanation may extend the application of SSI to the complex human movements. Moreover, it is possible that spatial and temporal heterogeneity of muscle fiber activation during voluntary contraction plays a role in reducing the stress concentration and partly explains the difference in shear modulus between conditions. In this case, the measurement of muscle shear modulus during voluntary contractions may provide information on the risk of muscle-tendon injuries, although further extensive studies are needed to address this intriguing possibility.

Conclusions. In the present study, we found that the shear modulus of the tetanized human tibialis anterior muscle measured along the axis of contraction increased with increasing of both fascicle length and contractile force. The results also indicated the linear association between muscle force and shear modulus, providing novel evidence that length-force relationship, one of the most fundamental characteristics of muscle, can be inferred from in vivo imaging of shear modulus in the tibialis anterior muscle. Furthermore, a similar length dependence of muscle shear modulus was observed during maximal voluntary contractions in which neural and mechanical interactions of multiple muscles are involved. We believe that these findings have important implications for the application of SSI in neuromuscular research and also for understanding the mechanisms of increased muscle stiffness (shear modulus) during contraction in vivo.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: K.S. and N.I. conception and design of research; K.S. and S.T. performed experiments; K.S. and S.T. analyzed data; K.S., S.T., and N.I. approved final version of manuscript.

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