Active muscle stiffness in the human medial gastrocnemius muscle in vivo

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Kubo K. Active muscle stiffness in the human medial gastrocnemius muscle in vivo. J Appl Physiol 117: 1020–1026, 2014. First published August 28, 2014; doi:10.1152/japplphysiol.00510.2014.—The aims of this study were to 1) directly assess active muscle stiffness according to actual length changes in muscle fibers (fascicles) during short range stretching; and 2) compare actual measured active muscle and tendon stiffness using ultrasonography with the stiffness of active (i.e., muscle) and passive (i.e., tendon) parts in series elastic component of plantar flexors using the alpha method. Twenty-four healthy men volunteered for this study. Active muscle stiffness in the medial gastrocnemius muscle was calculated according to changes in estimated muscle force and fascicle length during fast stretching after submaximal isometric contractions (10, 30, 50, 70, and 90% maximal voluntary contractions [MVC]). Using the variables measured during this fast stretch experiment, the stiffness of active (i.e., muscle) and passive (i.e., tendon) parts in plantar flexors was assessed using alpha method. Tendon stiffness was determined during isometric plantar flexion by ultrasonography. Active muscle stiffness increased with the exerted torque levels. At 30, 50, 70, and 90% MVC, there were no significant correlations between muscle stiffness using ultrasonography and stiffness of active part (i.e., muscle) by alpha method, although this relationship at 10% MVC was significant (r = 0.552, P = 0.005). In addition, no correlation was noted in tendon stiffness between the two different methods (r = 0.226, P = 0.209). The present study demonstrated that ultrasonography could quantify active muscle stiffness in vivo. Furthermore, active muscle stiffness and tendon stiffness using ultrasonography were not related to active (i.e., muscle) or passive (i.e., tendon) stiffness in series elastic component of plantar flexors by alpha method.

METHODS

Subjects. Twenty-four healthy men (age: 22.2 ± 3.6 yr, height: 172.3 ± 5.5 cm, body mass: 66.4 ± 8.1 kg, mean ± SD) volunteered for this study. Subjects were physically active, but had not participated in any organized program of regular exercise for at least 1 yr before testing. They were fully informed of the procedures to be utilized, as well as the purposes of the study. Written, informed consent was obtained from all subjects. This study was approved by the Ethics Committee for Human Experiments, Department of Life Science (Sports Sciences), University of Tokyo.

Passive muscle stiffness during slow stretching. A specially designed dynamometer (Applied Office, Tokyo, Japan) was used to measure external torque and ankle joint angles. Before the measurements, subjects sat on a chair to acclimate to the laboratory conditions for 20 min. All measurements were performed on the right lower limb. Subjects lay prone on a test bench, and their waist and shoulders were secured by adjustable lap belts and held in position. The right ankle joint was set at 100° (with the foot perpendicular to the tibia = 90° with angles more than 90° being in plantar flexion) with the knee joint at full extension, and the foot was securely strapped to a foot plate connected to the lever arm of the dynamometer. Subjects did not warm up before the stretch maneuver. The platform of the dynamometer, which was attached to the sole of the subject’s foot, was moved from 100 to 80°, with a constant velocity of 5°/s. During slow stretching, subjects were requested to relax completely and not offer muscle, and the other a torque-independent component (i.e., tendon). Cook and McDonagh (3) on the first dorsal interosseous muscle and Svantesson et al. (31) on the plantar flexor muscles applied the alpha method with electrostimulation. Unfortunately, these studies using electrostimulation included some drawbacks, e.g., pain and low intensity of force exerted. To overcome these limitations, Foure et al. (5) adapted the alpha method during submaximal voluntary contractions [from 30 to 90% maximal voluntary contraction (MVC)], and demonstrated plyometric and eccentric training-induced changes in both active (i.e., muscle) and passive (i.e., tendon) stiffness in the series elastic component (4, 6). However, we must note the limitation of this technique. The alpha method assumed that tendon stiffness remained constant; however, this has been rejected by studies using ultrasonography (e.g., Ref. 18). Furthermore, previous findings indicated that changes in the joint angle did not necessarily correspond to those in muscle fiber length (e.g., Ref. 7). Therefore, length changes in muscle fibers need to be directly determined during short-range stretching to assess active muscle stiffness in vivo.

The aims of this study were to 1) directly assess active muscle stiffness according to actual length changes in muscle fibers (fascicles) during short range stretching; and 2) compare actual measured active muscle and tendon stiffness using ultrasonography with estimated active (i.e., muscle) and passive (i.e., tendon) stiffness in the series elastic component by the alpha method. I hypothesized that actual measured muscle and tendon stiffness using ultrasonography may not be related to the values calculated by the alpha method.
any voluntary resistance. To minimize thixotropic effects as preconditioning (12, 26), we collected data during the sixth cycle after five cycles. Passive torque (TQ) during slow stretching was detected by the dynamometer. The TQ measured by the dynamometer during slow stretch was converted to muscle force (Fm) using the following equation:

\[ Fm = k \cdot Ft \]

\[ Ft = TQ \cdot MA^{-1} \]

where \( Ft \) and \( k \) represent the tendon force and relative contribution of the physiological cross-sectional area of the medial gastrocnemius muscle within plantar flexor muscles (8), respectively, and \( MA \) is the moment arm length of the triceps surae muscles at 90° of the ankle joint, which is estimated from the lower leg length of each subject (10).

During slow stretching, a real-time ultrasonic apparatus (SSD-6500, Aloka, Japan) was used to record continuously longitudinal ultrasonic images of the medial gastrocnemius muscle. At 30% of the distance from the center of the malleolus lateralis to the articular cleft between the femur and tibia condyles, the scanning probe (7.5-MHz wave frequency with an 80-mm scanning length; UX5047-S, Aloka, Tokyo, Japan) of the apparatus was secured with adhesive tape on the skin. Ultrasonic images were recorded on a videotape at 30 Hz and synchronized with recordings of a clock timer for subsequent analysis. Fascicle length was defined as the distance between the insertion of the fascicle into the superficial and deep aponeurosis. In the present study, the fascicle length was measured five times for the same images. The average value of the three measurements, excluding the largest and the smallest values, was proposed and used as a representative value. The coefficient of variation of three measurements ranged from 0 to 4.1%.

TQ, joint angle, fascicle length, and electromyographic (EMG) activity in the triceps surae muscles (see below) were continuously recorded over the entire range of stretch maneuvers. The slope of the portion of the passive muscle force-fascicle length curve, from 100 to 92°, was defined as passive muscle stiffness.

The repeatability of passive muscle stiffness measurements was investigated on 2 separate days in a preliminary study with nine young men. No significant differences were observed between the test and retest values for passive muscle stiffness. The test-retest correlation coefficient (\( r \)) and coefficients of variance were 0.89 and 5.6%, respectively.

Active muscle stiffness using ultrasonography during fast stretching. The posture of the subject and setup were similar to that for the measurement of passive muscle stiffness, as described above. After a standardized warm-up, the subjects performed two or three isometric MVCs at 100° of the ankle angle. The peak torque was recorded in every trial, and the highest MVC value (121.0 ± 23.1 N·m) was used to determine the target torque during the short-range stretch experiment (see below). Maximum dorsiflexion was also performed at the same ankle angle to normalize the antagonist muscle activation with respect to MVC (see below).

After a 5-min rest period, subjects performed the short-range stretch experiment using a previously described procedure (5). The ankle ergometer was programmed to apply dorsiflexion stretches from 100 to 80°. Subjects were instructed to relax as soon as ankle motion was perceived. A 60-ms period after the stretch was analyzed, because this time period was chosen to avoid any potential neural effects (1, 2, 5). During this period (60 ms), the range of motion was ~8°, and the angular velocity reached ~250°/s (Fig. 1). Before the experiment, subjects performed a familiarization to the short-range stretch experiments at 50% MVC. An additional measurement was conducted two times at 0% MVC (relaxed condition) before the short range stretch experiment for data correction purposes (see below). The averaged torque during the relaxed condition (caused by inertia and passive elasticity) was subtracted from the measured torque during each of the active stretch trials (2). Short-range stretching was performed at five levels of the submaximal torque in a random order (two tests at each 20% MVC from 10 to 90% MVC) with the visual aid of exerted torque on an oscilloscope. The measured values were the means of two trials.

During the short-range stretch experiment, fascicle length in the medial gastrocnemius muscle was determined using a real-time ultrasonic apparatus (Fig. 2). Ultrasonic images were stored at 98 Hz in the computer memory of the apparatus (32). An electric signal was superimposed on the images to synchronize them to the torque, joint angle, and EMG activity (see below). The location of the probe, analysis of fascicle length, and calculated muscle force were similar to those for the measurement of passive muscle stiffness, as described above. The slope of the muscle force-fascicle length curve between 100 and 92° was defined as active muscle stiffness.

The repeatability of active muscle stiffness was investigated on 2 separate days in a preliminary study with nine young men. No significant differences were observed between the test and retest values of active muscle stiffness for the measured torque levels (10, 30, 50, 70, and 90% MVC). The test-retest correlation coefficient (\( r \)) and the coefficients of variance were 0.911 and 11.8% for 10% MVC, 0.870 and 10.4% for 30% MVC, 0.865 and 10.3% for 50% MVC, 0.888 and 10.9% for 70% MVC, and 0.846 and 10.5% for 90% MVC, respectively.

Muscle and tendon stiffness by the alpha method. Previous studies (3, 5, 23) reported that the alpha method could separate muscle-tendon complex stiffness into two components. Joint compliance (i.e., the inverse of joint stiffness) was considered as the compliance of two springs placed in series, one representing the compliance of a torque-dependent component (muscle stiffness), and the other a torque-independent component (tendon stiffness). The inverted force and
change in length (from a calculated change in length of 7 mm of the total muscle-tendon complex during 60 ms) divided by the change in force were plotted for each torque level (Fig. 1 of Ref. 3). The slope calculated from this plot was defined as passive part (i.e., tendon) stiffness. Active part (i.e., muscle) stiffness values at each torque level were calculated by subtracting tendon stiffness from the total stiffness of the muscle-tendon complex (3, 5, 23).

**Stiffness of tendon structures using ultrasonography.** The posture of the subject (except for the ankle angle) and procedure used were similar to those for the measurement of passive muscle stiffness, as described above. The right ankle joint was set at 90° (anatomical position) with the knee joint at full extension. Before the test, the subject performed a standardized warm-up and submaximal contractions to become accustomed to the test procedure. Subjects were instructed to develop a gradually increasing force from a relaxed state to MVC within 5 s. The task was repeated two times per subject with at least 3 min between trials. A real-time ultrasonic apparatus was used to obtain a longitudinal ultrasonic image of the medial gastrocnemius muscle during the contraction. The tester visually confirmed echoes from the aponeurosis and fascicles. The point at which one fascicle is identifiable as the diagonal striae—fascicle is attached to the aponeurosis was visualized on the ultrasonic image. This cross-point moved proximally during the development of isometric torque up to the maximum (Fig. 1 of Ref. 15). Displacement of the cross-point was considered to indicate the lengthening of tendon structures (deep aponeurosis and distal tendon) (16, 18).

Tendon displacement has been attributed to both angular rotation and contractile tension, because any angular joint rotation occurs in the direction of ankle plantar flexion during an “isometric” contraction (e.g., Ref. 22). To monitor ankle joint angular rotation, an electrical goniometer (Penny and Giles, Biometrics, Gwent, UK) was placed on the lateral aspect of the ankle. Additional measurements were made under passive conditions to correct the measurements taken for the elongation of tendon structures. Displacement of the cross-point caused by rotation of the ankle from 100 to 80° was digitized in the sonographs taken, as described above. Thus displacement of the cross-point obtained from the ultrasound images of each subject could be corrected for that attributed to joint rotation alone (e.g., Ref. 22). Only values corrected for angular rotation have been reported in the present study.

Torque measured by the dynamometer during isometric plantar flexion was converted to muscle force by the same procedure used to measure passive muscle stiffness (see above). In this study, muscle force and elongation of the tendon structures above 50% of MVC were fit to a linear regression equation, the slope of which was adopted as tendon stiffness (16, 18). The reliability of tendon stiffness measurements using ultrasonography has been confirmed in our previous studies (16, 18).

**EMGs.** EMG activity was recorded during the measurements of passive muscle stiffness (slow stretch), active muscle stiffness (fast stretch), and tendon stiffness (ramp isometric contraction). Bipolar surface electrodes (5 mm in diameter) were placed over the bellies of the medial gastrocnemius, lateral gastrocnemius, soleus, and tibial anterior muscles with a constant interelectrode distance of 25 mm. EMG signals were transmitted to a computer at a sampling rate of 1 kHz. EMG values were normalized to the values obtained during MVC for a 100-ms period before and after the stretch (4). EMG was also full-wave rectified and averaged over two different phases, a 60-ms period just before the stretch (mEMGa) and a 60-ms period after the stretch (mEMGb).

**Statistics.** Descriptive data included means ± SD. A one-way ANOVA was used to detect significant effects of torque level (%MVC) on increments in force, changes in fascicle length, and active muscle stiffness. If the F-statistic of the analysis of variance was significant, differences between means were assessed using the Tukey post hoc test. Before the ANOVA analysis, Mauchly’s sphericity test was performed to assess homogeneity of variance and covariance. Greenhouse-Geisser correction was used to adjust the degree of freedom, when sphericity assumption was violated. A linear regression analysis was performed on the relationship between the measured variables. The level of significance was set at \( P < 0.05 \).
RESULTS

The increase in torque during stretching was enhanced as the torque levels exerted became higher ($P < 0.001$, Fig. 3A). Changes in the fascicle length during stretching decreased slightly with increases in the exerted torque levels, with differences in fascicle length between 70 and 90% being significant ($P = 0.047$, Fig. 3B). Active muscle stiffness increased as torque levels exerted became higher ($P < 0.001$, Fig. 3C). The correlation coefficients tended to be higher for active muscle stiffness and changes in the fascicle length than active muscle stiffness and changes in torque at all exerted torque levels (Table 1). Active part (i.e., muscle) stiffness by the alpha method also increased as the torque levels exerted became higher ($P < 0.001$, Fig. 4).

No significant relationships were observed between active muscle stiffness using ultrasonography and active part (i.e., muscle) stiffness by the alpha method at 30, 50, 70, and 90% MVC, whereas this relationship at 10% MVC was significant ($r = 0.552$, $P = 0.005$; Fig. 5). No correlation was noted in tendon stiffness between the two different methods ($r = 0.226$, $P = 0.002$).

Table 1. Correlation coefficients between active muscle stiffness using ultrasonography and changes in torque and fascicle length

<table>
<thead>
<tr>
<th>%MVC</th>
<th>10% MVC</th>
<th>30% MVC</th>
<th>50% MVC</th>
<th>70% MVC</th>
<th>90% MVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in torque</td>
<td>0.594†</td>
<td>0.667‡</td>
<td>0.439*</td>
<td>0.574†</td>
<td>0.469*</td>
</tr>
<tr>
<td>Change in fascicle length</td>
<td>0.708‡</td>
<td>0.766‡</td>
<td>0.821‡</td>
<td>0.673‡</td>
<td>0.784‡</td>
</tr>
</tbody>
</table>

MVC, maximal voluntary contraction. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.

Fig. 3. Change in torque (A), change in fascicle length (B), and active muscle stiffness (C) during the short-range stretch experiment. MVC, maximal voluntary contraction. Values are means ± SD. Significant difference from the preceding torque level exerted: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Fig. 4. Active part stiffness by the alpha method during the short-range stretch experiment. Values are means ± SD. Significant difference from the preceding torque level exerted: *$P < 0.05$, ***$P < 0.001$.

Fig. 5. Relationships between active muscle stiffness using ultrasonography and active part (i.e., muscle) stiffness by the alpha method at each torque level exerted. A–E: 10, 30, 50, 70, and 90% MVC, respectively.

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Passive muscle stiffness did not correlate with active muscle stiffness at any force level (Table 2).

Short- and long-latency stretch reflexes could not be identified at any exerted torque level (Fig. 7). mEMGb was significantly higher than mEMGa at 10, 30, and 50% MVC (all \( P < 0.001 \)), while no significant differences were observed between mEMGa and mEMGb at 70 and 90% MVC (\( P = 0.065 \) and \( P = 0.169 \)) (Table 3).

**DISCUSSION**

The main finding of this study was that active muscle stiffness and tendon stiffness using ultrasonography were not related to active (i.e., muscle) or passive (i.e., tendon) stiffness in the series elastic component by the alpha method. To the best of our knowledge, this is the first study to assess active muscle stiffness according to actual length changes in fascicles during short-range stretching in vivo.

When muscle stiffness is evaluated under active conditions, the effects of the stretch reflex on the muscle stiffness measured needs to be considered. In the present study, changes in torque and fascicle length were determined when the ankle joint commenced movement and then 60-ms thereafter. This time period was chosen to avoid any potential neural effects (1, 2, 5). In the present study, the neural stretch reflex could not be identified at any of the exerted torque levels (Fig. 7). However, mEMG values for a 60-ms period after stretching (mEMGb) were significantly higher than those for a 60-ms period before stretching (mEMGa) at 10, 30, and 50% MVC, while no significant difference was observed between mEMGa and mEMGb at the higher force levels (70 and 90% MVC) (Table 3). These results implied that a short-latency stretch reflex has affected mEMG values, but it could not be confirmed visually. The neural stretch reflex response is known to be enhanced when the exerted force increases up to intermediate levels (e.g., Ref. 25). Moreover, previous studies demonstrated that stretch reflex-induced stiffness decreased at high force levels (1, 30). Based on these findings, the active muscle stiffness values obtained in the present study included the neural stretch reflex, they would decrease at the higher torque levels. On the other hand, the present results showed that both the increase in torque and active muscle stiffness was enhanced as the torque levels exerted became higher (Fig. 3, A and C). Therefore, the active muscle stiffness values obtained in the present study were not affected by any potential neural effects.

An interesting finding of this study was that no significant relationships were observed between active muscle stiffness using ultrasonography and that by the alpha method at each torque level, except for 10% MVC. These results indicated that changes in fascicle length could not be estimated from those in the joint angle due to tendon compliance beyond 10% MVC.

Human muscle stiffness in vivo has only been examined under passive conditions (11, 24). Using this technique, previous studies reported changes in the stiffness of the overall muscle-tendon complex and muscle during and after various stretching procedures (24, 27). Naturally enough, the measured “muscle stiffness” by this technique just represented the properties of the muscle under passive conditions, but not active conditions. “Passive” muscle stiffness is known to be influenced by a lengthening deformation in the connective tissues of the endomysium, perimysium, and epimysium in the muscle belly (9). Although all three components of the connective tissue in the muscle belly contribute to resistance when a muscle is passively stretched, the larger amount of the perimysium has been identified as the tissue that is the major contributor to extracellular passive resistance to stretch (28). In the present study, passive muscle stiffness did not correlate with active muscle stiffness at any torque level exerted (Table 2). Therefore, passive and active muscle stiffness clearly represented respective mechanical properties. In future studies, we can assess acute and chronic changes in the mechanical properties of the series elastic component (mainly active muscle stiffness) and parallel elastic component (mainly passive muscle stiffness) within muscles separately.

The alpha method developed by Morgan (23) assumed that tendon stiffness remained constant throughout the range of force. However, previous studies using ultrasonography rejected this assumption. For example, Kubo et al. (18) reported that the ratio of the estimated muscle force to tendon elongation at every 10% MVC increased curvilinearly with an increase in force. The present results revealed no correlation in tendon stiffness between the two different methods (Fig. 6).

**Table 2. Correlation coefficients between passive muscle stiffness and active muscle stiffness using ultrasonography**

<table>
<thead>
<tr>
<th>Torque Level</th>
<th>10% MVC</th>
<th>30% MVC</th>
<th>50% MVC</th>
<th>70% MVC</th>
<th>90% MVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passive muscle stiffness</td>
<td>0.062</td>
<td>0.199</td>
<td>0.240</td>
<td>0.252</td>
<td>0.203</td>
</tr>
</tbody>
</table>
Similarly, Foure et al. (5) inferred in a preliminary study that there was no significant correlation \((n = 32, P > 0.05)\) between these two parameters. As the reason for these discrepancies, Foure et al. (5) explained the differences in the assessed structures (i.e., tendon-aponeurosis structures of the medial gastrocnemius muscle for the ultrasonographic method, passive structures of the series elastic component for the alpha method) and stretching velocity (i.e., low velocity for the ultrasonographic method, high velocity for the alpha method). Furthermore, passive part (i.e., tendon) stiffness by the alpha method affected active part (i.e., muscle) stiffness by the alpha method because the former was used to calculate the latter.

In the present study, I must draw the attention to large differences in muscle and tendon stiffness values between the two methods. The reason for these differences was attributed to the difference in calculation of force. I used the estimated muscle (medial gastrocnemius muscle) force for the active muscle stiffness and tendon stiffness using ultrasonography, because I investigated the fascicle length of the medial gastrocnemius muscle and elongation of its aponeurosis (including outer tendon). On the other hand, the previous studies using the alpha method used the tendon force (i.e., force exerted by the whole plantar flexor muscles) to calculate the active (i.e., muscle) and passive (i.e., tendon) stiffness in the series elastic component.

Table 3. Electromyographic activities of the plantar flexors 60 ms before and after stretching

<table>
<thead>
<tr>
<th></th>
<th>10% MVC</th>
<th>30% MVC</th>
<th>50% MVC</th>
<th>70% MVC</th>
<th>90% MVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>mEMGa</td>
<td>0.025 (0.013)</td>
<td>0.056 (0.026)</td>
<td>0.093 (0.036)</td>
<td>0.159 (0.083)</td>
<td>0.218 (0.101)</td>
</tr>
<tr>
<td>mEMGb</td>
<td>0.037 (0.017)*</td>
<td>0.067 (0.029)*</td>
<td>0.110 (0.046)*</td>
<td>0.169 (0.073)</td>
<td>0.228 (0.098)</td>
</tr>
</tbody>
</table>

Values are means (SD) in mV. mEMGa and mEMGb: electromyographic activities 60 ms before and after stretching, respectively. *Significantly different from mEMGa: \(P < 0.001\).
In addition, there may be the heterogeneity of changes in fascicle length within the muscle. In the present study, the fascicle length was measured five times for the same images. This procedure has been adopted in many previous studies concerning the dynamics of human fascicle during exercises (e.g., Refs. 17, 20). As far as we know, no reports have shown site differences in fascicle length changes during dynamic exercises. Kawakami et al. (14) reported that the fascicle length was almost uniform throughout the human medial gastrocnemius muscle, in both relaxed and submaximal isometric-contracted conditions. Therefore, I believed that the measured fascicle length in this study represented the dynamics of all fascicles within the medial gastrocnemius muscle.

In conclusion, the present study demonstrated that ultrasonography could quantify active muscle stiffness in vivo. Active muscle stiffness as assessed using ultrasonography could represent a new index of physical resources. Furthermore, the present method, as well as the measurement of tendon properties by ultrasonography (e.g., 16), could be used to assess the relationship between sport performance and muscle-tendon properties and their plasticity to various interventions.

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GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: K.K. conception and design of research; K.K. prepared figures; K.K. drafted manuscript; K.K. edited and revised manuscript; K.K. approved final version of manuscript.

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