Nonisometric behavior of fascicles during isometric contractions of a human muscle

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Ito, Masamitsu, Yasuo Kawakami, Yoshiho Ichinose, Senshi Fukashiro, and Tetsuo Fukunaga. Nonisometric behavior of fascicles during isometric contractions of a human muscle. J. Appl. Physiol. 85(4): 1230–1235, 1998.—Fascicle length, pennation angle, and tendon elongation of the human tibialis anterior were measured in vivo by ultrasonography. Subjects (n = 9) were requested to develop isometric dorsiflexion torque gradually up to maximal at the ankle joint angle of 20° plantarflexion from the anatomic position. Fascicle length shortened from 90 ± 7 to 76 ± 7 (SE) mm, pennation angle increased from 10 ± 1 to 12 ± 1°, and tendon elongation increased up to 15 ± 2 mm with graded force development up to maximum. The tendon stiffness increased with increasing tendon force from 10 N/mm at 0–20 N to 32 N/mm at 240–260 N. Young’s modulus increased from 157 MPa at 0–20 N to 530 MPa at 240–260 N. It can be concluded that, in isometric contractions of a human muscle, mechanical work, some of which is absorbed by the tendinous tissue, is generated by the shortening of muscle fibers and that ultrasonography can be used to determine the stiffness and Young’s modulus for human tendons.

ultrasound; tendon; stiffness; Young’s modulus

IN MOST SKELETAL MUSCLES, fibers attach to tendon plates, or aponeuroses, which then become more rounded tendons that extend off the muscle. When considering the joint action, which is the result of muscle fiber activity, the interaction between muscle fiber and tendons cannot be neglected. There have been some reports concerning tendon properties of human cadavers (16, 20). However, tendon properties have been mainly determined in animal experiments (13, 14, 21, 24, 25) because it has been impossible to measure directly in humans in vivo the length-tension relationship from which the elastic properties of tendon can be observed. Isolated tendon properties have been determined in detail biomechanically (1, 13, 21, 25). On the other hand, Hoffer et al. (9) and Griffiths (7) experimentally showed muscle shortening during tendon lengthening, thus indicating that it is not appropriate to consider either muscle or tendon properties in isolation when trying to understand their physiological roles. It has been also reported that tendons have a safety factor of from 5 to 30 (14, 16) and are highly nonlinear in their biomechanical behavior (1). The exact knowledge of length-tension characteristics of human tendons under physiological conditions will help us to understand the interaction between muscle fibers and tendons in human movements.

Force exerted by muscle fibers stretches compliant tendons before it is transmitted to the bone. Thus the length change in muscle fiber is not necessarily the same as that of the muscle-tendon complex. Isometric contraction literally means no change in muscle (fiber) length during contractions. However, because of tendon elastic properties, there could be some shortening of muscle fibers and lengthening of the tendon even during isometric contractions, i.e., when the total length of the muscle-tendon complex is kept constant. In fact, it has been confirmed in animal muscles that muscle fiber or sarcomere length changes in isometric contractions (2, 7, 17). During isometric contractions of hamster diaphragm muscle, sarcomere length decreased by up to 5% of its resting length value, while muscle length did not change (2). Muhl (17) demonstrated the active length-tension relationship, showing that the muscle fiber actually shortened during isometric contractions in rabbits. Griffiths (7) reported that muscle fibers of cats shortened by as much as 28% at the expense of the tendon during isometric tetanic contractions. These reports imply that muscle fibers produce mechanical work when there is no apparent work done by the whole muscle-tendon complex. This would happen in the muscle-tendon complex of humans, although experimental results focused on this matter have been lacking.

Recently developed imaging techniques, such as magnetic resonance imaging and ultrasonography, have made it possible to visualize muscle and tendon tissues in living humans. According to previous reports (5, 8, 12, 18), ultrasonography is a valid method for the measurement of muscle architecture (fascicle length (L_f) and pennation angles (θ)) as well as for the determination of tendinous movement during contractions (6). Fukashiro et al. (4) have recently reported the lengthening of the tendon during isometric ramp contractions, showing the possibility of observation of tendon characteristics in humans in vivo. By using ultrasonography, the behavior of fascicles and tendinous tissue in a human muscle in vivo could be observed quantitatively. However, those studies dealt predominantly with one of θ, fiber length, and tendinous movement or length change of the tendon, although these variables are dependent on each other.

In this report, we have studied by means of ultrasonography the relationships between architectural parameters, i.e., L_f and θ, and level of force exerted. We have also determined the stiffness and Young’s modulus for the human tendon by measuring tendon elongation (ΔX) during isometric contractions in vivo. The data presented here can give insights into in vivo behavior of human muscle fibers and tendons, as well as interactions between them.

METHODS

L_f, θ, and ΔX were measured during isometric contractions. Subjects were nine healthy volunteers (7 men and 2 women).
All subjects participated in $\Delta x$ measurements, and only six of them participated in $L_f$ and $\theta$ measurements. Their physiological characteristics are summarized in Table 1. After a detailed explanation of the purpose, procedure, and possible risks associated with the experiment, these individuals gave their informed consent. This study was approved by the ethical committee at the University of Tokyo.

The experimental setup is schematically shown in Fig. 1. A dynamometer (Myoret RZ-450; asics, Tokyo, Japan) was used to fix the ankle joint angle at 20° plantarflexion from the anatomic position and measure dorsiflexion torque (resolution $\pm$ 1.96 N·m). This ankle angle was selected because the average value of maximal voluntary contraction for all subjects was largest at this angle. Each subject lay supine on a bed, and the center of rotation of the dynamometer was visually aligned with the center of rotation of the ankle joint. The right foot was firmly attached to the footplate of the dynamometer with a strap. Subjects were instructed to develop gradually increasing isometric dorsiflexion torque up to their maximal efforts within 6 s, with a visual aid of the developed torque on an oscilloscope. Three trials were made for each subject, with a 2-min rest between trials. The measured values that are shown below are the means of these three trials.

An ultrasonic apparatus (SSD-2000; Aloka, Tokyo, Japan) with an electronic linear-array probe (5-MHz wave frequency with 80-mm scanning length; UST 5047-5, Aloka) was used to obtain sectional images of the tibialis anterior muscle (TA). The width and depth resolutions of ultrasonography with this probe are 1 and 0.62 mm, respectively. The probe was placed on the anterior aspect of the lower leg at 40% of the distance distal from the popliteal crease to the lateral malleolus, and longitudinal sections of TA were imaged (Fig. 2). The investigator visually confirmed the echoes reflected from the aponeuroses and interspaces among fascicles in TA on the ultrasonic images. They were displayed on a real-time basis on a monitor and recorded on videotape that was synchronized with a clock timer for subsequent analyses.

The TA is a bipennate muscle, the proximal end of which arises from the lateral condyle and superolateral shaft of the tibia, and inserts on the medial cuneiform and the base of the first metatarsal after passing under the extensor retinaculum (Fig. 1). Parallel echoes running diagonally represent the collagen-rich connective tissue between fascicles. The darker areas between the bands of echoes represent the fascicles (see Fig. 2 and Ref. 8). The echo that runs longitudinally in the middle of TA is from the central aponeurosis. The $\theta$ was defined as the angle between the aponeurosis and the tangent of fascicles at the points of attachment onto the aponeurosis; the angle was measured by using a motion-analysis system (Dig-98; DITECT, Tokyo, Japan). The length of the echo from the central aponeurosis to the proximal aponeurosis was considered the $L_f$ (5), which was measured with a digital curvimeter (Comcurve-8, Koizumi; Tokyo, Japan). The cross point of ultrasonic echoes from a fascicle and the central aponeurosis ($x$) was defined as the position where the fascicle attached to the aponeurosis, and the distance traveled by $x$ ($\Delta x$) was considered as the length change of tendon and aponeurosis during contraction (Fig. 2). Measurements of $\theta$, $L_f$, $\Delta x$, and $\Delta L_f$ were analyzed by Wilcoxon signed rank test.

### Table 1. Physical characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE</th>
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</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>31.7 ± 3.7</td>
<td>Body Height, cm</td>
<td>168.1 ± 2.0</td>
<td>Body Mass, kg</td>
<td>64.6 ± 2.5</td>
</tr>
<tr>
<td>Lower Leg Length, cm</td>
<td>38.0 ± 2.5</td>
<td>Foot Length, cm</td>
<td>24.8 ± 0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Schematic representation of experimental setup. A dynamometer was used to fix the ankle joint angle at 20° plantarflexion from the anatomic position and measure dorsiflexion torque. Right foot was firmly attached to the footplate of the dynamometer with a strap. An ultrasonic apparatus with an electronic linear-array probe was used to obtain sectional images of the tibialis anterior muscle (TA). Torque and ultrasonic data were synchronized by a timer. PC, personal computer; VTR, videotape recorder.

Fig. 2. Longitudinal ultrasonic images of TA (right, proximal) at 20° plantarflexion under relaxed (top) and moderately tensed (bottom) conditions. Subcutaneous adipose tissue, muscle, and central aponeurosis are shown. Fascicle length ($L_f$) was measured as length of the echo from central aponeurosis to proximal aponeurosis. Pennation angle ($\theta$) was defined as angle between lines of fascicles and aponeurosis. $L_f$ decreased and $\theta$ increased with application of force. Cross point of ultrasonic echoes from fascicle and central aponeurosis ($x$) was defined as position where fascicle attached to aponeurosis, and distance traveled by $x$ ($\Delta x$) was defined as length change of tendon and aponeurosis during contraction.
Lf and \( \Delta x \) were repeated five times on each ultrasonic image, and the average of the three measurements (after the largest and smallest values were excluded) was used as a representative for the image. The coefficients of variation of these measurements were 1–2%. Coefficients of variation of the three trials for \( \text{Lf} \) and \( \theta \) measurements were 2 and 7%, respectively.

When the force applied to the tendon (\( F_t \)) and the muscle force in the direction of the fascicle (\( F_m \)) were calculated, the following were assumed: 1) 49.8% of measured dorsiflexion torque was developed by TA based on physiological cross-sectional area (CSA; 26), 2) \( \theta \) of all fascicles at both sides of central aponeurosis were identical, and 3) \( \text{Lf} \) was identical throughout the muscle. Then \( F_t \) and \( F_m \) were calculated as

\[
F_t = \frac{\tau}{r} \times 0.498
\]

\[
F_m = \frac{F_t}{\cos \theta}
\]

where \( \tau \) and \( r \) represent measured dorsiflexion torque and the moment arm of TA, as reported by Rugg et al. (22), respectively.

Cross-sectional images of the distal tendon of TA were taken at two positions (one under the retinaculum and the other at 3 cm proximal from the retinaculum; Fig. 1), by using ultrasonography with a 7.5-MHz probe (UST 5710–7.5; Aloka). The width and depth resolutions of ultrasonography with this probe are 0.67 and 0.4 mm, respectively. CSAs of the tendon were measured in five different images at each position by using NIH Image (National Institutes of Health, Bethesda, MD) (Fig. 3). The average of three values (after the largest and smallest values were excluded) was calculated at each position. The average of CSAs at two positions was calculated as the representative of distal tendon CSA. Stress applied on the tendon was then estimated from \( F_t \) and CSA of the distal tendon of TA (\( F_t/\text{CSA} \)). Strain was also estimated from \( \Delta x \) and the initial length of the distal tendon (\( \Delta x/\text{initial tendon length} \)), which was estimated over the skin as the distance between the base of the first metatarsal and point \( x \).

To investigate the effect of force (10 levels) on \( \theta \), \( L_f \), and \( \Delta x \), one-way analysis of variance with repeated measures was done.
used. For those variables for which a significant effect was found, a Tukey post hoc test was used. A P < 0.05 level of confidence was set for all analyses.

RESULTS

From the ultrasonic image, it was observed that $L_f$ and $\theta$ changed during an isometric contraction of TA. $L_f$ during relaxation was $90 \pm 7$ (SE) mm. Changes in $L_f$, $\theta$, and $\Delta x$ were expressed as a function of relative torque (Fig. 4, n = 6 subjects). Each plot was the average over 10% of relative torque, i.e., 0–10 (plotted at 5%), 10–20 (plotted at 15%),... 90–100% (plotted at 95%). When the dorsiflexion torque increased, $L_f$ decreased from 87 ± 7 to 76 ± 7 mm, $\theta$ increased from 10 ± 1 to 12 ± 1°, and $\Delta x$ increased to 15 ± 2 mm. Changes in these three variables were curvilinear in fashion. Significant decreases in $L_f$ relative to 5% value were found over 15% torque levels. Significant increases in $\Delta x$ and $\theta$ relative to 5% values were also found over 25% torque levels. Increase in dorsiflexion torque was accompanied by shortening of fascicles and lengthening of the tendon and aponeurosis; this indicated that the muscle fibers produced mechanical work which was absorbed by the tendon even in the so-called isometric contractions. The mechanical work done by the muscle fiber, which could be calculated from change of $L_f$ and $F_m$, was $3.4 \pm 0.4$ J.

The tendon stiffness, i.e., $F_t$ for a given $\Delta x$, was related to $F_t$, (Fig. 5, n = 9 subjects). $F_t$ and $\Delta x$ were averaged over 20 N up to 260 N, i.e., >70% of their maximal $F_t$. For this condition, the tendon stiffness increased with increasing $F_t$ from 10 N/mm at 0–20 N to 32 N/mm at 240–260 N (Fig. 6). Estimation of initial tendon length (0.35 ± 0.01 m) and measurement of tendon CSA (21 ± 1 mm²) provided the stress-strain relationship of the tendon. It was calculated that the tendons were stretched at the mean strain rate of ~0.7%/s. Young's modulus increased from 157 MPa at 0–20 N to 530 MPa at 240–260 N (Fig. 6). Changes in stiffness and Young's modulus were larger in lower force regions.

DISCUSSION

The authors have recently described a method of using ultrasonography to determine architectural parameters of in vivo human muscles such as $\theta$ and $L_f$ (5, 12). With this method, one can observe changes in muscle architecture during contractions (4–6, 8, 18). In those studies, it was shown that the behavior of a muscle in terms of its architecture is different when the muscle is actively contracting compared with the resting condition (5, 18). In the present study, we observed shortening of fascicles and lengthening of the tendon during isometric contractions at a fixed joint. The fascicle shortened as much as 14 mm (16%) when the muscle was maximally activated. Because the muscle-tendon complex consists of contractile component (CC) and the elastic component (EC) (27), shortening of CC would have stretched the EC during isometric contractions. The change in $L_f$ would have been predominantly caused by the shortening of the distal tendon and distal aponeurosis. Slackness and compliance of the tendinous structure have been shown to allow $L_f$ and $\theta$ changes to occur during isometric contraction (11). The internal shortening of muscle fibers could cause a difference in the length-force relationships in active and passive conditions (17). Griffiths (7) used an ultrasound transit-time technique to measure the fiber length and found that muscle fibers in the medial head of the gastrocnemius muscle of cats shortened by 28%

Table 2. Tendon stiffness and Young's modulus observed in present study compared with data from previous reports

<table>
<thead>
<tr>
<th>Researchers</th>
<th>Ref. No.</th>
<th>Species</th>
<th>Muscle Used</th>
<th>Stiffness, N/mm</th>
<th>Young's modulus, MPa</th>
<th>Tendon Length, mm</th>
<th>Tendon CSA, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woo et al.</td>
<td>25</td>
<td>Swine</td>
<td>Digital extensors</td>
<td>65†</td>
<td>800†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ker</td>
<td>13</td>
<td>Sheep</td>
<td>Plantaris</td>
<td>1,650</td>
<td>25</td>
<td>10</td>
<td>1.84</td>
</tr>
<tr>
<td>Rack and Westbury</td>
<td>21</td>
<td>Cat</td>
<td>Soleus</td>
<td>450</td>
<td>10</td>
<td>2.11</td>
<td>0.118</td>
</tr>
<tr>
<td>Lieber et al.</td>
<td>15</td>
<td>Frog</td>
<td>Semitendinosus</td>
<td>10.5*</td>
<td>188</td>
<td>10.34</td>
<td>0.98</td>
</tr>
<tr>
<td>Treistik and Lieber</td>
<td>24</td>
<td>Frog</td>
<td>Gastrocnemius</td>
<td>146.7*</td>
<td>1,548</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rack and Ross</td>
<td>20</td>
<td>Human (cadaver)</td>
<td>Flexor pollicis longus</td>
<td>92†</td>
<td>1,017†</td>
<td>100</td>
<td>9.1</td>
</tr>
<tr>
<td>Loren and Lieber</td>
<td>16</td>
<td>Human (cadaver)</td>
<td>Wrist muscles</td>
<td>34.2–261.2*</td>
<td>438.1–726.1</td>
<td>47.0–182.1</td>
<td>14.2–27.4</td>
</tr>
</tbody>
</table>

CSA, cross-sectional area. *Calculated from Young's modulus, cross-sectional area, and length of tendon; †read (and calculated) from original plots.
during maximal isometric muscle contractions. The findings in this study are in agreement with those of the previous animal studies: there is a shortening of muscle fibers within the muscle-tendon complex, even during isometric contractions. The magnitude of internal shortening might differ between muscles, possibly due to architectural variations of the muscle-tendon complex. The architectural parameter that is a good predictor of sarcomere shortening is the tendon length-to-fiber length ratio (27). The larger this ratio is, the more compliant the muscle-tendon complex is, i.e., sarcomeres can shorten more during isometric contractions.

This is the first report that introduces in vivo determination of length-tension relation, stiffness, and Young's modulus of a living human tendon. The tendon stiffness and Young's modulus are summarized with data from previous reports in Table 2. The stiffness and Young's modulus obtained in this study were in the range previously reported for animals or human cadavers. In the present study, $F_t$ were estimated from dorsiflexion torque ($\tau$), physiological CSA ratio of TA to all the dorsiflexors, and previously reported moment arm ($r$, see Ref. 22). We used one moment arm length for all subjects, despite the fact that there must be variation in moment arms among subjects. Further research determining individual moment arms is necessary to calculate accurately the $F_t$ from measured torque. CSA used for stress calculations was of the tendons, whereas the $\Delta x$ used for strain calculations included lengthening of the aponeurosis as well as that of the tendon. The aponeurosis is reported to be more compliant than the tendons (15). Therefore, $\Delta x$ and strain presented here might be overestimated, and thus the stiffness and Young's modulus could be underestimated.

The tendon stiffness increased with increasing tendon force from 10 N/mm at 0–20 N to 32 N/mm at 240–260 N (Fig. 6). This is in accordance with previous reports (13–16, 20, 21, 24, 25). This means that the tendon is stretched more easily when the force is small and becomes less compliant with increment of force. The stiffness and Young's modulus tended to increase, although the changes in their values became less as $F_t$ increased. Tendons use their toe region of length-tension relationship in vivo. This means that, in physiological conditions, tendons are not subjected to the extreme strain which causes tendon failure. Stiffness and Young's modulus, which was obtained from experiments conducted to failure, were not suitable for consideration in regard to human movements. Contrary to the results of the present study, Griffiths (7) reported that there was a linear relationship between muscle fiber length and force during isometric muscle contractions of the cat gastrocnemius. However, considering the curvilinear nature of the length-tension relationship of the tendon, the smaller stiffness should allow larger change in fiber length at lower force. This discrepancy might be explained by the difference in initial tendon length. The $\theta$ change might also play a role in this matter. Although $\theta$ was not measured in that study, larger change in $\theta$ during an early stage of contraction probably attenuates length change of fibers.

Only a part of the force developed by muscle fibers is transmitted to the tendon by a factor of the cosine of the $\theta$. Thus the force transmission is less effective when the $\theta$ is larger. According to the present results, 1.4 and 2.2% of the force cannot be used in the longitudinal direction of the tendon in the relaxed and the maximal condition, respectively. The fascicle angle with respect to the aponeurosis has been referred to as $\theta$ in the present study as well as in the previous reports (5, 12, 18). When considering the geometry of the muscle-tendon complex, $\theta$ actually means the fascicle (fiber) angle with respect to the line of action of the muscle. These two angles may not be the same, because the aponeurosis angle with respect to the line of pull of the muscle is not taken into consideration in the former (10). It is presently impossible to determine the line of pull of the muscle in vivo.

Architectural data for human muscle have been collected mainly from cadaver experiments (26). Some have incorporated muscle architecture in muscle models to determine muscle functions (19). However, cadaver material cannot avoid morphological changes caused by fixation and treatment procedures (3, 23). Furthermore, measurements have been done under fixed conditions, with no activation of muscle fibers. As shown in the present study, $L_s$ shortened by 16% and $\theta$ increased by 20%, even during isometric contractions when TA was gradually contracted up to maximal effort. These results show that fibers produce work in spite of no work in the whole muscle-tendon complex. It is also concluded that tendons use the toe region of their length-tension relationship in physiological conditions, and it is necessary that stiffness and Young's modulus for human tendons should be determined in vivo.

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