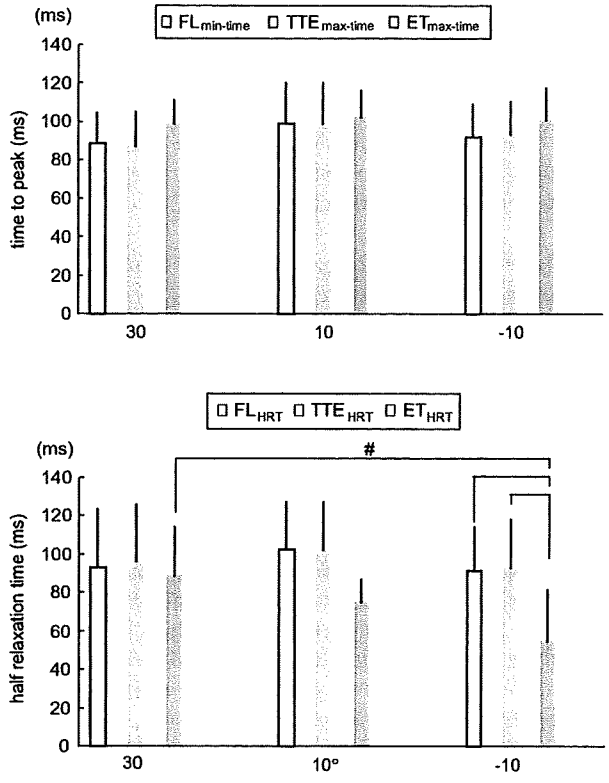

Fig. 2. Time to peak and half relaxation time of fascicle length (FL), tendinous elongation (TTE) and external torque (ET). #Significant difference ($p<0.05$).

Table 1
Descriptive data for the parameters of fascicles and tendinous tissues

Fascicles	30°		10°		−10°	
Resting FL (mm)	70.8	(6.0)	60.8	(4.5)#	54.8	(7.3)#
FL_{min} (mm)	66.2	(6.1)	55.7	(4.8)#	49.8	(7.5)#
Maximal FV (mm/s)	79.1	(17.4)	85.4	(16.6)	85.6	(18.3)
Resting PA (°)	11.0	(1.4)	12.8	(2.2)	14.5	(2.9)#
Maximal PA (°)	11.8	(1.4)	14.1	(2.3)	16.2	(3.7)#
Maximal FF (N)	53.9	(26.7)	38.2	(12.3)	16.9	(9.7)#
Tendinous tissues						
TEE_{max} (mm)	4.7	(1.2)	5.3	(0.9)	5.3	(1.0)
Maximal TTV (mm/s)	81.9	(17.9)	89.0	(16.9)	91.5	(19.5)
Maximal TF (N)	52.8	(26.3)	37.14	(12.1)	16.3	(9.3)#

Abbreviation: FL, fascicle length; FL_{min}, minimal fascicle length; FV , fascicle velocity; PA, pannation angle; FF, fascicle force; TEE_{max}, maximal elongation of tendinous tissues; TTV, velocity of tendinous tissues elongation; TF, tendon force.

#Significant difference compared to data at 30°: $p<0.05$.

resting PA and maximal PA differed between the 30° and −10° conditions. There were no significant differences between joint angles for TTE_{max}, maximal FV and maximal TTV. FF_{max} and TF_{max} were each significantly greater at 30° compared with −10°, and non-significantly greater than 10°.

3.3. Length–velocity–force properties

During the shortening phase of fascicles, the length–force properties of both fascicles (Fig. 3) and TT (Fig. 4) were curvilinear, while the velocity–force properties showed a loop-like pattern in all joint angles. The maximal force and absolute values of slopes of quasi-linear part in the length–force properties of both fascicles and TT, except for the first from 10 to 30 ms in which no force output occurred, tended to be greater in the plantar flexed positions than in the dorsi-flexed position (−15.0 N/mm at 30°, −12.2 N/mm at 10° and −5.9 N/mm at −10° for fascicles, and 14.3 N/mm at 30°, 11.7 N/mm at 10° and

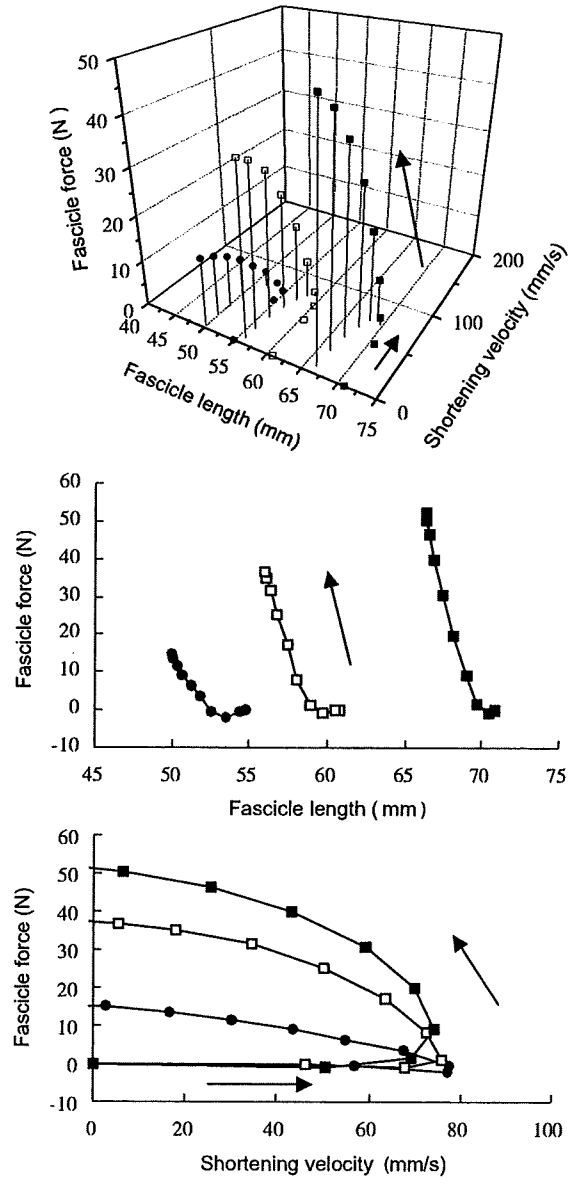

Fig. 3. Fascicle length–velocity–force property during twitch contraction (upper panel). Middle and bottom panels indicate the projections of length–force plane and velocity–force plane, respectively [(■) 30°, (□) 10°, and (●) −10°].

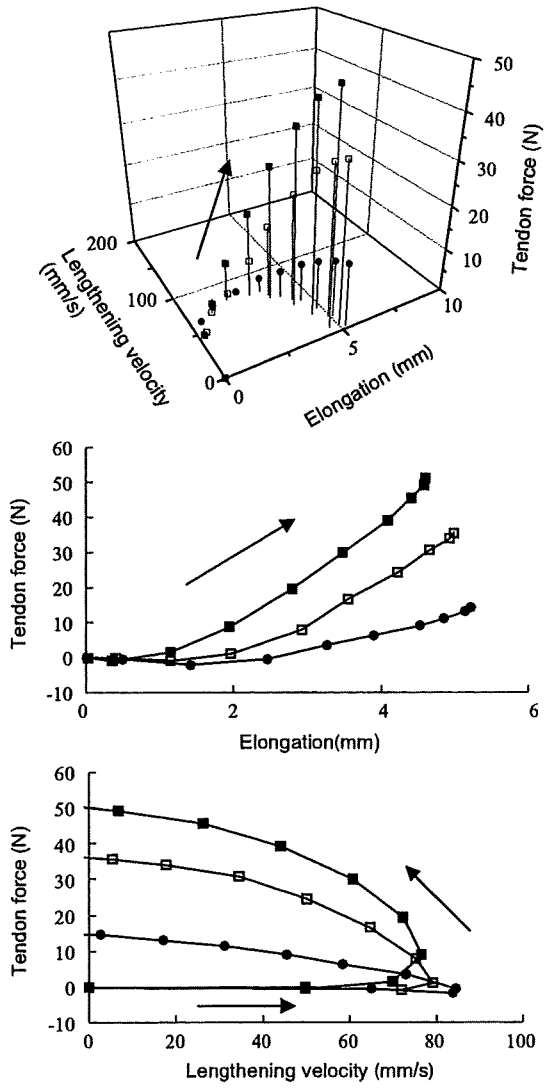

Fig. 4. Tendinous tissues length (elongation)–velocity–force property during twitch contraction (upper panel). Middle and bottom panels indicate the projections of length–force plane and velocity–force plane, respectively [(■) 30°, (□) 10°, and (●) −10°].

5.4 N/mm for −10° for TT). With regard to velocity–force properties, the time course of FV and TTV did not differ significantly between joint angles, although large FF and TF were observed in the plantar flexed position. As a result, larger power (force × velocity) was developed over the range of velocities in the plantar flexed position (mean power: 0.80 W at 30°, 0.49 W at 10°, 0.14 W at −10° for fascicles; and 0.81 W at 30°, 0.48 W at 10°, 0.14 W at −10° for TT).

4. Discussion

There are four key findings from the data collected. First, the time from stimulus to peak value did not differ between torque and length parameters both for fascicles and TT. Second, there was a delay of 10–30 ms between the onset of length change in each fascicles and TT, and the development of ET. Third, FL_{HRT} and TTE_{HRT} were significantly longer than the ET_{HRT} at −10°. Fourth, each time course of TTE, FV and TTV was similar between joint angles. Moreover, the curvilinear length–force properties and loop-like pattern velocity–force properties were observed both for the fascicles and TT.

4.1. The time course of torque and length changes in fascicles and tendinous tissues

In the present study, the time from stimulus to peak value did not differ between torque and length parameters. On the other hand, the 10–30 ms time delay in torque generated in the presence of fascicle shortening could to be due to the slack of TT (Ito et al., 2000; Muraoka et al., 2004). Concomitantly, slack TT has a very low passive tension (Muraoka et al., 2004). This would account for the early appearance of maximal FV (average 33 ± 16 ms), since large passive tension can become a mechanical resistance to fascicle shortening. If that is the case, relatively greater slack in the dorsi-flexed position would induce more delay. However, with the ultrasonography sampling frequency being relatively low (96.37 Hz), these small differences between the joint angles during the period from onset of length changes to force onset may not have been detected. In addition, because of the curvilinear length–force relations of both fascicles and TT, we expected that the half relaxation time of force and length is not equal. Indeed, we found the disparity that was greatest in the −10° condition (Fig. 2). Changes in joint angle would lead to differences in the initial length, their amount of slack and working range in the non-linear length–force relation of TT, which influenced onset and half relaxation time of twitch torque.

$ET_{max\text{-}time}$ and ET_{HRT} in the present study are comparable to those of previous research (Marsh et al., 1981; Connelly et al., 1999). The results on ET_{HRT} with respect to joint angle changes correspond to previous findings (Marsh et al., 1981), while the results on $ET_{max\text{-}time}$ do not. The joint angles used in their study differed from ours, and it is possible that their definition of time to peak force was not the same as ours. These factors may explain the discrepancies between studies.

Also, possible reasons for the identical time course of each TTE, FV and TTV over different joint positions and over different forces might include the length-dependent difference in stiffness of TT. In the plantar flexed position, TF was greater during contraction, but the stiffness of TT was also greater (filled symbols in Fig. 5). Conversely, in the dorsi-flexed condition, TF was smaller, but TT stiffness was also lower (blanked symbols in Fig. 5). In the former condition, the increased force capacity of the fascicles was mitigated by the increased stiffness of TT. In the latter condition, fascicle shortening was effectively increased by the lower stiffness of TT. This interaction between length changes and forces in both fascicles and TT

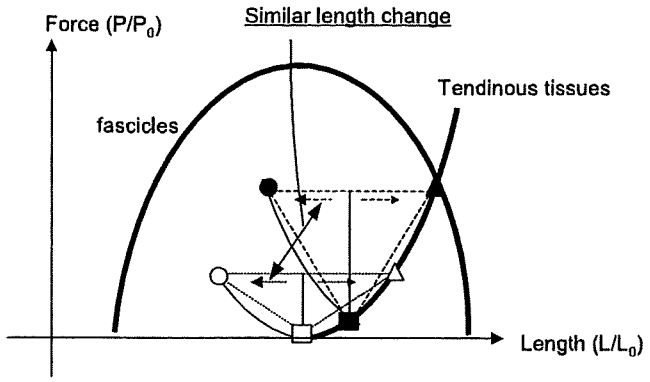

Fig. 5. A schematic representation of length–force relations at rest and peak force in the present study (symbols), combined with length–force relations during tetanic contraction (thick lines) on both fascicles and tendinous tissues. The square, triangle and circle demonstrate length–force relation of fascicles and tendinous tissues at rest, length–force relation of tendinous tissues at peak force and length–force relation of fascicles at peak force, respectively. In the plantar flexed position (filled symbols), the maximal force generated by the fascicles is high, but the stiffness—and therefore resistance to stretch—is also high. In the dorsi-flexed position (blank symbols), maximal force is lower, but the stiffness of tendinous tissues is also smaller. Because of the mechanical interaction, the time course of tendinous tissues elongation, and fascicle and tendinous velocities would be similar despite changes in joint angle.

(Kawakami and Lieber, 2000; Lieber et al., 1992) would result in no apparent changes between joint angles for TTE, FV and TTV.

4.2. Joint angle-dependent differences in fascicle and tendinous tissue length–velocity–force properties

Muscle fiber force is a function of its length, velocity and activation level, while the length change of muscle fibers is also affected by the mechanical properties of TT (Bobbert and Ingen Schenau, 1990; van Zandwijk et al., 1996, 1998). Due to the muscle–tendon interaction, the time course of FV has no joint-specific differences. Thus, assuming that the time course of fascicle activation is constant between joint angles, the differences in the shapes of length–force and velocity–force properties of both fascicles and TT between joint positions would be due to the difference in length-dependent force generating capacity of the fascicles. This suggests that FL is the most important contributor to the joint angle-dependent differences in the time course of twitch force.

4.3. Generating twitch force under muscle–tendon interaction

ET_{max} were observed when FV is zero, which is isometric state of fascicles. Before the peak force, fascicles interacting to TT shortened dynamically and generated force. Activation-dependent force generating capacity increased after the stimulus. Because of highly compliant nature of TT at low force levels, they are stretched rapidly (up to 91.5 mm/s). The fascicles in turn shorten at a corresponding velocity, as FV and TTV offset each other to keep identical total MTC length. Due to concentric force–velocity relation (Hill, 1939), this high shortening velocity keeps the force production of fascicles far below their isometric force production. As the force rises, TT undergoes more extension, and become less compliant. Thus TTV and FV decreases, allowing the force to rise, until finally reaching isometric state at the peak twitch force. Therefore, peak twitch force is dependent on the FF–length relation and the activation of the moment. FL decreases by 5–9% from the initial FL during this shortening. Collectively, the present results indicate that the whole time course of twitch force is affected not only by physiological properties in fascicles but also mechanical interaction between fascicles and TT.

In conclusion, the present study revealed that twitch torque was greatly affected by muscle–tendon interaction, depending on the slack and non-linearity of length–force relationship in compliant TT. We conclude that the mechanical interaction between fascicles and TT, are significant determinants of twitch torque and time characteristics.

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Effects of 6 months of walking training on lower limb muscle and tendon in elderly

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The purpose of this study was to investigate the effects of 6 months of walking training on muscle strength, muscle thickness and tendon stiffness on various parts of the lower limbs in the elderly. Subjects were assigned to training ($n = 35$) and control ($n = 10$) groups. Maximal isometric torque (MVC) and muscle thickness for knee extensors (KE), knee flexors (KF), dorsi flexors (DF) and plantar flexors (PF) were measured. Tendon stiffness for KE and PF was measured using ultrasonography while subjects performed isometric contraction. No significant changes occurred in any measured variables in the control group. In the training group, muscle thickness increased significantly for KF and DF, but not for PF. For KE, significant increases of muscle thickness at the proximal and medial sides were observed, although mean relative increase of the eight measured sites for KE was not significant. MVC increased significantly for KF, DF, and PF, but not for KE. In addition, tendon stiffness for KE and PF did not change after training. These results indicated that walking training brought about increments of muscle thickness and strength in most of the lower limbs in the elderly, but it did not result in any changes in tendon stiffness.

Walking is a popular form of moderate intensity physical activity for elderly individuals. A number of studies have been published regarding the effects of walking training on body composition and aerobic capacity (e.g., Hardman & Hudson, 1994; Murphy & Hardma, 1998). However, only a few studies have ever tried to investigate the effects of walking training on muscle mass (Sipila & Suominen, 1995). Sipila and Suominen (1995) reported that strength training of 18 weeks induced hypertrophy in the thigh muscles (quadriceps femoris muscle) in elderly women, whereas the effect of endurance training (walking) was negligible. Similarly, some cross-sectional studies showed that endurance training (i.e., walking, jogging, cycling) did not induce muscle hypertrophy and increase of muscle function (Klitgaard et al., 1990; Kuno et al., 1994). In these studies (Klitgaard et al., 1990; Kuno et al., 1994; Sipila & Suominen, 1995), however, the muscle cross-sectional area was measured for only one slice of computerized tomography and magnetic resonance imaging (MRI). This implied that the training-induced changes in muscle size might be overlooked, as muscle hypertrophy did not occur equally throughout the entire length of the muscle (Narici et al., 1996). Furthermore, information on the walking training-induced changes in the muscle strength and size of various parts in the lower limbs may provide significant knowledge on muscle function during the daily life of elderly individuals (e.g., going up the stairs, standing up).

Previous researchers have reported that the decreased muscle strength of elderly individuals was reversible by resistance training (e.g., Hakkinen et al., 2000). However, it was found that sports-related injuries were very common among elderly athletes (Pollock et al., 1991). As a main reason for this, it has been postulated that the tendon structures in elderly individuals are less able to cope with repetitive biomechanical stress during various activities. Recently, Reeves et al. (2003) reported that the stiffness of patella tendon in elderly individuals increased significantly after higher load resistance training. On the other hand, we found that the low-load resistance training (squat using body weight) led to an increase in the elasticity of tendon in knee extensors (KE) (Kubo et al., 2003a, b). Till date, however, no studies have ever tried to investigate the effects of walking training on the tendon

structures. The tendon structures for the KE and plantar flexors (PF) act as a spring that stores and releases elastic energy during walking and running (e.g., Alexander & Bennet-Clark, 1977). Therefore, it is hypothesized that the walking training during a given period time may affect the tendon structures for the KE and PF.

In the present study, we aimed to investigate the effects of 6 months of walking training on the muscle strength, muscle thickness, and tendon stiffness at various parts of the lower limbs in elderly individuals. We hypothesized that walking training brought about beneficial effects in the muscle and tendon in the lower limbs in the elderly.

Methods

Subjects

Fifty healthy men and women aged between 57 and 83 years volunteered to be subjects for this investigation. The subjects were assigned into a training group ($n = 39$) and a control group ($n = 11$) according to their own convenience and schedule. However, five subjects (training $n = 4$, control $n = 1$) dropped out during the course of the study. The reasons given were lack of time ($n = 2$), injury or illness ($n = 2$) and loss of interest ($n = 1$). Data are therefore presented for 45 subjects (57–77 years): training $n = 35$ (18 men), control $n = 10$ (4 men). The physical characteristics of both groups are presented in Table 1. The subjects were either sedentary, or mildly to moderately active, but none were involved in any type of resistance and/or endurance exercise programs at the time of the study. In the present study, the physical activity level of the subject was evaluated by the numbers of steps per day (see "Results"). These values were similar to the previous findings (e.g., Tudor-Locke & Bassett, 2004; Wyatt et al., 2005). They were in good health and free from cardiovascular disease and musculo-skeletal problem, as determined by the subjects' physicians' and medical history. The procedures, purpose and risks associated with the study were explained to all the subjects before they gave their written informed consent to participate in this investigation. This study was approved by the office of the Department of Sports Sciences, University of Tokyo, and complied with their requirements for human experimentation.

Walking program

Subjects were given a pedometer (FB-714, TANITA, Tokyo, Japan) to wear throughout the day for the 6-month walking program. For a 2-week period before beginning this program, the number of steps were measured to document pre-intervention daily lifestyle walking activity. Subjects put the pedometer on their belt or waistband as soon as they woke up each morning, removed it before going to bed every night and recorded the numbers of steps per day.

The training group ($n = 35$) undertook a progressive program of walking for 6 months. The first 2 weeks of the training consisted of walking for 15 min, three times per week. In the third to fourth week, the subjects were instructed to increase the duration of walking to 20–30 min per session. In the fifth to eighth week, they were asked to walk for 30 min, four times per week. From the ninth week, they were instructed to increase the duration of walking to 40 min per session, and maintained this level thereafter. Subjects were instructed to walk at a self-selected, comfortable pace, and were allowed to accumulate their steps in whatever pattern best fit their lifestyle. The walking time and the total numbers of steps each day was recorded on daily log sheets. Other than walking, the subjects were asked not to make any changes in their current lifestyle activities. In the present study, we checked the training status of subjects every month based on daily log sheets. All of the training sessions were preceded by a 10-min static stretching routine for the different muscles of the legs (hip flexors and extensors, KE, PF).

The control subjects ($n = 10$) were instructed to continue their daily routines and not to change their physical activity level. The number of steps taken was measured before and after training.

Table 1. Physical characteristics before and after training: mean (SD)

	Trained ($n = 35$)		Control ($n = 10$)	
	Before	After	Before	After
Age (year)	68.4 (5.6)		71.9 (2.7)	
Height (cm)	156.4 (6.6)	155.6 (6.7)	154.2 (5.5)	153.9 (5.7)
Body mass (kg)	58.4 (8.2)	57.3 (7.9)*	55.3 (9.7)	54.1 (9.5)*
Body fat (%)	26.5 (6.2)	26.9 (6.3)	28.2 (7.4)	28.5 (7.2)

*$P < 05$, significantly different from before.

Body composition

Body mass was measured to the nearest 0.1 kg using a calibrated scale, and height was measured to the nearest 0.1 cm. Percent body fat was assessed using a segmental multifrequency bioelectrical impedance analysis performed with an inBody II machine (Biospace, Tokyo, Japan) according to the procedure described by Kiyama et al. (2005).

Subcutaneous fat and muscle thickness

The subcutaneous fat and muscle thickness values for KE (eight anatomical sites), knee flexors (KF; two anatomical sites), dorsi flexors (DF; three anatomical sites) and PF (six anatomical sites) were measured with an ultrasonic apparatus (SSD-900, Aloka, Tokyo, Japan). The subjects remained in a supine position for the KE and DF measurements and a prone position for the KF and PF measurements with legs straight and the muscles relaxed. The anthropometric locations of the measurement sites were first precisely determined and marked by experienced technicians before the ultrasonic measurement. A transducer with a 7.5-MHz scanning head was coated with water-soluble transmission gel, which provided acoustic contact without depressing the dermal surface. The thickness of each site was measured to the nearest 0.1 mm using a vernier caliper. The anatomical sites for the measurements are noted below and presented in Fig. 1. The mean values of the subcutaneous fat and muscle thickness at all the measured sites were adopted as representative of each part. The accuracy and test–retest repeatability of the subcutaneous fat and muscle thickness measurements were certified in prior studies (e.g., Abe et al., 1994).

KE (eight sites): on the anterior central (included rectus femoris and vastus intermedius muscles), lateral (included vastus lateralis and vastus intermedius muscles) and medial surfaces (included vastus medialis and vastus intermedius) 30% (proximal; excluded medial site), 50% (middle), and

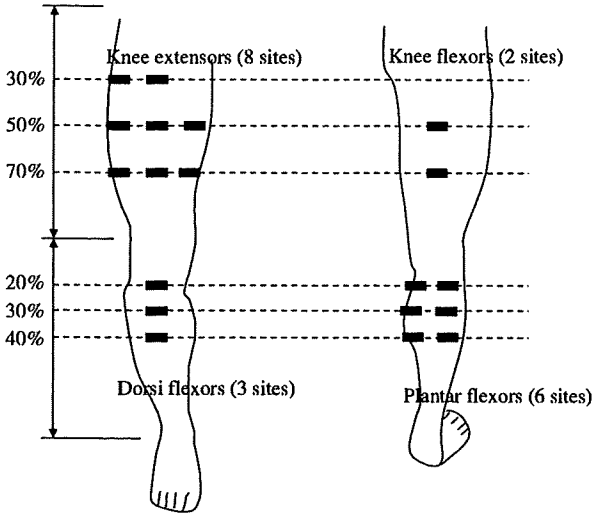

Fig. 1. Thick bars represent the locations of sonographic scanning sites for knee extensors (KE, eight sites), knee flexors (KF, two sites), dorsi flexors (DF, three sites), and plantar flexors (PF, six sites), respectively. The mean values of the subcutaneous fat and muscle thickness at all the measured sites were adopted as representative of each part.

70% (distal) between the lateral condyle of the femur and the greater trochanter.

KF (two sites): on the posterior central surfaces (included semimembranosus, adductor magnus and adductor longus muscles) 50% (middle), and 70% (distal) between the lateral condyle of the femur and the greater trochanter.

DF (three sites): on the anterior surfaces (included tibialis anterior muscle) 20% (proximal), 30% (middle), and 40% (distal) between the lateral malleous of fibula and the lateral condyle of the tibia.

PF (six sites): on the posterior medial (included medial gastrocnemis, soleus and flexor digitorum muscles) and lateral (included lateral gastrocnemius, soleus, and tibialis posterior muscles) surfaces 20% (proximal), 30% (middle), and 40% (distal) between the lateral malleous of fibula and the lateral condyle of the tibia.

The repeatability for measuring muscle thickness was assessed on two separate days with 33 young adult men and women (Kubo et al., 2003a, b). The test–retest correlation coefficient (r) was 0.995. The coefficient of variation was 2.5%.

Muscle strength

Maximal voluntary isometric torque (MVC) was measured by means of specially designed dynamometers (Vine, Tokyo, Japan) for knee joint and ankle joint, respectively. All measurements were performed on the right lower limb. Subjects visited the laboratory to become familiarized with the muscle strength measurements in both "pre" and "post" measurements. At each task, subjects exerted isometric torque from zero (relax) to MVC within 5 s. The subjects were encouraged verbally by the investigator to exert maximal effort throughout. During the knee extension and flexion tasks, subjects sat in an adjustable chair with support for the back and the hip joint flexed at an angle of 80° (full extension = 0°) to standardize the measurements and localize the action to the appropriate muscle group. During torque measurements, the hips and back were held tightly in the seat using adjustable lap belts. The axis of the knee joint was aligned with the axis of the lever arm of the dynamometer. The right ankle was firmly attached to the lever arm of the dynamometer with a strap and fixed with a knee joint flexed at an angle of 90° (full extension = 0°). During the dorsi and plantar flexion tasks, subjects sat on the chair of a dynamometer with their ankle at 90° (anatomical position) with the knee joint at full extension, and the foot was securely strapped to a foot plate. Before the test, the subject performed standardized warm-up and submaximal contractions to become accustomed to the test procedure. Each task was repeated two or three times per subject with at least 3 min between trials. The highest among these trials was recorded as the muscle strength for each.

Before training, the coefficient of variations (CV) of the three measurements were 6.1 ± 4.2% (0–17.8%) for the knee extension 6.4 ± 4.7% (0–19.1%) for the knee flexion, 3.2 ± 3.8% (0–16.6%) for the dorsi flexion, and 6.9 ± 4.2% (0–15.1%) for the plantar flexion, respectively. After training, these values were 4.6 ± 3.5% (0.5–17.9%) for the knee extension, 5.7 ± 3.3% (0.6–14.0%) for the knee flexion, 2.3 ± 1.9% (0–7.1%) for the dorsi flexion and 5.9 ± 4.5% (0–18.0%) for the plantar flexion, respectively.

Stiffness of tendon structures in KE and PF

Elongations of the tendon structures in KE and PF were also assessed during isometric knee extension and plantar flexion as mentioned above. An ultrasonic apparatus (SSD-2000, Aloka, Tokyo, Japan) with an electronic linear array probe (7.5 MHz wave frequency with 80 mm scanning length; UST 5047-5, Aloka) was used to obtain longitudinal ultrasonic images of vastus lateralis and medial gastrocnemius muscles by the procedures described previously (Kubo et al., 2004). The ultrasonic images were recorded on videotape at 30 Hz, synchronized with recordings of a clock timer for subsequent analyses. The point at which one fascicle was attached to the aponeurosis (P) was visualized on the ultrasonic images. The P moved proximally during isometric torque development up to maximum (see Fig. 1 of Kubo et al., 2004). The displacement of P (L) is considered to indicate the lengthening of the deep aponeurosis and the distal tendon (Kubo et al., 2004).

The displacements of tendon and aponeurosis can be attributed to both angular rotation and contractile tension, as any angular joint rotation occurs in the direction of knee extension and ankle plantar flexion during an "isometric" contraction (Magnusson et al., 2001). Thus, angular joint rotation needs to be accounted for to avoid an overestimation of tendon displacement during an isometric contraction. To monitor joint angular rotation, an electrical goniometer (Penny & Giles, Biometrics Ltd., Gwent, UK) was placed on the lateral aspect of each joint. To correct the measurements taken for the tendon and aponeurosis elongation, additional measurements were taken under passive conditions. The displacement of each site caused by rotating the knee and ankle from 110–70° was digitized in the sonographs taken. Thus, for each subject the displacement of each site obtained from the ultrasound images could be corrected for that attributed to joint rotation alone (Magnusson et al., 2001). In the present study, only values corrected for angular rotation are reported.

The measured torque (TQ) during isometric knee extension and plantar flexion were converted to force unit (F_m) by the following equations (Kubo et al., 2004):

$$F_m = k \cdot TQ \cdot MA^{-1}$$

where k is the relative contribution of the physiological cross-sectional area in each of vastus lateralis muscle within the KE and medial gastrocnemius muscle within the PF and MA is

the moment arm length in each of quadriceps femoris muscles at 90° and triceps surae muscle at 90°, which was estimated from the limb length of each subject. In the present study, the F_m and L values above 50% of MVC were fitted to a linear regression equation, whose slope was adopted as stiffness (Kubo et al., 2004).

The repeatability for the tendon stiffness measurement was investigated with eight young males (Kubo et al., 2002). The test–retest correlation coefficient (r) was 0.89 for the tendon stiffnes. The CV was 5.6% for the tendon stiffness.

Statistics

Descriptive data included means ± SD. A two-way (treatment group × time) analysis of variance (ANOVA) with repeated measures was used to analyze the numbers of steps and physical characteristics (height, body mass and body fat). A three-way (treatment group × time × measured site) ANOVA with repeated measures was used to analyze the subcutaneous fat and muscle thickness, MVC and the tendon properties (maximal elongation and stiffness). The F ratio for main effects and interactions were considered significant at $P<0.05$. Significant differences among means at $P<0.05$ were detected by *post-hoc* test using the Scheffe procedure.

Results

There was no difference in the numbers of steps per day before training between the training (7025 ± 2492 steps/day) and control groups (6692 ± 2152 steps/day). A two-way ANOVA on the numbers of steps showed no significant effect ($P = 0.126$) of group × test time interaction. In the control group, the numbers of steps did not change after 6 months (+7.1%; $P = 0.756$). In the trained group, the numbers of step increased 43.8% from 7025 ± 2492 steps/day to 9915 ± 5287 steps/day ($P<0.001$). The actual walking duration was 45.0 ± 15.6 min/day, and the actual participation was 5.4 ± 1.1 days/week.

Body mass decreased after training (main effect of time, $P<0.001$), although the percent body fat remained for both groups (Table 1). For these variables, however, the interactions of group × test time were not significant ($P = 0.971$ for body mass, $P = 0.992$ for the percent body fat). The main effect for time in the thickness of subcutaneous fat was also significant, but the interaction of time group × measured site was not significant ($P = 0.998$). In the control group, there were no changes in the thickness of subcutaneous fat in all the sites [+1.3% for KE, −4.2% for KF, +4.5% for DF, −2.7% for PF; Table 2, Fig. 2(a)]. In the trained group, the thickness of subcutaneous fat decreased −5.5% for KE ($P = 0.003$), −13.9% for KF ($P<0.001$) and −5.5% for PF ($P<0.001$), except for DF (+2.9%, $P = 0.263$) [Table 2, Fig. 2(a)].

Table 2 and Fig. 2(b) show the muscle thickness in all the sites and their relative changes, respectively. There was no three-way interaction, group × test time × measured site ($P = 0.988$). In the control group, there were no changes in the muscle thickness in all the sites [−0.7% for KE, +1.5% for KF, +2.6% for DF, −1.1% for PF; Table 2, Fig. 2(b)]. In the trained group, the muscle thickness increased for KF (+7.6%, $P<0.001$) and DF (+4.9%, $P = 0.004$), but not for KE (+3.1%, $P = 0.089$) and PF (+0.5%, $P = 0.928$) [Table 2, Fig. 2(b)]. In the trained group, the muscle thickness of KE increased for the proximal part of the central site (+7.7%, $P = 0.011$) and middle (+6.4%, $P = 0.047$) and distal (+8.7%, $P = 0.006$) of the medial site (Fig. 3).

Table 3 and Fig. 4 show the MVC in all the tasks and their relative changes, respectively. There was no three-way interaction, group × test time × measured site ($P = 0.943$). In the control group, there were no changes in the MVC in all the tasks (+4.5% for KE, +5.6% for KF, +2.1% for DF, +6.1% for PF; Table 3, Fig. 4). In the trained group, the MVC values increased for KF (+19.6%, $P = 0.048$), PF (+22.5%, $P<0.001$) and DF (+6.4%, $P<0.001$), but not for KE (+2.2%, $P = 0.681$) (Table 3, Fig. 4).

For both groups, there were no differences in the L values at any force levels (not showing data in the control group) (Fig. 5). In addition, there was no three-way interaction, group × test time × measured

Table 2. Subcutaneous fat and muscle thickness (mean values of each site) before and after training: mean (SD)

	Trained ($n = 35$)		Control ($n = 10$)	
	Before	After	Before	After
Subcutaneous fat thickness (mm)				
Knee extensors	8.2 (2.9)	7.6 (2.9)**	8.3 (2.5)	8.5 (2.2)
Knee flexors	6.6 (2.9)	5.5 (2.4)***	6.7 (2.1)	6.4 (2.4)
Dorsi flexors	3.1 (1.7)	2.8 (1.2)	3.0 (1.2)	3.2 (1.1)
Plantar flexors	4.8 (1.9)	4.5 (1.8)***	5.0 (1.7)	4.7 (1.8)
Muscle thickness (mm)				
Knee extensors	28.3 (5.6)	29.5 (5.1)	25.6 (4.5)	25.1 (4.8)
Knee flexors	52.8 (6.3)	56.8 (5.5)***	55.6 (3.4)	56.7 (5.3)
Dorsi flexors	24.4 (3.1)	25.4 (3.2)**	23.8 (2.9)	24.7 (3.3)
Plantar flexors	57.5 (5.6)	57.6 (5.1)	54.8 (3.4)	54.1 (5.1)

**$P<0.01$, ***$P<0.001$, significantly different from before.

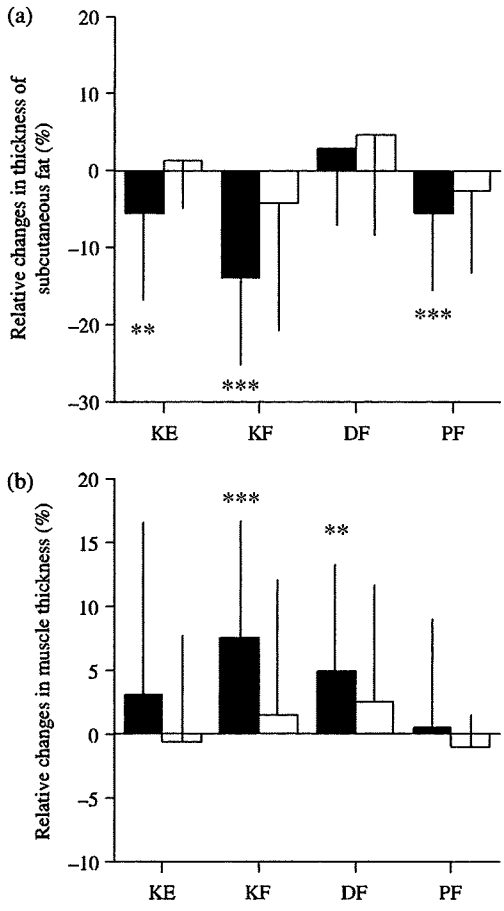

Fig. 2. (a) Relative changes (after 6 months of walking training) in the thickness of subcutaneous fat for knee extensors (KE), knee flexors (KF), dorsi flexors (DF) and plantar flexors (PF); (b) Relative changes (after 6 months of walking training) in muscle thickness for KE, KF, DF and PF. Training and control groups are shown by closed and open bars, respectively. Values are the mean ± SD. $^{**}P<0.01$, $^{***}P<0.001$.

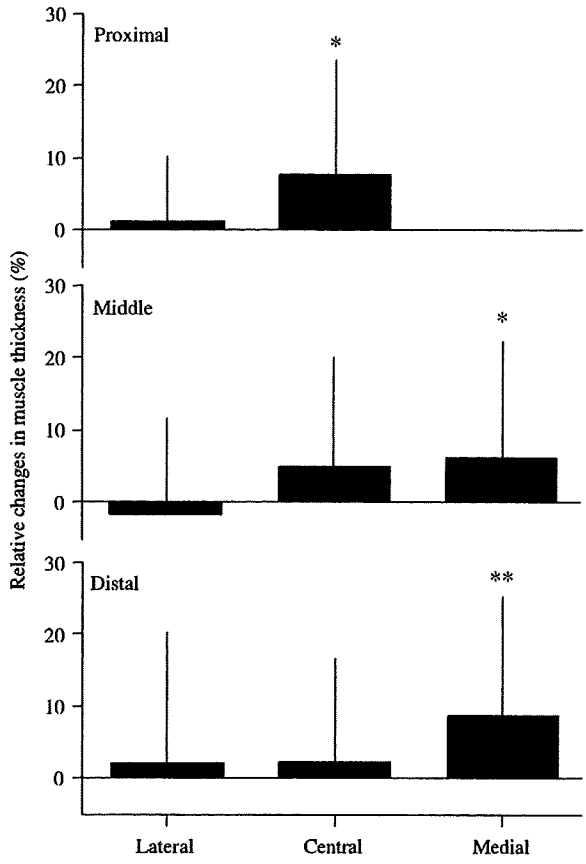

Fig. 3. Relative changes (after 6 months of walking training) in muscle thickness of eight sites for knee extensors (KE) in the training (closed) and control (open) groups. Values are the mean ± SD. $^{*}P<0.05$, $^{**}P<0.01$.

site ($P = 0.849$ for the maximal elongation, $P = 0.498$ for the stiffness). No changes in maximal elongation and stiffness of tendon structures were found (Table 4).

Discussion

One of the interesting findings was that the muscle thickness in the center proximal site and the medial distal site for KE increased significantly after walking training (Fig. 3). Previous studies showed that endurance training did not increase muscle strength and size (Klitgaard et al., 1990; Kuno et al., 1994; Sipila & Suominen, 1995). According to cross-sectional studies (Klitgaard et al., 1990; Kuno et al., 1994), strength-trained master athletes had a larger muscle size of quadriceps femoris muscles compared with controls of the same age, although no difference was found between the an endurance master athletes and sedentary indivduals. Sipila and Suominen (1995) also reported that endurance (walking) training of 18 weeks did not induce thigh muscle (quadriceps femoris muscle) hypertrophy in elderly women. The discrepancy between these previous findings and the present result may be caused by the difference in the measured sites of muscle size. In the previous studies (Klitgaard et al., 1990; Kuno et al., 1994; Sipila & Suominen, 1995), the muscle cross-sectional area was measured for only one slice of computerized tomography and MRI. However, some previous studies showed that muscle hypertrophy did not occur equally throughout the entire length of the muscle (Narici et al., 1996). In the present study, the muscle thickness at the center proximal and medial sites in KE increased significantly, although that at the middle of the thigh (measured in these previous studies) did not (Fig. 3). Therefore, it is entirely fair to say that the procedure used in the previous studies

Table 3. Muscle strength (mean values of each site) before and after training: mean (SD)

	Trained ($n=35$)		Control ($n=10$)	
	Before	After	Before	After
Knee extensors (N m)	124.1 (33.7)	125.5 (35.0)	121.5 (41.3)	119.3 (47.5)
Knee flexors (N m)	48.9 (20.5)	53.5 (18.2)*	46.9 (18.0)	47.8 (13.2)
Dorsi flexors (N m)	20.2 (5.1)	21.3 (5.0)***	18.6 (3.8)	19.0 (4.1)
Plantar flexors (N m)	55.7 (20.4)	67.1 (25.7)***	53.5 (19.0)	56.5 (19.7)

*$P<0.05$, ***$P<0.001$, significantly different from before.

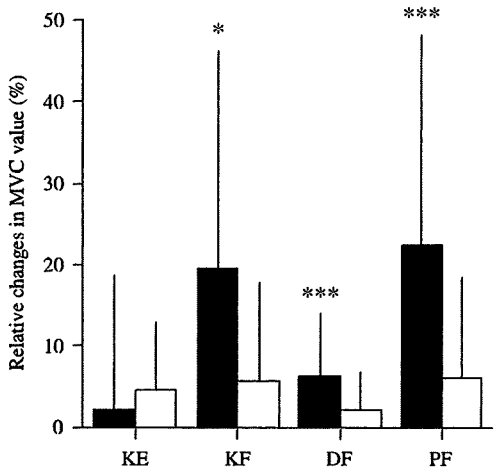

Fig. 4. Relative changes (after 6 months of walking training) in maximal voluntary contraction (MVC) for knee extensors (KE), knee flexors (KF), dorsi flexors (DF) and plantar flexors (PF) in the training (closed) and control (open) groups. Values are the mean ± SD. *$P<0.05$, ***$P<0.001$.

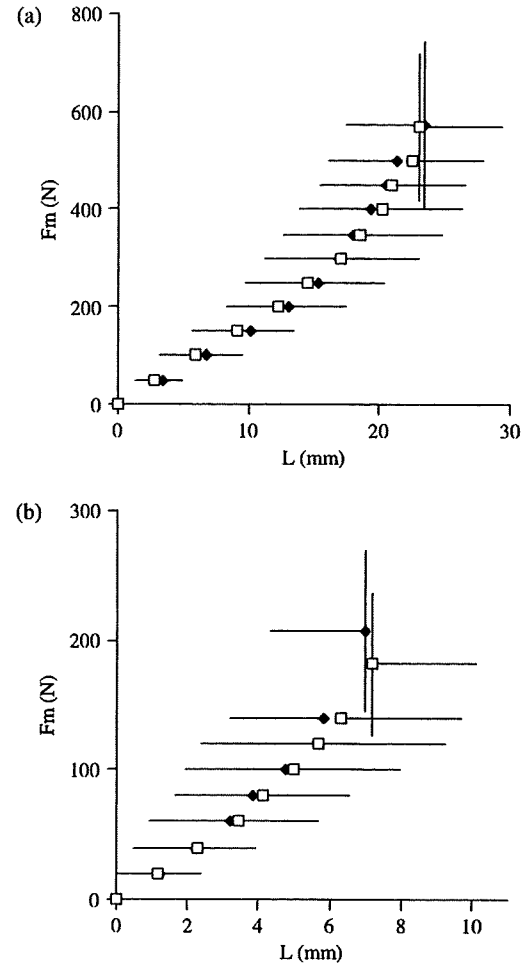

Fig. 5. Relationship between muscle force (F_m) and tendon elongation (L) before (open) and after (closed) walking training for 6 months [(a) knee extensors, (b) plantar flexors]. Values are the mean ± SD.

overlooked the changes in the proximal and/or distal sites of the KE.

The increments of muscle thickness at center proximal and medial sites for KE implied that the thickness of the rectus femoris and vastus medialis muscles increased significantly after training. The rectus femoris muscle is a two-joint muscle crossing both the hip and knee joints. Andersson et al. (1997) stated that the hip flexors dominated leg swing during level walking. Ericson et al. (1986) also indicated that the rectus femoris muscle contributed to accelerate the lower limb forward during the start of the swing phase. Therefore, we may say that the leg swing movements during walking training would give an impulse to the rectus femoris muscle. For the vastus medialis muscle, some previous studies demonstrated that injury- and surgery-related atrophy in the quadriceps femoris muscles was predominantly found in this muscle in orthopedics and rehabilitation research (Gerber et al., 1985). This finding tempts us to assume that the size of vastus medialis muscle may be more changeable by training and disuse than other KE muscles. Regardless, further investigations are needed to clarify this point.

Maximal knee extension torque did not change after training in spite of the significant increase in the thickness of rectus femoris and vastus medialis muscles. This discrepancy might be explained by the phenomenon of "joint angle specificity" (e.g., Thepaut-Mathieu et al., 1988). Namely, it is well

Table 4. Tendon properties of knee extensors and ankle plantar flexors for two groups: mean (SD)

	Trained ($n = 35$)		Control ($n = 10$)	
	Before	After	Before	After
Knee extensors				
Maximal L (mm)	22.9 (6.3)	23.3 (5.9)	20.6 (6.2)	21.2 (5.7)
Stiffness (N/mm)	22.7 (9.7)	25.8 (9.6)	25.5 (9.9)	27.9 (9.6)
Plantar flexors				
Maximal L (mm)	7.1 (2.9)	7.0 (2.7)	7.1 (3.0)	7.2 (3.2)
Stiffness (N/mm)	12.7 (8.4)	14.1 (8.9)	12.2 (8.2)	13.4 (7.2)

known that strength increases induced by strength training are specific to the joint angle at the point where exercise is performed (e.g., Thepaut-Mathieu et al., 1988). During the stance phase of walking, the knee joint moves between 0° and 30° of flexion (Winter
et al., 1990). Therefore, no significant increase in knee extension torque would be attributed to the difference in knee joint angle between walking (0–30°) and torque measurements (90°).

Some previous studies indicated that the relative activation level (to MVC) of the PF would be higher than that of the KE during normal walking (Ericson et al., 1986; Winter & Yack, 1987). For example, Ericson et al. (1986) stated that the important role of the triceps surae during walking was reflected in the comparatively high muscular activity at push-off. This result supported the opinion that the PF were important muscles for normal walking. Furthermore, Bendall et al. (1989) reported that the walking speed was significantly correlated to the muscle strength of PF. Therefore, it is possible to build up a hypothesis that the muscle strength and thickness of PF increase after walking training. In the present result, however, the muscle thickness of the PF did not change after walking training, although the muscle strength increased significantly (+22.5%). This increase in the plantar flexor strength was in agreement with the previous findings concerning the resistance training (Kubo et al., 2002; Scaglioni et al., 2002). On the other hand, some previous studies demonstrated that the increases of muscle size for PF were lower than other parts (Weiss et al., 1996; Kubo et al., 2002). For example, Weiss et al. (1996) showed that 8 weeks of heavy resistance training involving the triceps surae muscles increased isotonic muscle strength by about 13% without a concurrent increase in muscularity. Taking the present findings into account along with these previous findings, it is likely that no increase in the muscle thickness of PF can be attributed to the lower plasticity of the PF.

In the present study, the walking training provided a sufficient stimulus to elicit improvements in muscle strength and thickness of the KF. As far as we know, no studies have ever tried to study the effect of endurance training on the KF in elderly people. The KF (i.e., hamstrings) are two-joint muscles and act as hip extensors as well as KF. During walking, the hamstrings play the roles of hip extensors to study the the body mass ahead during the stance phase and work as KF to fold the legs during the swing phase, respectively. Furthermore, before heel contact, the hamstrings as well as the quadriceps femoris muscles were activated during normal walking (Hortobagyi et al., 2005). This result indicates that increased hamstrings coactivity was useful to stabilize the knee by increasing the compressive force (Hagood et al., 1990). In fact, Sipila and Suominen (1995) reported that the number of endurance (walking) training sessions was significantly related to the change in the cross-sectional area of KF. Accordingly, it seems reasonable to suppose that the walking training in elderly individuals has an effect on the function and size of hamstrings.

Similarly, the muscle strength and thickness of DF increased significantly after the walking training (Figs 2(b) and 4). Ericson et al. (1986) showed that the tibialis anterior muscle was active during the whole period of swing phase. At the end of the swing phase, this muscle increased its activity in preparation for the heel strike. On the other hand, previous studies reported that the dorsi flexion angle of ankle at the heel strike was smaller in elderly than in younger individuals (e.g, Winter et al., 1990). This fact would be mainly caused by the decline in the strength and flexibility of DF with aging. Furthermore, this would lead to fall accidents in elderly individuals (Winter et al., 1990). The present result indicates that the walking training increases the function of DF and may be useful to prevent falls in elderly individuals.

The maximal elongation of tendon structures was 22 mm for KE and 7 mm for PF, respectively. These values were lower compared with the reported previously in younger subjects (Magnusson et al., 2001; Kubo et al., 2004). This result agreed with some previous findings using animal and human cadavers (e.g., O'Brien, 1992). The tendon structures in elderly individuals (less elasticity) are weaker than those in younger ones and are more likely to tear or

suffer from overuse injury (Pollock et al., 1991). Hansen et al. (2003) reported that aerobic training (running) did not induce the changes in dimension and mechanical properties of Achilles tendon in young subjects. However, our recent study showed that the low-load resistance training (squat using body weight) made the maximal elongation of tendon in KE increase (Kubo et al., 2003a, b). Based on this previous finding, we expected that the decreased elasticity of tendon structures in elderly individuals was reversible by the walking training, as the load imposed on the muscle and tendon in the lower limbs during walking would be as low as during the squat training using body weight. In the present study, however, the tendon properties in elderly individuals did not change after 6 months of walking training. We found that the isometric training regimen using a higher internal muscle force increased the tendon stiffness, whereas that using a lower force level did not (Kubo et al., 2006). Reeves et al. (2003) also reported that the stiffness of patella tendon in elderly individuals increased significantly after higher load resistance training. Taking the present finding into account together with the above-quoted findings, greater mechanical stress on the tendon structures than aerobic training (walking, jogging) would be necessary to bring about changes in the tendon properties in elderly individuals.

However, we must draw attention to a limitation in the present study. Certainly, it would be difficult to measure the daily activity "accurately" using the pedometer. However, the main purpose of this study was to investigate the effects of walking training on muscle and tendon. We considered that the numbers of step at least during walking training were measured accurately. Because the subjects were asked not to make any changes in their current lifestyle activities, we considered that the increment of number of step would cause by "walking training." In the present study, however, the subjects were instructed to walk at a self-selected and comfortable pace. Unfortunately, we cannot investigate the effects of the training intensity, i.e., walking speed in the present results. We considered that the present findings would be related to the actual training volume, i.e. walking duration and distance. Regardless, further investigations are needed to clarify this point.

Perspectives

In this study, we investigated the effects of 6 months of walking training on muscle strength, muscle thickness and tendon stiffness on various parts of the lower limbs in elderly. These results suggested that a moderate walking training brought about increments of muscle thickness and strength in most of lower limbs in the elderly, but it did not result in any changes in tendon stiffness. Furthermore, these results implied that the easy, flexible and unsupervised program of walking training had a sufficient effect on the decline of muscle function and mass with aging in elderly individuals.

Key words: aging, ultrasonography, leg muscle, stiffness.

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Age-Related Differences in the Properties of the Plantar Flexor Muscles and Tendons

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ABSTRACT

KUBO, K., M. MORIMOTO, T. KOMURO, N. TSUNODA, H. KANEHISA, and T. FUKUNAGA. Age-Related Differences in the Properties of the Plantar Flexor Muscles and Tendons. *Med. Sci. Sports Exerc.*, Vol. 39, No. 3, pp. 541–547, 2007. **Purpose:** The purpose of this study was to determine age-related differences in the human plantar flexor muscles and tendon. **Methods:** Four age groups—a 20-yr group (20–27 yr, $N = 19$), 30-yr group (31–38 yr, $N = 15$), 50-yr group (46–57 yr, $N = 10$) and 70-yr group (62–77 yr, $N = 15$)—volunteered to take part in the present study. Muscle thickness, strength, and activation level (using twitch-interpolation technique) of plantar flexor muscles were measured. Elongation of the Achilles tendon was determined using ultrasonography while subjects performed ramp isometric plantar flexion up to the voluntary maximum. **Results:** No significant difference in relative muscle thickness (to limb length) was observed among the four age groups. Muscle strength and activation level of the 20-yr group were significantly higher than those of the 50- and 70-yr groups (activation levels were not measured in the 70-yr group), and maximal strain (elongation/initial tendon length) of the Achilles tendon decreased with aging. Although there were no differences in muscle strength and activation levels between the 20- and 30-yr groups, maximal strain of the Achilles tendon of the 30-yr group was already lower than that of the 20-yr group ($P = 0.062$). **Conclusion:** These results suggest that the processes of age-related changes in the muscle and tendon are different. Furthermore, the differences in age-related changes of muscle and tendon might play a role in the frequency of Achilles tendon ruptures among men in their 30s. **Key Words:** TENDON, ELONGATION, AGING, ULTRASONOGRAPHY, MUSCLE

It is well known that muscle strength decreases with aging (1,9), and it has also been suggested that a steeper decline of muscle strength begins after the age of 50 yr (35,38). According to previous findings (1,25), the decline of muscle strength with aging has been ascribed to the declines of muscle mass and neural drive to muscle. Some previous researches demonstrated that a decline in muscle thickness and cross-sectional area with aging starts after the age of 60 yr (9,24). On the other hand, no studies have ever tried to investigate when the age-related changes in the neural drive to muscle starts, although some previous researchers have made comparison of the neural drive to muscle between younger and elderly (25).

Previous findings obtained from animal and human cadaver experiments showed that the ultimate strength and Young's modulus of the tendons decreased with specimen age (5,11,20). Recently, studies have demonstrated the age-related changes in the human tendon properties *in vivo* (14,18,30). For example, Onambele et al. (30) report that middle-aged and older individuals (46 ± 1 yr, 68 ± 1 yr) had lower stiffness and Young's modulus of the Achilles tendon than did younger individuals (24 ± 1 yr). We have observed that the maximal strain of the human tendon structures in knee extensors decreased significantly with aging (18). However, according to these previous studies, which have used both human cadaver and *in vivo* experiments, it is not clear at which age this impairment begins.

In recent years, the number of middle-aged and elderly people who participate in sports has been increasing in those societies with the longest average life expectancy. As a result, injuries from overuse are becoming recognized in middle-aged and elderly people (13). In particular, the human Achilles tendon is the most common tendon to rupture during sports and daily activities (12). Furthermore, some previous researchers have indicated that the rupture of this tendon often occurs in men in their 30s (10,19,29). However, the reason for this phenomenon is not clear at present.

It remains an unsettled question whether the process of age-related changes in the morphology and the function of human muscle and tendon are the same. The purpose of this study was to determine age-related changes in the

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plantar flexor muscles and Achilles tendon in 59 healthy men aged between 20 and 77 yr.

METHODS

Subjects. A total of 59 male subjects agreed to participate in the present study. The subjects were distributed into four age groups: a 20-yr group (ages 20–27 yr, $N = 19$), a 30-yr group (ages 31–38 yr, $N = 15$), a 50-yr group (ages 46–57 yr, $N = 10$), and a 70-yr group (ages 62–77 yr, $N = 15$). The physical characteristics of the subjects are shown in Table 1. The subjects in the study were either sedentary or mildly to moderately active men, but none were involved in any type of resistance exercise program at the time of the study. The procedures, purpose, and risks associated with the study were explained to all the subjects before they gave their written informed consent to participate in this investigation. This study was approved by the office of the department of sports sciences, University of Tokyo, and complied with their requirements for human experimentation.

Numbers of steps. For a 2-wk period, the numbers of steps of each subject were measured to document their physical activity levels during daily life. Subjects put the pedometer (FB-714, TANITA, Tokyo, Japan) on their belt or waistband as soon as they woke up each morning, removed it before going to bed every night, and recorded their number of steps each day. The total numbers of steps each day were recorded on daily log sheets. In the present study, the mean numbers of steps during 2 wk were used as an index of physical activity level during daily life.

Muscle thickness. The muscle thickness of the plantar flexors was measured with an ultrasonic apparatus (SSD-2000, Aloka, Japan) at six anatomic sites: on the posterior medial and lateral surfaces, 20, 30, and 40% between the lateral malleous of fibula and the lateral condyle of the tibia. The anatomic sites for the measurements are presented in Figure 1. Each subject remained in a prone position with the legs straight and the muscles relaxed. The anthropometric locations of the measurement sites were precisely determined and marked by experienced technicians before the ultrasonic measurement. A transducer with a 7.5-MHz scanning head was coated with water-soluble transmission gel, which provided acoustic contact without depressing the dermal surface. The muscle thickness of each site was measured to the nearest 0.1 mm using a vernier caliper. The mean values of muscle thickness at all measured sites were adopted as their representative of the muscle size of the plantar flexor muscles.

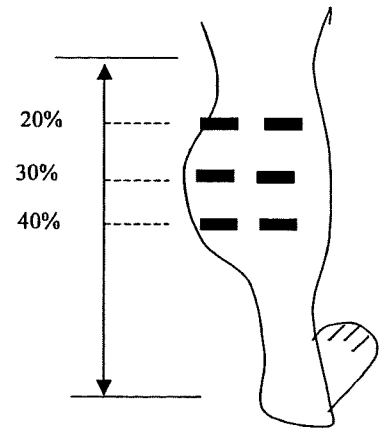

FIGURE 1—*Thick bars* represent the locations of sonographic scanning sites for plantar flexors (six sites). The mean values of muscle thickness at all measured sites were adopted as their representative of muscle thickness.

Muscle strength and central neuromuscular activation. Maximal voluntary isometric strength (MVC) of the plantar flexor muscles was determined using an electrical dynamometer (Myoret, Asics, Japan). The subject lay prone on a test bench, and the waist and shoulders were secured by adjustable lap belts and were held in position. The right ankle joint was set at 90° (anatomic position) 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. Before the test, the subject performed a standardized warm-up and submaximal contractions to become accustomed to the test procedure. The subjects performed more than two trials within a 1-min rest period. The highest among two trials was accepted as the muscle strength.

During MVC, evoked twitch contractions were imposed by supramaximal electric stimulations. The experimental procedures have been described in detail previously (4). The stimulating lead electrodes were placed on the skin of the right popliteal fossa and were oriented longitudinally to the estimated path of the tibial nerve with the anode distal. A high-voltage stimulator (SEN-3301, with a specially

TABLE 1. Physical characteristics and steps numbers.

	20 yr ($N = 19$)	30 yr ($N = 15$)	50 yr ($N = 10$)	70 yr ($N = 15$)
Age (yr)	24.5 (2.1)	35.2 (2.2)	51.4 (4.4)	69.7 (4.5)
Height (cm)	171.6 (3.9)	171.5 (4.8)	172.4 (4.3)	159 (6.3)*
Body mass (kg)	69.7 (10.3)	72.4 (8.6)	73.9 (7.0)	59.4 (8.0)*
Lower-leg length (cm)	39.4 (1.5)	39.0 (1.6)	38.9 (1.8)	35.4 (1.9)*
Step numbers (steps per day)	7712 (2415)	7884 (3081)	7676 (2883)	7025 (2492)

Mean (SD).
* Significantly different from 20-, 30-, and 50-yr groups.

modified isolator SS-1963, Nihon-Koden, Japan) generated rectangular pulses (triple stimuli, with a 500-μs duration for one stimulus and an interstimulus interval of 10 ms). The stimulation intensity was confirmed by setting the output of the stimulator to a level at which there was no further increase in twitch torque. In all subjects, the stimuli increased the force during MVC at the appropriate latency. Shortly (within 1–2 s) after MVC, when the potentiation effect of the contraction still persisted, the same stimulation was given to the muscle at rest (control twitch). The voluntary force at the instant of stimulation was used as the MVC force. Two separate efforts were made routinely, and a third extension was performed if more than a 5% difference existed. The measured values that are shown below are the means of two trials. The twitch force (difference between peak twitch force and MVC force) was measured, and from this, the level of muscle activation with voluntary effort (% activation) was assessed from the following equation (twitch interpolation technique (4)): % activation = {1 − (twitch force during MVC/control twitch force)} × 100 (%), where control twitch represents the twitch imposed on the resting muscle after MVC. In the present study, the activation level for the 70-yr group was not measured because of ethical issues and scheduling limitations.

Elongation of the Achilles tendon. The experimental setup has previously been described in detail (16,17). The subject was instructed to develop a gradually increasing force from a relaxed state to maximal voluntary contraction (MVC) within 5 s. The task was repeated two times per subject, with at least 3 min between trials. The measured values shown below are the means of two trials. An ultrasonic apparatus (SSD-2000, Aloka, Tokyo, Japan) with an electronic linear-array probe (7.5-MHz wave frequency with 80-mm scanning length; UST 5047-5, Aloka) was used to obtain longitudinal ultrasonic images of the medial gastrocnemius muscle. The probe was longitudinally attached to the dermal surface with adhesive tape, which restrained the probe from sliding. A marker (black arrows in Fig. 2) was placed between the skin and the ultrasonic probe as the landmark to confirm that the probe did not move during measurements. To evaluate the elongation of the Achilles tendon, the displacement of the distal myotendinous junction (dL; Fig. 2) of the medial gastrocnemius muscle in the transition from rest to MVC was measured (16,26). In the present study, the Achilles tendon was defined as the distance between the Achilles tendon insertion and the distal myotendinous junction of the medial gastrocnemius muscle (16,26). The ultrasonic images were recorded on videotape at 30 Hz and were synchronized with force recordings using a clock timer for subsequent analyses.

The tendon displacement can be attributed to both angular rotation and contractile tension, because any angular joint rotation occurs in the direction of ankle plantarflexion during an isometric contraction (16,21,26).

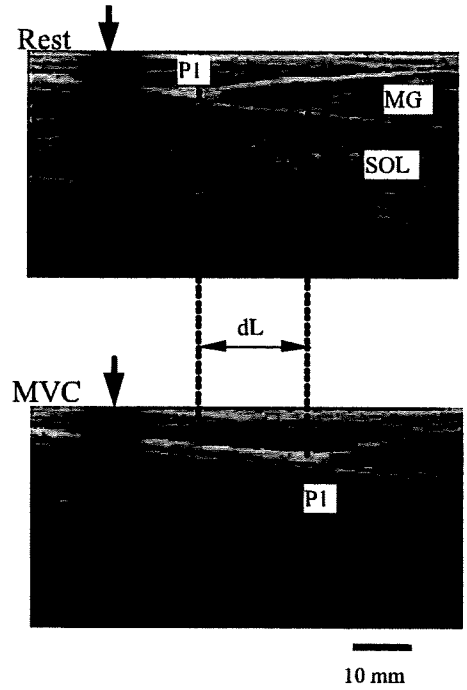

FIGURE 2—Ultrasonic images of longitudinal sections of the Achilles tendon at rest and MVC. The *black arrows* point to the shadow generated by an echo-absorptive marker attached by adhesive to the skin. P1 and P2 moved proximally during isometric torque development from rest to MVC. The distance traveled by P1 and P2 (dL) was defined as the elongation of the Achilles tendon during contraction.

To monitor ankle-joint angular rotation, an electrical goniometer (Penny and Giles) was placed on the lateral aspect of the ankle. To correct the measurements taken for the elongation of the Achilles tendon, additional measurements were taken under passive conditions. The displacement of the myotendinous junction of the medial gastrocnemius muscle caused by rotating the ankle from 90 to 70° was digitized in sonographs taken as described above. Thus, for each subject, the displacement of the myotendinous junction obtained from the ultrasound images could be corrected for that attributed to joint rotation alone (16,21). In the present study, only values corrected for angular rotation are reported.

To calculate the strain values from the measured elongation, we measured the respective length of the Achilles tendon, from the myotendinous junction (position of the probe) to the insertion of Achilles tendon (16,26). In a preliminary study, the coefficient of variation (100 × SD/mean) for repeated measurements of the maximal elongation of the Achilles tendon in one subject was 7.4%.

Statistics. Descriptive data include means ± SD. One-way analysis of variance (ANOVA) was used for the comparison among the four groups. If the F-statistic of the analysis of variance was significant, differences between groups were assessed by the Scheffe test. The level of significance was set at $P < 0.05$.

RESULTS

Height, body mass, and lower-leg length in the 70-yr group were significantly lower than those of the other groups (all $P < 0.01$), whereas no significant differences were observed among the 20-, 30-, and 50-yr groups (Table 1). There was no significant difference in the number of steps per day among all the groups.

The absolute muscle thickness of the 70-yr group (59.4 ± 5.6 mm) was significantly lower than that of the other group (all $P < 0.05$), whereas no significant differences were observed among the 20-yr (63.8 ± 5.1 mm), 30-yr (64.4 ± 3.9 mm), and 50-yr (64.7 ± 4.5 mm) groups (Fig. 3A). There was no significant difference in relative muscle thickness (to limb length) among all the groups (Fig. 3B).

The absolute MVC of the 70-yr group (63.4 ± 18.4 N·m) was significantly lower than that of the other groups (all $P < 0.05$), and that of the 50-yr group (105 ± 24 N·m) was significantly lower than that of the 20-yr group ($P = 0.031$) (Fig. 4A). The difference in MVC between the 20-yr (124 ± 24 N·m) and 30-yr (119 ± 21 N·m) groups was not significant ($P = 0.599$). The relative MVC (to body mass) did not alter these observations, although the differences among groups were smaller (Fig. 4B).

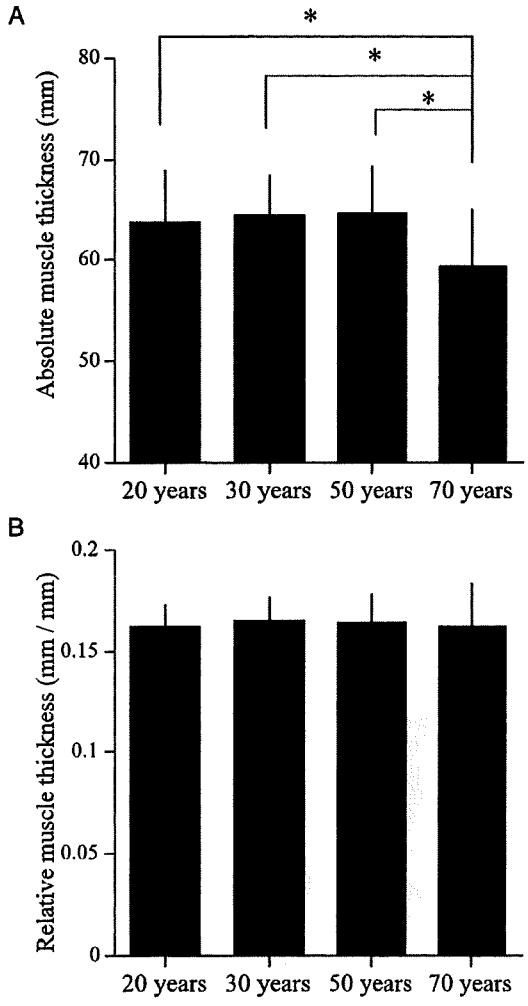

FIGURE 3—Absolute (*upper*) and relative (to limb length; *lower*) muscle thickness of plantar flexor muscles from the four age groups. Values are means ± SD. * $P < 0.05$.

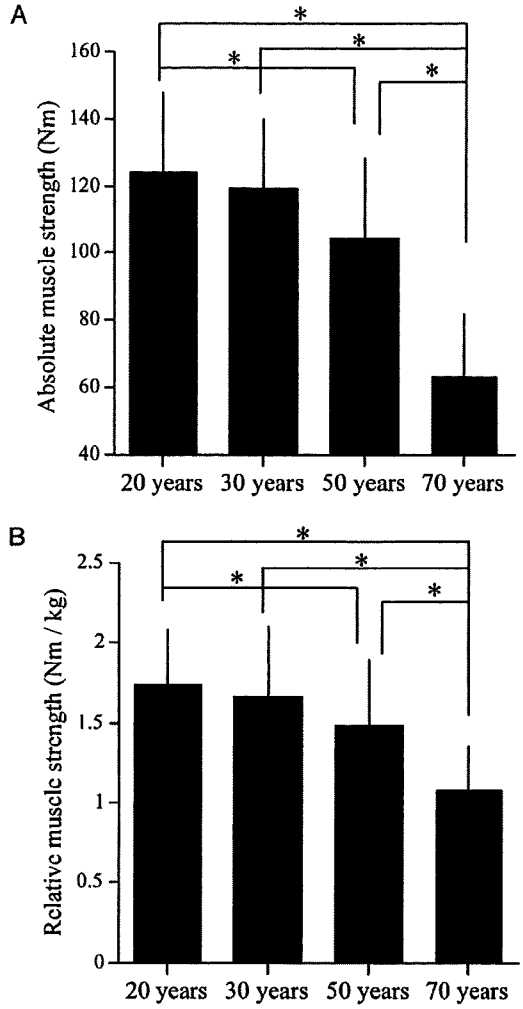

FIGURE 4—Absolute (*upper*) and relative (to body mass; *lower*) maximal strength of plantar flexor muscles from the four age groups. Values are means ± SD. * $P < 0.05$.

The activation level of the 50-yr group (83.9 ± 6.7%) was significantly lower than that of the 20-yr (94.1 ± 4.7%; $P < 0.001$) and 30-yr (93.0 ± 7.8%; $P = 0.037$) groups, and the difference in activation level between the 20- and 30-yr groups was not significant ($P = 0.607$) (Fig. 5).

The maximal strain of the Achilles tendon of the 70-yr group (3.1 ± 0.7%) was significantly lower than that of the other groups (all $P < 0.01$), and that of the 50-yr group (4.0 ± 0.5%) was also lower than that of the 20-yr group ($P = 0.003$) (Fig. 6). Furthermore, the maximal strain of the Achilles tendon of the 30-yr group (4.4 ± 0.8%) tended

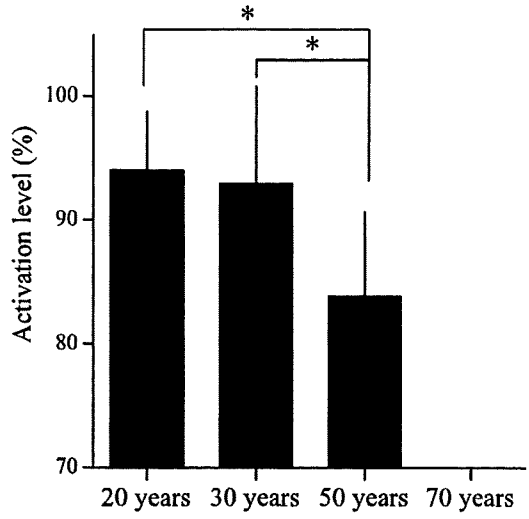

FIGURE 5—Activation level of plantar flexor muscles from the three age groups, except for the 70-yr group. Values are means ± SD. * $P < 0.05$.

to be lower than that of the 20-yr group (5.1 ± 0.8%) ($P = 0.062$).

DISCUSSION

The present study has shown the age-related decrease in the maximal strain of the Achilles tendon (Fig. 6). Recently, we have also reported that the maximal strain of tendon structures in the knee extensors decreased significantly with aging (18). On the other hand, other researchers have shown different findings concerning age-related changes in human tendon properties (14,28,30). For example, Karamanidis and Arampatzis (14) have reported that there were no significant differences in maximal strain stiffness of the Achilles tendon between young (21–32 yr) and elderly subjects (60–69 yr). Onambele et al. (30) have shown that the maximal gastrocnemius tendon strain values of middle-aged (46 ± 1 yr) and older (68 ± 1 yr) individuals were significantly greater than those of younger subjects (24 ± 1 yr). Unfortunately, we cannot say the reasons for this discrepancy. On the other hand, previous findings obtained from human cadaver experiments have shown that ultimate strength and Young's modulus of the tendons decreased with specimen age (5,11,20). With regard to the collagen fibers of tendons, the mean area and diameter of collagen fibers have been shown to decrease with aging (27,34). The density and structure of cross-links and the fibril morphology in collagenous tissues have been shown to change with aging, in a manner that could be correlated with age-related changes in mechanical properties (32). Furthermore, age-related increases in connective tissue and collagen cross-linking have been reported that might decrease the tendon extensibility during muscle contractions (3). In fact, some previous studies using animals have shown that the maximal strain of rat-tail tendon decreases during aging (36,37). According to these previous findings, however, it is not clear at which age this impairment begins. An interesting finding of this study was that the maximal strain of the Achilles tendon in the 30-yr group tended to be lower than in the 20-yr group ($P = 0.062$). Therefore, the age-related changes of the Achilles tendon (i.e., decline in tendon extensibility) was already observed in men in the 30-yr group.

We should present a stress–strain relationship to compare the tendon properties of the different age groups accurately. In addition to the tendon extensibility, therefore, it would be necessary to consider the age-related difference in the morphological characteristics of tendon (i.e., cross-sectional area). Recently, Magnusson et al. (22) have demonstrated that elderly women had a greater Achilles tendon cross-sectional area compared with young women. Magnusson et al. (22) state that a greater tendon size may reduce the risk of injury to the tendon in elderly individuals. On the contrary, Onambele et al. (30) have reported that the cross-sectional area of the Achilles tendon was significantly smaller in older men than in young men. Thus, the data concerning the age-related difference in the human tendon size *in vivo* seem inconsistent and inconclusive.

In the present study, there was no significant difference in the relative muscle thickness (to limb length) of the plantar flexors among the four age groups (Fig. 3B). This result is inconsistent with the previous findings that declines in muscle thickness and cross-sectional area with aging start after the age of 60 yr (9). In these previous studies, however, age-related difference in the knee extensor and knee flexor muscles were investigated. According to our previous finding (15), there was no significant difference in the relative muscle thickness of

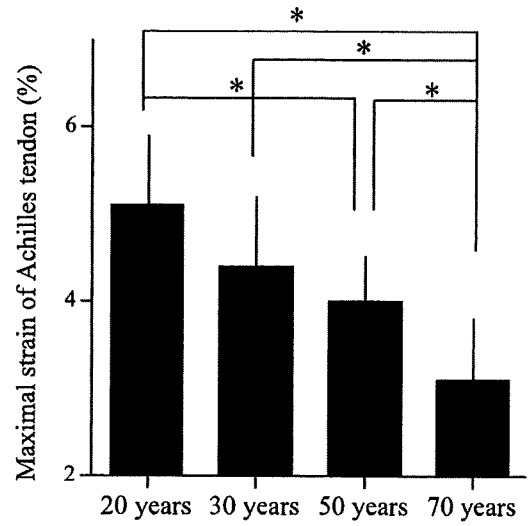

FIGURE 6—Maximal strain of the Achilles tendon from the four age groups. Values are the mean ± SD. * $P < 0$.

medial gastrocnemius muscle between young and elderly groups, although in vastus lateralis muscle, the relative muscle thickness of the elderly group was significantly lower than that of the younger group. The reasons for the differences in the declines in muscle thickness with aging are unclear, but several possibilities exist. These discrepancies may be attributable to differences in the daily activity levels between the knee extensors and plantar flexors. We have considered that the relative activation level of plantar flexors would be higher than that of knee extensors during normal walking. In fact, Ericson et al. (6) state that the important role of the plantar flexors during walking was reflected in the comparatively high muscular activity at push-off. In any case, further investigations are needed to clarify this point.

It is well known that muscle strength decreases with aging, and this decrease is especially pronounced in populations beyond 50 yr (35,38). The age-related decrease in muscle force-generating capacity may be attributable to muscle atrophy and decreases in activation level (1,24). In the present study, the muscle strength and activation levels of the 50-yr group were significantly lower than those of the 20-yr group. In addition, according to the findings of Morse et al. (25), who used a procedure similar to that of the present study, the activation level of the elderly group (age 74 ± 4 yr) was 78%, and this value tended to be lower than that of the 50-yr group (84%) in the present study. On the other hand, there was no difference in relative muscle thickness among the four age groups (Fig. 3B). Considering these points, these results indicate that the decreases in muscle strength observed in the 50- and 70-yr groups were mainly caused by the age-related decrease in the activation level.

No significant differences in the muscle strength and activation level of the plantar flexor muscles were observed between the 20- and 30-yr groups (Figs. 4 and 5). Previous studies have indicated that under normal conditions, human muscle strength reaches its peak between the ages of 20–30 yr, after which time it remains more or less unchanged for more than 20 yr (31,35,38). Therefore, it seems reasonable to suppose that the force-generating capacity and activation level of the plantar flexor muscles remain steady until almost 40 yr.

On the other hand, the age-related changes of the Achilles tendon (i.e., decline in the tendon extensibility) was already observed in men in their 30s, as mentioned above. Similarly, Sargon et al. (33) have reported that the diameter of the collagen fibers taken from the 30- to 39-yr group was smaller than that of the 20- to 29-yr group. They stated that the decreases in diameter of collagen fibers in the 30- to 39-yr group would play a role in the frequency of Achilles tendon ruptures. It is well known that the tendons play a role in storing and releasing elastic energy during walking and running (2). In addition, tendon elasticity acts as a mechanical buffer, protecting the muscle from damage during high-intensity contractions (8). From this point of view, the present results imply that Achilles tendons in people in their 30s are apt to be injured during various activities. The Achilles tendon is one of the most frequently injured tendons in the human body (12). In particular, there is a peak in the occurrence of the Achilles tendon rupturing in men in their 30s (10,19,29). Taking the present results into account, together with the previous findings cited above (31,33,35,38), it is likely that a main reason for the high frequency of Achilles tendon ruptures in men in the 30-yr group is that the Achilles tendons of men in this age group are less able to cope with repetitive biomechanical stress because of the decrease in the tendon elasticity, although the muscle strength and activation levels in the 30-yr group were similar to those of the 20-yr group.

In the present study, the Achilles tendon was defined as the distance between the Achilles tendon insertion and the distal myotendinous junction of the medial gastrocnemius muscle (16,26). However, "Achilles tendon" as defined in this study included the aponeurosis of soleus muscle. According to some previous findings (7,23), the strain of aponeurosis in plantar flexor muscles was lower than that in free Achilles tendon. Therefore, the maximal strain of the free Achilles tendon would be greater than that of the present study. In addition, if the ratio of soleus muscle mass to gastrocnemius muscle mass increased with aging, the main results of this study (decline in maximal strain with aging) would be affected by the stiffer aponeurosis of soleus muscle. As far as we know, however, there have been no studies concerning these points. Therefore, we believe that the definition of Achilles tendon in the present study is valid for comparisons across different age groups, because we used the same methods for all subjects.

In conclusion, the age-related changes in human plantar flexor muscles and in the Achilles tendon were different from each other. In particular, the age-related changes of the Achilles tendon (i.e., decline in the tendon extensibility) was observed in men in their 30s, whereas there were no differences in muscle strength and activation levels between the 20- and 30-yr groups. These results indicate that the high frequency of Achilles tendon ruptures in men in the 30-yr group was caused by these differences in the aging processes of muscle and tendon.

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