A Cross-Sectional Study of the Plantar Flexor Muscle and Tendon during Growth

Abstract

The purpose of this study was to investigate growth changes in human plantar flexor muscle and tendons. In addition, we ascertained whether growth changes in muscle and tendon were more closely related to skeletal age than chronological age. 22 elementary school children (ESC), 19 junior high school students (JHS), and 23 young adults (ADT) men participated in this study. Maximal strain and hysteresis of tendon structures and cross-sectional area of Achilles tendon were measured using ultrasonography. In addition, skeletal age was assessed using Tanner-Whitehouse III method. Maximal strain of ESC was significantly greater than that of other groups, while no significant difference was observed between JHS and ADT. There was no difference in hysteresis among 3 groups. Relative cross-sectional area (to body mass^{2/3}) of ADT was significantly smaller than that of other groups. For ESC and JHS, measured variables of muscle and tendon were significantly correlated to both chronological and skeletal ages. These results suggested that immature musculoskeletal system was protected by more extensible and larger tendon structures in ESC and only by larger tendon structures in JHS, respectively. Furthermore, there were no differences in correlation coefficient values between measured variables of muscle and tendon and chronological or skeletal ages.

Introduction

It is well known that each tissue type in the body does not always change the same way with growth. This point must to be given attention in order for growing children to perform various kinds of exercise and training. However, few studies have ever attempted to investigate growth changes in tendon structures, compared to the other tissues, e.g., muscle, bone [14–16]. On the other hand, it is well established that there is an age-related change in muscle function and performance e.g., [1]. Information on the growth changes in the human tendon structures in vivo is necessary for understanding the changes in the various muscle functions, since the viscoelastic properties of tendon structures affect performance during human movements e.g., [21]. To date, however, a few reports have been available regarding the changes in the tendon structures with growth when tested experimentally in humans. Previous findings obtained from animal experiments showed that the tendons became less extensible, stiffer and stronger during the period of growth [30, 34]. Recent studies have demonstrated the growth changes in the elastic properties of human tendon structures in vivo [22, 31, 37]. According to these previous findings, tendon structures of children (pre-pubertal) were more compliant than those of adults. In addition, we reported that the knee extensor tendon properties of junior high school student (14.8±0.3 years) were already similar to those of adults [22]. Notably, our previous finding on tendon structures in knee extensors [22] suggested that more compliant tendon structures in pre-pubertal boys (10.8±0.9 years) played a role in protecting them from athletic injuries associated with musculoskeletal immaturity, e.g., Osgood Schlatters disease [9,25]. However, the musculoskeletal disorder during a period of growth also appears frequently in the ankle joint, e.g., calcaneal apophysitis [11,36] as well as the knee joint. To date, no studies have ever tried to investigate growth changes in plantar flexor tendon properties in pre-pubertal and pubertal children.
On the other hand, loading and unloading curves during the cyclic tensile test of tendon structures produced a loop (hysteresis) since tendon structures are viscoelastic materials [4]. The hysteresis of tendons affected the performances during stretch-shortening cycle exercises [7, 21]. Furthermore, previous studies have shown that the hysteresis of human tendons changes with aging, training, and immobilization [7, 19, 24]. Among these changes, we reported that the hysteresis of tendon structures in knee extensors increased with aging [24]. This result implied that for elderly individuals, the percentage of energy dissipated during a stretch-shortening cycle exercise is higher than that occurring in the young. According to the finding of animal experiments [34], the tendon hysteresis was greater in newborn pigs than in mature ones. However, no studies have been performed to investigate growth changes in the hysteresis of human tendon structures in vivo.

It is well known that the biological maturation (e.g., skeletal age) of children varies widely among a group of the same chronological age. According to a previous study [3], the fairly high percentage of variations in body dimensions between 12 and 16 years of age was explained by skeletal age, but not by chronological age. Furthermore, previous researchers have indicated that successful young athletes were of advanced skeletal maturation [14, 28]. Malina et al. [28] showed that the height, body mass and skeletal age of elite youth soccer players were slightly higher than those of untrained ones. Kanehisa et al. [14] also observed that the thickness of the quadriceps femoris muscle for elite junior Olympic weight lifters was highly correlated to skeletal age. However, no report has so far been available regarding the relationships between growth changes in human tendon properties and chronological or skeletal ages. Considering these previous findings on muscle and body size, it seems reasonable to suppose that growth changes in tendon properties are related to skeletal age rather than chronological age.

In the present study, we aimed to compare the morphological and viscoelastic properties of human plantar flexor muscle and tendon among elementary school children (pre-pubertal), junior high school students (pubertal) and adults. In addition, we ascertained whether growth changes in muscle and tendon were more closely related to skeletal age than chronological age.

### Materials and Methods

#### Subjects

22 elementary school children (ESC), 19 junior high school students (JHS), and 23 young adults (ADT) men participated in this study. The ages and physical characteristics of each group are shown in Table 1. The ages and physical characteristics of the subjects. Data are presented as mean (SD).

<table>
<thead>
<tr>
<th></th>
<th>ESC (n = 22)</th>
<th>JHS (n = 19)</th>
<th>ADT (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>11.2 (1.1)</td>
<td>13.8 (0.6)</td>
<td>22.2 (2.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>141.9 (6.1)</td>
<td>162.2 (7.8)</td>
<td>170.6 (5.8)</td>
</tr>
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<td>Body mass (kg)</td>
<td>33.7 (4.8)</td>
<td>48.7 (6.5)</td>
<td>68.1 (10.1)</td>
</tr>
<tr>
<td>Lower leg length (cm)</td>
<td>32.8 (1.9)</td>
<td>37.4 (2.3)</td>
<td>38.6 (2.0)</td>
</tr>
</tbody>
</table>

ESC: Elementary school children, JHS: Junior high school students, ADT: Adults

*significant difference between ESC and the other 2 groups

*significant difference between JHS and ADT

#### Viscoelastic properties of tendon structures

The maximal voluntary isometric strength (MVC) of plantar flexor muscles was determined using a specially designed dynamometer (Applied Office, Tokyo, Japan). The subject lay prone on a test bench, and the waist and shoulders were secured by adjustable lap belts and held in position. The right ankle joint was set at 90° (anatomical 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. Prior to the test, the subject performed a standardized warm-up and submaximal contractions to become accustomed to the test procedure. Subjects were instructed to develop a gradually increasing force from a relaxed state to MVC within 5 s, followed by gradual relaxation within 5 s. The task was repeated 2 times per subject with at least 3 min between trials. A real-time ultrasonic apparatus (SSD-6500, Aloka, Japan) was used to obtain a longitudinal ultrasonic image of the medial gastrocnemius muscle (MG) at the level of 30% of the lower leg length, from the popliteal crease to the center of the lateral malleolus. Ultrasonic images were recorded on a videotape at 30Hz, and were synchronized with recordings of a clock timer for subsequent analyses. The tester visually confirmed the echoes from the aponeurosis and fascicles. The point at which one fascicle was attached to the aponeurosis (P) was visualized on the ultrasonic image. P moved proximally during isometric torque development up to the maximum [Fig. 1 of 20]. The cross-point between the superficial aponeurosis and fascicles did not move. Therefore, the displacement of P was considered to indicate the lengthening of tendon structures (deep aponeurosis and distal tendon) [20, 23].

Tendon displacement has been attributed to both angular rotation and contractile tension, since any angular joint rotation occurs in the direction of ankle plantar flexion during an “isometric” contraction e.g., [26]. To monitor ankle joint angular rotation, an electrical goniometer (Penny and Giles, Biometrics Ltd, Gwent, UK) was placed on the lateral aspect of the ankle. To correct the measurements taken for the elongation of tendon structures, additional measurements were made under passive conditions. The displacement of P caused by rotating the ankle from 100 to 80° deg was digitized in sonographs taken as described above. Thus, for each subject, the displacement of P obtained from the ultrasound images could be corrected for that attributed to joint rotation alone e.g., [26]. In the present study, only values corrected for angular rotation have been reported.
The torque (TQ) measured by the dynamometer during the isometric plantar flexion was converted to muscle force (Fm) by the following equation:

\[ F_m = k \cdot F_t \]

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

where \( F_t \) and \( k \) represent the tendon force and the relative contribution of the physiological cross-sectional area of MG within plantar flexor muscles [8], and MA is the moment arm length of the triceps surae muscles at 90° of the ankle joint, which is estimated from the lower leg length of each subject [10]. To calculate strain values from the measured elongation, we measured the initial length of tendon structures, from the measured site (position of the probe) to the insertion of Achilles tendon [23]. The area within the force–elongation loop, as a percentage of the area beneath the curve during the ascending phase, was calculated as hysteresis [20, 23].

To estimate co-activation level of the tibialis anterior muscle, an electromyographic activity (EMG) of the tibialis anterior muscle (TA) was measured during plantar flexion contraction. To determine maximal activation of the TA, a maximal dorsiflexion isometric contraction was performed at the same angle (90° of the ankle joint). We normalized the integrated EMG value of TA with respect to the integrated EMG value of TA at the same angle when acting as an agonist at maximal effort. We confirmed that there was no significant difference in co-activation level among ESC (12.9±5.3%), JHS (13.6±4.8%) and ADT (13.0±4.3%). This result agreed with the previous findings [18, 29, 32]. In the present study, we did not use these data to estimate tendon force, because the main purpose of this study was to compare the measured variables among the 3 groups.

The repeatability of the measurements of maximal strain and hysteresis was investigated on 2 separate days in a preliminary study with 6 young males. There were no significant differences between the test and retest values of the maximal strain and hysteresis of tendon structures. The coefficient of variation (CV) was 7.8% for maximal strain and 10.7% for hysteresis.

**Muscle thickness and tendon cross-sectional area**

The subject’s posture was the same as for the measurement of tendon properties described above. The ultrasonic apparatus with a 7.5 MHz linear-array probe was used to determine the muscle thickness of the plantar flexor muscles, i.e., MG, lateral gastrocnemius muscle (LG), and soleus muscle (SOL). Cross-sectional images were obtained at the proximal levels of 30% (MG and LG) and 50% (SOL) of the distance from the popliteal crease to the center of the lateral malleolus. The scanning head of the probe was coated with a transmission gel to obtain acoustic coupling and oriented along the mid-sagittal axis of each muscle. At that level, the mediolateral widths of MG and LG were determined over the skin surface, and the position of one-half of this width was used as the measurement site for each muscle. For SOL, the position of the greatest thickness in the lateralf hal of the muscle was measured at the level mentioned above. For MG and LG, the subcutaneous adipose tissue-muscle (MG or LG) interface and muscle (MG or LG)-SOL interface were identified from ultrasonic images. For SOL, the muscle (LG)-muscle (SOL) interface and muscle (SOL)-muscle (flexor hallucis longus) interface were identified from the ultrasonic image. The distances from the adipose tissue-MG interface to the MG-SOL interface, from the adipose tissue-LG interface to the LG-SOL interface and from the LG-SOL interface to the SOL-muscle (flexor hallucis longs) interface were adopted as representative of the thickness of MG, LG and SOL, respectively. In addition, the mean values of the muscle thickness of MG, LG and SOL were adopted as representative of the muscle size of the plantar flexor muscles.

Immediately following the measurement of muscle thickness, the cross-sectional area of the Achilles tendon was also measured by an ultrasonic apparatus at the height of lateral malleolus of the Achilles tendon (Fig. 1). From the axial image, an outline of the tendon was traced, and the traced image was transferred to a computer for calculating the cross-sectional area of the tendon using open-source image analysis software (Image J, NIH, Bethesda, MD). All images were investigated 3 times, with the mean being used for further analysis. The repeatability of the measurements of muscle thickness and tendon cross-sectional area was investigated on 2 separate days in a preliminary study with 6 young males. There were no significant differences between the test and retest values of the muscle thickness and tendon cross-sectional area. CV was 2.5% for muscle thickness and 3.8% for tendon cross-sectional area.

**Skeletal age**

Skeletal age was estimated from X-rays of the left hand and wrist of each subject using the radius-ulna-short bone (RUS) score according to the Tanner-Whitehouse method [35]. At first, the RUS score was rated by an experienced analyst as a coauthor of this study (Dr. Hirose). The RUS score was calculated into skeletal age using the Tanner-Whitehouse III method [13].

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The re-evaluation of the skeletal age was performed for all subjects. There were no significant differences between the test and retest values of the skeletal age. The test-retest correlation coefficient (r) was 0.996.

Statistics
Descriptive data included means ± SD. One-way analysis of variance (ANOVA) was used for comparisons among the 3 groups. If the F statistic of the analysis of variance was significant, differences between groups were assessed by a Tukey-post hoc test. A linear regression analysis was performed on the relationship between the measured variables of muscle and tendon and chronological or skeletal age. The level of significance was set at p < 0.05.

Results
The height and body mass were the greatest in ADT and the lowest in ESC among the 3 groups. The lower leg length of ESC was significantly lower than that of the other groups, while no significant differences were observed between JHS and ADT (Fig. 3).

The MVC and muscle thickness (absolute and relative) of the plantar flexors are presented in Table 2. The absolute MVC and muscle thickness were greatest in ADT and the lowest in ESC among the 3 groups. There was no difference in the relative MVC (to body mass) among the 3 groups. The relative muscle thickness (to body mass$^{1/3}$) of ADT was significantly greater than that of the other groups, while no significant differences were observed between ESC and JHS.

The morphological and viscoelastic properties of tendon structures are presented in Table 3. There was no difference in the maximal elongation of tendon structures among the 3 groups (Fig. 2). The maximal strain of tendon structures of ESC was significantly greater than that of the other groups, while no significant difference was observed between JHS and ADT (Fig. 3).

There was no difference in the hysteresis of tendon structures among the 3 groups. The absolute tendon cross-sectional area of ESC was significantly smaller than that of the other groups, while the relative tendon cross-sectional area (to body mass$^{2/3}$) of ADT was significantly smaller than that of the other groups. For ESC and JHS, the measured variables of muscle (MVC and muscle thickness) and tendon (maximal strain, cross-sectional area) were significantly correlated to the chronological or skeletal age (p = 0.003–p < 0.001, Table 4). For all the variables, there were no differences in the correlation coefficient values between the 2 relations (p = 0.834 for MVC, p = 0.764 for muscle thickness, p = 0.764 for maximal strain, p = 0.603 for tendon cross-sectional area).

Discussion
The main findings of this study were that 1) the tendon structures in plantar flexors were more extensible in ESC than in JHS and ADT; 2) the relative tendon cross-sectional area (to body mass$^{2/3}$) of ESC and JHS was larger than that of ADT; 3) there were no differences in the correlation coefficient values between the measured variables of muscle and tendon and chronological or skeletal ages.

Previous studies showed that the tendon structures of children (pre-pubertal) were more compliant than those of adults [22, 31, 37]. In the present study, the maximal strain of tendon structures of ESC was greater than that of ADT. The present result on the maximal strain of tendon structures agreed with the previous findings on knee extensor tendon structures [22] and patellar tendon [31]. Previous findings obtained from animal experiments also showed growth changes in the viscoelastic properties of tendons [30, 34]. Nakagawa et al. [30] reported that the strain value at a given stress level of immature rabbits was greater than that of adults, and thus the Young's modulus of immature rabbits was lower than that of adults. Furthermore, previous researchers demonstrated that the wave crimping angle of collagen fibers within tendons decreased during growth [5, 17, 33]. Accordingly, the more extensible tendon structures of

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<tr>
<td>MVC (Nm)</td>
<td>54.9 (9.8)</td>
<td>84.4 (17.3)</td>
<td>118.2 (26.1)</td>
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<td>relative MVC to body mass (Nm·kg$^{-1}$)</td>
<td>1.64 (0.29)</td>
<td>1.74 (0.31)</td>
<td>1.75 (0.37)</td>
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<td>muscle thickness (mm)</td>
<td>14.0 (1.3)</td>
<td>15.6 (1.6)</td>
<td>20.0 (2.7)</td>
</tr>
<tr>
<td>relative muscle thickness to (body mass)$^{1/3}$ (mm·kg$^{-1/3}$)</td>
<td>4.34 (0.03)</td>
<td>4.28 (0.38)</td>
<td>4.90 (0.57)</td>
</tr>
</tbody>
</table>

ESC; Elementary school children, JHS; Junior high school students, ADT; Adults
* significant difference between ESC and the other 2 groups
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<tr>
<td>initial length (mm)</td>
<td>229.1 (15.2)</td>
<td>263.9 (17.5)</td>
<td>275.1 (20.8)</td>
</tr>
<tr>
<td>maximal elongation (mm)</td>
<td>15.9 (2.1)</td>
<td>15.6 (3.4)</td>
<td>16.5 (3.0)</td>
</tr>
<tr>
<td>maximal strain (%)</td>
<td>6.98 (1.06)</td>
<td>5.95 (1.29)</td>
<td>6.02 (1.09)</td>
</tr>
<tr>
<td>hysteresis (%)</td>
<td>18.8 (9.8)</td>
<td>20.0 (12.8)</td>
<td>17.9 (11.5)</td>
</tr>
<tr>
<td>cross-sectional area (mm$^2$)</td>
<td>60.1 (13.6)</td>
<td>76.9 (16.7)</td>
<td>74.7 (14.7)</td>
</tr>
<tr>
<td>relative cross-sectional area to (body mass)$^{2/3}$ (mm$^2$·kg$^{-2/3}$)</td>
<td>5.73 (1.20)</td>
<td>5.79 (1.62)</td>
<td>4.48 (0.68)</td>
</tr>
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ESC; Elementary school children, JHS; Junior high school students, ADT; Adults
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$ significant difference between ADT and the other 2 groups
ESC would be related to the greater crimp angles of collagen fibers within tendon structures, although no difference in the range of the toe region was found among the 3 groups (\( Fig. 2, 3 \)). Moreover, the more compliant tendon structures of ESC may contribute to decreasing the excessive stress on the insertion of Achilles tendon due to a rapid bone growth.

On the other hand, the present (plantar flexors) and our previous (knee extensors; 22) studies have shown that the extensibility of tendon structures of junior high school students (13.8 ± 0.6 years in the present study, 14.8 ± 0.3 years in [22]) was already similar to that of adults. Many previous studies demonstrated that the muscle strength and size increased rapidly during a period of junior high school, i.e., during and after puberty e.g., [15]. Previously, we suggested that more compliant tendon structures in elementary school children (10.8 ± 0.9 years) played a role in providing protection against athletic injuries associated with musculoskeletal immaturity, e.g., Osgood Schlatters disease [22]. It is therefore likely that the immature musculoskeletal system of junior high school students, unlike that of elementary school children, is protected by another factor (discussed later) and not by compliant tendon structures.

During stretch-shortening cycle exercises (e.g., running, jumping), the hysteresis of tendon structures represented the energy lost as heat due to internal damping, while the area under the unload curve was the energy recovered in the elastic recoil [4]. Only few studies have ever tried to investigate growth changes in tendon hysteresis [34]. Shadwick [34] reported that porcine tendon hysteresis decreased during growth (24.5% in newborn and 9.2% in mature pigs). In the present study, however, there was no difference in the hysteresis of tendon structures among the 3 groups. The discrepancy between the finding of Shadwick [34] and the present result could be explained by the difficulty of measuring hysteresis of human tendons. More recently, Finni et al. [6] stated that the large variability in hysteresis of human tendons was due to the difficulty in controlling the relaxation phase, a low sampling frequency of ultrasound images, etc. In the future study, we need to introduce a reliable technique to measure the hysteresis of human tendons in vivo.

Another interesting finding of this study was that the relative cross-sectional area of the Achilles tendon was larger in ESC and JHS than in ADT. Magnusson et al. [27] also reported that the cross-sectional area of the Achilles tendon was greater in elderly than in young individuals, which may reduce the risk of injury to the tendons of elderly people. Therefore, it seems reasonable to suppose that the larger tendons of both children and the elderly contribute to preventing tendon injuries. In particular, the increase in muscle strength and decrease in tendon extensibility occurred simultaneously during the period of junior high school. Considering these points, we may say that the immature musculoskeletal system of JHS is protected through reduction of the imposed stress through increased tendon size.

A previous study indicated that a fairly high percentage of variations in body dimensions could be explained by skeletal age, but not by chronological age [3]. Furthermore, Kanehisa et al. [14] reported that the thickness of the quadriceps femoris muscle in elite junior Olympic weight lifters was highly correlated to skeletal age. However, there were no differences in the correlation coefficient values between the measured variables of muscle (muscle strength and thickness) and tendon (maximal strain, cross-sectional area) and chronological or skeletal age (\( Table 4 \)). As a reason for this difference in the results between the present and previous findings, the characteristics of subjects may be involved. In the present study, all subjects of ESC and JHS were ordinary students, thereby yielding a high correlation between chronological and skeletal age (\( r=0.88, \ p<0.001 \)). On the other hand, previous studies demonstrated that successful young athletes were of advanced skeletal maturation [14,28]. Hence, it is likely that if elite athletes are included, growth changes in the muscle and tendon are related to those of skeletal age rather than chronological age. These discussions require additional data for clarification.

We should notice that there are some limitations of the methodology followed. Firstly, the Fm–L relationship of the tendon structures should be converted to a stress–strain relationship to accurately compare the tendon properties between children and adults. However, we could not calculate the Young’s modulus from the stress–strain relationship of tendon, since we did not measure the viscoelastic properties of the outer tendon (i.e., Achilles tendon), but rather those of the tendon structures.
(including outer tendon and aponeurosis). In the present study, we used the maximal strain of tendon structures as a main measured variable of the tendon properties. Furthermore, we considered that the dynamics of muscle fibers and muscle function would be more closely related to the viscoelastic properties of tendon structures (including outer tendon and aponeurosis) than those of outer tendons, although this point has not yet been proven experimentally. Secondly, the maximal strain of tendon structures may be affected by the activation level of each subject during the measurement of tendon properties. Previous studies indicated that the activation level of children was lower than that of adults [2,32]. In the present study, we did not use the interpolated twitch technique to measure the activation level for ESC and JHS due to ethical concerns. The difference in maximal strain of tendon structures between ESC and ADT may increase in the present study, if the activation level of ESC was lower than that of ADT. In other words, the obtained result (the maximal strain was greater in ESC than in ADT) did not change, if the activation level of ESC was lower than that of ADT. In addition, it would be untrue to say that the activation level of JHS was lower than that of ESC and ADT. Therefore, we considered that this point did not affect the main result (on the maximal strain of tendon structures) of this study.

In conclusion, the tendon structures in plantar flexors were more extensible in elementary school children than in junior high school students and adults. In addition, the relative tendon cross-sectional area of elementary school children and junior high school students was greater than that of adults. These results suggested that the process of growth changes in the plantar flexor muscle and tendon were different. Furthermore, there were no differences in the correlation coefficient values between the measured variables of muscle and tendon and chronological or skeletal ages. Finally, these observations on the growth changes in the human tendon structures in vivo may contribute to planning appropriate exercise interventions for growing children.

Acknowledgements
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