

## Original Article

# A Longitudinal Study of the Physical Characteristics, Muscle-Tendon Structure Properties, and Skeletal Age in Preadolescent Boys

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## Abstract

**Objectives:** The purpose of this study was to examine longitudinal growth changes in physical characteristics, muscle-tendon structure properties, and skeletal age in preadolescent boys and to compare the relationship between the changes in physical characteristics and muscle-tendon properties and the changes in chronological and skeletal ages. **Methods:** Fourteen prepubescent boys ( $10.9 \pm 1.1$  years old at the onset of the study) participated in this study over two years (yearly). Maximal muscle strength and maximal strain of tendon structure during ramp isometric contraction and muscle and tendon thickness for knee extensors and plantar flexors were measured. In addition, skeletal age was assessed using Tanner-Whitehouse three method. **Results:** Changes in height, thigh length, and lower leg length were highly correlated with changes in skeletal age but not chronological age. However, changes in the morphological and mechanical properties of muscle and tendon structure were not significantly associated with changes in chronological and skeletal ages. **Conclusion:** The present preliminary results suggest that longitudinal growth changes in the long-axis of the body are highly correlated with skeletal age change, whereas those in the muscle-tendon structure properties were not.

**Keywords:** Growth, Knee Extensor, Plantar Flexor, Skeletal Age, Ultrasonography

## Introduction

It is known that during early adolescence, there is a rapid increase in height, resulting in an imbalance in the growth of various body tissues<sup>1,2</sup>. This imbalance in the growth of various body tissues is thought to cause disorders specific to the growth period, although experimental data has not so far been provided to support this notion. According to the findings of several cross-sectional studies<sup>3-5</sup>, tendons in children,

compared to adults, may protect the fragile skeletal system by being more extensible and having a relatively large cross-sectional area. Furthermore, our previous study<sup>6</sup> indicated that children had the above characteristics in their Achilles tendon but not in their patellar tendon and that this regional difference in growth changes of tendons was associated with a regional difference in the frequency of growth-specific disorders, i.e., Osgood-Schlatter disease was more frequent than Seaver's disease<sup>7,8</sup>. However, all these previous studies compared tendon properties between children and adults, and there was limited knowledge of growth changes in muscle-tendon properties during the prepubertal period (approximately 9 to 12 years of age) when height increases rapidly<sup>4,9,10</sup>.

Skeletal age is considered to represent the body's biological maturity<sup>11</sup>. Therefore, growth changes in physical characteristics and muscle-tendon properties are expected to be more closely related to changes in skeletal age than chronological age. Beunen et al.<sup>12</sup> reported that skeletal

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**Table 1.** Age and physical characteristics in one-year intervals over two years. Mean (sd).

|                         | First year  | Second year     | Third year         | Effect of time | Effect size    |
|-------------------------|-------------|-----------------|--------------------|----------------|----------------|
| Chronological age (yrs) | 10.9 (1.1)  | 12.0 (1.1) ***  | 13.0 (1.1) ***###  | p<0.001        | $\eta^2=1.000$ |
| Skeletal age (yrs)      | 10.4 (1.4)  | 11.5 (1.5) ***  | 12.7 (1.6) ***###  | p<0.001        | $\eta^2=0.857$ |
| Height (cm)             | 141.1 (6.6) | 147.5 (8.1) *** | 154.5 (8.7) ***### | p<0.001        | $\eta^2=0.935$ |
| Body mass (kg)          | 33.8 (5.6)  | 37.6 (6.6) ***  | 43.2 (8.0) ***###  | p<0.001        | $\eta^2=0.909$ |
| Thigh length (cm)       | 32.6 (2.2)  | 34.3 (2.3) ***  | 35.9 (2.4) ***###  | p<0.001        | $\eta^2=0.863$ |
| Lower leg length (cm)   | 32.5 (2.2)  | 34.2 (2.3) ***  | 36.0 (2.7) ***###  | p<0.001        | $\eta^2=0.903$ |

*Significantly different from the first year (\*\*\*) p<0.001. Significantly different between the second and the third years (### p<0.001).*

age, not chronological age, explained the relatively large proportion of variability in body dimensions between 13 and 16 years. According to Malina et al.<sup>13</sup>, elite youth soccer players had somewhat greater heights, body masses, and skeletal ages than untrained adolescents. In addition, Kanehisa et al.<sup>14</sup> noted a strong correlation between skeletal age and quadriceps femoris muscle thickness in elite junior weightlifters. However, only an earlier cross-sectional study within our group<sup>4</sup> has examined the relationship between skeletal age and growth changes in tendon properties. In this previous study, there were no differences in correlation coefficient values between the measured variables of muscle and tendon and chronological or skeletal ages.

All previous studies cited in the above two paragraphs are cross-sectional studies. Therefore, no conclusions can be drawn regarding growth changes in muscle-tendon properties since cross-sectional studies involve other factors besides growth. To date, longitudinal studies on growth changes in muscle-tendon properties have been limited<sup>15-18</sup>. A series of studies by Mersmann et al. found increased muscle strength and muscle volume and no change in tendon extensibility (maximal strain) for knee extensors and plantar flexors in adolescent boys (16 to 18 years of age)<sup>15,16</sup>. However, to our knowledge, the only longitudinal study in preadolescence, when the growth changes in each tissue were unbalanced due to the rapid increase in height, was Pentidis et al.<sup>18</sup>, which examined growth changes in plantar flexors in 9-year-old subjects every three months for one year. Therefore, to prevent disorders specific to preadolescence, knowledge from longitudinal studies of growth changes in muscle-tendon properties during this period (especially in the knee extensors and plantar flexors, where growth-specific disorders develop) is considered essential.

In this study, we aimed to examine longitudinal growth changes in physical characteristics, morphological and mechanical properties of muscle and tendon structure in preadolescent children and to compare the relationship between the changes in physical characteristics and muscle-tendon properties and the changes in chronological and skeletal ages. We hypothesized that growth changes in physical characteristics and muscle-tendon structure properties would be more strongly associated with changes in skeletal age than with changes in chronological age.

## Materials and Methods

### Participants

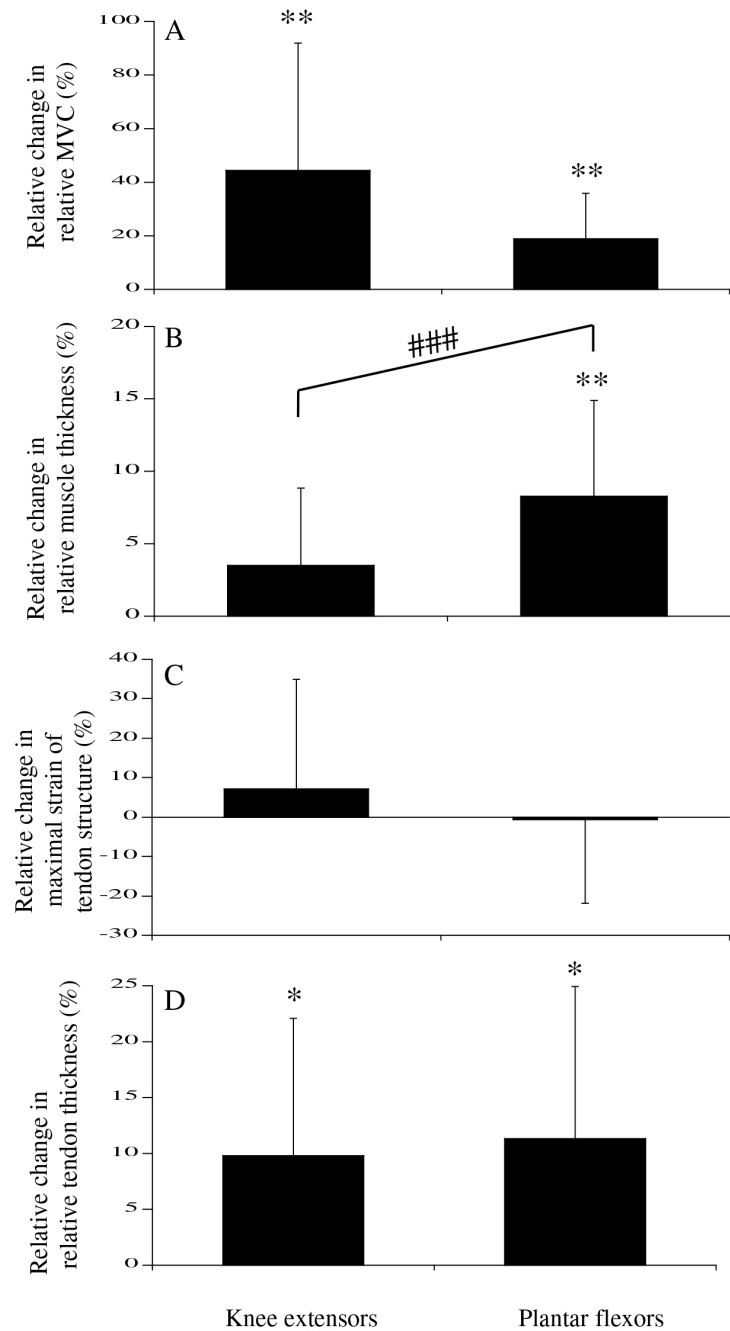
Fourteen early pubescent boys participated in this study over two years (yearly). All measurements (see below) were taken at approximately the same time of year (from November to December), with physical characteristics, muscle-tendon properties, and skeletal age measured on the same day. The ages and physical characteristics at the time of each measurement are shown in Table 1. Preadolescent boys were not involved in any physical training program beyond their regular school curriculum activities (less than 3 hours of physical activity per week during physical education classes at school).

### Physical characteristics

Height was measured to the nearest 0.1 cm using a stadiometer (TK-11253, Takei Scientific Instruments, Japan), and body mass was measured to the nearest 0.1 kg using a weight meter (YK-150D, YAGAMI, Japan). Limb lengths were measured to the nearest 0.5 cm using a flexible metal tape (Flat rule, KDS, Japan) based on anatomical landmarks<sup>e.g.,19</sup>: thigh length, the distance between the greater trochanter and the lateral condyle of the femur; lower leg length, the distance between the lateral condyle of the tibia and the lateral malleolus of the fibula.

### Mechanical properties of tendon structure

Maximal voluntary isometric contraction (MVC) was measured using custom-made dynamometers (Applied Office, Tokyo, Japan) for knee extension and plantar flexion, respectively. All measurements were performed on the right lower limb. After a standard warm-up and several submaximal contractions, participants were asked to exert isometric torque from relaxation to MVC within 5 s. The test was repeated twice for each person, with at least 3 minutes between trials. The highest torque among the trials was adopted to analyze the elongation of tendon structure (including outer-tendon and aponeurosis). During knee extension, the hips and back were held securely in the seat by lap belts. The ankle was fixed to the dynamometer's lever arm using a strap and secured with the knee joint flexed at 90 deg



**Figure 1.** Comparison of the relative changes from the first year to the third year in relative MVC (A), relative muscle thickness (B), maximal strain of tendon structure (C), and relative tendon thickness (D) between knee extensors and plantar flexors. Significantly different from the first year to the third year (\*  $p < 0.05$ , \*\*  $p < 0.01$ ). Significantly different between knee extensors and plantar flexors (###  $p < 0.001$ ).

(full extension = 0 deg). During plantar flexion, participants lay prone on a test bench and the waist and shoulders were securely held by lap belts. The ankle joint was fixed at 90 deg with the knee joint fully extended, and the foot was tightly strapped to a footplate attached to the dynamometer's lever arm by two straps.

Elongation of tendon structure (L) for knee extensors and plantar flexors was measured during isometric contractions, as mentioned above. By following the procedures described previously<sup>6</sup>, an ultrasonic apparatus (SSD-6500, Aloka, Tokyo, Japan) with an electronic linear array probe was used to obtain longitudinal ultrasonic images of the vastus

**Table 2.** Muscle strength and thickness in one-year intervals over two years. Mean (sd).

|                 |                                                      | First year  | Second year     | Third year        | Effect of time | Effect size        |
|-----------------|------------------------------------------------------|-------------|-----------------|-------------------|----------------|--------------------|
| Knee extensors  | MVC (Nm)                                             | 53.3 (20.8) | 73.9 (26.9) *** | 93.6 (36.9) ***#  | p<0.001        | $\rho\eta^2=0.793$ |
|                 | Relative MVC (Nm · kg <sup>-1</sup> )                | 1.55 (0.50) | 1.95 (0.55) **  | 2.12 (0.57) **    | p<0.001        | $\rho\eta^2=0.679$ |
|                 | Muscle thickness (mm)                                | 29.9 (2.3)  | 31.4 (2.9) **   | 33.5 (3.5) ***##  | p<0.001        | $\rho\eta^2=0.709$ |
|                 | Relative muscle thickness (mm · kg <sup>-1/3</sup> ) | 9.3 (0.6)   | 9.4 (0.6)       | 9.6 (0.7)         | p=0.034        | $\rho\eta^2=0.229$ |
| Plantar flexors | MVC (Nm)                                             | 53.5 (11.9) | 69.4 (13.8) *** | 80.5 (17.6) ***## | p<0.001        | $\rho\eta^2=0.810$ |
|                 | Relative MVC (Nm · kg <sup>-1</sup> )                | 1.60 (0.31) | 1.85 (0.24) **  | 1.86 (0.25) **    | p<0.001        | $\rho\eta^2=0.539$ |
|                 | Muscle thickness (mm)                                | 14.1 (1.2)  | 14.8 (1.2)      | 16.5 (1.5) ***### | p<0.001        | $\rho\eta^2=0.720$ |
|                 | Relative muscle thickness (mm · kg <sup>-1/3</sup> ) | 4.4 (0.2)   | 4.5 (0.4)       | 4.7 (0.4) **#     | p=0.001        | $\rho\eta^2=0.398$ |

MVC; maximal voluntary contraction. Significantly different from the first year (\*\* p<0.01, \*\*\* p<0.001). Significantly different between the second and the third years (# p<0.05, ## p<0.01, ### p<0.001).

**Table 3.** Mechanical and morphological properties of tendon structure in one-year intervals over two years. Mean (sd).

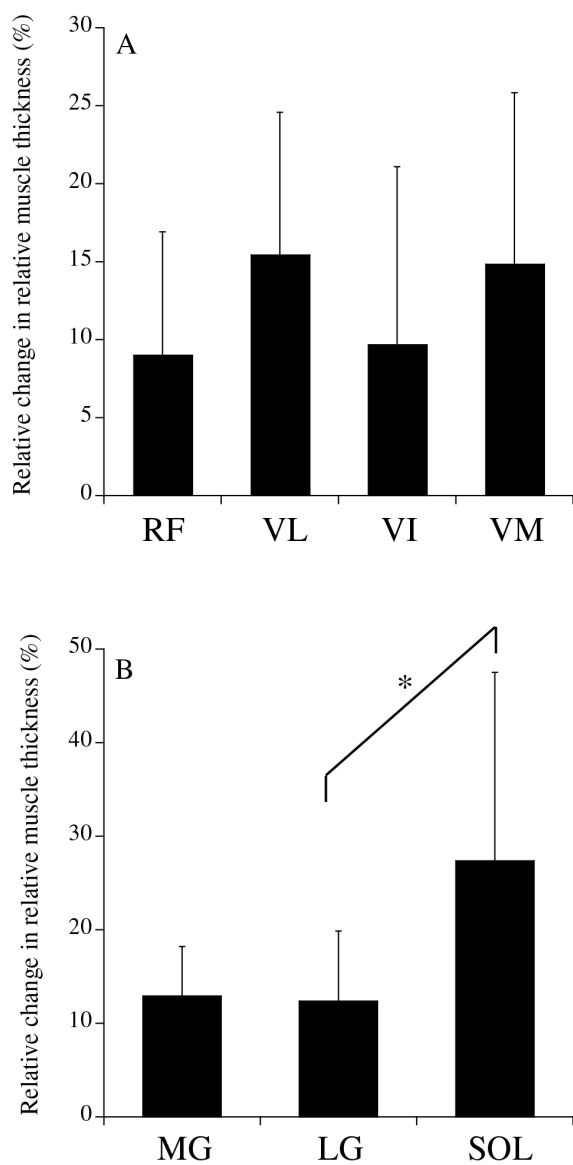
|                 |                                               | First year   | Second year      | Third year         | Effect of time | Effect size        |
|-----------------|-----------------------------------------------|--------------|------------------|--------------------|----------------|--------------------|
| Knee extensors  | Initial length (mm)                           | 280.1 (17.8) | 299.1 (21.0) *** | 307.5 (22.7) ***#  | p<0.001        | $\rho\eta^2=0.764$ |
|                 | Maximal elongation (mm)                       | 17.7 (3.6)   | 20.4 (4.4) *     | 20.5 (5.4)         | p=0.024        | $\rho\eta^2=0.248$ |
|                 | Maximal strain (%)                            | 6.3 (1.2)    | 6.8 (1.3)        | 6.6 (1.6)          | p=0.406        | $\rho\eta^2=0.067$ |
|                 | Stiffness (N · mm <sup>-1</sup> )             | 22.5 (15.5)  | 31.9 (14.9)      | 36.3 (15.2) *      | p=0.013        | $\rho\eta^2=0.285$ |
|                 | Thickness (mm)                                | 2.6 (0.4)    | 2.9 (0.4) ***    | 3.1 (0.4) ***      | p<0.001        | $\rho\eta^2=0.604$ |
|                 | Relative thickness (mm · kg <sup>-1/3</sup> ) | 0.81 (0.09)  | 0.87 (0.10) *    | 0.88 (0.09) *      | p=0.006        | $\rho\eta^2=0.323$ |
| Plantar flexors | Initial length (mm)                           | 226.4 (15.5) | 240.7 (18.0) *** | 255.7 (21.5) ***## | p<0.001        | $\rho\eta^2=0.790$ |
|                 | Maximal elongation (mm)                       | 15.9 (2.4)   | 16.6 (2.9)       | 17.5 (3.2)         | p=0.258        | $\rho\eta^2=0.099$ |
|                 | Maximal strain (%)                            | 7.1 (1.2)    | 6.9 (1.4)        | 6.9 (1.2)          | p=0.880        | $\rho\eta^2=0.010$ |
|                 | Stiffness (N · mm <sup>-1</sup> )             | 22.0 (10.4)  | 22.9 (7.8)       | 24.9 (9.2)         | p=0.649        | $\rho\eta^2=0.033$ |
|                 | Thickness (mm)                                | 4.7 (0.4)    | 5.2 (0.4) ***    | 5.6 (0.5) ***#     | p<0.001        | $\rho\eta^2=0.649$ |
|                 | Relative thickness (mm · kg <sup>-1/3</sup> ) | 1.45 (0.13)  | 1.56 (0.12) **   | 1.61 (0.17) *      | p=0.007        | $\rho\eta^2=0.386$ |

Significantly different from the first year (\* p<0.05, \*\* p<0.01, \*\*\* p<0.001). Significantly different between the second and the third years (# p<0.05, ## p<0.01).

lateralis muscle (VL) at 50% of the thigh length and medial gastrocnemius muscle (MG) at 30% of the lower leg length. Ultrasonic images were captured on videotape at 30 Hz and synchronized with recordings of a clock timer for further analysis. The echoes from the aponeurosis and fascicles were visually confirmed by the tester. The displacement of the point at which one fascicle was attached to the aponeurosis is considered to indicate the lengthening of tendon structure<sup>e.g.,20</sup>. During isometric contractions, however, any angular joint rotation occurred in the direction of knee extension and ankle plantar flexion. To measure joint angular rotation, an electrical goniometer (Penny and Giles, Biomechanics Ltd., Gwent, UK) was attached to the lateral aspect of each joint. Additional measurements were taken under passive conditions in order to correct the measurements taken for the elongation of tendon

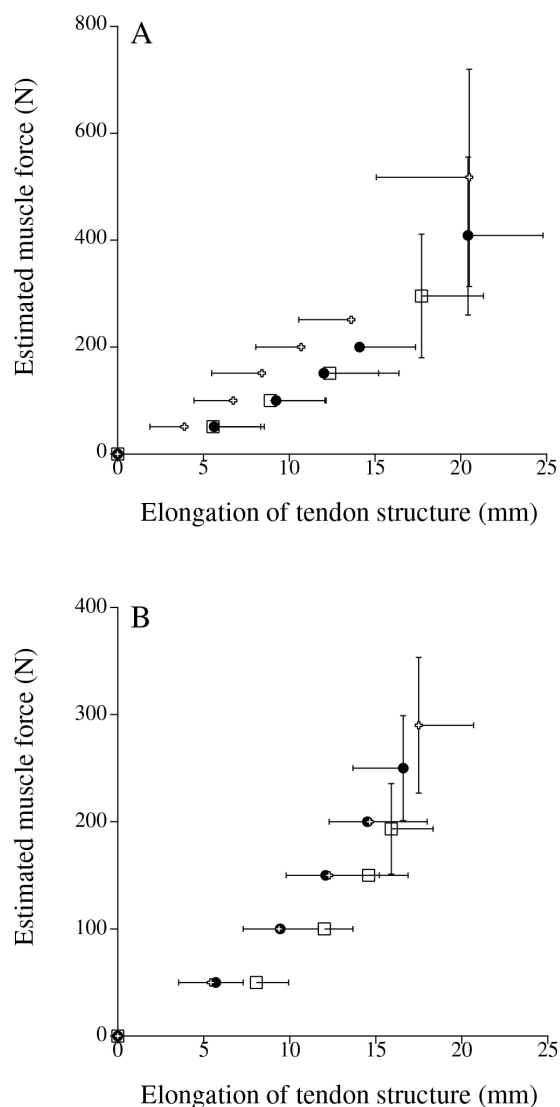
structure. The displacement of each site caused by rotating the knee and ankle from 100 deg to 80 deg was digitized in sonographs taken. Under passive conditions, the movement of this point was subtracted from the recorded elongation of tendon structure during isometric contractions<sup>e.g.,21</sup>. In this study, only values corrected for angular rotation are reported.

The following equation was used to convert torque (TQ) recorded during isometric contractions to the estimated muscle force (Fm)<sup>6</sup>:  $F_m = k \cdot TQ \cdot MA^{-1}$ , where k is the ratio of the physiological cross-sectional area of the vastus lateralis muscle within knee extensors<sup>22</sup> and the medial gastrocnemius muscle within plantar flexors<sup>23</sup>, and MA is the moment arm length in each knee extensors at 90 deg and plantar flexors at 90 deg, which was estimated from thigh length<sup>24</sup> and lower leg length<sup>25</sup>, respectively. In this study, the



**Figure 2.** Comparison of relative changes in relative muscle thickness among synergistic muscles for knee extensors (A) and plantar flexors (B). RF; rectus femoris muscle, VL; vastus lateralis muscle, VI; vastus intermedius muscle, VM; vastus medialis muscle, MG; medial gastrocnemius muscle, LG; lateral gastrocnemius muscle, SOL; soleus muscle. \*significantly different among synergistic muscles (\*  $p < 0.05$ ).

slope of Fm and L above 50% of MVC was defined as stiffness of tendon structure<sup>e,g,20</sup>. In addition, maximal elongation of tendon structure at MVC was converted to maximal strain of tendon structure by the following equation<sup>6</sup>:  $\text{Strain (\%)} = L \cdot \text{TL}^{-1} \cdot 100$ , where TL is the length of the tendon structure at rest (initial length of the tendon structure), calculated over the skin as the distance between the measured site (probe position) and the insertion of the patella and Achilles tendons



**Figure 3.** The relationship between the estimated muscle force and elongation of tendon structure in the first (open square), second (closed circle), and third (open cross) years for knee extensors (A) and plantar flexors (B).

(verified using ultrasonography). The repeatability of the measurements of maximal strain and stiffness of tendon structure was confirmed in our previous studies<sup>e,g,6</sup>.

#### Muscle and tendon thickness

An ultrasonic device was used to measure the muscle thickness of the knee extensors and plantar flexors. Participants remained in a supine position for the measurement of knee extensors and a prone position for the measurement of plantar flexors with legs straight and the muscles relaxed. The anthropometric locations of the measurement sites were precisely identified and marked

**Table 4.** Correlation coefficient values between the relative changes in chronological or skeletal ages and the measured variables of muscle and tendon structure.

|                 |                                    | vs Chronological age | vs Skeletal age |
|-----------------|------------------------------------|----------------------|-----------------|
| Knee extensors  | Relative MVC                       | 0.226                | -0.407          |
|                 | Relative muscle thickness          | -0.349               | 0.081           |
|                 | Maximal strain of tendon structure | -0.067               | -0.073          |
|                 | Stiffness of tendon structure      | 0.281                | -0.429          |
|                 | Relative tendon thickness          | 0.285                | -0.086          |
| Plantar flexors | Relative MVC                       | 0.014                | 0.455           |
|                 | Relative muscle thickness          | 0.131                | 0.407           |
|                 | Maximal strain of tendon structure | -0.026               | -0.037          |
|                 | Stiffness of tendon structure      | -0.040               | 0.165           |
|                 | Relative tendon thickness          | 0.336                | 0.218           |

*MVC; maximal voluntary contraction. All  $p > 0.05$ .*

before the ultrasonic measurement. For knee extensors, cross-sectional images on the central (rectus femoris muscle; RF and vastus intermedius muscle; VI), lateral (VL and VI), and medial surfaces (vastus medialis muscle; VM) were obtained at proximal level of 50% of the thigh length. For VI, the mean value of the central and lateral parts was adopted. For plantar flexors, cross-sectional images were obtained at proximal levels of 30% (MG and lateral gastrocnemius muscle; LG) and 50% (soleus muscle; SOL) of the lower leg length. The mean of the thickness of all synergistic muscles was adopted as the muscle thickness for knee extensors and plantar flexors, respectively. After measuring muscle thickness, the thickness of the patellar and Achilles tendons was measured using an ultrasonic equipment at 50% of patella tendon length and the height of the Achilles tendon's lateral malleolus. In the present study, absolute and relative (to body mass<sup>1/3</sup>) muscle and tendon thickness were presented<sup>6</sup>. The repeatability of the measurements of muscle and tendon thickness was confirmed in our previous studies<sup>e,g,6</sup>.

#### Skeletal age

Skeletal age, which indicated the individuals' biological maturation, was estimated from X-ray films of the hand and wrist using the radius-ulna-short bone (RUS) score according to the Tanner-Whitehouse method<sup>11</sup>. Radiographic images of the left hand and wrist of each participant were taken using X-ray image diagnostic device (DFW-10B/15D, Toshiba Medical Systems Corporation, Japan). The radiation dose *per session* was less than 1 mSv. Initially, the RUS score was determined by an experienced analyst who was also a collaborator of this work (N.H.). The Tanner-Whitehouse three method was used to convert the RUS score to skeletal age<sup>1</sup>. All subjects had their skeletal ages re-evaluated. There were no significant differences between the test and retest values of the skeletal age. The intraclass correlation coefficient between tests was 0.966.

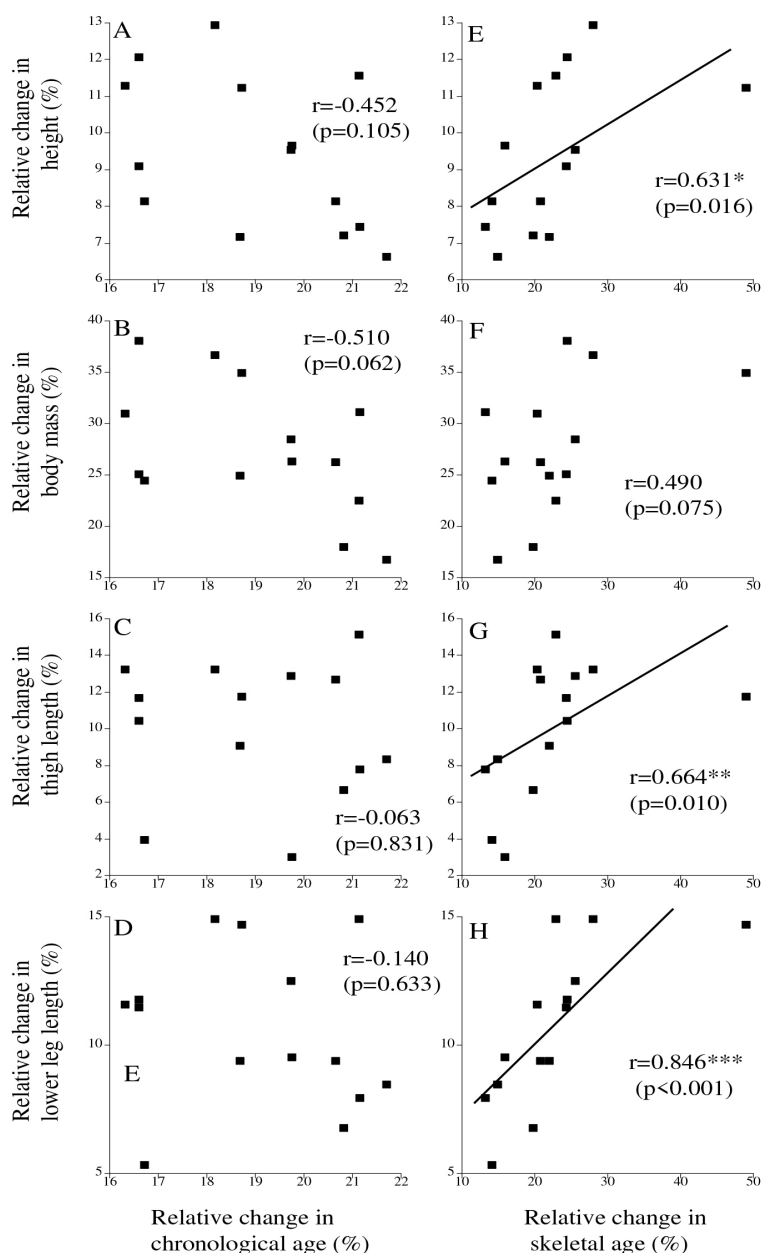
#### Statistical analysis

Descriptive data included means  $\pm$  SD. Normal distribution of the measured variables was tested using the Shapiro-Wilk test. One-way ANOVA with repeated measures was used to detect significant differences in the measured variables. In the event of significant values of F in the ANOVA, the Bonferroni post-hoc test of critical differences was used to assess the significance between means. Mauchly's sphericity test was employed in ANOVA to evaluate the homogeneity of variance. Where the sphericity assumption was violated, the Greenhouse-Geisser correction was used. Moreover, significant differences in the relative changes in the measured variables between knee extensors and plantar flexors were analyzed by a paired t-test. The effect size was calculated using partial eta-squared ( $\eta^2$ ) for one-way ANOVA and Cohen's d formula for a paired Student's t-test. Pearson's or Spearman's correlation coefficient based on data distribution was computed in order to determine the relationships among measured variables. The level of significance was set at  $p < 0.05$ .

#### Results

Height ( $9.4 \pm 2.1\%$ ), body mass ( $27.5 \pm 6.4\%$ ), thigh length ( $10.0 \pm 3.7\%$ ), and lower leg length ( $10.6 \pm 3.0\%$ ) significantly increased during the 2-year experimental period (Table 1). No significant correlation was found between the changes in chronological and skeletal ages ( $r = -0.393$ ,  $p = 0.164$ ).

Growth changes in muscle strength and thickness are shown in Table 2. For both knee extensors and plantar flexors, relative MVC significantly increased from the first year to the second year ( $p = 0.005$  for knee extensors,  $p = 0.002$  for plantar flexors), and no change was seen from the second



**Figure 4.** The relationship between the relative changes in chronological (left) or skeletal (right) ages and the physical characteristics.

year to the third year ( $p=0.544$  for knee extensors,  $p=1.000$  for plantar flexors). The increase in relative MVC from the first year to the third year tended to be higher for knee extensors ( $44.5 \pm 47.5\%$ ) than for plantar flexors ( $18.9 \pm 16.9\%$ ) ( $p=0.079$ ,  $d=0.795$ ; Figure 1A). No significant difference in relative muscle thickness for knee extensors was detected in the post-test (the effect of time was significant), whereas relative muscle thickness for plantar flexors significantly increased from the second year to the third year ( $p=0.036$ ). The increase in relative muscle thickness from the first year

to the third year was significantly higher for plantar flexors ( $8.3 \pm 6.6\%$ ) than for knee extensors ( $3.5 \pm 5.4\%$ ) ( $p<0.001$ ,  $d=0.800$ ; Figure 1B). In addition, among the plantar flexors, the increase in relative muscle thickness of the SOL ( $27.3 \pm 20.2\%$ ) was more pronounced than that of MG ( $13.0 \pm 5.3\%$ ) and LG ( $12.4 \pm 7.5\%$ ) ( $p=0.022$ ,  $\eta^2=0.324$ ), whereas relative muscle thickness increases in the gastrocnemius muscles and knee extensors (RF, VL, VI, and VM) were similar (Figure 2).

Growth changes in the morphological and mechanical

properties of tendons are shown in Table 3. For knee extensors and plantar flexors, there was a tendency for the Fm-tendon elongation relationship to shift to the left (Figure 3), but no change in maximal strain of tendon structure was observed during the 2-year experimental period (Figure 1C). Stiffness of tendon structure significantly increased for knee extensors but not for plantar flexors in the third year. For knee extensors and plantar flexors, relative tendon thickness significantly increased from the first year to the second year ( $p=0.023$  for the patellar tendon,  $p=0.001$  for the Achilles tendon), and no change was seen from the second year to the third year ( $p=1.000$  for the patellar tendon,  $p=0.759$  for the Achilles tendon). No difference in the increase in relative tendon thickness in the third year was found between patellar ( $9.8 \pm 12.3\%$ ) and Achilles ( $11.3 \pm 13.6\%$ ) tendons ( $p=0.758$ ,  $d=0.116$ ; Figure 1D).

Changes in muscle-tendon structure properties were not significantly correlated with both those in chronological and skeletal ages (Table 4). Changes in height, thigh length, and lower leg length were highly correlated with changes in skeletal age but not chronological age (Figure 4).

## Discussion

The present study examined longitudinal growth changes in physical characteristics, muscle-tendon structure properties, and skeletal age over two years (yearly) in preadolescent boys. The main results of this study were that 1) the increase in relative muscle strength (to body mass) tended to be higher for knee extensors, whereas the increase in relative muscle thickness (to body mass<sup>1/3</sup>) was higher for plantar flexors, 2) relative tendon thickness (to body mass<sup>1/3</sup>) increased similarly for knee extensors and plantar flexors, 3) maximal strain of tendon structure was unchanged for knee extensors and plantar flexors, 4) increases in height and limb lengths were closely associated with changes in skeletal age but not chronological age, while growth changes in any of muscle-tendon structure properties were not associated with either age change.

For knee extensors and plantar flexors, relative MVC and relative muscle thickness significantly increased between 11 and 13 years of age on average. A 1-year longitudinal study on average age of 9 years old, who are slightly younger than the participants in this study, also reported an increase in the relative strength of plantar flexors<sup>18</sup>. On the other hand, according to our cross-sectional research comparing two groups, an average of 11.2 years and 13.8 years<sup>4</sup>, there were no differences in relative MVC and relative muscle thickness for plantar flexors between the two groups. This discrepancy may be because the cross-sectional study failed to detect an actual difference between the two age groups. A new finding in this study was that the increase in relative muscle thickness was more pronounced for plantar flexors than knee extensors (Figure 1B). Furthermore, the increase in SOL thickness was remarkable among plantar flexors, and the increases in MG and LG thickness were about the

same as in the four knee extensors (Figure 2). These results indicated that the hypertrophy of slow-twitch muscle fibers was remarkable in the early adolescent (approximately 9-12 years old).

Some cross-sectional studies demonstrated that tendons were more extensible in children than in adults<sup>3-5</sup>, whereas other studies showed no difference in tendon extensibility between children and adults<sup>9,10</sup>. Furthermore, our previous study showed no difference between children and adults in the maximal tendon strain of knee extensors, but the maximal tendon strain of plantar flexors was higher in children than in adults<sup>6</sup>. The present study found no significant change in maximal strain of tendon structure during the two years of early adolescence, a narrower age range than the previous studies described above. None of the limited longitudinal studies of growth changes in tendon extensibility have found changes in maximal tendon strain<sup>15-18</sup>, and the results of this study are consistent with these previous findings.

A cross-sectional study found no significant difference in the Achilles tendon cross-sectional area between elementary (average age of 11 years old) and junior high school students (average age of 13 years old)<sup>4,9</sup>. In the present study, however, longitudinal studies during the same age period showed similar increases in relative tendon thickness of both patellar and Achilles tendons, which differed from the results of the cross-sectional studies cited earlier. One reason for this discrepancy is that the cross-sectional studies failed to detect actual differences between the two different age groups in the same way as with relative muscle strength and muscle thickness. Furthermore, the increases in relative muscle and tendon thickness were similar for plantar flexors, whereas the increase in tendon thickness (+9.8%) exceeded that of muscle thickness (+3.5%) for knee extensors (Figure 1BD). Although the mechanism for this regional difference is unknown, Mersmann et al.<sup>16</sup> similarly examined longitudinal growth changes in muscle-tendon size from 16 to 18 years old. They showed that the increase in patellar tendon cross-sectional area (+24%) exceeded that of quadriceps muscle cross-sectional area (approximately +6%).

One of the interesting findings of this study is that growth changes in the long-axis direction of the body (height and limb lengths) were highly associated with the change in skeletal age (Figure 4). Skeletal age theoretically indicates biological maturity, and indeed several cross-sectional studies have shown significant associations between skeletal age and body size<sup>12,13,26,27</sup>. To the best of our knowledge, this is the first longitudinal study to show an association between the growth changes in skeletal age and long-axis direction of the body. Previous studies on heritability and genetic polymorphisms have shown that height is more highly determined by heredity than other parameters such as body mass index and physical fitness<sup>28,29</sup>. The results of this study support the findings of previous studies on these genetic determinants. On the other hand, skeletal age changes were not significantly correlated with any of the muscle-tendon structure properties changes. Therefore, it is possible that the growth changes in the long-axis direction of the body are determined based on



genetic factors, whereas the growth changes in body mass and muscle-tendon structure properties are primarily due to acquired factors such as exercise history and nutritional status.

We should be aware that the methodology used has some limitations. Firstly, the sizes of muscles and tendons were evaluated in terms of thickness in the present study. Several previous studies showed a high correlation between muscle thickness and muscle volume (or cross-sectional area)<sup>30,31</sup>. For tendons, Finni et al.<sup>32</sup> showed using ultrasonography that the tendon thickness was significantly correlated with the tendon cross-sectional area. Therefore, the muscle and tendon thicknesses employed in this study represent the size of the respective tissues. Secondly, we used a previously reported the ratio of the physiological cross-sectional area of each muscle within the synergistic muscles and moment arm length estimated from limb length in order to calculate the muscle force. Unfortunately, it is not known whether the ratio of the physiological cross-sectional area among the synergistic muscles and the relationship between moment arm length and limb length in children is the same as in adults. However, since the main data for the mechanical properties of tendon structure in this study was the maximal strain, this should not significantly affect the main results. Thirdly, we determined the elongation of tendon structure (including outer-tendon and aponeurosis), but not the outer-tendon. Several studies showed that the mechanical properties of the outer-tendon were different from those of aponeurosis<sup>33,34</sup>. In future studies, a longitudinal study of growth changes in outer-tendon should be conducted. Fourthly, all participants were boys in the present study. It is known that puberty begins earlier in girls than boys<sup>35</sup>. Therefore, if the participants were girls in this study, the results should differ from those of the present study. Fifthly, all participants were ordinary students in the present study. Previous studies reported that elite junior athletes had higher skeletal age and body size than ordinary children<sup>13,14</sup>. Thus, changes in skeletal age and physical characteristics in preadolescence may be more pronounced than in ordinary children. If so, not only the growth changes in the long-axis direction of the body but also the growth changes in muscle-tendon structure properties may be significantly related to the change in skeletal age when elite junior athletes are used as participants.

In conclusion, the results of this study showed that longitudinal growth change in the long-axis of the body (height and limb length) was highly correlated with skeletal age change, not chronological age change, whereas those in the morphological and mechanical properties of muscle and tendon structure were not associated with both age changes.

#### Ethics approval

The study was approved by the office of the Department of Sports Sciences, University of Tokyo, and complied with their requirements for human experimentation (approval number: 312).

#### Consent to participate

All participants and their parents were informed of the purpose and the procedures to be used, and they gave their written informed consent to participate in this study.

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