

## Training- induced Achilles tendon adaptation: a distinct population within adolescence

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### Abstract

The Achilles tendon functions as a load-bearing component crucial for movement performance and swiftly adapts to changes in body mass and strength, particularly during periods of growth spurts. Mechanical and morphological attributes of the Achilles tendon were assessed in 41 participants (20 boys and 21 girls, comprising 22 athletes and 19 nonathletes) over a span of 18 months, employing ramped isometric plantar flexion. Longitudinal variations were analyzed using linear mixed modeling. This longitudinal study aimed to explore the combined effects of sport participation, maturation, and sex on the mechanical characteristics of the Achilles tendon across various muscle-tendon lengths. While the rate of stiffness increase post peak height velocity was comparable across all groups, a noteworthy finding was that boy-athletes displayed a more pronounced and earlier rate of Achilles tendon stiffness augmentation twelve months prior to PHV. Additionally, a distinctive mechanical property alteration was identified solely in boy-athletes at the 0° ankle angle. This elevation in Achilles tendon stiffness among boy-athletes was linked to greater peak force of the plantar flexors and a reduction in elongation. In summary, our study introduces novel longitudinal evidence indicating that sport participation interacts with sex and maturation, yielding a distinct adaptive response in Achilles tendon stiffness, specifically observed at the neutral position. These findings suggest a nuanced adaptive model wherein elevated force levels preceding PHV instigate heightened mechanical loading in specific muscle-tendon lengths, thereby inducing increased stiffness throughout adolescence. Specifically, the Achilles tendon stiffness exhibited a more substantial rate of increase in athletes compared to nonathletes and girls, leading to the conclusion that boy-athletes represent a distinct population during the maturation process.

**Keywords:** sex, maturation, sport participation, tendon stiffness, peak force

### Introduction

The Achilles tendon (AT), which plays a vital role as a key load-bearing element, holds significant importance in enhancing movement performance and displays swift responsiveness to changes in body mass and strength, especially around growth-spurt (Malina & Bouchard, 1991; Malina et al., 2004), which results in rapid increases in muscle force and higher mechanical loading on tendons (Lefevre et al., 1990; Lindgren, 1978). Furthermore, the mechanical attributes of the Achilles tendon are notably influenced by both sex disparities (Kubo et al., 2003) and variations in growth rates (Neugebauer & Hawkins, 2012). A significant body of evidence suggests that a swifter escalation in stiffness preceding the age of peak height velocity (PHV) in boys signifies distinct adaptive responses between sexes. Moreover, sports participation during adolescence has the potential to trigger adaptations in tendons (Heinemeier et al., 2016; O'Brien et al., 2010; Waugh et al., 2012) closely tied to amplified force capacity. Furthermore, the force-generating potential of plantar flexors varies across different lengths due to the length-tension relationship (Kawakami et al., 1998). However, the intricate interplay between these contributing factors remains inadequately comprehended in the context of children. It is widely acknowledged that muscle development occurs concomitantly with bone growth (Weide et al., 2015), as evidenced by the 6% annual increase in AT length (Benard et al., 2011; Mogi et al., 2018), coupled with alterations in AT stiffness arising from heightened body mass and strength (Waugh et al., 2012).

Discrepancies in Achilles tendon mechanical properties between adult males and females are apparent (Kubo et al., 2003), while growth rates exhibit variability in adolescents (Neugebauer & Hawkins, 2012). Recent investigations by G. Chalatzoglidis, A. J. Blazeovich, et al. (2021) reported accelerated increases in AT stiffness among boys due to heightened strength capacity, and in girls due to augmented strength and body mass around PHV. Likewise, Mogi et al. (2018) revealed heightened stiffness at and/or following PHV due to shifts in mechanical tendon attributes. Conversely, Neugebauer and Hawkins (2012), found no substantive alterations in AT strain, stiffness, or Young's modulus over a six-month span. Furthermore, no discernible correlation with sex, growth rate, or levels of physical activity emerged among girls aged 10-12 years and boys aged 12-14 years.

Although divergent growth rates may potentially influence AT mechanical properties, it is plausible that factors beyond maturation and sex contribute to mechanical transformations.

The interplay between sports participation, maturation, and their impact on the mechanical and morphological traits (such as rest length and cross-sectional area) of the AT cannot be underestimated. Waugh et al. (2014) observed a notable increase in AT stiffness following ten weeks of resistance training in pre-adolescent children. Correspondingly, Mersmann, Charcharis, et al. (2017) documented comparable findings among adolescents, signifying alterations in the internal tendon structure and collagen composition (Waugh et al., 2014). Reports of hypertrophy in both the AT and patellar tendon due to engagement in specific sports have also emerged (Cassel et al., 2016).

Recent longitudinal studies unveiled augmented stiffness normalized to force/mass among adolescent athletes (George Chalatzoglidis et al., 2021; G. Chalatzoglidis, A. J. Blazeovich, et al., 2021), whereas Pentidis et al. (2019) discerned no disparity in AT stiffness between pre-adolescent athletes and non-athletes following plyometric training. Thus, sports participation should also be considered an important factor regarding the force capacity and tendon properties during adolescence and the possible interaction with the sex. Therefore, a comprehensive understanding of the dynamic interrelation between sex and sports participation is imperative to mitigate injury risks and optimize athletic development within adolescence.

The foundation of strength capacity relies on the force-length relationship (Kawakami et al., 1998). However, insights into the force-length relationship among children are scant and primarily derive from cross-sectional or adult-comparison studies. For instance, Kannas et al. (2010) highlighted greater elongation in adults compared to children for identical force levels, despite similarities in fascicle length and pennation angle patterns. Both groups exhibited reduced fascicle length and heightened pennation angle as generated force was increased. Additionally, Wang et al. (2021) established that the free tendon contributes to over half of total musculotendinous unit (MTU) lengthening during stretches in adults. Similarly, Arabatzi (2018) demonstrated that distinct plyometric protocols exert analogous effects on both plantar flexor torque production and AT mechanical traits across various joint angles.

Hence, evaluating MTU length at different angles offers valuable insights into the interplay of muscle, tendon, and joint dynamics during force generation, particularly among demographics like boys and girls or athletes and non-athletes. However, there is still a noticeable lack of longitudinal data on the force-length relationship during maturation. Addressing this pivotal research gap, we present a longitudinal study exploring potential variations in the force-length relationship due to sports participation in relation to sex.

This study aims to investigate the repercussions of sex and sports participation on AT properties around the time of PHV. We also proposed the hypothesis that sports participation among adolescent boys and girls might result in unique patterns of adaptation in the mechanical characteristics of the Achilles tendon across different lengths.

## Materials and Methods

### *Participants*

A cohort of forty-one participants was assembled for this study, consisting of 20 boys (mean age:  $12.6 \pm 0.4$  years, weight:  $48.7 \pm 7.2$  kg, height:  $157.6 \pm 5.1$  cm) and 21 girls (mean age:  $10.6 \pm 0.5$  years, weight:  $36.6 \pm 6.8$  kg, height:  $146.4 \pm 8.1$  cm). All participants were selected within proximity to the age of peak height velocity (PHV; Table 1). Predictions of PHV were generated employing the anthropometric characteristics and age data, utilizing the equation developed by Mirwald et al. (2002).

The correlation coefficients (ICC) between the predicted and observed ages of PHV were notably high, measuring 0.97 ( $p < .001$ ) for boys and 0.77 ( $p < .001$ ) for girls, surpassing the significance threshold of  $>0.75$  (Koo & Li, 2016). Hence, the predicted PHV age demonstrated remarkable alignment with the observed PHV age. Participants were categorized as athletes or non-athletes based on their levels of physical activity. Athletes encompassed individuals engaged in track and field disciplines such as running, sprinting, and long jumping, characterized by high physical activity. In contrast, non-athletes reported engaging in less than 3 hours of sports activity weekly within the school setting and lacked supplementary sports training. The study cohort comprised 22 athletes (average age:  $13.1 \pm 1.1$  years) and 19 non-athletes (average age:  $12.8 \pm 1.1$  years). Participants were recruited from public schools, ensuring good health without any disabilities or leg injuries. Ethical consent was secured from both the children and their parents, aligning with the study's purpose and measurement protocol. The Local Ethics Research Committee granted formal approval for the study, adhering steadfastly to the ethical guidelines outlined in the Declaration of Helsinki.

Anthropometric measurements, covering parameters such as standing height, sitting height, leg length, body mass, and age, were meticulously recorded in intervals of six months across four sessions over a span of 1.5 years. These measurements were instrumental in delineating each participant's maturation stage, utilizing the methodology elucidated by Mirwald et al. (2002). The progression of maturation was systematically divided into four stages: 12 months preceding PHV, 6 months prior to PHV, the PHV period, and 6 months post-PHV (designated as the 1st, 2nd, 3rd, and 4th time points of measurement, respectively).

**Table 1.** Participant anthropometric data across different maturity stages (measured in months from the anticipated age of PHV) included age (years), body mass (kg), and height (cm). Notably, a noteworthy alteration in height (\*:  $p < 0.01$ ) was evident during the period spanning from one year before the projected age of PHV to the actual PHV age.

| Maturity stage  |           | -12         | -6          | 0             | +6           |
|-----------------|-----------|-------------|-------------|---------------|--------------|
| Boys<br>(N=21)  | Age       | 12.6 ± 0.4  | 13.4 ± 0.4  | 13.9 ± 0.4    | 15.2 ± 0.7   |
|                 | Body mass | 48.7 ± 7.2  | 53.2 ± 7.3  | 59.1 ± 7.2    | 61.3 ± 8.3   |
|                 | Height    | 175.6 ± 5.1 | 162.1 ± 7.2 | 169 ± 7.5 *   | 173.2 ± 10.7 |
| Girls<br>(N=20) | Age       | 10.6 ± 0.5  | 11.5 ± 0.5  | 12 ± 0.5      | 13.05 ± 0.5  |
|                 | Body mass | 36.6 ± 6.8  | 41.6 ± 7.9  | 46.8 ± 8      | 50.0 ± 8.5   |
|                 | Height    | 146.4 ± 8.1 | 151.4 ± 7.9 | 158.1 ± 6.9 * | 162.2 ± 4.0  |

#### *Procedure/Test protocol/Measure/Instruments*

Before testing, each participant engaged in a warm-up protocol that encompassed relaxed running and lower limb stretching to mitigate potential injury risks associated with maximal isometric contractions. Participants assumed a prone position, with their knees fully extended, atop a dynamometer bench (Cybex Humac Norm, CSMI, MA, USA). Ensuring optimal positioning, the right foot was placed perpendicular to the tibia (knee fully extended) and stationed on the dynamometer's footplate, conforming to established protocols (Kubo et al., 2014; Maganaris, 2002; Muramatsu et al., 2001; Muraoka et al., 2004). Torque measurements were meticulously captured at angles of  $-15^\circ$ ,  $0^\circ$ , and  $+15^\circ$ , relative to the neutral position ( $0^\circ$ ). The devised experimental setup and protocol remained consistent with methodologies detailed in prior literature (G. Chalatzoglidis, A. J. Blazeovich, et al., 2021).

The Achilles tendon's moment arm ( $MA$ ) was operationally defined as the perpendicular distance spanning the center of rotation and the line of AT action. The calculation of this  $MA$  entailed the utilization of the excursion method (Fath et al., 2013; Ito et al., 2000), consistent with prior investigations (G. Chalatzoglidis, A. J. Blazeovich, et al., 2021). A meticulous fitting of a third-order polynomial function was performed to clarify the relationship between angular position and AT elongation. From this polynomial, the derivative was extracted to provide an understanding of the slope of the curve that depicts the moment arm across varying angles ( $-15^\circ$ ,  $0^\circ$ , and  $+15^\circ$ ).

During measurements, observations revealed instances of heel movement and shifts in ankle joint angles. Detection of potential heel displacement relied on a method elucidated in preceding studies (Arampatzis et al., 2008; G. Chalatzoglidis, A. J. Blazeovich, et al., 2021). To offset potential impacts stemming from ankle joint rotation, this supplementary displacement was subtracted from the recorded elongation data ( $\Delta L$ ). The force of the tendon was determined through the equation  $F = M / d$ , where  $M$  denotes the plantarflexion moment and  $d$  stands for the length of the moment arm of the Achilles tendon. The opposing moment of the tibialis anterior (TA) was calculated using a method previously outlined (Mademli et al., 2004). The highest plantar flexor moment was adapted by factoring in the values of the opposing moment. This involved adding up the resulting joint moment and opposing moment to establish the plantar flexor moment.

Tendon stiffness ( $k$ ) in N/mm was determined as the slope of the linear portion of the force ( $F$ ) - elongation ( $\Delta L$ ) correlation. To establish this correlation, data from force-time calculations and data from the muscle-tendon junction (MTJ) position - time correlation were combined for each ramped isometric plantar flexion measurement. Subsequently, muscle force (N) and MTJ length change (mm) data were synchronized, allowing for the calculation of the gradient of the  $F$ - $\Delta L$  correlation:  $k = dF / dL$  (Maganaris et al., 2000; Waugh et al., 2012).

#### *Statistical analysis*

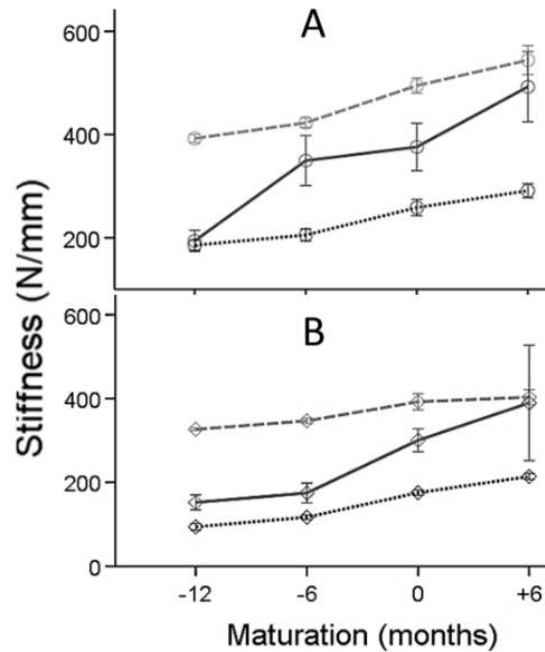
Statistical analysis was conducted using SPSS (IBM Corp., Version 29.0, Armonk, New York, USA). The independent variables included ankle angle ( $-15^\circ$ ,  $0^\circ$ ,  $+15^\circ$ ), maturation stages (-12, -6, 0, +6 months from PHV), gender (boys and girls), and sports participation (athletes vs. non-athletes). The dependent variables encompassed peak force, AT stiffness, and AT elongation. We employed a linear mixed model (LMM) analysis to explore potential variations in the dependent variables (peak force, AT stiffness, AT elongation) across the four maturity stages due to changes in ankle angle, gender, sports participation, and their interactions. Bonferroni confidence interval adjustment was applied for pairwise comparisons between main effects and interactions to determine mean differences between groups. Data are presented as mean ± SD, and statistical significance was determined at a p-value < .05.

#### **Results**

Table 1 presents the combined data's mean ± SD values for age and body mass across different stages of maturity.

The impact of ankle angle on AT stiffness emerged as significant ( $b=180.90$ , 95% CI 25.20 to 336.60,  $p=0.023$ ). Specifically, stiffness at  $-15^\circ$  ( $M=405.98 \pm 7.74$  N/mm-1) surpassed that at  $0^\circ$ , and  $0^\circ$  stiffness

( $M=290.01 \pm 7.32$ ) exceeded that at  $+15^\circ$  ( $M=187.65 \pm 7.44$ ) across maturity stages ( $p < .001$ ; see Fig. 1). A notable distinction arose between boys and girls around PHV, showcasing superior stiffness among boys ( $M=303.39 \pm 6.62$ ) compared to girls ( $M=256.03 \pm 5.97$ ) at both  $0^\circ$  and  $\pm 15^\circ$  ( $p < .001$ ). Athletes also exhibited heightened stiffness ( $M=333.07 \pm 6.67$ ) around PHV in the  $\pm 15^\circ$  angle in contrast to nonathletes ( $M=285.71 \pm 6.92$ ) ( $p < .049$ ).



**Fig. 1.** Main effect of angle on AT stiffness across maturity stages. A: Boy-athletes (circles), B: Girl-athletes (rombuses), Ankle angle:  $-15^\circ$  (dashed line),  $0^\circ$  (solid line),  $+15^\circ$  (dotted line).

We conducted a comparison of the average rate of AT stiffness augmentation at  $-15^\circ$ ,  $0^\circ$ , and  $+15^\circ$ . Considering distinct observation numbers across maturation stages, a linear mixed model (LMM) scrutinized the interaction's effect among maturation, sex, and sport participation. LMM analysis uncovered a significant interaction among maturation, sport involvement, and sex, assessed at  $0^\circ$  ( $b=83.07$ , [14.72 to 151.42],  $p=0.018$ ). This indicated a higher rate of AT stiffness increase in boy-athletes compared to other groups. The mean rate was defined as  $r =$

$\text{mean} \left[ \frac{(k_{\text{post}} - k_{\text{pre}})}{\Delta t} \right]$  where  $r$  signifies the mean rate of increase (in  $\text{N mm}^{-1} \text{ month}^{-1}$ ),  $k_{\text{pre}}$ ,  $k_{\text{post}}$  denotes AT stiffness (Table 2; refer to Fig. 2A). No rate of change disparity over time emerged among all groups at  $\pm 15^\circ$ .

**Table 1.** The mean rate of AT stiffness's increase per stage of maturity at the neutral position (months from predicted age of PHV). Notably, a significant greater rate ( $*: p < .05$ ) was discerned in the interval between 12 months before and 6 months before the predicted age of PHV in boy-athletes.

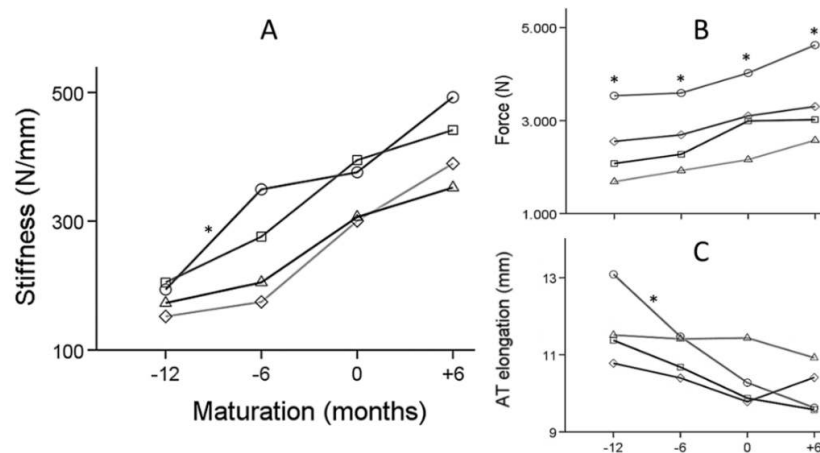
| Rate (AT Stiffness) | Mean $\pm$ SD ( $\text{N mm}^{-1} \text{ month}^{-1}$ ) |                  |                 |
|---------------------|---|------------------|-----------------|
|                     | -12 up to -6  | -6 up to 0       | 0 up to 1       |
| Boy-athletes        | $19.57 \pm 1.8^*$                                       | $10.37 \pm 5.7$  | $19.30 \pm 4.5$ |
| Boy-nonathletes     | $12.29 \pm 1.7$   | $18.30 \pm 5.76$ | $11.07 \pm 3.1$ |
| Girl-athletes       | $10.05 \pm 3.6$   | $18.14 \pm 9.4$  | $17.05 \pm 4.6$ |
| Girl-nonathletes    | $10.10 \pm 2.2$   | $16.20 \pm 5.1$  | $15.63 \pm 3.7$ |

#### Peak Force

A substantial effect of sex surfaced regarding peak force ( $b=1045.72$ , [143.51 to 1947.92,  $p=0.023$ ]), evidencing that boy exhibited higher force ( $M=3169.18 \pm 42.24$  N) compared to girl ( $M=2467.62 \pm 37.91$ ) around PHV at  $0^\circ$  and  $\pm 15^\circ$  ( $p < .001$ ). Athletes displayed significantly superior peak force ( $M=3169.18 \pm 42.24$ ) than nonathletes across maturity stages in all three ankle positions. It's noteworthy that boy-athletes, when compared to all groups, demonstrated elevated peak force starting from 12 months before PHV onward at  $0^\circ$  ( $p < .001$ ; Fig. 2B).

#### AT elongation

Intriguingly, significant variations in elongation were confined to the  $0^\circ$  angle. Although boy-athletes showcased no elongation discrepancies with other groups, the reduction rate of elongation was more pronounced 12 months before up to PHV compared to all groups ( $b=-3.313$ , [-7.230 to -.163,  $p=.032$ ). Notably, girl-athletes exhibited identical elongation reduction to boy-nonathletes up to PHV. Worth noting is the elongation increase post-PHV among girl-athletes, while nonathletes (both boys and girls) exhibited elongation decline after PHV (Fig. 2C).



**Fig II.** The critical findings: a pronounced (\*) rate of increase in AT stiffness 12 up to 6 months before PHV (A), the notably elevated peak force among boy-athletes (B), and the enhanced (\*) rate of decrease in the elongation of AT 12 up to 6 months before PHV during ramp plantar flexion isometric contraction at the neutral (0°) position (C). The groups depicted are boy-athletes (circles), boy-nonathletes (squares), girl-athletes (rhombuses), and girl-nonathletes (triangles). Statistical significance (\*) is indicated ( $p < .05$ ).

## Discussion

This longitudinal investigation delves into the intricate interplay of sport participation, sex, and maturation concerning Achilles tendon (AT) mechanical properties across varying ankle angles, particularly during the proximity of peak height velocity (PHV). The comprehensive analysis reveals distinctive patterns, most notably observed among athlete boys, who exhibit elevated stiffness and enhanced force capacity, leading to distinct stiffness profiles at the 0° angle during maturation, setting them apart from the remaining three participant groups. Notably, the boy-athletes exhibit a greater rate of stiffness increase compared to other groups, during the 12-month period preceding PHV and from PHV (time point 0) to 6 months post-PHV (time point 1). This divergence is particularly pronounced after PHV, where the stiffness values of the boy-athletes rise sharply, suggesting an intriguing adaptation. Although the stiffness patterns were similar between the groups at the other two ankle angles (-15° and 15°), the boy-athletes always showed greater stiffness and force capacity. The current findings expand our understanding of the impact of sports participation on tendon stiffness across various phases of biological maturation, and these outcomes are explored in the subsequent discussion.

### Implications for Boy-Athletes

Our findings, based on longitudinal measurements, unequivocally highlight a distinctive pattern among boy-athletes participants compared to girls (both athletes and non-athletes) and non-athlete boys. Specifically, we identified a more pronounced increase in tendon stiffness during the 12 months preceding PHV and the 6 months following PHV for athletes compared to the other groups. This trend aligns with our recent study (G. Chalatzoglidis, A. J. Blazevich, et al., 2021), which indicated rapid AT stiffness augmentation around PHV at the neutral ankle position for both boys and girls. The present data clarify the effect of interaction between sport participation and sex on tendon adaptation around PHV. As previously discussed, adult Achilles tendon adaptation arises from cyclic elongation during force production (Arampatzis et al., 2007), while in adolescents, training-induced strength gains and maturation also impact tendon properties (Quatman et al., 2008; Waugh et al., 2012). However, our study suggests that the rise in stiffness among boy-athletes is more linked to the reduction in tendon elongation rather than an increase in force. This pattern leads to the notable surge in stiffness slope 12 months before PHV, possibly attributable to the robust force capacity of boy-athletes (Arampatzis et al., 2010) during training and their effects on mechanoreceptor function.

These insights align with previous research underscoring the role of training-induced strain and its effects on tendon properties (Arampatzis et al., 2010; Kjaer, 2004). Moreover, our results concur with prior studies demonstrating adolescent athletes' radial growth of the patellar tendon without alterations in elastic modulus (Mersmann, Charcharis, et al., 2017). Furthermore, our findings echo the significance of isometric strength as an indicator of tendon properties (Waugh et al., 2012). Additionally, the nuances of short and long-term neuromuscular training during adolescence seem to result in neuromuscular adaptations before inducing greater force capacity, (G. Chalatzoglidis, F. Arabatzi, et al., 2021; Faude et al., 2017; Steib et al., 2017; Williams et al., 2021), thus explaining the delayed response of tendon tissue compared to muscle leading to greater force capacity resulting in the delayed response of the tendon tissue compared to muscle (Mersmann, Bohm, et al., 2017; Mersmann et al., 2019). Given these observations, our longitudinal results underscore the pivotal role of mechanical loading during training in shaping tendon adaptations, particularly one year before PHV. Sports participation could potentially expedite tendon property development, aiding adjustment to the high force capacity typical of male athlete participants, evident in the greater force levels observed.

#### *Post-PHV Alterations and Determinants*

Subsequent to PHV, we noted concurrent alterations in tendon and muscle tissues among male athlete participants. Our longitudinal exploration revealed a secondary increase in the produced force slope within this specific population, accompanied by a more significant rise in stiffness rate. This prompts the question: “Which is the determinate factor for the increase of Achilles tendon stiffness at a greater rate in sport participant children during maturation?”. Known factors like force level and body mass as loading influencers exert separate and cumulative effects on tendon stiffness throughout maturity. (Waugh et al., 2012). Although the precise mechanics of tendon adaptation during maturation remain to be fully elucidated, it's evident that the heightened rate of tendon stiffness in children is closely associated with greater force capacity. In line with our recent work (G. Chalatzoglidis, F. Arabatzi, et al., 2021), structural changes in the Achilles tendon were more prominent in athletes compared to non-athletes, linked to peak force. This finding supports the assertion that the rate of stiffness increase is notably higher among athletes in the months preceding PHV and six months post-PHV. This increase is primarily linked to greater force capacity and elongation reduction rate during the initial period, and an increase in force rate six months post-PHV. This alignment with previous intervention studies involving pre-pubertal children (Waugh et al., 2014) and late adolescence athletes (Mersmann, Bohm, et al., 2017), suggesting that muscle molecular signaling and force level appear to be a determinate factor after the PHV. Nevertheless, the training stimulus and the preceded greater capacity of the athletes could provide the trigger stimulus to alter the material properties in the Achilles tendon (Arampatzis et al., 2007; Kongsgaard et al., 2007). The concept of sport participation fostering significant alterations in tendon properties, leading to a unique adaptive model, gains support from these observations. Notably, the distinctive pattern of Achilles tendon stiffness observed in male athletes is prominent 12 months prior to PHV and extends to six months after PHV, marking a sex-specific interaction between sport participation and maturation.

#### *Sex Differences and Uniform Adaptation*

In terms of sex differences, our study uncovered similar patterns of tendon stiffness alteration during maturation for both non-athlete boys and girls, along with athlete girls. These patterns persisted despite variations in stiffness levels. All groups exhibited increased stiffness from six months prior to six months post-PHV, highlighting the intertwined relationship between growth and sport involvement. Notably, boys consistently displayed greater stiffness across all age stages compared to girls, suggesting that the interaction between sport participation and maturity has a sex-specific effect. In girls, stiffness changes could be attributed to concomitant increases in force capacity and body mass, while in boys, maximal plantar flexor force emerges as a predominant trigger. Our findings corroborate our recent longitudinal study (G. Chalatzoglidis, A. J. Blazeovich, et al., 2021), expanding our understanding of the interaction between sex and maturation. Muscle strength and body mass stimuli appear to drive tendon adaptation, with boys experiencing these adaptations earlier and to a greater extent than girls. These observations align with previous research (Mersmann et al., 2020; Pentidis et al., 2019) but not with others that showed no differences in AT between boys and girls cross-sectionally (Kubo et al., 2003) or over a short time period (Neugebauer & Hawkins, 2012). Our analysis further underscores that non-athlete boys and girls, along with female athlete participants, display comparable stiffness and force capacity patterns, suggesting a uniform adaptation of the muscle-tendon system.

#### *The effect of different ankle angles on the AT stiffness*

The unique change pattern of AT stiffness in boy athletes was evident only at 0° compared to -15° and 15°. This result supports the hypothesis that stiffness change is also an angle specific adaptation through the maturation. Although there is limited data about stiffness levels at different angles in adolescence, our findings based on longitudinal observations, clearly proposed that sports participation for boys could be beneficial not only in greater magnitudes (G. Chalatzoglidis, F. Arabatzi, et al., 2021), but also in earlier adaptations of tendon properties due to the increased level of force when imposed on the specific MTU length. One explanation might be that plantar flexors muscle fibers work nearly isometrically producing greater forces near their optimal length region (at neutral angle) supported by the AT gearing (Bohm et al., 2019). Furthermore, an increase in muscle strength with a corresponding change in tendon stiffness at the optimal length region, would undergo synergistic growth and adaptation during maturation in order to optimize muscle-tendon congruity (G. Chalatzoglidis, A. J. Blazeovich, et al., 2021). Thus, a link between muscle molecular signaling through the training and tendon adaptation may exist. This concept is consistent with the present data showing that the greater force levels for boy-athletes 12 months before PHV, at the neutral ankle angle, might trigger the tendon adaptation in response to mechanical loading. After PHV, muscle force increases again leading to a rapid increase of tendon stiffness at the same angle. Regarding our results at the different ankle's angle, the stiffness level increases with the force capacity of plantar flexors, showing greater stiffness at -15° compared to the other two angles in all maturation stages for all groups. At this angle (-15°), more force is needed to cause the same elongation during maximum isometric contraction.

These results might reflect a lesser adaptation around PHV on the extreme angles where the loads and requirements are greater, speculatively resulting from their lower force levels. In addition, the tendon adaptation at these ankle angles seems to be task specific and they are rarely used in daily activities. For the first time longitudinally, sex, sport and maturity-related adaptations of AT are recorded at different MTU lengths during



the growth spurt, leading to the suggestion that the immature system acts in favor to the myotendinous system congruity through balanced interaction of maturation and mechanical loading.

#### *Limitations and Future Directions*

This study did not examine the hormonal changes during adolescence, which could potentially lead to distinct alterations in stiffness (Cassel et al., 2016) with maturity, sex and sport participation. The assumption was made that the foot remained rigid. As a result, the potential impact of foot deformation on alterations in MTU length during contractions was deemed negligible (G. Chalatzoglidis, A. J. Blazevich, et al., 2021), and this factor was not taken into account in our measurements. Therefore, in a future study should measure the range of foot deformation in adolescents to avoid any systematic error. Finally, our findings are limited to adolescents and not adults. Future studies should determine the interaction between sport participation and sex on tendon's properties in adults.

#### **Conclusions**

In conclusion, our longitudinal study shows that sport participation interacts with sex and maturation during adolescence and induces a unique adaptive model of Achilles tendon in boy-athletes. Specifically, the Achilles tendon stiffness increased at a greater rate in athletes than nonathletes and girls leading to the conclusion that boy-athletes are a different population during maturation. These findings provide original longitudinal evidence that the greater force capacity of the boy-athletes, 12 months before PHV, triggers the adaptations of the tendon earlier than the other groups where the changes were found 6 months before to 6 months after the PHV and associated with the maturation progress. Although stiffness was increased in all groups after PHV, boy-athletes showed more rapid change with greater magnitude. These mechanical changes were observed in athletes at 0° ankle angle while all groups showed similar pattern of tendon properties change, despite the differences in the stiffness level, in both 15° and -15°. Muscle-tendon length at the extreme angles ( $\pm 15^\circ$ ) might not be suitable to record the initial alterations in tissue properties. These adaptive changes likely result in optimal muscle-tendon congruity and improve tendon efficiency.

**Conflict of interest:** The authors declare no conflict of interest.

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