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# Musculoskeletal and estrogen changes during the adolescent growth spurt in girls

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## Abstract

Introduction: The adolescent growth spurt is associated with rapid growth and hormonal changes, thought to contribute to the increased anterior cruciate ligament injury risk in girls. However, relatively little is known about these musculoskeletal and estrogen changes during the growth spurt in girls. Purpose: To investigate the longitudinal changes in estrogen as well as anterior knee laxity and lower limb strength and flexibility throughout the adolescent growth spurt in girls. Methods: Thirty-three healthy girls, age 10-13 yr, in Tanner stage II and 4-6 months from their peak height velocity were recruited. Participants were tested up to four times during the 12 months of their growth spurt, according to the timing of their maturity offset (test 1: maturity offset = -6 to -4 months; test 2: maturity offset = 0 months; test 3: maturity offset = +4 months; test 4: maturity offset = +8 months). During each testing session, anterior knee laxity, lower limb flexibility, and isokinetic strength as well as saliva measures of estradiol concentration were measured. Results: A significant ($P = 0.002$) effect of time on anterior knee laxity was found from the time of peak height velocity, although no changes in estradiol concentration were displayed over time ($P = 0.811$). Participants displayed a significant increase ($P < 0.05$) in isokinetic quadriceps strength over time, with no apparent increase in isokinetic hamstring strength. Conclusions: We speculate that increased quadriceps strength, combined with increased knee laxity and no accompanying hamstring strength development during the adolescent growth spurt in girls, might contribute to a decrease in their knee joint stability during landing tasks. These musculoskeletal changes could potentially increase anterior cruciate ligament injury risk at a time of rapid height and lower limb growth.

## Keywords

changes, girls, estrogen, growth, during, musculoskeletal, spurt, adolescent

## Disciplines

Arts and Humanities | Life Sciences | Medicine and Health Sciences | Social and Behavioral Sciences

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**Title:**

# Musculoskeletal and estrogen changes during the adolescent growth spurt in girls.

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**Running Head:**

Musculoskeletal changes during the growth spurt

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## Abstract

The adolescent growth spurt is associated with rapid growth and hormonal changes, thought to contribute to the increased anterior cruciate ligament (ACL) injury risk in girls. However, relatively little is known about these musculoskeletal and estrogen changes during the growth spurt in girls. **Purpose:** To investigate the longitudinal changes in estrogen, as well as anterior knee laxity, and lower limb strength and flexibility throughout the adolescent growth spurt in girls. **Methods:** Thirty-three healthy girls, aged 10-13 years, in Tanner Stage II and 4-6 months from their peak height velocity (PHV), were recruited. Participants were tested up to four times during the 12 months of their growth spurt, according to the timing of their maturity offset (Test 1: maturity offset = -4 to -6 months, Test 2: maturity offset = 0 months, Test 3: maturity offset = +4 months, Test 4: maturity offset = +8 months). During each testing session anterior knee laxity, lower limb flexibility and isokinetic strength, as well as saliva measures of estradiol concentration were measured. **Results:** A significant ($p = 0.002$) effect of time of anterior knee laxity was found from the time of PHV, although no changes in estradiol concentration were displayed over time ($p = 0.811$). Participants displayed a significant increase ($p < 0.05$) in isokinetic quadriceps strength over time, with no apparent increase in isokinetic hamstring strength. **Conclusions:** We speculate that increased quadriceps strength, combined with increased knee laxity and no accompanying hamstring strength development during the adolescent growth spurt in girls, might contribute to a decrease in their knee joint stability during landing tasks. These musculoskeletal changes could potentially increase ACL injury risk at a time of rapid height and lower limb growth.



**Key Words:** PUBERTY, INJURIES, LOWER LIMB, ACL.

## Introduction

**Paragraph 1:** From the onset of puberty girls incur a high incidence of non-contact anterior cruciate ligament (ACL) ruptures, which contribute to 37% of all their recorded knee injuries [30]. This high ACL rupture rate in girls is not apparent prior to puberty [30], whereby puberty is defined as the transitional period from childhood to adulthood, accompanied by the appearance of secondary sex characteristics and the adolescent growth spurt [21, 38]. It has been speculated that the rapid growth, as well as the musculoskeletal and hormonal changes, that occur during puberty may be factors contributing to this increased injury risk in girls [38].

**Paragraph 2**: A major event marking pubertal onset in girls is the large influx of estrogen [21]. Estrogen has the potential to directly affect the structure and composition of the human ACL [19], inevitably affecting the mechanical properties of the ligament, including laxity [38]. Cyclic fluctuations in estrogen throughout the menstrual cycle have been shown to contribute to significant increases in anterior knee joint laxity from 0.7-1.5 mm in women [13]. Due to the increasing influx of estrogen in girls from the onset and throughout puberty [21], it could be assumed that pubescent girls would also display an increase in knee laxity. However, when researchers have investigated changes in knee laxity throughout puberty, mixed results have been reported. For example, some researchers have demonstrated an increase in knee laxity (from 1-2 mm) throughout puberty in girls [7, 27], whilst others have reported a decrease (approximately 2 mm) or no change in knee laxity throughout puberty [5, 10]. The lack of consistency, however, with respect to the methods used to classify participant developmental stage, such as chronological age [5, 7], Tanner stage [10, 27] or menarche status [1, 7], is a major limitation when trying to compare the results of these studies, and highlights the need for further investigation in this field.

**Paragraph 3:** In addition to a large hormonal influx, puberty is also accompanied by the adolescent growth spurt [21]. During the adolescent growth spurt girls grow approximately 25 cm in height from the onset to the cessation of growth [35]. In fact, during the period of most rapid growth in height (peak height velocity; PHV), girls have been shown to grow at a rate of approximately 8-10 $cm.yr^{-1}$ [35]. Interestingly, the peak velocity for lower limb growth in girls (4.3 $cm.yr^{-1}$) occurs before the time of PHV, whereas the peak velocity for torso growth (4-4.5 $cm.yr^{-1}$) occurs after the time of PHV. Furthermore, differential timing of growth exists within the lower limb itself, whereby the more distal segments such as the foot, experience their peak growth velocity before the more proximal segments such as the shank and thigh [34]. This differential timing of segment growth, and consequent rapid changes in lower limb moment of inertia, is thought to contribute to altered lower limb flexibility and strength during the adolescent growth spurt, increasing the potential for lower limb injuries [12, 38].

**Paragraph 4:** The rapid increases in lower limb moment of inertia throughout puberty [16] require greater strength for a given movement in order to accelerate and decelerate the lower limb segments during movements such as jumping and landing, in which ACL injuries commonly occur [12, 22]. In particular, the quadriceps and hamstring muscles play a vital role in controlling the knee during landing [8]. Research has shown that, from the time of PHV and throughout the growth spurt, males display a defined acceleration in the development of their quadriceps [26, 28] and hamstring muscle strength [4, 15], which is not apparent in girls [4, 15, 26, 28]. This lack of a muscle strength spurt, particularly of the hamstring muscles [4, 15, 26, 28], could result in insufficient muscular torque being available to protect the ACL when girls perform dynamic movement tasks, potentially contributing to a greater injury risk. However, further research is required to provide greater insight into muscle strength development in girls during their growth spurt.

**Paragraph 5:** Due to the rapid increase in height and lower limb growth around the time of PHV, girls typically display a reduction in lower limb flexibility at this time [14, 20, 23, 37], potentially increasing the risk of lower limb injuries [39]. Whilst there is thought to be an optimum range of joint flexibility that may prevent injury in the event that muscles or joints are overstretched during an activity [2], limited research pertaining to lower limb flexibility in adolescent girls exists. In fact, most researchers quantify lower limb flexibility during puberty by asking participants to touch their fingertips to the floor or past their toes [14, 20, 37]. This measure of flexibility is likely to be confounded by the differential changes in lower limb and torso growth and, therefore, may not directly measure lower limb flexibility. Furthermore, most of these studies have been cross-sectional in design [20, 37], with only one being longitudinal [14], highlighting the need to longitudinally investigate joint specific range of motion and flexibility of the lower limbs throughout the adolescent growth spurt in girls, particularly with respect to changes in height velocity.

**Paragraph 6:** There is a paucity of research pertaining to the lower limb musculoskeletal and estrogen changes experienced by girls throughout the adolescent growth spurt. Within the limited published literature in this field, there is a lack of research examining changes longitudinally [7, 10, 14, 28], with most studies being cross-sectional in design [1, 4, 5, 15, 20, 23, 26, 27, 37]. Furthermore, most studies investigating lower limb musculoskeletal changes during puberty have selected participants based purely on chronological age [4, 5, 14, 20, 23, 26], which is not an accurate indicator of maturity in this population [24]. Therefore, the purpose of this study was to investigate the longitudinal changes in estrogen, as well as anterior knee laxity, and lower limb strength and flexibility throughout the adolescent growth spurt in girls. Based on the literature, it was hypothesized that girls would display a consistent increase in hamstring and quadriceps muscle strength without an apparent muscular strength 'spurt', and a decrease in lower limb flexibility around the time of PHV, as well as a rapid increase in estrogen and anterior knee laxity throughout the growth spurt.

**Methods**

*Participants*

**Paragraph 7:** Seventy-one healthy, female volunteers aged between 10-13 years were initially screened for their Tanner stage of pubertal development [34], as well as their estimated time from PHV (maturity offset), to determine pubertal onset [21]. Due to ethical constraints and participant burden, each girl's Tanner stage was self-assessed, with the assistance of a parent and/or guardian, using simplified Tanner stage line drawings of pubic hair development; a valid and reliable measurement of pubertal development and sexual maturity (within 88% agreement) [36]. Maturity offset was estimated using a sex-specific multiple regression equation based on each participant's body mass, standing and sitting height, lower limb length and chronological age [24]. This method can estimate maturity offset within ±1 year 95% of the time [24].

**Paragraph 8:** Thirty-three girls satisfied the initial inclusion criteria for Test 1 (healthy female, aged 10-13 years, Tanner Stage II of pubic hair development, maturity offset = -4 to -6 months) and were recruited as participants. Girls were excluded if they did not satisfy the developmental inclusion criteria, had a lower limb injury that prevented them from completing the experimental task, or had begun menstruating. The remaining 38 volunteers who did not satisfy the initial inclusion criteria for Test 1 were re-screened approximately 6 months later. Thirteen of these girls satisfied the inclusion criteria for Test 2 (Tanner Stage II-III and maturity offset = 0 months) and the remaining 25 girls were excluded from the study (see Figure 1). A complete outline of the study design is depicted in Figure 1. Due to cyclic fluctuations in hormones and knee laxity [13], participants who reached menarche (onset of menstruation) at any point during the 12-month testing period were excluded from the study at this point, such that all participants tested were pre-menarche, and, because of participant availability, 15 participants did not complete Test 4 (see Figure 1). Based on the method of Bach and Sharpe [3], the sample size was shown to provide sufficient statistical power (> 80%) to detect significant main effects at $p \leq 0.05$, when comparing the longitudinal changes in the dependent variables over time, when standing height was used as the outcome measure (standard deviation of the differences in measurements = 0.75). All participants were recruited through schools from the Illawarra region, as well as through local newspaper advertisements in order to ensure the participants represented a general, pubescent female cohort, with minimal sample bias. The University of Wollongong Human Research Ethics Committee (HE08/281) approved all study procedures and the participants and their parents/guardians provided informed written and verbal consent before the girls participated in the study.

< Insert Figure 1 here >

*Experimental Protocol*

**Paragraph 9:** Participants were tested in the Biomechanics Research Laboratory up to four times over the 12-month period that encompassed their adolescent growth spurt (see Figure 1). The timing of each laboratory testing session was based around maturity offset, estimated using the regression equation described previously [24] (see Figure 2; Test 1: maturity offset = -4 to -6 months, Test 2: maturity offset = 0 months, Test 3: maturity offset = +4 months, Test 4: maturity offset = +8 months). During each laboratory test session, each participant's estrogen levels, body mass, standing and sitting height, lower limb length, anterior knee joint laxity, lower limb flexibility, and isokinetic strength of the dominant lower limb (defined as the landing limb each participant used when asked to perform a vertical jump, taking off from two legs and landing on one leg [33]) were measured. In addition, each participant's body mass, standing and sitting height, lower limb length (used to predict the timing of Test 2 and retrospectively calculate height velocity), as well as their hamstring flexibility and isometric strength of the dominant limb (hereafter referred to the test limb) were tracked monthly in each participant's home. The chief investigator [CYW] performed all anthropometric, flexibility, knee laxity and strength measurements during both the laboratory and monthly tracking sessions, after confirming she was reliable (ICC > 0.9) in taking these measurements.

< Insert Figure 2 here >

*Anthropometric, Flexibility and Laxity Measurements*

**Paragraph 10:** During each laboratory and monthly tracking session, each participant's standing height, sitting height, lower limb length (Seca Corp, Hanover, MD, USA) and body mass (A&D Personal Precision Scales, A&D Company Ltd., Tokyo, Japan) were quantified using the procedures described by Mirwald et al. [24]. These values were then input into the regression equation to estimate maturity offset

[24], as well as to track changes in these measurements throughout the adolescent growth spurt (see Table 1 for participant characteristics). As the stature measurements were crucial to the estimation of maturity offset [24], the chief investigator [CYW] performed all measurements at approximately the same time of day for each participant. An estimate of height velocity was retrospectively calculated using the individual height data that were collected each month for the 19 participants who completed all four testing sessions, so as to obtain a height velocity value (cm.yr$^{-1}$). This was calculated as the change in height two months prior to (this included the screening measurements when calculating height velocity at Test 1) and after each laboratory testing session divided by the change in time, multiplied by 12 months.

< Insert Table 1 here >

**Paragraph 11:** Goniometric measurements (Model 01135; Lafayette Instrument Co., Inc, USA) of knee (passive knee extension for the hamstrings and Modified Thomas Test for the quadriceps) and hip (Modified Thomas Test for illiopsoas) joint range of motion [11] were recorded for each participant's test limb to represent changes in the flexibility of their hamstrings, quadriceps and illiopsoas muscles over time (a higher angle for hamstring flexibility indicates a decrease in flexibility, whereas a higher angle for quadriceps and illiopsoas flexibility indicates an increase in flexibility). Hamstring flexibility (using the passive knee extension test) [11] was also measured during the monthly tracking sessions to determine the month-to-month changes in hamstring muscle extensibility throughout the growth spurt.

**Paragraph 12:** Passive anterior knee laxity of each participant's test limb was measured using a Dynamic Cruciate Tester (DCT; Smith & Nephews Richards, Australia) and following the procedures of Steele et al. [32], which have been shown to be reliable (ICC > 0.9). In brief, the participants were seated in an adjustable chair, with their thigh supported, their knee flexed between 20-30° and their tibia in neutral rotation [32]. The ankle was firmly secured with a strain-gauged strap of the DCT, which was placed superior to the malleoli to limit vertical movement of the test limb, and the DCT tibial sensor was positioned on the participant's tibial tuberosity. In order to limit muscle guarding during testing, participants were encouraged to remain as relaxed as possible. This was enhanced by gently shaking the thigh and leg muscle bellies prior to each test, as well as by placing the contralateral leg in external rotation [32]. Following one to two familiarization trials, three passive anterior drawer tests were performed. Anterior tibial translation (mm) and the force applied by the participant's test limb against the ankle strap (N) were simultaneously recorded as force-displacement curves. For each participant, the anterior tibial displacement, recorded at a force value that was consistent throughout all testing sessions (approximately 80-100% of peak force), was averaged over the three trials to enable the changes in anterior knee laxity over time to be compared. All knee laxity data were analyzed at the completion of all testing sessions to limit any bias during future tests.

*Isokinetic Lower Limb Strength*

**Paragraph 13:** After completing a standardized 5-10 minute warm-up on a cycle ergometer (Monark Model 818E, Sweden), each participant's hamstring and quadriceps muscle strength were assessed using an isokinetic dynamometer (KinCom, Chattanooga Inc., USA) following standardized procedures [9]. In brief, each participant was adequately familiarized with the strength testing procedures by performing a series of both concentric and eccentric quadriceps and hamstring muscle strength tests (with minimal effort), until each participant was confident with performing the tasks. Participants then performed four separate tests at $180°.s^{-1}$ from

10-90° of knee flexion to assess concentric and eccentric hamstring and quadriceps muscle strength. An angular velocity of 180°.s$^{-1}$ was selected to replicate the angular knee velocity displayed during landing, and which has been safely used previously with a similar cohort [29]. During each test, the lever arm moved back and forth for a total of six cycles. During the first two cycles, participants were asked to relax (0% effort), during cycles 3-4 participants were asked to exert 25% effort, and during cycles 5-6 participants were asked to exert 100% effort. Participants performed one test for each of the strength measures (concentric hamstrings, eccentric hamstrings, concentric quadriceps and eccentric quadriceps), resulting in four tests performed by each participant. Each strength test was performed in a randomized order and the gravity corrected peak torque was recorded [18]. Participants were allowed adequate rest between each trial to reduce the effects of fatigue on peak torque values.

*Isometric Hamstring Strength*

**Paragraph 14:** Using modified, reliable procedures (ICC > 0.9, 95% confidence intervals [31]), month-to-month isometric hamstring strength was recorded by having participants sit with their knee and hip joints at 90° and a strap, connected to a tensiometer placed around their ankle, superior to the malleoli. With their arms crossed across their chest, participants were asked to pull their leg back (posteriorly) as hard as they could for 3 seconds, and then to relax. Participants performed three trials, with 1-minute rest between each trial, and the highest strength reading (kg) was recorded.

*Salivary Levels of Estrogen*

**Paragraph 15:** Due to ethical constraints, as well as to reduce participant burden, estradiol (the most physiologically active form of estrogen) concentration was measured by collecting saliva samples. One hour prior to each testing session, participants refrained from eating. Participants rinsed their mouth thoroughly with water 10 minutes before each sample was collected, to minimize food particles compromising the results, and saliva samples were then collected employing the unstimulated passive drool method. Participants were instructed to allow saliva to pool in their mouth (this involved the participants 'imagining' their favourite food). Then, with their head tilted forward, participants allowed their saliva to passively move down a 5 cm-long straw and into a disposable tube (Eppendorf Inc; North America, USA; safe-lock tubes, 2.0 mL). Three 1 mL samples were collected over a 2 hour period and all samples were dated and stored at -20°C for later analysis.

**Paragraph 16:** For analysis, the saliva samples were completely thawed, vortexed and centrifuged at 1500 x g (at 3000 rpm) for 15 minutes at room temperature (approximately 22°C). Estradiol concentration was determined by enzyme immunoassay (EIA) using a high sensitivity EIA kit (Salivary 17β-Estradiol EIA kit; Item no. 1-3702; Salimetrics., PA, USA; calibrator range: 1-32 pg/mL; serum correlation: 0.80). The assay plate (containing all standards, controls and unknowns) was then read in a plate reader (PowerWave x340, Bio-Tek., Victoria, Australia; 450 nm). Using data reduction software (GraphPad Prism 4; Version 4.03, 2005), the concentration of estrogen in the controls and unknowns was determined through extrapolation of the standard curve.

*Statistical Analyses*

**Paragraph 17:** Means and standard errors of the anthropometric, flexibility, anterior knee laxity, strength and estrogen variables were calculated for each laboratory-based testing session, as well as the hamstring flexibility and strength measures for each monthly tracking session. A linear mixed model was used (repeated covariance type = compound symmetry; correlated residuals within the random effects) to determine any significant ($p \leq 0.05$) main effects of time on the dependent variables, controlling for growth variables (height, body mass, lower limb length) as covariates (which were time dependent). The linear mixed model is a direct likelihood approach, which has been shown to be a suitable method for analysing longitudinal data with missing values, whereby the method assumes that data are missing at random [6]. Post-hoc comparisons were performed using a *t*-test with a Bonferroni adjustment. All statistical procedures were performed using SPSS (Version 20; SPSS Inc., Chicago, IL).

**Results**

**Paragraph 18:** A significant main effect of time was displayed for the anthropometric variables (see Table 1), whereby standing height, lower limb length, body mass, chronological age and Tanner stage all significantly increased during each testing session throughout the 12 months. A significant main effect of time on standing height, lower limb length and sitting height growth velocity was also found (see Figure 2), whereby post-hoc analyses revealed that the increase in standing height was faster during Test 2 (at the time of PHV) compared to the other test sessions ($p < 0.001$). Post-hoc analyses also revealed that peak lower limb growth velocity was attained prior to PHV (Test 1; $p < 0.001$), and peak sitting height growth velocity, a reflection of torso growth, occurred after PHV (Test 3; $p < 0.003$).

**Paragraph 19:** Despite the rapid and differential timing of standing height, lower limb length and sitting height growth, there was no significant main effect of time on hamstring, quadriceps or illiopsoas flexibility over the four laboratory testing sessions (see Table 1). However, a significant ($p = 0.024$) effect of time on the month-to-month measures of hamstring flexibility was found (see Figure 3), whereby post-hoc analyses revealed participants displayed significantly ($p = 0.001$) lower hamstring flexibility during Month 4 (1 month prior to PHV, Test 2) compared to Month 13 (Test 4).

< Insert Figure 3 here >

**Paragraph 20:** Interestingly, there was no significant change in estradiol concentration throughout the 12 months ($p = 0.811$; Figure 4). However, a significant effect of time on anterior knee laxity was found, whereby participants displayed an increase in knee laxity from Test 1 to 2 ($p = 0.001$) and Test 1 to 3 ($p = 0.029$).

< Insert Figure 4 here >

**Paragraph 21:** Participants displayed no change in either concentric ($p = 0.539$) or eccentric ($p = 0.249$) hamstring muscle strength over time (see Figure 5). However, participants displayed a significant increase in concentric quadriceps muscle strength from Test 1 to 3 ($p = 0.011$), Test 1 to 4 ($p < 0.001$), and Test 2 to 4 ($p = 0.002$). Participants also displayed significantly greater eccentric quadriceps strength at Test 4 compared to Test 1 ($p = 0.017$), Test 2 ($p = 0.003$), and Test 3 ($p = 0.024$). Interestingly, a significant ($p < 0.001$) effect of time on the month-to-month changes in isometric hamstring strength was also found (see Figure 3), such that post-hoc analyses revealed that participants displayed a significant ($p < 0.023$) increase in hamstring strength from Month 10 (1 month after Test 3) to Month 13 (Test 4).

< Insert Figure 5 here >

## Discussion

**Paragraph 22:** It has been speculated that the rapid growth, as well as the musculoskeletal and hormonal changes that occur during puberty, may be factors contributing to the reported increased ACL injury risk in girls at this time [38]. This study investigated the longitudinal changes in anthropometry, estrogen and knee laxity, as well as lower limb strength and flexibility during the adolescent growth spurt in girls, in order to provide a greater understanding of changes in these variables during this time. As hypothesized, rapid changes in height, flexibility and knee laxity occurred throughout the growth spurt, which are detailed below.

**Paragraph 23:** As would be expected during a growth spurt, the participants displayed a significant increase in all growth parameters over time, including height, lower limb length and body mass, as well as chronological age and Tanner stage (see Table 1). Participants also experienced different rates of growth in standing and sitting height, as well as lower limb length throughout the 12 months (see Figure 2). This compares to previously reported height velocity curves in adolescent girls in terms of both the magnitude and timing of each peak growth velocity [24], offering further confirmation of current knowledge that peak lower limb growth occurs prior to the time of PHV, whereas peak torso growth (sitting height) occurs after PHV. Furthermore, the PHV attained by participants is comparable to values reported in the literature of approximately 8-10 $cm.yr^{-1}$ [35]. The Tanner stage (II-III) of the participants at the time of PHV is also comparable to previously reported values [17, 24].

**Paragraph 24:** Bones grow faster than the developing musculature, and so it may be assumed that the rapid increase in lower limb length and height velocity at the time of Test 1 and 2 would be accompanied by an associated decrease in lower limb flexibility during this time [2], as has been reported previously [14, 20, 23]. When the month-to-month changes in hamstring flexibility were analyzed, a significant decrease in hamstring flexibility was noted just prior to the time of PHV (Test 2), around the time of peak lower limb length growth (see Figure 2). This finding is comparable to the results of Loko et al. [20] and, whilst further research is warranted to confirm this notion, may suggest that rapid growth of the lower limbs just before

PHV contributes to a reduction in hamstring flexibility, and potentially increased injury risk [39].

**Paragraph 25:** In contrast to previous research [14, 20, 23, 37], the present study found no associated changes in hamstring, quadriceps or illiopsoas flexibility throughout the growth spurt when measured in the laboratory every 4 months, despite significantly changing height velocity throughout the 12 months. Heras-Yague et al. [14] measured the changes in maximal trunk flexion in girls twice annually for 3 years, reporting a decrease around the time of PHV and an increase in flexibility after PHV. Although involving a larger cohort than the present study (453 girls aged between 10-13 years), it is thought that only two measurements per year cannot accurately characterize the changes in flexibility, particularly when it is not aligned with changes in lower limb and sitting height growth. In addition, many studies [14, 20, 23] assess trunk flexion flexibility as 'touching the fingertips to the floor' or using the sit-and-reach test. Based on the anthropometric results from the present study, increases in trunk flexion, like those displayed by Heras-Yague et al. [14] after PHV, may not be due to improved flexibility or range of motion at all, but merely due to an increase in torso length (sitting height) and a slowing down of lower limb growth (see Figure 2). Unlike these previous studies, we assessed the participant's flexibility using joint range of motion tests, making comparisons to current literature difficult. However, as no changes in hamstring, quadriceps or illiopsoas muscle flexibility were noted over time when measured every 4 months, whereas significant changes in hamstring flexibility were found when assessed monthly, we recommend that regular monitoring of girls during their growth spurt is required to detect changes in lower limb flexibility, and potential 'at risk' times during the adolescent growth spurt.

**Paragraph 26:** Growth in length of the lower limbs of approximately 5 $cm.yr^{-1}$ leading up to, and around the time of PHV (Test 1 and 2), results in changes in the inertial properties of these segments [12]. This requires an analogous increase in lower limb muscular strength, particularly around the time of Test 1 (peak lower limb growth velocity), in order to effectively accelerate and decelerate the limbs for given movements, such as landing [12]. The importance of the quadriceps and hamstring muscles during dynamic landing movements has been highlighted, particularly with respect to stabilizing the knee joint and protecting the ACL [38]. Similar to the results reported by Barber-Westin et al. [4], participants in the present study displayed a significant increase in isokinetic (concentric and eccentric) quadriceps strength after PHV, with no apparent acceleration in the development of isokinetic (concentric and eccentric) hamstring muscle strength over time. This demonstrates a lag in development of hamstring muscle strength relative to quadriceps muscle strength over time, particularly with respect to the rapidly growing lower limbs around the time of Test 1. Further research is recommend to determine whether this lag in hamstring strength development negatively affects how girls perform dynamic landing movement and, in turn, their risk of ACL injury.

**Paragraph 27:** Heitz et al. [13] reported an association between increased anterior knee laxity and peak rises in both estrogen and progesterone throughout the menstrual cycle. This suggests that increases in estrogen may contribute to greater ligament laxity which, in turn, has been shown to decrease knee joint stability [25] and ultimately, increase ACL injury risk. Interestingly, participants in the present study displayed a significant increase in anterior knee laxity from the time of PHV, which is comparable to the results of previous studies [7, 10], but with no associated increase in estradiol concentration (see Figure 4). Whilst the estradiol concentration values in the present study are significantly lower than the concentrations reported previously [13], these previous values were of serum measurements of regular menstruating women. In fact, the reported estradiol concentrations (2-4 pg/mL) in the present study are comparable to values reported in a similar non-menstruating pubescent female population [21]. Therefore, we speculate the longitudinal changes in lower limb flexibility, strength and laxity displayed in the present study may be attributed to factors outside of estrogen influences. Further research is warranted, however, to determine whether the increase in knee laxity displayed by girls from the time of PHV in the present study, combined with a lag in development of hamstring relative to quadriceps strength, decreases knee joint stability during dynamic landing tasks [25], potentially placing girls at an increased risk of non-contact ACL injuries during sport.

**Paragraph 28:** Whilst this study provides greater insight into the musculoskeletal and estrogen changes during puberty, particularly when measured longitudinally, limitations of the study are acknowledged. Despite our findings being consistent with previous studies of non-menstruating girls, we propose that hormonal measurements every 4 months may not have been sensitive enough to detect subtle changes in estrogen during the growth spurt in girls. Therefore, research that investigates the changes in other hormones associated with puberty, such as testosterone and progesterone, together with estrogen, measured on a more regular basis (i.e. daily), would provide further insight into the effects of hormones during the adolescent growth spurt. Furthermore, in the present study it was assumed that peak lower limb growth occurred at the time of Test 1. We acknowledge, however, that this may not be the actual peak, and thus recommend further investigation to determine the precise timing of peak lower limb growth. Whilst it is acknowledged that the best indicator of biological age is through x-rays to calculate skeletal age, due to ethical constraints placed on the present study, use of skeletal age was not possible. Instead, Tanner staging and maturity offset were used to characterize pubertal and biological age. Finally, the lack of physical activity data of each participant presents as a limitation as seasonal changes in sporting activity may have contributed to an increase in variability of the results, suggesting the need for future studies to include these variables as covariates.

**Paragraph 29:** Overall, it was found that from the time of PHV, participants displayed significantly increased anterior knee laxity, accompanied by a significant increase in isokinetic quadriceps strength over time, with no accompanying increase in isokinetic hamstring muscle strength or estrogen concentration. A significant decrease in hamstring flexibility just prior to PHV, around the time of peak lower limb growth velocity, was also evident. We speculate that this combination of changes during the adolescent growth spurt in girls might contribute to a decrease in their knee joint stability during landing tasks, potentially increasing ACL injury risk at this time of rapid height and lower limb growth. Therefore, further research is recommended to determine whether these musculoskeletal and growth variables affect the way girls land at critical stages of the adolescent growth spurt.

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**Conflict of Interest**

No conflict of interest exists.

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**Figure Captions:**

**Figure 1:** Participant selection protocol outlining the number of volunteers screened and re-screened, participant numbers during each testing session, as well as the attrition of participants due to menarche.

**Figure 2:** Timing of each of the four testing sessions based around maturity offset, estimated using a regression equation. Results are displayed as the mean ± SE for standing height, lower limb length and sitting height velocity over time, calculated retrospectively (height velocity data are attained from the 19 participants completing the 12-month study; * indicates a significant main effect of time on growth velocity; $p < 0.001$).

**Figure 3:** Means ± SE for the month-to-month changes in hamstring flexibility and isometric hamstring strength over 12 months (note that an increase in angle indicates a decrease in hamstring flexibility; * indicates a significant main effect of time on the dependent variable; $p < 0.05$).

**Figure 4:** Means ± SE for anterior knee laxity (mm) and estradiol concentration (pg/mL) over the four laboratory test sessions (* indicates a significant main effect of time on knee laxity; $p = 0.002$).

**Figure 5:** Means ± SE for the concentric and eccentric hamstrings and quadriceps torque (Nm) over the four laboratory test sessions (* indicates a significant main effect of time on the strength variable; $p < 0.05$).

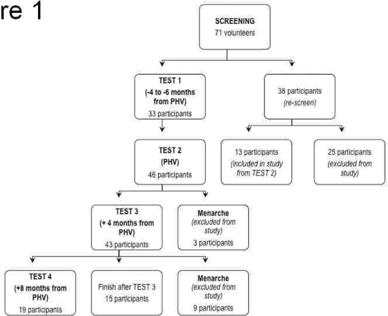
Table

**Table 1:** Means ± SE and *p*-values for the participant characteristics and the goniometric measures of hamstring, quadriceps and illiopsoas flexibility recorded during the four laboratory test sessions.

| Variable | Test 1 (n = 33) | Test 2 (n = 46) | Test 3 (n = 43) | Test 4 (n = 19) | *p*-value |
|---|---|---|---|---|---|
| Standing height (cm) | 149.7 ± 0.8 | 152.7 ± 0.8 | 155.2 ± 0.8 | 157.9 ± 0.8 | <0.001 |
| Lower limb length (cm) | 70.6 ± 0.6 | 72.4 ± 0.6 | 73.5 ± 0.6 | 74.7 ± 0.6 | <0.001 |
| Body mass (kg) | 40.1 ± 0.8 | 42.2 ± 0.8 | 44.2 ± 0.8 | 46.7 ± 0.8 | <0.001 |
| Age (yr) | 11.4 ± 0.1 | 11.8 ± 0.1 | 12.1 ± 0.1 | 12.5 ± 0.1 | <0.001 |
| Tanner Stage | II | II-III | III | III-IV | <0.001 |
| Hamstrings (°)* | 23.7 ± 2.3 | 25.3 ± 2.1 | 24.1 ± 2.2 | 21.4 ± 2.5 | 0.295 |
| Quadriceps (°) | 68.0 ± 1.6 | 64.2 ± 1.4 | 64.4 ± 1.4 | 66.2 ± 2.0 | 0.112 |
| Illiopsoas (°) | 11.2 ± 1.4 | 13.1 ± 1.2 | 12.1 ± 1.3 | 12.9 ± 1.8 | 0.644 |

* Note that a higher angle for hamstring flexibility indicates a decrease in flexibility, whereas a higher angle for quadriceps and illiopsoas flexibility indicates an increase in flexibility.

# Figure 1


SCREENING
71 volunteers
TEST 1
(-4 to -6 months from PHV)
33 participants
38 participants
(re-screen)
TEST 2
(PHV)
46 participants
13 participants
(included in study from TEST 2)
25 participants
(excluded from study)
TEST 3
(+ 4 months from PHV)
43 participants
Menarche
(excluded from study)
3 participants
TEST 4
(+8 months from PHV)
19 participants
Finish after TEST 3
15 participants
Menarche
(excluded from study)
9 participants