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Gender Comparisons and Associations between Lower Limb Muscle Activation Strategies and Resultant Knee Biomechanics during Single Leg Drop Landings

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Abstract: (1) Background: We aimed to compare gender differences in knee biomechanics and neuromuscular characteristics, and to determine the relationships between lower limb muscle pre-activations and knee biomechanics during a single leg drop landing, in order to identify riskier landing patterns to prevent injury and intervene properly. (2) Methods: Descriptive laboratory cross-sectional study on 38 healthy untrained subjects with low to moderate physical activity status. (3) Results: During the initial-contact phase of landing, females demonstrated greater peak vertical ground reaction force (GRF) normalized to body weight (49.12 ± 7.53 vs. 39.88 ± 5.69 N/kg; $p < 0.001$; Hedge's g = 1.37), peak knee anterior reaction force normalized to body weight (0.23 ± 0.04 vs. 0.17 ± 0.05 N/kg; $p < 0.001$; Hedge's g = 1.33), and decreased pre-activation of the semitendinosus (45.10 ± 20.05% vs. 34.03 ± 12.05%; $p = 0.04$; Hedge's g = 0.67). The final regression equation was peak knee anterior reaction force = 0.024 + 0.025 (peak knee flexion moment) − 0.02 (semitendinosus-to-vastus lateralis pre-activation ratio) + 0.003 (peak vertical GRF) ($R^2 = 0.576$, $p < 0.001$). (4) Conclusions: Overall, the data provided in this study support that a reduced semitendinosus-to-vastus lateralis pre-activation ratio predicted an increase in knee anterior reaction force and potentially an increase in ACL forces. Female non-athletes had gender-specific landing characteristics that may contribute to ACL injury. Future studies are warranted to consider more possible predictors of non-contact ACL injury.

Keywords: anterior cruciate ligament; knee; biomechanics; sEMG; landing; injury mechanism

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1. Introduction

Anterior cruciate ligament (ACL) injury continues to be a significant health concern worldwide. It is estimated that more than 2 million ACL injuries occur each year, which leads to a considerable financial burden on healthcare systems [1]. These injuries are especially devastating to athletes because they result in long recovery times, difficulty in return to pre-injury level, and even disability in later life [2]. Individuals who experienced an ACL injury also have an increased risk of developing osteoarthritis (OA) [3], resulting in the early onset of OA between the ages of 30 and 50 years. Most non-contact ACL injuries occur in the period following the initial contact of the foot with the ground in tasks that involve sudden decelerations or rapid directional changes, such as landing, cutting, or pivoting movements [4]. Unilateral landings are more prone to cause ACL rupture compared with bilateral landings, due to a decreased base of support and increased demand on the musculature of a single leg to absorb the impact [4].

Female athletes are 2–6 times more likely to suffer ligament rupture compared to age-matched males with comparable athletic ability [5]. However, the precise mechanism of non-contact ACL injury remains controversial based on current clinical and biomechanical evidence. One of the joint forces that primarily increases ACL strain leading to ligament injury is proximal tibia anterior shear force, as it represents the most direct loading mechanism of the ligament [6,7]. An internal tibial torque in the transverse plane also contributes to ACL loading, but only at small knee flexion angles (0–30°) [8,9]. An injury video analysis reported female soccer players had higher knee flexion angles at initial contact and injury frame compared to male players [10]. However, gender comparisons observed during laboratory tasks, such as single leg landings, show equivocal findings. For example, in some studies, females tend to show a more extended knee angle during single leg landings in laboratory tasks [11,12], with larger flexion moments [13] and greater vertical ground reaction forces [11,12,14], while other studies found no significant differences during unilateral landings in knee flexion [14–16] or ground reaction force based on sex [15]. The potential reason for the inconsistency in research evidence might be due to male and female participants partaking in different types of sports training, which specifically change their landing pattern. The differences in muscular strength of the lower limb might influence the landing performance [17]. Based on the current evidence, it is difficult to conclude upon a gender-specific difference in single-leg landing patterns exhibited by a cohort with similar training level.

Recent evidence shows a significant correlation between neuromuscular control strategies and knee biomechanics [18]. Increased quadriceps activation during the deceleration phase of landing might cause anterior tibial translation, thereby increasing excessive loads within the ACL [19]. A decreased hamstring to quadriceps myoelectric pre-activation ratio was reported to be a risk for ACL injuries [20]. However, there is a controversy as to whether reduced hamstring activation or excessively elevated quadriceps activation are better predictors of ACL injury [18,21]. In addition, it remains unclear whether females have decreased pre-activation of hamstrings, compared to males, and whether this activation pattern is correlated with riskier landing biomechanics.

Regarding the neuromuscular controls, regulation of stiffness is achieved by the agonist and antagonist muscle co-activation before ground contact, and during the deceleration phase [22]. It seems that most falls occur during 25–50 ms after initial contact with the ground, and most non-contact ACL injuries occur within 40 ms after initial contact. [23,24]. However, since the suggested latency of muscular reflexes elicited by electrical stimulations of ACL is 80–100 ms, it seems unlikely that there is time for mechanosensory feedback to directly protect against injury [25]. This indicates that muscle pre-activation is critical for dynamic joint stability during fast movements such as drop landings. The magnitude of the muscle pre-activation before ground contact is influenced by motor learning experience [26]. Thus, understanding the pre-activation neuromuscular patterns related to high-risk knee joint biomechanical variables in a cohort with similar training experience may provide an additional concept to predict the risk of injury or the effectiveness of training in later life.

The effectiveness of any prophylactic intervention relies on a reasonable underlying etiological rationale of the associated conditions. However, the mechanisms of non-contact ACL injury are not fully understood, and it is therefore important to identify riskier landing patterns in order to intervene properly and reduce the risk of injury. Explicit knowledge of neuromuscular and biomechanical characteristics that can predict dangerous loading patterns may provide important evidence for the future development of injury prevention training programs. Therefore, the first aim of this study was to compare gender differences in knee biomechanics and neuromuscular characteristics during a single leg drop landing task. The second aim was to determine the relationships between lower limb muscle pre-activation patterns and knee joint biomechanics during a single leg drop landing task.

The variables tested included knee flexion angle, external knee flexion moment, ground reaction force, knee anterior reaction force, and pre-activation of the semitendinosus (ST) and vastus lateralis (VL) muscles. Based on epidemiological investigations supporting that females are more likely to suffer ACL injuries, as well as previous studies conducting biomechanical observations on gender related differences, it was hypothesized that females would have a larger peak knee anterior reaction force, decreased knee flexion angle, greater knee flexion moment, and greater ground reaction force than males during the single leg drop landing task. It was also hypothesized that there would be a linear regression model predicting the knee anterior reaction force based on these biomechanical variables.

2. Materials and Methods

Ethical approval for the study was granted by the hospital ethics committee. An a priori power calculation indicated a minimum of 17 participants in each group was needed to achieve an effect size >1, an α level of 5%, and a power of 80%. This effect size was calculated based a between-group difference of 0.15 of EMG in ST:VL neuromuscular pre-activation [20]. For the linear multiple regression analysis, it was estimated that 38 participants were needed to get an $R^2 > 0.3$, an α level of 5% and a statistical power of 80% [27]. Hence, 18 healthy males and 20 healthy females with similar low–-moderate habitual physical activity levels assessed by the International Physical Activity Questionnaire-Short Form (IPAQ-SF) [28] provided written informed consent and voluntarily took part in the study. To minimize the potentially confounding effects of training background on landing performance, and particularly neuromuscular pattern, exclusion criteria included any: (1) previous training experience in a strength, power, or aerobic program during the last two years; (2) any recent injury to the lower extremities in the previous six months; (3) suffered from any disorder that impacted on sensory input, musculoskeletal function, or motor function; (4) unable to provide written informed consent prior to their participation in the study. After signing informed consent, their anthropometric parameters were recorded, including age, height, body mass index (BMI), and characteristics of the dominant leg, defined as the leg with which they could kick a ball the furthest.

Kinematic and kinetic data describing the performance of each landing were collected using a 3-D motion capture system that included eight cameras, capturing at 100 Hz (VICON Motion Systems Ltd. Oxford, UK) and a force plate at 1000 Hz (AMTI, Advanced Mechanical Technology INC, Watertown, MA, USA), respectively. Seventeen reflective markers (14 mm diameter spheres) were placed on anatomical landmarks according to the Plug-In Gait marker set on the second metatarsal head, posterior calcaneus, lateral malleolus, lateral shank, lateral knee, lateral thigh, anterior superior iliac spine, posterior superior iliac spine and sacrum. Surface electromyographic data were recorded using a wireless EMG device at 2000 Hz (Noraxon, Inc., Scottsdale, AZ, USA). Surface EMG electrodes (bipolar surface electrodes, 272 S, Noraxon, Inc., Scottsdale, AZ, USA) with a 10-mm diameter and 1-cm inter-electrode distance, were placed parallel to the presumed orientation of the muscle fibers of vastus lateralis (VL) and semitendinosus (ST) in accordance with the SENIAM guidelines [29]. Each reference electrode was placed on a nearby bony area. Before placement, the skin under each electrode site was shaved and cleaned with alcohol. The markers and sEMG electrodes were placed by a trained physiotherapist and then confirmed by a second trained physiotherapist. Before data collection, the subjects followed a 5 min standardized warm-up program (5-min treadmill jogging followed by stretching) and practiced the drop landing task until comfortable with the action. The subjects were instructed to prepare on a 30-cm high box with single leg standing and hands on the waist. Then, they were asked to perform a single leg drop-off and land on the force plate positioned 30 cm from the front of the box for three successful trials. Any landing that was not stable and required extra steps or hops after touch-down was repeated. Successful trials were defined as those in which the participant landed with their foot entirely on the force place without having to extra steps of hops. Three successful trials were recorded for each participant.

The kinematic and kinetic variables were computed using the Vicon's plug-in gait model (PiG; Vicon Motion Systems, Oxford, UK), which is developed on the basis of the 'Newington' model [30,31]. The definitions of kinetics parameters are shown in Figure 1. Kinematics were calculated for each joint using Euler angles. Moments and forces are then calculated using inverse dynamics and anthropometric properties. From Newton's second law, $\sum F = ma$. By summing horizontal forces,

$$F_{xproximal} + F_{xdistal} = m \times a_x, \quad (1)$$

The ankle proximal force was calculated first. The ankle proximal force is equal and opposite to the shank distal force because of Newton's third law. Equation (1) can then be applied to the shank to estimate the horizontal knee resultant force (knee anterior reaction force). The muscle forces that are generated from actively contracting muscles should be incorporated in estimating tibia anterior shear force [32], but the knee anterior reaction force that we used in this paper is a resultant force that includes all muscular, ligamentous, and contact forces applied at the knee, and does not represent shear force loading on the ACL. The Dempster's body segment parameters were applied in inverse dynamics analysis.

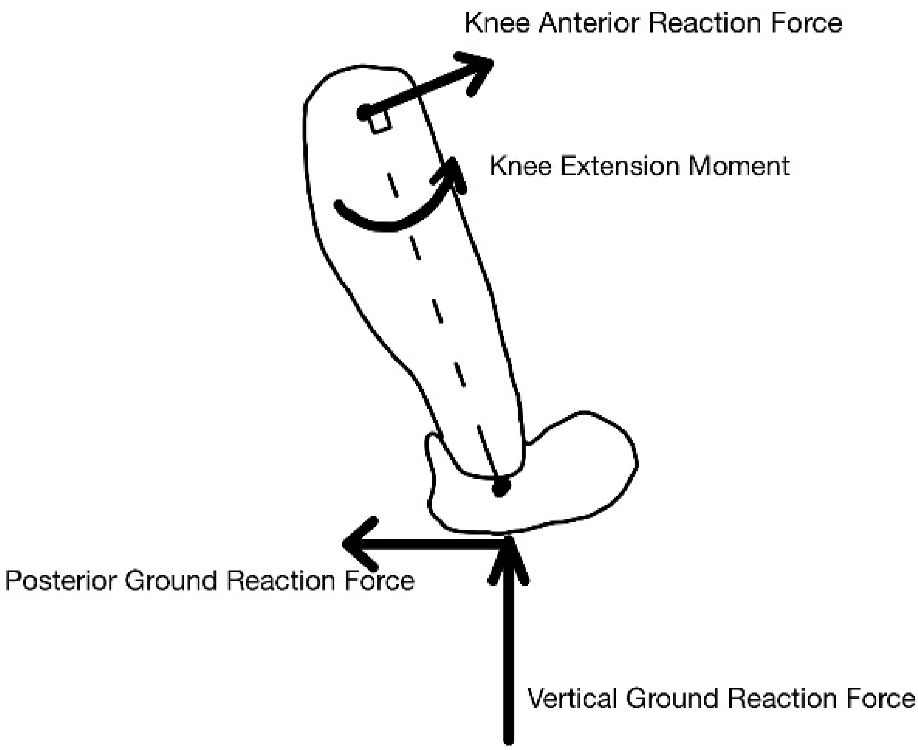

Figure 1. Kinetics definition on the landing leg in the sagittal plane.

The sEMG data were filtered by a 10–500 Hz band pass filter to attenuate movement artifacts. Root mean square signal processing was used with a 50-ms moving window. The pre-activation phase was considered to occur 100 ms prior to initial contact. Initial contact (IC) was determined when the force vector (vertical GRF) first exceeded 10 N. The reason for using the 100 ms prior to initial contact as pre-landing timing is due to the pre-activation of the muscles and the electromechanical delay, which is reportedly between 50 ms [33] and 120 ms [34]. The IC was determined at the first frame when the force vector (vertical GRF) exceeded 10 N for a width of at least 40 samples [35]. Observations by a researcher were then applied to further confirm the determination of the IC events. The average muscle activation amplitude over 100 ms prior to initial contact was normalized to the peak amplitude of the respective muscle from the three trials of landing. Simultaneous semitendinosus to vastus lateralis (ST:VL) pre-activation ratio was used to calculate the muscle pre-activation ratio using a formula: ST:VL pre-activation ratio = (averaged ST normalized to peak amplitude within 100 ms prior to IC)/(averaged VL normalized to peak amplitude within 100 ms prior to IC).

Between-gender differences in biomechanical and neuromuscular variables were assessed by independent-sample t-tests using SPSS (26.0, IBM, Chicago, IL, USA). An alpha level of 0.05 was used to determine the significance of differences between the groups. All data were expressed as mean and standard deviation. Hedge's g indicated the magnitude of effect size [36] ($|g| < 0.2$ 'negligible', $|g| < 0.5$ 'small', $|g| < 0.8$ 'medium', $|g| > 0.8$ 'large'). The Shapiro–Wilk test was applied before the analysis to examine the normal distribution of the data. Levene's test was performed to evaluate the equality of variances between two groups. Stepwise multiple regression was used to determine the neuromuscular and biomechanical variables that significantly predicted knee anterior reaction force during the deceleration phase of landing. The predictor variables included peak posterior/vertical GRF normalized to body weight, knee flexion angle, knee abduction angular excursion, knee flexion moment, pre-activation of the vastus lateralis and semitendinosus, and sex. Measurements of ST:VL pre-activation ratio were examined by regression analysis to assess their correlation with knee flexion moment and peak knee flexion angle within 100 ms. All assumptions of the multiple linear regression were checked. An alpha level of 0.05 was selected to identify the predictor variables to be included in the final equation and for determining the significance of the model in predicting the response variable.

3. Results

There was no difference in the mean age of the males (age: 23.9 ± 3.0; mass 68.5 ± 10.9 kg; height 1.74 ± 0.04 m) and females (age: 23.1 ± 2.0; mass 54.6 ± 6.9 kg; height 1.63 ± 0.05 m), whereas significant differences between mass and height existed ($p < 0.001$, $p < 0.001$, respectively). There was no difference in IPAQ-SF levels between the two genders (Pearson's chi-square = 2.620; $p = 0.106$), indicating similar low–moderate physical activity status. The intraclass correlation coefficient (ICC) of GRF and knee flexion angle is 0.90–0.99; with a coefficient of variation (CV) of 5.44–10.39% demonstrating high reliability in the kinematic and kinetic measurement. The pre-activation of VL and ST has large but there is acceptable data variability with an ICC of 0.88–0.84 and CV of 21%. There is one outlier in the pre-activation of the ST:VL with a z-score of 4.52, but the outlier was not influential in statistical analysis (see supplementary notes).

The means and standard deviations for each of the neuromuscular and biomechanical variables are reported in Table 1. The Shapiro-Wilk test was applied before the analysis to examine the normal distribution of the data, and all variables except 'Pre-activation of the ST:VL 100 ms before IC' are normally distributed, which would be a limitation in statistical analysis. During the post-contact phase of a single leg landing, females demonstrated greater peak vertical GRF ($p < 0.001$) and peak knee anterior reaction force ($p < 0.001$. A significant decrease in pre-activation of the semitendinosus muscle before initial contact ($p = 0.04$) was observed in the females. However, no other gender differences in muscle activation were found. There was also no gender-based difference in peak knee flexion moment or peak flexion angle.

Table 1. Biomechanical and neuromuscular data (means and standard deviations) for all of the subjects, the male subjects, the female subjects and the gender difference.

Variables	Total (*n* = 38)	Male (*n* = 18)	Female (*n* = 20)	*p* Value	g
Peak posterior GRF (N/kg)	6.89 ± 1.34	6.50 ± 1.11	7.24 ± 1.46	0.09	0.57
Peak vertical GRF (N/kg)	44.75 ± 8.11	39.88 ± 5.69	49.12 ± 7.53	<0.001 *	1.37
Peak knee anterior reaction force (N/kg)	0.20 ± 0.05	0.17 ± 0.05	0.23 ± 0.04	<0.001 *	1.33
Peak knee flexion moment (N*m/kg)	2.72 ± 0.54	2.61 ± 0.65	2.83 ± 0.42	0.21	0.41
Peak knee flexion angle (degrees)	55.60 ± 10.54	57.76 ± 10.44	53.65 ± 10.51	0.23	0.39
Peak knee flexion angle within 100 ms after IC (degrees)	48.00 ± 6.46	47.99 ± 5.97	48.00 ± 7.03	0.99	0.002
Pre-activation of the VL 100 ms before IC (%)	33.51 ± 11.86	33.74 ± 12.74	33.31 ± 11.35	0.91	0.04
Pre-activation of the ST 100 ms before IC (%)	39.28 ± 17.05	45.10 ± 20.05	34.03 ± 12.05	0.04 *	0.68
Pre-activation of the ST:VL 100 ms before IC	1.35 ± 0.88	1.54 ± 1.10	1.18 ± 0.61	0.22	0.41

GRF: ground reaction force. IC: Initial contact. Pre-activation: The EMG values of each subphase were normalized to the peak EMG during landing from 30 cm height. VL: vastus lateralis. ST: semitendinosus. g: Hedge's g effect sizes. * $p < 0.05$.

Pearson's correlation coefficients between the response variables and the potential predictor variables are listed in Table 2. By reducing the family wise error rates, Bonferroni correction was applied and the significant correlations at an adjusted alpha level were indicated. Peak knee anterior reaction force significantly correlated with pre-activation of the semitendinosus, ST:VL pre-activation ratio, knee angular excursion on the frontal plane, peak vertical GRF, peak posterior GRF, peak knee flexion moment, and gender. Of those variables, peak knee flexion moment, ST:VL pre-activation ratio, and peak vertical GRF were shown to be better predictors in the multiple regression model ($p < 0.001$) summarized in Table 3. This model accounted for 57.6% of the variance in the peak knee anterior reaction force during the single leg landing task. The regression equation was Y = 0.024 + 0.025 (peak knee flexion moment) − 0.02 (ST:VL pre-activation ratio) + 0.003 (peak vertical GRF). Pre-activation of the semitendinosus muscle was found to be a significant predictor of peak knee flexion moment ($R^2 = 0.345$, $p < 0.001$). Specifically, a 10% increase in semitendinosus pre-activation predicted a 0.20 N*m/kg decrease in peak knee flexion moment. A significant portion of the variance in the knee flexion angle within 100 ms after initial contact was explained by the ST:VL pre-activation ratio ($R^2 = 0.351$, $p < 0.001$) (Figure 2), with a one unit increase in the ST:VL pre-activation ratio predicting a 4.33° increase in knee flexion angle.

Table 2. Pearson's correlations among the response variables (peak knee anterior reaction force) and the predictor variables.

Variable	1	2	3	4	5	6	7
1. Peak knee anterior reaction force (N/kg)	-	**−0.562 ****	**−0.482 ****	0.302 *	**0.663 ****	0.358 *	**0.457 ****
2. Pre-activation of the ST 100ms before IC (%)		-	**0.563 ****	-0.379 *	**−0.542 ****	-0.178	**−0.588 ****
3. Pre-activation of the ST:VL 100ms before IC			-	−0.089	−0.365 *	−0.142	−0.088

4. Knee abduction angular excursion (degrees)	-	**0.528** **	0.053	0.196
5. Peak vertical GRF (N/kg)		-	0.39 *	0.324 *
6. Peak posterior GRF (N/kg)			-	0.228
7. Peak knee flexion moment (N*m/kg)				-

Bold indicates correlation is significant at alpha level adjusted by Bonferroni correction. GRF: ground reaction force. IC: Initial contact. Knee abduction angular excursion = peak knee varus (+) − peak knee valgus (-). Pre-activation: The EMG values of each subphase were normalized to the peak EMG during landing from 30 cm height. VL: vastus lateralis. ST: semitendinosus. * $p < 0.05$. ** $p < 0.005$.

Table 3. Regression coefficients for predicting peak knee anterior reaction force.

Variable	B	95%CI	β	t	*p*
Peak proximal knee flexion moment (N*m/kg)	0.025	[0.014, 0.036]	0.28	2.367	0.024
Pre-activation of the ST:VL 100ms before IC	−0.016	[−0.023, −0.009]	−0.287	−2.39	0.023
Peak vertical GRF (N/kg)	0.003	[0.002, 0.004]	0.468	3.703	0.001
Constant	0.024	[−0.018, 0.066]	-	0.580	0.566

GRF: ground reaction force. VL: vastus lateralis. ST: semitendinosus. $R^2 = 0.576$ (N = 38, $p = 0.000$). CI = confidence interval for B.

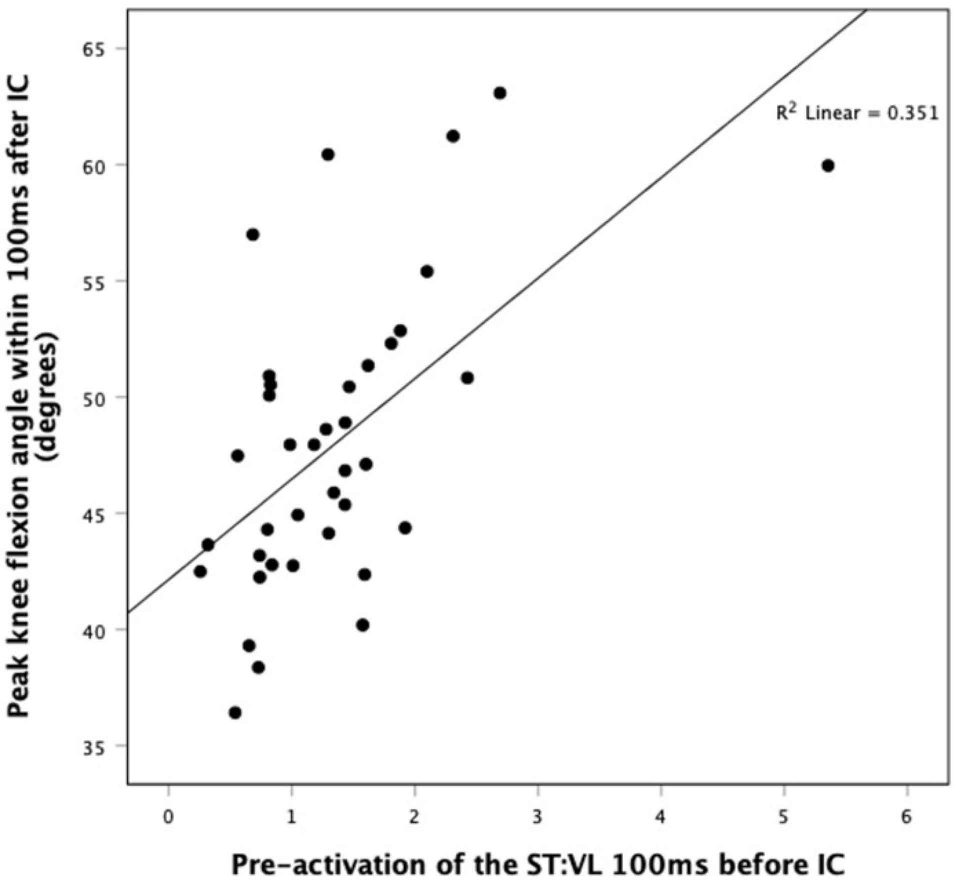

Figure 2. Relationship between peak knee flexion angle and pre-activation ratio of the semitendinosus to vastus lateralis.

4. Discussion

The findings of this study partially supported our hypothesis. We found that females had a higher knee anterior reaction force, vertical GRF, and lower pre-activation of the semitendinosus compared to males. Our findings also suggested that a linear regression model with an equation shows the relationship between predictor variables (peak vertical GRF, peak knee flexion moment, and ST:VL pre-activation ratio) and peak knee anterior reaction force. The results of our study preliminarily showed differences in landing biomechanics between genders and riskier landing movements, which may have implications for future research on these high-riskier landing biomechanics and injury prevention on young untrained sports enthusiasts.

Proximal tibia anterior shear force has been reported to positively correlate with knee anterior drawer force and exerted anterior tibial translation [6,7]. While a proxy measure of forces acting on the ACL, this variable can provide a useful, measurable indicator of ACL strain, which could provide a measurable risk factor for non-contact ACL injury. Findings suggested that females experienced a greater knee anterior reaction force compared to males, a finding consistent with those of previous studies [27,37], though the magnitude of differences found in this study as 35% higher for females was smaller than those previously reported a 50% difference [27,37]. A possible reason for the lower knee anterior reaction force in the current study is the shorter landing distance (30 cm) than that in the stop-landing study (~69 cm) [27]. Evidence shows that knee anterior reaction force increased significantly as jump distance increased [38]. A study with 10 cm drop-landing distance showed a similar reaction force as the current study [39]. We provide additional evidence for higher proximal tibia anterior shear force in females, compared to males, which, as discussed later, could provide a key risk factor for ACL injury.

The gender-related differences in knee anterior reaction force stem from neuromuscular control differences. In agreement with previous research [18], our study suggests that females exhibit a lower pre-activation of the semitendinosus and subsequent increases in knee anterior reaction force. Despite the supporting 'quadriceps dominance' theory, our results show that a low ST:VL pre-activation ratio in females was mainly determined by reduced hamstrings activation rather than excessive quadriceps during the pre-landing phase, a finding inconsistent with [40,41]. In fact, an association between reduced pre-activation of the medial hamstring and risk of injury in non-contact ACL injury has been reported [20,21,42–44]. Additionally, female athletes who performed exercises with a more balanced quadriceps and hamstring ratio showed medial hamstring activation in various risk movement tasks [20,42–44]. Thus, increasing medial hamstring activation may benefit ACL rehabilitation and injury-prevention programs rather than simply inhibiting quadriceps activation. However, the high variability within a population of subjects should be noted. Neuromuscular fatigue could be a potential consideration [45]. Each subject in the current study repeatedly performed an average of 5.4 ± 1.5 times to obtain three successful landings. Given the difficulty in landing on single-leg, additional alterations in motor control and motor unit recruitment can occur [46]. Further variability may be attributed to biomechanical factors. Given the variation in landing movement each time, the VL and ST muscles are susceptible to changes in length after changes to hip or knee angle [47]. Subsequent differences in muscle length and tension can result in EMG activity changes [48]. Generalizability for estimating the pre-activation could be limited, due to the high variation within each population. However, in addressing the aims, medium effect size in gender comparison of the pre-activation of ST and a significant correlation between pre-activation of ST and knee anterior reaction force were found in the current study.

The reduced co-activation between the hamstrings and quadriceps in women may result in greater knee joint instability than that observed in men. As evidenced by our results, a decrease in semitendinosus pre-activation contributed to an increase in peak knee flexion moment during the deceleration phase of landing. The underlying rationale of stabilizing the knee might be that hamstrings create a posteriorly directed force on the

ACL that counteracted the anteriorly directed stress forces on the tibia [22]. Anterior shear force is generated primarily from active quadriceps contraction force, which is most pronounced at less flexed knee joint angles of 0–30 degrees of flexion [19]. Regarding the knee flexion angle, the current finding was unable to conclude that knee flexion angle during the deceleration phase has gender specific difference or a relationship with knee anterior reaction forces. Our finding supports previous evidence [14–16], while it is in conflict with the concept that females have decreased knee flexion angles [13,40]. The possible explanation for the discrepancy is that anatomical risk factors of ACL injury, such as femoral intercondylar notch width [49], patellar tendon tibial shaft angle [50], and tibial slope, can be different between the genders [51], and thereby contribute to different knee flexion angle and ACL loading. Given the evidence that athletes with non-contact ACL injury had less knee flexion angle in matches compared with healthy control [52], the current evidence showing a greater knee flexion angle associated with an increase in ST:VL pre-activation ratio provides a potential practical intervention method to reduce non-contact ACL injury. Additionally, a proper H:Q muscular co-contraction to produce the joint compression [22,53] would provide proprioceptive feedback control via mechanoreceptors embedded in the ACL tissue to further improve knee stability [54]. However, it remains unclear why females have a decreased pre-activation of the hamstrings during high-risk movements associated with non-contact ACL injuries. One potential explanation for this occurrence might be that the decreased amplitude of H:Q muscular pre-activation facilitates more explosive jumping and landing movements, in which the knee extensors would more efficiently provide high concentric push-off and eccentric landing force and power.

The inclusion of peak vertical ground reaction force in the final regression model suggested that peak vertical GRFs were associated with ACL injuries, a finding consistent with those of previous studies [52,55]. In contrast, some studies reported the opposite results on vGRF in those men who had a greater peak vGRF than that measured in women during landing [15,56]. It should be noted that landing biomechanics can be affected by different types of landing task [56]. The vertical drop landing in our study might have led to increased peak vGRF [56], revealing different biomechanical mechanisms of ACL injury. Evidence has indicated that ground reaction force is influenced by the ability of postural control or/and ankle stability [57,58], and therefore baseline control of postural stability is required in future studies. Similar to vGRF, our results also indicated that an increase in external knee flexion moment was associated with an increase in knee anterior reaction forces. A knee flexion moment is required to balance a knee extension moment generated by the quadriceps [59]. The quadriceps are needed to control knee flexion, which generates an anterior force on the tibia by pulling the patellar ligament [59]. Although our findings showed that a decrease in ST pre-activation predicted an increase in a peak knee flexion moment during the deceleration phase of landing, without knowledge of the muscle forces, it is difficult to confirm if the increased internal quadriceps moment is due to an elevated quadriceps force and/or a reduced hamstrings force.

One of the factors that may influence the landing performance is the muscular strength of the lower limb [17]. The current study did not control the lower limb strength, but a recent study with a similar cohort as our study showed that males have higher lower limb strength than females in non-athletes [60]. The females had a smaller muscle cross-sectional area, leading to the less strength compared to males in the general population [61]. We cannot provide evidence that the cause of differences is purely due to gender without controlling for relative strength levels or morphological characteristics. However, it is rather difficult to purposefully recruit stronger females in order to obtain a strength-matched cohort, without compromising the generalization of the study. In the current study, an untrained cohort with low–moderate physical activity status was chosen, which reduced the confounding effects that are modifiable through training, such as strength, sporting, and movement.

There are certain limitations associated with the method. It should be noted that the current study established an association between various biomechanical parameters with peak knee anterior reaction force, but we cannot directly conclude that those biomechanical parameters correlate with ACL loads. Anterior shear force has been established as a loading factor on ACL strain [6,7], but the knee anterior reaction force, estimated by inverse dynamics in this study does not represent shear force loading on the ACL or shear force exerted by the patellar tendon. The muscle forces that are generated from actively contracting muscles should be incorporated into the model in future research to predict forces loading on the ACL. More variables can be incorporated into the regression equation, such as muscle strength, anatomical influences, lower limb joint stability, postural control, and different types of landing tasks, as these variables would have mixed effects on the result. Laboratory simulation study cannot fully mimic the landing scenario in a real-life event, as the knee joint forces would be more complex to cope with unanticipated activities that involve landing in real life, and thereby different from those in laboratory. The current study was unable to control for the same drop height. The subjects drop-landed from a 30 cm box, but the box height and drop height are typically different [62,63]. However, we could ensure that all of our participants did not receive any professional landing- or jumping-related training, which could reduce the learning effects on the results. Future studies are therefore warranted to consider more possible predictors of non-contact ACL injury in non-sports populations to help explain why females have a decreased pre-activation of the hamstrings associated with non-contact ACL injuries.

5. Conclusions

The results of our analysis in female non-athletes demonstrated higher peak knee anterior reaction force, vertical GRF, and lower pre-activation of the semitendinosus. Increased peak vertical ground reaction force, knee flexion moment, and a reduced ST:VL pre-activation ratio predicted an increase in knee anterior reaction force and potentially an increase in ACL forces. The semitendinosus pre-activation correlated negatively with peak knee flexion moment. The ST:VL pre-activation ratio was positively correlated with knee flexion angle. Our findings therefore provide additional knowledge on the neuromuscular patterns of lower limbs and suggest a potential mechanism for non-contact ACL injury in non-athletes. The results of our study have implications for future research in terms of screening for non-contact ACL injury risks and the development of prophylactic training programs to prevent these injuries.

Practical implications:

- Increased pre-activation of the semitendinosus rather than decrease vastus lateralis may decrease proximal tibia anterior shear force.
- Higher proximal tibia anterior shear force may be related to a higher risk of ACL injury in females.
- Knee flexion angle appears not to show gender-specific differences during single leg drop landing tasks.
- Vertical rather than posterior ground reaction force may be related to proximal tibia anterior shear force in drop landing tasks.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomechanics2040044/s1.

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References

1. Renström, P.A. Eight clinical conundrums relating to anterior cruciate ligament (ACL) injury in sport: Recent evidence and a personal reflection. *Br. J. Sports Med.* **2013**, *47*, 367–372.
2. Toale, J.P.; Hurley, E.T.; Hughes, A.J.; Withers, D.; King, E.; Jackson, M.; Moran, R. The majority of athletes fail to return to play following anterior cruciate ligament reconstruction due to reasons other than the operated knee. *Knee Surg. Sports Traumatol. Arthrosc.* **2021**, *29*, 3877–3882.
3. Wang, L.-J.; Zeng, N.; Yan, Z.-P.; Li, J.-T.; Ni, G.-X. Post-traumatic osteoarthritis following ACL injury. *Arthritis Res. Ther.* **2020**, *22*, 1–8.
4. Hewett, T.E.; Myer, G.D.; Ford, K.; Paterno, M.V.; Quatman, C.E. Mechanisms, prediction, and prevention of ACL injuries: Cut risk with three sharpened and validated tools. *J. Orthop. Res.* **2016**, *34*, 1843–1855.
5. Stanley, L.E.; Kerr, Z.Y.; Dompier, T.P.; Padua, D.A. Sex differences in the incidence of anterior cruciate ligament, medial collateral ligament, and meniscal injuries in collegiate and high school sports: 2009–2010 through 2013–2014. *Am. J. Sports Med.* **2016**, *44*, 1565–1572.
6. Markolf, K.L.; Burchfield, D.M.; Shapiro, M.M.; Shepard, M.F.; Finerman, G.A.M.; Slauterbeck, J.L. Combined knee loading states that generate high anterior cruciate ligament forces. *J. Orthop. Res.* **1995**, *13*, 930–935.
7. Fleming, B.C.; A Renstrom, P.; Beynnon, B.D.; Engstrom, B.; Peura, G.D.; Badger, G.J.; Johnson, R.J. The effect of weightbearing and external loading on anterior cruciate ligament strain. *J. Biomech.* **2001**, *34*, 163–170.
8. Ball, S.; Stephen, J.M.; El-Daou, H.; Williams, A.; Amis, A.A. The medial ligaments and the ACL restrain anteromedial laxity of the knee. *Knee Surg. Sports Traumatol. Arthrosc.* **2020**, *28*, 3700–3708.
9. Kittl, C.; El-Daou, H.; Athwal, K.K.; Gupte, C.M.; Weiler, A.; Williams, A.; Amis, A.A. The Role of the Anterolateral Structures and the ACL in Controlling Laxity of the Intact and ACL-Deficient Knee. *Am. J. Sports Med.* **2016**, *44*, 345–354.
10. Lucarno, S.; Zago, M.; Buckthorpe, M.; Grassi, A.; Tosarelli, F.; Smith, R.; Della Villa, F. Systematic Video Analysis of Anterior Cruciate Ligament Injuries in Professional Female Soccer Players. *Am. J. Sports Med.* **2021**, *49*, 1794–1802.
11. Schmitz, R.J.; Kulas, A.S.; Perrin, D.H.; Riemann, B.L.; Shultz, S.J. Sex differences in lower extremity biomechanics during single leg landings. *Clin. Biomech.* **2007**, *22*, 681–688.
12. Zhao, X.; Gu, Y. Single leg landing movement differences between male and female badminton players after overhead stroke in the backhand-side court. *Hum. Mov. Sci.* **2019**, *66*, 142–148.
13. Weinhandl, J.T.; Irmischer, B.S.; Sievert, Z.A. Sex differences in unilateral landing mechanics from absolute and relative heights. *Knee* **2015**, *22*, 298–303.
14. Ali, N.; Rouhi, G.; Robertson, G. Gender, Vertical Height and Horizontal Distance Effects on Single-Leg Landing Kinematics: Implications for Risk of non-contact ACL Injury. *J. Hum. Kinet.* **2013**, *37*, 27–38.
15. Orishimo, K.F.; Kremenic, I.; Pappas, E.; Hagins, M.; Liederbach, M. Comparison of Landing Biomechanics Between Male and Female Professional Dancers. *Am. J. Sports Med.* **2009**, *37*, 2187–2193.
16. Sinclair, J.; Brooks, D.; Stainton, P. Sex differences in ACL loading and strain during typical athletic movements: A musculoskeletal simulation analysis. *Eur. J. Appl. Physiol.* **2019**, *119*, 713–721.
17. Suchomel, T.J.; Nimphius, S.; Stone, M.H. The Importance of Muscular Strength in Athletic Performance. *Sports Med.* **2016**, *46*, 1419–1449.
18. Bencke, J.; Aagaard, P.; Zebis, M.K. Muscle Activation During ACL Injury Risk Movements in Young Female Athletes: A Narrative Review. *Front. Physiol.* **2018**, *9*, 445.
19. Englander, Z.A.; Cutcliffe, H.C.; Utturkar, G.M.; Taylor, K.A.; Spritzer, C.E.; Garrett, W.E.; DeFrate, L.E. In vivo assessment of the interaction of patellar tendon tibial shaft angle and anterior cruciate ligament elongation during flexion. *J. Biomech.* **2019**, *90*, 123–127.
20. Zebis, M.K.; Andersen, L.L.; Brandt, M.D.; Myklebust, G.; Bencke, J.; Lauridsen, H.B.; Bandholm, T.; Thorborg, K.; Hölmich, P.; Aagaard, P. Effects of evidence-based prevention training on neuromuscular and biomechanical risk factors for ACL injury in adolescent female athletes: A randomised controlled trial. *Br. J. Sports Med.* **2016**, *50*, 552–557.
21. Zebis, M.K.; Andersen, L.L.; Bencke, J.; Kjaer, M.; Aagaard, P. Identification of athletes at future risk of anterior cruciate ligament ruptures by neuromuscular screening. *Am. J. Sports Med.* **2009**, *37*, 1967–1973.
22. Li, G.; Rudy, T.; Sakane, M.; Kanamori, A.; Ma, C.; Woo, S.-Y. The importance of quadriceps and hamstring muscle loading on knee kinematics and in-situ forces in the ACL. *J. Biomech.* **1999**, *32*, 395–400.

23. Koga, H.; Nakamae, A.; Krosshaug, T. Mechanisms for noncontact anterior cruciate ligament injuries: Knee joint kinematics in 10 injury situations from female team handball and basketball. *Am. J. Sports Med*. **2010**, *38*, 2218–2225.
24. Krosshaug, T.; Nakamae, A.; Bahr, R.; Engebretsen, L.; Smith, G.; Slauterbeck, J.R.; Hewett, T.E. Mechanisms of anterior cruciate ligament injury in basketball: Video analysis of 39 cases. *Am. J. Sports Med.* **2007**, *35*, 359–367.
25. Dyhre-Poulsen, P.; Krogsgaard, M.R. Krogsgaard, Muscular reflexes elicited by electrical stimulation of the anterior cruciate ligament in humans. *J. Appl. Physiol.* **2000**, *89*, 2191–2195.
26. Markström, J.L.; Grip, H.; Schelin, L.; Häger, C.K. Dynamic knee control and movement strategies in athletes and non-athletes in side hops: Implications for knee injury. *Scand. J. Med. Sci. Sports* **2019**, *29*, 1181–1189.
27. Sell, T.C.; Ferris, C.M.; Abt, J.P.; Tsai, Y.-S.; Myers, J.B.; Fu, F.H.; Lephart, S.M. Predictors of proximal tibia anterior shear force during a vertical stop-jump. *J. Orthop. Res*. **2007**, *25*, 1589–1597.
28. Lee, P.H.; Macfarlane, D.J.; Lam, T.H.; Stewart, S.M. Validity of the international physical activity questionnaire short form (IPAQ-SF): A systematic review. International *J. Behav. Nutr. Phys. Act.* **2011**, *8*, 115.
29. Stegeman, D.; Hermens, H. Standards for surface electromyography: The European project Surface EMG for non-invasive assessment of muscles (SENIAM). *Enschede Roessingh Res. Dev.* **2007**, *10*, 108–112.
30. Kadaba, M.P.; Ramakrishnan, H.K.; Wootten, M.E.; Gainey, J.; Gorton, G.; Cochran, G.V.B. Repeatability of kinematic, kinetic, and electromyographic data in normal adult gait. *J. Orthop. Res.* **1989**, *7*, 849–860.
31. Davis, R.B.; Õunpuu, S.; Tyburski, D.; Gage, J.R. A gait analysis data collection and reduction technique. *Hum. Mov. Sci.* **1991**, *10*, 575–587.
32. Li, G.; Kawamura, K.; Barrance, P.; Chao, E.Y.S.; Kaufman, K. Prediction of muscle recruitment and its effect on joint reaction forces during knee exercises. *Ann. Biomed. Eng.* **1998**, *26*, 725–733.
33. Cavanagh, P.R.; Komi, P.V. Electromechanical delay in human skeletal muscle under concentric and eccentric contractions. *Eur. J. Appl. Physiol. Occup. Physiol.* **1979**, *42*, 159–163.
34. Blackburn, J.T.; Bell, D.R.; Norcross, M.F.; Hudson, J.D.; Engstrom, L.A. Comparison of hamstring neuromechanical properties between healthy males and females and the influence of musculotendinous stiffness. *J. Electromyogr. Kinesiol.* **2009**, *19*, e362–e369.
35. Tirosh, O.; Sparrow, W. Identifying heel contact and toe-off using forceplate thresholds with a range of digital-filter cutoff frequencies. *J. Appl. Biomech.* **2003**, *19*, 178–184.
36. Hedges, L.V. Distribution theory for Glass's estimator of effect size and related estimators. *J. Educ. Stat.* **1981**, *6*, 107–128.
37. Fain, A.C.; Lobb, N.J.; Seymore, K.D.; Brown, T.N. Sex and limb differences during a single-leg cut with body borne load. *Gait Posture* **2019**, *74*, 7–13.
38. Sell, T.C.; Akins, J.S.; Opp, A.R.; Lephart, S.M. Relationship between tibial acceleration and proximal anterior tibia shear force across increasing jump distance. *J. Appl. Biomech*. **2014**, *30*, 75–81.
39. Gheidi, N.; Sadeghi, H.; Talebian, S.; Farhad, M.; Ghoshe, T.; Kernozek, T.W. Kinematics and kinetics predictor of proximal tibia anterior shear force during single leg drop landing. *Phys. Treat. Specif. Phys. Ther. J.* **2014**, *4*, 102–108.
40. Chappell, J.D.; Creighton, R.A.; Giuliani, C.A.; Yu, B. Kinematics and electromyography of landing preparation in vertical stop-jump: Risks for noncontact anterior cruciate ligament injury. *Am. J. Sports Med*. **2007**, *35*, 235–241.
41. Shultz, S.J.; Nguyen, A.-D.; Leonard, M.D.; Schmitz, R.J. Thigh strength and activation as predictors of knee biomechanics during a drop jump task. *Med. Sci. Sports Exerc.* **2009**, *41*, 857–866.
42. Letafatkar, A.; Rajabi, R.; Tekamejani, E.E.; Minoonejad, H. Effects of perturbation training on knee flexion angle and quadriceps to hamstring cocontraction of female athletes with quadriceps dominance deficit: Pre–post intervention study. *Knee* **2015**, *22*, 230–236.
43. Nagano, Y.; Ida, H.; Akai, M.; Fukubayashi, T. Effects of jump and balance training on knee kinematics and electromyography of female basketball athletes during a single limb drop landing: Pre-post intervention study. *Sports Med. Arthrosc. Rehabil. Ther. Technol.* **2011**, *3*, 14–18.
44. Zebis, M.K.; Bencke, J.; Andersen, L.L.; Døssing, S.; Alkjær, T.; Magnusson, S.P.; Kjær, M.; Aagaard, P. The effects of neuromuscular training on knee joint motor control during sidecutting in female elite soccer and handball players. *Clin. J. Sport Med*. **2008**, *18*, 329–337.
45. Behm, D.G. Force maintenance with submaximal fatiguing contractions. *Can. J. Appl. Physiol*. **2004**, *29*, 274–290.
46. Fauth, M.L.; Petushek, E.J.; Feldmann, C.R.; E Hsu, B.; Garceau, L.R.; Lutsch, B.N.; Ebben, W.P. Reliability of surface electromyography during maximal voluntary isometric contractions, jump landings, and cutting. *J. Strength Cond. Res*. **2010**, *24*, 1131–1137.
47. Kellis, E.; Konstantinidou, A.; Ellinoudis, A. Muscle Length of the Hamstrings Using Ultrasonography Versus Musculoskeletal Modelling. *J. Funct. Morphol. Kinesiol.* **2021**, *6*, 26.
48. Mohamed, O.; Perry, J.; Hislop, H. Relationship between wire EMG activity, muscle length, and torque of the hamstrings. *Clin. Biomech*. **2002**, *17*, 569–579.
49. Uhorchak, J.M.; Scoville, C.R.; Williams, G.N.; Arciero, R.A.; Pierre, P.S.; Taylor, D.C. Risk factors associated with noncontact injury of the anterior cruciate ligament: A prospective four-year evaluation of 859 West Point cadets. *Am. J. Sports Med*. **2003**, *31*, 831–842.
50. Nunley, R.M.; Wright, D.; Renner, J.B.; Yu, B.; Garrett, W.E. Gender comparison of patellar tendon tibial shaft angle with weight bearing. *Res. Sports Med.* **2003**, *11*, 173–185.

51. Beynnon, B.D.; Hall, J.S.; Sturnick, D.R.; DeSarno, M.J.; Gardner-Morse, M.; Tourville, T.W.; Smith, H.C.; Slauterbeck, J.R.; Shultz, S.J.; Johnson, R.J.; et al. Increased slope of the lateral tibial plateau subchondral bone is associated with greater risk of noncontact ACL injury in females but not in males: A prospective cohort study with a nested, matched case-control analysis. *Am. J. Sports Med*. **2014**, *42*, 1039–1048.
52. Leppänen, M.; Pasanen, K.; Kujala, U.; Vasankari, T.; Kannus, P.; Äyrämö, S.; Krosshaug, T.; Bahr, R.; Avela, J.; Perttunen, J.; et al. Stiff Landings Are Associated With Increased ACL Injury Risk in Young Female Basketball and Floorball Players. *Am. J. Sports Med*. **2017**, *45*, 386–393.
53. Navacchia, A.; Ueno, R.; Ford, K.R.; DiCesare, C.A.; Myer, G.D.; Hewett, T.E. EMG-Informed musculoskeletal modeling to estimate realistic knee anterior shear force during drop vertical jump in female athletes. *Ann. Biomed. Eng.* **2019**, *47*, 2416–2430.
54. Criss, C.R.; Melton, M.S.; Ulloa, S.A.; Simon, J.E.; Clark, B.C.; France, C.R.; Grooms, D.R. Rupture, reconstruction, and rehabilitation: A multi-disciplinary review of mechanisms for central nervous system adaptations following anterior cruciate ligament injury. *Knee* **2021**, *30*, 78–89.
55. Aizawa, J.; Hirohata, K.; Ohji, S.; Ohmi, T.; Yagishita, K. Limb-dominance and gender differences in the ground reaction force during single-leg lateral jump-landings. *J. Phys. Ther. Sci.* **2018**, *30*, 387–392.
56. Peebles, A.T.; Dickerson, L.C.; Renner, K.E.; Queen, R.M. Sex-based differences in landing mechanics vary between the drop vertical jump and stop jump. *J. Biomech.* **2020**, *105*, 109818.
57. Niu, W.; Feng, T.; Wang, L.; Jiang, C.; Zhang, M. Effects of prophylactic ankle supports on vertical ground reaction force during landing: A meta-analysis. *J. Sports Sci. Med.* **2016**, *15*, 1–10.
58. Paillard, T.; Noé, F. Techniques and Methods for Testing the Postural Function in Healthy and Pathological Subjects. *Biomed. Res. Int.* **2015**, *2015*, 891390.
59. Yu, B.; Garrett, W.E. Mechanisms of non-contact ACL injuries. *Br. J. Sports Med.* **2007**, *41* (Suppl. 1), i47.
60. Ben Mansour, G.; Kacem, A.; Ishak, M.; Grélot, L.; Ftaiti, F. The effect of body composition on strength and power in male and female students. *BMC Sports Sci. Med. Rehabil.* **2021**, *13*, 150.
61. Miller, A.E.J.; MacDougall, J.D.; Tarnopolsky, M.A.; Sale, D.G. Gender differences in strength and muscle fiber characteristics. *Eur. J. Appl. Physiol. Occup. Physiol.* **1993**, *66*, 254–262.
62. Costley, L.; Wallace, E.; Johnston, M.; Kennedy, R. Reliability of bounce drop jump parameters within elite male rugby players. *J. Sports Med. Phys. Fit.* **2018**, *58*, 1390–1397.
63. McMahon, J.; Lake, J.; Stratford, C.; Comfort, P. A Proposed Method for Evaluating Drop Jump Performance with One Force Platform. *Biomechanics* **2021**, *1*, 178–189.