Risk factors for non-contact anterior cruciate ligament injury in elite female footballers: A prospective study

Abstract

Background: Female soccer and Australian Rules Football players are at high risk of anterior cruciate ligament (ACL) injury. Risk of ACL injury is thought to be related to a players lower limb strength and biomechanics.

Purpose: To determine if a pre-season field-based test battery was prospectively associated with non-contact ACL injury in elite female footballers.

Study design: Prospective cohort study.

Methods: In total, 322 elite senior and junior female Australian Rules Football and soccer players had their isometric hip adductor and abductor strength, eccentric knee flexor strength, countermovement jump (CMJ) kinetics, and single-leg hop kinematics assessed during the 2019 pre-season. Demographic and injury history details were also collected. Footballers were subsequently followed for 18 months for ACL injury.

Results: 15 non-contact ACL injuries occurred during the follow-up period. Prior ACL injury (odds ratio [OR] = 9.68, 95% confidence interval [95%CI] = 2.67-31.46), a lower isometric hip adductor to abductor strength ratio (OR=1.98, 95%CI=1.09-3.61), greater CMJ peak take-off force (OR=1.74, 95%CI=1.09-3.61), and greater single-leg triple vertical hop average dynamic knee valgus (OR=1.97, 95%CI=1.06-3.63) and ipsilateral trunk flexion (OR=1.60, 95%CI=1.01-2.55) were independently associated with increased risk for subsequent ACL injury. A multivariable prediction model consisting of CMJ peak take-off force, dynamic knee valgus, and ACL injury history that was internally validated classified ACL injured from uninjured footballers with 78% total accuracy. Between-leg asymmetry in lower limb strength and CMJ kinetics were not associated with subsequent ACL injury risk.

Conclusion: Pre-season field-based measures of lower limb muscle strength and biomechanics were associated with future non-contact ACL injury in elite female footballers.

Clinical Relevance: These risk factors can be used to guide ACL injury screening practices and inform the design of targeted injury prevention training in elite female footballers.

Key Terms: ACL, Injury prevention, Physical therapy/Rehabilitation, & Female athlete.

What is known about the subject: A number of ACL strength and biomechanical injury risk factors have been proposed in the literature, however, there is often a limited amount of evidence or conflicting results between prospective studies. Many of these studies have been performed in adolescent athletes, and no studies have been performed within elite Australian soccer or Australian Rules Football players.

What this study adds to existing knowledge: This study provides evidence for a number of non-contact ACL injury risk factors using a field-based testing battery. Risk factors included a lower isometric hip adductor to abductor strength ratio, greater countermovement jump take-off forces, and greater single-leg triple vertical hop knee valgus and lateral trunk flexion. Further, a multivariable prediction model correctly identified footballers who sustained a future ACL injury with acceptable accuracy. We also demonstrated that between-leg asymmetry in lower limb strength and countermovement jump kinetics were not associated with subsequent ACL injury risk.

Introduction

Anterior cruciate ligament (ACL) ruptures are among the most catastrophic injuries in soccer and Australian Rules Football, and occur 3-6 times more frequently in female than male footballers.[24, 44] Even after ACL reconstructive surgery, two-thirds of female footballers fail to return to the same level of competition, and one in three go on to sustain a second ACL injury.[42] The treatment and surgery costs associated with ACL injury, combined with the potential for early onset knee osteoarthritis, generates a large health care-related financial burden.[11] Therefore, efforts to prevent ACL injury in female footballers are imperative.

In football, ACL injuries most commonly occur due to a non-contact mechanism when changing direction, decelerating, or landing on a single-leg.[20] Prior ACL injury is the most consistently identified risk factor for subsequent ACL injury.[6] Biomechanical factors suggested to be associated with increased risk of ACL injury include greater knee valgus angles, moments and peak vertical ground reaction force during double-leg drop vertical jumps,[14, 19] two-dimensional[14, 19] frontal plane knee valgus and trunk lateral flexion during single-leg drop vertical jumps,[9] and hip flexion and knee internal rotation angles at initial contact during sidestepping.[46] However, until recently, these factors have been difficult to measure outside of the laboratory. Between-leg asymmetry in jumping kinetics is also used a tool to monitor ACL rehabilitation,[36] yet it is unknown whether asymmetries are associated with increased ACL injury risk.

Lower limb muscle strength may also contribute to ACL injury risk. For example, hip muscle weakness (in all planes) is associated with greater dynamic knee valgus during single-leg landing in females,[10] which may increase ACL strain.[16] Further, the hip abductors and hamstrings may be important for opposing ACL loads during side-step cutting by applying a knee varus moment, or posterior shear force to the knee, respectively.[22] In theory, hamstrings and hip muscle weakness, or asymmetry between legs, may expose a player to greater risk of ACL injury, although this has never been explored in elite female footballers.[32]

An improved understanding of risk factors for ACL injury in female football is needed to identify at-risk players and inform the design of targeted injury prevention strategies. To date, limited research has examined female only cohorts, and no study has been conducted in women's Australian Rules Football, despite this cohort having the highest rates of ACL injury in the world.[44] Previous research has also been limited by univariable approaches to injury risk profiling, which may not capture the complex, multifactorial nature of sport injuries.[40] Measuring several physical characteristics, including strength and biomechanics, may provide a better estimation of injury risk than a single test. Further, for any test to be widely adopted, it is important that equipment is quick and easy to use, inexpensive, and able to be used in a wide range of settings. As such, field-based testing devices may provide suitable substitutes to traditional laboratory measures, which remain inaccessible for many teams.

The primary aim of this study was to determine if a pre-season, field-based test battery consisting of hip strength, knee flexor strength, jump-landing kinetics, and hop kinematics was associated with future non-contact ACL injury in elite female footballers. A secondary aim was to determine the ability of multivariable prediction models to estimate ACL injury risk.

Methods

Participants & study design

This prospective cohort study was conducted from 2019 to 2021. Data were collected from 322 elite senior and junior Australian Rules Football and soccer players. Teams were recruited via club contacts, and all players within the training squad were invited to participate in the study. This study was approved by the University's human research ethics committee (reference number: 2019/423), and all players provided written informed consent prior to data collection. The parents/guardians of players under 18 years of age also provided written informed consent.

At the beginning of their pre-season (between November-December 2019), players completed a questionnaire detailing their demographics, injury history (including any lower limb injuries over the prior 12 months and lifetime history of ACL or other knee injuries), contraceptive use, and the Knee Injury and Osteoarthritis Outcome Score (KOOS) 'Pain' and 'Sport and Recreation' (Sport/Rec) sub-scales. Subsequently, players underwent assessments of 1) isometric hip adductor and abductor strength, 2) eccentric knee flexor strength during the Nordic hamstring exercise (NHE), 3) bilateral countermovement jump (CMJ) kinetics, and 4) single-leg triple vertical hop kinematics. Details of any prospective ACL injuries sustained in the subsequent 18 months were reported to the research team. This study was designed and conducted in coordination with team medical staff and the sporting national governing bodies. The screening battery was purposefully designed to take <10 mins per player, as established during pilot testing, and utilized equipment/tests that were routinely employed by the included teams. Data and study findings were shared with all participating teams to guide future practice.

Hip adductor and abductor strength

Isometric hip adductor and abductor strength were measured for the left and right leg independently during bilateral contractions using uniaxial load cells (sampling rate of 50 Hz) attached to a rigid frame (ForceFrame, Vald Performance, Brisbane) (Figure 1A). Players were tested in a supine position, with the hip in neutral and the knee fully extended, and the load cells aligned with the ankle malleoli.[30] Players performed three maximal effort trials of approximately five second isometric contractions, alternating between hip abduction and adduction with 5-10 seconds rest in between efforts. Similar testing protocols with the same device have shown excellent reliability (intraclass correlation coefficient [ICC]=0.94).[41] The maximum force produced during three trials was used in the analysis. In addition, the ratio of hip adductor to hip abductor force for each leg was calculated.

Eccentric knee flexor strength

Eccentric knee flexor strength was measured from the left and right legs independently during the NHE using two uniaxial load cells (sampling rate of 50 Hz) attached immediately superior to the ankle malleoli (NordBord, Vald Performance, Brisbane) (Figure 1B). From a kneeling position, players were instructed to lower themselves to the ground as slowly as possible, while keeping the trunk and hips in a neutral position. Players performed three maximal effort trials separated by 5-10 seconds of rest. Similar testing protocols with a similar device have shown moderate-to-high reliability (ICC=0.83 for left and 0.90 for right leg).[31] The maximum force produced during three trials was used in the analysis. To remove the relationship between force and body weight in the current cohort (r=0.58), eccentric knee flexor strength was also reported allometrically scaled ($N.kg^{-k}$, where ^{k} was 0.68).[3]

Countermovement jump-landing kinetics

Bilateral CMJs were performed on dual force plates that independently recorded left and right vertical ground reaction forces at 1000 Hz (ForceDecks, FDLite, Vald Performance, Brisbane) (Figure 1C). Players were instructed to jump as high as possible, using a countermovement to a self-selected depth, and to keep their hands on their hips throughout jumping and landing. Players performed three maximal effort trials separated by 5-10 seconds of rest. Peak forces and impulses measured using similar protocols and the same device have demonstrated excellent reliability (ICC>0.90).[13] Data were extracted using

ForceDecks software (Version 2.0.7). Variables were selected based on previous research in athletes with ACL reconstructions,[36] and included peak take-off force (concentric phase), take-off impulse (total eccentric + concentric phase), rate of force development (RFD) (eccentric phase), peak landing force, and landing RFD. Other variables were excluded because they were highly correlated with the selected variables and offered little independent information (Supplementary 1). All CMJ variables were normalized to body weight in Newtons.

Single-leg triple vertical hop landing kinematics

Single-leg hopping kinematics were recorded during a novel 'single-leg triple vertical hop' task using a three-dimensional markerless motion capture system (HumanTrak, Vald Performance, Brisbane) (Figure 1D), consisting of a Kinect camera (v2, Microsoft, WA, United States) and Microsoft artificial intelligence to track joint trajectories. The single-leg triple vertical hop test involved three consecutive single-leg vertical jumps for maximal height in a continuous motion. Players were instructed to jump as high as they could with every jump, while minimizing ground contact time. Players completed three trials for the left leg, followed by three trials for the right leg (i.e., 9 hops per leg). The single-leg triple vertical hop was designed to require high levels of exertion, while challenging single-leg knee and trunk control. Kinematics were extracted (using R Studio, version 4.0.5, Boston, US) from the landing phase defined as the phase between estimated ground contact (center of mass below standing height) and the lowest position of the center of mass. Variables selected for analysis were based on previous ACL risk factor studies in female athletes,[9, 19] and included average knee flexion, two-dimensional dynamic knee valgus, trunk flexion, and trunk ipsilateral flexion angles during landing. Measurement validity compared to a gold standard three-dimensional motion capture system (Vicon, Oxford, UK) ranged from 2 to 9°

(root mean square error) and reliability was moderate to good (ICCs=0.58 to 0.87) for all examined variables (Supplementary 2).

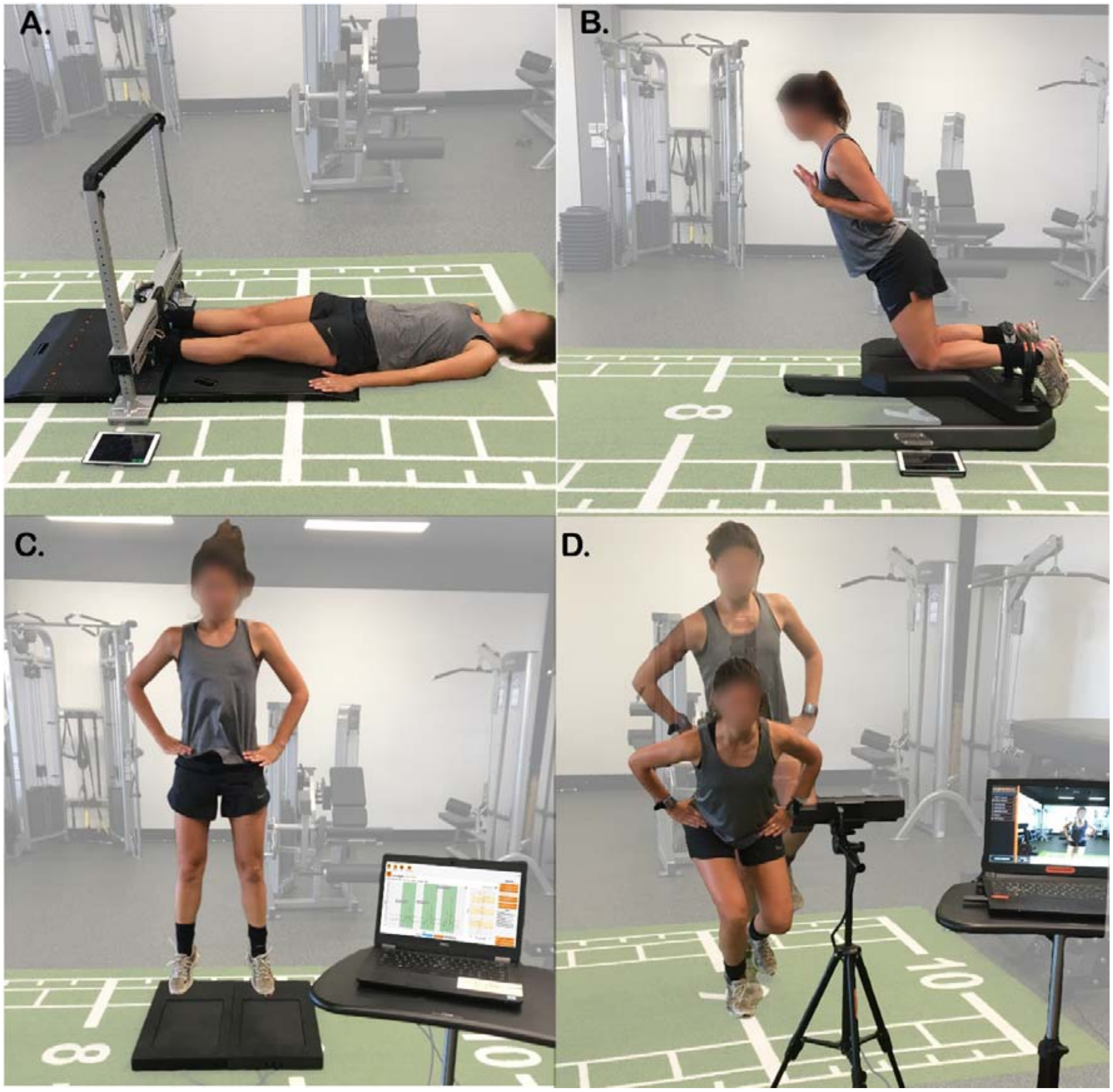


Figure 1 Demonstration of the **s**trength and biomechanics field-based testing battery. A) isometric hip adductor and abductor strength; B) eccentric knee flexor strength during the Nordic hamstring exercise; C) Bilateral countermovement jump on portable force plates; D) single-leg triple vertical hop kinematics using a markerless motion capture system.

Between-leg asymmetry

Directional between-leg asymmetry (%) was calculated for lower limb strength and CMJ variables using the equation *(leg maximum–leg minimum)/(leg maximum)*100* and made positive when the injured leg (or right leg for uninjured players) was greater than the uninjured leg (or left leg for uninjured players). Non-directional asymmetry was also analyzed by taking the absolute value of the directional between-leg asymmetry measure (i.e. making all values positive).

ACL injury reporting

All ACL injuries were reported to the researchers during the subsequent 18 months, which included two professional league playing seasons (2019/20 and 2020/21) and one regular season/COVID-19 break (2020). Only full ruptures of the ACL (both index and recurrent injuries) due to a non-contact injury mechanism were included in the analysis. For players competing in a national professional league, ACL injuries were recorded by team medical staff using a standardized form that included injury diagnosis, time-loss, mechanism of injury, and place of injury. For under-17 soccer players who did not have regular team medical staff, ACL injuries and related questions were self-reported by players via SMS or email at the end of each season.

Statistical analysis

Statistical analysis was performed using R Studio (version 4.0.5, Boston, US). A list of packages used are presented in Supplementary 3. The number of players and ACL injury characteristics were summarized by sport cohort using frequencies (n) and proportions (%) (Table 1). Group data for players who sustained a subsequent ACL injury (‘ACL injured’) and those who did not (‘Uninjured’) were summarized with descriptive statistics (Table 2) and compared using Glass’s Delta effect sizes and independent two-tailed t-tests for normally distributed variables, and Wilcoxon effect sizes (*r*) and Wilcoxon rank-sum tests for non-parametric variables. Categorical variables were compared between groups using Fisher's exact test. Statistical significance was set at $P<0.05$.

Incomplete data differed by 0-19% per variable mainly due to availability of equipment at testing sessions (Supplementary 4). Therefore, data were considered missing at random, and for subsequent analysis was handled using multiple imputation ($m = 20$) with predictive mean matching (Multivariate Imputation by Chained Equations, *mice* package).[43] Injury risk estimates were analyzed using logistic regression and presented as odds ratios (Table 3). To aid the comparison between variables with different units, standardized odds ratios that represent the change in odds of sustaining an ACL injury per 1 standard deviation change in each variable were used, and odds ratios <1 were inverted so that higher odds ratios were always associated with greater ACL injury risk. Wald 95% confidence intervals (95%CI) for odds ratios were calculated, with intervals that did not contain one considered statistically significant. The influence of confounding factors on ACL injury risk were explored by including potential covariates in logistic regression models. Covariates included prior ACL injury and age. Variables with significant odds ratios were further explored by plotting the predicted probability of sustaining a future ACL injury as a function of strength/biomechanics (Figure 2). Predicted probabilities were determined using unadjusted logistic regression models (Table 3), as models adjusted for covariates showed very small differences in odds ratios. Without considering strength or biomechanics, the risk of sustaining an ACL injury in this cohort was calculated as 15/277 = 5.4%. Based on this, values of strength and biomechanics with a predicted injury probability of less than 5.4% were considered to reduce injury risk, and values above 5.4% were considered to increase the risk of injury.

To evaluate the joint ability of tests to assess ACL injury risk, a multivariable prediction model was developed in accordance with guidelines for prognostic studies.[4] Missing data were handled using multiple imputation (m = 20). The number of predictor variables was determined using the *pmsampsize* package.[37] Assuming a Cox-Snell R^2 of 0.09, with an ACL injury proportion of 5%, and target shrinkage of less than 10%, a maximum of 3 predictor variables were deemed appropriate.[38] All variables analyzed in the univariable analysis were considered, and the model with the highest accuracy is presented in the results, with remaining high performing models presented in Supplementary 5. Models were created using multiple logistic regression with penalized coefficients (ridge regression) to reduce overfitting. Lambda (λ) was determined by selecting the smallest value that minimized model deviance using 10-fold cross-validation (*glmnet* package). The final model was formed by averaging coefficients across imputed datasets and evaluated on the original training data set to obtain apparent predictive performance (sensitivity, specificity, and area under the curve [AUC]).[26] Internal validation was performed by determining optimism adjusted predictive performance using bootstrapping resampling with replacement (B = 200),[12] which incorporated multiple imputation within each bootstrap sample.[26] Calibration plots were used to assess the agreement between predicted and observed probability of sustaining a subsequent ACL injury, with risk estimates determined using a rolling mean (window = 50 observations) due to a low number of injured to uninjured players.

Results

Participant and injury characteristics

Of the 322 players who underwent data collection, 277 (86%) completed the 18-month injury follow-up (Table 1). The main reason for players not completing the injury follow-up was not replying to SMS/email (n=28) (see Supplementary 4 for flow diagram). Of the players who did not complete the 18-month injury follow-up (n=45), 51% were junior soccer players and 40% were senior players. The players who did not complete the 18-month follow-up were significantly younger, had less body mass, and had lower eccentric knee flexor strength during the NHE (*P*<.05, d=0.36 to 0.44) than those who did.

In total, 15 of the 277 prospectively followed players sustained an ACL injury within the 18-month follow-up period (Table 1). The median time from testing to injury was 100 days (range=58 to 453 days, interquartile range=184 days). Ten of the 15 injuries (67%) were index injuries. Of the five players who sustained a second ACL injury, one (7%) was a reinjury to the surgically reconstructed leg, and four (27%) were sustained on the contralateral side. For 12 injuries (80%), the primary injury mechanism was change of direction and 10 (67%) occurred during match play (Table 1).

Table 1. Number of players and anterior cruciate ligament (ACL) injury characteristics by sport cohort. Data are total number (percentage relative to column total).

	Australian Rules Football (AFLW)	**Senior soccer (W-League)**	**Junior soccer (under-17 state)**	**Total**
Total players	**153**	**62**	**107**	**322**
Completed follow-up	149 (97%)	44 (69%)	84 (79%)	277 (86%)
ACL injury group	**12**	**1**	**2**	**15**
Dominant leg	2 (17%)	1 (100%)	2 (100%)	5 (33%)
Non-dominant leg	10 (83%)	0 (0%)	0 (0%)	10 (67%)
Index injury	8 (67%)	1 (100%)	1 (50%)	10 (67%)
Reinjury (same side)	1 (8%)	0 (0%)	0 (0%)	1 (7%)
Contralateral injury	3 (25%)	0 (0%)	1 (50%)	4 (27%)
Change of direction	9 (75%)	1 (100%)	2 (100%)	12 (80%)
Tackling	2 (17%)	0 (0%)	0 (0%)	2 (13%)
Landing from a jump	1 (8%)	0 (0%)	0 (0%)	1 (7%)
Match	8 (67%)	1 (100%)	1 (50%)	10 (67%)
Training	4 (33%)	0 (0%)	1 (50%)	5 (33%)

AFLW = Australian Football League Women's; W-League = National Australian women's soccer league. Note: Six junior soccer players were also playing for a W-league team and are counted only in the junior soccer group.

Group demographic, strength, and biomechanical differences

Players who went on to sustain an ACL injury were more likely to have a prior ACL injury (33% of injured group vs 5% of uninjured group, *P* = .001, odds ratio [OR] = 9.68, 95% CI =

2.67-31.46), had lower KOOS pain scores (47% of injured players <100 vs 32% of uninjured players <100, *P* = .046), had a lower isometric hip adductor to abductor strength ratio (*d* = 0.56, *P* = .022), greater CMJ peak take-off force (*d* = 0.70, *P* = .013), greater dynamic knee valgus (*d* = 0.63, *P* = .030), and greater ipsilateral trunk flexion (*d* = 0.38, *P* = .046) average angles during triple vertical hop landing phase (Table 2).

Table 2. Group comparison of players who sustained an anterior cruciate ligament (ACL) injury (ACL injured leg) and players who did not (average of legs) during the 18-month follow up period.

	ACL injured players ($n \leq 15$)	Uninjured players ($n \leq 265$)	Effect size	*P* value
Demographic				
Age (years) $^{\alpha}$	20.1 (3.8)	20.4 (9.7)	0.02	.682
Height (m)	1.77 (0.06)	1.78 (0.07)	0.09	.781
Mass (kg)	67.8 (10.9)	65.6 (8.7)	0.20	.351
Playing experience (years)	8.1 (2.7)	8.4 (5.1)	0.11	.819
Contraceptive use (n [%])	2 (13%)	55 (21%)		.743
Injury history $^{\gamma}$				
Prior ACL injury (n [%])	5 (33%)	13 (5%)		**.001***
Prior knee (any) injury (n [%])	2 (13%)	16 (6%)		.253
Prior hamstring injury (n [%])	2 (13%)	20 (8%)		.338
Prior hip/groin injury (n [%])	1 (7%)	15 (6%)		.600
KOOS				
Pain $^{\alpha}$	100 (9.7)	100 (0)	0.12	**.046***
Sport/Rec $^{\alpha}$	100 (5)	100 (0)	0.08	.201
Strength				
Isometric hip adductor force (N)	135 (26)	145 (27)	0.41	.149
Isometric hip abductor force (N)	147 (26)	145 (24)	0.11	.658
Isometric hip ADD:ABD force ratio	0.93 (0.17)	1.01 (0.14)	0.51	**.022***
Eccentric knee flexor force (N)	293 (35)	277 (55)	0.46	.302
Eccentric knee flexor force allometrically scaled ($N.kg^{-k}$)	16.4 (2.1)	15.8 (2.8)	0.28	.458
CMJ kinetics				
Peak take-off force (BW)	1.23 (0.11)	1.15 (0.13)	0.75	**.013***
Take-off positive impulse (BW.s)	0.37 (0.04)	0.39 (0.05)	0.44	.174

Take-off eccentric RFD (BW.s^{-1}) $^{\alpha}$	4.2 (2.5)	3.3 (2.5)	0.11	.083
Peak landing force (BW) $^{\alpha}$	2.22 (0.79)	2.31 (0.64)	0.01	.876
Landing RFD (BW.s^{-1}) $^{\alpha}$	38.9 (25.7)	39.9 (26.5)	0.02	.759
Hop kinematics (average angle)				
Knee flexion angle (°)	40.7 (9)	43.4 (9.9)	0.30	.310
Dynamic knee valgus (+°)/varus (-°)	1.5 (5.8)	-2.6 (7.2)	0.71	**.030***
Trunk flexion angle (°)	18.1 (5.3)	17.5 (5.8)	0.11	.702
Ipsilateral trunk flexion (°)	9.1 (4.2)	7.8 (2.3)	0.31	**.046***

* indicates statistically significant, $P < .05$. Note: missing data differs by variable see Supplementary 4. Unless indicated, data presented as mean (standard deviation), Glass's Delta effect size, and independent two-tail t-test *P* value.

$^{\alpha}$ non-parametric variable, presented as median (interquartile range), Wilcoxon effect size and Wilcoxon rank-sum test *P* value. $^{\beta}$ categorical variable, Fisher's exact test. $^{\gamma}$ Prior knee, hamstring, and hip/groin injury in previous 12 months.

BW = body weights; KOOS = Knee Injury and Osteoarthritis Outcome Score; RFD = rate of force development.

Strength and biomechanical ACL injury risk factors

For the unadjusted logistic regression analysis (Table 3), a lower isometric hip adductor to abductor strength ratio (1.98 increase in odds per 0.14 decrease in ratio, 95% CI = 1.09-3.61), greater CMJ peak take-off force (1.74 increase in odds per 0.13 BW increase, 95% CI = 1.09-2.77), greater dynamic knee valgus (1.97 increase in odds per 7.2 ° increase, 95% CI = 1.06-3.63), and ipsilateral trunk flexion average angles during single-leg triple vertical hop landing (1.60 increase in odds per 2.4 ° increase, 95% CI = 1.01-2.55) were independently associated with increased risk of subsequent ACL injury. Ipsilateral trunk flexion was not statistically significant when adjusting for prior ACL injury (95% CI = 0.85-2.26).

Table 3. Anterior cruciate ligament (ACL) injury risk estimates expressed as standardized odds ratios (increase in odds per 1 standard deviation change in each variable) with and without the inclusion of potential covariates (prior ACL injury and age).

		Odds ratio (95% CI)		
	1 SD change	Unadjusted	Adjusted for prior ACL	Adjusted for age
Strength				
Isometric hip adductor force	-27 N	1.51 (0.85-2.68)	1.61 (0.90-2.90)	1.46 (0.82-2.6)
Isometric hip abductor force	25 N	1.13 (0.67-1.88)	1.03 (0.61-1.75)	1.18 (0.69-2.02)
Isometric hip ADD:ABD force ratio	-0.14	**1.97 (1.08-3.58)***	**1.85 (1.00-3.44)***	**1.96 (1.08-3.59)***
Eccentric knee flexor force	54 N	1.33 (0.76-2.33)	1.15 (0.64-2.06)	1.44 (0.81-2.54)
Eccentric knee flexor force allometrically scaled	2.8 N.kg-k	1.25 (0.69-2.25)	1.11 (0.62-2.01)	1.27 (0.71-2.3)
CMJ kinetics				
Peak take-off force	0.13 BW	**1.77 (1.11-2.82)***	**1.67 (1.02-2.72)***	**1.73 (1.08-2.78)***
Take-off positive impulse	-0.05 BW.s	1.48 (0.84-2.60)	1.39 (0.78-2.47)	1.42 (0.80-2.53)
Take-off eccentric RFD	1.9 BW.s-1	1.43 (0.91-2.26)	1.36 (0.84-2.18)	1.39 (0.87-2.22)
Peak landing force	-0.57 BW	1.00 (0.59-1.70)	1.00 (0.58-1.72)	1.02 (0.60-1.72)
Landing RFD	-36.8 BW.s-1	1.05 (0.58-1.90)	1.12 (0.56-2.21)	1.03 (0.58-1.84)
Hop kinematics (average angle)				
Knee flexion angle	-9.9 °	1.35 (0.77-2.38)	1.38 (0.78-2.46)	1.30 (0.73-2.3)
Dynamic knee valgus	7.2 °	**1.96 (1.06-3.64)***	**2.17 (1.12-4.23)***	**1.88 (1.01-3.5)***
Trunk flexion angle	5.8 °	1.10 (0.65-1.86)	1.01 (0.57-1.78)	1.16 (0.69-1.98)
Ipsilateral trunk flexion	2.4 °	**1.60 (1.01-2.55)***	1.39 (0.85-2.26)	**1.62 (1.02-2.59)***

Note: missing data imputed using multiple imputation. * indicates statistically significant 95% confidence interval (95% CI). Odds ratios <1 have been inverted and are indicated by a negative SD change. BW = body weights; RFD = rate of force development; SD = standard deviation.

Without considering the effect of strength or biomechanics, an individual's probability of sustaining an ACL injury ('base risk') was determined to be 5.4% (15 ACL injuries in 277 players). Based on univariable logistic regression model predicted probabilities (Figure 2), an isometric hip adductor to abductor strength ratio less than 0.97, CMJ peak take-off force greater than 1.19 BW, dynamic knee valgus greater than 0 ° (i.e. valgus not varus), or ipsilateral trunk flexion greater than 8 ° increased ACL injury risk beyond base risk (>5.4%). Conversely, increasing/decreasing values in the opposite direction of these cut-offs were indicative of a reduction in ACL injury risk relative to the expected base risk (<5.4%).

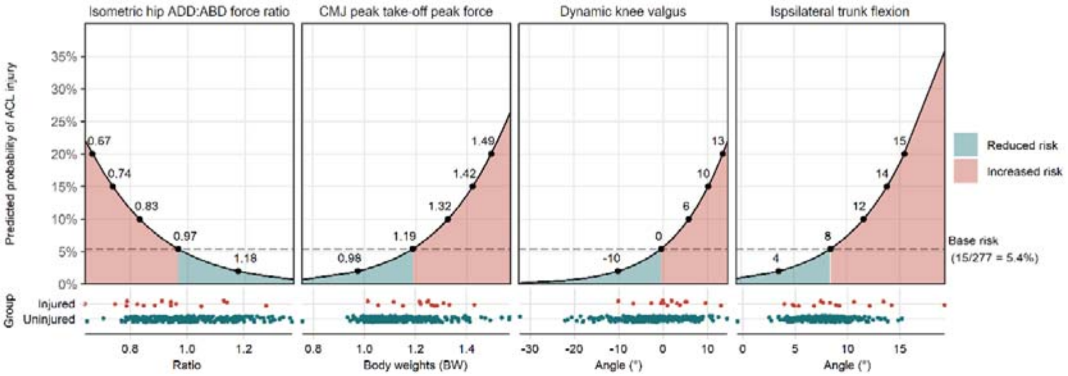


Figure 2 Predicted proabability of sustaining an ACL injury over a range of strength and biomechanics values. Predicted probabilities derived from univariable (unadjusted) logistic regression models. Horizontal dashed line indicates base risk (5.4%), and the intersection with predicted probability indicates the value at which ACL injury risk increases/decreases relative to base risk. Values coresponding with 2%, 5.4%, 10%, 15%, and 20% predicted probabilities of ACL injury are highlighted on the curve with black dots. Bottom panel displays the disitribution of individual ACL injured and uninjured data.

Between-leg asymmetry risk factors

Directional between-leg asymmetry (i.e. injured leg is lower or greater) in isometric hip adductor/abductor strength, eccentric knee flexor strength, or CMJ kinetics were not associated with ACL injury risk (OR ranging from 1.00 to 1.03 per 1% change in asymmetry)

(Figure 3). However, non-directional between-leg asymmetry (i.e. either leg lower or greater) resulted in a significant odds ratio for eccentric knee flexor strength asymmetry (OR = 1.10 increase in odds per 1% increase, 95% CI = 1.03-1.17).

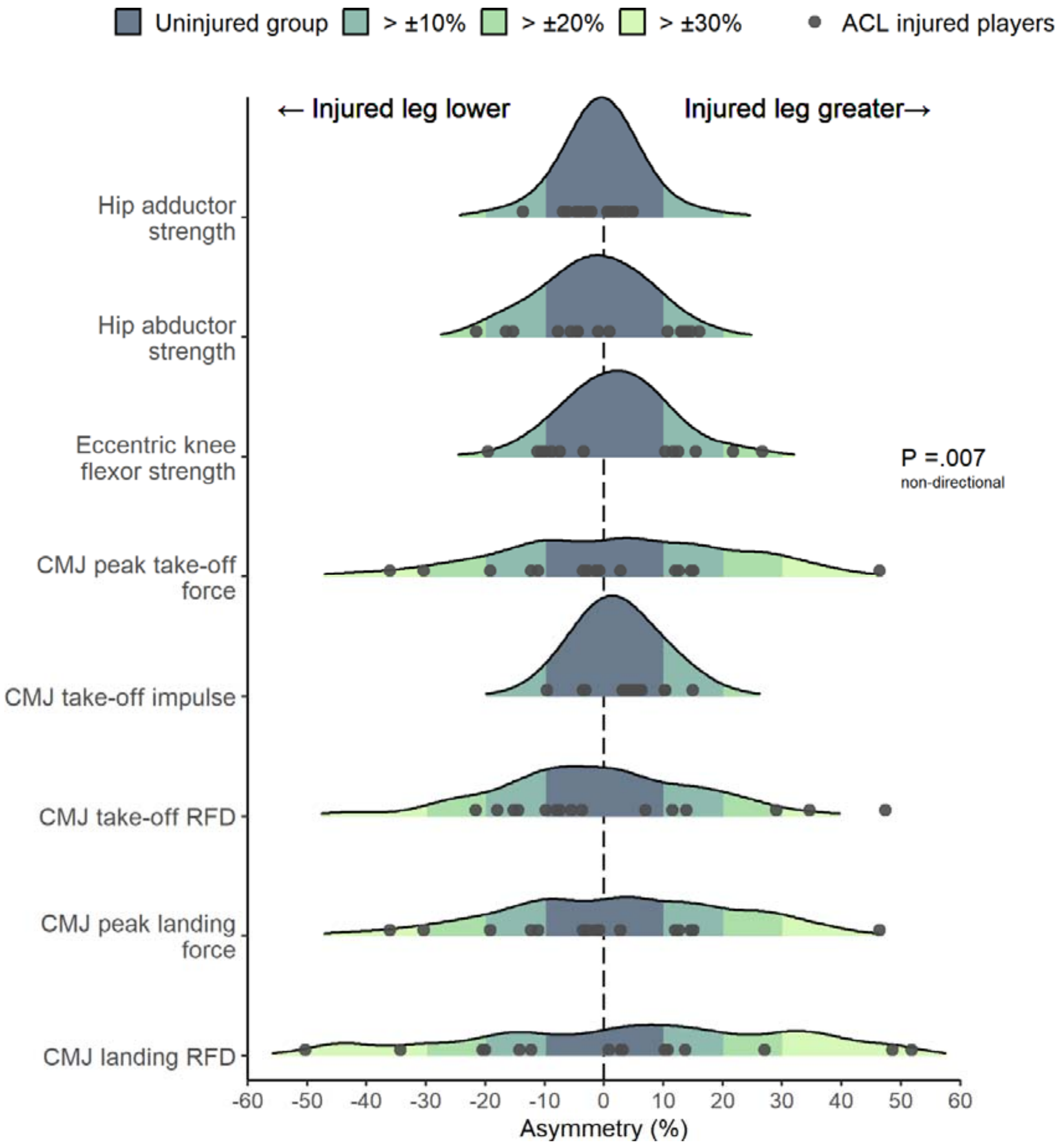
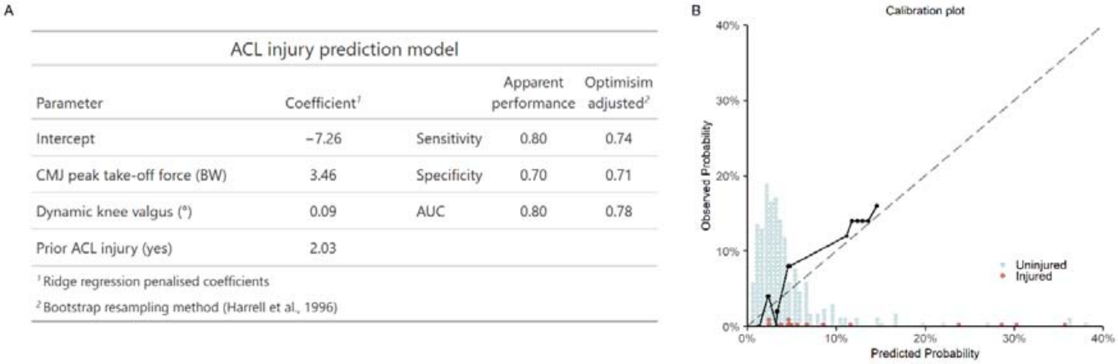


Figure 3 Between-leg asymmetry in hip strength, knee flexor strength, and countermovement jump (CMJ) kinetics for players who sustained an anterior cruciate ligament injury (ACL injured players) and players who did not (uninjured group). Uninjured players (n = 265) are represented by density curve, with shaded areas indicating increasing amounts of asymmetry.

Players who sustained an ACL injury are presented with black dots. Significant variables indicated with *P* value. RFD = rate of force development.

Multivariable ACL injury prediction model

The multivariable ACL injury prediction model with the highest prediction accuracy included CMJ peak take-off force, single-leg triple vertical hop dynamic knee valgus, and prior ACL injury (Figure 4A). This model predicted future ACL injury in the training dataset (apparent performance) with sensitivity of 0.80, specificity of 0.70, and had an AUC of 0.80. When adjusting for optimism, it was estimated that this model would identify ACL injury in unseen data with sensitivity of 0.74, specificity of 0.71, and an AUC of 0.78. Model calibration (Figure 4B) generally showed a small amount of underestimation of ACL injury probability. There were three other multivariable model combinations that performed similarly to the best model (Optimism adjusted AUCs = 0.75), with the additional use of ipsilateral trunk flexion and the isometric hip adduction to abduction ratio (Supplementary 5).



ACL injury prediction model

Parameter	Coefficient[1]		Apparent performance	Optimisim adjusted[2]
Intercept	−7.26	Sensitivity	0.80	0.74
CMJ peak take-off force (BW)	3.46	Specificity	0.70	0.71
Dynamic knee valgus (°)	0.09	AUC	0.80	0.78
Prior ACL injury (yes)	2.03			

[1] Ridge regression penalised coefficients

[2] Bootstrap resampling method (Harrell et al., 1996)

Figure 4 Best performing anterior cruciate ligament (ACL) injury prediction model. A) Multiple logistic regression model with ridge regression penalized coefficients, and model predictive performance using training dataset (apparent) and bootstrapped samples (optimism adjusted). B) Calibration plot comparing model predicted probability of sustaining an ACL injury and observed ACL injury probability (black line) for a rolling window of 50 observations. The grey dashed line indicates perfect agreement between model predictions and observed data. Calibration line is superimposed over the distribution of model predicted probabilities for players who subsequently sustained an ACL injury (red dots) and those who did not (blue dots).

Discussion

This prospective study was the first to investigate if a pre-season, field-based test battery consisting of lower limb muscle strength and jump-landing biomechanics was associated with future non-contact ACL injury in elite female footballers in Australia. We found that 1) prior ACL injury, 2) lower hip adductor to abductor strength ratios, 3) greater dynamic knee valgus and ipsilateral trunk flexion during a single-leg triple vertical hop, and 4) greater CMJ peak-take off force all independently increased the risk for subsequent non-contact ACL injury. Using these variables together, a multivariable model was able to predict ACL injury with 78% accuracy. These results may be used to improve injury screening and inform the design of targeted injury prevention training in elite female footballers.

Hip muscle strength

Lower isometric hip adductor to abductor strength ratios were associated with greater risk of ACL injury. Individually, absolute isometric hip adductor or abductor strength were not significantly associated with ACL injury risk. However, by observing the underlying data, lower hip adductor to abductor strength ratios were predominantly driven by lower hip adductor strength. Strength of the adductors relative to the abductors may influence lower limb coordination arising from the hip during high-risk maneuvers (e.g., single-leg landings and decelerations).[39] The hip adductor muscles also provide small contributions to knee varus moments early in single-leg landing that may support the ACL against knee valgus moments.[21] Further, adductor magnus is a strong hip extensor, particularly when the hip is flexed,[2] and weakness of this muscle may limit the ability to absorb energy at the hip, thereby increasing loads applied to the knee.[23] Nevertheless, the mechanism linking hip adductor function and ACL injury risk is not well understood and warrants exploration in future studies.

Single-leg triple vertical hop

Players who sustained a future ACL injury performed single-leg triple vertical hops with greater dynamic knee valgus and ipsilateral trunk flexion (i.e. towards stance leg) during landing than uninjured players. Dynamic knee valgus (hip internal rotation and adduction, knee abduction, and ankle eversion) is observed more frequently in females than males in a range of landing tasks,[5] but evidence for an association with ACL injury risk is unclear.[14, 28] Conflicting evidence may relate to biomechanical differences in bilateral versus single-leg landing tasks. Studies using single-leg drop vertical jumps in other female populations have found dynamic knee valgus to be associated with future ACL injury risk,[9, 29] however this appears to not be the case in studies employing double-leg drop vertical jumps.[19, 28] Previous work has also reported that the combination of knee valgus and trunk lateral flexion during single-leg drop vertical jumps was associated with non-contact knee injuries in female athletes.[9] The ability to control lateral trunk displacement during a perturbation task has also been highlighted as a risk factor for ACL injury.[45] Lateral trunk flexion towards the stance leg shifts the moment arm of the trunk lateral to the knee joint center, and therefore increases the knee abduction moment, which may contribute to ACL injury.[15] Training female footballers to perform high-risk single-leg movements with less dynamic knee valgus and ipsilateral trunk flexion[8] may represent an effective strategy to mitigate the risk of future ACL injury.

Countermovement jump kinetics

Those who produced greater CMJ peak take-off force were at greater risk of sustaining a future ACL injury. The ability to generate high force during a CMJ may be a general indicator of (i.e. correlated with) a player's ability to generate knee loads in other dynamic tasks. For example, ACL loading during a land-and-cut task is predominantly generated by quadriceps and gastrocnemius muscle forces,[27] which are prime contributors to jumping vertical ground reaction forces.[33] Reducing peak CMJ force to mitigate ACL injury risk is likely counterproductive for players who aim to maximize power during jumping. Instead, players with a high-risk CMJ kinetic profile may benefit from interventions targeted at altering other modifiable factors, such as improving kinematics and decreasing joint moments associated with injury or strengthening muscles capable of unloading the ACL. Further, between-leg asymmetries in CMJ kinetics were not associated with ACL injury risk. The amount of asymmetry varied widely between variables (7 to 28% per standard deviation), indicating no single asymmetry threshold provides a clear indication of injury risk.[35]

Prior ACL injury

In the present study, players with a history of ACL reconstruction were 9.7-times more likely to sustain a future ACL injury than those without. Prior ACL injury has consistently been identified as a risk factor for future ACL injury in female athletes from a wide-range of field and court sports.[6] Although the specific mechanism(s) by which prior injury predisposes to reinjury is not fully understood, risk factors for ACL reinjury include greater dynamic knee valgus during a drop vertical jump, postural instability,[34] as well as quadriceps weakness and sagittal plane single-leg landing biomechanics.[17] Females with a history of ACL injury also demonstrate persistent deficits in lower limb strength and biomechanics that may contribute to the subsequent injury.[25] Interestingly, KOOS was not associated with ACL injury risk in the current study, with both ACL injured and uninjured players reporting much higher (and often perfect) pain and sport/rec scores compared to previous studies.[1] Higher KOOS scores are likely reflective of our elite level cohort and those returning from ACL injury having better knee related symptoms and function than the general population.

Between-leg asymmetry

Directional between-leg asymmetry in strength and CMJ kinetics was not associated with an increase in ACL injury risk. Within the players who sustained a future ACL injury, no consistent asymmetry was observed (e.g. lower values in the injured compared to uninjured leg) (Figure 3) and asymmetries were within similar ranges of uninjured players (~10-20% for strength and ~10-50% for CMJ kinetic variables). However, non-directional asymmetry for eccentric knee flexor strength was significantly associated with increased ACL injury risk. This finding can be explained by a previous study in the same cohort of players[7] which found that those with a history of ACL injury demonstrate long lasting eccentric knee flexor strength asymmetries. In the current study, the same players were found to be at high risk of sustaining a second ACL injury. Therefore, eccentric knee flexor strength asymmetry is associated with prior ACL injury and may not be an independent risk factor, particularly given that the stronger leg was injured as frequently as the weaker leg. These findings question the utility of between-leg symmetry for evaluating ACL injury risk and as a return to play criterion. However, the results should be interpreted with caution given the mix of first time (two-thirds) and second time (one-third) ACL injuries included in the analysis.

Multivariable prediction model

Together, CMJ peak take-off force, single-leg triple vertical hop dynamic knee valgus, and ACL injury history predicted subsequent ACL injury risk with acceptable accuracy (AUC = 0.78), successfully classifying 74% of all ACL injuries (sensitivity = 0.74) and 71% of all uninjured players (specificity = 0.71).The predictive ability of the current model performed better than a previous model built using medial knee displacement during a bilateral drop vertical jump (AUC = 0.60),[18] and similarly to one built using dynamic knee valgus and lateral trunk flexion during single-leg drop vertical jumps (AUC = 0.80).[9] Prediction performance is likely to vary widely between studies depending on cohort characteristics, sample size, study design, and model development. Importantly, the current study is one of the first in the ACL injury literature to apply model regularization techniques to reduce overfitting and hence increase model generalizability to future data. The overall ACL injury prediction performance of 78% shows promise for estimating player injury risk. Such predication models may be useful for deciding which players require additional targeted injury risk reduction training.

Limitations

Associations with injury risk were drawn from measures of strength and biomechanics taken at a single time-point (i.e. pre-season), while ACL injuries occurred months after the time of testing. More frequent in-season assessments of strength and biomechanics may provide greater insight into ACL injury risk. Although our injury rate (5.4%) and total number of injuries (15) was consistent with previous ACL injury risk factor studies,[6] this may have limited our ability to detect small to moderate effect sizes. Injury follow-ups were predominantly performed using team medical staff, however, where this was not possible, players were contacted directly via mobile or email and asked to self-report knee injuries. Although self-report may result in inaccurate recall for some injuries, it is likely most elite players were accurately diagnosed via medical imaging and aware they had an ACL rupture. Given the exploratory nature of this study, no corrections for multiple comparisons were made, and further sufficiently powered studies with strict Type 1 error rate control are required to confirm the findings.

Conclusion

Pre-season measures of hip strength, single-leg triple vertical hop frontal plane knee and trunk kinematics, and CMJ kinetics were independently associated with future non-contact ACL injury, supporting the use of a field-based test battery at the outset of pre-season. A prediction model built using a combination of these measures provided acceptable levels of accuracy at identifying players who went on to sustain ACL injury. These results may be used to guide ACL injury screening practices and inform the design of targeted injury prevention training in elite female footballers.

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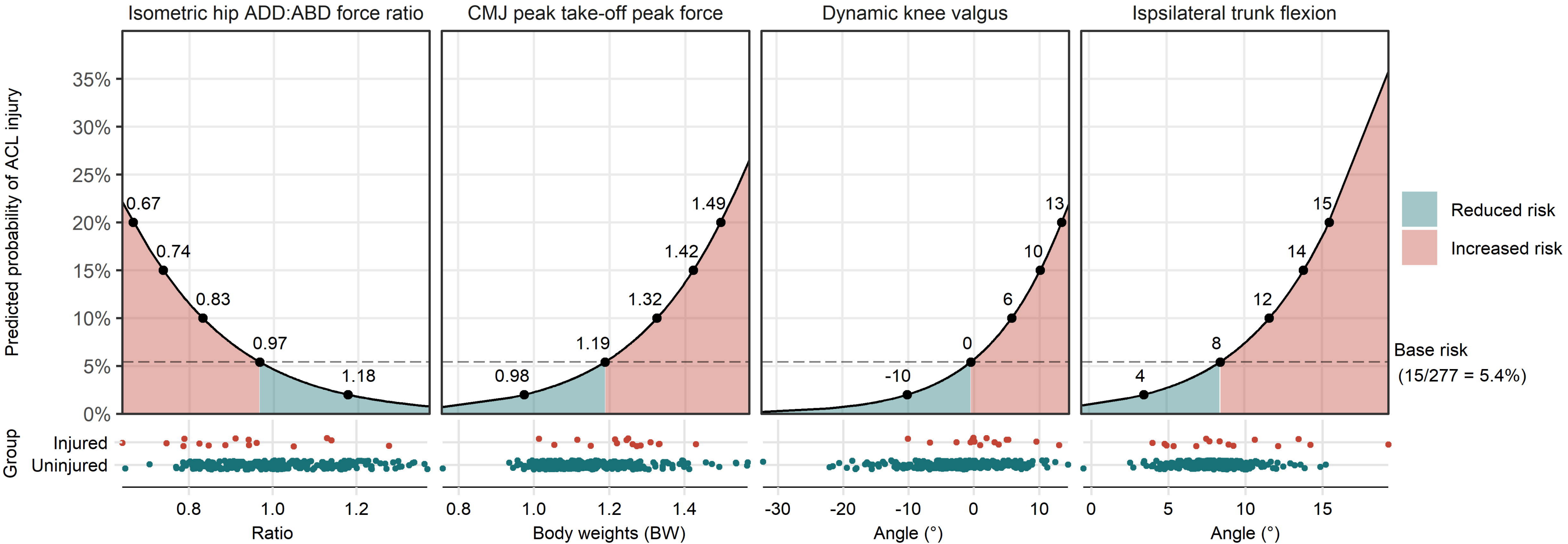
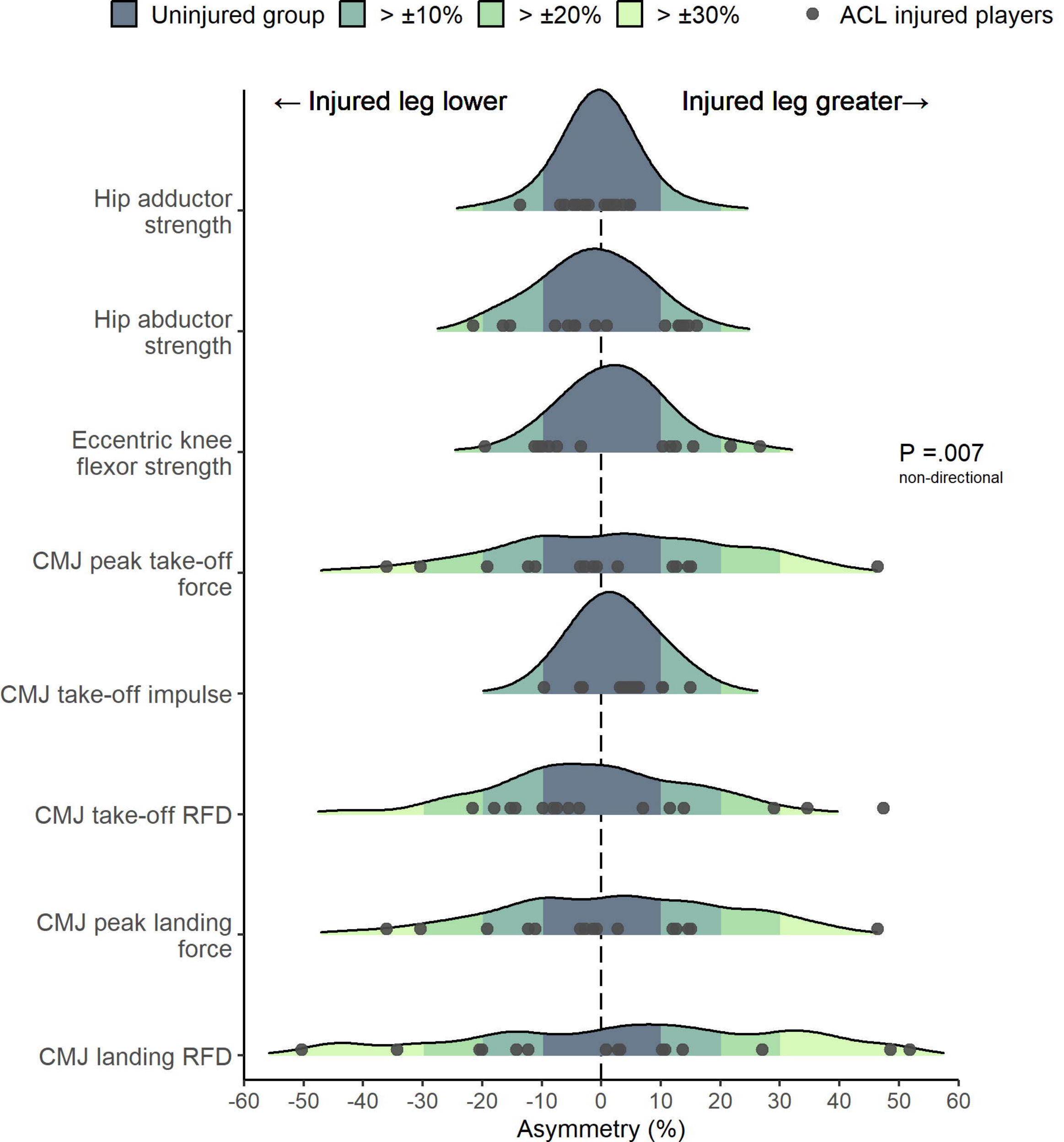


Isometric hip ADD:ABD force ratio
CMJ peak take-off peak force
Dynamic knee valgus
Ispsilateral trunk flexion
Predicted probability of ACL injury
35%
30%
25%
20%
15%
10%
5%
0%
0.67
0.74
0.83
0.97
1.18
0.98
1.19
1.32
1.42
1.49
-10
0
6
10
13
4
8
12
14
15
Reduced risk
Increased risk
Base risk
(15/277 = 5.4%)
Group
Injured
Uninjured
0.8
1.0
1.2
Ratio
0.8
1.0
1.2
1.4
Body weights (BW)
-30
-20
-10
0
10
Angle (°)
0
5
10
15
Angle (°)


Uninjured group
> ±10%
> ±20%
> ±30%
ACL injured players
← Injured leg lower
Injured leg greater→
Hip adductor strength
Hip abductor strength
Eccentric knee flexor strength
P =.007
non-directional
CMJ peak take-off force
CMJ take-off impulse
CMJ take-off RFD
CMJ peak landing force
CMJ landing RFD
-60
-50
-40
-30
-20
-10
0
10
20
30
40
50
60
Asymmetry (%)

A

ACL injury prediction model

Parameter	Coefficient[1]		Apparent performance	Optimisim adjusted[2]
Intercept	−7.26	Sensitivity	0.80	0.74
CMJ peak take-off force (BW)	3.46	Specificity	0.70	0.71
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