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Differences in Kinematic and Muscle Activity between ACL Injury Risk and Healthy Players in Female Football: Influence of Change of Direction Amplitude. Cross-Sectional Case-Control Study.

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Abstract: Background and Objectives: Anterior cruciate ligament (ACL) injury rates remain 17 high and have a significant impact on female football players. This study aims to evaluate 18 knee kinematics and lower limb muscle activity in players at risk of ACL injury compared 19 to healthy players during three side-cutting tests. It also investigates how the amplitude 20 of change of direction influences stabilization parameters. Materials and Methods: A cross-21 sectional case-control study was conducted with 16 participants (23.93 ± 5.16 years), di-22 vided into Injured (n=8) and Healthy groups (n=8). Injured players had a history of non-23 contact knee injury involving valgus collapse, without undergoing surgical intervention. 24 Three change of direction tests: Change of Direction and Acceleration Test (CODAT), Go 25 Back Test (GOB), and Turn test (TURN) were used for evaluation. The peak and range of 26 knee joint angles and angular velocities across three planes, along with the average recti-27 fied and peak envelope EMG signals of the Biceps Femoris (BF), Semitendinosus (ST), 28 Vastus Medialis (VM), and Gastrocnemius Lateralis (GL), were recorded during the prep-29 aration and load phases. Group differences were analyzed using two-factor mixed-model 30 ANOVA with pairwise comparisons. Statistical significance was set at p < 0.05. Results: 31 Injured players demonstrated lower external tibial rotation angular velocity and a greater 32 range of motion in tibial external rotation compared to Healthy players. Additionally, the 33 Injured showed significantly higher average rectified muscle activity in VM and GL both 34 increased by 4% during the load phase. The CODAT and TURN tests elicited higher Bf 35 and VM muscle activity, compared to the GOB test. The TURN test also showed greater 36 extension angular velocity in the sagittal plane. Conclusions: The results revealed differ-37 ences in knee kinematics and muscle activity between players at risk of ACL injury and 38 healthy players, influenced by the amplitude of directional changes. Players altered trans-39 verse plane mechanics and increased VM and LG activation during LOAD may reflect a 40 dysfunctional motor pattern, while the greater sagittal plane angular velocity and VM and 41 BF activation from CODAT and TURN highlight their higher potential to replicate ACL 42 injury mechanisms compared to GOB. 43

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Clinical Implications: This study highlights the importance of assessing kinematics in 44 conjunction with specific muscle activity during functional tests that replicate ACL injury 45 mechanisms, in order to better determine player risk profiles and design more effective prevention programs.

Keywords: Anterior cruciate ligament; biomechanics; electromyography; female football; 48 injury prevention 49

1. Introduction

Anterior cruciate ligament (ACL) injury rates disproportionately affect females, who 52 are 2-6 times more likely to suffer from an ACL injury compared to males with 0.7 injuries 53 per squad per season and 0.1 injuries per 1,000 hours of play [1–3]. ACL injuries are sig-54 nificantly burdensome for the player with, on average, 38 days lost per 1,000 hours of 55 exposure and 117 days of recovery [1]. Furthermore, 25-35% of players face re-injury 56 within 2-5 years with females having a higher re-injury risk compared to males [4], with 57 only 81% returning to their prior competition level post-rehabilitation [5,6]. 58

Due to the severity and long-term impact of ACL injuries, current research focuses 59 on identifying ACL risk factors to develop prevention strategies aimed at lowering injury 60 thresholds [7]. Since non-contact mechanisms persist as the primary cause of ACL injuries, 61 kinematic and kinetic analyses have traditionally focused on identifying biomechanical 62 risk factors. Recent research has recognized the importance of addressing valgus collapse, 63 characterized by hip adduction and internal rotation, and knee abduction, incorporating 64 the function analysis of the transverse plane in prevention programs [8–10]. While most 65 studies link increased internal tibial rotation to a higher risk of ACL injury, recent research 66 suggests that excessive transverse plane movement may be the primary risk factor[11–17]. 67 However, to date, there is no consensus whether excessive internal or external tibial rota-68 tion is a risk factor [16]. Consequently, recent studies advocate for incorporating new out-69 comes, such as angular velocity, into kinematic analyses, as it reflects the speed of joint 70 movement, closely linked to motor control [11,12,16]. Therefore, it serves to characterize 71 the direction and quality of control, based on movement velocity during the stabilization 72 task. 73

Sensorimotor control, driven by muscle activity, directly influences the kinematic 74 and kinetic factors associated with ACL injury mechanics [18]. Recent research has 75 demonstrated altered muscle activity is related to ACL injured risk [10,13,19,20]. Most 76 prevalent injury mechanism occurs during defensive actions, particularly during front-77 facing pressing situations, where the player must rapidly change direction to follow of-78 fensive opponent [21,22]. 21Therefore, recent studies suggest that functional tasks, such 79 as change of direction tests, can be an effective strategy for assessing risk by replicating 80 the mechanisms of ACL injuries [23,24]. However, it remains unclear which specific am-81 plitude of directional change presents the greatest challenge to knee stabilization, thereby 82 placing the maximum stress on its functional mechanisms [22]. 83

Notably, most of ACL stabilization loading occurs in the sagittal plane, primarily in-84 volving the hamstrings, quadriceps, and gastrocnemius muscles [13,18,25]. Hamstrings 85 play a crucial role as ACL synergist by counteracting anterior tibial translation during 86 change of direction stabilization maneuvers [10,13,19]. Previous research highlights that 87 altered hamstring activity, prior to initial ground contact, is associated with an increased 88 risk of ACL injury [10,13,19,20,26]. Semitendinosus (ST) is particularly significant due to 89 its role as a 'knee adductor', contributing to medial joint compartment compression and 90 preventing valgus collapse [10,19,26]. Valgus collapse is often associated with increased 91 quadricep activity, which has been suggested to increase anterior shear forces and places92excessive strain on the ACL, particularly during the load phase when players re-establish93their stability [13,19,26]. Additionally, recent studies have analyzed the role of the gas-94trocnemius in the stabilization process, identifying its function as ACL antagonist [18,25].95It may perform a posterior displacement of the femur that may contribute to anterior tibial96translation, thereby enhancing the action triggered by quadricep contraction during the97load phase [18,25].98

Given the combined impact of the ACL incidence and its burdensome, along with 99 the persistent sex-related prevalence disparities, improving evaluation strategies in fe-100 male players remains essential [1,3]. Although previous studies have combined kinematic 101 and motor control assessments, there is still a need to enhance sensitivity of movement 102 quality evaluations and the specificity of muscle activity analysis during tasks that repli-103 cate ACL injury mechanisms. In this context, incorporating new kinematic variables, for 104 example, angular velocity, which is more closely linked to motor control, alongside tradi-105 tional joint angle measurements could enhance assessment specificity [11,12,16]. Simi-106 larly, by focusing on key muscles such as hamstrings and quadriceps and considering 107 secondary muscles including gastrocnemius to analyze functionality, may help distin-108 guish motor patterns between players at risk of ACL injury and healthy players [13,19,25]. 109 Therefore, the first objective of this study was to evaluate angular velocity and joint angle 110 kinematics of the knee, as well as muscle activity in hamstrings, quadriceps and gas-111 trocnemius, in both players at risk of ACL injury and healthy players during three change 112 of direction tests. The second objective was to evaluate how the amplitude of the change 113 of direction angle involved in each test influences the knee stabilization pattern of the 114 player, based on kinematic and muscle activity variables. It was hypothesized that players 115 at risk of ACL injury will show differences in kinematic and muscle activity patterns com-116 pared to healthy players. Additionally, this study suggests that such differences will 117 emerge during change of direction tasks and be influenced by amplitude of directional 118 change. 119

2. Materials and Methods

2.1. Participants

Potential participants were identified from professional female futsal teams (Real Federación de Fútbol de Ceuta). They were eligible if they held an active national futsal license, train for over 8 hours per week, competed in the Second Spanish National Futsal Division, and actively participated in competitions at the time of the study. Participants were excluded from the study if they had any lower limb injury which may affect the outcomes of this study or had received knee surgery and/or any other lower limb surgery. 127

A cross-sectional case-control study was conducted, and the recruitment was during 128 2022/2023 season. Participants were allocated to groups based on their clinical history. 129 Injured players were defined as those who had previously sustained a non-contact knee 130 injury resulting from a valgus collapse mechanism, without having undergone ACL sur-131 gical intervention. All injuries had to be fully recovered through conservative treatment 132 by the time of the study in accordance with criteria used in previous research [4,9,27,28]. 133 The control group consisted of players that were injury free and had not previously sus-134 tained a knee injury. Eight players were allocated in each group. The Research Ethics 135 Committee of the Community of Aragón approved this study (code PI20/127), which ad-136 hered to the ethical principles of the Declaration of Helsinki [29]. All participants provided 137 written informed consent prior to data collection commencement. For those participating 138 players who were minors, it was also signed by their legal guardian. 139

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2.2. Procedure

Participants took part in a single testing session and following a 10 minute warm up 142 consisting of mobility exercises and variable intensity running with change of direction 143 drills, were required to complete one trial under three different change of direction tasks; 144 (1) CODAT [27,30,31], (2) GOB [32], and (3) TURN [24,33] (see Figure 1). These tests re-145 quire performing a change of direction at maximal velocity, but each test involves differ-146 ent amplitudes of directional change. In CODAT and TURN, a 90° change of direction was 147 recorded. However, in CODAT, this occurs after an initial 45° directional change and is 148 followed by two additional consecutive changes of direction, requiring the player to con-149 tinually adapt her trajectory. In contrast, the TURN test involves a single 90° change of 150 direction, after which the player continues running straight to complete the task. In GOB 151 test, a 180° change of direction was recorded, which includes a forward braking phase 152 followed by running back towards where the participant started the task. Injured partici-153 pants were instructed to use their injured limb and the healthy participants used their 154 dominant limb as the stance limb for each change of direction test as the support limb, to 155 replicate the injury mechanism associated with non-contact injuries [21]. Prior to data col-156 lection, participants completed a familiarization period to avoid learning bias [27]. Partic-157 ipants were given a unique anonymised study code to minimise any bias during data 158 analysis. 159



Figure 1. Functional change of direction test: CODAT test, GOB test and TURN test. The initial 161 point of the test is drawn with a ball and the cross identifies the change of direction task that was 162 recorded. (A) Change of Direction and Acceleration Test (CODAT), this test combines sprinting me-163 chanics with the stabilization and acceleration required for change of direction movements. It con-164 sists of four diagonal change of direction tasks: two at 45° and two at 90°, interspersed with 3-meter 165 sprints and culminating in a 10-meter sprint. (B) Go and Back test (GOB), this test involves a 10 m 166 frontal sprint at maximum possible speed, followed by deceleration and a final backward sprint. It 167 simultaneously incorporates a change of direction and a deceleration task, both of which are com-168 mon mechanisms associated with ACL injuries. (C) TURN test, this test is a modified version of 169 the T-test. It involves a frontal sprint followed by a single pre-planned 90° change of direction, per-170 formed once in each direction. 171

2.3. Marker and EMG sensor Placement

A total of twenty-six markers were placed on the anterior and posterior superior iliac 173 spines, bilaterally on the greater trochanters, medial and lateral femoral epicondyles and 174 medial and lateral malleoli. Non-orthogonal tracking clusters comprising of four markers 175

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were positioned on the lateral thighs and lateral shanks. The feet were modelled as single 176 segments with four markers attached to each foot on the calcanei, 1st metatarsal, 5th met-177 atarsal and midfoot. Kinematic data were captured at 250 Hz using a nine-camera 3D mo-178 tion capture system (Qualisys AB, Göteborg, Sweden).

Surface EMG were recorded using four Trigno Avanti (Delsys Inc., USA) wireless 180 sensors sampled at 1000 Hz positioned over the Biceps Femoris (BF), ST, Vastus Medialis 181 (VM) and Lateral Gastrocnemius (LG) following the SENIAM guidelines [19,34]. 182 Throughout data collection, regular checks of the signal-to-noise ratio were conducted to 183 ensure good signal quality. 184

2.4. Data Analysis

Marker trajectories and EMG data were exported to C3D and imported into Visual 187 3D (C-Motion Inc., Germantown, USA) for analysis. Kinematic data were filtered using a 188 4th order zero-lag, 8Hz low-pass Butterworth filter [35]. Knee joint kinematics were cal-189 culated from the shank relative to the thigh using an XYZ cardan sequence [36]. Peak knee 190 kinematics in the sagittal, coronal, and transverse planes were extracted [36]. EMG signals 191 were filtered using a second-order Butterworth high-pass filter with a cut off frequency 192 of 40 Hz to minimize movement artifacts [37] and then full wave rectified and low-pass 193 filtered with a 15Hz cut off frequency [37]. The maximum observed signal from the fil-194 tered data across all trials and muscles was used to normalized the average and peak EMG 195 signals during the preparation (PREP) phase, defined as 100ms prior to ground contact to 196 the frame immediately before initial contact and the loading (LOAD) phase defined as 197 initial contact to maximum knee flexion [13,38]. 198

2.5. Sample Size

The sample size was calculated based on a minimum expected difference of 0.16 (SD 0.11) in BF muscle activity during PREP phase in a change of direction maneuver [38] using the GRANMO 8.0 calculator, considering an alpha risk of 0.05, a beta risk of 0.20, and a two-sided test. A target sample of 16 participants, eight per group, was required.

2.6. Statistical Analysis

Sharpiro-Wilk tests were performed to explore data normality. For normal distrib-207 uted data, two factor Mixed Methods (2x2) ANOVA tests were used to explore kinematic 208 differences between groups (Injured and Healthy) during the three different movement 209 tasks (CODAT, GOB and TURN). For EMG measures, separate Mixed Methods ANOVAs 210 were run for the PREP and LOAD phases. For significant main effects, pairwise compari-211 sons were conducted to explore differences between groups and tasks. For significant in-212 teractions, separate one-way ANOVAs were performed. The significance level was set at 213 p < 0.05 and all statistical analysis were performed using SPSS software v.25 (SPSS Inc., 214 Chicago, IL, USA). 215

3. Results

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Eight participants were allocated to the Injured group, and eight to the Healthy 218 group, based on their clinical history. The average age was 23.93 ± 5.16 years with an av-219 erage height of 1.61 ± 0.05 m, demographic characteristics are summarized in Table 1. 220

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| able 1 . Demographic characteristics of the players |
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| | Total (n=16) | Injured (n=8) | Healthy (n=8) |
|---|-------------------|-------------------|-------------------|
| Aged (years) | 23.93 ± 5.16 | 23.00 ± 4.04 | 24.75 ± 6.13 |
| Position (Goalkeper : Back: Wing : Pivot) | 3:4:7:2 | 1:3:3:1 | 2:1:4:1 |
| Height (cm) | 161.24 ± 5.41 | 162.71 ± 5.87 | 159.95 ± 4.97 |
| Limb dominance (Right : Left) | 13:3 | 6:2 | 7:1 |
| Football Experience | | 15.75 ± 1.98 | 16.63 ± 1.19 |
| Injury limb (Dominant Limb : Non-Dominant Limb) | 4:4 | 4:4 | |

Table 2 shows the descriptive statistics, main effects and interactions for sagittal, cor-227 onal and transverse plane knee kinematics. No differences were reported in the coronal 228 plane for task or group. In the sagittal plane, Mixed Methods ANOVA revealed a signifi-229 cant main effect of task for peak knee flexion-extension angular velocity (p = 0.049) (Table 230 2). Post-hoc pairwise comparisons showed the CODAT and GOB demonstrated signifi-231 cantly higher knee extension angular velocity compared to TURN task (p = 0.035, and p =232 0.046, respectively) (Table 5). 233

Table 2. Mean (SDs), and the two-factor mixed linear model statistics for peak sagittal, coronal and transverse plane knee kinematics during the CODAT, GOB and Turn tasks. 235

| | COI | DAT | G | OB | TU | RN | Task p value | Injured p value | Interaction effect |
|------------------------------|---------|---------|------------|---------|---------|---------|-----------------|--------------------|-----------------------|
| | Injured | Healthy | Injured | Healthy | Injured | Healthy | | | |
| Variables | | | | | | | | | |
| | | 5 | Sagittal I | Plane | | | | | |
| Joint Angle | | | | | | | | | |
| Minimum Vaca Elevier Angle | 19.06 | 23.64 | 20.10 | 21.22 | 22.65 | 23.83 | 0 500 | 0.207 | 0 741 |
| Minimum Knee Flexion Angle | ±4.49 | ±8.77 | ±4.49 | ±11.96 | ±2.26 | ±9.11 | 0.599 | 0.307 | 0.741 |
| Peak Knee Flexion-Extension | -53.64 | 54.30 | -62.26 | 59.46 | -60.01 | 58.41 | 0.150 | 0.000 | 0.004 |
| Angle | ±8.46 | ±12.04 | ±10.18 | ±8.18 | ±10.69 | ±14.88 | 0.150 | 0.090 | 0.004 |
| Knee Flexion-Extension Angle | -34.59 | 30.66 | 42.16 | 38.23 | 37.36 | 34.59 | 0 124 | 0.265 | 0.096 |
| ROM | ±9.05 | ±11.17 | ±10.04 | ±10.91 | ±11.57 | ±9.05 | 0.134 | 0.205 | 0.980 |
| Angular Velocity | | | | | | | | | |
| Minimum Knee Flexion Angu- | -517.30 | -501.68 | -462.53 | -471.95 | -536.92 | -485.78 | 0.280 | 0 556 | 0.710 |
| lar Velocity | ±127.17 | ±126.55 | ±64.01 | ±11.96 | ±109.41 | ±124.02 | 0.360 | 0.556 | 0.710 |
| Peak Knee Flexion-Extension | 46.24 | 143.81 | 11.79 | 58.63 | 7.32 | -3.85 | 0.049* | 0.201 | 0.164 |
| Angular Velocity | ±140.89 | ±224.30 | ±50.65 | ±77.11 | ±18.88 | ±19.01 | 0 | 0.201 | 0.164 |
| Knee Flexion-Extension Angu- | 562.06 | 643.98 | 471.74 | 524.55 | 534.60 | 464.36 | 0 194 | 0 6 7 9 | 0.264 |
| lar Velocity ROM | ±201.87 | ±197.75 | ±77.73 | ±163.86 | ±110.51 | ±119.65 | 0.104 | 0.020 | 0.204 |
| | | | Coronal I | Plane | | | | | |

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| Ioint Angle | | | | | | | | | |
|------------------------------|---------|---------|-----------|---------|---------|---------|----------------|--------|--------------|
| Minimum Knee Abduction | -6.67 | -7.87 | -4.42 | -8.80 | -7.07 | -6.65 | | | |
| Angle | +5.59 | +4 24 | +5.37 | +6.96 | +5.81 | +4 99 | 0.942 | 0.289 | 0.511 |
| Peak Knee Abduction/Adduc- | 0.90 | -2.25 | 2.68 | 2.91 | 0.67 | 1.76 | | | |
| tion Angle | ±3.39 | ±3.99 | ±5.72 | ±7.55 | ±3.54 | ±6.80 | 0.173 | 0.698 | 0.385 |
| Knee Abduction-Adduction | 7.58 | 5.62 | 7.09 | 11.71 | -7.73 | 8.41 | | | |
| Angle ROM | ±4.37 | ±2.52 | ±2.78 | ±5.50 | ±2.92 | ±3.87 | 0.152 | 0.320 | 0.086 |
| Angular Velocity | | | | | | | | | |
| Minimum Knee Abduction | -118.01 | -114.89 | -138.28 | -133.43 | -154.99 | -105.27 | | | |
| Angular Velocity | ±76.41 | ±37.91 | ±77.59 | ±45.78 | ±137.08 | ±51.92 | 0.672 | 0.405 | 0.721 |
| Peak Knee Adduction Angular | 196.10 | 140.87 | 183.62 | 175.43 | 244.57 | 179.37 | | | |
| Velocity | ±73.84 | ±98.07 | ±73.92 | ±85.87 | ±97.19 | ±91.62 | 0.388 | 0.097 | 0.601 |
| Knee Abduction-Adduction | 314.11 | 255.76 | 321.90 | 308.86 | 399.56 | 284.64 | | | |
| Angular Velocity ROM | ±123.82 | ±127.85 | ±148.60 | ±92.26 | ±223.63 | ±108.42 | 0.557 | 0.145 | 0.636 |
| | | T | ransverse | Plane | | | | | |
| Joint Angle | | | | | | | | | |
| Minimum Knee External Rota- | -1.55 | -0.97 | -6.89 | -4.18 | -6.16 | -2.44 | | | |
| tion Angle | ±8.45 | ±8.48 | ±9.86 | ±9.51 | ±9.22 | ±7.35 | 0.386 | 0.365 | 0.867 |
| Peak Knee Internal Rotation | 11.55 | 8.19 | 6.54 | 7.92 | 11.15 | 6.19 | o (o (| | a 19- |
| Angle | ±7.96 | ±7.33 | ±6.65 | ±8.63 | ±6.23 | ±5.76 | 0.626 | 0.269 | 0.435 |
| Knee Internal-External Rota- | 13.11 | 9.16 | 13.43 | 12.10 | 17.31 | 8.63 | 0 ==0 | | |
| tion Angle ROM | ±5.21 | ±4.40 | ±5.09 | ±5.54 | ±7.37 | ±5.14 | 0.558 | 0.006* | 0.223 |
| Angular Velocity | | | | | | | | | |
| Minimum Knee External Rota- | -96.61 | -164.32 | -173.90 | -254.06 | -109.27 | -176.00 | 0.100 | 0.024* | 0.000 |
| tion Angular Velocity | ±92.06 | ±145.82 | ±120.09 | ±134.48 | ±58.56 | ±96.89 | 0.129 | 0.034* | 0.983 |
| Peak Knee Internal Rotation | 253.16 | 215.68 | 270.14 | 233.24 | 366.58 | 215.80 | 0 0 | 0.071 | 0.471 |
| Angular Velocity | ±155.06 | ±126.86 | ±124.14 | ±115.39 | ±200.39 | ±88.08 | 0.552 | 0.071 | 0.461 |
| Knee Internal-External Rota- | 349.77 | 380.00 | 444.04 | 487.30 | 475.85 | 391.81 | 0 411 | 0.050 | 0 54 |
| tion Angular Velocity ROM | ±225.48 | ±249.73 | ±116.70 | ±221.62 | ±206.39 | ±141.91 | 0.411 | 0.952 | 0.564 |

*denotes significance

In the transverse plane, Mixed Methods ANOVA showed a significant main effect of 237 group on knee internal-external rotation range of motion (p = 0.006) and minimum knee 238 external rotation angular velocity (p = 0.034). Post-hoc pairwise comparisons revealed that 239 the Injured demonstrated significantly increased knee internal-external rotation range of 240 motion (p=0.006) and significantly decreased external rotation angular velocity (p=0.034). 241 (Table 5). These kinematic post-hoc pairwise comparisons were graphically represented 242 in Figure 2, in the sagittal plane for task comparison, and in Figure 3, in the transverse 243 plane for group comparison. 244

7 of 17

100

50

0

-50

-100

Velocity ([°]/s)



KINEMATIC LOAD PHASE - Sagittal Plane

0 25 50 75 100 Phase Time (%) - INJURED MEAN ----- HEALTHY MEAN

Figure 3. Transverse plane for Injured and Healthy players based on mean data from functional test 249 (CODAT, GOB and TURN) players angular velocity in the CODAT test during the LOAD phase. 250

Table 3 and 4 show the Mixed Methods ANOVA results for average and peak muscle 251 activity for the PREP and LOAD phases, respectively. There were no significant between 252 group differences or main effect of task for EMG measures during the PREP phase (p > 253

0.05) (Table 3). In the LOAD phase, Mixed Model ANOVA showed a significant interaction between group and task for peak lateral gastrocnemius muscle activity (p = 0.025). 255 Post-hoc one way ANOVA revealed peak LG muscle activity was significantly higher for 256 Injured compared to Healthy under the TURN task (p = 0.022) (Table 6). There were no 257 between group differences either the GOB or CODAT tasks for LG muscle activity (p>0.05) 258 (Table 6). 259

Table 3. Mean (SDs) and the two-factor mixed linear model statistics for peak and average muscle260activity for Biceps Femoris, Semitendinosus, Vastus Medialis and Lateral Gastrocnemius during the261CODAT, GOB and Turn tasks during preparation phase.262

| | COI | DAT | G | OB | TU | RN | Task p value | Group p value | Interaction effect |
|-------------------------------|---------|---------|---------|---------|---------|---------|-----------------|------------------|-----------------------|
| | Injured | Healthy | Injured | Healthy | Injured | Healthy | | | |
| Variables | | | | | | | | | |
| Average Bigens Femaric | 0.14 | 0.13 | 0.12 | 0.10 | 0.15 | 0.14 | 0.226 | 0.482 | 0.802 |
| Average Biceps Femoris | ±0.05 | ±0.11 | ±0.04 | ±0.07 | ±0.05 | ±0.12 | 0.320 | 0.403 | 0.893 |
| Paak Bicons Fomoric | 0.63 | 0.59 | 0.51 | 0.38 | 0.68 | 0.53 | 0 1 2 2 | 0.206 | 0.842 |
| Teak Diceps Femoris | ±0.24 | ±0.37 | ±0.12 | ±0.25 | ±0.26 | ±0.42 | 0.122 | 0.200 | 0.042 |
| Average Semitondinosus | 0.17 | 0.15 | 0.19 | 0.16 | 0.19 | 0.14 | 0 791 | 0.097 | 0 887 |
| Average Semilentinosus | ±0.09 | ±0.05 | ±0.05 | ±0.06 | ±0.08 | ±0.07 | 0.771 | 0.077 | 0.007 |
| Poak Somitondinosus | 0.71 | 0.75 | 0.68 | 0.63 | 0.78 | 0.63 | 0.656 | 0.403 | 0 576 |
| i eak Semittenumosus | ±0.33 | ±0.23 | ±0.20 | ±0.22 | ±0.24 | ±0.18 | 0.050 | 0.405 | 0.570 |
| Average Vastus Medialis | 0.11 | 0.08 | 0.08 | 0.07 | 0.10 | 0.07 | 0 335 | 0.092 | 0.867 |
| Average vastus methans | ±0.06 | ±0.05 | ±0.03 | ±0.04 | ±0.05 | ±0.05 | 0.000 | 0.072 | 0.007 |
| Poak Vastus Modialis | 0.49 | 0.43 | 0.47 | 0.33 | 0.53 | 0.46 | 0.420 | 0 107 | 0.850 |
| I eak vastus meutans | ±0.18 | ±0.31 | ±0.21 | ±0.18 | ±0.23 | ±0.25 | 0.420 | 0.197 | 0.850 |
| Average Lateral Gastrocnem- | 0.08 | 0.08 | 0.06 | 0.07 | 0.05 | 0.05 | 0.217 | 0.618 | 0.959 |
| ius | ±0.06 | ±0.06 | ±0.04 | ±0.04 | ±0.02 | ±0.04 | 0.217 | 0.010 | 0.959 |
| Posk Lateral Castrogramius | 0.40 | 0.48 | 0.26 | 0.35 | 0.38 | 0.31 | 0 274 | 0.612 | 0.633 |
| i eak Laterai Gastrocheillius | ±0.18 | ±0.31 | ±0.19 | ±0.25 | ±0.25 | ±0.29 | 0.274 | 0.012 | 0.033 |

*denotes significance

Table 4. Mean (SDs) and the two-factor mixed linear model statistics for peak and average muscle264activity for Biceps Femoris, Semitendinosus, Vastus Medialis and Lateral Gastrocnemius during the265CODAT, GOB and Turn tasks during load phase.266

| | CODAT GOB | | TURN | | Task p value | Group p value | Interaction effect | | |
|--------------------------|-----------|---------|---------|---------|-----------------|------------------|-----------------------|-------|-------|
| | Injured | Healthy | Injured | Healthy | Injured | Healthy | | | |
| Variables | | | | | | | | | |
| Awara ao Pisona Formaria | 0.16 | 0.13 | 0.10 | 0.08 | 0.15 | 0.13 | 0.042* | 0.000 | 0 001 |
| Average Biceps Femoris | ±0.06 | ±0.09 | ±0.03 | ±0.07 | ±0.04 | ±0.08 | 0.042 | 0.206 | 0.004 |
| Peak Biceps Femoris | 0.78 | 0.69 | 0.59 | 0.47 | 0.71 | 0.67 | 0.188 | 0.376 | 0.946 |

| | ±0.25 | ±0.31 | ±0.25 | ±0.40 | ±0.26 | ±0.34 | | | |
|-----------------------------|-------|-------|-------|-------|-------|-------|--------|---------|--------|
| | 0.11 | 0.14 | 0.10 | 0.10 | 0.13 | 0.11 | 0.264 | | 0 (52 |
| Average Semitendinosus | ±0.06 | ±0.07 | ±0.02 | ±0.05 | ±0.07 | ±0.05 | 0.264 | 0.755 | 0.653 |
| | 0.64 | 0.74 | 0.52 | 0.63 | 0.59 | 0.73 | 0.205 | 0 1 4 2 | 0.000 |
| reak Semitendinosus | ±0.35 | ±0.24 | ±0.12 | ±0.28 | ±0.27 | ±0.26 | 0.395 | 0.143 | 0.969 |
| Average Vastus Medialis | 0.20 | 0.12 | 0.13 | 0.10 | 0.17 | 0.16 | 0.050* | 0.021* | 0.152 |
| | ±0.05 | ±0.07 | ±0.05 | ±0.07 | ±0.06 | ±0.09 | 0.052* | 0.031* | 0.153 |
| D 1. M 1 M 1 1. | 0.91 | 0.73 | 0.68 | 0.49 | 0.71 | 0.81 | 0.051* | 0.050 | 0.045 |
| Peak Vastus Medialis | ±0.14 | ±0.31 | ±0.25 | ±0.32 | ±0.23 | ±0.32 | 0.051* | 0.253 | 0.245 |
| Average Lateral Gastrocnem- | 0.15 | 0.14 | 0.12 | 0.08 | 0.15 | 0.08 | 0 174 | 0.02(* | 0 422 |
| ius | ±0.07 | ±0.07 | ±0.07 | ±0.04 | ±0.08 | ±0.04 | 0.174 | 0.036* | 0.433 |
| | 0.69 | 0.86 | 0.52 | 0.60 | 0.79 | 0.46 | 0.007 | 0 770 | 0.005* |
| reak Lateral Gastrochemius | ±0.28 | ±0.23 | ±0.31 | ±0.35 | ±0.26 | ±0.25 | 0.096 | 0.770 | 0.025* |

*denotes significance

There was also significant main effects of task on average BF muscle activity (p = 268 0.042) between tasks. Post-hoc pairwise comparisons that CODAT and TURN tasks sig-269 nificantly increased average BF (p = 0.034 and p = 0.040, respectively) compared to GOB 270 (Table 4). 271

There was also significant main effect of group on average VM and peak LG muscle activity (p = 0.031 and 0.036, respectively) (Table 3). For group condition, post-hoc pairwise comparisons showed that the Injured demonstrated significantly increased average 274 VM and LG muscle activity compared to Healthy (p = 0.031 and p = 0.036, respectively) 275 (Table 4). 276

Table 5. Knee kinematic and EMG pairwise comparisons for significant main effects of injured con-277 dition and task. 278

| | | Juce | e | 95% Confidence Intervals for | |
|--|---------------------|---------------|--------|---------------------------------|----------------|
| Variable – Knee kinematic | | Aean ferer | valu | Diffe | rences |
| | | N Dif | Р | Lower Bound | Upper Bound |
| Sagittal Plane (Significant Main effects of Task) | | | | | |
| | CODAT and GOB | 59.82 | 0.247 | -46.12 | 165.76 |
| Peak Knee Flexion-Extension Angular Velocity | CODAT and TURN | 93.29 | 0.035* | 3.55 | 82.00 |
| | GOB and TURN | 33.47 | 0.046* | 2.18 | 208.61 |
| Transverse Plane (Significant Main effects of Group) | | | | | |
| Knee Internal-External Rotation Angle ROM | Injured and Healthy | 4.66 | 0.006* | 1.42 | 7.89 |
| Minimum Knee External Rotation Angular Velocity | Injured and Healthy | 71.54 | 0.034* | 5.92 | 137.15 |
| | | | | | |

Variable - Muscle Activity EMG

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| (Significant Main effects of Task) | | | | | |
|------------------------------------|----------------|-------|--------|-------|-------|
| | CODAT and GOB | 0.05 | 0.034* | 0.00 | 0.10 |
| Average Biceps Femoris Load Phase | CODAT and TURN | 0.01 | 0.812 | -0.05 | 0.06 |
| | GOB and TURN | -0.05 | 0.040* | -0.09 | -0.00 |
| Average Vastus Medialis Load Phase | CODAT and GOB | 0.04 | 0.030* | 0.01 | 0.08 |

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| | CODAT and TURN | -0.01 | 0.711 | -0.05 | 0.04 |
|--|---------------------|-------|--------|-------|-------|
| | GOB and TURN | -0.05 | 0.046* | -0.10 | -0.00 |
| | CODAT and GOB | 0.24 | 0.018* | 0.05 | 0.43 |
| Peak Vastus Medialis Load Phase | CODAT and TURN | 0.06 | 0.535 | -0.13 | 0.25 |
| | GOB and TURN | -0.18 | 0.080 | -0.39 | 0.02 |
| (Significant Main effects of Group) | | | | | |
| Average Vastus Medialis Load Phase | Injured and Healthy | 0.04 | 0.031* | 0.00 | 0.08 |
| Average Lateral Gastrocnemius Load Phase | Injured and Healthy | 0.04 | 0.036* | 0.00 | 0.08 |
| | | | | | |

*denotes significance

Table 6. Pairwise comparison for significant main effect of limb for each task for Peak Lateral Gas-280trocnemius during the load phase.281

| Variable Peak Lateral Gastrocnemius load phase | | Mean Difference | p value | 95% Confidence | |
|--|---------------------|--------------------|---------|---------------------------|-------|
| | | | | Intervals for Differences | |
| | | | | Lower | Upper |
| | | | | Bound | Bound |
| CODAT | Injured and Healthy | -0.17 | 0.203 | -0.45 | 0.10 |
| GOB | Injured and Healthy | -0.09 | 0.616 | -0.44 | 0.27 |
| TURN | Injured and Healthy | 0.33 | 0.022* | 0.05 | 0.60 |

*denotes significance

4. Discussion

This study investigated the differences in kinematic and muscle activity outcomes 285 associated with ACL injury mechanisms during functional change of direction tasks, com-286 paring players at risk of ACL injury with healthy players. Additionally, it examined 287 whether these differences were influenced by the amplitude of the angle involved in each 288 different change of direction test. The findings support the hypothesis that players at risk 289 of ACL injury, defined as those with a history of valgus collapse-related knee injury, 290 demonstrate altered functional motor pattern compared to healthy players, as reflected 291 by movement quality and motor control. Moreover, both knee kinematics and muscle ac-292 tivity were also influenced by the amplitude of the angle required in each functional 293 change of direction test. 294

Injured showed significant differences for kinematics exclusively in the transverse 295 plane, where exhibited higher internal-external tibial rotation range of motion and de-296 creased external rotation angular velocity compared to Healthy. This altered kinematic 297 pattern amplifies the rotational load on the ACL, especially in the internal rotation direc-298 tion, which is critical during pivot shift maneuvers that involve knee abduction and inter-299 nal tibial rotation, thereby increasing ACL injury risk [39,40]. This aligns with Bates et al., 300 who explained that the ACL primarily stabilizes anterior tibial translation in the sagittal 301 plane (87%) while also resists torsional forces (13%) from movements in the transverse 302 and coronal planes [39,41]. Additionally, Hewett et al. outlined how axial forces increase 303 compression on the lateral knee side during valgus collapse [42], which combined with 304 the posterior slope of the lateral tibial plateau, enhances internal tibial rotation [15]. This 305 increased internal rotation corresponds with the motor pattern observed in Injured, high-306 lighting kinematic dysfunctions directly associated with the ACL injury mechanism. Our 307 findings align with these studies, as the Injured group demonstrated reduced external 308

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tibial rotation angular velocity, particularly during the early LOAD phase (Figure 3), 309 when main stabilization adjustments are essential to avoid excessive strain in the ACL 310 [13]. These are consistent with research that identified greater rotation motion, impaired 311 in internal rotation direction, in players at risk of ACL [12,43,44]. Consequently, Injured 312 may be more likely to exhibit high risk ACL kinematic profiles, particularly in the transverse plane, compared to Healthy. 314

Group differences in muscle activity were observed exclusively during the LOAD 315 phase. Kinematic profile differences, particularly in the transverse plane, may influence 316 muscle activity during load absorption, explaining the lack of differences during the air-317 borne PREP phase. Injured players demonstrated significantly higher average VM and LG 318 muscle activity, 4% more than Healthy, and only during the TURN, 33% higher peak LG 319 activity. Quadriceps-dominant strategies during LOAD phase increase anterior tibial 320 shear forces, contributing to excessive ACL strain and higher injury risk [10,13]. As Mur-321 phy et al. explains VM, as a uni-articular muscle, plays a key role in knee stabilization [45]. 322 Therefore, the increased VM activity in Injured may reflect greater neuromuscular de-323 mand for rapid stabilization, potentially resulting from impaired motor control. In addi-324 tion, LG anatomical position enables posterior femoral translation and posterior knee 325 compression [13,18,25], which, in synergy with quadriceps activity, generates anterior tib-326 ial shear forces, further increasing ACL strain [13,18,25]. As noted by Nasseri et al., this 327 interaction reaches its peak early in the LOAD phase, along with the previously men-328 tioned kinematic imbalances [13] (Figures 2 and 3). Our findings highlight this dysfunc-329 tional neuromuscular mechanism in Injured, where elevated VM and LG activation pro-330 motes anterior tibial displacement, thereby increasing ACL strain and significantly raising 331 injury risk. This Injured pattern, according to Picot et al., could be attributed to muscle 332 compensation related to dysfunctional hamstrings muscle activity, key stabilizers of knee 333 rotation [10,19]. Despite the absence of significant differences in hamstring muscle activity 334 between groups or between BF and ST, the presence of transverse plane kinematic differ-335 ences suggest that Injured compensates through VM and LG EMG to maintain rotational 336 stability, highlighting a potentially dysfunctional strategy that could underline the in-337 creased ACL injury risk explained. This pattern may raise immediate ACL injury risk and 338 contribute to long-term vulnerability [4,13,19]. 339

Tasks analysis indicates that the amplitude of directional change significantly im-340 pacts kinematic strategies, particularly in the sagittal plane. This highlights this plane load 341 component as the primary involved in change of direction tasks, supporting functional 342 test specificity, as most ACL loading occurs in the sagittal plane during the LOAD phase 343 [13,20,25,39]. The results revealed that both CODAT and GOB exhibited significantly 344 higher knee extension angular velocity compared to TURN. In our study the maximum 345 knee flexion marking the end of the LOAD phase [13,38,46]. In this way, CODAT and GOB 346 highly extension angular velocity appears at the end of LOAD phase (Figure 2). According 347 to Thomas et al., increased knee flexion during the LOAD phase aids in achieving an op-348 timal body position at final contact, characterized by a lower center of mass, which en-349 hances force absorption and control of external disturbances [46]. The higher extension 350 angular velocity observed in CODAT and GOB during this phase suggests a more efficient 351 transition from stabilization, increased then decreased flexion angular velocity, to propul-352 sion, extension angular velocity. In contrast, TURN appears to present a greater stabiliza-353 tion challenge, potentially delaying this transition and reducing extension angular veloc-354 ity. Therefore, TURN may better replicate functional injury mechanisms in defense foot-355 ball context, as it allows players to perform the maneuver at high intensity, preceded and 356 followed only by straight-line sprinting [22]. 357

Significant differences in muscle activity between tasks were observed only during 358 the LOAD phase. This suggests that the amplitude of directional change during the 359

functional change of direction maneuver does not influence muscle activity in the PREP 360 phase. Both CODAT and TURN tasks resulted in higher average BF and VM muscles com-361 pared to GOB. Murphy et al. highlight that the hamstrings and quadriceps are the primary 362 controllers of knee stability [45]. This is because most of the knee stabilization load occurs 363 in the sagittal plane, which is the primary axis of action for the hamstrings and quadriceps 364 functioning in an agonist-antagonist balance [10,13]. Therefore, CODAT and TURN, that 365 involve greater activation of these muscle groups, suggest that 90° change of direction 366 maneuvers implicates a greater stabilization challenge for the knee compared to other task 367 as GOB that involving other amplitude angle. Supporting this, Markström et al., report 368 that 90° directional changes are associated with more specific and demanding functional 369 patterns than other directional amplitudes [40]. These results, when considered alongside 370 the kinematic data, suggest that tasks involving 90° directional changes may offer a more 371 effective means of evaluating knee stability. Overall, the increased muscle demands and 372 coordination required during these tasks may better reflect functional challenges faced 373 during high-risk movements, such as those related to ACL injury mechanisms. 374

Future research should investigate the transferability of these findings to other 375 sports, as change of direction is a common functional task across various disciplines. It 376 would be valuable to examine whether similar kinematic and muscle activity patterns are 377 present in athletes exposed to valgus collapse knee injury mechanisms in different sport-378 ing contexts. This exploration could enhance the development of cross-sport prevention 379 strategies. Additionally, further research should focus on the design and implementation 380 of specific training programs based on the kinematic and muscle activity patterns identi-381 fied in players at risk of ACL injury. These interventions should aim to reduce the magni-382 tude of these risk-related variables, reverse the dysfunctional knee stabilization patterns 383 observed, and ultimately prevent ACL injuries. 384

4.1. Limitations

This study presented some limitations. The assessor(s) in the present study were not 387 blinded during data collection. However, participants were given a unique, anonymized 388 study code to mitigate potential bias during data and statistical analyses. Although this 389 study was powered appropriately, the sample size small and the results should be inter-390 preted with caution. However, these findings do support the use of kinematics, angular 391 velocity and muscle activity to explore differences between those at risk of ACL injury 392 and healthy participants during change of direction tests. Additionally, potential covari-393 ates such as player age, years of sport participation, and injury type were not included in 394 the a priori statistical plan. Future research may want to appropriately power a study to 395 explore these factors. Methodological limitation of this study is its exclusive focus on knee 396 kinematics, as knee motion is influenced by the kinematics of the entire lower limb. Future 397 research may want to include hip and ankle kinematics and/or muscle activity to gain a 398 more comprehensive understanding of functional differences between players at risk of 399 ACL injury. Furthermore, incorporating gluteal muscle activity analysis would be valua-400 ble, as these muscles play a key role in controlling hip movement, which in turn affects 401 knee stability, particularly in the transverse plane. 402

4.2. Clinical contributions

This study highlights the growing trend of incorporating muscle activity analysis, as 405 neuromuscular factor related to motor control, into ACL injury risk identification studies. 406 In synergy with kinematic variables such as angular velocity may provide more sensitive 407 information to identify players at risk of ACL injury, based on quality of movement analysis. Furthermore, since the study involved active players who had previously suffered 409

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an injury due to valgus collapse and had fully recovered, it is plausible that the observed 410 risk patterns reflect dysfunctional compensatory mechanisms. Our findings show that 411 players at risk of ACL injury exhibited greater angular velocity and range of motion in the 412 transverse plane, along with heightened VM and LG muscle activity compared to healthy 413 players. These alterations in movement quality and motor control reinforce the hypothesis 414 of a dysfunctional compensatory motor pattern. Therefore, these risk patterns should be 415 leveraged to design more targeted injury prevention programs that address these move-416 ment strategies and mitigate long-term risk factors. 417

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5. Conclusions

This study identified differences in knee angular velocity, and muscle activity be-420 tween players at risk of ACL injury and healthy players. These differences were influ-421 enced by the amplitude and direction of the change of direction indicating that CODAT 422 and TURN tasks, that involving 90° directional changes, may offer a more effective means 423 of evaluating knee stability. Players at risk of ACL injury exhibit increased range of mo-424 tion and angular velocity in the transverse plane, along with elevated VM and LG muscle 425 activity during the LOAD phase, compared to healthy players. Conversely, the TURN and 426 CODAT tests are characterized by greater angular velocity in the sagittal plane, which is 427 associated with increased activation of the VM and BF muscles. Therefore, the increased 428 angular velocity and range of motion in the transverse plane, along with elevated VM and 429 LG muscle activity during the LOAD phase, may reflect an underlying dysfunctional mo-430 tor pattern. This highlights the importance of assessing kinematics alongside specific mus-431 cle activity during functional tests, replicating ACL injury mechanisms, to better deter-432 mine player risk profiles and design more effective prevention programs. 433

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Abbreviations

The following abbreviations are used in this manuscript:

| ACL | Anterior Cruciate Ligament |
|------|----------------------------|
| EMG | Electromyography |
| ST | Semitendinosus |
| BF | Biceps Femoris |
| VM | Vastus Medialis |
| LG | Lateral Gastrocnemius |
| PREP | Preparation phase |
| LOAD | Load phase |
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