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Comparative Analysis of Neuromuscular Activation Patterns Associated with Force between Semi-Professional Female Soccer Players with Previous Anterior Cruciate Ligament Surgery and Healthy Players in Thigh Musculature Related to Valgus Collapse

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Abstract: This study investigates electromyography activation and force development differences in key lower limb muscles between female football players with previous anterior cruciate ligament injuries compared with healthy players. Twenty-two semi-professional players were divided into ACL-injured (n = 11) and non-injured groups (n = 11). Participants underwent maximal voluntary isometric contractions while electromyography activation, peak and average, and peak torque of force were measured. Results indicated significant differences in electromyography activation patterns between anterior cruciate ligament players and non-injured players, particularly in biceps femoris and gluteus maximus muscles. These differences were also evident when comparing between limbs within anterior cruciate ligament players. Interestingly, both groups exhibited similar peak torque of force during maximal contractions, suggesting a compensatory neuromuscular strategy that supports a return to sport based on kinetic and kinematic factors. However, these findings underscore persistent muscle integration imbalances potentially contributing to the high rate of anterior cruciate ligament reinjury. In conclusion, this study highlights the importance of evaluating electromyography activation alongside force development in understanding neuromuscular adaptations post anterior cruciate ligament injury. These insights emphasize the need for comprehensive rehabilitation strategies that address muscle imbalance to mitigate the risk of recurrent anterior cruciate ligament injuries in female football players.

Keywords: anterior cruciate ligament; electromyography; isometric test; football; risk factor

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1. Introduction

The professionalization of women's football has led to an improvement in the physical condition of female players, equalizing the total injury rate per season between female and male players [1,2]. The prevention of anterior cruciate ligament (ACL) injuries has been extensively studied for years. Nonetheless, it remains one of the most prevalent and severe injuries, with 0.7 being the average count of ACL injuries per season in a squad. In addition, sex-based differences in ACL-specific incidence persist, occurring 3 to 6 times more frequently in female football players than in male players [2]. The incidence rate of ACL tears stands at 0.2 per 1000 h, with the highest burden observed among all injuries,

from 38 to 59 days lost per 1000 h [1,3–7]. Therefore, only 33% to 61% of players return to the same competition level after an ACL reconstruction due to a lack of self-confidence or kinesiophobia, and the risk of reinjury is 20–27% higher in females than in males [8–10].

The ACL injury mechanism is usually promoted by a non-contact mechanism, particularly during a change of direction task, with a 48% incidence rate due to a failure in the motion pattern [1,5,6]. Consequently, identifying potential biomechanical and neuromuscular risk factors has become a primary focus in the research studying the prevention of ACL injuries [3,11,12]. Most of this research has developed a methodology focused on the kinematic and kinetic pattern [3–5,10,12–14]. However, despite prevention efforts focused on kinematics and kinetic factors, gender disparity in non-contact ACL injury rates persists in football, and it is higher than in other injuries [1,2,15,16]. Additionally, recent studies suggest that solely focusing on kinematic and kinetic mechanisms for prevention may not be sufficient [17]. Thus, the last evidence promotes the study of the risk factors associated with the neuromuscular function, suggesting that they might be more sensitive for injury prediction in elite trained players [11,18].

Neuromuscular function evaluation is frequently conducted by calculating the maximal voluntary isometric contraction because it ensures the highest central nervous system recruitment of motor units [8,10,19,20]. Nevertheless, after an ACL injury, it has been shown that neural alterations occur [21,22]. Specifically, previous ACL-injured people have a lesser ability to generate descending action potentials from the motor cortex, lower motor output, and higher activation of the brain areas responsible for motor processing [22]. Moreover, McPherson et al. [21] demonstrated alterations in quadriceps (Q) and hamstrings (H) motor unit action potentials in both injured and uninjured limbs of previous ACL-injured subjects compared to healthy controls. It seems that players who have suffered an ACL injury exhibit reduced effective strength due to inefficient contractions, as they struggle to generate more force despite heightened motor unit activation. This could explain the high rate of ACL reinjury due to the fact that in return-to-play tests for medical clearance, effective strength is evaluated, but simultaneous neuromuscular activation patterns are not analyzed [23,24]. For that reason, following rehabilitation, the muscles inhibited by the injury and surgical intervention may not have fully recovered. The balance strength symmetry index might, therefore, be a result of compensation by the adjacent muscles [25].

Isometric H/Q ratios have been widely analyzed in ACL injury prevention programs to monitor strength imbalances. It has been considered that a co-contraction developed by H as an antagonist of anterior Q force during the stance phase in the change of direction task is necessary [26,27]. Female players have exhibited Q dominance activation during this eccentric phase compared to males [28–31]. This has also been associated with increased anterior shear forces of the tibia that can elevate ACL strain [30,32]. Therefore, ACL acts as an antagonist of Q during the stabilization phase, restricting anterior shear associated with Q activation [32]. Moreover, the addition of high-intensity rotational forces during a sidecutting task promotes a valgus collapse position. The valgus collapse position involves knee abduction, hip adduction, and internal rotation, along with limited lower limb triple flexion and trunk bending in the opposite direction during the stabilization phase in a sidecutting task [3,13,14,28,33]. The valgus collapse generates medial displacement of the ground reaction force vector, which is then centered medial and posterior on the tibial plateau. This results in relative anterior and lateral shear of the tibia with increased ACL load. This position may lead to impingement of the ACL on the lateral femoral condyle [13,14,30,32,34]. Thus, players who develop this pattern have been shown to be more susceptible to suffering an ACL injury [14,30,32].

Meanwhile, Hs play a crucial role as synergists of the ACL [27,35]. Hs counteract the two main vectors of ACL strain, the anterior component and the lateral component; therefore, they prevent strain damage of the ACL [27,29,36]. Females have exhibited lower electromyographical activation (actEMG) ratios of the H during stabilization actions and have shown greater H inhibition due to fatigue compared to males [27,35]. Furthermore, recent evidence has explained that biceps femoris (BF) and semitendinosus (ST) activations

reveal functional differences controlling knee stability [11,30,37]. ST develops three main functions to decrease ACL injury risk. Due to its anatomical positioning, it limits maximal hip internal rotation, thus controlling relative external tibial rotation. The ST also functions as a medial knee stabilizer, compressing the medial knee compartment to counteract excessive knee valgus [11,38]. Finally, ST has a main role as a "knee adductor", avoiding knee abduction that might appear during the valgus collapse in high-intensity tasks [11].

The valgus collapse mechanism is the primary ACL risk factor. Hip abductors and external rotator muscles, such as gluteus maximus (GMax) and gluteus medius (Gm), might be the other keys protecting the ACL, as they act as primary controllers of this mechanism [29,39]. Nonetheless, little is known about their maximal voluntary isometric contraction torque or about their muscle pattern recruitment or alterations after ACL injury or reconstruction. The study of the GMax and Gm as potential controllers of the knee in the transversal and frontal plane and of the H and Q in the sagittal plane may provide reference neuromuscular data to identify neuromuscular thresholds in relation to the ACL injury risk. These data may increase the effectiveness of ACL prevention programs. In addition, recent studies have introduced an innovation. They consider laterality and its role in the incidence of ACL injury mediated by the valgus collapse mechanism. The study by DeLang et al. explains that football players typically develop more injury risk factors in their dominant limb (DL) (defined by the preferred kicking limb) than in their non-dominant limb (NDL) [40]. They argue that two main interdependent factors contribute to this: fatigue and the sports context, which together determine which limb the player uses for stabilization. Consequently, the DL tends to experience fatigue due to its involvement in kicking during matches, without typically serving the stabilization role [14].

Most ACL injury rehabilitation and knee injury prevention programs focus on correcting postural or kinematic alignment to prevent valgus collapse mechanisms. They are based on muscle-strengthening exercises that reproduce general movement patterns. These programs fail to fully explain the ACL injury mechanism and consequently fail to provide a solution to the continuous increase in incidence. Thus, this study aims to (a) evaluate differences in actEMG and force between female football players with previous ACL injury and healthy players in BF, ST, vastus medialis (VM), vastus lateralis (VL), GMax, and Gm during a maximal voluntary isometric contraction and (b) determine if differences occur based on the laterality of the injured limb.

2. Materials and Methods

2.1. Study Design

This study has an analytical cross-sectional design. Allocation depended on clinical history: previous ACL injury, followed by surgical intervention. The no-injury (NI) group served as the control group. Among players with previous ACL injury, a distinction was made between those with injury in the DL and in the NDL, allowing for a comparative analysis conditioned by laterality. The Research Ethics Committee of the Community of Aragón approved this study (code PI20/127. Date of approval: 18 March 2020), which adhered to the ethical principles of the Declaration of Helsinki [41].

2.2. Participants

Professional female football players from clubs affiliated with the Hungarian University of Sport Science and the University of Zaragoza were contacted through personal meetings. They held an active national football license, attended football training for more than 8 h per week, and competed in the same division. ACL players must have undergone ACL surgery and received an ST graft. The player must have completed supervised injury rehabilitation by a healthcare professional. They must have been medically cleared by a healthcare professional from the national football federation of each country at least 4 months prior. Players were excluded if they had sustained an injury incompatible with regular training in the last 4 months. All players provided written informed consent before

participating. For those participating players who were minors, the informed consent was signed by their legal guardian and the player, as well.

According to the sample size calculation presented at the end of the Section 2, 22 players were recruited. A convenience sampling method was adopted. When the participants provided informed consent, inclusion and exclusion criteria were checked. According to their previous individual clinical history, they were included in the ACL group (n = 11) or the NI group (n = 11).

Table 1 shows that the ACL group and NI group were similar, considering age and anthropometric characteristics. The average age was 21.72 ± 3.59 years, height was 1.63 ± 0.05 m, and weight was 60.45 ± 5.40 kg.

Table 1. Comparative analysis between ACL group and NI group considering age and anthropometric characteristics.

	ACL (n = 11)	**NI (n = 11)**	***p*-Value**
	Mean ± SD	**Mean ± SD**	
Age (years)	22.36 ± 3.56	20.63 ± 3.58	0.287
Weight (kg)	59.72 ± 5.80	61.99 ± 6.54	0.408
Height (cm)	162.68 ± 7.05	163.17 ± 6.09	0.869

2.3. Procedure

The evaluation started with the registration of the individual clinical history.

The recorded information included age, height, weight, and lower limb dominance. Limb dominance criteria were defined by the preferred kicking limb [40]. If the subject pertained to the ACL group, they were asked about the laterality of the injured limb, the injury mechanism, and the treatment received. All data collection was completed in one session. Before the EMG and force data collection, the players developed a 5 min aerobical warm-up consisting of relaxed running [8,42]. The players also trained on the tests three times before the real data collection to avoid learning bias.

A surface electromyography system (Trigno Avanti, Delsys Europe, Manchester, UK) was used to record data on muscle activation patterns, and a load cell (Trigno Avanti, Delsys Europe, Manchester, UK) was used to measure the muscular force. Muscle activation patterns and muscular force were registered synchronously (Figure 1). Zielinski et al. instructions for systematizing were followed to ensure the repeatability of the electromyographic examination [43]. Sensors were placed on the skin by a specific sticker after cleaning it with alcohol and cotton wool and waxing the area if necessary [44]. Allocation of EMG sensors was performed following the European Recommendations for Surface Electromyography (SENIAM) [45]. Sensors were placed bilaterally in BF, ST, VM, VL, GMax, and Gm (Figure 2). EMGworks Acquisition software 4.8.0 (Delsys Europe, Manchester, UK) was employed to collect the data. Before performing the tests, all the sensors were calibrated to avoid recording noise and to obtain a relaxed muscle signal between 10 and −10 μV. EMGworks Analysis software (Delsys Europe, Manchester, UK) was used to analyze the EMG signal.

Players performed the MVIC of the hamstrings (BF and ST simultaneously), the Q (VL and VM simultaneously), the GMax, and the Gm for 3 s. The MVIC contractions from each muscle were repeated three times. Subjects rested for 30 s between each repetition to prevent muscle fatigue [8,42,46]. During the test, players were encouraged, and a computer screen was used to show the signal and provide biofeedback.

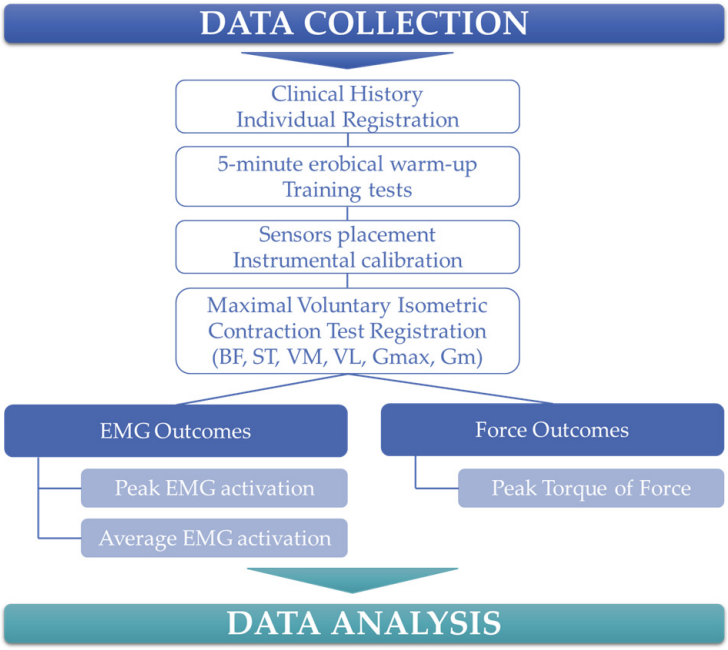

Figure 1. Flow diagram explaining the procedure. (BF) Biceps Femoris, (ST) Semitendinosus, (VM) Vastus Medialis, (VL) Vastus Lateralis, (GMax) Gluteus Maximus and (Gm) Gluteus Medius.

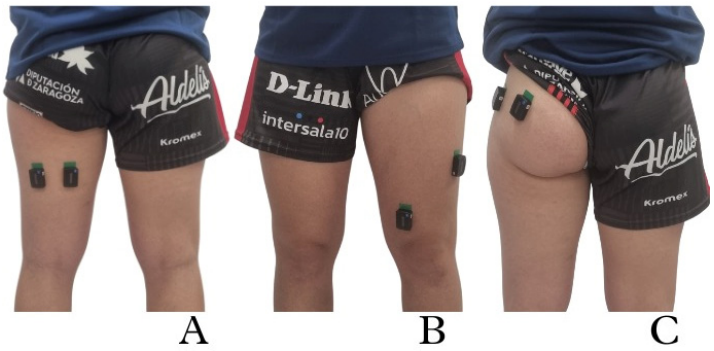

Figure 2. Location of the EMG sensors: biceps femoris and semitendinosus (**A**), vastus medialis and vastus lateralis (**B**), and gluteus maximus and gluteus medius (**C**).

The maximal voluntary isometric contractions were performed as depicted in Figure 3. For the Gm, the players were side-lying with the hips in a neutral position with both knees extended. They were instructed to abduct the limb. One fixation was placed on the lateral aspect of the thigh to allow the MVIC [47]. GMax was assessed with the players lying prone, with the hip flexed 30° and the knee flexed 90°. The fixation was placed on the posterior side of the thigh. The players were instructed to elevate the limb to the ceiling [48]. Hs were measured with the players in the prone position with the knee flexed 45°. The fixation was placed on the posterior aspect of the ankle, and they were asked to bend the knee [42,46]. Finally, for the Q, players were seated with the hips and the knees flexed 90°. The fixation was placed on the anterior aspect of the ankle. The players were asked to extend the knee [42,46,48].

Figure 3. Positions while measuring maximal voluntary isometric contraction of the gluteus medius (**A**), gluteus maximus (**B**), hamstrings (**C**), and quadriceps (**D**).

2.4. Outcome Variables

The study variables were divided into two domains, delineating the two components evaluated synchronously. One domain comprised the variables related to actEMG, while the other domain included those associated with muscular force torque.

2.4.1. EMG Outcome Variables

Two variables were extracted from the actEMG analysis: the peak actEMG and the average actEMG during the MVIC of each muscle tested. The sampling rate registration was 2000 Hz, and the common mode rejection ratio was 100 dB. The raw EMG signal was processed to enable the comparison of the statistical means. The band-pass filter ranged from 10 to 500 Hz, the full wave was rectified, and the root-mean-square (RMS) of the signal was derived [18,49]. The signal normalization was performed relative to the maximum of maximum values [50]. To determine this maximum of maximum, the highest value observed for each muscle across three MVIC repetitions was selected. To calculate peak actEMG variables, the mean of peak actEMG values was calculated, and then it was divided by the maximum of maximum value, selected before, and multiplied by 100. Thus, the peak actEMG variable is expressed as the percentage of maximal activation. To calculate average actEMG, the mean of all activation values during each maximal voluntary isometric contraction was calculated and divided by the maximum of maximum value, selected before, and multiplied by 100. In this way, the average actEMG variable is also expressed as the percentage of maximal activation.

2.4.2. Force Outcome Variables

Peak torque of force was evaluated during each maximal voluntary isometric contraction. Peak torque was calculated by selecting the maximal force value of each maximal voluntary isometric contraction and averaging over the three cycles. Peak torque value was measured in newton meter per kg (Nm/kg) [34].

2.5. Sample Size

The sample size was calculated based on the outcomes from Bencke and Zebis [27]. The main variable used for sample size calculation was the peak actEMG in BF muscle. The sample size was calculated with the GRANMO 8.0 calculator, with an alpha risk of 0.05, a beta risk of 0.20, and the two-sided test. We used a common standard deviation of 16% for

peak actEMG and a minimum expected difference of 22% for peak actEMG, estimating the losses of follow-up in 20%. A total sample of 11 subjects per group was obtained.

2.6. Data Analysis

Data were analyzed with SPSS software v.25 (SPSS Inc., Chicago, IL, USA). Normality was determined by the Shapiro–Wilk test. To determine differences in actEMG variables and peak torque of force during the maximal voluntary isometric contraction for each muscle tested between the ACL and NI groups, the independent samples *t*-test or the U-Mann–Whitney test was used, based on the distribution of the data. Statistical analysis was composed of two parts: (a) Comparative analysis between ACL and NI, considering the laterality of the injured limb. Thus, the DL of players with the ACL injury in DL was compared with the DL of NI players, and the NDL of players with the ACL injury in NDL was compared with the NDL of NI players. (b) Comparative analysis between the limbs within each player. Thus, players were analyzed separately in three categories: players from the ACL group with an injury in the DL (ACL_DL), players from the ACL group with an injury in the NDL (ACL_NDL), and players from the NI group. The mean, the standard deviation (SD), the 95% confidence interval (CI) for the difference in means, and the effect size were obtained. Effect size of actEMG and torque variables between groups and between limbs was determined using Sawilowsky classification [51]. The following values were used to distinguish the levels of effect size: 0.01 to 0.19 (very small), 0.20 to 0.49 (small), 0.50 to 0.79 (medium), 0.80 to 1.19 (large), 1.20 to 1.99 (very large), and more than 2.00 (huge).

The level of statistical significance was set at $p < 0.05$.

3. Results

Twenty-two semi-professional players participated in the stud, with eleven in the ACL group and eleven in the NI group. The ACL group was divided into two groups for secondary analysis. Six players were allocated to the ACL_DL group and five to the ACL_NDL group.

3.1. Comparative Analysis between Groups, ACL and NI, Considering DL or NDL

The comparative analysis in peak actEMG for DL and NDL showed no significant differences between groups. However, strong to excellent effect sizes for NDL were observed in VM (d = 1.075) and in GMax (d = 1.124). For NDL, VM peak actEMG was higher in NI players, while in GMax peak actEMG was higher in ACL players (Table 2).

Table 2. Comparative analysis of peak actEMG outcomes during maximal voluntary isometric contraction between groups, ACL and NI, considering DL or NDL.

	DOMINANT LIMB (DL)					NO DOMINANT LIMB (NDL)				
	ACL	NI	*p*-Value	Cohen's d	Confidence Interval (95%)	ACL	NI	*p*-Value	Cohen's d	Confidence Interval (95%)
	Mean ± SD	Mean ± SD				Mean ± SD	Mean ± SD			
BF_peak actEMG (%)	85.81 ± 85.81	90.06 ± 5.05	0.157	0.73	(−10.33 to 1.83)	91.22 ± 5.59	93.61 ± 5.45	0.464	0.43	(−9.29 to 4.52)
ST_peak actEMG (%)	83.79 ± 83.79	89.38 ± 7.65	0.320	0.49	(−17.29 to 6.11)	91.34 ± 6.98	86.44 ± 5.96	0.204	0.75	(−3.07 to 12.86)
VL_peak actEMG (%)	87.04 ± 87.04	89.18 ± 8.69	0.647	0.23	(−11.86 to 7.59)	86.14 ± 8.59	89.39 ± 5.16	0.407	0.46	(−11.55 to 5.05)
VM_peak actEMG (%)	82.68 ± 82.68	87.44 ± 10.10	0.347	0.47	(−15.19 to 5.68)	78.84 ± 7.34	86.78 ± 7.43	0.087	1.07	(−17.23 to 1.35)
GMax_peak actEMG (%)	87.26 ± 87.26	90.08 ± 6.39	0.509	0.34	(−11.76 to 6.11)	94.37 ± 4.67	88.09 ± 6.38	0.085	1.12	(−1.01 to 13.58)
Gm_peak actEMG (%)	89.68 ± 89.68	88.04 ± 6.16	0.611	0.26	(−5.11 to 8.39)	91.59 ± 4.86	91.08 ± 6.16	0.879	0.09	(−6.67 to 7.69)

The comparative analysis in average actEMG for NDL obtained statistical signification between groups in BF (p = 0.023) and GMax (p = 0.038). Moreover, for NDL, these outcomes showed strong to excellent effect sizes in BF (d = 1.384) and GMax (d = 1.351), as well. For NDL, the BF average actEMG was higher in NI players, while the GMax average actEMG was higher in ACL players (Table 3).

Table 3. Comparison of average actEMG outcomes during maximal voluntary isometric contraction between groups, ACL and NI, considering DL or NDL.

	DOMINANT LIMB (DL)					NO DOMINANT LIMB (NDL)				
	ACL	NI	*p*-Value	Cohen's d	Confidence Interval (95%)	ACL	NI	*p*-Value	Cohen's d	Confidence Interval (95%)
	Mean ± SD	Mean ± SD				Mean ± SD	Mean ± SD			
BF_average actEMG (%)	57.64 ± 57.64	63.60 ± 13.94	0.315	0.50	(−18.17 to 6.25)	49.49 ± 10.01	60.56 ± 5.28	0.023 *	1.38	(−20.30 to −1.84)
ST_average actEMG (%)	48.57 ± 48.57	60.21 ± 15.26	0.145	0.75	(−27.76 to 4.49)	54.77 ± 10.99	53.55 ± 6.38	0.803	0.14	(−9.27 to 11.70)
VL_average actEMG (%)	58.95 ± 58.95	55.97 ± 12.32	0.543	0.30	(−7.26 to 13.23)	55.57 ± 8.25	57.92 ± 7.76	0.615	0.29	(−12.31 to 7.62)
VM_average actEMG (%)	54.13 ± 54.13	53.94 ± 17.31	0.979	0.01	(−15.79 to 16.18)	47.07 ± 15.42	51.83 ± 10.47	0.518	0.36	(−6.88 to 11.61)
GMax_average actEMG (%)	48.86 ± 48.86	55.83 ± 10.88	0.178	0.71	(−17.53 to 3.58)	59.82 ± 5.54	52.25 ± 5.66	0.038 *	1.35	(0.52 to 14.62)
Gm_average actEMG (%)	56.79 ± 56.79	58.57 ± 18.71	0.801	0.13	(−16.68 to 13.12)	60.09 ± 5.37	60.02 ± 8.34	0.986	0.01	(−9.20 to 9.36)

* Statistical significance $p < 0.05$. (BF) biceps femoris, (ST) semitendinosus, (VL) vastus lateralis, (VM) vastus medialis, (GMax) gluteus maxims, (Gm) gluteus medius, (actEMG) EMG activation.

No significative differences were observed for DL or NDL in the comparative analysis of peak torque of force between ACL and NI groups. However, for DL, strong to excellent effect sizes were obtained for peak torque of force in Gm (d = 0.942) (Table 4).

Table 4. Comparison of peak torque of force outcomes during maximal voluntary isometric contraction between groups, ACL and NI, considering DL or NDL.

	DOMINANT LIMB (DL)					NO DOMINANT LIMB (NDL)				
	ACL	NI	*p*-Value	Cohen's d	Confidence Interval (95%)	ACL	NI	*p*-Value	Cohen's d	Confidence Interval (95%)
	Mean ± SD	Mean ± SD				Mean ± SD	Mean ± SD			
H_peak_Torque (Nm/kg)	1.55 ± 1.55	1.50 ± 0.50	0.831	0.11	(−0.47 to 0.58)	1.25 ± 0.91	1.47 ± 0.57	0.596	0.29	(−1.11 to 0.67)
Q_peak_Torque (Nm/kg)	2.86 ± 2.86	2.69 ± 0.98	0.640	0.23	(−0.59 to 0.94)	2.54 ± 0.97	2.50 ± 1.08	0.958	0.03	(−1.27 to 1.34)
GMax_peak_Torque (Nm/kg)	3.15 ± 3.15	2.93 ± 1.12	0.175	0.22	(−0.85 to 1.29)	2.71 ± 0.93	2.99 ± 1.10	0.649	0.27	(−1.58 to 1.03)
Gm_peak_Torque (Nm/kg)	2.22 ± 2.22	1.61 ± 0.65	0.092	0.94	(−0.11 to 1.32)	1.78 ± 0.78	1.52 ± 0.68	0.540	0.35	(−0.64 to 1.16)

3.2. Analysis between Limbs in ACL and NI Groups Considering DL or NDL

Peak actEMG analysis between limbs obtained statistical significance in ACL players, while in NI players, no statistical differences were obtained. In ACL_DL players, peak actEMG in BF was significantly higher for NDL, which was the non-injured limb group, than for DL, which was the injured limb (p = 0.041). ACL_NDL players showed significantly higher peak actEMG in GMax for NDL, the injured one, compared to DL, the non-injured one (p = 0.043). Moreover, ACL_DL players revealed strong to excellent effect sizes in peak actEMG in Gm (d = 1.035). Thus, peak actEMG in Gm was higher for DL, the injured limb, compared to NDL, the non-injured one. In addition, ACL_NDL players demonstrated strong to excellent effect sizes in peak actEMG in BF (d = 1.140). Thus, peak actEMG in BF was higher for NDL, the injured limb, compared to DL (Table 5).

No statistical significance was obtained in the comparative analysis of average actEMG between limbs for any group. However, ACL_DL players showed strong to excellent effect

sizes in average actEMG in GMax (d = 0.808) and Gm (d = 0.926). In ACL_DL players, the average actEMG in GMax was higher for NDL, the non-injured limb, while in Gm, it was higher for DL, the injured one. ACL_NDL players showed strong to excellent effect sizes in average actEMG in GMax (d = 0.912) in favor of NDL, the injured limb (Table 6).

Statistical significance was obtained in ACL_NDL players in H peak torque of force (p = 0.011). H peak torque of force was higher in DL (non-injured limb). No other significant difference was obtained in ACL players. In NI players, significant differences were obtained between the limb's peak torque of force in Q (p = 0.017). They showed higher values in DL. In ACL_DL players, strong to excellent effect sizes were observed in peak torque of force in GMax (d = 0.830). Thus, they exhibited higher values for DL, the injured limb (Table 7).

Table 5. Comparison of peak actEMG outcomes during maximal voluntary isometric contraction between limbs in ACL and NI groups, considering DL or NDL.

	ACL INJURED DOMINANT LIMB (ACL_DL)					**ACL INJURED NO DOMINANT LIMB (ACL_NDL)**					**NO INJURED (NI)**				
	DL (Injured)	**NDL (No Injured)**	***p* Value**	**Cohen's d**	**Confidence Interval 95%**	**DL (No Injured)**	**NDL (Injured)**	***p* Value**	**Cohen's d**	**Confidence Interval 95%**	**DL**	**NDL**	***p* Value**	**Cohen's d**	**Confidence Interval 95%**
	Mean ± SD	**Mean ± SD**				**Mean ± SD**	**Mean ± SD**				**Mean ± SD**	**Mean ± SD**			
BF_peak actEMG (%)	85.81 ±6.50	91.29 ±5.53	0.041 *	0.91	(−10.67 to −0.30)	80.45 ±12.14	91.22 ±5.59	0.151	1.14	(−27.65 to 6.10)	90.06 ±5.05	93.61 ±5.45	0.227	0.67	(−9.88 to 2.78)
ST_peak actEMG (%)	83.79 ±83.79	90.39 ±6.80	0.290	0.60	(−20.04 to 6.83)	84.86 ±84.86	91.34 ±6.98	0.402	0.53	(−25.66 to 12.71)	89.38 ±89.38	86.44 ±5.96	0.356	0.42	(−4.09 to 9.96)
VL_peak actEMG (%)	87.04 ±87.04	88.21 ±8.75	0.827	0.12	(−13.11 to 10.77)	83.14 ±83.14	86.14 ±8.59	0.409	0.37	(−12.04 to 6.05)	89.18 ±89.18	89.39 ±5.16	0.949	0.03	(−7.84 to 7.42)
VM_peak actEMG (%)	82.68 ±82.68	89.68 ±8.77	0.201	0.74	(−18.59 to 4.59)	86.08 ±86.08	78.84 ±7.34	0.298	0.85	(−9.59 to 24.05)	87.44 ±87.44	86.78 ±7.43	0.855	0.07	(−7.52 to 8.83)
GMax_peak actEMG (%)	85.83 ±85.83	89.27 ±4.97	0.642	0.36	(−22.50 to 15.61)	86.02 ±86.02	94.37 ±4.67	0.043 *	1.21	(−18.12 to 1.42)	90.08 ±90.08	88.09 ±6.38	0.502	0.31	(−4.67 to 8.67)
Gm_peak actEMG (%)	92.27 ±92.27	85.17 ±9.05	0.126	1.03	(−3.11 to 17.31)	92.92 ±92.92	91.59 ±4.86	0.663	0.30	(−6.52 to 9.18)	88.04 ±88.04	91.08 ±6.16	0.411	0.49	(−11.25 to 5.17)

* Statistical significance $p < 0.05$. (BF) Biceps Femoris/(ST) Semitendinosus/(VL) Vastus Lateralis/(VM) Vastus Medialis/(GMax) Gluteus Maximus/(Gm) Gluteus Medius/(actEMG) EMG activation.

Table 6. Comparison between limbs in both groups Injured and No Injured considering DL or NDL on average actEMG outcomes during maximal voluntary isometric contraction.

	ACL INJURED DOMINANT LIMB (ACL_DL)					**ACL INJURED NO DOMINANT LIMB (ACL_NDL)**					**NO INJURED (NI)**				
	DL (Injured)	**NDL (No Injured)**	***p* Value**	**Cohen's d**	**Confidence Interval 95%**	**DL (No Injured)**	**NDL (Injured)**	***p* Value**	**Cohen's d**	**Confidence Interval 95%**	**DL**	**NDL**	***p* Value**	**Cohen's d**	**Confidence Interval 95%**
	Mean ± SD	**Mean ± SD**				**Mean ± SD**	**Mean ± SD**				**Mean ± SD**	**Mean ± SD**			
BF_average actEMG (%)	57.64 ±57.64	57.68 ±6.27	0.984	0.01	(−4.32 to 4.24)	51.17 ±51.17	49.49 ±10.01	0.851	0.15	(−21.64 to 25.00)	63.60 ±63.60	60.56 ±5.28	0.525	0.29	(−7.72 to 13.80)
ST_average actEMG (%)	48.57 ±48.57	54.23 ±9.75	0.311	0.43	(−17.73 to 6.41)	52.30 ±52.30	54.77 ±10.99	0.73	0.17	(−20.99 to 16.05)	60.21 ±60.21	53.55 ±6.38	0.215	0.57	(−4.89 to 18.20)
VL_average actEMG (%)	58.95 ±58.95	54.63 ±6.50	0.184	0.63	(−2.53 to 11.18)	51.12 ±51.12	55.57 ±8.25	0.175	0.43	(−11.95 to 3.06)	55.97 ±55.97	57.92 ±7.76	0.749	0.19	(−15.81 to 11.91)
VM_average actEMG (%)	54.13 ±54.13	54.20 ±5.89	0.991	0.01	(−11.86 to 11.73)	52.80 ±52.80	47.07 ±15.42	0.472	0.49	(−14.31 to 25.76)	53.94 ±53.94	51.83 ±10.47	0.760	0.15	(−13.53 to 17.73)
GMax_average actEMG (%)	47.33 ±47.33	54.44 ±6.36	0.347	0.81	(−25.65 to 11.43)	53.40 ±53.40	59.82 ±5.54	0.090	0.91	(−14.41 to 1.57)	55.83 ±55.83	52.25 ±5.66	0.501	0.41	(−8.35 to 15.51)
Gm_average actEMG (%)	59.51 ±59.51	52.30 ±10.86	0.202	0.93	(−5.93 to 20.36)	57.25 ±57.25	60.09 ±5.37	0.404	0.51	(−11.30 to 5.62)	58.57 ±58.57	60.02 ±8.34	0.829	0.10	(−16.69 to 13.80)

Table 7. Comparison between limbs in both groups ACL and NI considering DL or NDL on peak torque force outcomes during maximal voluntary isometric contraction.

	ACL INJURED DOMINANT LIMB (ACL_DL)					ACL INJURED NO DOMINANT LIMB (ACL_NDL)					NO INJURED (NI)				
	DL (Injured)	NDL (No Injured)	*p* Value	Cohen's d	Confidence Interval 95%	DL (No Injured)	NDL (Injured)	*p* Value	Cohen's d	Confidence Interval 95%	DL	NDL	*p* Value	Cohen's d	Confidence Interval 95%
	Mean ± SD	Mean ± SD				Mean ± SD	Mean ± SD				Mean ± SD	Mean ± SD			
H_peak_ Torque (Nm/kg)	1.55 ±1.55	1.50 ±0.39	0.720	0.12	(−0.29 to 0.40)	1.72 ±1.72	1.25 ±0.91	0.011 *	0.56	(0.18 to 0.77)	1.50 ±1.50	1.47 ±0.57	0.770	0.05	(−0.19 to 0.25)
Q_peak_ Torque (Nm/kg)	2.86 ±2.86	2.55 ±0.53	0.138	0.63	(−0.12 to 0.73)	2.92 ±2.92	2.54 ±0.97	0.270	0.32	(−0.45 to 1.21)	2.69 ±2.69	2.50 ±1.08	0.017 *	0.18	(0.04 to 0.32)
GMax_peak_ Torque (Nm/kg)	3.15 ±3.15	2.34 ±1.09	0.066	0.83	(−0.07 to 1.70)	2.67 ±2.67	2.71 ±0.93	0.877	0.04	(−0.60 to 0.54)	2.93 ±2.93	2.99 ±1.10	0.865	0.05	(−0.78 to 0.67)
Gm_peak_ Torque (Nm/kg)	2.22 ±2.22	1.80 ±1.07	0.245	0.47	(−0.37 to 1.20)	1.77 ±1.77	1.78 ±0.78	0.984	0.01	(−0.80 to 0.79)	1.61 ±1.61	1.52 ±0.68	0.305	0.14	(−0.11 to 0.30)

* Statistical significance $p < 0.05$. (H) hamstrings, (Q) quadriceps, (GMax) gluteus maximus, (Gm) gluteus medius.

4. Discussion

The aim of this study was to evaluate differences in actEMG and force between female football players with previous ACL injury and healthy players in BF, ST, VM, VL, GMax, and Gm during a maximal voluntary isometric contraction. Additionally, the study aimed to determine if differences occur based on the laterality of the injured limb.

In relation to BF, NI players develop a muscle functional pattern that involves a significantly higher value in average actEMG in the BF compared to ACL players during the maximal voluntary isometric contraction for NDL. In the comparison between limbs, the ACL_DL players obtained significantly higher values in peak actEMG in BF for NDL, the non-injured limb, compared with DL, the injured limb. In NI players, no significant difference was obtained between limbs in the biceps or in any other muscle. Therefore, ACL players demonstrated reduced actEMG in the BF muscle compared to NI players. This inhibition of BF actEMG was also evident in the injured limb compared to the non-injured limb in ACL players.

Recent studies have demonstrated that altered motor unit activity occurs after ACL surgery due to muscle inhibition. This mechanism explains changes in EMG signal resulting from the inflammatory components of the joint post-surgery, which promote presynaptic reflex inhibition of muscles surrounding the knee [8,19]. McPherson et al. have shown that ACL players exhibit reduced motor unit rate coding and recruitment size in Q and H muscles [21]. This mechanism, combined with women's lower H actEMG during pre-contact high-intensity actions and the use of the ST muscle as a graft in ACL surgery, contribute to greater dysfunction and muscle imbalances, particularly in women [27,34,35,52]. In our study, ACL players showed lower average actEMG in BF compared to NI. Additionally, ACL_DL players exhibited lower peak actEMG in BF on the injured side compared to the non-injured side. These findings support the high risk of imbalance described in the literature by H inhibition in women and show that it could be higher in ACL players [27,35]. This deficiency is associated with the Q dominance stabilization pattern observed in women [31,53], which results in an imbalance favoring the Q in the H/Q ratio [26]. Q contraction generates anterior shear force, which is typically countered by the H. If the H fails, the ACL endures increased tensile stress, acting as the sole antagonist to anterior shear forces [35]. During high-intensity stabilization tasks, if the Hs are insufficiently activated or inhibited while the Q torque of force is maximal, inadequate co-contraction may fail to counteract the action of Q, increasing the risk of ACL rupture.

In relation to peak torque of force, no differences were observed between groups, ACL and NI, nor in DL or NDL in any muscle. However, in the comparison between limbs, the ACL_NDL players showed a significantly higher peak torque of force in H for the DL, the non-injured limb, compared to NDL, the injured one.

Few studies have explored the functional relationship between actEMG and force outcomes; however, previous limited research has indicated a linear relationship between both during maximal voluntary isometric contraction [54]. This relationship results from the need to increase motor unit firing rates to overcome constant resistance in isometric tests [21,55]. Therefore, achieving maximum actEMG should be the physiological response. Consequently, higher actEMG correlates with greater peak torque of force production. In our study, there were no statistical differences in torque development in Hs between ACL and NI players. However, in ACL_NDL players, significantly higher values in peak torque of force development were obtained for DL, the non-injured limb. Moreover, as has been shown before, ACL players developed significantly less average actEMG in BF during maximal voluntary isometric contraction. Consequently, proximal musculature is likely compensating for the inhibited role of the H muscles, leading to dysfunctional adaptations that may not be detected by return-to-sport common test [21]. This could explain the high incidence and recurrence rates of ACL injuries, given that all participants in the study were actively competing. Thus, in high-intensity stabilization tasks, the ACL injury mechanism might be triggered.

Recent research has shown that BF muscle inhibition persists even one year post-ACL reconstruction [56,57]. Our study found that ACL players who had been playing for at least 4 months post-clearance still exhibited significant differences in peak and average actEMG between limbs compared to NI players, indicating BF muscle inhibition. However, there was no force imbalance between groups. Despite the similar functions of the BF and ST, their histological structures and anatomical dispositions create functional differences, leading to an imbalance in the inhibition pattern post-ACL reconstruction [56–59]. The BF has powerful contractility because it contains a higher quantity of fast-twitch type II fibers, which develop explosive efforts such as maximal voluntary isometric contraction [58]. However, these fibers require higher motor unit rate coding and recruitment, which, as previously explained, are decreased in ACL players [21]. These types of fibers fatigue quickly, which may contribute to the condition of BF inhibition observed in our study during maximal voluntary isometric contraction in ACL players. Furthermore, Wang et al. highlight the protective role of the BF in preventing knee abduction, which can cause valgus collapse and ACL rupture [59]. They suggest that the short head of the biceps femoris enhances knee stabilization during high-intensity tasks and exhibits a stronger contractile response when the hip is externally rotated, reducing the risk of valgus collapse [59]. Valgus collapse is typically defined by internal hip rotation [13,14]. BF muscle inhibition during peak and average actEMG in ACL players underscores the need to evaluate neuromuscular activation patterns during return-to-play tests, as balanced force might mask compensatory actions, contributing to high ACL injury recurrence rates.

In relation to GMax, ACL players obtained significantly higher values in average actEMG in GMax for NDL compared to NI players. Moreover, the ACL players developed higher values in peak actEMG in GMax for NDL compared to NI players with a strong-to-excellent effect size. In the comparison between limbs, the ACL_NDL players showed significantly higher values in peak actEMG in GMax for NDL, the injured limb. In addition, the ACL_NDL players developed higher values in average actEMG in GMax for NDL, the injured limb, compared to the DL, with a strong-to-excellent effect size. Therefore, ACL players obtained elevated values in peak and average actEMG in GMax compared to NI players for NDL. This increase was also evident in ACL_NDL players in peak and average actEMG in GMax for the injured limb compared to the non-injured one.

Many ACL injury prevention studies incorporate gluteal muscles into their analysis due to their role in stabilizing the knee through hip control. Maniar et al. highlight that while the Hs are crucial for counteracting anterior imbalances in the sagittal plane, gluteal muscles are vital for preventing valgus collapse in the frontal plane during high-intensity tasks as the main hip abductor when the hip is highly flexed [39,60]. Additionally, it serves as a protective muscle in the transverse plane as a hip external rotator [39]. Valgus collapse, defined by hip adduction and hip internal rotation, underscores the importance of GMax as an essential protective muscle counteracting this mechanism [13,14]. Our results show that ACL players exhibited significantly higher average actEMG values for the GMax compared to NI players during maximal voluntary isometric contraction. Moreover, ACL_NDL players demonstrate higher peak GMax actEMG values in the injured limb compared to the non-injured limb. However, there were no differences observed between ACL and NI players in the peak torque of force developed. Similarly, ACL_NDL players did not show differences in the peak torque of force between their limbs. As previously explained, during an isometric test, higher motor unit rate coding and recruitment must be associated with a higher rate of force development [54,55]. Nevertheless, in this case, this pattern could be the result of a dysfunctional adaptation in which the GMax exhibits an inefficient attempt at motor unit recruitment. Because of isometric resistance, the GMax tried to recruit a high rate of type I fibers, which comprise 52–68% of the muscle's fibers, oriented for endurance [61]. However, these fibers do not respond correctly or become prematurely inhibited. This could be due to proprioceptive failure resulting from compensatory muscular reactions aimed at overcoming the post-surgical weakness of this muscle. Consequently, the firing rate of the motor unit must increase to recruit type II fibers, enhancing effective force. Nevertheless,

this situation of hyperreactivity in the GMax may be compensating for the actEMG deficit of the BF due to their functional synergistic relationship. This may likely lead to early muscle fatigue, preventing the GMax from acting as a protector during high-intensity actions when the ACL is at risk of rupture.

No other muscles obtained significant differences in peak or average actEMG between ACL and NI players for DL or NDL nor in the comparison between limbs. In fact, most of the effect sizes observed between groups are small or medium, particularly in the VL. This may be because all players in the study, both ACL and NI, were competing at a high level at the time of the study. As explained by McPherson et al., 12 months post-surgery, actEMG in the Q tends to equalize, while actEMG in the H continues to show a significant imbalance between the injured and non-injured limbs in ACL players [8]. Similarly, Zebis et al. found in their study that actEMG differences in the VL between ACL-injured and NI players are not statistically significant [11].

In summary, the laterality analysis revealed that the NDL exhibited greater actEMG muscle imbalances both in the comparison between ACL and NI players and in the comparison between limbs in ACL players, DL and NDL.

Since players typically have better proprioceptive muscle representation in the DL, it may be more difficult to physiologically recover the actEMG when ACL injury affects the NDL limb [62,63]. Suh et al. emphasized that ACL players injured in the NDL showed weaker strength compared to those injured in the DL up to 6 months post-surgery, with differences being compensated by 12 months [63]. This suggests that players injured in the DL may more readily develop compensation strategies, assisting in the correction of biomechanical imbalances and facilitating their return to play.

Finally, in the current study, in the analysis between limbs, NI players obtained significantly higher values in peak torque of force in the Q in the DL compared to the NDL. This imbalance could result from two combined mechanisms. Firstly, as previously explained, players typically have better proprioceptive muscle representation in DL [62,63]. Secondly, football is a sport conditioned by ball contact through kicking. This situation favors the predominance of DL, which is usually preferred for kicking [40,64]. Female football players have been shown to perform between an average of 50 and 110 kicks during an elite soccer match [65]. This functional movement involves knee extension mediated by the Q. Additionally, female players perform between 70 and 190 sprints during a match, which usually involves hip flexion, also mediated by the quadriceps [64]. This high rate of kicking and sprinting activities promotes the overdevelopment of the Q.

4.1. Limitations

The measurements employed in this study utilized the maximal voluntary isometric contraction, which does not simulate the functional demands of the ACL injury mechanism. The maximal voluntary isometric contraction primarily aims to generate maximal muscle force, assuming the player possesses a well-integrated proprioceptive motor pattern. However, players typically do not engage imbalanced muscles such as the H or gluteal muscles with maximal isolated contraction during routine sports training, so they are not accustomed to this level of contraction. As a result, players may demonstrate different actEMG values if assessment tests simulate functional tasks similar to those related to the ACL injury mechanism. Moreover, isolated maximal voluntary isometric contraction may be a non-specific test to evaluate muscle condition because it is easily compensated by synergistic muscles involved in the same joint action.

Therefore, conducting new studies that consider these factors may provide valuable insights.

4.2. Clinical Implications

The main clinical question was whether isolated return-to-play tests that only assess the range of movement and joint position are sufficient to evaluate an ACL player's capability to recover and compete at a high level. Range of movement and joint position

may depend on different neuromuscular recruitment patterns. Consequently, while a player might perform well enough to pass a return-to-play test, they may still be using incorrect muscles, perpetuating imbalances. Thus, evaluating imbalances in neuromuscular recruitment patterns could be a useful tool to determine if muscles are resynchronized enough to develop a physiological motor pattern during functional sports tasks.

Therefore, in the future, it is important to develop methods that evaluate the neuromuscular motor pattern during functional tasks. This approach aims to prevent situations such as these observed in our study, where active semi-professional players competing after an ACL injury exhibited dysfunctional neuromuscular activation patterns. These patterns may perpetuate muscle imbalances, leading to fatigue and inhibition, thus maintaining the ACL injury mechanism.

5. Conclusions

The present study identified differences in actEMG in BF and GMax muscles between players with a previous ACL injury and NI players and between limbs in ACL players.

ACL players exhibited lower average actEMG in BF in the NDL compared to NI players and lower peak actEMG in BF in the injured DL compared to the non-injured limb. ACL_NDL players showed lower peak torque in H in the injured limb compared to the non-injured limb.

Our results showed that ACL players in the NDL exhibited significantly higher average actEMG values in GMax compared to NI players. Moreover, ACL_NDL players demonstrated higher peak actEMG in GMax values for the injured limb compared to the non-injured limb.

Other outcomes showed no significant differences between groups or limbs. Moreover, small-to-medium effect sizes were observed in most of the other outcomes, particularly in VL actEMG.

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Abbreviations

ACL	anterior cruciate ligament
actEMG	electromyographical activation
NI	no injury
BF	biceps femoris
ST	semitendinosus
VL	vastus lateralis

VM	vastus medialis
GMax	gluteus maximus
Gm	gluteus medius
DL	dominant limb
NDL	no dominant limb
H	hamstrings
Q	quadriceps

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