J Musculoskelet Neuronal Interact 2022; 22(2):179-192

Journal of Musculoskeletal and Neuronal Interactions JMNI

Original Article

The muscle contractile properties in female soccer players: inter-limb comparison using tensiomyography

Armin H. Paravlic[1,2,3], Zoran Milanović[2,3,4], Ensar Abazović[5], Goran Vučković[1], Darjan Spudić[1], Ziva Majcen Rošker[1], Maja Pajek[1], Janez Vodičar[1]

[1]Faculty of Sport, Institute of Kinesiology, University of Ljubljana, Ljubljana, Slovenia
[2]Science and Research Centre Koper, Institute for Kinesiology Research, Koper, Slovenia
[3]Faculty of Sports Studies, Masaryk University, Brno, Czech Republic;
[4]Faculty of Sport and Physical Education, University of Nis, Nis, Serbia;
[5]Faculty of Sport and Physical Education, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Abstract

Objective: The present study aimed to: i) determine the contractile properties of the major lower limb muscles in female soccer players using tensiomyography; ii) investigate inter-limb differences; and iii) compare inter-limb differences between different selections and playing positions. **Methods**: A total of 52 female soccer players (A team; U19 and U17) were recruited. The vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF), biceps femoris (BF), gastrocnemius medialis (GM), lateralis (GL) and tibialis anterior (TA) of both lower limbs were evaluated. **Results**: When the entire sample was assessed regardless of selection or playing position, there were significant inter-limb differences in all measured muscles except BF. Compared to the non-dominant limb, the dominant limb had higher delay time in VL (p=0.008), while showing lower values in VM (p=0.023), GL (p=0.043) and GM (p=0.006). Contraction time was lower in the RF of the dominant limb (p=0.005) and VM (p=0.047), while showing higher values in VL (p=0.036) and TA (p<0.001) as compared to the non-dominant limb. **Conclusion**: Given the differences found between the limbs in the whole sample studied, it is necessary to examine both limbs to gather a more in-depth understanding of underlying mechanisms related to neuromuscular functions in female soccer players. **Level of evidence**: Prognostic study, Level II.

Keywords: Asymmetry, Neuromuscular Function, Skeletal Muscle, TMG, Women's Football

Introduction

The popularity of female soccer has increased significantly in recent years[1]. Along with the increased popularity and number of female participants, an increasing number of injuries can be expected due to the complex nature of this sports discipline. Several injury prevention strategies have been proposed[2–4], however, a key component of injury prevention practice is a regular screening of athlete health and neuromuscular function[5]. Typically, various proxies of neuromuscular function and performance-based measures have been introduced to address injury risk. Most commonly, lateral and functional asymmetries in strength, power, balance, flexibility, and electromyographic muscle activity were considered[6].

Strength asymmetries have been observed in a variety of different sports[7–9], but have been the most studied in soccer[10–13]. Although asymmetries have been previously confirmed in soccer players[14–16], these asymmetries do not necessarily affect soccer performance[12,17,18], but can be interpreted as potentially problematic[19–22] in terms of injury occurrence. Hence, they can be considered as a valuable indicator for planning and programming future training activities and to provide guidelines for necessary improvements[23,24].

The assessment of inter-limb asymmetries can be often difficult in a real-life setting, due to time-consuming and complex methodology involved, coupled with robust and expensive equipment used. The tensiomiography (TMG)

The authors have no conflict of interest.

Corresponding author: Armin H. Paravlic, Faculty of Sport, Institute of Kinesiolgy, University of Ljubljana, Ljubljana, Slovenia; Science and Research Centre Koper, Institute for Kinesiology Research, Slovenia; Faculty of Sports Studies, Masaryk University, Brno, Czech Republic
E-mail: armin.paravlic@hotmail.com

Edited by: G. Lyritis
Accepted 30 January 2022

represents a relatively new method for assessing the contractile properties of superficial skeletal muscles by evaluating lateral muscle deformation induced by electrical stimuli. Compared to other measurement methods representing the gold standard in neuromuscular function evaluation, such as isokinetic dynamometry and/or force plate, neuromuscular assessment with TMG is independent of motivation or volitional effort, which are moderators of athletic performance[25].

TMG has been extensively used to measure muscle adaptations in different settings[26–28]. Although several time and distance related parameters of muscle contraction could be derived from TMG response, the contraction time (Tc) and maximal displacement amplitude i.e., displacement measure (Dm) proved to be the most reliable[29–31] and clinically relevant[26,28,32]. Decreased Tc values, would indicate a muscles with predominance of fast-twitch muscle fibres[33,34], while Dm provides an information about the muscle structure i.e., increased Dm correlates well with decreased muscle stiffness[26]. Additionally, alterations in Dm and the half-relaxation time (Tr) showed to be the most sensitive measures of muscle fatigue[35,36], with higher values indicating fatigued state[36] and/or in case of pathology such as anterior cruciate ligament (ACL) injury, it may indicate a muscles less resistant to fatigue[37]. Thus, TMG can be reliably used for non-invasive estimation of predominant skeletal muscle fibres[32], muscle fatigue monitoring[36,38], training and rehabilitation induced adaptations[27,28,39–41] and lastly, for neuromuscular risk factors assessment of ACL injury[37,42].

A few studies conducted on soccer players demonstrated the absence of bilateral asymmetry in elite and sub-elite male futsal players[43] and soccer players[18,44,45]. For example, Gill et al.[44] didn't find any difference in TMG parameters between dominant and non-dominant legs in Brazilian elite soccer players. Similarly, except for vastus medialis (VM) Tc, rectus femoris (RF) Tr and sustain time (Ts), and biceps femoris (BF) Ts, Alvarez-Diaz[45] found no significant difference for the majority of TMG variables assessed in injury-free, competitive Spanish soccer players. Thus, it can be argued that male soccer players with no history of musculoskeletal injuries leg dominance have no significant effect on TMG derived parameters[18,43–45].

On the other hand, TMG was shown to be a valuable tool for assessing neuromuscular risk factors in ACL injuries[37]. The authors compared lower extremity TMG parameters between the healthy side of ACL-injured subjects and those of the gender- and sport-matched healthy control group[37]. It was found that time-related parameters in the vastus lateralis (VL) and RF muscles, such as Tr, were 71% and 61% higher in ACL-injured subjects compared to controls. In addition, RF showed 7% and 31% higher Tc and Ts in ACL-injured subjects compared to controls, respectively. Finally, Dm of the BF was found to be 48% higher in ACL-injured subjects. Overall, the later results suggest that fatigue resistance and muscle stiffness of hamstring muscles may be risk factors for ACL injury[37].

It is well known that the requirements of a soccer game are gender-specific[46]. This could be due to the same characteristics of a soccer field and the same rules that apply regardless of anthropometric and physiological differences between the sexes[46]. Female soccer players cover less total distance during a match, less distance during high-intensity runs and sprints[47], and generally run slower than men[48]. Consequently, these factors may lead to different neuromuscular performance patterns of the major muscles that act in soccer[49,50], as well as greater load on their musculoskeletal system[46], resulting in different injury patterns[51] or a greater injury incidence in female than male soccer players[51–53].

To the best of the authors' knowledge, no TMG study with female soccer players can be found in the literature. Therefore, the purpose of the current study is threefold. First, we aimed to identify TMG-derived parameters for the major lower limb muscles in female soccer players; second, to investigate inter-limb differences; and third, to compare inter-limb differences across different selections and playing positions.

Methods

Subjects

The sample comprised of 52 female soccer players (average age: 18 ± 4 years; body height 168.46 ± 6.76 cm; body mass 60.98 ± 7.12 kg; body mass index 21.44 ± 1.71 kg/m^2) of the Slovenian National Team that were assessed at the beginning of the new 2021/2022 soccer season. One week before the actual data collection, players were informed about the measurement procedures and detailed study protocol. They were advised not to have a strenuous workout for at least 48 hours before the assessment, which was monitored by the team staff. Before the initial assessment, a brief meeting was held to explain a study protocol in detail where written consent of each athlete was obtained as well. All players were physically healthy, without acute pain, and serious lower limb injury-free for at least one year. This study was approved by the Ethical Committee of Faculty of Sport (University of Ljubljana, Slovenia).

Experimental approach to the problem

A cross-sectional cohort study was conducted to investigate the inter-limb differences in neuromuscular performance of female soccer players using TMG. To address this question, the 52 female soccer players from the three Slovenian National Team selections (A team [A], N=18; under 19 years of age [U19], N=11; and under 17 years of age [U17], N=23) were assessed at the beginning of the upcoming season. These included, 15 central defenders (CD), 10 fullbacks (FB), 18 midfielders (MF), and 9 forwards (FW). All players typically have 4 to 7 soccer-specific training sessions and one to three strength and power-based training sessions per week, depending on the specific periods of the season. There were nine left-sided dominant and 43 right-sided dominant soccer players. Leg

dominance was defined as the preferred leg the players are kicking the ball with [54]. All measurements were performed by the well experienced professionals.

All procedures were carried in the following order:

Anthropometry

Body mass and height were measured using a stadiometer and scale anthropometer (GPM, Model 101, Zurich, Switzerland) to the nearest 0.1 cm, while body mass was assessed with multifrequency bioelectrical impedance (InBody 720: Biospace, Tokyo, Japan) to the nearest of 0.05 kg. Additionally, fat mass, body fat percentage, skeletal muscle mass, fat free mass and total body water were calculated using manufacturer's algorithm.

Tensiomyography assessment

The contractile properties of the individual muscles were assessed by the non-invasive TMG method. We measured superficial muscles surrounding the knee joint. Therefore, BF assessment was performed while prone at rest at a knee angle set at 5° of knee flexion; whereas the vastus lateralis VL, vastus medialis (VM), and RF were measured while supine at rest at a knee angle set at 30° knee flexion. Foam pads were used for leg support in both positions. We used the well-established methodology previously described[28,29] In brief, following an electrically induced isometric twitch, the radial displacement of the muscle belly was recorded at the skin surface using a sensitive digital displacement sensor (TMG-BMC, Ljubljana, Slovenia). The sensor was set perpendicular to the skin normal plane above the muscle belly as recommended[55]. The rounded (5-cm diameter) self-adhesive cathode and anode (Axelgaard, Aarhus, Denmark) were set 5 cm distally and 5 cm proximally to the measuring point, on all muscles assessed. Electrical stimulation was applied through a TMG-100 System electro stimulator (TMG-BMC d.o.o., Ljubljana, Slovenia) with a pulse width of 1 ms and an initial amplitude of 20 mA. During each measurement, the amplitude was progressively increased by 20 mA increments until there was no further increase in the amplitude of the TMG response (Dm), which was usually accompanied by the maximal stimuli of 110 mA. Rest periods between two stimuli of 30 s were given between each stimulus to minimize the effects of fatigue and potentiation. More detailed testing procedures were previously described elsewhere[29,30]. From two maximal twitch responses, several TMG parameters were calculated, as follows: delay time (Td) as time from an electrical impulse to 10% of the Dm; Tc as time from 10% to 90% of Dm; Ts as time from 50% to 50% of Dm; and Tr as time from 90% to 50% of Dm. Additionally, from the TMG-derived parameters a myosin heavy chain (MHC) I (MHC-I) proportion (in %) was estimated as proposed by Šimunić et al.[32] for VL muscle only (Equation 1).

Equation 1:

$$MHC\text{-}I\ (\%) = 2.829 \left(\frac{\%}{ms}\right) * Td + 2.98 \left(\frac{\%}{ms}\right) * Tc + 1.27 \left(\frac{\%}{ms}\right) * Tr - 121.023\%$$

where *MHC – I* proportion represents MHC type I proportion in VL muscle, *Td* TMG-derived delay time, *Tc* TMG-derived contraction time, and *Tr* TMG-derived half relaxation time of VL muscle.

Moreover, the TMG proposed the algorithm for calculating both the lateral and functional symmetries which were implemented in the current investigation (Equations 2 and 3).

Equation 2:

$$LS = 0.1 \times \left(\frac{MIN(TdR;TdL)}{MAX(TdR;TdL)}\right) + 0.6 \times \left(\frac{MIN(TcR;TcL)}{MAX(TcR;TcL)}\right) + 0.1 \times \left(\frac{MIN(TsR;TsL)}{MAX(TsR;TsL)}\right) + 0.2 \times \left(\frac{MIN(DmR;DmL)}{MAX(DmR;DmL)}\right) \times 100$$

where LS represents the lateral symmetry, MIN – the minimum, MAX– the maximum, R – right leg parameters and L – left leg parameters.

Equation 3:

$$FS = 0.1 \times \frac{MIN(AVERAGE(TdRF;TdVL;TdVM);TdBF)}{MAX(AVERAGE(TdRF;TdVL;TdVM);TdBF)} + 0.8 \times \frac{MIN(AVERAGE(TcRF;TcVL;TcVM);TcBF)}{MAX(AVERAGE(TcRF;TcVL;TcVM);TcBF)} + 0.1 \times \frac{MIN(AVERAGE(TrRF;TrVL;TrVM);TrBF)}{MAX(AVERAGE(TrRF;TrVL;TrVM);TrBF)} \times 100$$

where FS represents a functional symmetry, MIN – the minimum, MAX– the maximum, R – right leg parameters and L – left leg parameters.

Statistical analysis

All data are presented as mean ± SD. Statistical analysis was performed using SPSS statistical software (version 27.0, IBM Inc, Chicago, USA). Descriptive statistics were used to summarize player general characteristics and all outcome measures. Normality was confirmed by visual inspection and using the Shapiro-Wilk test, while homogeneity of variances was tested using Levene's test for all dependent variables. Student t-test for paired samples was used to assess inter-limb differences (dominant vs. non-dominant limb). Main effects were studied with a repeated-measures General Linear Model with a limb (dominant vs. non-dominant) as within-subject factor, while selection (A vs. U19 vs. U17) and playing position (CD vs. FB vs. MF vs. FW) were used as between-subject factors. Where significant effects were found for limb, selection, or playing position (two-way interactions were excluded from the analysis), post-hoc pairwise comparisons were performed for each variable independently. Furthermore, one-way ANOVA with Bonferroni test corrections was used to identify differences for lateral symmetries between selections ($p \leq 0.017$) and playing positions ($p \leq 0.10$). In addition, partial Eta squared (η^2) effect size was reported for identified main and interaction effects, while percent difference (PD) was used to describe significant differences between limbs identified by the student t-test[56]. The criteria for effect size were small (η^2=.01), medium (η^2=.06), and large (η^2=.14)[57]. Statistical significance was accepted at $p \leq 0.05$ unless otherwise stated in the case of post-hoc tests with Bonferroni corrections.

Results

A total of 52 female soccer players were assessed, with the right leg defined as dominant in 43 players. Players in A team, U19 and U17 significantly differed on average, in

Table 1. Body height, weight and composition across different national team selections of female soccer players.

	Team							
	A (n = 18)		U19 (n = 11)		U17 (n = 23)		One-way ANOVA	
	Mean	SD	Mean	SD	Mean	SD	F value	P value
Age (years)	23.1	3.7[b,c]	16.9	.8[a]	15.0	.8[a]	66.157	**<0.001**
Training experience (years)	15.7	5.8[b,c]	8.5	2.7[a]	8.5	2.0[a]	20.769	**<0.001**
Height (cm)	169.6	6.8	166.9	5.6	167.1	6.6	0.919	0.406
Weight (kg)	63.9	7.1	58.3	6.8	58.2	4.5	5.196	**0.009**
Body mass index (kg/m^2)	22.2	1.4[c]	20.9	1.7	20.9	1.7[c]	3.933	**0.026**
Skeletal muscle mass (kg)	29.5	3.3[b,c]	26.0	3.0[a]	26.4	2.3[a]	7.614	**0.001**
Body fat mass (kg)	11.3	4.5	11.6	3.4	10.7	2.7	0.298	0.744
Body fat (%)	17.5	6.2	19.6	4.5	18.3	4.1	0.597	0.555
Fat free mass (kg)	52.6	5.7[b,c]	46.8	5.0[a]	47.4	3.9[a]	7.346	**0.002**
Total body water (%)	60.4	4.4	58.8	3.3	59.6	3.1	0.687	0.508
Irregular menstrual status (%)	5.6		9.1		17.4			

Bold value – significant difference; [a] - significantly different than A team, [b] – significantly different than U19 team; [c] – significantly different than U17 team.

training experience (p<0.001), body mass index (p=0.009), skeletal muscle mass (p=0.009) and fat free mass (p=0.002) (Table 1).

Inter-limb asymmetries regardless of team or playing position

At first, we confirmed the validity of TMG-derived parameters assessment, showing that the mean Tc values of thigh muscles were highest at BF, followed by RF, VM, and VL.

When the entire sample was assessed regardless of selection or playing position, there were significant inter-limb differences in all measured muscles except BF (Table 2 and Table 3). Compared to the non-dominant limb, the dominant limb had higher Td in VL (p=0.008; PD=3%), while showing lower values in VM (p=0.023; PD=-2%), GL (p=0.043; PD=2%) and GM (p=0.006; PD=2%). Tc was lower in the dominant limb RF (p=0.005; PD=5%) and VM (p=0.047; PD=-2%), while it showed higher values in VL (p=0.036; PD=2%) and TA (p<0.001; PD=5%) as compared to the non-dominant limb. In addition, lower Ts were observed in the dominant limb only in RF (p=0.005; PD=-5%) compared to the non-dominant limb. Tr was higher only in RF (p=0.014; PD=35%), while Dm showed a tendency to significantly higher values (p=0.059; PD=5%) in the dominant limb compared to the non-dominant limb only at VL. MHC-I% showed to be significantly higher in the dominant limb (p=0.009; PD=3%). Finally, functional symmetry of the ankle was higher in the dominant limb (p=0.011; PD=3%).

Inter-limb asymmetries between selections

There was significant main effect on limb dominance at almost all muscles assessed for TMG derived variables as follows (Table 2 and Table 3): RF - Tc (p=0.023; η^2=0.102); Ts (p=0.016; η^2=0.112); Tr (p=0.023; η^2=0.118); VL - Td (p=0.009; η^2=0.131); Tc (p=0.041; η^2=0.083); MHC-I% (p=0.008; η^2=0.134); GL – Td (p=0.024; η^2=0.099); GM – Td (p=0.006; η^2=0.144); TA – Tc (p<0.001; η^2=0.225); and functional ankle symmetry (p = 0.027; η^2 = 0.096). However, limb*selection interaction was identified for GM, Td variable only (p=0.037; η^2=0.126). Post-hoc analysis showed that A selection has higher GM Td (p=0.041, PD=4%) asymmetry when compared to U19 selection.

Inter-limb asymmetries between playing positions

There was significant main effect on limb dominance at almost all muscles assessed for TMG derived variables as follows (Table 4 and Table 5): RF - Tc (p=0.009; η^2=0.135); Ts (p=0.039; η^2=0.086); Tr (p=0.037; η^2=0.088); VL - Td (p=0.005; η^2=0.151); Tc (p=0.044; η^2=0.082); MHC-I% (p=0.007; η^2=0.144); VM - Tc (p=0.047; η^2=0.080); GL – Td (p=0.049; η^2=0.078); GM – Td (p=0.018; η^2=0.111); TA – Tc (p=0.001; η^2=0.205); and functional ankle symmetry (p=0.007; η^2=0.141). However, limb*playing position interaction was identified for VM Tr (p=0.017; η^2=0.189); GM Td (p=0.011; η^2=0.206); and functional ankle symmetry (p=0.030; η^2=0.168). The post hoc analysis did not reveal significant differences between the playing positions for any of the variables examined.

Discussion

The present study examined values of muscle contractile properties in female soccer players. We found significant inter-limb differences for at least one parameter in all muscles assessed, except for BF. There is no unique pattern of higher and/or lower values of muscle contractile properties based

Table 2. Comparisons between limbs of TMG-derived parameters between selections for thigh muscles only.

			Team										RM ANOVA			
			Total sample				A		U19		U17		Main effect		Interaction	
			Mean	SD	t value	p value	Mean	SD	Mean	SD	Mean	SD	F value	p value	F value	p value
Biceps Femoris (BF)	Td (ms)	ND	25.4	3.6	1.044	0.301	27.7	4.4	24.2	1.8	24.3	2.5	0.876	0.3540	0.026	0.974
		DOM	25.1	3.4			27.3	4.2	23.9	1.6	23.9	2.5				
	Tc (ms)	ND	29.8	5.1	1.007	0.319	29.3	6.1	29.5	3.9	30.3	5.0	1.1250	0.294	0.320	0.727
		DOM	29.2	4.9			28.1	4.6	28.8	4.6	30.2	5.2				
	Ts (ms)	ND	200.0	54.0	0.231	0.818	218.5	58.0	218.2	62.6	176.8	36.9	0.5860	0.448	1.831	0.171
		DOM	198.5	37.5			223.9	32.3	192.2	19.5	181.5	37.8				
	Tr (ms)	ND	62.3	48.7	1.281	0.206	54.3	49.5	88.3	66.0	56.2	34.6	3.3580	0.073	2.015	0.144
		DOM	52.7	26.9			50.6	25.2	50.4	14.6	55.6	32.8				
	Dm (mm)	ND	5.3	2.1	0.709	0.481	5.2	1.6	5.8	2.3	5.1	2.3	0.2010	0.656	0.413	0.664
		DOM	5.2	2.1			5.0	1.6	6.0	2.3	5.0	2.2				
Rectus Femoris (RF)	Td (ms)	ND	25.0	2.8	0.342	0.733	25.5	2.9	23.1	1.8	25.4	2.8	0.007	0.932	0.328	0.722
		DOM	24.9	2.1			25.5	2.2	23.5	2.0	25.0	1.8				
	Tc (ms)	ND	29.5	4.6	2.912	**0.005**	29.7	4.5	26.8	2.7	30.6	5.0	5.537	**0.023**	1.039	0.361
		DOM	28.2	4.2			28.4	3.8	26.6	3.7	28.7	4.6				
	Ts (ms)	ND	66.3	29.9	-2.432	0.019	70.5	31.9	55.3	25.6	68.3	30.2	6.1790	**0.016**	0.820	0.446
		DOM	79.5	40.4			91.9	46.8	70.8	40.0	74.1	34.2				
	Tr (ms)	ND	30.7	26.8	-2.545	**0.014**	32.0	27.9	23.5	22.3	33.0	28.3	6.553	**0.014**	0.861	0.429
		DOM	43.8	36.3			53.7	42.5	37.5	37.3	39.2	30.1				
	Dm (mm)	ND	7.3	2.0	-0.409	0.684	6.8	2.0	6.6	1.3	7.9	2.1	0.0990	0.754	0.137	0.872
		DOM	7.3	2.0			7.1	2.1	6.6	1.9	7.9	1.8				
Vastus Lateralis (VL)	Td (ms)	ND	21.5	1.4	-2.761	**0.008**	22.0	1.7	20.9	0.7	21.3	1.2	7.4000	**0.009**	0.163	0.850
		DOM	22.1	1.6			22.5	1.7	21.8	1.6	21.9	1.6				
	Tc (ms)	ND	20.7	1.7	-2.152	**0.036**	20.9	1.9	19.8	1.2	21.0	1.7	4.4080	**0.041**	0.893	0.416
		DOM	21.2	1.9			21.0	2.2	20.5	1.4	21.7	1.9				
	Ts (ms)	ND	65.1	33.0	0.505	0.616	72.1	34.5	42.7	15.7	70.3	34.4	0.0810	0.777	0.208	0.813
		DOM	62.3	38.0			69.7	41.1	46.2	36.6	64.2	35.3				
	Tr (ms)	ND	35.8	25.4	0.224	0.824	42.5	27.7	20.2	14.1	38.0	25.5	0.0120	0.914	0.206	0.814
		DOM	34.7	31.3			37.5	29.3	23.7	35.9	37.8	30.8				
	Dm (mm)	ND	5.0	1.2	-1.931	0.059	5.3	1.4	4.8	1.1	5.0	1.1	4.1740	0.046	0.562	0.574
		DOM	5.3	1.2			5.4	1.1	5.3	1.3	5.2	1.2				
Vastus Medialis (VM)	Td (ms)	ND	23.2	1.7	2.346	**0.023**	24.2	2.2	22.6	1.3	22.6	1.0	3.8730	0.055	0.643	0.530
		DOM	22.8	1.4			23.7	1.6	22.6	1.0	22.3	1.1				
	Tc (ms)	ND	23.6	2.0	2.038	**0.047**	24.3	2.3	23.3	2.2	23.3	1.5	2.4700	0.122	0.766	0.471
		DOM	23.1	2.1			23.8	2.4	23.3	1.7	22.4	1.9				
	Ts (ms)	ND	178.2	42.3	-0.152	0.88	205.1	54.1	168.8	33.3	161.6	21.5	0.0050	0.945	0.916	0.407
		DOM	179.4	37.5			192.4	37.4	172.3	25.6	172.6	40.9				
	Tr (ms)	ND	80.9	46.3	0.64	0.525	56.9	36.5	111.6	50.2	85.1	42.8	0.6630	0.419	0.309	0.735
		DOM	76.0	51.6			49.0	22.9	97.8	45.4	86.7	62.3				
	Dm (mm)	ND	7.1	1.3	0.972	0.336	6.4	1.6	7.7	1.3	7.4	0.9	0.5250	0.472	0.746	0.480
		DOM	7.0	1.2			6.5	1.4	7.6	1.3	7.1	0.9				
LS (%)	Biceps Femoris		88.9	6.6			88.3	6.6	90.3	6.2	88.8	7.0	0.3090	0.735		
	Rectus Femoris		88.9	5.6			88.3	5.0	89.8	5.7	88.9	6.3	0.2470	0.782		
	Vastus Lateralis		89.8	4.4			88.6	4.7	89.6	4.2	90.7	4.1	1.2770	0.288		
	Vastus Medialis		91.6	3.3			91.8	3.7	92.5	3.2	90.9	3.0	0.9090	0.410		

Table 2. *(Cont. from previous page).*

			Team										RM ANOVA			
			Total sample				A		U19		U17		Main effect		Interaction	
			Mean	SD	t value	p value	Mean	SD	Mean	SD	Mean	SD	F value	p value	F value	p value
FS (%)	Knee	ND	81.6	10.0	0.991	0.326	83.3	10.3	77.6	10.5	82.1	9.3	0.6470	0.425	0.099	0.906
	Knee	DOM	80.3	7.4			82.3	6.8	77.2	4.9	80.3	8.4				
MHC-I% (VL)	ND		6.0	9.8	-2.718	**0.009**	9.5	9.1	10.9	-0.2	5.3	6.6	7.5830	**0.008**	0.894	0.416
	DOM		9.2	10.3			10.8	10.2	11.8	5.1	6.6	10.4				

Bold value – significant difference; FS – functional symmetry; LS – lateral symmetry; MHC – I% - myosin heavy chain isoforms.

Table 3. Comparisons between limbs of TMG-derived parameters between selections for lower leg muscles only.

			Team										RM ANOVA			
			Total sample				A		U19		U17		Main effect		Interaction	
			Mean	SD	t value	p value	Mean	SD	Mean	SD	Mean	SD	F value	p value	F value	p value
Gastrocnemi Lateralis (GL)	Td (ms)	ND	20.0	1.5	2.075	**0.043**	20.8	1.6	19.9	1.6	19.4	1.1	5.3990	**0.024**	1.211	0.307
		DOM	19.6	1.4			20.2	1.5	19.3	1.2	19.3	1.4				
	Tc (ms)	ND	19.6	2.6	0.232	0.818	20.1	2.5	19.5	3.2	19.4	2.4	0.0110	0.916	0.962	0.389
		DOM	19.6	2.3			19.7	2.4	20.1	2.9	19.3	1.8				
	Ts (ms)	ND	207.4	34.6	-0.974	0.335	231.7	41.6	197.5	17.5	193.2	23.5	0.8040	0.374	0.653	0.525
		DOM	220.1	108.5			264.9	177.5	199.5	12.6	194.8	19.2				
	Tr (ms)	ND	28.1	20.2	0.636	0.528	36.8	32.1	23.7	5.4	23.3	6.1	0.1660	0.685	0.577	0.566
		DOM	26.6	10.6			32.5	14.5	26.1	6.4	22.3	5.4				
	Dm (mm)	ND	3.0	0.9	-0.633	0.530	3.1	1.1	3.2	0.8	2.8	0.7	0.0640	0.802	0.685	0.509
		DOM	3.1	1.0			3.2	1.2	3.1	0.9	3.0	0.9				
Gastrocnemi Medialis (GM)	Td (ms)	ND	22.6	2.4	2.855	**0.006**	24.0	3.0	21.6	1.6	22.0	1.8	8.2360	**0.006**	3.523	**0.037**
		DOM	22.1	2.0			22.8	2.5	21.4	1.2	21.9	1.7				
	Tc (ms)	ND	22.5	2.1	0.838	0.406	22.4	2.0	22.7	1.9	22.5	2.3	0.4260	0.517	1.170	0.319
		DOM	22.3	2.3			21.7	2.0	23.0	2.0	22.5	2.5				
	Ts (ms)	ND	158.7	59.5	-1.249	0.218	201.9	39.3	139.7	47.5	133.9	60.4	1.9150	0.173	1.285	0.286
		DOM	168.6	69.8			227.3	65.7	152.1	43.1	130.7	51.6				
	Tr (ms)	ND	53.7	38.6	-1.709	0.094	47.9	25.9	68.3	45.3	51.3	43.1	2.7910	0.101	2.8250	0.069
		DOM	66.2	45.4			83.4	54.3	70.4	40.8	50.8	35.3				
	Dm (mm)	ND	3.1	1.0	0.760	0.451	3.4	1.3	3.3	0.8	2.9	0.8	0.656	0.422	0.8010	0.455
		DOM	3.1	1.0			3.1	1.2	3.3	0.9	2.9	0.9				
Tibialis Anterior (TA)	Td (ms)	ND	20.5	1.8	-0.075	0.941	21.6	1.8	20.0	1.8	19.9	1.4	0.0010	0.970	1.9210	0.157
		DOM	20.5	1.9			22.1	1.9	19.7	1.1	19.6	1.2				
	Tc (ms)	ND	18.0	2.2	-3.888	**<0.001**	16.9	1.8	18.5	1.9	18.7	2.3	14.2640	**<0.001**	0.6230	0.540
		DOM	18.9	2.2			18.1	2.1	19.4	2.4	19.3	2.1				
	Ts (ms)	ND	199.6	36.4	-0.935	0.354	218.1	22.9	187.5	19.4	191.0	45.4	0.4860	0.489	0.3540	0.704
		DOM	213.7	108.8			221.4	33.0	189.2	24.4	219.4	161.0				

Table 3. *(Cont. from previous page).*

			Team										RM ANOVA			
			Total sample				A		U19		U17		Main effect		Interaction	
			Mean	SD	t value	p value	Mean	SD	Mean	SD	Mean	SD	F value	p value	F value	p value
	Tr (ms)	ND	32.5	25.9	-1.130	0.264	27.8	15.7	30.1	10.2	37.3	35.7	0.7230	0.399	0.3590	0.700
		DOM	49.3	102.6			36.7	13.4	30.7	10.2	67.9	153.4				
	Dm (mm)	ND	1.9	0.6	-0.997	0.323	1.8	0.6	1.8	0.8	2.0	0.6	0.9210	0.342	1.0080	0.372
		DOM	2.7	5.7			4.2	9.6	2.0	0.7	1.8	0.7				
LS (%)	Gastrocnemius Lateralis		91.7	5.3			91.4	91.4	5.5	91.3	3.5	92.2	6.0	0.1420	0.868	
	Gastrocnemius Medialis		91.2	4.4			91.4	91.4	3.6	90.8	5.7	91.1	4.6	0.0550	0.947	
	Tibialis Anterior		89.4	5.2			88.3	88.3	6.2	89.6	4.9	90.2	4.5	0.6770	0.513	
FS (%)	Ankle	ND	85.2	7.4	-2.641	**0.011**	82.3	7.7	87.7	6.9	86.1	6.9	5.2060	**0.027**	0.8240	0.445
		DOM	87.9	6.4			86.6	6.3	88.4	7.2	88.6	6.1				

Bold value – significant difference; FS – functional symmetry; LS – lateral symmetry; MHC – I% - myosin heavy chain isoforms.

Table 4. Comparisons between limbs of TMG-derived parameters between positions for thigh muscles only.

			Position											RM ANOVA		
			CD		FB		MF		FW		Main effect			Interaction		
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	F value	p value	Eta S	F value	p value	Eta S
Biceps Femoris (BF)	Td (ms)	ND	27.1	5.1	23.1	1.8	25.5	2.7	24.9	1.6	1.3060	0.259		2.2580	0.094	
		DOM	25.7	3.8	23.2	2.3	26.0	3.9	24.4	1.7						
	Tc (ms)	ND	31.5	6.7	27.0	4.7	29.8	4.0	29.7	4.0	1.2940	0.261		0.7080	0.552	
		DOM	30.0	6.2	26.8	4.0	30.3	4.8	28.2	2.3						
	Ts (ms)	ND	192.0	40.5	183.9	49.6	209.2	63.9	212.9	58.6	0.1790	0.674		0.3550	0.785	
		DOM	198.1	35.7	181.8	28.4	208.4	44.8	197.5	31.3						
	Tr (ms)	ND	59.6	43.8	63.2	46.8	64.6	54.0	61.4	55.6	1.8150	0.184		0.3360	0.799	
		DOM	44.6	17.4	43.4	11.2	64.2	34.8	53.7	29.0						
	Dm (mm)	ND	5.5	2.3	4.5	1.6	5.4	2.3	5.6	1.7	0.4760	0.493		0.1290	0.942	
		DOM	5.4	2.6	4.2	1.4	5.3	1.9	5.6	2.0						
Rectus Femoris (RF)	Td (ms)	ND	25.3	2.5	24.9	4.4	24.7	2.2	25.0	2.5	0.3700	0.546		0.5170	0.673	
		DOM	25.4	2.0	23.9	2.0	25.0	2.2	24.7	2.0						
	Tc (ms)	ND	29.1	4.7	29.7	6.0	29.9	2.4	29.1	6.4	7.5180	**0.009**	0.135	0.2920	0.831	
		DOM	27.8	4.0	27.6	5.2	28.6	3.7	28.4	4.6						
	Ts (ms)	ND	54.5	12.1	81.6	37.5	70.9	33.6	60.0	28.5	4.5030	**0.039**	0.086	0.2080	0.891	
		DOM	69.4	34.2	86.2	43.1	87.6	47.8	72.9	31.0						
	Tr (ms)	ND	19.7	7.1	45.2	37.7	33.1	28.5	27.9	25.3	4.6230	**0.037**	0.088	0.2670	0.849	
		DOM	34.2	30.2	52.3	41.9	51.4	42.5	35.4	23.0						
	Dm (mm)	ND	6.4	1.7	7.9	2.6	7.5	2.0	7.6	1.4	0.493	0.486		1.4550	0.239	
		DOM	6.9	2.0	8.0	2.1	7.0	1.9	8.0	1.9						

Table 4. *(Cont. from previous page).*

			Position								RM ANOVA					
			CD		FB		MF		FW		Main effect			Interaction		
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	F value	p value	Eta S	F value	p value	Eta S
Vastus Lateralis (VL)	Td (ms)	ND	22.0	1.5	21.1	1.5	21.4	1.2	21.0	1.1	8.5600	**0.005**	0.151	1.5620	0.211	
		DOM	21.9	1.5	21.9	2.0	22.4	1.5	22.3	1.7						
	Tc (ms)	ND	20.6	1.6	20.5	1.8	20.8	1.6	20.7	2.4	4.2810	**0.044**	0.082	0.1920	0.901	
		DOM	21.3	2.2	20.8	1.5	21.2	1.9	21.5	2.3						
	Ts (ms)	ND	72.5	35.1	60.4	32.2	64.9	32.5	58.2	34.7	0.1450	0.705		0.3160	0.814	
		DOM	67.8	43.7	68.6	44.2	60.0	32.6	50.8	33.5						
	Tr (ms)	ND	42.3	28.5	30.4	20.7	34.8	24.3	32.9	29.0	0.0070	0.935		0.7580	0.523	
		DOM	35.8	29.8	43.6	41.6	33.1	27.8	26.2	30.6						
	Dm (mm)	ND	4.9	1.1	4.7	0.9	5.1	1.4	5.6	0.9	3.3420	0.074		0.2810	0.839	
		DOM	5.0	1.3	4.9	1.0	5.4	1.3	5.9	0.5						
Vastus Medialis (VM)	Td (ms)	ND	23.6	2.2	22.3	1.0	23.6	1.2	22.7	2.1	3.7090	**0.060**	0.072	0.6840	0.566	
		DOM	23.3	1.6	22.1	1.4	22.9	1.3	22.6	1.2						
	Tc (ms)	ND	23.2	1.8	22.9	1.6	24.7	1.7	23.0	2.7	4.1650	**0.047**	0.080	0.5400	0.657	
		DOM	23.2	1.6	21.9	2.0	23.9	2.0	22.4	2.5						
	Ts (ms)	ND	192.0	59.3	163.6	28.4	179.5	34.2	168.8	33.7	0.0490	0.825		0.7090	0.551	
		DOM	179.2	40.0	182.4	51.4	185.5	24.8	164.0	39.3						
	Tr (ms)	ND	78.7	52.2	66.7	30.4	91.1	50.7	80.1	43.7	0.0080	0.930		3.7260	**0.017**	0.189
		DOM	63.3	39.9	110.5	75.0	70.5	42.5	69.6	46.2						
	Dm (mm)	ND	6.7	1.4	6.9	0.7	7.4	1.4	7.6	1.6	0.5500	0.462		0.4390	0.726	
		DOM	6.7	1.4	7.0	0.9	7.0	1.1	7.4	1.7						
LS (%)	Biceps Femoris (%)		85.3	7.3	89.6	7.0	90.5	5.8	91.1	4.6	2.3570	0.083				
	Rectus Femoris (%)		90.2	5.6	87.7	7.0	87.7	5.5	90.3	4.3	0.8640	0.466				
	Vastus Lateralis (%)		89.7	3.5	91.8	3.3	88.8	5.4	89.6	4.4	1.0390	0.384				
	Vastus Medialis (%)		92.1	3.0	91.3	3.7	91.7	3.6	90.8	3.2	0.3020	0.824				
FS (%)	Knee	ND	77.4	10.9	87.7	6.7	82.4	10.0	79.9	9.3	0.396	0.532		1.383	0.259	
		DOM	77.7	6.8	85.1	7.6	78.6	7.1	82.8	6.4						
MHC-I% (VL)	ND		8.3	10.2	3.7	9.3	6.2	8.7	4.3	12.3						
	DOM		8.9	11.4	8.6	8.5	9.8	10.5	9.4	11.7	8.068	**0.007**	0.144	0.739	0.534	

Bold value – significant difference; FS – functional symmetry; LS – lateral symmetry; MHC – I% - myosin heavy chain isoforms.

Table 5. Comparisons between limbs of TMG-derived parameters between positions for lower leg muscles only.

			Position								RM ANOVA					
			CD		FB		MF		FW		*Main effect*			*Interaction*		
			Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*F value*	*p value*	*Eta S*	*F value*	*p value*	*Eta S*
Gastrocnemi Lateralis (GL)	Td (ms)	*ND*	20.1	1.2	19.4	1.3	20.4	1.9	19.6	1.3	4.0730	**0.049**	0.049	2.1090	0.111	
		DOM	19.1	1.4	19.3	0.9	20.4	1.7	19.3	1.1						
	Tc (ms)	*ND*	19.4	2.9	19.4	1.7	20.0	2.8	19.6	2.7	0.1570	0.694		0.4010	0.753	
		DOM	19.2	2.3	19.6	1.9	20.1	2.7	19.1	1.9						
	Ts (ms)	*ND*	211.0	36.1	197.0	19.6	205.4	24.3	216.8	58.2	0.4530	0.504		1.4540	0.239	
		DOM	264.9	196.2	185.5	12.9	208.0	22.3	207.9	26.3						
	Tr (ms)	*ND*	25.4	6.4	21.3	3.9	27.6	7.3	41.1	46.1	0.7020	0.406		1.0110	0.396	
		DOM	25.4	13.5	23.8	6.0	26.9	8.2	31.3	13.2						
	Dm (mm)	*ND*	2.8	1.0	2.6	0.5	3.1	0.7	3.5	0.9	0.6000	0.442		0.4220	0.738	
		DOM	2.9	1.0	2.8	1.0	3.1	1.0	3.5	0.9						
Gastrocnemi Medialis (GM)	Td (ms)	*ND*	23.3	2.5	21.3	1.3	23.2	3.0	21.9	1.3	6.0150	**0.018**	0.111	4.1420	**0.011**	0.206
		DOM	21.9	1.8	21.6	1.5	22.8	2.5	21.6	1.0						
	Tc (ms)	*ND*	22.7	1.9	22.6	1.7	22.9	2.3	21.4	2.2	0.5760	0.452		2.1310	0.109	
		DOM	21.5	1.8	22.6	3.2	23.2	2.1	21.4	1.3						
	Ts (ms)	*ND*	179.9	61.4	156.6	61.8	148.2	63.0	146.7	44.6	1.0880	0.302		1.8650	0.148	
		DOM	185.6	107.3	133.1	51.1	170.7	46.6	175.7	34.5						
	Tr (ms)	*ND*	53.2	32.7	64.6	48.6	45.3	32.1	59.4	49.3	1.5980	0.212		1.1870	0.325	
		DOM	74.8	48.6	50.3	35.4	66.4	40.2	69.2	60.8						
	Dm (mm)	*ND*	2.9	1.2	3.0	0.8	3.4	1.0	3.2	0.9	0.552	0.461		0.7130	0.549	
		DOM	2.9	1.0	3.1	1.1	3.3	1.1	2.9	0.7						
Tibialis Anterior (TA)	Td (ms)	*ND*	20.8	1.8	19.3	1.6	21.1	1.6	20.0	1.8	0.0170	0.896		1.4080	0.252	
		DOM	21.0	2.2	20.0	0.9	20.9	1.8	19.6	1.9						
	Tc (ms)	*ND*	17.8	2.8	18.9	1.7	17.9	1.6	17.7	2.6	12.3600	**0.001**	0.205	0.8400	0.479	
		DOM	19.2	2.2	19.3	2.2	18.7	2.4	18.4	2.0						
	Ts (ms)	*ND*	203.2	35.4	206.9	57.9	195.3	27.8	194.3	25.9	0.3230	0.573		0.6260	0.601	
		DOM	214.9	36.3	189.8	43.8	234.7	178.6	196.2	33.5						
	Tr (ms)	*ND*	27.0	17.2	55.5	47.8	26.8	11.9	27.4	8.3	0.5750	0.452		0.6920	0.561	
		DOM	35.7	19.6	44.9	37.4	71.8	171.9	31.6	13.6						
	Dm (mm)	*ND*	1.6	0.6	1.9	0.4	1.9	0.7	2.2	0.8	0.6350	0.430		0.8140	0.492	
		DOM	4.4	10.6	1.9	0.5	2.0	0.7	2.1	0.7						
LS (%)	Gastrocnemius Lateralis		79.33	92.7	5.2	91.5	5.5	92.6	5.6	88.7	4.4	1.3500	0.269			
	Gastrocnemius Medialis		89.33	90.3	2.1	91.7	4.1	92.1	5.6	90.1	5.4	0.6800	0.568			
	Tibialis Anterior		91.00	87.1	7.3	90.2	3.9	89.8	4.1	91.7	3.0	1.7640	0.167			
FS (%)	Ankle	*ND*	83.5	8.8	89.6	5.8	85.1	5.7	83.2	8.3	7.8620	**0.007**	0.141	3.2390	**0.030**	0.168
		DOM	87.9	6.5	87.6	6.4	86.7	6.8	90.3	5.2						

Bold value – significant difference; FS – functional symmetry; LS – lateral symmetry; MHC – I% - myosin heavy chain isoforms; VL – Vastus lateralis.

on the limb dominance (e.g., Tc was lower in the dominant limb RF and VM, while it showed higher values in VL and TA compared to the non-dominant limb). Furthermore, the MHC-I proportion showed to be significantly higher in the dominant limb, as well as functional symmetry of the dominant limb ankle. Finally, non-significant inter-limb differences were observed for most of the variables assessed when different selections or playing positions were compared.

To the best of our knowledge, this is the first study to show the muscle contractile properties in female soccer players. Overall, we confirmed the validity of the TMG measurements by showing that the mean Tc values of the thigh muscles were highest at BF (29.2 ms), followed by RF (28.2 ms), VM (23.1 ms), and VL (21.2 ms)[26,28,29]. Since, the neuromuscular system has been shown to be affected by an individual's physical activity level[58–60] in exercise modality dependent manner[61], it was not surprising that muscle contractile properties in female soccer players differ from those reported in older symptomatic population[28], habitually inactive adults[61], recreationally active adults[39], endurance[62] and/or power trained athletes[63]. For example, Tc values in BF, showed that female soccer players have shorter contraction times (29.3 ms) than highly trained amateur road cyclists (42.5 ms)[62], but longer compared to power trained athletes (14.3 ms)[63] and/or male professional soccer players (26.3 ms)[64]. The shorter Tc value would indicate muscles with a predominance of fast-twitch muscle fibres[33,34], and thus shorter Tc in power athletes could most likely be due to training specificity[65–67], resulting in a greater proportion of fast-twitch fibers i.e., MHC IIa and IIx[65], compared to the levels observed in the general population (71% vs. 58%)[68]. Fast-twitch muscle fibers tend to shorten faster due to higher myosin ATPase activity and thus can generate more force[69]. In the present study, players were evaluated two weeks before the start of the preparation period for the upcoming season. Therefore, any observed difference between the results of our study examining only female players and those reported for male soccer players could likely be attributed to the study period (pre-season vs. in-season)[64,70], as MHC content does not differ between genders[68], but could be altered by training interventions[39,67,71]. Nevertheless, a soccer game demands are known to be sex-specific[46]. Female soccer players covers less total distance during a match and less distance during high intensity and sprint runs[47], while generally running slower than males[48], which may lead to different neuromuscular performance patterns of the major muscles acting in soccer[49,50].

We found significant differences between limbs for the vast majority of variables and muscles examined. In addition to the higher MHC-I content in VL and the functional symmetry of the ankle of the dominant limb, individual TMG-derived parameters such as Td, Tc, and Tr also differed between the dominant and non-dominant limb. Thus, the current study does not support previous findings showing no significant bilateral differences in male professional soccer players[44,45,72,73] and futsal players[43]. Limb dominance in the present study was defined as that with which players kick the ball[54]. Therefore, repetitive activity consisting of high-speed contraction movements such as ball kicking, jumping, and sprinting, in conjunction with other training modalities, may lead to positive neuromuscular adaptation seen through greater recruitment of fast-twitch muscle fibres[64,71,74]. Interestingly, Tc values were higher in the dominant limb VL, while lower in RF and VM compared to the non-dominant limb muscles. Further testing using the multiple linear regression model to estimate MHC-I content showed that the dominant limb VL has a 3% higher MHC-I content compared to the non-dominant limb. These results correlate quite well with previous findings where unequal changes in TMG responses were found in BF and RF dominant limb after soccer-specific training[71,75].

TMG has been extensively used to measure muscle adaptations in different settings[26–28]. Although several time and distance-related parameters of muscle contraction could be derived from TMG response, Tc and Dm proved to be the most reliable[29–31] and clinically relevant[26,28,32]. Shorter Tc values would indicate a muscles with predominance of fast-twitch muscle fibres[33,34], whereas Dm provides an information about the muscle structure i.e., increased Dm correlates well with decreased muscle stiffness[26]. Present investigation failed to find any differences in Dm regardless of muscle assessed. However, consistent with previous findings conducted in male soccer players[45], we found higher Tr in the dominant limb RF compared to the non-dominant limb. Changes in Dm and Tr were found to be the most sensitive measures of muscle fatigue[35,36], with higher values indicating a fatigued state[36] and/or, in the case of pathology such as ACL injury, may indicate a less fatigue-resistant muscle[37]. Therefore, the present results suggest that the dominant limb RF may be prone to fatigue earlier during exercise[37]. The RF plays a very important role in soccer-specific movements such as sprinting and kicking the ball[74,76]. It is the only muscle within the quadriceps muscle group that acts across two major lower body joints and thus regulates knee flexion and/or or hip extension. Given a high velocity of movements involved in, RF showed to be the most frequently injured muscle of the quadriceps muscle group[77,78], with long return to play time ranging from four[79] to eight months[80]. The clinical significance of the current findings has been previously confirmed[41], showing that the Tr of the ACL-injured limb of RF was greater than that of the non-injured side after ACL reconstruction surgery. The extent to which these findings, collected from injury-free female soccer players, may have clinical significance as a valuable indicator for prevention of future injuries remains to be determined. Unlike other research carried out in male soccer players[64], we did not find any significant difference for inter-limb asymmetries between selections and/or playing positions. The reason for this observation may lie in the study period, i.e. before the start of the preparation period for the upcoming season, when neuromuscular performance normally declines regardless of age and or

trainability level[81] making it difficult to distinguish between highly trained and less trained athletes.

We are aware that our research may have some limitations. We used technology that measures neuromuscular performance under static conditions in the supine position, which is different from soccer-specific requirements. However, the assessment of an individual skeletal muscle belly, providing multiple contractile parameters from a single assessment, has provided new insights in the study of asymmetries in female soccer. Future studies aimed at investigating muscle contractile properties between different training periods, the value of TMG-derived parameters for injury prediction, and the relationship between muscle contractile properties and other sport-related performance measures in female soccer players are warranted.

Conclusion

Given the differences found between the limbs in the whole sample studied, it is necessary to examine both limbs to gather a more in-depth understanding of underlying mechanisms related to neuromuscular functions in female soccer players. We found that Tc levels in female soccer players were higher than those observed in male soccer players in the published literature. This is likely due to the study period, training specificity, and different match demands, which should be investigated in future studies examining differences in neuromuscular profiles between female and male soccer players. Finally, we found no differences in the asymmetry of TMG parameters between different selections and playing positions. Thus, future studies investigating TMG parameters with other well-established measures of neuromuscular function such as isokinetic strength, jumping ability and running speed in female soccer players are warranted.

Funding

Measurements were performed in the framework of the national project funded by the Ministry of Education, Science and Sport (project ID: C3330-21-406065) and Slovenian Sports Foundation (project ID: D5-21-024). Also, this project was supported by the Slovenian Research Agency (ARRS) (project ID: P5-0147 entitled "The kinesiology of mono-structured, poly-structured and conventional sports," project manager: Prof. dr. Matej Supej).

Acknowledgements

The authors would like to thank the participants enrolled in the study.

References

1. Datson N, Hulton A, Andersson H, et al. Applied physiology of female soccer: An update. Sport Med 2014;44(9):1225-1240.
2. McCall A, Dupont G, Ekstrand J. Injury prevention strategies, coach compliance and player adherence of 33 of the UEFA Elite Club Injury Study teams: A survey of teams' head medical officers. Br J Sports Med 2016;50(12):725-730.
3. Talpey SW, Siesmaa EJ. Sports Injury Prevention: The Role of the Strength and Conditioning Coach. Strength Cond J 2017;39(3):14-19.
4. Fanchini M, Steendahl IB, Impellizzeri FM, et al. Exercise-Based Strategies to Prevent Muscle Injury in Elite Footballers: A Systematic Review and Best Evidence Synthesis. Sport Med 2020;50(9):1653-1666.
5. Hughes T, Sergeant JC, van der Windt DA, Riley R, Callaghan MJ. Periodic Health Examination and Injury Prediction in Professional Football (Soccer): Theoretically, the Prognosis is Good. Sport Med 2018; 48(11):2443-2448.
6. Bishop C, Turner A, Read P. Effects of inter-limb asymmetries on physical and sports performance: a systematic review. J Sports Sci 2018;36(10):1135-1144.
7. Ueberschär O, Fleckenstein D, Warschun F, Kränzler S, Walter N, Hoppe MW. Measuring biomechanical loads and asymmetries in junior elite long-distance runners through triaxial inertial sensors. Sport Orthop Traumatol 2019;35(3):296-308.
8. Fohanno V, Nordez A, Smith R, Colloud F. Asymmetry in elite rowers: effect of ergometer design and stroke rate. Sport Biomech 2015;14(3):310-322.
9. Pajek MB, Hedbávný P, Kalichová M, Čuk I. The asymmetry of lower limb load in balance beam routines. Sci Gymnast J 2016;8(1):5-13.
10. Fousekis K, Tsepis E, Vagenas G. Lower limb strength in professional soccer players: Profile, asymmetry, and training age. J Sport Sci Med 2010;9(3):364-373.
11. Ruas C V., Minozzo F, Pinto MD, Brown LE, Pinto RS. Lower-extremity strength ratios of professional soccer players according to field position. J Strength Cond Res 2015;29(5):1220-1226.
12. Loturco I, Pereira LA, Kobal R, et al. Do asymmetry scores influence speed and power performance in elite female soccer players? Biol Sport 2019;36(3):209-216.
13. Pardos-Mainer E, Casajús JA, Gonzalo-Skok O. Adolescent female soccer players' soccer-specific warm-up effects on performance and inter-limb asymmetries. Biol Sport 2019;36(3):199-207.
14. Ergün M, Işlegen Ç, Taşkiran E. A cross-sectional analysis of sagittal knee laxity and isokinetic muscle strength in soccer players. Int J Sports Med 2004;25(8):594-598.
15. Magalhães J, Oliveira J, Ascensão A, Soares J. Concentric quadriceps and hamstrings isokinetic strength in volleyball and soccer players. J Sports Med Phys Fitness 2004;44(2):119-125.
16. Voutselas V, Papanikolaou Z, Soulas D, Famisis K. Years of training and hamstring-quadriceps ratio of soccer players. Psychol Rep 2007;101(3 I):899-906.
17. Lockie R, Callaghan S, Berry S, et al. Relationship between unilateral jumping ability and asymmetry on multidirectional speed in team-sport athletes. J Strength Cond Res 2014;28(12):3557-3566.
18. Loturco I, Pereira LAR, Abad K, et al. Functional

Screening Tests: Interrelationships and Ability to Predict Vertical Jump Performance. Int J Sports Med 2018; 39(3):189-197.
19. Barber SD, Noyes FR, Mangine RE, McCloskey JW, Hartman W. Quantitative assessment of functional limitations in normal and anterior cruciate ligament-deficient knees. Clin Orthop Relat Res 1990;(255):204-214.
20. Bishop C. Interlimb Asymmetries: Are Thresholds a Usable Concept? Strength Cond J 2021;43(1):32-36.
21. Knapik JJ, Bauman CL, Jones BH, Harris JM, Vaughan L. Preseason strength and flexibility imbalances associated with athletic injuries in female collegiate athletes. Am J Sports Med 1991;19(1):76-81.
22. Noyes FR, Barber SD, Mangine RE. Abnormal lower limb symmetry determined by function hop tests after anterior cruciate ligament rupture. Am J Sports Med 1991;19(5):513-518.
23. Maloney S. The Relationship Between Asymmetry and Athletic Performance: A Critical Review. J Strength Cond Res 2019;33(9):2579-2593.
24. Souissi S, Chaouachi A, Burnett A, et al. Leg asymmetry and muscle function recovery after anterior cruciate ligament reconstruction in elite athletes: a pilot study on slower recovery of the dominant leg. Biol Sport 2020;37(2):175-184.
25. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev 2001;81(4):1725-1789.
26. Pišot R, Narici MV, Šimunič B, et al. Whole muscle contractile parameters and thickness loss during 35-day bed rest. Eur J Appl Physiol 2008;104(2):409-414.
27. Zubac D, Paravlić A, Koren K, Felicita U, Bostjan S. Plyometric exercise improves jumping performance and skeletal muscle contractile properties in seniors. J Musculoskelet Neuronal Interact 2019;19(1):38-49.
28. Paravlić, Pisot R, Simunic B. Muscle-specific changes of lower extremities in the early period after total knee arthroplasty: Insight from tensiomyography. J Musculoskelet Neuronal Interact 2020;20(3):390-397.
29. Šimunič B. Between-day reliability of a method for non-invasive estimation of muscle composition. J Electromyogr Kinesiol 2012;22(4):527-530.
30. Paravlić, Zubac D, Šimunič B. Reliability of the twitch evoked skeletal muscle electromechanical efficiency: A ratio between tensiomyogram and M-wave amplitudes. J Electromyogr Kinesiol 2017;37:108-116.
31. Tous-Fajardo J, Moras G, Rodríguez-Jiménez S, Usach R, Doutres DM, Maffiuletti NA. Inter-rater reliability of muscle contractile property measurements using non-invasive tensiomyography. J Electromyogr Kinesiol 2010;20(4):761-766.
32. Šimunic B, Degens H, Rittweger J. Noninvasive Estimation of Myosin Heavy Chain Composition in Human Skeletal Muscle. Med Sci Sport Exerc Sport Exerc 2011;d(February):27-30.
33. Valenčič V, Knez N. Measuring of skeletal muscles' dynamic properties. Artif Organs 1997;21(3):240-242.
34. Dahmane R, Djordjevič S, Šimunič B, Valenčič V. Spatial fiber type distribution in normal human muscle: Histochemical and tensiomyographical evaluation. J Biomech 2005;38(12):2451-2459.
35. Križaj D, Šimunič B, Žagar T. Short-term repeatability of parameters extracted from radial displacement of muscle belly. J Electromyogr Kinesiol 2008;18(4):645-651.
36. García-Manso JM, Rodríguez-Ruiz D, Rodríguez-Matoso D, de Yves S, Sarmiento S, Quiroga M. Assessment of muscle fatigue after an ultra-endurance triathlon using tensiomyography (TMG). J Sports Sci 2011;29(6):619-625.
37. Alentorn-Geli E, Alvarez-Diaz P, Ramon S, et al. Assessment of neuromuscular risk factors for anterior cruciate ligament injury through tensiomyography in male soccer players. Knee Surgery, Sport Traumatol Arthrosc 2015;23(9):2508-2513.
38. Hunter, Galloway SDR, Smith IJ, et al. Assessment of eccentric exercise-induced muscle damage of the elbow flexors by tensiomyography. J Electromyogr Kinesiol 2012;22(3):334-341.
39. Zubac D, Šimunič B. Skeletal Muscle Contraction Time and Tone Decrease After 8 Weeks of Plyometric Training. J Strength Cond Res 2017;31(6):1610-1619.
40. Seijas R, Marín M, Rivera E, et al. Gluteus maximus contraction velocity assessed by tensiomyography improves following arthroscopic treatment of femoroacetabular impingement. Knee Surgery, Sport Traumatol Arthrosc 2018;26(3):976-982.
41. Alvarez-Diaz P, Alentorn-Geli E, Ramon S, et al. Effects of anterior cruciate ligament reconstruction on neuromuscular tensiomyographic characteristics of the lower extremity in competitive male soccer players. Knee Surgery, Sport Traumatol Arthrosc 2015;23(11):3407-3413.
42. Alentorn-Geli E, Alvarez-Diaz P, Ramon S, et al. Assessment of gastrocnemius tensiomyographic neuromuscular characteristics as risk factors for anterior cruciate ligament injury in male soccer players. Knee Surgery, Sport Traumatol Arthrosc 2015;23(9):2502-2507.
43. López-Fernández J, García-Unanue J, Sánchez-Sánchez J, Colino E, Hernando E, Gallardo L. Bilateral asymmetries assessment in elite and sub-elite male futsal players. Int J Environ Res Public Health 2020;17(9):1-11.
44. Gil S, Loturco I, Tricoli V, et al. Tensiomyography parameters and jumping and sprinting performance in Brazilian elite soccer players. Sport Biomech 2015; 14(3):340-350.
45. Alvarez-Diaz P, Alentorn-Geli E, Ramon S, et al. Comparison of tensiomyographic neuromuscular characteristics between muscles of the dominant and non-dominant lower extremity in male soccer players. Knee Surgery, Sport Traumatol Arthrosc 2014;24(7):2259-2263.
46. Pedersen AV, Aksdal IM, Stalsberg R. Scaling demands

of soccer according to anthropometric and physiological sex differences: A fairer comparison of men's and women's soccer. Front Psychol 2019;10(APR):1-11.
47. Bradley PS, Dellal A, Mohr M, Castellano J, Wilkie A. Gender differences in match performance characteristics of soccer players competing in the UEFA Champions League. Hum Mov Sci 2014;33(1):159-171.
48. Baumgart C, Freiwald J, Hoppe M. Sprint Mechanical Properties of Female and Different Aged Male Top-Level German Soccer Players. Sports 2018;6(4):161.
49. Lehnert M, Croix MDS, Zaatar A, Lipinska P, Stastny P. Effect of a simulated match on lower limb neuromuscular performance in youth footballers - a two year longitudinal study. Int J Environ Res Public Health 2020;17(22):1-17.
50. Reilly T, Drust B, Clarke N. Muscle fatigue during football match-play. Sport Med 2008;38(5):357-367.
51. Brophy R, Silvers HJ, Gonzales T, Mandelbaum BR. Gender influences: The role of leg dominance in ACL injury among soccer players. Br J Sports Med 2010;44(10):694-697.
52. Giza E, Mithöfer K, Farrell L, Zarins B, Gill T. Injuries in women's professional soccer. Br J Sports Med 2005; 39(4):212-216.
53. Ireland ML. The female ACL: Why is it more prone to injury? J Orthop 2016;13(2):A1-A4.
54. Abazovíc E, Kovəcevíc E, Kovəc S, Bradíc J. The effect of training of the non-dominant knee muscles on ipsi- and contralateral strength gains. Isokinet Exerc Sci 2015;23(3):177-182.
55. Delagi E, Iazzetti J, Perotto A, Morrison D. Anatomical Guide for the Electromyographer: The Limbs and Trunk.; 2011.
56. Cole TJ, Altman DG. Statistics Notes: What is a percentage difference? BMJ 2017;358(August):3663.
57. Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2nd Editio. Lawrence Erlbaum Associates; 1988.
58. Milanović Z, Pantelić S, Trajković N, Sporiš G, Kostić R, James N. Age-related decrease in physical activity and functional fitness among elderly men and women. Clin Interv Aging 2013;8:549-556.
59. Grimby G, Saltin B. The ageing muscle. Clin Physiol 1983;3(3):209-218.
60. Šimunič B, Koren K, Rittweger J, et al. Tensiomyography detects early hallmarks of bed-rest-induced atrophy before changes in muscle architecture. J Appl Physiol 2019;126(4):815-822.
61. Šimunic B, Pišot R, Rittweger J, Degens H. Age-related Slowing of Contractile Properties Differs between Power-, Endurance- and non-athletes; a Tensiomyographic Assessment. J Gerontol A Biol Sci Med Sci 2018;1.
62. García García O. Preseason Neuromuscular Profile of Knee Extensor and Flexor Muscles in Elite Amateur Road Cyclist's Assessment through Tensiomyography. Ann Sport Med Res 2015;2(January):1-6.
63. Loturco I, Gil S, Laurino CFDS, Kobal HRR, Abad CCC, Nakamura FY. Differences in muscle mechanical properties between elite power and endurance athletes: A comparative study. J Strength Cond Res 2015;29(6):1723-1728.
64. Rey E, Lago-Peñas C, Lago-Ballesteros J. Tensiomyography of selected lower-limb muscles in professional soccer players. J Electromyogr Kinesiol 2012;22(6):866-872.
65. Trappe S, Luden N, Minchev K, Raue U, Jemiolo B, Trappe TA. Skeletal muscle signature of a champion sprint runner. J Appl Physiol 2015;118(12):1460-1466.
66. Trappe S, Williamson D, Godard M, Porter D, Rowden G, Costill D. Effect of resistance training on single muscle fiber contractile function in older men. J Appl Physiol 2000;89(1):143-152.
67. Andersen JL, Aagaard P. Effects of strength training on muscle fiber types and size; consequences for athletes training for high-intensity sport. Scand J Med Sci Sport 2010;20(SUPPL. 2):32-38.
68. Staron RS, Hagerman FC, Hikida RS, et al. Fiber type composition of the vastus lateralis muscle of young men and women. J Histochem Cytochem 2000;48(5):623-629.
69. Bárány M. ATPase activity of myosin correlated with speed of muscle shortening. J Gen Physiol 1967;50(6).
70. Garcia-Garcia O, Cancela-Carral JM, Martinez-Trigo R, Serrano-Gomez V. Differences in the contractile properties of the knee extensor and flexor muscles in professional road cyclists during the season. J Strength Cond Res 2013;27(10):2760-2767.
71. Rusu LD, Cosma GG, Cernaianu SM, et al. Tensiomyography method used for neuromuscular assessment of muscle training. J Neuroeng Rehabil 2013;10(1):1-8.
72. García-García O, Serrano-Gómez V, Hernández-Mendo A, Morales-Sánchez V. Baseline Mechanical and Neuromuscular Profile of Knee Extensor and Flexor Muscles in Professional Soccer Players at the Start of the Pre-Season. J Hum Kinet 2017;58(1):23-34.
73. Garcia-Garcia O, Serrano-Gómez V, Cuba-Dorado A. Evolution of the lateral symmetry of the limbs of professional footballers during the season. Strength Cond 2011;25(March).
74. Manolopoulos E, Papadopoulos C, Kellis E. Effects of combined strength and kick coordination training on soccer kick biomechanics in amateur players. Scand J Med Sci Sport 2006;16(2):102-110.
75. Loturco I, Pereira LA, Kobal R, et al. Muscle contraction velocity: A suitable approach to analyze the functional adaptations in elite soccer players. J Sport Sci Med 2016;15(3):483-491.
76. Kellis E, Katis A. Biomechanical characteristics and determinants of instep soccer kick. J Sport Sci Med 2007;6(June):154-165.
77. Woods C, Hawkins R, Hulse M, Hadson A. The Football Association medical research programme: An audit of

injuries professional football - analysis of preaseason injuries. Br J Sports Med 2002;36:a436-441.
78. Orchard J, Seward H. Epidemiology of injuries in the Australian Football League, seasons 1997-2000. Br J Sports Med 2002;36(1):39-44.
79. García VV, Duhrkop DC, Seijas R, Ares O, Cugat R. Surgical treatment of proximal ruptures of the rectus femoris in professional soccer players. Arch Orthop Trauma Surg 2012;132(3):329-333.
80. Irmola T, Heikkilä JT, Orava S, Sarimo J. Total proximal tendon avulsion of the rectus femoris muscle. Scand J Med Sci Sport 2007;17(4):378-382.
81. Clemente FM, Ramirez-Campillo R, Sarmento H. Detrimental Effects of the Off-Season in Soccer Players: A Systematic Review and Meta-analysis. Sport Med 2021;51(4):795-814.