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

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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 years1. 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 proposed2–4, however, a key component of injury prevention practice is a regular screening of athlete health and neuromuscular function5. 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 considered6.

Strength asymmetries have been observed in a variety of different sports7–9, but have been the most studied in soccer10–13. Although asymmetries have been previously confirmed in soccer players14–16, these asymmetries do not necessarily affect soccer performance12,17,18, but can be interpreted as potentially problematic19–22 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 improvements23,24.

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 tensiomyography (TMG)
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 performance25.

TMG has been extensively used to measure muscle adaptations in different settings26–28. 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 reliable29–31 and clinically relevant28,29,32. Decreased Tc values, would indicate a muscles with predominance of fast-twitch muscle fibres, while Dm provides an information about the muscle structure i.e., increased Dm correlates well with decreased muscle stiffness26. Additionally, alterations in Dm and the half-relaxation time (Tr) showed to be the most sensitive measures of muscle fatigue, with higher values indicating fatigued state and/or in case of pathology such as anterior cruciate ligament (ACL) injury, it may indicate a muscles less resistant to fatigue. Thus, TMG can be reliably used for non-invasive estimation of predominant skeletal muscle fibres, muscle fatiguing32, training and rehabilitation induced adaptations and, lastly, for neuromuscular risk factors assessment of ACL injury.

A few studies conducted on soccer players demonstrated the absence of bilateral asymmetry in elite and sub-elite male footballers and soccer players. For example, Gill et al. 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 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.

On the other hand, TMG was shown to be a valuable tool for assessing neuromuscular risk factors in ACL injuries. 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. 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.

It is well known that the requirements of a soccer game are gender-specific. 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. Female soccer players cover less total distance during a match, less distance during high-intensity runs and sprints, and generally run slower than men. Consequently, these factors may lead to different neuromuscular performance patterns of the major muscles that act in soccer, as well as greater load on their musculoskeletal system, resulting in different injury patterns or a greater injury incidence in female than male soccer players.

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. 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. 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. 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 stimulable 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. From two maximal twitch responses, several TMG parameters were calculated, as follows: delay time (Td) as time from an electrical impulse to 10% of 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. for VL muscle only (Equation 1).

Equation 1:

\[ \text{MHC-I(\%)} = 2.829 \left( \frac{\text{Ds}}{\text{ms}} \right) + 2.98 \left( \frac{\text{Td}}{\text{ms}} \right) + 1.27 \left( \frac{\text{Tc}}{\text{ms}} \right) + 121.023\% \]

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:

\[ \text{LS} = 0.1 x \left( \frac{\text{MIN}(\text{TdR};\text{TdL})}{\text{MAX}(\text{TdR};\text{TdL})} \right) + 0.6 x \left( \frac{\text{MIN}(\text{TsR};\text{TsL})}{\text{MAX}(\text{TsR};\text{TsL})} \right) + 0.2 x \left( \frac{\text{MIN}(\text{DmR};\text{DmL})}{\text{MAX}(\text{DmR};\text{DmL})} \right) \times 100 \]

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

Equation 3:

\[ \text{FS} = 0.1 x \left( \frac{\text{MIN}(\text{AVG}(\text{TrR};\text{TrL});\text{TsR};\text{TsL}))}{\text{MAX}(\text{AVG}(\text{TrR};\text{TrL});\text{TsR};\text{TsL}))} \right) + 0.8 x \left( \frac{\text{MIN}(\text{AVG}(\text{DmR};\text{DmL}))}{\text{MAX}(\text{AVG}(\text{DmR};\text{DmL}))} \right) \times 100 \]

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<0.017) and playing positions (p<0.10). In addition, partial Eta squared (η²) 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. The criteria for effect size were small (η²=0.01), medium (η²=0.06), and large (η²=0.14). Statistical significance was accepted at p≤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
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; \( \eta^2=0.102 \); Ts (p=0.016; \( \eta^2=0.112 \)); Tr (p=0.023; \( \eta^2=0.118 \)); VL - Td (p=0.009; \( \eta^2=0.131 \)) ; TC (p=0.041; \( \eta^2=0.083 \)) ; MHC-I% (p=0.008; \( \eta^2=0.134 \)) ; GL - Td (p=0.024; \( \eta^2=0.099 \)) ; GM - Td (p=0.006; \( \eta^2=0.144 \)); TA - Tc (p<0.001; \( \eta^2=0.225 \)); and functional ankle symmetry (p = 0.027; \( \eta^2=0.096 \)). However, limb*selection interaction was identified for GM, Td variable only (p=0.037; \( \eta^2=0.126 \)). 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; \( \eta^2=0.135 \)); Ts (p=0.039; \( \eta^2=0.086 \)); Tr (p=0.037; \( \eta^2=0.088 \)); VL - Td (p=0.005; \( \eta^2=0.151 \)); TC (p=0.044; \( \eta^2=0.082 \)); MHC-I% (p=0.007; \( \eta^2=0.144 \)); VM - Tc (p=0.047; \( \eta^2=0.080 \)); GL - Td (p=0.049; \( \eta^2=0.078 \)); GM - Td (p=0.018; \( \eta^2=0.111 \)); TA - Tc (p=0.001; \( \eta^2=0.205 \)); and functional ankle symmetry (p=0.007; \( \eta^2=0.141 \)). However, limb*playing position interaction was identified for VM Tr (p=0.017; \( \eta^2=0.189 \)); GM Td (p=0.011; \( \eta^2=0.206 \)); and functional ankle symmetry (p=0.030; \( \eta^2=0.168 \)). 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           |                  |                  |                  |                  |                  |                  |                  |
|                |      |                       | Mean  | SD    | t value | p value | Mean  | SD    | t value | p value | Mean  | SD    | t value | p value |
| Vastus Medialis (VM) | ND   | 25.4                  | 3.6    | 1.044 | 0.301  | 27.7  | 4.4    | 1.8    | 0.239 | 24.3  | 2.5    | 0.076  | 0.354  | 0.026  | 0.974  |
|                 | DOM  | 25.1                  | 3.4    | 1.007 | 0.319  | 27.3  | 4.2    | 1.6    | 0.239 | 23.9  | 2.5    | 0.125  | 0.294  | 0.320  | 0.727  |
| Vastus Lateralis (VL) | ND   | 29.8                  | 5.1    | -2.761| 0.008  | 22.0  | 1.7    | 1.2    | 0.023 | 19.0  | 0.7    | 0.009  | 0.163  | 0.850  | 1.4080 |
|                 | DOM  | 29.5                  | 4.6    | -2.152| 0.036  | 20.9  | 1.9    | 1.6    | 0.014 | 20.5  | 1.4    | 0.040  | 0.097  | 0.893  | 0.416  |
| Rectus Femoris (RF) | ND   | 65.3                  | 33.0   | 0.505 | 0.516  | 69.7  | 41.1   | 3.6    | 0.014 | 70.8  | 4.0    | 0.081  | 0.777  | 0.208  | 0.813  |
|                 | DOM  | 35.8                  | 25.4   | -1.931| 0.059  | 37.5  | 29.3   | 3.5    | 0.012 | 37.5  | 3.1    | 0.012  | 0.914  | 0.206  | 0.814  |
| Biceps Femoris | ND   | 23.7                  | 1.7    | 2.346 | 0.023  | 24.2  | 2.2    | 1.3    | 0.055 | 22.6  | 1.0    | 0.040  | 0.055  | 0.064  | 0.530  |
|                 | DOM  | 23.6                  | 2.0    | 2.038 | 0.047  | 24.3  | 2.3    | 2.2    | 0.047 | 23.3  | 1.2    | 0.073  | 0.122  | 0.766  | 0.471  |
| Vastus Lateralis (VL) | ND   | 178.2                 | 42.3   | -0.152| 0.88   | 205.1 | 54.1   | 3.3    | 0.005 | 192.4 | 41.5   | 0.050  | 0.945  | 0.96   | 0.407  |
|                 | DOM  | 179.4                 | 37.5   | 0.64  | 0.525  | 56.9  | 36.5   | 5.02   | 0.066 | 52.9  | 4.5    | 0.060  | 0.419  | 0.309  | 0.735  |
| Vastus Medialis | ND   | 7.1                   | 1.3    | 0.972 | 0.336  | 6.4   | 1.6    | 1.3    | 0.052 | 7.7   | 1.3    | 0.520  | 0.472  | 0.746  | 0.480  |
|                 | DOM  | 7.0                   | 1.2    | -1.544| 0.148  | 5.6   | 1.4    | 1.3    | 0.012 | 7.1   | 1.3    | 0.018  | 0.520  | 0.746  | 0.480  |
Table 2. (Cont. from previous page).

| 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-% (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 = MHC-1% - myosin heavy chain isoforms.

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

| Team | Td (ms) | Md (mm) | Tc (ms) | Ts (ms) | Tr (ms) | Dm (mm) | Team | Td (ms) | Md (mm) | Tc (ms) | Ts (ms) | Tr (ms) | Dm (mm) |
|------|---------|---------|---------|---------|---------|---------|------|---------|---------|---------|---------|---------|---------|
|      | ND      | DOM     | ND      | DOM     | ND      | DOM     |      | ND      | DOM     | ND      | DOM     | ND      | DOM     |
|      | Mean    | Mean    | Mean    | Mean    | Mean    | Mean    |      | Mean    | Mean    | Mean    | Mean    | Mean    | Mean    |
|      | SD      | SD      | SD      | SD      | SD      | SD      |      | SD      | SD      | SD      | SD      | SD      | SD      |
|      | t value | p value | t value | p value | t value | p value |      | t value | p value | t value | p value | t value | p value |
| Total sample | | | | | | | | | | | | | |
| Mean | SD | t value | p value | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| A   | U19 | U17 |     |     |     |     |     |     |     |     |     |     |     |
|      |     |     |     |     |     |     |     |     |     |     |     |     |     |

RM ANOVA

| Main effect | Interaction | F value | p value | F value | p value | F value | p value |
|-------------|-------------|---------|---------|---------|---------|---------|---------|
|             |             |         |         |         |         |         |         |

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Table 3. (Cont. from previous page).

| Team       | Total sample | A | U19 | U17 | RM ANOVA |         |         |
|------------|--------------|---|-----|-----|----------|---------|---------|
|            | Mean | SD   | t value | p value | Mean | SD   | t value | p value | Mean | SD   | t value | p value | 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 | -0.997 | 0.323 | 1.8  | 0.6  | 1.8  | 0.8  | 2.0  | 0.6  | 0.9210 | 0.342 | 1.0080 | 0.372 |
| Dm (mm)    | ND   | 1.9  | 0.6  | -0.550 | 0.592 | 4.2  | 0.9  | 2.0  | 0.7  | 1.8  | 0.7  | 0.5170 | 0.517 | 1.6000 | 0.132 |
|            | DOM  | 2.7  | 5.7  | -0.550 | 0.592 | 4.2  | 0.9  | 2.0  | 0.7  | 1.8  | 0.7  | 0.5170 | 0.517 | 1.6000 | 0.132 |
| LS (%)     | ND   | 91.7 | 5.3  | 91.4  | 91.4 | 5.5  | 91.3 | 3.5  | 92.2 | 6.0  | 0.0550 | 0.947 |
| Gastrocnemius Lateralis | 91.4 | 91.4 | 5.5  | 91.3 | 3.5  | 92.2 | 6.0  | 0.0550 | 0.947 |
| Tibialis Anterior | 89.4 | 5.2  | 88.3 | 89.3 | 6.2  | 90.6 | 4.9  | 90.2 | 4.5  | 0.6770 | 0.513 |
| FS (%)     | 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 |
| Ankle      | DOM  | 87.9 | 6.4  | -2.641 | 0.011 | 86.6 | 6.3  | 88.4 | 7.2  | 88.6 | 6.1  | 0.9420 | 0.694 | 1.3060 | 0.259 |
| 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        | CD | FB | MF | FW | Main effect | RM ANOVA |         |         |
|-----------------|----|----|----|----|-------------|----------|---------|---------|
|                 | Mean | SD | Mean | SD | Mean | SD | F value | p value | F value | p value | F value | p value | Eta S | F value | p value | Eta S |
| Biceps Femoris (BF) | 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 | 1.2940 | 0.261 | 0.7080 | 0.552 |
|                 | ND | 31.5 | 6.7 | 27.0 | 4.7 | 29.8 | 4.0 | 29.7 | 4.0 | 0.1790 | 0.674 | 0.3550 | 0.785 |
|                 | DOM | 30.0 | 6.1 | 26.8 | 4.0 | 30.3 | 4.8 | 28.2 | 2.3 | 0.0760 | 0.804 | 0.1440 | 0.785 |
|                 | ND | 198.1 | 35.7 | 181.8 | 49.6 | 209.2 | 63.9 | 212.9 | 58.6 | 0.1790 | 0.674 | 0.3550 | 0.785 |
|                 | DOM | 196.1 | 35.7 | 181.8 | 49.6 | 209.2 | 63.9 | 212.9 | 58.6 | 0.1790 | 0.674 | 0.3550 | 0.785 |
|                 | ND | 69.6 | 43.8 | 63.2 | 46.8 | 64.6 | 54.0 | 61.4 | 55.6 | 1.8150 | 0.184 | 0.3360 | 0.799 |
|                 | DOM | 67.4 | 43.8 | 63.2 | 46.8 | 64.6 | 54.0 | 61.4 | 55.6 | 1.8150 | 0.184 | 0.3360 | 0.799 |
|                 | 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 | 0.4760 | 0.493 | 0.1290 | 0.942 |
| Rectus Femoris (RF) | 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 | 0.3700 | 0.546 | 0.5170 | 0.673 |
|                 | ND | 29.1 | 4.7 | 29.7 | 6.0 | 29.9 | 2.4 | 29.1 | 6.4 | 7.5800 | 0.009 | 0.135 | 0.831 |
|                 | DOM | 29.4 | 4.0 | 29.7 | 5.2 | 28.6 | 3.7 | 28.4 | 4.6 | 4.5030 | 0.039 | 0.086 | 0.891 |
|                 | ND | 63.2 | 32.2 | 63.8 | 32.3 | 63.8 | 32.3 | 63.8 | 32.3 | 4.6230 | 0.037 | 0.088 | 0.849 |
|                 | DOM | 63.2 | 32.2 | 63.8 | 32.3 | 63.8 | 32.3 | 63.8 | 32.3 | 4.6230 | 0.037 | 0.088 | 0.849 |
|                 | ND | 6.4  | 1.7  | 7.9  | 2.6 | 7.5  | 2.0 | 7.6  | 1.4 | 0.4930 | 0.486 | 1.4550 | 0.239 |
|                 | DOM | 6.9  | 2.0  | 8.0  | 2.1 | 7.0  | 1.9 | 8.0  | 1.9 | 0.4930 | 0.486 | 1.4550 | 0.239 |
Table 4. (Cont. from previous page).

| Position | Main effect | Interaction |
|----------|-------------|-------------|
|          | CD          | FB          | MF          | FW          |
|          | Mean |SD |Mean |SD |Mean |SD |Mean |SD |
|          |          |          |          |          |          |          |          |          |
| Td (ms)  | ND 22.0 |1.5 |21.1 |1.5 |21.4 |1.2 |21.0 |1.1 |
|          | 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 |
|          | 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 |
|          | 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 |
|          | 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 |
|          | DOM 5.0  |1.3 |4.9  |1.0 |5.4  |1.3 |5.9  |0.5 |
|          |          |          |          |          |          |          |          |          |
| Td (ms)  | ND 23.6 |2.2 |22.3 |1.0 |23.6 |1.2 |22.7 |2.1 |
|          | 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 |
|          | 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 |
|          | 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 |
|          | 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 |
|          | DOM 6.7  |1.4 |7.0  |0.9 |7.0  |1.1 |7.4  |1.7 |
|          |          |          |          |          |          |          |          |          |
| Biceps Femoris (%) | 85.3 |7.3 |89.6 |7.0 |90.5 |5.8 |91.1 |4.6 |
|       | MHC-I% (VL) | ND 8.3 |10.2 |3.7 |9.3 |6.2 |8.7 |4.3 |
|       | DOM 8.9  |11.4 |8.6  |8.5 |9.8  |10.5 |9.4  |11.7 |

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 | CD      | FB      | MF      | FW      | **Main effect** | **RM ANOVA** |
|--------------------|----------|---------|---------|---------|---------|-----------------|--------------|
|                    |          | Mean    | SD      | Mean    | SD      | Mean            | **F value**  | p value | Eta S | **F value** | p value | Eta S |
| **Gastrocnemius Medialis (GM)** |          |         |         |         |         |                 |              |         |       |              |         |       |
| Td (ms)            |          | 23.3    | 2.5     | 21.3    | 1.3     | 23.2            | 6.0150       | 0.018   | 0.111 | 4.1420       | 0.011   | 0.206 |
|                   | DOM      | 21.9    | 1.8     | 21.6    | 1.5     | 22.8            | 0.5760       | 0.452   |       | 2.1310       | 0.109   |       |
|                   | ND       | 22.7    | 1.9     | 22.6    | 1.7     | 22.9            | 1.0880       | 0.302   |       | 1.8650       | 0.148   |       |
| Ts (ms)            |          | 179.9   | 61.4    | 156.6   | 61.8    | 148.2           | 1.5980       | 0.212   |       | 1.1870       | 0.325   |       |
|                   | DOM      | 185.6   | 107.3   | 133.1   | 51.1    | 170.7           | 0.0552       | 0.461   |       | 0.7130       | 0.549   |       |
|                   | ND       | 53.2    | 32.7    | 64.6    | 48.6    | 45.3            | 0.552        | 0.461   |       | 0.7130       | 0.549   |       |
| Dm (mm)            |          | 74.8    | 48.6    | 50.3    | 35.4    | 66.4            | 0.552        | 0.461   |       | 0.7130       | 0.549   |       |
|                   | DOM      | 2.9     | 1.2     | 3.0     | 0.8     | 3.4             | 0.552        | 0.461   |       | 0.7130       | 0.549   |       |
|                   | ND       | 2.9     | 1.0     | 3.1     | 1.1     | 3.3             | 0.552        | 0.461   |       | 0.7130       | 0.549   |       |
| **Tibialis Anterior (TA)** |          |         |         |         |         |                 |              |         |       |              |         |       |
| Td (ms)            |          | 20.8    | 1.8     | 19.3    | 1.6     | 21.1            | 0.0010       | 0.896   |       | 1.4080       | 0.252   |       |
|                   | DOM      | 21.0    | 2.2     | 20.0    | 0.9     | 20.9            | 0.0010       | 0.896   |       | 1.4080       | 0.252   |       |
|                   | ND       | 17.8    | 2.8     | 18.9    | 1.7     | 17.9            | 12.3600      | 0.001   | 0.205 | 0.8400       | 0.479   |       |
| Ts (ms)            |          | 203.2   | 35.4    | 206.9   | 57.9    | 195.3           | 0.3230       | 0.573   |       | 0.6260       | 0.601   |       |
|                   | DOM      | 214.9   | 36.3    | 189.8   | 43.8    | 234.7           | 0.5750       | 0.452   |       | 0.6920       | 0.561   |       |
|                   | ND       | 35.7    | 19.6    | 44.9    | 37.4    | 71.8            | 0.6350       | 0.430   |       | 0.8140       | 0.492   |       |
| Dm (mm)            |          | 1.6     | 0.6     | 1.9     | 0.4     | 1.9             | 0.6350       | 0.430   |       | 0.8140       | 0.492   |       |
|                   | DOM      | 4.4     | 10.6    | 1.9     | 0.5     | 2.0             | 0.6350       | 0.430   |       | 0.8140       | 0.492   |       |
| **GS (%)**         |          | 79.33   | 92.7    | 5.2     | 91.5    | 5.5             | 4.4          | 1.3500  | 0.269 | 7.8620       | 0.007   | 0.141 |
| **LS (%)**         |          | 89.33   | 90.3    | 2.1     | 91.7    | 4.1             | 5.4          | 0.6800  | 0.568 | 7.8620       | 0.007   | 0.141 |

*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)25,28,29. Since, the neuromuscular system has been shown to be affected by an individual’s physical activity level in exercise modality dependent manner61, it was not surprising that muscle contractile properties in female soccer players differ from those reported in older symptomatic population28, habitually inactive adults61, recreationally active adults39, endurance62 and/or power trained athletes63. 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)44. The shorter Tc value would indicate muscles with a predominance of fast-twitch muscle fibres33,34, and thus shorter Tc in power athletes could most likely be due to training specificity65–67, resulting in a greater proportion of fast-twitch fibers i.e., MHC IIa and IIx65, 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 force69. 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. Nevertheless, a soccer game demands are known to be sex-specific46. Female soccer players covers less total distance during a match and less distance during high intensity and sprint runs67, while generally running slower than males48, which may lead to different neuromuscular performance patterns of the major muscles acting in soccer49,50.

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 players44,45,72,73 and futsal players43. Limb dominance in the present study was defined as that with which players kick the ball44. 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 fibres64,71,74. 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 training71.75.

TMG has been extensively used to measure muscle adaptations in different settings26–28. Although several time and distance-related parameters of muscle contraction could be derived from TMG response, Tc and Dm proved to be the most reliable29–31 and clinically relevant26,28,32. Shorter Tc values would indicate a muscles with predominance of fast-twitch muscle fibres33,34, whereas Dm provides an information about the muscle structure i.e., increased Dm correlates well with decreased muscle stiffness25. Present investigation failed to find any differences in Dm regardless of muscle assessed. However, consistent with previous findings conducted in male soccer players45, 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 fatigue35,36, with higher values indicating a fatigued state35 and/or, in the case of pathology such as ACL injury, may indicate a less fatigue-resistant muscle37. Therefore, the present results suggest that the dominant limb RF may be prone to fatigue earlier during exercise37. The RF plays a very important role in soccer-specific movements such as sprinting and kicking the ball74,76. 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 group7,78, with long return to play time ranging from four79 to eight months80. The clinical significance of the current findings has been previously confirmed41, 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 players64, 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\(^1\) 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.

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