


# Comparison of the effect of passive recovery and self-myofascial release in post-match recovery in female soccer players

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## ABSTRACT

The self-myofascial release is often included in football training routines the day after games to help athletes recover, but its effects when performed at this time have not yet been investigated. This study aimed to investigate the effect of a myofascial self-release protocol on post-match recovery in female professional soccer players. Ten players were included in the study, and all athletes underwent two study conditions: self-myofascial release (SMFR) and passive recovery (control). The SMFR was performed on the quadriceps, adductors, hamstrings, iliotibials, gluteus, and gastrocnemius bands, lasting approximately 25 minutes, on the day after the match. The study monitored various recovery markers, including the Total Quality Recovery Scale (TQR), delayed onset muscle soreness (DOMS), mood state, BRAMS (fatigue and vigour), vertical jump, countermovement jump (CMJ), 10 and 20m sprint, and creatine kinase (CK), before the game, 24 and 48 h post-match. The results showed no significant differences between the passive recovery and SMFR for any of the variables monitored. The results of this study indicate that a single session of self-myofascial release (SMFR), performed 24 hours after a female soccer match, has comparable efficacy to passive recovery for post-match.

**Keywords:** Physical education, Foam roller, Fatigue, Recovery strategy, Post-match recovery, Female soccer.

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## INTRODUCTION

The demands of soccer matches can lead to muscle damage, dehydration, and glycogen depletion, causing acute and residual fatigue that can negatively affect physical performance for several hours to days after a match (Andersson et al., 2008; Nédélec et al., 2012). It may take more than 72 hours for a complete recovery of athletes (Andersson et al., 2008), which poses a challenge for teams that often play multiple matches per week, congested schedule (Abaïdia & Dupont, 2018). This short recovery time may lead to chronic reductions in training and game performance and an increased risk of injury (Barnett, 2006). Therefore, implementing recovery strategies to accelerate the athletes' recovery process is crucial (Barnett, 2006; Nédélec et al., 2013). One such strategy commonly used by soccer players is self-myofascial release (SMFR) (Rey et al., 2019).

SMFR is a technique where an individual massage the myofascial tissue by applying pressure to the muscles using a foam roller (Cheatham et al., 2015). SMFR may have physiological effects, such as improving arterial and vascular endothelial functions and increasing parasympathetic nervous system activity, which is useful for the recovery process (Beardsley & Škarabot, 2015). Previous research has demonstrated that performing SMFR immediately after exercise can yield multiple benefits. These include reducing muscle pain (Macdonald et al., 2014; Pearcey et al., 2015; Rey et al., 2019), perception of recovery (Rey et al., 2019), increase range of motion (Macdonald et al., 2014; Grabow et al., 2018; Hodgson et al., 2019), and improve performance measures, such as sprint time (Pearcey et al., 2015; Kaya et al., 2021), agility (D'Amico & Gillis, 2019; Rey et al., 2019), vertical and horizontal jump (Macdonald et al., 2014; Pearcey et al., 2015), and positive effects on removal blood lactate (Adamczyk et al., 2020; Ali, Rahimi et al., 2020).

Despite the benefits of performing self-myofascial release (SMFR) immediately after exercise on the recovery of athletes, there is still a debate about the optimal timing of its application as a recovery strategy, and further investigation is needed in football. Particularly on match days, there may not be sufficient time to perform SMFR immediately after the match due to logistical and timing constraints. Instead, SMFR is often included in soccer training routines on the day following the match to aid athletes' recovery. Rey et al., (2019) demonstrated that SMFR performed immediately after soccer training can positively impact athletes' recovery. However, the effects of SMFR on recovery when performed 24 hours after a match have not been studied. Furthermore, some studies (Arabaci, 2008) showed that massage has a significant detrimental effect on athletic performance such as vertical jump, sprint time and acceleration. Freiwald et al., (2016), suggest that SMFR may cause harmful side effects due to the high load mechanical induced in the underlying tissue during foam rolling, which could potentially damage connective tissue, nerves, vessels, and bones. Therefore, investigating the potential positive or negative effects of SMFR on recovery can guide coaches and practitioners in selecting the most appropriate interventions to accelerate post-match recovery. Additionally, given that current evidence is limited in considering SMFR as a recovery tool (Wiewelhove et al., 2019), it is even more crucial to examine its effects.

Thus, this study aims to investigate whether SMFR performed 24 hours after an official women's soccer match interferes with athletes' recovery. The hypothesis is that SMFR will positively impact post-match recovery in female, compared to passive recovery, even when performed the day after the match.

## MATERIAL AND METHODS

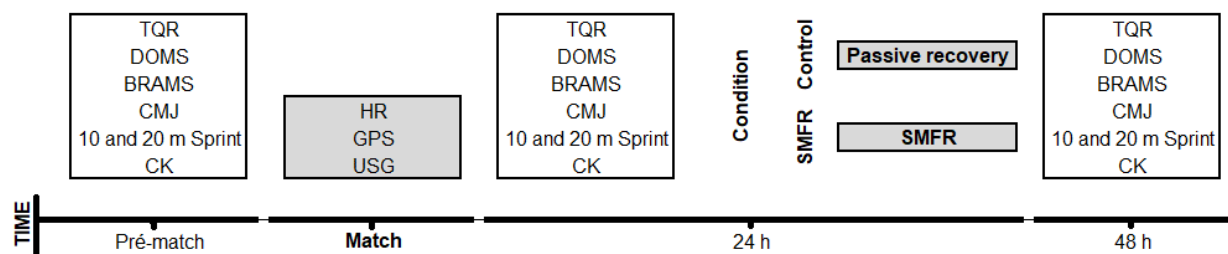
### Participants

Ten Brazilian professional female soccer players (age  $23.6 \pm 5.4$  years, height  $161.7 \pm 6.5$  cm, body mass  $58.5 \pm 6.4$  kg, body fat  $18.7 \pm 3.1\%$ ,  $VO_{2max}$   $42.3 \pm 1.7$  ml.kg<sup>-1</sup>.min<sup>-1</sup>) participated in this study. The athletes were regularly training five times per week for approximately 2-3h per training session and participated in regional and national competitions organized by the Brazilian Football Confederation. Data collection was conducted in the second half of the season, during which the athletes played the main competition of the year and one match per week, without a congested calendar. The study was approved by the ethics committee of the Universidade Federal de Minas Gerais, UFMG - Brazil (approval reference number 13546619.6.0000.5149), and all participants provided both verbal and written informed consent forms prior to participation.

### Procedures

This study compared two conditions, self-myofascial release (SMFR) and passive recovery (control), to assess their impact on athletes' perceptual, physiological, and performance measures pre, 24h and 48h post-match. The study used a within-subject cross-over design, with professional female soccer players being monitored during official championship matches held on Sundays between 10:00 and 11:00 am. Pre-match variables were collected two hours before the game, and performance tests were conducted two days before the match due to their lengthy nature. All tests were repeated 24h and 48h post-match. Athletes assigned to the SMFR condition performed an SMFR protocol after the 24h post-match tests, while those in the control condition underwent passive recovery (Figure 1).

The study was conducted in the same environment and at the same time of the day to reduce the effect of daytime variations on the monitored parameters.



Note. TQR = total quality recovery; DOMS = delayed onset muscle soreness; BRAMS = Brazilian mood scale; CMJ = countermovement jump; SMFR = self-myofascial release protocol; CK = creatine kinase; HR = heart rate; GPS = global positioning satellite; USG = urine specific gravity.

Figure 1. Experimental design.

One week before the competition, athletes underwent an anthropometric assessment and completed the Yo-Yo Intermittent Recovery Test - level 1 (Bangsbo et al., 2008). They were also familiarized with self-myofascial release protocol, vertical jump and sprint tests. During the competition, athletes wore a Polar® Team Pro System which included an integrated heart rate monitor, GPS device with a 10 Hz sampling frequency, and a 200 Hz 3D accelerometer, gyroscope, and magnetometer. The internal load variable, peak heart rate was recorded, along with external load variables such as total distance, distance covered at different speed ranges, (<7.9 km/h; 8 to 11.9 km/h; 12 to 17.9 km/h; >18 km/h and sprints: >25 km/h) number of sprints, accelerations, and decelerations (2.0 m/s<sup>2</sup> and - 2.0 m/s<sup>2</sup> respectively). Pre-match hydration status

was analysed using a portable RTP 20 ATC refractometer (Instrutherm, São Paulo - Brazil), with values ranging from 1.013 to 1.029 g/ml considered normal and values  $\geq 1.030$  g/ml classified as dehydration (Lustosa et al., 2017). Body mass was measured pre and post-match using a digital scale (model Glass 10, G-TECH, China). Ad libitum water intake during the match was not recorded. Environmental temperature and relative humidity were measured every 15 minutes using a digital thermo-hygrometer (TTH100, Incoterm, Porto Alegre - Brazil).

### **Perceptual variables**

The study employed the Total Quality Recovery Scale (TQR), which was validated by Kenttä e Hassmén, (1998) and utilizes a 6-20 scale to measure participants' subjective perception of their recovery process. The level of Delayed Onset Muscle Soreness (DOMS) was assessed using a visual analogue scale (VAS) with mild pain (0) and severe pain (10) as endpoints. The Brazilian Mood Scale (BRAMS) a validated instrument consisting of 24 mood indicators and six subscales (anger, confusion, depression, fatigue, tension, and vigour), was used to evaluate participants' mood state (Miranda et al., 2008). Respondents rated each item on a Likert scale ranging from nothing (0) to extremely (4) to indicate their current emotional state. The fatigue and vigour subscales were the focus of the analysis since they were the most commonly reported mood states by the participants.

### **Performance variables**

Athletic performance was evaluated using Countermovement Jump (CMJ) tests, which consisted of three submaximal jumps followed by four maximal jumps with 20 seconds of rest between each trial. The CMJ test was conducted on a Plataforma Jumptest® contact mat and measured using Multisprint® software. The test's reliability was high, with an ICC of 0.96 and a SEM of 0.6 cm. Sprint times over 10m and 20m were also recorded using Multisprint® software and three photocells. Athletes completed two trials after a low-intensity running warm-up with a 2-minute rest interval. The 20m sprint time had an ICC of 0.87 and SEM of 0.04s, while the 10m sprint time had an ICC of 0.85 and SEM of 0.021s.

### **Physiological variable**

Creatine Kinase (CK) total - 200µL of peripheral blood was collected into a heparinized capillary tube. It was centrifuged at 2000 rpm for 5 minutes (BMC macro centrifuge, model 1123, Benfer, Brazil), and plasma was collected and frozen at -80°C until analysis. CK total was determined using a colorimetric method, with a semi-automatic biochemical analyser BIO 2000 (Bioplus, São Paulo - Brazil) and the CK-NAC kit for kinetic method – UV (Ebram, São Paulo - Brazil).

### **Self-myofascial release protocol**

The athletes in this study followed a standardized SMFR protocol using a rigid, cylindrical roller with a uniform diameter of 14.5 cm and a height of 33.5 cm. The SMFR technique was applied unilaterally to the quadriceps, adductors, hamstrings, iliotibial, gluteus, and gastrocnemius muscle groups. Athletes were instructed to begin at the most distal part of the muscle and roll to the most proximal part, using body weight to apply pressure to the local musculature. Each muscle group was stimulated for 2 sets of 60 seconds on both right and left limbs, totalling approximately 25 minutes (Macdonald et al., 2014). Rolling motions were performed at a cadence of 1 every 1.2 seconds, as indicated by an audible signal from the Soundbrenner metronome application, version 1.2, for Android (Pearcey et al., 2015).

### **Statistical analysis**

The data were reported as means and standard deviations. The normality of the data was verified using the Shapiro-Wilk test. Mauchly's test was used to check for violations of sphericity, and Greenhouse-Geisser

correction was applied if necessary. To compare internal and external match loads between conditions, a dependent samples t-test was used. A two-way (3x2) repeated-measures analysis of variance (ANOVA) was used to examine differences in dependent variables between the SMFR and control conditions, as well as changes over time (pre-match, 24h, and 48h post-match). The effect size (ES) for ANOVA was calculated using partial eta squared ( $\eta^2$ ), with threshold values defined as small ( $\geq 0.01$ ), medium ( $\geq 0.059$ ), and large ( $\geq 0.138$ ) (Fritz et al., 2012; Rey et al., 2019). Bonferroni post hoc testing was conducted when significant differences were found. The significance level was set at  $\alpha = .05$ . For paired comparisons, Cohen's d was calculated to determine effect size (Hopkins, 2006). Statistical analyses were conducted using Statistical Package for the Social Sciences version 21.0 (IBM, Chicago, USA) and Prism 6.0 software (GraphPad Software, San Diego, USA).

## RESULTS

Mean environmental temperature and humidity during matches were  $25.1 \pm 2.8^\circ\text{C}$  and  $59.7 \pm 14.9\%$ , respectively. Pre-match USG corresponded to  $1.016 \pm 0.008$  g/ml and  $1.017 \pm 0.008$  g/ml for control and SMFR conditions, respectively ( $p = .55$ ,  $d = 0.17$ ). Post-match body mass change was not significantly different between control ( $1.48 \pm 0.75\%$ ) and SMFR ( $0.97 \pm 0.60\%$ ) conditions ( $p = .53$ ,  $d = -0.56$ ).

Table 1 presents the values for external and internal loads during the match for both the control and SMFR conditions. There were no significant differences observed between the conditions.

Table 1. External and internal match loads during Control and SMFR conditions.

Match characterization	Control		SMFR		$p < .05$	ES
	Mean	$\pm$ SD	Mean	$\pm$ SD		
Playing time (min)	87.0	$\pm 9.7$	86	$\pm 10.6$	.66	-0.18
Total distance (m)	7610.0	$\pm 815.3$	7607.8	$\pm 738.5$	.99	0.00
Average speed/minute (m/min)	87.0	$\pm 7.3$	88.2	$\pm 5.6$	.49	0.15
Distance >25.0 km/h (m)	33.4	$\pm 46.7$	24.4	$\pm 46.3$	.57	-0.18
Distance >18.0 km/h (m)	411.7	$\pm 183.7$	362.0	$\pm 183.1$	.11	-0.25
Distance 12.0 to 17.9 km/h (m)	1356.3	$\pm 366.9$	1359.6	$\pm 308.9$	.95	0.01
Distance 8.0 to 11.9 km/h (m)	2068.7	$\pm 499.7$	2083.4	$\pm 222.0$	.91	0.03
Distance < 7.9 km/h (m)	3772.8	$\pm 405.6$	3802.9	$\pm 563.4$	.87	0.07
Sprint > 25.0 km/h (n°)	3.1	$\pm 3.3$	2.2	$\pm 3.4$	.43	-0.25
Accelerations >3.0 m/s <sup>2</sup> (n°)	7.9	$\pm 6.1$	8.6	$\pm 3.5$	.71	0.10
Accelerations > 2.0 m/s <sup>2</sup> (n°)	62.5	$\pm 18.8$	64.1	$\pm 20.9$	.75	0.23
Decelerations <-3.0 m/s <sup>2</sup> (n°)	14.6	$\pm 9.0$	14.8	$\pm 8.3$	.87	0.02
Decelerations <-2.0 m/s <sup>2</sup> (n°)	69.3	$\pm 20.6$	71.2	$\pm 24.5$	.76	0.08
Peak heart rate (bpm)	194.0	$\pm 10.9$	191.0	$\pm 10.6$	.08	-0.23

Note. (\*) represents significantly different between conditions ( $p < .05$ ); ES = Cohens' d effect size.

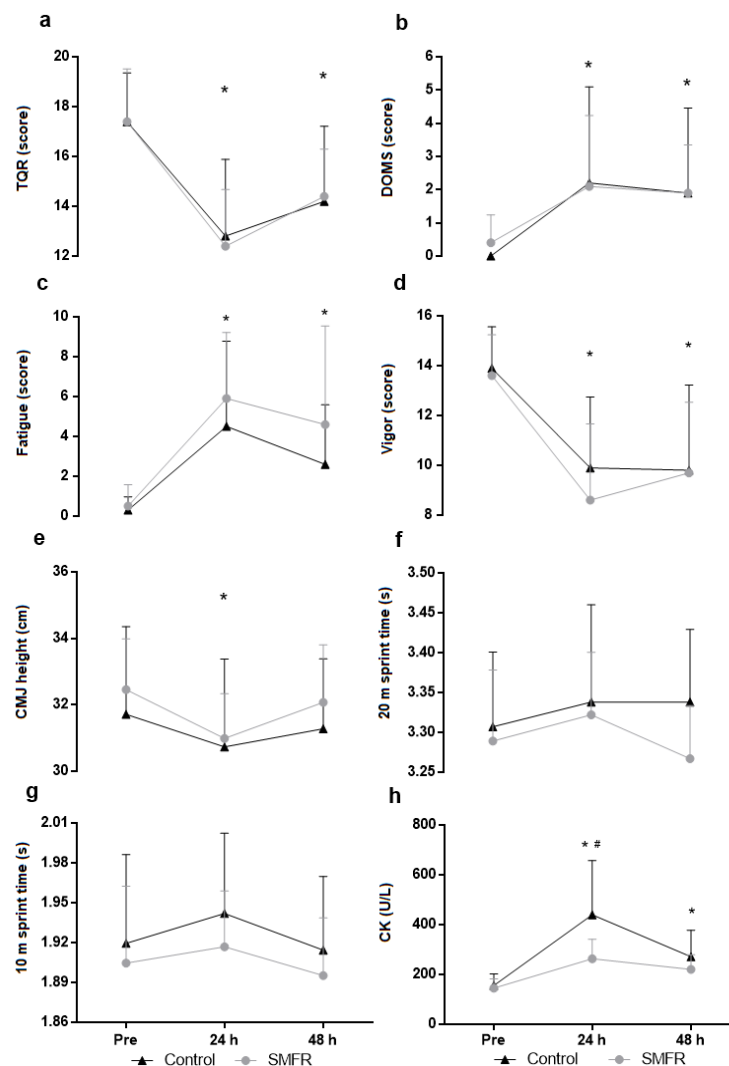
Table 2 presents the results of the repeated measures ANOVA for TQR, DOMS, BRAMS (Fatigue, Vigor), CMJ, 20m and 10m Sprint, and CK. The analysis did not reveal any significant condition x time interactions or main effects for condition on TQR, DOMS, BRAMS (Fatigue, Vigor), CMJ, 20m and 10m Sprint. However, a significant difference was observed for CK, where higher values were found for the control condition compared to SMFR at 24h post-match.

Figure 2 displays the mean values and standard deviations of the perceptual, performance, and physiological variables under both control and SMFR conditions.

Table 2. Results ANOVA of repeated measures.

Parameters	Interaction condition x time			Effect for condition			Effect for time		
	F	p	$\eta p^2$	F	p	$\eta p^2$	F	p	$\eta p^2$
TQR	0.419	.664	0.044	0.220	.885	0.002	23.330	.001*	0.722
DOMS	1.70	.845	0.190	0.35	.856	0.004	8.111	.003*	0.474
Fatigue	0.705	.447	0.073	4.658	.059	0.341	9.597	.001*	0.516
Vigor	0.451	.644	0.048	0.549	.478	0.057	17.661	.001*	0.662
CMJ	0.240	.789	0.026	1.226	.297	0.120	6.401	.008*	0.416
20m Sprint	2.968	.770	0.248	2.337	.161	0.206	2.404	.119	0.211
10m Sprint	1.486	.253	0.142	0.776	.401	0.079	4.264	.056	0.321
CK	10.696	.001*	0.543	8.979	.015*	0.499	18.049	.001*	0.677

Note. (\*) represents significantly different ( $p < .05$ );  $\eta p^2$  = partial eta squared; TQR = total quality recovery; DOMS = delayed onset muscle soreness; Fatigue and Vigor = Brazilian mood scale; CMJ = countermovement jump; CK = creatine kinase.



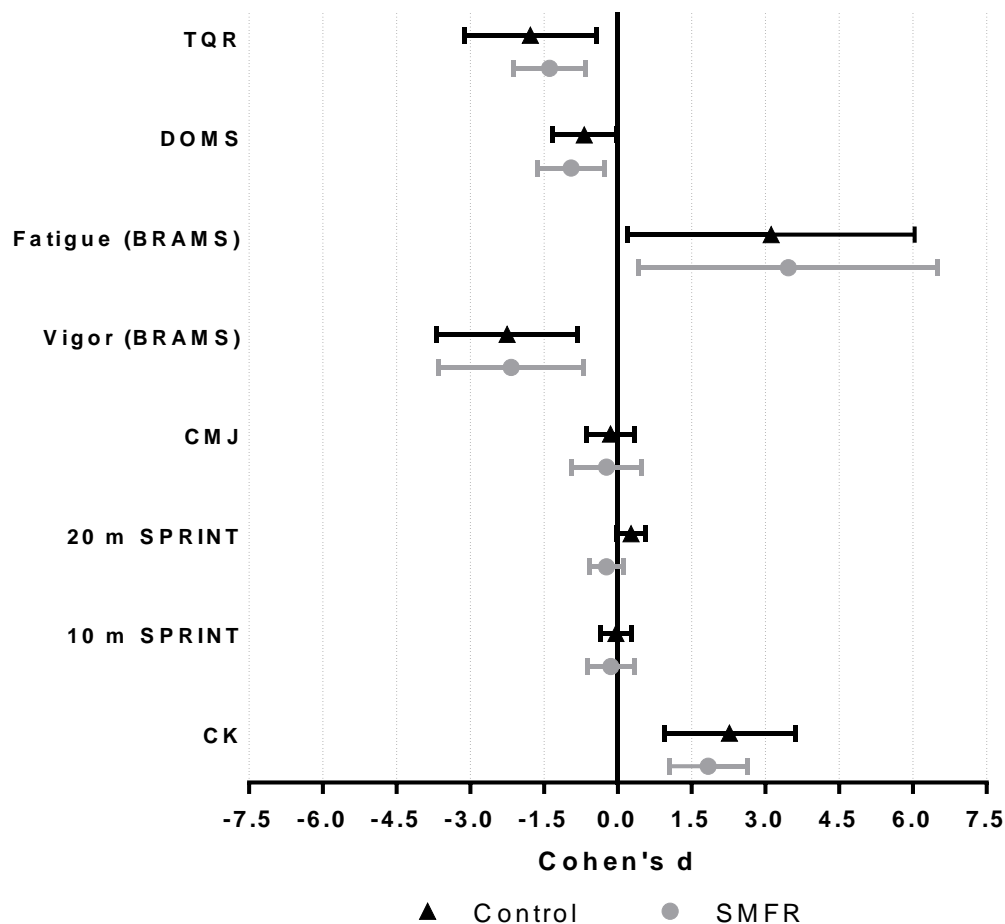
Note. Values are as mean and standard deviation, a. Total quality recovery; b. Delayed onset muscle soreness; c. Fatigue; d. Vigor; e. Countermovement jump; f. 20m sprint; g. 10 m sprint; h. CK. (\*) represents significantly different from pre for all conditions (time main effect) ( $p < .05$ ). (#) represents significantly different between conditions at 24h ( $n = 10$ ,  $p < .05$ ).

Figure 2. Perceptual, performance and physiological responses for Control and SMFR conditions at pre, 24h and 48h post-match.



The present study demonstrates significant time main effects for several parameters. Specifically, 24h and 48h post-match, TQR was significantly reduced for two conditions ( $p = .002$ ,  $d = -2.15$  and  $p = .005$ ,  $d = -1.50$ , respectively), and DOMS was significantly higher for two conditions ( $p = .032$ ,  $d = 3.04$  and  $p = .035$ ,  $d = 2.65$ , respectively) compared to pre-match (Figure 2a-b). Additionally, fatigue was significantly higher ( $p = .007$ ,  $d = 5.22$ ) and vigour was lower ( $p = .002$ ,  $d = -2.67$ ) at 24h post-match for all conditions (Figure 2c-d). At 48h post-match, fatigue remained significantly higher ( $p = .043$ ,  $d = 3.48$ ) and vigour remained significantly lower ( $p = .002$ ,  $d = -2.37$ ) for all conditions (Figure 2c-d). Furthermore, CMJ height was significantly reduced at 24h ( $p = .030$ ,  $d = -0.55$ ) for two conditions (Figure 2e), but not significantly different at 48h ( $p = .669$ ,  $d = -0.18$ ). No significant time main effect was observed for 20m and 10m sprints (Figure 2f-g). However, a significant time main effect was observed for CK, which was higher at 24h ( $p = .005$ ,  $d = -0.45$ ) and 48h ( $p = .005$ ,  $d = 1.84$ ) post-match for two conditions (Figure 2h). The effect sizes values were similar between control and SMFR conditions for all investigated parameters.

The effect size changes from pre to 48h were similar between control and SMFR conditions for all investigated parameters (Figure 3).



Note. Values are effect size (ES) and confidence interval (CI) change from pre to 48h post-match for control and SMFR. TQR = total quality recovery; DOMS = delayed onset muscle soreness; BRAMS = Brazilian mood scale; CMJ = countermovement jump; CK = creatine kinase.

Figure 3. Effect size changes from pre to 48h post-match for all variables in Control and SMFR conditions.

## DISCUSSION

The present study aimed to investigate the effects of a self-myofascial release protocol on the recovery of athletes following a professional women's soccer match. The study found that a single session of myofascial self-release performed 24 hours after the match did not accelerate or impair the athletes' perceptual responses, physical performance, or physiological parameters 48 hours post-match. Therefore, SMFR did not interfere with the athletes' recovery, contrary to the initial hypothesis of the study.

TQR did not show significant differences between SMFR and control conditions, with large effect sizes for both conditions when comparing pre-and 48 hours post-match. Likewise, Barrenetxea-García et al., (2023) did not find improvements in variables related to recovery when using FR after water polo training in athletes of both sexes. The authors suggest that acute adaptations differ considerably from those observed in land and high-speed sports due to the nature of the aquatic sports environment. Contrastingly, a study by Rey et al., (2019) reported a large effect size on athletes' perception of recovery 24 hours post-SMFR. Differences in the timing of SMFR, gender differences, and stimulus type can explain this discrepancy in results. In the present study, SMFR occurred 24 hours after an official women's soccer match, while Rey et al., (2019) used the protocol immediately after a men's soccer training session. Therefore, the effect of SMFR may be conditioned to some of these factors.

The peak delayed onset muscle soreness (DOMS) value occurred 24 hours post-match and remained elevated at 48 hours with no differences between the control and SMFR conditions. Therefore, in the present study, self-myofascial release did not decrease athletes' perception of soreness. However, previous studies by Macdonald et al., (2014); Pearcey et al., (2015); Rey et al., (2019), have reported a decrease in the perception of muscle pain when using a foam roller protocol immediately after exercise. These results suggest that the benefits of self-myofascial release on recovery may be time-dependent.

Hart et al., (2005) also reported no effects on DOMS when performing a massage intervention 24 hours after an eccentric exercise protocol in healthy male and female college-aged individuals. Biochemical processes related to inflammation begin to act a few hours after muscle damage. Therefore, performing an SMFR protocol 24 hours post-match might be too late to provide relief from signs or symptoms of muscle pain.

Fatigue was higher and vigour was lower during the 48 hours post-match with no significant differences between control and SMFR conditions. Furthermore, very large effect sizes were observed between pre and 48 hours for both conditions. The fatigue, control ( $d = 3.12$ ) and SMFR ( $d = 3.47$ ), and vigour, control ( $d = -2.25$ ) and SMFR ( $d = -2.17$ ). Of note, no studies investigating the effect of self-myofascial release on mood were found, making comparisons difficult. Nevertheless, SMFR did not change athletes' mood profiles.

The present study showed no significant differences in CMJ height between control and SMFR conditions, with small effect sizes observed from pre to 48h ( $d = -0.15$  and  $d = -0.23$ , respectively). Consistent with these findings, previous studies have reported no significant differences in vertical jump performance following SMFR interventions after a soccer training (Rey et al., 2019) or a repeated sprint protocol (D'Amico & Gillis, 2019). However, other studies have shown beneficial effects of foam rolling on vertical jump performance after a squat protocol (10x10 repetitions, 60% 1RM), with authors suggesting that the improvements may be related to accelerated recovery of connective tissue, leading to enhanced communication of afferent receptors and the preservation of natural patterns of muscle sequencing and recruitment (Macdonald et al., 2014; Pearcey et al., 2015). Given the greater complexity of movements during soccer matches, including



multiple jumps, changes in direction, accelerations, decelerations, and sprints, the efficacy of SMFR may depend on the specific exercise protocol used to induce fatigue.

The SMFR did not influence the 20m sprint recovery, with a small effect size from pre to 48h for both control ( $d = -0.27$ ) and SMFR ( $d = -0.23$ ) conditions. Contrastingly, Pearcey et al., 2015, reported improvement in the 30m sprint performance at 24 and 72h post-exercise, when the SMFR protocol was performed immediately, 24 and 48h post-exercise, compared to the control condition. According to these authors, the improvement in recovery was likely due to a reduction in late muscle pain. In the present study, performing the myofascial release technique 24h post-match did not result in muscle pain relief and did not promote performance improvements in the 20m sprint. Further, SMFR did not change the 10m sprint performance, with no significant difference and trivial effect sizes from pre to 48h for both control ( $d = -0.04$ ) and SMFR ( $d = -0.14$ ) conditions. Similarly, Rey et al., (2019), found no significant differences for 5 and 10m sprint performance when a SMFR protocol was used immediately post-training in male soccer players. Of note, in the present study, similar sprint times were observed between pre and post-match, suggesting match loads did not reduce speed performance. Thus, SMFR may not promote beneficial effects on short-distance speed tests, since the assessed capacity shows a complete recovery.

The CK total is a commonly used parameter by soccer teams to indirectly assess muscle damage and recovery status of athletes (Ascensão et al., 2008). In the current investigation, CK levels were found to be significantly higher in the control condition as compared to SMFR, only at the 24h post-match time-point. Although the external match load was similar in both groups, no reports of athlete trauma or shocks were observed during the study, which could be a potential explanation for this observed difference (Brancaccio et al., 2007). To date, no other studies have specifically evaluated the effect of self-myofascial release on CK levels, thereby making it difficult to draw any definitive comparisons. However, Kargarfard et al., (2016) reported reduced CK values at 48 and 72h post-eccentric exercise with massage therapy. Therefore, massage therapy may offer potential benefits in assisting with athlete recovery, though the efficacy of SMFR remains unclear.

## CONCLUSIONS

The results of this study indicate that a single session of self-myofascial release (SMFR), performed 24 hours after a female soccer match, has comparable efficacy to passive recovery for post-match. Although SMFR did not accelerate the recovery of perceptual, physical, and physiological variables, it did not impair the recovery process either. Therefore, the present results do not discourage the use of the SMFR technique, but its benefits when used 24h post-match in female athletes remain uncertain. The discrepancies with previous studies may be attributed to the moment of carrying out the SMFR post-exercise. Future research should investigate the effects of SMFR immediately after a women's soccer match and in male soccer players, thus providing additional information on the effectiveness of SMFR in promoting recovery in soccer players. The results of this study contribute to the growing knowledge about recovery techniques and can help coaches optimize their athletes' post-match recovery process.

## AUTHOR CONTRIBUTIONS

Study design and methodology: G.C., K.G. and B.C. Data collection: G.C., K.G, E.P. and B.C. Laboratory analyses: G.C., K.G., S.F. and K.B.G. Data analysis: G.C., K.G and B.C. All authors participated in the writing and review.

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## DISCLOSURE STATEMENT

No potential conflict of interest were reported by the author.

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