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Intensity demands and peak performance of elite soccer referees during match play

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ABSTRACT

Objectives: This study examined the peak physical and physiological (heart rate) performance intensities and associated decrements in elite soccer referees during match play.

Design: Longitudinal study.

Methods: Physical performance variables and heart rate were analyzed during 457 matches across two seasons. Differences between halves, and the rate of decline in peak performance intensities across moving average durations of 1–10 minutes were assessed using linear mixed models and power-law analysis, respectively.

Results: Large significant differences were observed between halves for mean total distance, mean velocity, mean heart rate, and percentage of maximal heart rate ($p \leq 0.05$; $r = 0.51$ – 0.64). Peak intensities ($p \leq 0.05$; $r = 0.15$ – 0.17) and the rate of decline ($p < 0.001$; $r = 0.17$ – 0.37) were significantly higher in the 2nd half compared to the 1st half, for relative total distance, relative high-intensity running and mean velocity. The rate of decline was significantly greater in the 2nd half than the 1st half for relative distance covered by high-intensity acceleration (>2 m/s²/min), deceleration (<-2 m/s²/min), and relative mean heart rate ($p < 0.001$; $r = 0.28$ – 0.61). Elite soccer referees might have experienced transient fatigue during match play, as relative high-intensity running immediately following the most intense 5-minute period significantly declined by 61.2% ($p < 0.001$; $r = 0.94$), and was 16.2% lower than the mean 5-minute period ($p < 0.001$; $r = 0.34$).

Conclusions: Increased physical and physiological demands during match play, with associated declines in the second half and transient signs of fatigue throughout the match, supports the inclusion of high-intensity interval and endurance training programs to prepare soccer referees for the intensity demands and peak performance outcomes of match play.

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Practical implications

- The load and strain demands experienced by elite soccer referees are predominantly aerobic, while high-intensity running and high-intensity actions may account for anaerobic demands during match play.
- Elite referees may employ pacing strategies that allow them to keep pace with the match intensity as established by the players. As such, any effects on the physical and physiological performance of elite referees, between halves and throughout the match, may become evident when the performed work is characterized in terms of relative duration and therefore relative intensity.

- Prescribing training and monitoring of performance intensity can be designed according to the application of the power-law analysis, based on match demands, during training as a function of time.

1. Introduction

During the match, soccer referees need to make critical decisions to enforce the laws of the game and to control the behavior of players during match play.¹ They need to move unrestricted throughout the field of play to ensure optimal positioning with the ball and identify any infringements to the laws of the game. Thus, soccer referees must possess the physical and physiological capacities required to keep up with the match tempo through maintaining pace and intensity with players and the ball throughout match play.^{2–4} Therefore, a comprehensive

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understanding of the physical and physiological demands imposed on referees during competitive matches is essential for designing and planning effective physical preparation programs.

Previous studies have demonstrated that elite soccer referees experience high physical and physiological loads during the match.^{2,5} The intermittent mode of exercise is predominantly aerobic, while anaerobic demands are manifested through short duration repeated high-intensity running (HIR) and actions including short-distance sprints, accelerations, and decelerations.^{5–7}

Given that the majority of the intermittent nature of referee match activity is performed at low to medium intensities, analyzing the amount of HIR performed represents a more relevant requisite for assessing referee match demands and demanding periods of a match underpinning the development of fatigue.^{2,6} Decrements in physical and physiological parameters that represent high intensity activities have been demonstrated to occur between halves,^{3,5} throughout the match,² as well as following the most intense period of activity.^{3,4} Such observations suggest that, similar to professional soccer players, referees experience various fatigue patterns during the match following an intense period of activity and toward the end of the match.^{8,9} However, in a recent study by Riiser et al.⁷, it was demonstrated that HIR ($>13 \text{ km}\cdot\text{h}^{-1}$) frequency and distance performance are relatively stable throughout a match, similar between halves. Also, despite the decline in both HIR and acceleration performance following the most intense period of activity, Riiser et al.⁷ concluded that referees do not experience transient fatigue, as such periods were not significantly different when compared to the mean 5-minute period of activity. In fact, there are equivocal findings in studies, whereby a decrease in total distance covered was observed in elite Italian referees,⁶ but no differences were detected between halves in elite Danish referees.¹⁰

Given the stochastic nature of a soccer match, the most intense periods of activity within a match have been previously characterized using a moving average analysis. Such an approach allows identification of fluctuations in match intensity based on the specific duration of the rolling average.^{11–13} More recently, Delaney et al., following a power-law analysis approach proposed a method to measure the peak intensity and the rate of decline in various velocity-based metrics across moving average durations of 1–10 min in both elite rugby¹⁴ and professional soccer players.¹¹ The findings from Delaney et al.¹¹ present an effective and robust method for coaches and practitioners to design effective training sessions that allow monitoring intensity, based on match data, as a function of time. Nevertheless, such an approach has not been applied in the analysis of match performance of elite soccer referees.

Therefore, the current study aimed to examine the peak intensities and associated rates of decline of defined physical and physiological parameters as a function of time in elite soccer referees during official matches. For that, a comparison between 1st and 2nd halves, and the comparison between 5-minute peak, post and mean periods of match-play were developed. We hypothesized to observe differences between halves, with the 2nd half revealing lower values than the 1st half. Also, we expected to observe the effect of transient fatigue after the peak 5-min of the match, with the decrease in the values of post 5-min in comparison with the mean 5-min periods.

2. Methods

A retrospective observational study was conducted to determine the overall demands, as well as the peak intensities and the rate of decline in physical and physiological variables in elite Portuguese soccer referees during competitive matches across two seasons (2018/19 and 2019/20). Peak running and heart rate intensities, and their associated rates of decline, were measured as a function of time across rolling average durations of 1–10 min during the match and each half. Decrements in relative HIR were also assessed during the match and for each half during (5'-Peak) and immediately following (5'-Post) the most intense 5-

minute period, as well as the mean 5-minute period, minus the 5'-peak (5'-Mean).

Physical performance data was collected from 23 referees (age 37.9 ± 4.6 years; body mass 76.0 ± 5.3 kg; height 180.8 ± 5.6 cm) across 363 competitive matches ($n = 726$ observations; median of 14 matches per referee; range of 2–38 matches) of the Portuguese 1st league ($n = 196$ matches), 2nd league ($n = 106$ matches), the Portugal Cup ($n = 38$ matches), and the Portugal League Cup ($n = 23$ matches). From the 23 referees of the sample, heart rate measures were collected from 10 across 94 competitive matches ($n = 188$ observations; median of 8 matches per referee; range of 2–20 matches). Prior to the start of the investigation and obtaining their written consent, all participants were informed of all experimental procedures and associated risks. The study was approved by the ethics committee of the University Institute of Maia (UIM5/2020) and conducted in accordance with the Declaration of Helsinki.

Match activity profiles were collected using global positioning system (GPS) devices (10 Hz; STATSport Apex, Northern Ireland), and heart rate (HR) measures were collected telemetrically (Polar, Kempele, Finland). The reliability and validity of the GPS units, and their use to assess sprint performance and peak velocity in team sports, have previously been demonstrated.¹⁵ Throughout the study, each referee wore the same GPS unit to avoid inter-unit variation between the shoulder blades in tightly fitted vests throughout the match.

Raw GPS and HR data were downloaded and processed using respective proprietary software and analyzed using customized Microsoft Excel spreadsheets and Python programming software. A duration of 45 min for each half, excluding any extra time, was analyzed. Data was analyzed for total running distance (TD), mean (AvgVel; $\text{km}\cdot\text{h}^{-1}$) and maximal velocity (MaxVel; $\text{km}\cdot\text{h}^{-1}$), and selected locomotor categories and acceleration ranges classified based on previous studies^{3,16,17}: high-intensity running (HIR; $>13 \text{ km}\cdot\text{h}^{-1}$), high speed running (HSR; $>18 \text{ km}\cdot\text{h}^{-1}$), and sprinting (SPR; $>25.2 \text{ km}\cdot\text{h}^{-1}$) distances, as well as high intensity acceleration (Acc; $>2 \text{ m}\cdot\text{s}^{-2}$) and high intensity deceleration (Dec; $<-2.0 \text{ m}\cdot\text{s}^{-2}$) distances. A rolling average method was used to determine relative HIR during the 5'-Peak, 5'-Post, and 5'-Mean periods of the match and within each half.¹² Mean HR (HRavg; $\text{beats}\cdot\text{min}^{-1}$), peak HR (HRpeak; $\text{beats}\cdot\text{min}^{-1}$), and percentage of peak HR (%HRmax) were also measured during the match and for each half. Peak HR was the peak HR achieved during the match as per previous studies.^{10,18}

Linear mixed models (LMM) were used to assess differences between halves for GPS-derived variables and heart-rate measures, as well as for peak intensities and associated rates of declines (slope). In all models referees were included as random effects, while halves and 5-min periods were defined as fixed effects. Residuals of the LMM were analyzed for normal distribution using a combination of Q-Q plots and the Kolmogorov–Smirnov test. In cases where normality was not obtained, the non-parametric Wilcoxon test for paired samples was conducted. The magnitude of mean differences was assessed using the t statistics derived from the LMM and converted to effect size correlations (r), and interpreted based on the following criteria: <0.1 , trivial; $0.1–0.3$, small; $0.3–0.5$, moderate; $0.5–0.7$, large; $0.7–0.9$, very large; $0.9–0.99$, almost perfect; and 1.0 , perfect.^{19,20} Effect size (r) for the Wilcoxon signed-rank test for paired samples was calculated based on the Z-score divided by the square root of the sample number, and interpreted as 0.1 small, 0.3 average, and 0.5 large effect.²¹ Data are presented as mean \pm 95 % confidence intervals (CIs), unless otherwise stated. Significance level was set at $p \leq 0.05$. Statistical analyses were performed using IBM SPSS for Windows statistics version 25.0 (IBM Corp., Armonk, NY, United States).

3. Results

Physical and physiological performances during the match and each half are presented in Table 1. Large differences were observed for total distance, mean velocity, mean heart rate, and peak heart rate, with

Table 1
Physical performance and physiological measures during the match, 1st half, and 2nd half. Data are presented as mean ± 95 % CI [95 % CI].

	Match	1st half	2nd half	Sig.	ES	Qualitative interpretation
TD (m)	9353.4 ± 245.4	4807.1 ± 43.3	4632.7 ± 40.4*	0.00	0.51	Large
HIR (m)	2504.6 ± 166.5	1287.7 ± 31.3	1276 ± 31.4	0.30	0.05	Trivial
HSR (m)	436.8 ± 57.9	220.5 ± 10.3	229.1 ± 11.6	0.07	0.10	Small
Sprinting (m)	22.5 ± 11	12 ± 5.7	10.9 ± 5.1	0.34	0.04	Trivial
Accelerations (>2 m·s ⁻²)	1097 ± 103	566 ± 53	527 ± 49.5*	0.00	0.12	Small
Decelerations (<-2 m·s ⁻²)	1069 ± 101	553 ± 52	513 ± 48.5*	0.00	0.13	Small
Max Vel (km·h ⁻¹)	27 ± 0.8	27.1 ± 0.8	26.9 ± 0.8	0.28	0.04	Trivial
Avg Vel (km·h ⁻¹)	6.2 ± 0.2	6.4 ± 0.1	6.2 ± 0.1*	0.00	0.51	Large
HRavg (b·min ⁻¹)	155.3 ± 5.8	158.3 ± 1.8	154.7 ± 2*	0.00	0.64	Large
HRpeak (b·min ⁻¹)	183.4 ± 5.2	184.6 ± 1.6	183.4 ± 1.7*	0.00	0.34	Moderate
%HRmax	84.6 ± 1.5	84.3 ± 0.6	85.7 ± 0.5*	0.00	0.54	Large

TD = total distance; HIR = high-intensity running; HSR = high-speed running; SPR = sprinting; ACC = acceleration; DEC = deceleration; Max Vel = maximal velocity; Avg Vel = mean velocity; HRavg = mean heart rate; HRpeak = peak heart rate; %HRmax = percentage of peak heart rate; 1stH = first half; 2ndH = second half; Sig. = significance; ES = effect size.
* Significant difference compared to the 1st half, p < 0.001.

decrements of 3.6 %, 3.6 %, 2.3 %, and 0.67 %, respectively, in the 2nd half compared with the 1st half (p < 0.001; r = 0.51–0.64). The percentage of peak HR increased moderately by 1.6 % in the 2nd half compared to the 1st half (p < 0.001; r = 0.34). Total distances covered with high-intensity accelerations and decelerations decreased by 6.9 % and 7.2 %, respectively, in the 2nd half compared to the 1st half, demonstrating small, but significant differences between halves (p < 0.001; r = 0.12–0.13). Similar values were observed between halves for distances covered by HIR, HSR, SPR, as well as maximal velocity (p > 0.05).

Peak intensities and rates of decline for physical and physiological performance variables during the match and each half are presented in Table 2. Small increases of 3.1 % and 3.6 % in both the peak intensity and rate of decline, respectively, in relative HIR were observed in the 2nd half compared to the 1st half (p < 0.01; r = 0.15–0.17). Peak relative TD and relative AvgVel intensities increased by only 1.4 % (p < 0.001; r = 0.16–0.17), while their respective rates of decline increased moderately by 8.3 % (p < 0.001; r = 0.36) and 8.9 % (p < 0.001; r = 0.37), in the 2nd half compared to the 1st half. The rates of decline increased moderately by 6.9 % and 6.8 % for relative distance covered by high-intensity accelerations and decelerations, respectively, in the 2nd half (p < 0.01; r = 0.28–0.30). A large increase of 24.1 % was observed for the rate of decline in relative HRavg in the 2nd half compared to the 1st half (p < 0.001; r = 0.61). No significant differences in peak intensities or rates of decline were observed for relative HSR and SPR distances between halves (p > 0.05).

Relative HIR declined by 61.2 % in the 5'-Post period compared to the 5'-Peak period of the match (5'-Peak: 55.9 ± 2.0 m·min⁻¹, 5'-Post: 21.69 ± 2.1 m·min⁻¹; p < 0.001; r = 0.94), corresponding to a 16.2 % reduction compared to the 5'-Mean period (5'-Mean: 25.90 ± 1.9 m·min⁻¹; p < 0.001; r = 0.38) (Fig. 1a). Similarly, relative HIR performance declined in the 5'-Post period in the 1st half by 59.5 %, and by 59.8 % in the 2nd half, compared to 5'-Peak, respectively (1st half: 5'-Peak: 51.31 ± 1.9 m·min⁻¹, 5'-Post: 20.80 ± 1.9 m·min⁻¹; 2nd half: 5'-Peak: 51.15 ± 1.9 m·min⁻¹, 5'-Post: 20.64 ± 1.9 m·min⁻¹; p < 0.001; r = 0.95). The 5'-Post periods were also lower than the 5'-Mean periods by 16.8 % in the 1st half, and 12.9 % in the 2nd half (1st half: 5'-Mean: 25.02 ± 1.8 m·min⁻¹, 5'-Post: 24.86 ± 1.8 m·min⁻¹; p < 0.001; r = 0.43). No significant interactions were observed for 5'-periods (5'-Peak, 5'-Post, and 5'-Mean) × halves (1st half and 2nd half) (p > 0.05) (Fig. 1b).

4. Discussion

The current study aimed to examine the peak intensities and associated rates of decline of defined physical and physiological parameters as a function of time in elite soccer referees during official matches. According to our expectations, the findings in the current study demonstrated fluctuations in running intensity across increasing 1–10 min moving average durations within and between halves, as well as transient fatigue in relative HIR performance in the 5-min period immediately following the most intense 5-min period during the match.

Table 2
Peak intensity (intercept) and rate of decline (slope) for relative physical performance and physiological measures across rolling average 1–10 min durations during the match, 1st half, and 2nd half. Data are presented as mean ± 95 % CI [95 % CI].

		Match	1stH	2ndH	Sig.	ES	Qualitative interpretation
TD (m)	Intercept	186.0 ± 1.8	178.5 ± 1.7	180.9 ± 1.9*	<0.001	0.16	Small
	Slope	-0.19 ± 0.004	-0.17 ± 0.003	-0.18 ± 0.004*	<0.001	0.36	Moderate
HIR (m)	Intercept	116.4 ± 2.3	104.9 ± 2.2	108.1 ± 2.3*	0.01	0.15	Small
	Slope	-0.45 ± 0.01	-0.42 ± 0.01	-0.44-0.01*	<0.001	0.17	Small
HSR (m)	Intercept	53.2 ± 1.7	44.1 ± 1.6	45.5 ± 1.7	0.15	<0.1	Trivial
	Slope	-0.69 ± 0.012	-0.66 - 0.1	-0.67-0.1	0.09	<0.1	Trivial
Sprinting (m)	Intercept	16.5 ± 1.4	6.6 ± 1.1	5.7 ± 1.0	0.07	0.10	Small
	Slope	-0.92 ± 0.012	-0.7 ± 0.04 ^a	-0.66 ± 0.04 ^a	0.18	0.07	Small
Accelerations (>2 m·s ⁻²)	Intercept	30.4 ± 1.1	27.0 ± 0.79	27.0 ± 0.83	0.98	<0.1	Trivial
	Slope	-0.28 ± 0.01	-0.26 ± 0.01	-0.28 ± 0.005*	<0.001	0.30	Small
Decelerations (<-2 m·s ⁻²)	Intercept	30.1 ± 1.1	28.3 ± 1.1 ^a	27.9 ± 1 ^a	0.21	0.07	Small
	Slope	-0.29 ± 0.01	-0.26 ± 0.01	-0.28 ± 0.01*	0.00	0.28	Small
HRavg (b·min ⁻¹)	Intercept	181.3 ± 1.7	178.5 ± 1.86	177.6 ± 1.97	0.09	0.18	Small
	Slope	-0.04 ± 0.002	-0.033 ± 0.002	-0.041 ± 0.002*	0.00	0.61	Large
Avg Vel (km·h ⁻¹)	Intercept	11.2 ± 0.1	10.7 ± 0.1	10.9 ± 0.12*	0.00	0.17	Small
	Slope	-0.19 ± 0.004	-0.17 ± 0.0035	-0.18 ± 0.003*	0.00	0.37	Mod

TD = total distance; HIR = high-intensity running; HSR = high-speed running; SPR = sprinting; ACC = acceleration; DEC = deceleration; Max Vel = maximal velocity; AvgVel = mean velocity; HRavg = mean heart rate; 1stH = first half; 2ndH = second half; Sig. = significance; ES = effect size.

^a Wilcoxon test [median (interquartile range)].
* Significant difference compared to the 1st half, p < 0.001.

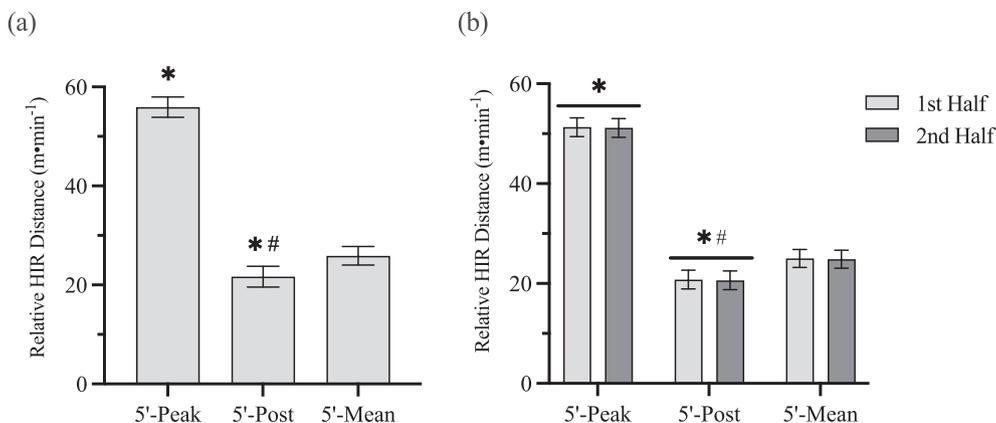


Fig. 1. Relative high-intensity running (HIR; >13 km·h⁻¹) distance (m·min⁻¹) during the most intense 5-min period (5'-Peak), the immediately following period (5'-Post), and mean 5-min period (5'-Mean) in (a) the match and (b) each half. Data are presented as mean ± 95% CI. *Significantly ($p < 0.001$) different than 5'-Peak. #Significantly ($p < 0.001$) different than 5'-Mean.

In the present study, we showed that mean physical and physiological performance changes between halves do not necessarily correspond to those observed for relative intensity and rates of decline. For example, both mean TD and AvgVel decreased in the 2nd half compared to the 1st half. However, the peak and associated rate of decline in relative TD and AvgVel intensities were greater in the 2nd half than in the 1st half. Changes in mean TD between halves have been previously reported by Weston et al.²² and D'Ottavio and Castagna⁶. However, analyzing distances covered by HIR has been suggested to be more representative of the demands of the match and may therefore be more significant in relation to fatigue.^{2,8,22}

In terms of HIR, our findings align with previous studies, whereby no significant changes were observed between halves, despite a significantly greater peak relative intensity and rate of decline in the 2nd half compared to the 1st half. Although, the mean amount of HIR covered by elite English and Italian soccer referees was greater than those reported in the current study.^{6,22,23} In general, the results support previous evidence that the majority of soccer referee activity is performed at low to medium intensity, and as such, similar high-intensity actions were observed between halves, as noted with HIR, HSR, and SPR.^{5,7,18} Nevertheless, increased peak relative HIR intensity in the 2nd half may occur due to changes in the match intensity and dynamics toward the end of the match when teams may adjust their tactical performance by adopting longer passing distances and increased intensity to change the match outcome,¹ or changes in the pacing strategy of players, resulting in changes in ball displacement and velocity.^{3,4,24} In effect, referees must maintain pace with the match intensity, which may underpin the increased peak relative HIR performance in the 2nd half. The associated rate of decline in relative HIR may then be related to physiologically mediated fatigue accumulated throughout the match and related to the work performed during the 1st half.²⁵ Similar interpretations can be extended to high-intensity acceleration and decelerations, and mean HR.

Alternatively, discrepancies between mean volume and peak relative intensity performance between halves could also be underpinned by referees employing a sparing behavior.²⁴ When analyzing the activity profile of referees, it is difficult to conclusively ascertain any decrements in performance to be related to either the fitness level of the referee or the onset of fatigue as referees are tasked with keeping up with play and are therefore not actively setting the pace of the match. For example, Weston et al.²² demonstrated that the amount of HIR (>19.8 km·h⁻¹) performed by referees is partly related to that of players. In fact, our findings of the peak relative HIR performance are similar to those reported by Delaney et al.¹¹ for central defenders, wingers, and wide midfielders.

Furthermore, differences in overall demands between referee cohorts may also be attributed to differences in the fitness status of

referees^{3,5} and differences in the standard of competition.^{7,16,18} Differences in the physical preparation regimen of referees, as well as the amount of HIR performed by players during a match based on the competition standards can therefore influence the amount of physical exertion by referees due to the relatedness between the activity profiles of referees and match intensity.^{4,8}

Interestingly, analysis of the peak intensity and the associated rate of decline as a function of time through power-law, revealed very interesting findings. The peak intensities and the rates of decline in the relative values of TD, HIR and AvgVel were greater in the 2nd half than in the 1st half. Taken together, such findings provide a greater insight into the dynamics of intensity when expressed in relative terms. That is, while absolute values were lower in the 2nd half for each respective variable, the relative intensities were greater. When considering their respective rates of decline, it may be suggested that the referees may be experiencing greater fatigue in the 2nd half, due to the greater rates of decline as a function of time from the respective intensities. It may be that increased intensity with increased slope may in fact suggest the increased intensity of the match in the 2nd half.^{4,22,25}

The identification and characterization of the most-intense period of HIR during a match, with specific rolling average epochs, have been previously studied in football players.^{11,12,26} Analyzing the most-intense period and the immediately following period provides insight into the extent of performance impairment that may be associated with the onset of transient fatigue during match play.^{8,9} In terms of analyzing intensity and the associated fatigue patterns, analysis of relative HIR in 5'-Peak revealed that elite soccer referees experience transient fatigue during match play. The use of the 5-min epoch window period is supported by previous studies that have demonstrated large correlations between 5-min peak running distance and YYIR2 performance, as well as skeletal muscle Na⁺-K⁺ ATPase protein and subunit expression.^{26,27} These findings suggest that the predominance of anaerobic metabolism during such periods predates the ability to perform high-intensity exercise and may underpin the development of fatigue in the subsequent 5-min period.²⁶ Previous studies in elite soccer players have also demonstrated such transient fatigue patterns during match play.^{8,9,26} Nevertheless, the methods of analysis used here corroborate that while soccer referees experienced transient fatigue in the most intense 5-min periods in both halves, there were no differences between halves. Taken together, the results demonstrate that while there was no difference in absolute values, the relative amount of HIR performed by referees was greater in the 2nd half, with a higher rate of decline in intensity. Definitely ascribing such observations to the onset of fatigue may be misleading due to the numerous previous studies that have demonstrated that the physical demands of referees are highly correlated with those of professional players and further that the workload performed in

the 1st half is related to that in the 2nd half.^{4,25} Furthermore, the intensity of player activity and ball displacement, which underpin match tempo influence referee demands. Therefore, further investigation is required to determine whether such activities and observed differences between halves are due to the physical fitness of referees, match tempo (player and match intensity), or the onset of fatigue. Future studies may benefit from temporal analysis of referee and player activities to draw more definite conclusions.

5. Conclusion

Our findings have demonstrated that elite soccer referees experienced high physical load and physiological strain during match play. That load and strain are predominantly aerobic, while high-intensity running and high-intensity actions may account for anaerobic demands during match play. Reporting of average match demands provides insight into the volume of work performed, but the characterization of such activities in terms of relative duration allows the interpretation of the performed work in terms of intensity. Furthermore, this is the first study to demonstrate, by using GPS technology, that elite soccer referees may experience transient fatigue during match play, as well as during each half, through significant reductions in HIR in the 5-min period immediately following the most intense period, which is also lower when compared to the mean 5-min HIR periods. Future studies would benefit from determining the contextual characteristics of such activity profile, as well the occurrence of such periods throughout the match.

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No funding was received for the completion of the study.

Confirmation of ethical compliance

Prior to the start of the investigation and obtaining their written consent, all participants were informed of all experimental procedures and associated risks. The study was approved by ethics committee of the University Institute of Maia (UIM5/2020) and conducted in accordance with the Declaration of Helsinki.

Declaration of interest statement

None.

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