

1 **Intra-individual variability of sleep and nocturnal cardiac autonomic**  
2 **activity in elite female soccer players during an international**  
3 **tournament**

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19 **Running title:** Sleep and nocturnal heart rate variability individual profiles in elite female  
20 soccer.

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22 **Keywords:** overnight measurements, parasympathetic system, sleep accelerometer,  
23 recovery, women football

24

## 25 **Abstract**

26           **Purpose:** This study provides insights into the individual sleep patterns and  
27 nocturnal cardiac autonomic activity responses of elite female soccer players during an  
28 international tournament. **Materials and methods:** Twenty elite female soccer players  
29 (aged  $25.2 \pm 3.1$  years) wore wrist actigraph units and heart rate (HR) monitors during  
30 night-sleep throughout 9 consecutive days (6 day-time training sessions [DT], 2 day-time  
31 matches [DM], and 1 evening-time match [EM]) of an international tournament. Training  
32 and match loads were monitored using the session-rating of perceived exertion (s-RPE)  
33 and wearable 18-Hz GPS (total distance covered [TD], training and match exposure time,  
34 and high-speed running [HSR]) to characterize training and match loads. **Results:**  
35 Individually, s-RPE, TD, exposure time, and HSR during training sessions ranged from  
36 20 to 680 arbitrary units (AU), 892 to 5176 m, 20 to 76 min, and 80 to 1140 m,  
37 respectively. During matches, s-RPE, TD, exposure time, and HSR ranged from 149 to  
38 876 AU, 2236 to 11210 m, 20 to 98 min, and 629 to 3213 m, respectively. Individually,  
39 players slept less than recommended ( $<7$  hours) on several days of the tournament,  
40 especially after EM ( $n=8$ ; TST ranging between 6:00-6:54 h). Total sleep time coefficient  
41 of variation (CV) ranged between 3.1 and 18.7%. However, all players presented good  
42 sleep quality (i.e., sleep efficiency  $\geq 75\%$ ; individual range between: 75-98%) on each  
43 day of the tournament. Most of the players presented small fluctuations in nocturnal  
44 cardiac autonomic activity (individual nocturnal heart rate variability [HRV] ranged from  
45 3.91-5.37 ms and HRV CV ranged from 2.8-9.0%), while two players presented higher  
46 HRV CV (11.5 and 11.7%; respectively). **Conclusion:** Overall, elite female soccer  
47 players seemed to be highly resilient to training and match schedules and loads during a  
48 9 day international tournament.

## 49 **Introduction**

50 *Paragraph 1.* Elite soccer players are constantly exposed to multiple high  
51 physiological demands due to an elevated number of training sessions and matches played  
52 in National and international competitions, often with congested match calendars [1]. In  
53 some women's competitive tournaments, only one to two days of recovery are given  
54 between matches [2]. In this scenario, optimizing recovery is required to reduce the risk  
55 of transitioning into a state of excessive fatigue as well as to reduce the risk of injury [3].

56 *Paragraph 2.* One of the most critical aspects of the recovery *continuum* for  
57 elite athletes is obtaining a sufficient quantity and quality of sleep [4]. In fact, athletes  
58 and coaches from several sports including soccer have ranked sleep as the most important  
59 recovery strategy [5]. A minimum of 7-9 hours of total sleep time (TST) per night is  
60 generally recommended to promote optimal health and cognitive function among adults  
61 aged 18 to 60 years old [6]. Although there is no general consensus regarding the amount  
62 of sleep an elite athlete should obtain to maintain optimal performance [7], athletes who  
63 obtain less than 7 hours of sleep per night might have an increased likelihood of injury  
64 [4, 8]. In fact, some studies have found sleep durations of less than 7 hours in elite athletes,  
65 especially in soccer teams. Sargent et al. [7], for example, found that athletes (from  
66 individual and team sports) obtained an average of 6.5 hours sleep per night, ranging from  
67 5 to 8 hours. Lastella et al. [9] confirmed these results, finding that average sleep duration  
68 for elite athletes (including elite soccer players) was 6.8 hours, ranging from 5.5 hours to  
69 8 hours. Based on these results, it seems that athletes are probably not getting sufficient  
70 sleep. Therefore, and importantly, athletes that might be sleeping for less than 7 hours  
71 [10, 11] may require an extension of sleep time. In fact, extended TST (commonly used  
72 as sleep quantity index [4]) can lead to better psychomotor accomplishment and technical  
73 accuracy [12], with likely positive effects on competitive performance [13]. Besides sleep

74 quantity analyses, sleep efficiency (SE) is recommended for monitoring sleep patterns as  
75 a sleep quality variable [4, 14], especially in elite athletes [15]. According to the National  
76 Sleep Foundation report [16],  $SE \geq 85\%$  is generally recommended as an appropriate  
77 indicator of good sleep quality, whereas a sleep efficiency  $\leq 74\%$  indicates inappropriate  
78 sleep quality for young adults/adults. As already mentioned, athletes are often unable to  
79 achieve  $\geq 7$  hours of TST and  $\geq 85\%$  SE during training and competition [4]. However,  
80 these results are especially concerning when interpreted as group mean, suggesting that  
81 athletes may achieve these recommendations, where and more likely are included  
82 individuals and/or nights that do not achieved [4].

83 **Paragraph 3.** Due to a variety of essential immunological and metabolic  
84 processes which occur during sleep, it seems that a conceptual relationship exists between  
85 the quantity and quality of sleep and the capacity of athletes to recover and perform [17].  
86 However, the majority of research available examining the sleep of athletes, especially in  
87 women, has typically averaged data across several nights, providing a mean estimate of  
88 usual sleep [7, 9, 18-20]. While such an approach is useful to provide basic insight into  
89 sleep in athletes, it lacks details of how sleep may vary across multiple nights [21].  
90 Moreover, individual variability can reflect differences within individuals over time [22],  
91 with high intra-individual sleep variability indicating the need for individualized sleep  
92 education strategies and interventions to promote appropriate sleep [21]. Additionally,  
93 the coefficient of variation (CV) of sleep parameters (e.g.  $TST_{CV}$ ), classified as a measure  
94 of intra-individual sleep variability [23], has been calculated to measure nocturnal sleep  
95 variability [24]. In this respect, it may be important to include the presentation of sleep  
96 data by encompassing individual responses, in addition to general group means [21]. In  
97 addition, special attention should be given to the sleep behavior of elite athletes (e.g.,

98 TST<sub>CV</sub>) during international tournaments (a period of highly congested fixtures) since  
99 sleep deficits can impair performance [25].

100 **Paragraph 4.** Although sleep is considered a restorative behavior, heart rate  
101 HR variability (HRV) has become one of the most practical and popular methods to  
102 monitor positive and negative training adaptations in athletes [26]. Recently, there has  
103 been growing interest in the use of HRV measurements during sleep to evaluate exercise-  
104 induced disturbances in allostatic load (i.e., adaptive processes that maintain homeostasis  
105 through the production of mediators such as adrenalin, cortisol, and other chemical  
106 messengers) [27], and recovery from daily training and other sources of stress [19, 20,  
107 28]. In fact, it is currently accepted that overnight sleep measurements over consecutive  
108 days are appropriate for tracking the recovery of HRV following high-intensity exercise  
109 [29]. However, standardized training programs within team sport settings have often  
110 produced sparse adaptive results, with high responders and low responders often getting  
111 lost in averaged data reports [30]. As a consequence, an increased desire for training  
112 individualization in team sport settings has given rise to a variety of athlete-monitoring  
113 strategies, enabling coaches to better manage fatigue and manipulate training prescription  
114 on an individual basis [31].

115 **Paragraph 5.** Vagal indices of HRV, such as the logarithm of the root mean  
116 square of successive R-R interval differences (lnRMSSD), reflecting cardiac  
117 parasympathetic modulation, are sensitive to fatigue and have been useful in evaluating  
118 individual training adaptation in soccer players [32, 33]. Furthermore, the weekly (4-7  
119 days) CV of lnRMSSD (lnRMSSD<sub>CV</sub>) may provide valuable information concerning  
120 training-induced perturbations in homeostasis, i.e., can reflect the day-to-day variations  
121 in cardiac parasympathetic activity [32, 34, 35]. In general, athletes with a lower  
122 lnRMSSD<sub>CV</sub> are more aerobically fit and seem to cope better with training and match

123 loads [36-38]. Thus, athletes with high TST and SE and lnRMSSD are expected to  
124 experience less perturbation in sleep patterns [23] and cardiac autonomic activity [26].

125 *Paragraph 6.* Although data exist on the role of sleep in recovery and on the  
126 impact of various interventions (e.g. competitions, time of day for training, training and  
127 match loads) on sleep quality/quantity, there is a lack of individual variability sleep  
128 analysis during international tournaments, especially in elite female athletes. These  
129 investigations may identify potential factors related to disturbed sleep and nocturnal  
130 HRV, which could assist in better defining and recommending appropriate sleep hygiene  
131 strategies and more adequately managing fatigue, as well as manipulation of training  
132 prescription and post-match recovery on an individual basis. Therefore, the aim of this  
133 study was to describe sleeping patterns and nocturnal HRV individual profiles of elite  
134 female soccer players from a National team during an international tournament.

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## 136 **Materials & methods**

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### 138 **Subjects.**

139 *Paragraph 7.* Twenty elite outfield female soccer players (age:  $25.2 \pm 3.1$   
140 years; height:  $167.2 \pm 4.8$  cm; body mass:  $57.8 \pm 3.8$  kg; mean  $\pm$  SD) competing in the  
141 Portuguese National team volunteered to participate in the study, during an international  
142 tournament (Algarve Cup 2018). The study design was carefully explained to the subjects,  
143 and written informed consent was obtained. The study followed the Declaration of  
144 Helsinki and was approved by the Ethics Committee of the Faculty of Sports, University  
145 of Porto (CEFADE 03.2017).

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## 147 **Study design.**

148 *Paragraph 8.* The study followed a descriptive, observational design,  
149 highlighting the individual sleep and overnight HRV responses of elite female soccer  
150 players during an international competition. Data collection was performed throughout 9  
151 consecutive days (encompassing 6 day-time training sessions; DT [start ranged between  
152 11:00AM–5:30 PM], 2 day-time matches; DM [both started at 3:00 PM], and 1 evening-  
153 time match; EM [started at 7:00 PM) of an international tournament (Table 1). Players’  
154 sleeping patterns and nocturnal cardiac autonomic activity were monitored every night  
155 throughout the tournament. Players wore wrist actigraph units and heart rate (HR)  
156 monitors during night-sleep. Training and match loads were quantified by session-rating  
157 of perceived exertion (s-RPE), total distance covered (TD), training and match exposure  
158 time (volume), and high-speed running (HSR) to characterize training practices and  
159 competition demands during the observation period.

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170 **Table 1. Data collection during 9 consecutive days of an international tournament in elite female soccer players.** Training load (TL) was  
 171 assessed each day-training (DT), day-match (DM), and evening-match (EM). Cardiac autonomic activity and actigraphy were assessed during  
 172 night-sleep, and after each training session and match (represented as grey area). Scheduled time and duration of training and matches are also  
 173 illustrated.

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Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday
TL (RPE, GPS) Cardiac autonomic activity Actigraphy	TL (RPE, GPS) Cardiac autonomic activity Actigraphy	TL (RPE, GPS) Cardiac autonomic activity Actigraphy	TL (RPE, GPS) Cardiac autonomic activity Actigraphy	TL (RPE, GPS) Cardiac autonomic activity Actigraphy	TL (RPE, GPS) Cardiac autonomic activity Actigraphy	TL (RPE, GPS) Cardiac autonomic activity Actigraphy	TL (RPE, GPS) Cardiac autonomic activity Actigraphy	TL (RPE, GPS) Cardiac autonomic activity Actigraphy
<b>DT1</b> 5:30 PM (65 ± 2 min)	<b>DT2</b> 11:30 AM (70 ± 17 min)	<b>DM1</b> 3:00 PM (67 ± 26 min)	<b>DT3</b> 4:30 PM (34 ± 11 min)	<b>DM2</b> 3:00 PM (79 ± 23 min)	<b>DT4</b> 4:00 PM (71 ± 20 min)	<b>DT5</b> 4:00 PM (57 ± 2 min)	<b>EM3</b> 7:00 PM (63 ± 27 min)	<b>DT6</b> 4:00 PM (51 ± 10 min)

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178 Abbreviations: RPE, rating of perceived exertion; GPS, global positioning system

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185                    *Paragraph 9.* Throughout the study (i.e., 9 consecutive days of the  
186 tournament), the subjects were hosted in the same hotel. The players slept in twin rooms  
187 with separate beds. All meals were eaten at the same hotel restaurant (i.e., breakfast,  
188 lunch, and dinner). Similarly, all training sessions were conducted within the hotel's  
189 sports complex. All training sessions and matches were performed on an outdoor natural  
190 grass pitch. Ambient temperature ranged from 16–18°C during the day and 10–12°C  
191 during the night. The competitive matches were held in 3 different stadiums located in  
192 the same district (Faro, Portugal; the furthest stadium from the hotel was ~1h by bus).  
193 Therefore, long journeys and the consequences (e.g., travel fatigue and jet leg) were  
194 avoided. Training schedules were set at the discretion of the team coaching staff. There  
195 was no interference by the research team in the athletes' regular training schedule or  
196 sleep/wake patterns. The athletes were free to consume snacks, nutritional supplements,  
197 and caffeine during the data collection period.

198                    *Paragraph 10.* Technical problems and/or player compliance resulted in  
199 some missing data points (sleep variables: valid cases, n=142 [92%], and missing cases,  
200 n=13 [8%]; nocturnal HRV indices: valid cases, n=137 [88%], and missing cases, n=18  
201 [12%]; TD, exposure time and HSR: valid cases, n=137 [88%], and missing cases, n=18  
202 [12%]; s-RPE: valid cases, n=103 [67%], and missing cases, n=52 [34%]).

203

## 204 **Measurements.**

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206                    *Paragraph 11. Training and match load monitoring.* Individual training  
207 and match exposure time was routinely recorded by a member of the team's medical staff.  
208 Subsequently, the recorded exposure time was used to select the actual global positioning

209 system (GPS) training data. The same procedure was used during matches, based on the  
210 individual effective playing time, including substitute players. The amount of activities  
211 performed during the tournament was monitored using wearable 18-Hz GPS units  
212 (STATSports Apex, Northern Ireland). The accuracy of this device has been previously  
213 examined, reporting a nearly perfect criterion validity to measure distance during team  
214 sport specific movements (ICC = 0.98)[39]. A special vest was tightly fitted to each  
215 player, with the receiver placed between the scapulae. All devices were activated 15-min  
216 before the data collection to allow acquisition of satellite signals in accordance with the  
217 manufacturer's instructions. In addition, in order to avoid inter-unit error, each player  
218 wore the same GPS unit throughout the tournament [40]. Data were subsequently  
219 downloaded and adjusted to training and match exposure using corporate software  
220 (STATSports Apex, Northern Ireland). TD covered was adopted as the measure of  
221 training and match volume. High-speed running (HSR, >12.6 km·h<sup>-1</sup>) was adopted as the  
222 measure of high-intensity activity performed, according to a recent study in top-level  
223 female players [41].

224 **Paragraph 12.** Psychophysiological response to exercise was quantified  
225 using the session-rating of perceived exertion method (s-RPE). Throughout the  
226 tournament, the players reported individual RPE using the Borg category ratio scale  
227 (CR10) via a customized mobile application, approximately 30 min after each training  
228 session or match. The CR10 score (perceived intensity) was subsequently multiplied by  
229 individual exposure time (training and match volume), thus providing an overall load  
230 quantification of the session or match [42].

231 **Paragraph 13. Sleep monitoring.** Night-sleep was assessed using 3-axial  
232 accelerometers (Actigraph LLC wGT3X-BT, Pensacola, USA) worn on the non-  
233 dominant wrist. Wrist-worn accelerometers have been used to monitor sleep in elite

234 athletes [15], and validated against polysomnography (PSG; [43]). Data were analysed  
235 using corporate software (ActiLife LLC Pro software v6.13.3, Pensacola, USA). The  
236 sampling frequency was 50 Hz and the epoch of activity counts was 60 s [44]. All sleep  
237 variables were determined every night throughout the tournament using the Sadeh's  
238 algorithm [44]. Objective sleep measures included total sleep time (total amount of sleep  
239 obtained), time in bed (time between lying down until getting up the next day), wake-up  
240 time (time between the last minute of sleep and getting up from bed), sleep onset time  
241 (time of the first epoch of sleep between time of trying to initiate sleep and time at wake  
242 up), wake after sleep onset (number of min awake after sleep onset), sleep fragmentation  
243 index (sum of mobility and immobility accesses in one minute, divided by the number of  
244 immobility accesses), latency (time in minutes attempting to fall asleep), and sleep  
245 efficiency (percentage of time in bed that was spent asleep) [44]. It is important to  
246 mention that although some studies have reported  $SE \geq 85\%$  as insufficient sleep quality  
247 [4], according to the latest National Sleep Foundation report [16], SE ranging from 75-  
248 84% is considered uncertain for young adults/adults, whereas  $SE \leq 74\%$  indicates  
249 inappropriate sleep quality for the respective age category. Therefore, sleep quantity  
250 (TST) and sleep quality (SE) were analysed according to the Sleep National Foundation  
251 report (i.e., TST <7h; 420 min as an indicator of inappropriate sleep quantity, and SE  
252  $\leq 74\%$  as inappropriate sleep quality) [16].

253 ***Paragraph 14. Cardiac autonomic activity monitoring.*** The slow-wave  
254 sleep episode (SWSE) method, which accounts for the deep stage of sleep [32], was used  
255 to analyse the cardiac autonomic activity during night-sleep [45]. This method records 10  
256 minutes of normal RR intervals, considering the criteria proposed by Brandenberger et  
257 al., (2005). HR monitors (Firstbeat Bodyguard2®, Firstbeat Technologies, Finland) were  
258 used during sleep. This device has been validated against standard electrocardiogram

259 equipment to detect heartbeats [46]. The RR intervals analyzed in time domain measures  
260 included mean RR interval (mRR), mean HR, RMSSD (square root of the mean of the  
261 sum of the squares of differences between adjacent normal RR intervals; vagal  
262 modulation index), and SDNN (standard deviation of all NN [RRintervals] interval). RR  
263 intervals were also used to produce the Poincaré plot  $SD_1$  (short-term beat-to-beat  
264 variability) and  $SD_2$  (long-term beat-to-beat variability) values. Fast Fourier Transform  
265 (Welch's periodogram: 300-s window with 50% overlap)[47] was used to obtain  
266 measures of nocturnal cardiac autonomic activity in the frequency domain, considering  
267 both LF (0.004–0.15 Hz) and HF (0.15–0.4 Hz) indices. The ratio (i.e., LF/HF) index was  
268 calculated from the non-transformed LF and HF data [47]. In addition, to reduce any  
269 potential non-uniformity or skewness in HRV, data were log-transformed by taking the  
270 natural logarithm (ln) before conducting any statistical analyses [48]. In all cases, HRV  
271 was calculated using Kubios HRV 3.0.0® software (Kubios Oy, Kuopio, Finland).

272 **Paragraph 15.** Buchheit [32] proposed 3% as the fixed smallest worthwhile  
273 change (SWC) to detect eventual changes related to positive and negative adaptations in  
274 HRV-derived indices. Given that both  $0.5 \times CV$  [32] and  $1 \times CV$  have been acceptably used  
275 to account for within-athlete variations in HRV-derived indices, it is possible to state that  
276 using 3% as proposed by Buchheit [32] is acceptable and conservative for lnRMSSD,  
277 especially for soccer players [26].

278 **Paragraph 16. Statistical Analysis.** Sample distribution was tested using the  
279 Shapiro–Wilk test for sleep patterns, nocturnal cardiac autonomic activity indices (HRV),  
280 and training and match load variables for each day of the tournament. Sleep patterns,  
281 nocturnal autonomic activity, and training load variables displayed during the 9 days of  
282 the tournament are presented as mean  $\pm$  SD for the indices that displayed normal  
283 distribution and presented as median (interquartile range) for data that did not present

284 normal distribution. The coefficient of variation (CV;  $CV = ([SD/mean] \times 100)$ ) was  
285 calculated for the whole group and also intra-individually for TST and lnRMSSD indices  
286 across 9 days of the international tournament. The SWC was calculated from the intra-  
287 individual CV of lnRMSSD ( $\ln RMSSD_{CV}$ ), considering the 9 days of the tournament  
288 [32].

289 *Paragraph 17.* A within-subjects linear mixed model analysis was  
290 performed to examine differences in TST, SE, and nocturnal lnRMSSD variables across  
291 9 days of the tournament. An  $\alpha$ -level of 0.05 was set as the level of significance for  
292 statistical comparisons. The days of the tournament (i.e., DT, DM, and EM) were  
293 included as a fixed effect and player identity (subject ID) as the random effect.  
294 Furthermore, among the recommended variance-covariance structure models, compound  
295 symmetry was selected according to the smallest Akaike Information Criterion  
296 assessment [49], based on the Restricted Maximum Likelihood method. Pairwise  
297 comparisons (Bonferroni) were used to show the day-to-day mean differences for TST,  
298 SE, and nocturnal lnRMSSD indices.

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## 301 **Results**

302 *Paragraph 18. Training and match load variables.* As a group, the s-RPE,  
303 TD, exposure time, and HSR during training sessions ranged from 131 to 360 arbitrary  
304 units (AU), 2201 to 4284 m, 34 to 76 min, and 130 to 756 m, respectively. During matches  
305 these variables ranged from 504 to 602 AU, 7012 to 7746 m, 64 to 83 min, and 1678 to  
306 1888 m, respectively. These data are summarized in Figure1 (Fig 1. A, B, C, and D).

307

308 **Figure 1. Individual subject session of perceived exertion (A), total distance (B),**  
309 **exposure time (C), and high-speed running (D) responses during 9 consecutive days**  
310 **of an international tournament in elite female soccer players.** Group mean  $\pm$  95%  
311 confidence interval are presented (black lines) for s-RPE and HSR, and median  
312 (interquartile range) for TD and exposure time (black lines) for each day in elite female  
313 soccer players. Abbreviations: s-RPE, session-rating of perceived exertion; TD, total  
314 distance; HSR, high-speed running; DT, day-training; DM, day-match; EM, evening-  
315 match. Note: s-RPE: DT<sub>1</sub> (n=15); DT<sub>2</sub> (n=14); DM<sub>1</sub> (n=10); DT<sub>3</sub> (n=17); DM<sub>2</sub> (n=7);  
316 DT<sub>4</sub> (n=8); DT<sub>5</sub> (n=13); EM<sub>3</sub> (n=7); and DT<sub>6</sub> (n=11). TD, exposure time, and HSR: DT<sub>1</sub>  
317 (n=16); DT<sub>2</sub> (n=18); DM<sub>1</sub> (n=9); DT<sub>3</sub> (n=18); DM<sub>2</sub> (n=11); DT<sub>4</sub> (n=14); DT<sub>5</sub> (n=20); EM<sub>3</sub>  
318 (n=13); and DT<sub>6</sub> (n=18).

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320 **Paragraph 19.** Individually, the s-RPE, TD, exposure time, and HSR during  
321 training sessions ranged from 20 to 680 AU, 892 to 5176 m, 20 to 76 min, and 80 to 1140  
322 m, respectively. During matches these values ranged from 149 to 876 AU, 2236 to 11210  
323 m, 20 to 98 min, and 629 to 3213 m, respectively. These data are summarized in Figure 1  
324 (Fig 1. A, B, C, and D).

325 **Paragraph 20. Sleep variables.** As a group, player TST ranged between  
326 7:41 $\pm$ 0:48 and 8:26 $\pm$ 0:41 hours during all DT and both DM of the tournament, Table 2.  
327 However, a lower duration of TST was observed after EM (6:47 $\pm$ 0:58 hours) compared  
328 to all days of the tournament ( $p<0.001$ ), especially when compared to both DM<sub>1</sub> and DM<sub>2</sub>  
329 (-1:39 $\pm$ 0:17 hours and -1:28 $\pm$ 0:12 hours, respectively;  $p<0.001$ ). In addition, a lower SE  
330 was found after EM compared to all days of the tournament ( $p<0.001$ ), especially when  
331 compared to both DM<sub>1</sub> and DM<sub>2</sub> (-6 $\pm$ 3% and -4 $\pm$ 2%, respectively;  $p<0.001$ ). All the  
332 sleep-related variables are presented in Table 2.

333 **Table 2. Actigraphy sleep responses during 9 days comprising an international tournament in elite female soccer players.** Values are mean  
 334  $\pm$  standard deviation (SD) and median (interquartile range).

	DT <sub>1</sub> (n=20)	DT <sub>2</sub> (n=20)	DM <sub>1</sub> (n=13) missing (n=1; 7%)	DT <sub>3</sub> (n=19) missing (n=1; 5%)	DM <sub>2</sub> (n=13) missing (n=1; 7%)	DT <sub>4</sub> (n=12) missing (n=2; 14%)	DT <sub>5</sub> (n=17) missing (n=3; 15%)	EM <sub>3</sub> (n=13) missing (n=2; 13%)	DT <sub>6</sub> (n=15) missing (n=3; 16%)
Sleep onset time (h:min)	23:31 $\pm$ 0:23*	23:12 $\pm$ 0:36*	23:14 $\pm$ 0:34*	23:26 $\pm$ 0:34*	23:19 $\pm$ 0:56*	23:48 $\pm$ 0:39*	23:30 $\pm$ 0:33*	0:45 $\pm$ 0:32	23:35 $\pm$ 0:39*
Wake up time (h:min)	8:27 $\pm$ 0:31	8:39 (0:36)	9:02 (1:01)	8:38 (0:37)	8:25 $\pm$ 0:22	8:14 $\pm$ 0:27	8:37 $\pm$ 0:27	8:56 $\pm$ 0:34	8:39 (0:43)
TIB (h:min)	9:06 $\pm$ 0:45	9:15 (0:58)	9:42 $\pm$ 0:28	9:03 $\pm$ 0:32	9:08 $\pm$ 1:11	8:21 $\pm$ 0:44	8:54 $\pm$ 0:56	7:50 $\pm$ 0:46	8:34 $\pm$ 0:33
TST (h:min)	7:56 $\pm$ 0:47*	7:58 $\pm$ 0:54*	8:26 $\pm$ 0:41*	7:54 $\pm$ 0:36*	8:15 $\pm$ 1:08*	7:41 $\pm$ 0:48*	8:02 $\pm$ 0:57*	6:47 $\pm$ 0:58	7:55 $\pm$ 0:38*
WASO(min)	44 (32)	59 (38)	61 $\pm$ 32	45 (37)	41 (35)	42 $\pm$ 25	53 $\pm$ 33	65 $\pm$ 35	63 (74)
SFI (%)	26 $\pm$ 9	26 $\pm$ 9	26 $\pm$ 8	29 $\pm$ 11	27 $\pm$ 10	23 $\pm$ 10	27 $\pm$ 9	32 $\pm$ 10	24 (18)
SE (%)	91 $\pm$ 6*	91 $\pm$ 6*	87 $\pm$ 4*	91 $\pm$ 6*	85 $\pm$ 5*	90 $\pm$ 6*	92 $\pm$ 6*	81 $\pm$ 7	91 $\pm$ 4*
Sleep latency(min)	4 $\pm$ 3	3 (2)	7 $\pm$ 2*	3 (2)	9 $\pm$ 4*	4 $\pm$ 3	3 (2)	20 $\pm$ 9	4 $\pm$ 3

335 \*Significantly different compared to EM (P<0.001)

336 Abbreviations: DT, day-training; DM, day-match; EM, evening-match; TIB, time in bed; TST, total sleep time; WASO, wake after sleep onset;

337 SFI, sleep fragmentation index; SE, sleep efficiency.

338 Note: The mean values represent only the players that trained and played on the respective day of the tournament (goalkeepers were excluded).

339

340 **Paragraph 21.** Individually, some players slept less than recommended (<7  
341 hours; 420 min) after DT<sub>1</sub> (n=4; player 8,13,19 and 20), DT<sub>2</sub> (n=3; player 6, 13 and 20),  
342 DM<sub>1</sub> n=0, DT<sub>3</sub> (n=1; player 12), DM<sub>2</sub> (n=6; 8, 10, 16 and 20), DT<sub>4</sub> (n=2; player 12 and  
343 13), DT<sub>5</sub> (n=2; player 2 and 12), EM<sub>3</sub> (n=8; player 5, 6, 8, 10, 12, 16, 17 and 20), and DT<sub>6</sub>  
344 (n=1; player 20); especially after EM (TST range between 6:00-6:54 h). TST<sub>CV</sub> ranged  
345 between 3.1 and 18.7 % (Figure 2). Overall, all players presented good sleep quality (i.e.,  
346 sleep efficiency ≥75%; individual range between 75-98%) across all days of the  
347 tournament (Figure 2).

348

349 **Figure 2. Group and individual subject (n=20) total sleep time (TST) and sleep**  
350 **efficiency (SE) displayed during 9 days of an international tournament.** Group and  
351 individual black and grey dots represent daily changes in TST and SE, respectively. TST  
352 and SE averages (with max-min values) are also presented. The black dashed  
353 circumferences represent the days that TST was lower than recommended (i.e., <420 min;  
354 <7 hours). The exposure time (min) for each day is also presented on all days of the  
355 tournament (x axis). Abbreviations: DT, day-training; DM, day-match; EM, evening-  
356 match; CV, coefficient of variation.

357

358 **Paragraph 22. Cardiac autonomic activity variables.** As a group, nocturnal  
359 autonomic cardiac activity was not affected during the 9 days of the tournament.  
360 Overnight lnRMSSD ranged from 4.19±0.88 to 4.54±0.42 ln[ms] ( $p>0.05$ ). Nocturnal  
361 autonomic cardiac activity data are presented in Table 3.

362



363 **Table 3. Overnight cardiac autonomic activity (SWSE method) responses during 9 days of the international tournament in elite female**  
 364 **soccer players.** Values are mean  $\pm$  standard deviation (SD) and median (interquartile range).

	DT <sub>1</sub> (n=19) missing (n=1; 5%)	DT <sub>2</sub> (n=18) missing (n=2; 10%)	DM <sub>1</sub> (n=13) missing (n=1; 7%)	DT <sub>3</sub> (n=19) missing (n=1; 5%)	DM <sub>2</sub> (n=12) missing (n=2; 14%)	DT <sub>4</sub> (n=12) missing (n=2; 14%)	DT <sub>5</sub> (n=16) missing (n=4; 20%)	EM <sub>3</sub> (n=12) missing (n=3; 20%)	DT <sub>6</sub> (n=15) missing (n=3; 17%)
Heart rate (bpm)	46.5 $\pm$ 5.2	47.4 $\pm$ 7.0	46.1 $\pm$ 7.3	46.9 $\pm$ 6.9	48.3 $\pm$ 4.7	46.0 $\pm$ 6.5	46.9 $\pm$ 6.0	48.8 $\pm$ 5.6	47.5 $\pm$ 7.1
RR interval (ln[ms])	7.2 $\pm$ 0.1	7.2 $\pm$ 0.2	7.2 $\pm$ 0.2	7.2 $\pm$ 0.1	7.1 $\pm$ 0.1	7.2 $\pm$ 0.1	7.2 $\pm$ 0.1	7.1 $\pm$ 0.1	7.2 $\pm$ 0.2
RMSSD (ln[ms])	4.4 $\pm$ 0.7	4.2 $\pm$ 0.9	4.4 $\pm$ 0.7	4.3 $\pm$ 0.7	4.4 $\pm$ 0.6	4.5 $\pm$ 0.4	4.3 $\pm$ 0.5	4.4 $\pm$ 0.7	4.5 $\pm$ 0.4
SDNN (ln[ms])	4.0 $\pm$ 0.7	3.9 $\pm$ 0.8	4.0 $\pm$ 0.6	3.9 $\pm$ 0.7	4.1 $\pm$ 0.6	4.2 $\pm$ 0.3	4.0 $\pm$ 0.4	4.1 $\pm$ 0.6	4.2 $\pm$ 0.3
SD1 (ln[ms])	4.0 $\pm$ 0.7	3.8 $\pm$ 0.9	4.0 $\pm$ 0.7	4.0 $\pm$ 0.7	4.1 $\pm$ 0.6	4.2 $\pm$ 0.4	4.0 $\pm$ 0.5	4.1 $\pm$ 0.7	4.2 $\pm$ 0.4
SD2 (ln[ms])	3.9 $\pm$ 0.6	3.8 $\pm$ 0.7	4.0 $\pm$ 0.5	3.9 $\pm$ 0.9	4.0 $\pm$ 0.5	4.1 $\pm$ 0.3	3.9 $\pm$ 0.3	4.0 $\pm$ 0.5	4.1 $\pm$ 0.3
LF (ln[ms <sup>2</sup> ])	6.5 $\pm$ 1.2	6.3 $\pm$ 1.4	6.9 $\pm$ 1.1	6.5 $\pm$ 1.5	6.8 $\pm$ 0.9	7.0 $\pm$ 0.6	6.4 $\pm$ 0.6	6.9 $\pm$ 1.0	6.9 $\pm$ 0.7
HF (ln[ms <sup>2</sup> ])	7.4 $\pm$ 1.4	7.1 $\pm$ 1.7	7.6 $\pm$ 1.5	7.3 $\pm$ 1.5	7.5 $\pm$ 1.2	7.7 $\pm$ 0.8	7.3 $\pm$ 0.9	7.5 $\pm$ 1.4	7.7 $\pm$ 0.8
LF/HF	0.7 (0.5)	0.6 (0.5)	0.8 (0.5)	0.6 (0.5)	0.6 $\pm$ 0.4	0.6 (0.5)	0.5 (0.4)	0.7 $\pm$ 0.5	0.4 (0.2)

365 Abbreviations: DT, day-training; DM, day-match; EM, evening-match; RR interval, variations between consecutive heart beats (beat to beat);  
 366 RMSSD, square root of the mean of the sum of the squares of differences between adjacent NN intervals; SDNN, standard deviation of all NN  
 367 interval; SD1, short-term beat-to beat variability; SD2, long-term beat-to-beat variability; LF, low frequency; HF, high frequency; LF/HF, ratio of  
 368 the low-to high frequency power.

369 Note: The mean values represent only the players that trained and played on the respective day of the tournament (goalkeepers were excluded).

370

371

372           **Paragraph 23.** Individually, two players (players 6 and 7) appeared to  
373 present higher  $\ln\text{RMSSD}_{\text{CV}}$  (11.7 and 11.5%; respectively), which occurred  
374 simultaneously with a reduced  $\ln\text{RMSSD}$  average (3.66 and 2.73 ms; respectively),  
375 throughout the tournament, in contrast to the remaining team (individual  $\ln\text{RMSSD}$   
376 ranging between 3.91 and 5.37 ms, and  $\ln\text{RMSSD}_{\text{CV}}$  ranging between 2.8 and 9%)  
377 (Figure 3).

378

379 **Figure 3. Group and individual subject (n=20) cardiac parasympathetic activity**  
380 **displayed during 9 days of an international tournament.** Group and individual black  
381 dots represent daily changes in natural logarithm of the root mean square of successive  
382 RR intervals ( $\ln\text{RMSSD}$ ).  $\ln\text{RMSSD}$  averages (with max-min values) are also presented.  
383 The grey area represents the smallest worthwhile change zone (3%) [32]. The exposure  
384 time (min) for each day is also presented on all days of the tournament (x axis).  
385 Abbreviations: DT, day-training; DM, day-match; EM, evening-match; CV, coefficient  
386 of variation.

387

## 388 **Discussion**

389           **Paragraph 24.** The main finding from the sleep results was that some players  
390 presented less TST than recommended during different days of the tournament (i.e.,  
391 independently of being DT, DM and EM). However, after EM, a higher number of  
392 athletes slept for less than 7 hours in contrast to the remaining days of the tournament  
393 during which they presented adequate sleep duration. Nevertheless, all players displayed  
394 good sleep quality.

395                   **Paragraph 25.** The main finding from overnight HRV results was that most  
396 players seemed to present small fluctuations in nocturnal cardiac autonomic activity,  
397 while two players appeared to present higher  $\ln\text{RMSSD}_{\text{CV}}$ , occurring simultaneously with  
398 a reduced  $\ln\text{RMSSD}$  average during the tournament, compared to the remaining players  
399 of the team.

400                   **Paragraph 26.** Overall, as a group, players accumulated adequate sleep  
401 quantity and presented good sleep quality on all training days and DM of the tournament.  
402 However, significantly decreased durations of TST and SE were observed after EM  
403 compared to DM of the tournament. These results are in accordance with recent studies  
404 showing that soccer players presented sleep duration within the appropriate healthy range  
405 on training days and match days concluded before 6 PM, but slept significantly less,  
406 delayed bedtime, and presented a lower SE after matches starting after 6 PM [50, 51]. In  
407 fact, the impact of night matches (i.e., schedule time) on subsequent sleep is well  
408 established [52, 53]. For example, Sargent and Roach (2016) examined the sleep of elite  
409 Australian football players on the night immediately following a day match or following  
410 an evening match. During the night immediately after the evening match, the players  
411 initiated sleep 2.5 hours later and obtained 2.1 hours less sleep when compared to sleep  
412 immediately following the day match. In the present study, following EM, female soccer  
413 players initiated sleep much later compared to  $\text{DM}_1$  and  $\text{DM}_2$ , and obtained less sleep  
414 quantity compared to  $\text{DM}_1$  and  $\text{DM}_2$ . Another interesting result was that higher sleep  
415 latency was found after EM compared to  $\text{DM}_1$  and  $\text{DM}_2$ . These results are in accordance  
416 with a large epidemiological survey that reported that 24% of respondents perceived  
417 difficulty in initiating sleep after late-evening exercise compared to 7% after early  
418 evening exercise [54]. Recently, it was also shown that sleep latency in high-level female  
419 soccer players was negatively affected after night-training sessions (start 9:00 PM)

420 compared to resting days and match days (3:00 PM) [18]. These results suggest that late-  
421 evening exercise, occurring close to bedtime sleep, can impose a high risk of obstructing  
422 falling asleep at night. Thus, an acceptable explanation for the results observed in the  
423 present study could be the time scheduling of the matches, suggesting the potential value  
424 for sleep education strategies and interventions to promote appropriate sleep and recovery,  
425 especially after night competitions, since athletes are often unable to achieve  
426 recommended TST and SE, with lower values on the night of competition compared with  
427 the previous night [4].

428 **Paragraph 27.** A main finding from the individual sleep analysis was that  
429 some players slept less than recommended during the tournament, especially after EM.  
430 Importantly, seven out of eight players who played the EM also presented shorter TST  
431 on the night after EM (start 7 PM) compared with the previous night (DT). As already  
432 mentioned, these results are in accordance with a recent study [4] in elite athletes that  
433 showed nocturnal TST was shorter on the night after the match (start  $\geq 6$  PM) compared  
434 with the previous night (training and match or rest day).

435 **Paragraph 28.** Besides the training schedule, another possible explanation  
436 for the sleep results could be the training and match loads that players were exposed to  
437 during the tournament, especially during matches [4]. In fact, on days DM<sub>2</sub> and EM, more  
438 players that participated during the match slept less than recommended, with both days  
439 presenting the highest values of external load (e.g., HSR) and exposure time during the  
440 tournament. Interestingly, no sleep disturbances were found for any players that played  
441 DM<sub>1</sub>. Nonetheless, despite the external training and match loads that players were  
442 exposed to, each player seemed to present good sleep quality across all days of the  
443 tournament. These results could also be supported by the appropriate values of sleep  
444 latency observed for all players during the tournament. Additionally, in the current study,

445 the within-player variability in TST (CV=7.4%) was relatively low compared with a  
446 recent study that found a good within-player consistency of sleep (CV=15.2%), across 8  
447 days of rest (without exercise) and 8 days of night-training sessions in highly trained  
448 female soccer players [19]. Thus, even under stress imposed by tournament scheduling  
449 and training and match loads, the players maintained relatively good consistency in sleep  
450 habits to recover from the training sessions and matches.

451 **Paragraph 29.** Notably, six players seemed to have constantly “compensated”  
452 the low TST after participating in matches (independently of being DM or EM), including  
453 one player that played all matches (player 10), by extending sleep duration on the next  
454 day, probably as a “self-sleep hygiene” strategy. These findings are relatively consistent  
455 with previous data suggesting that training and competition schedules dictate the  
456 sleep/wake behavior of elite athletes [7, 9, 55]. Unfortunately, the strategies that athletes  
457 used to increase their sleep duration during the day (e.g., naps) were not recorded which  
458 is recognized as a limitation of the current study. Therefore, the findings from the present  
459 study indicate that, if given the opportunity, elite female soccer players will compensate  
460 by extending their sleep the following day to ameliorate any sleep loss and fatigue [11].  
461 Taken together, these findings are of considerable importance for athletes and coaches  
462 because nights of reduced sleep (i.e., 5–7 h of sleep per night) can lead to deficits in  
463 neurobehavioural performance and increases in subjective feelings of sleepiness and  
464 fatigue [56]. Therefore, if the training or competition schedule provides sufficient space  
465 for recovery strategies, extending bedtime to achieve prolonged sleep duration appears to  
466 be a beneficial approach. As prevalence of sleep restriction among athletes seems to be  
467 high [57], athletes are encouraged to implement 30 to 60 minutes of additional sleep each  
468 night as a ‘self-experiment’ which should be monitored and supported by staff members  
469 [58]. In addition, when feasible, athletes may also achieve additional sleep time by

470 implementing daytime naps. Furthermore, habitual napping can be generally encouraged,  
471 whereas timing (i.e., preferably in the early afternoon) and duration (i.e., < 30 min) should  
472 be appropriately planned [59]. However, naps shorter than one hour are recommended,  
473 and not too close to bedtime as it may interfere with sleep [59].

474 **Paragraph 30.** In the current study, no significant changes in HRV were  
475 noticed across 9 days of an international tournament (independently of being DT, DM, or  
476 EM). Regarding the training and match loads of the investigated soccer team, it appears  
477 that the s-RPE of training sessions and matches during the international tournament were  
478 not high enough to cause overnight changes in the cardiac autonomic system in this  
479 specific National team. Our study showed that, as a group, elite female soccer players are  
480 able to tolerate up to  $\approx 3000$  AU across 9 days or  $\approx 600$  AU per day of s-RPE during an  
481 international tournament, without presenting signs of severe nocturnal cardiac autonomic  
482 perturbation. Accordingly, Costa et al., 2018 [20] found in highly trained female soccer  
483 players during a 7-day period of the competitive season that late-night soccer training did  
484 not affect nocturnal HRV indices in comparison with rest days. The authors suggest that  
485 the late-night training loads, as measured by training impulse (range between: 77.5 [36.5]  
486 and 110.8 [31.6] AU) and s-RPE (range between: 281.8 [117.9] and 369.0 [111.7] AU),  
487 were not high enough to disturb the cardiac autonomic function during sleep hours.  
488 Although National team players might be more resilient to sustained high levels of  
489 training and match load without presenting signs of severe nocturnal cardiac autonomic  
490 perturbation, this needs to be further investigated.

491 **Paragraph 31.** The  $\ln\text{RMSSD}_{\text{CV}}$  has also been assessed in studies involving  
492 highly-trained athletes as a marker of weekly variation in daily assessed  $\ln\text{RMSSD}$  [60].  
493 In a recent study [61], the authors suggested that a high diurnal  $\ln\text{RMSSD}_{\text{CV}}$  (subject 3  
494  $\text{CV}=12.8\%$  and subject 8  $\text{CV}=11.9\%$ ) was positively associated with perceived fatigue

495 and negatively associated with the physical fitness of female soccer players. Moreover,  
496 another study found that diurnal  $\ln\text{RMSSD}_{\text{CV}}$  measured in swimmers can increase to  
497 values  $>10\%$  during overload periods [62]. In our study, as a group,  $\ln\text{RMSSD}$  derived  
498 from the SWSE method displayed low average CV (6%). In fact, most of the  $\ln\text{RMSSD}$   
499 values across all days of the tournament were located within the SWC range (3%) [32].  
500 However, individually, two players (players 6 and 7) appeared to present higher  
501  $\ln\text{RMSSD}_{\text{CV}}$ , which occurred simultaneously with a reduced average  $\ln\text{RMSSD}$  during  
502 the tournament, contrasting with the remaining players of the team. Thus, overnight HRV  
503 during the international tournament was more sensitive to variation in these two players.  
504 Furthermore, it could be speculated that higher  $\ln\text{RMSSD}_{\text{CV}}$  associated with reduced  
505 average  $\ln\text{RMSSD}$  during training and matches may be interpreted as a sign of overload  
506 [61]. However, additional measures (e.g., well-being ratings) are needed to contextualize  
507 and better interpret changes in HRV [61, 63]. Unfortunately, due to time and technical  
508 constraints, well-being, fitness level, and other types of perceived stress were not  
509 determined, and we recognize this as an important limitation of the current study to better  
510 understand the HRV values of each player during the tournament.

511 **Paragraph 32.** With the purpose of monitoring athletes, measurements  
512 performed during consecutive days throughout the week are very useful to interpret  
513 adaptations [64]. In our study, players seemed to display lower perturbation of nocturnal  
514 cardiac autonomic activity, as expressed by the  $\ln\text{RMSSD}_{\text{CV}}$  [64]. Although the study  
515 comprised only 9 days of observation, it can be globally inferred that players were coping  
516 well with the training sessions and matches. In fact, a reduced  $\ln\text{RMSSD}_{\text{CV}}$  without a  
517 clear decrease in the  $\ln\text{RMSSD}$ , which occurred for most of these female soccer players,  
518 may reflect the possibility of a high level of readiness to perform [60] during the  
519 international tournament. This finding corroborates previous studies assessing HRV

520 during the day, in athletes who were awake [26, 62]. Finally, coaches and technical staff  
521 should give great attention to player 6 who presented the highest CV for both TST and  
522 lnRMSSD (12.5% and 11.7%, respectively) during the 9 days of the international  
523 tournament.

524 **Paragraph 33. Limitations.** Some limitations of the current study should be  
525 noted. Given that this study was conducted in a field setting (i.e., real-world scenario),  
526 there were many uncontrolled factors that may have affected the athletes' sleep, other  
527 than match/training scheduling and load (e.g. use of caffeine; social media after lights  
528 out, electronic devices, level of light exposure during daytime, and perceived stress/well-  
529 being). In addition, caution should be applied when interpreting the CV values for sleep  
530 and HRV, since on some days there were missing data. Furthermore, this study was  
531 limited by the fact that a "real baseline" was not evaluated for possible sleep and nocturnal  
532 HRV comparisons across the 9 days of the tournament. Finally, while the session-RPE  
533 method may be simple, valid, and reliable, in this study we did not use other types of  
534 internal training and match load monitoring (e.g. HR monitors), which could provide  
535 objective information for the interpretation of the physiological responses of the players.

536

## 537 **Conclusions**

538 **Paragraph 34.** The present observational study is the first to systematically  
539 analyse consistent individual sleep and nocturnal HRV responses in elite female soccer  
540 players during a congested match schedule. Individually, some players presented less  
541 TST than recommended after some days of the tournament. However, the highest number  
542 of athletes sleeping less than 7 hours was found after EM compared with the remaining  
543 days of the tournament. Nevertheless, all players displayed good sleep quality for each  
544 day of the tournament. Additionally, most players seemed to present small fluctuations in



545 nocturnal cardiac autonomic activity. Overall, elite female soccer players from a National  
546 team appeared to be highly resilient to training and match schedules and loads during an  
547 international tournament.

548

## 549 **Practical applications**

550 *Paragraph 35.* Our observational analysis of sleep and nocturnal HRV  
551 responses of elite female soccer players could assist coaches and practitioners to identify  
552 sleep and HRV disturbances during official competitions, especially during periods of  
553 highly congested fixtures, as occurs during international tournaments. Moreover, this  
554 study highlights the substantial individual variability in sleep and HRV, suggesting the  
555 adoption of an individual approach to sleep (e.g. sleep hygiene), load monitoring, and  
556 recovery interventions in team sports.

557

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565

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Fig 1.

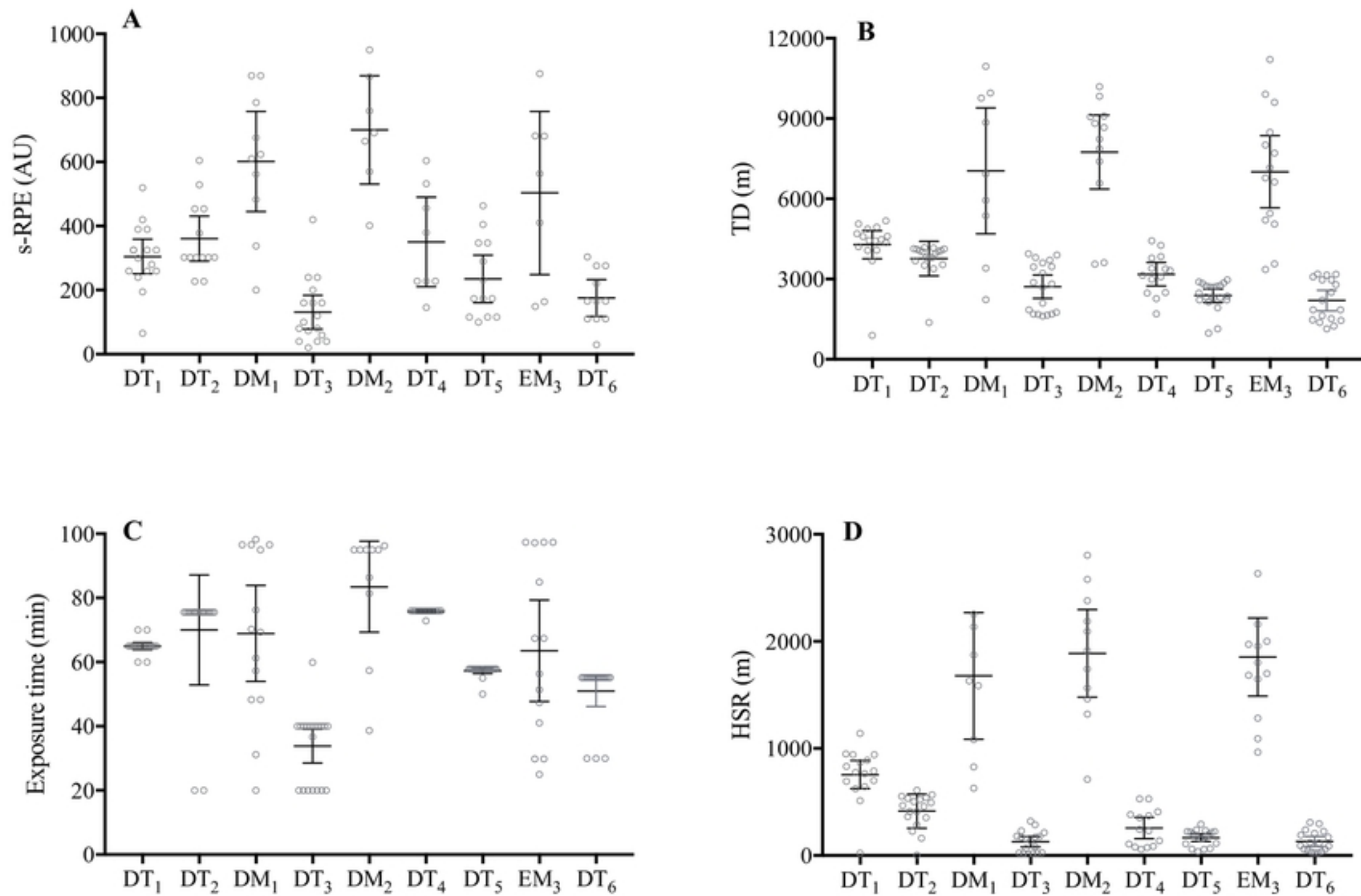


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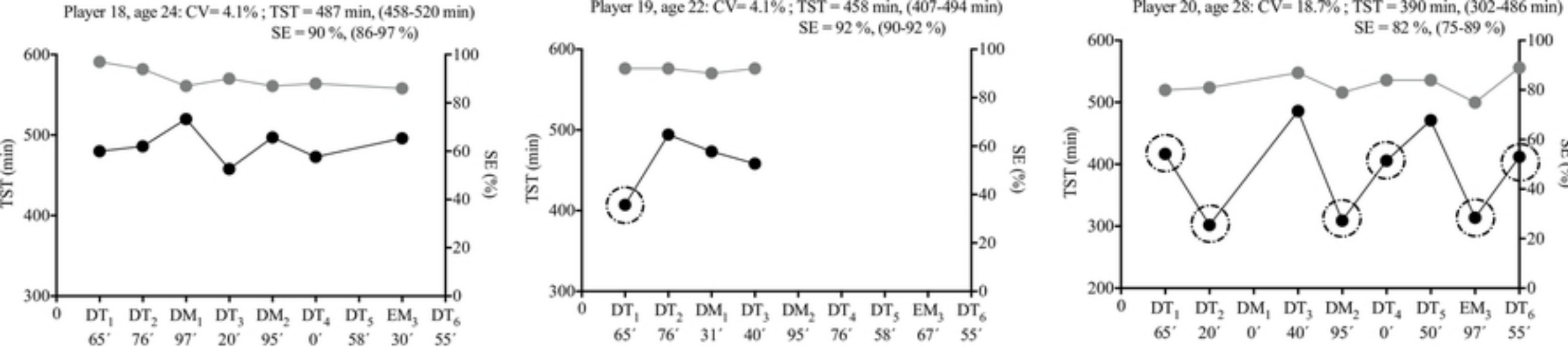


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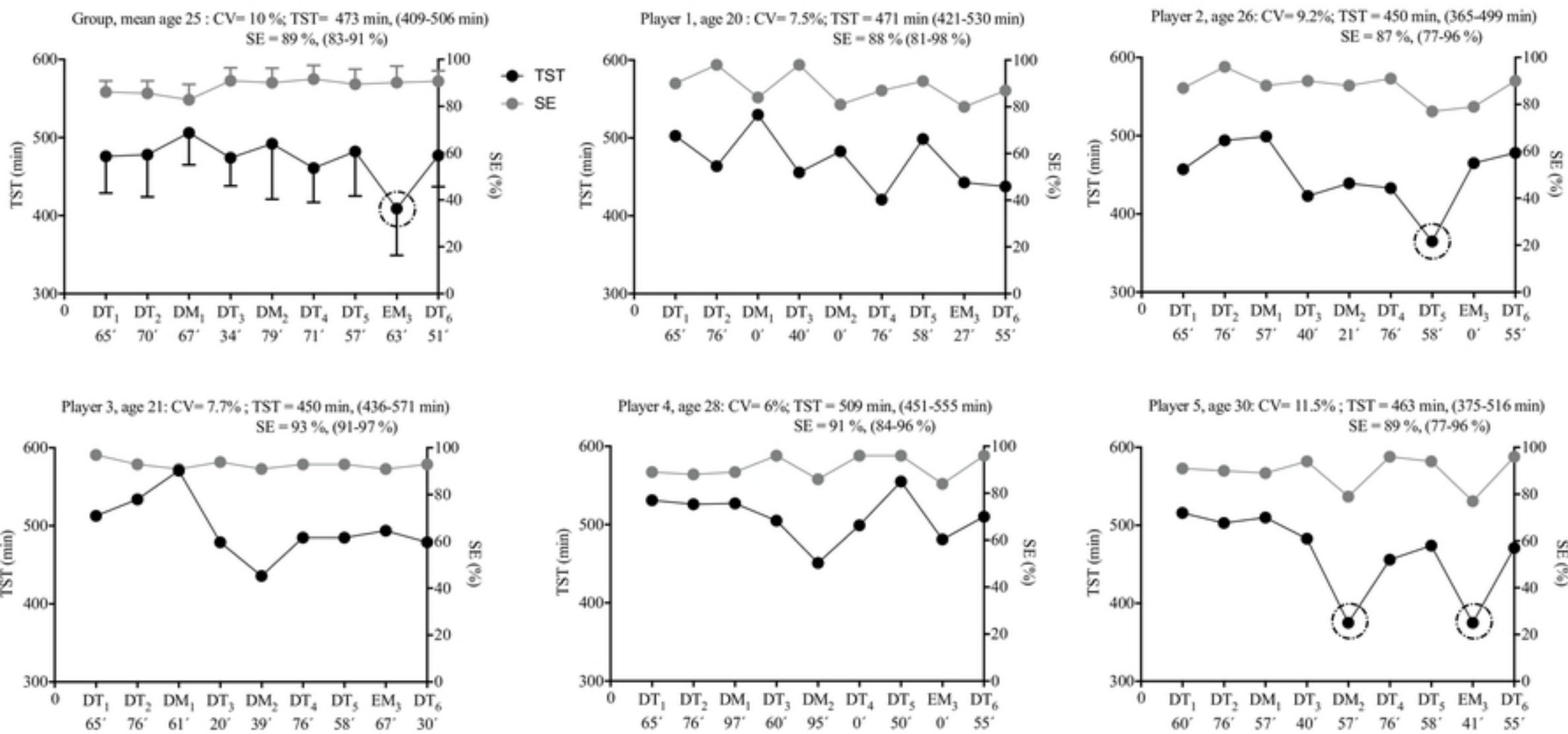


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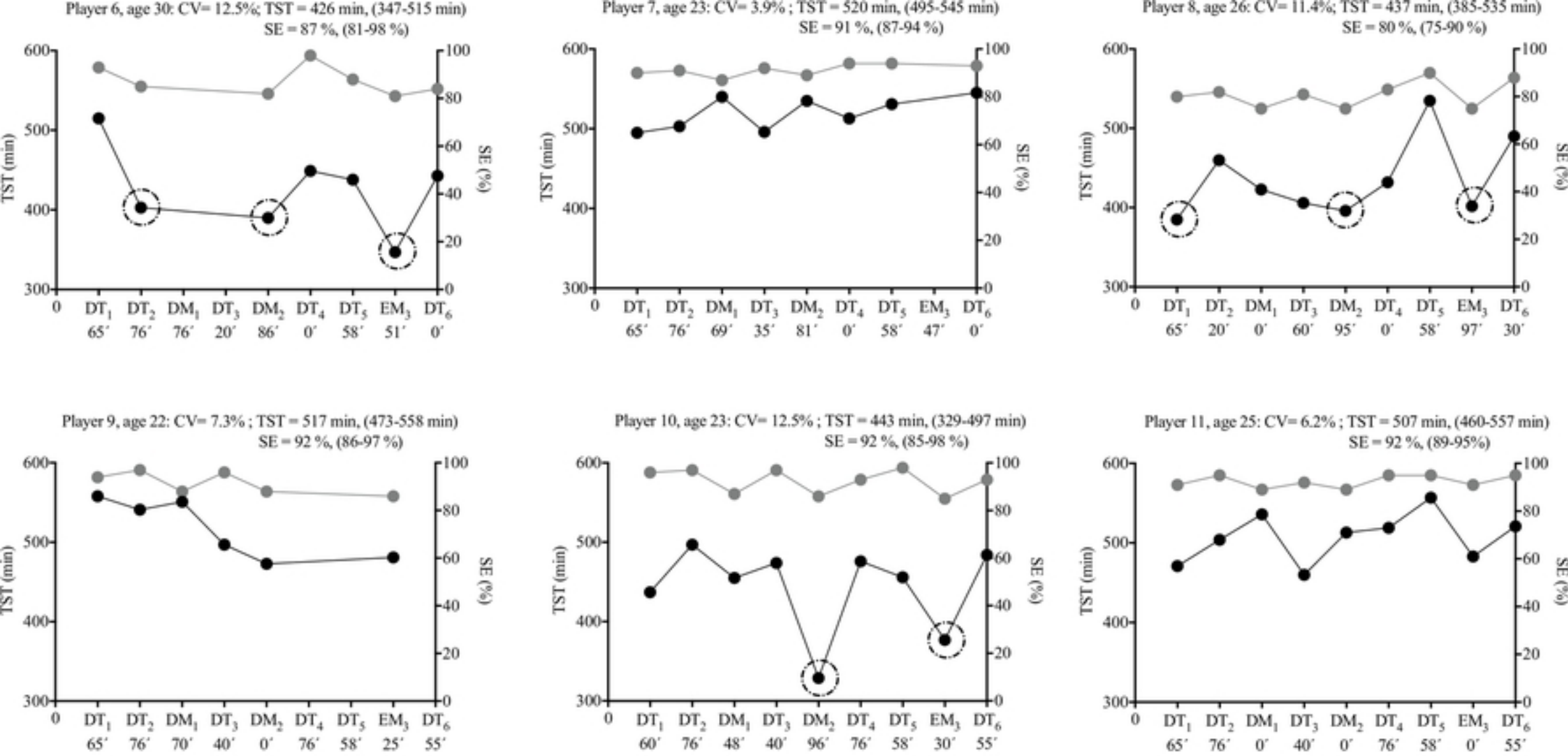


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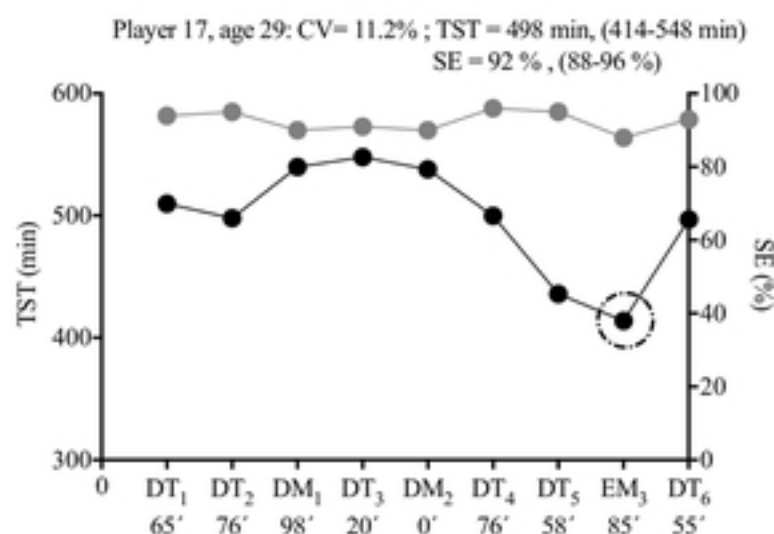
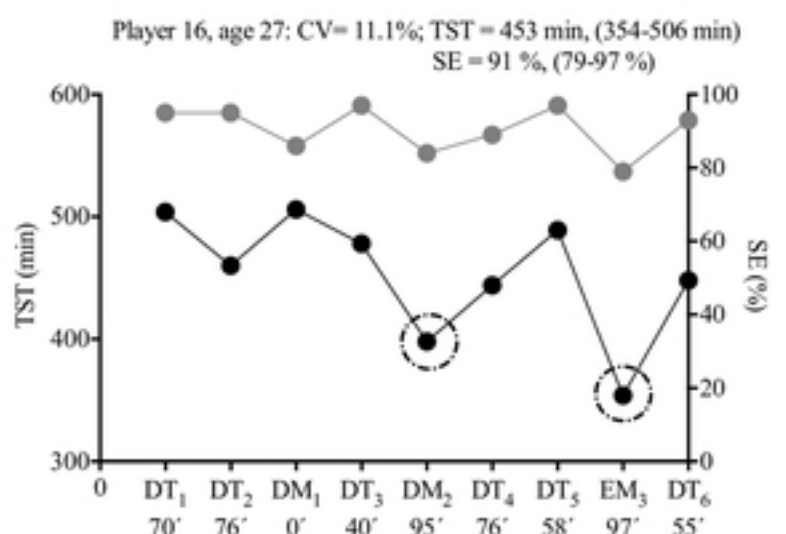
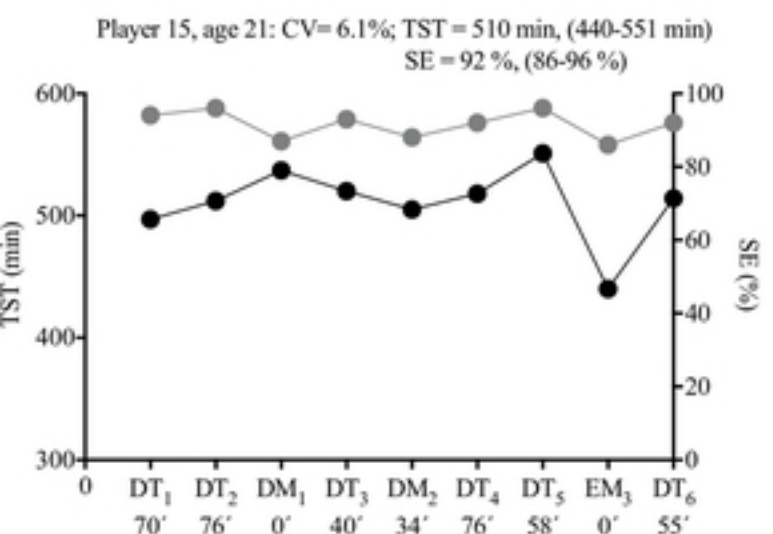
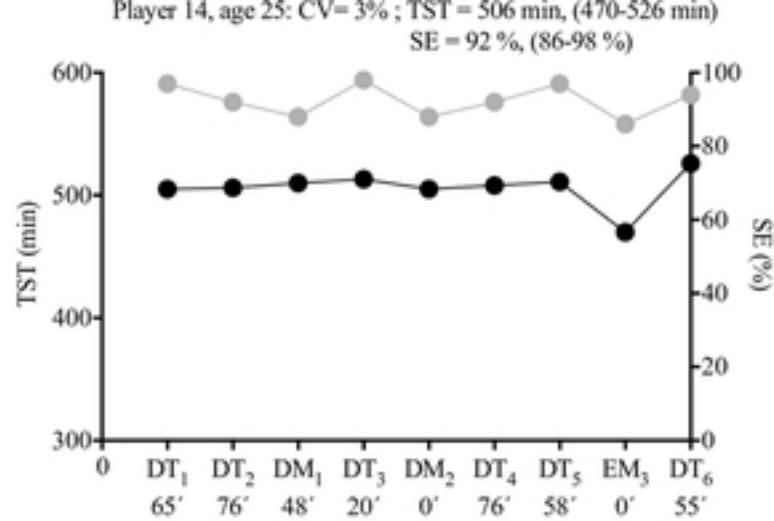
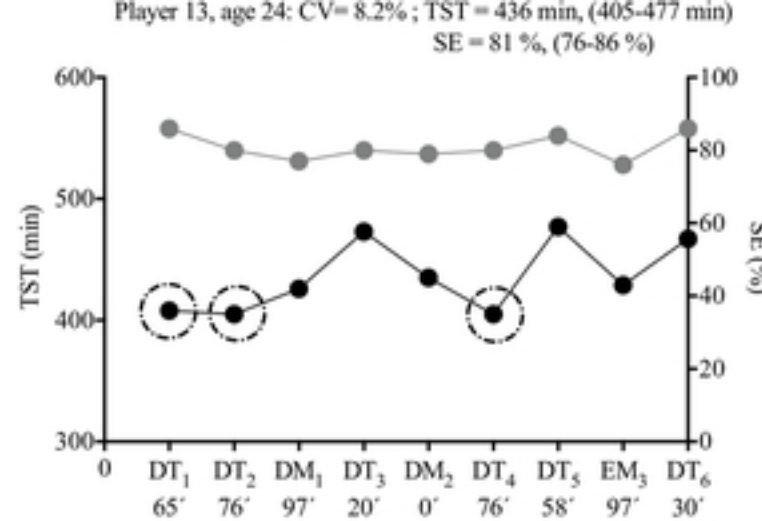
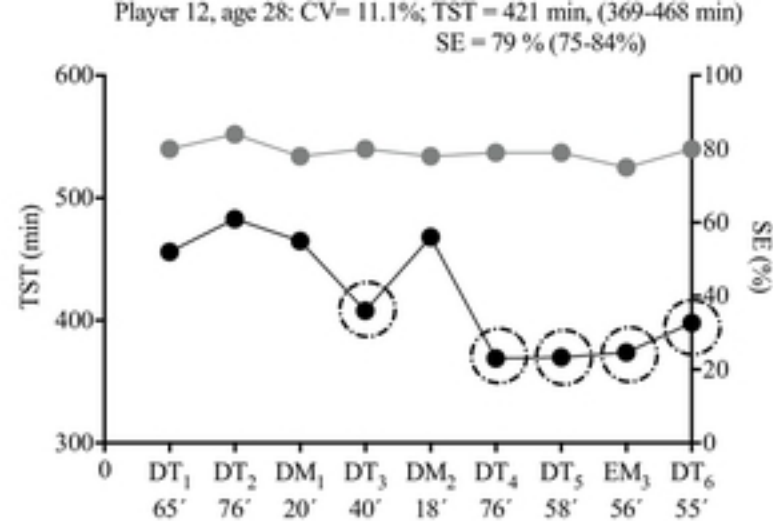


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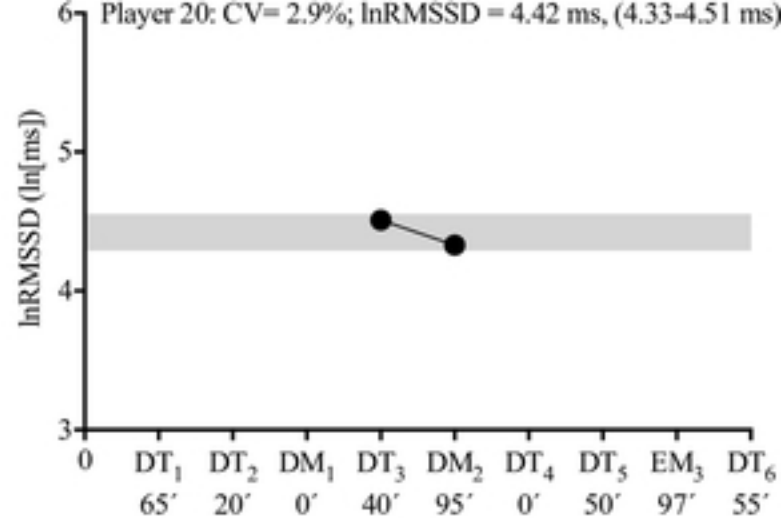
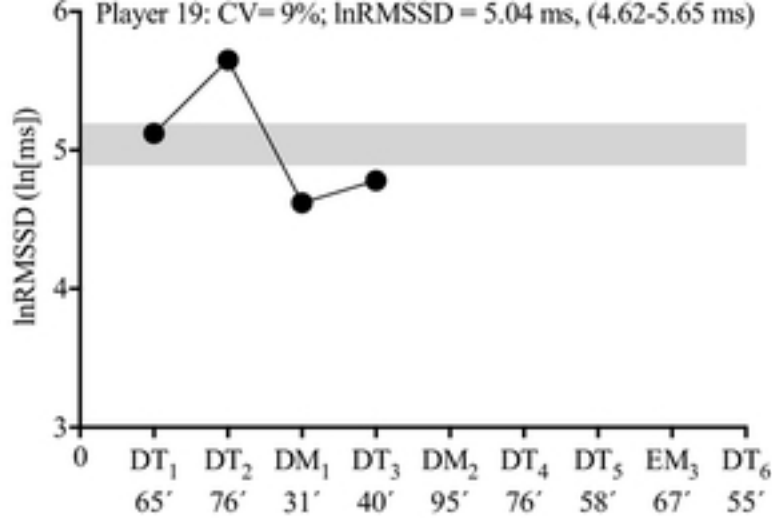
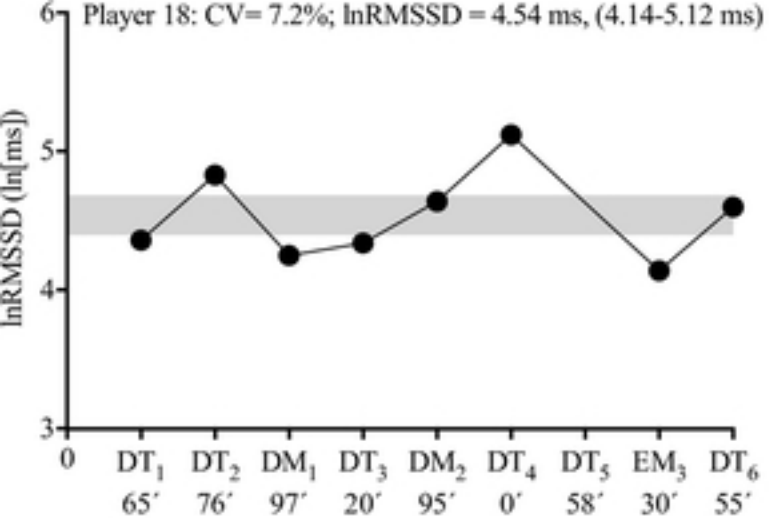


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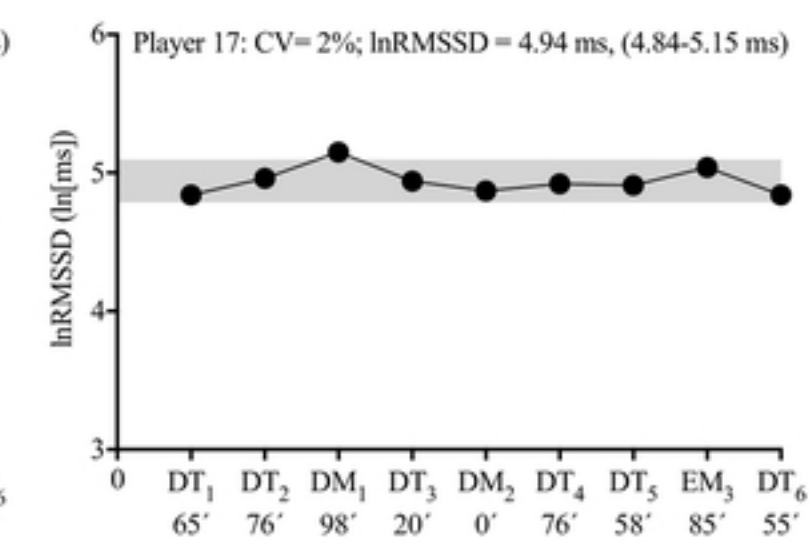
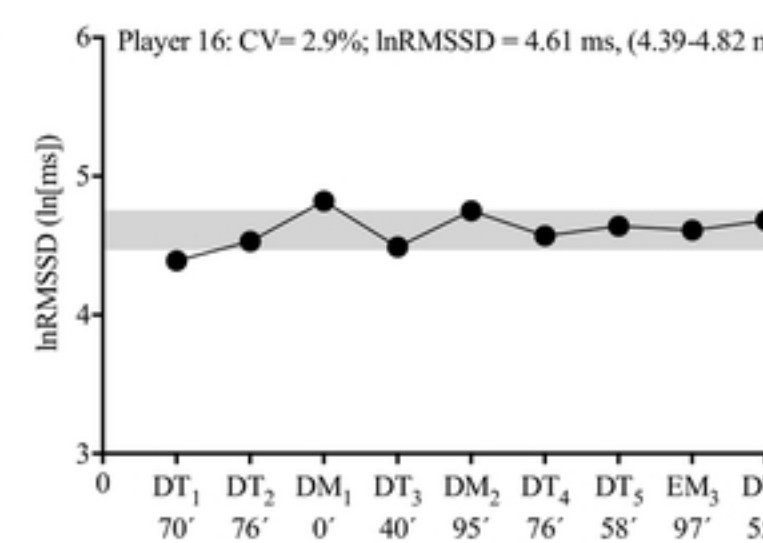
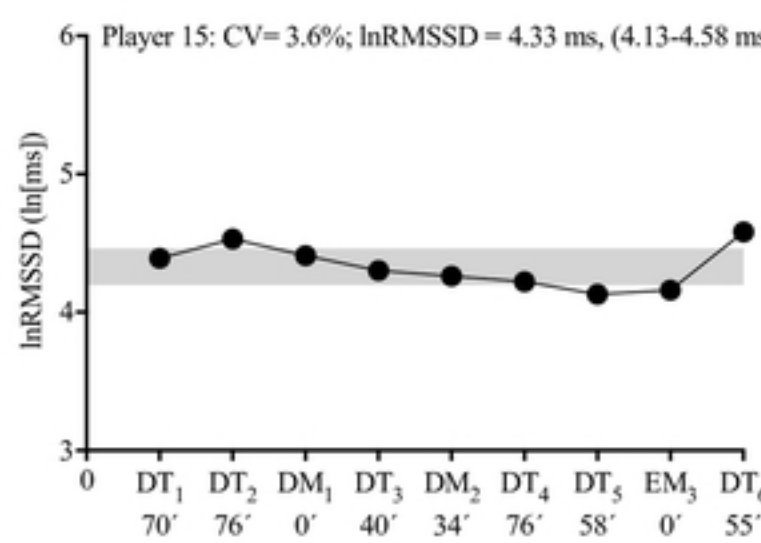
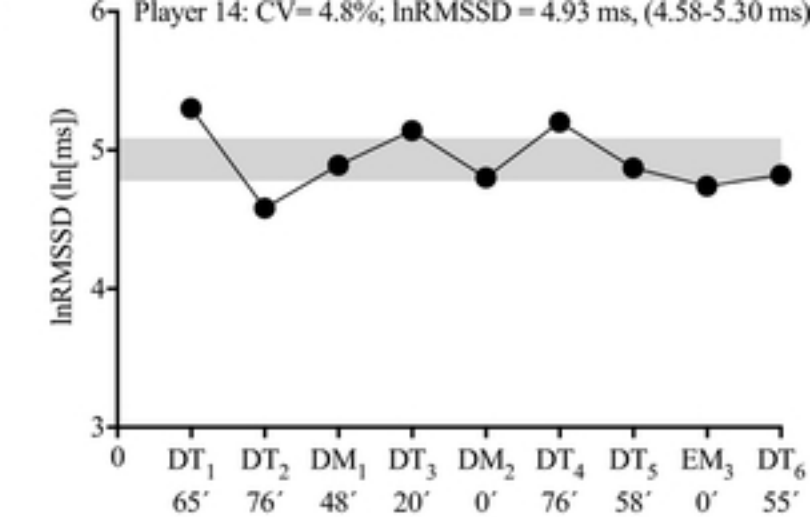
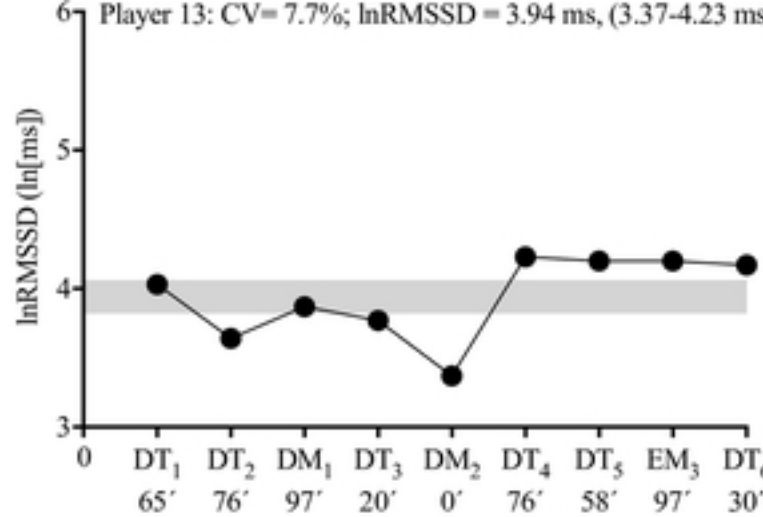
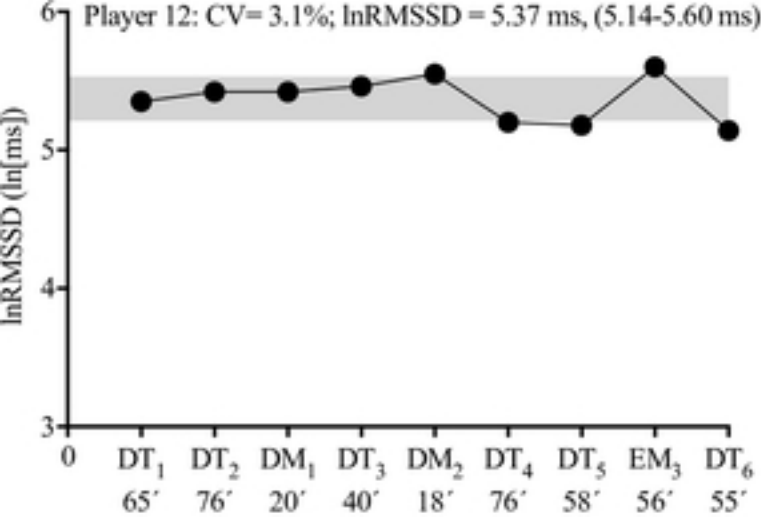


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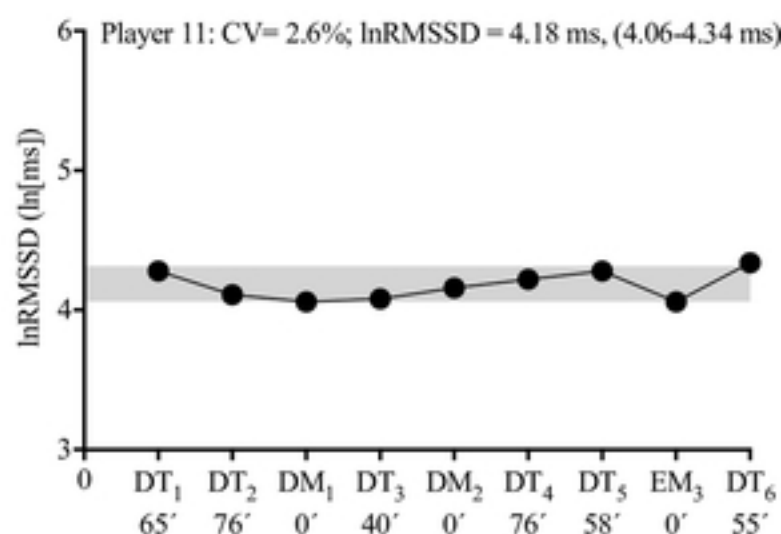
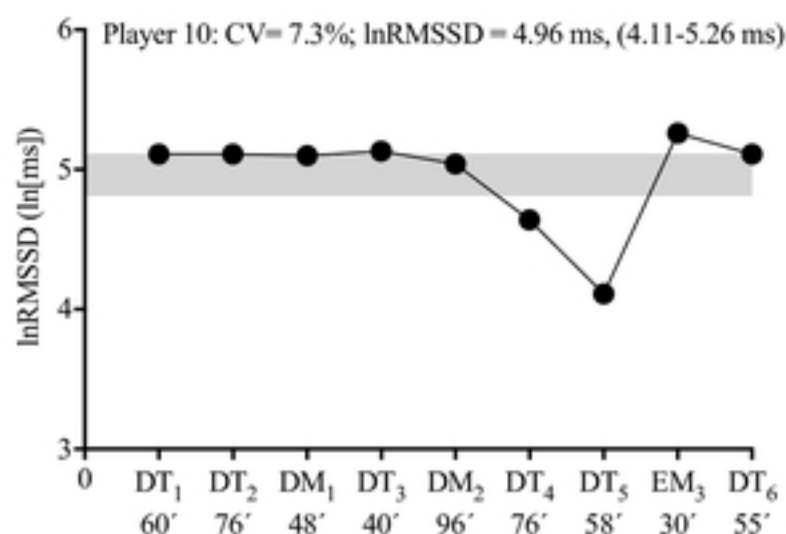
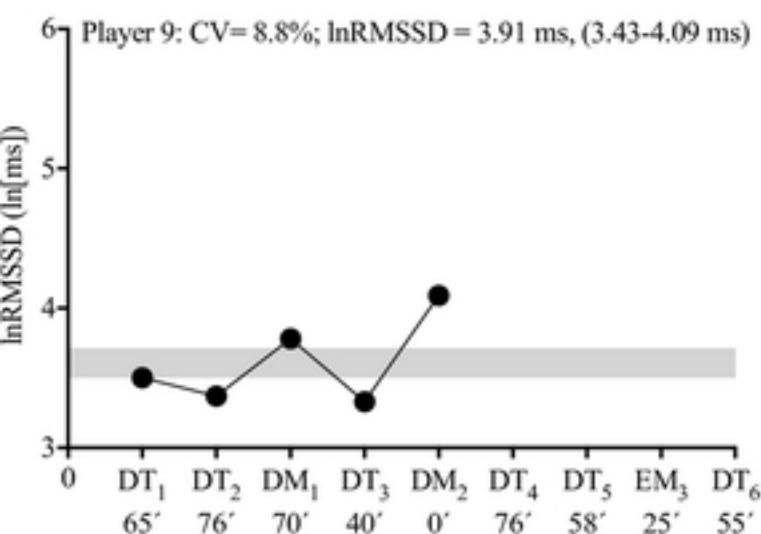
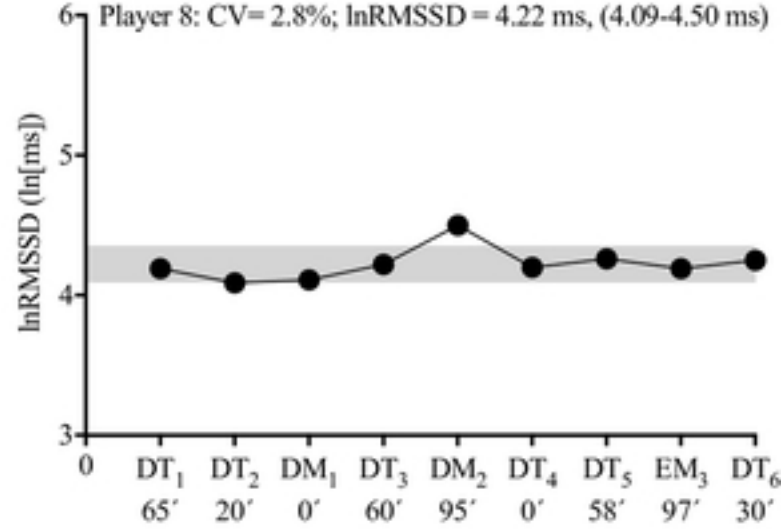
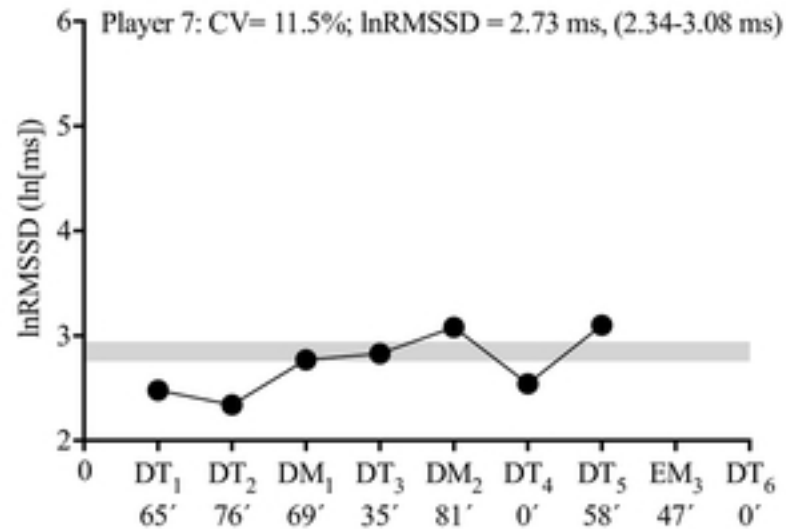
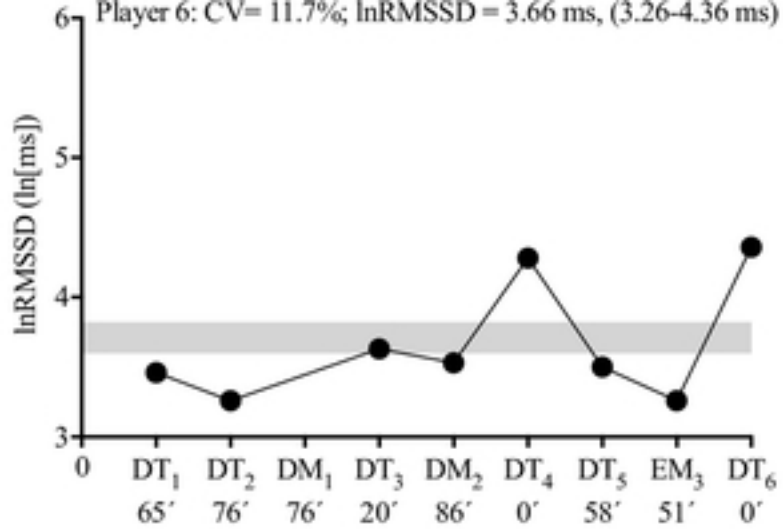


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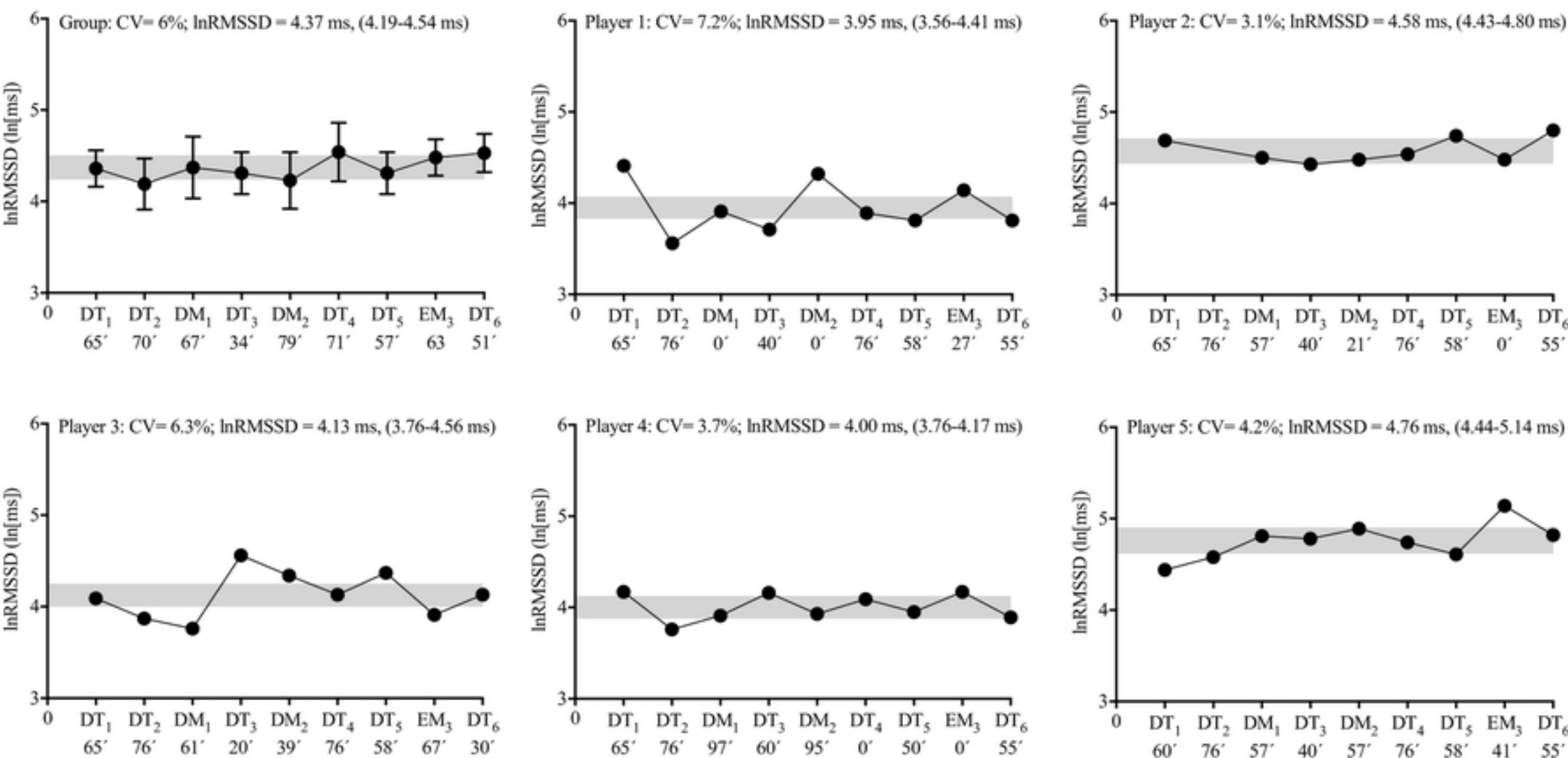


Figure 3.