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1 **Biochemical markers and wellness status during a congested match play**
2 **period in elite soccer players**

3 Original Investigation

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22
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28 **Abstract**

29 **Objectives:** To analyze biochemical markers, wellness status and physical fitness in elite soccer
30 players in relation to changes in training and match exposure during a congested period of
31 match play. **Methods:** Fourteen elite soccer players were evaluated three times (T1, T2, and
32 T3) over 12 weeks ([T1-T2: six weeks regular period of match play], [T2-T3: six weeks
33 congested period of match play]). Players performed vertical jump tests, the repeated shuttle
34 sprint ability test (RSSA), and the Yo-Yo intermittent recovery test (YYIR1) at T1, T2, and T3.
35 Plasma C-reactive protein (CRP), creatinine, and creatine kinase (CK) were analyzed at T1, T2,
36 and T3. Wellness status was measured daily using Hopper questionnaires (delayed onset of
37 muscle soreness [DOMS], stress, fatigue, sleep quality). Training session rating of perceived
38 exertion (sRPE) was also recorded on a daily basis. **Results:** A significant increase was found
39 in stress, fatigue, DOMS scores, and Hopper index during the congested period (between T2-
40 3) as compared with the regular period (between T1-2), ($0.001 < p < 0.008$, $0.8 < ES < 2.3$).
41 Between T2-3, significant relationships were found between the $\Delta\%$ of CRP, and $\Delta\%$ of CK
42 with the Hopper index and the $\Delta\%$ of fatigue score. In addition, the $\Delta\%$ of fatigue score and
43 $\Delta\%$ of DOMS score correlated with $\Delta\%$ YYIR1 and $\Delta\%$ RSSA_{best} ($0.49 < r < p < 0.01$).
44 **Conclusions:** An intensive period of congested match play significantly compromised elite
45 soccer players' physical fitness and wellness status. Elite soccer players' wellness status reflects
46 declines in physical fitness during a congested period of match play while biochemical changes
47 do not.

48 **Key words:** training, congested calendar, overtraining, overreaching, recovery.

49

50 **Introduction**

51 The physiological demands of soccer have increased over the past decades due to a higher
52 density of soccer matches, and an increase in mean intensity of matches.¹ During periods when
53 the schedule is particularly congested (i.e., two matches per week over several weeks), the
54 recovery time between successive matches is only 2-3 days, which may be insufficient to allow
55 full recovery of the players.¹⁻² As a result, players may experience acute and chronic fatigue
56 potentially leading to underperformance and an increased injury risk.²⁻³
57 To prevent imbalances and overtraining during congested schedules, biochemical assessments
58 are commonly used.⁴ This procedure could be used in soccer clubs as it typically involves the
59 assessment of blood markers indicating inflammation and muscle damage.⁵⁻⁶ Creatine kinase
60 (CK), C-reactive protein (CRP), and creatinine are biochemical markers that are often used to

61 quantify muscle damage and inflammation.⁷ These biochemical parameters are sensitive to
62 training periods differing in volume and intensity and frequency of matches during the
63 competitive period.⁸

64 Coaches and sports scientists conceptualized soccer as a psycho-physiological complex
65 sport⁹ where subtle changes in training load could affect wellness status and, ultimately physical
66 performance.³⁻¹⁰ Therefore, several studies reported the usefulness of psychological
67 questionnaires for monitoring changes in training-related stress, wellness, strain and recovery
68 to detect early signs of tiredness and potential overtraining in high-performance sport
69 programs.¹¹⁻¹² Recently, Thorpe et al.¹⁰ have reported that daily training load had affected these
70 wellness measurements in elite soccer players of the English Premier League. In addition to
71 training load, there are various contextual aspects, particularly those related to environmental
72 conditions such as social factors, personality trait, moment of the season, league ranking, and
73 match importance, that could influence players' status and, hence, their perceived wellness
74 level.¹³⁻¹⁴

75 However, to the best of our knowledge, there is little information concerning the relation
76 between biochemical markers, players' wellness, and physical fitness in elite soccer players
77 during different competitive periods of a soccer season. Therefore, the aims of this study were
78 to examine fluctuations in inflammatory and muscle damage markers, wellness status
79 (including perceived ratings of sleep, fatigue, stress, and muscle soreness), and physical fitness
80 during a competitive soccer season, which included a congested period of match play and to
81 examine the inter-relation between all of these parameters. Based on relevant literature data,³⁻⁹⁻
82 ¹⁰⁻¹⁴ it was hypothesized that biochemical parameters and wellness status may be altered after
83 the congested periods of competition, which, in turn, could cause negative changes in players'
84 physical fitness which would be evident through the associations between selected measures.

85

86 **Methods**

87 **Participants**

88 Initially, 22 elite male soccer players from the same soccer club within the first soccer division
89 of Tunisia were enrolled in this study. Over the course of the study, eight players were injured
90 and did not complete all training sessions. Athletes who attended less than 85% of the scheduled
91 training sessions and matches were therefore excluded from the study. Finally, 14 players (age
92 20.9 ± 0.8 y; height: 177 ± 5 cm; mass: 72.4 ± 5.2 kg) were eligible for inclusion in this study.
93 The enrolled players had the longest match exposure over the experimental period (accumulated
94 match time = 1032.50 ± 161.05 minutes). All participants signed informed consent forms

95 outlining commitment, benefits, potential risks, and the study's procedures, which were
96 approved by the local Ethics Committee of the medical staff of the soccer club and conformed
97 to the Declaration of Helsinki.

98

99 **Procedures**

100 The study was carried out during a period of 12 weeks within the competitive season, and was
101 designed to analyze inflammation, muscle damage, workload, physical fitness, and wellness
102 status to changes in training and match exposure during a congested period of match play.
103 Testing of players started 6 weeks after the beginning of the second competitive period
104 following the winter break. During this period, players trained five times per week with one
105 match. All players were evaluated three times during the study; T1 was in the middle of the
106 regular period (uncongested) (week 1); T2 was at the end of the regular period (before the
107 congested period; week 6). T3 was completed after the congested period (week 12; after the
108 season) (**figure 1**).

109 The assessments were performed over three days in the same order during the T1, T2 and T3
110 testing sessions. On the first day, blood samples were collected at each time point to monitor
111 muscle damage (creatine kinase [CK]) and inflammation (C-reactive protein [CRP] and
112 creatinine). On the second day, participants performed three physical fitness tests (the squat
113 jump (SJ), the countermovement jump (CMJ), and the repeated shuttle sprint ability test
114 (RSSA). On the third testing day, participants performed the Yo-Yo intermittent recovery test
115 level 1 (YYIR1). In order to minimize diet-induced performance changes, players were asked
116 to standardize and follow the same nutritional plan 24 hours before each test session. Before
117 each training session and match, the Hopper index was evaluated to monitor sleep, stress,
118 delayed onset of muscular soreness (DOMS), and fatigue. Session ratings of perceived exertion
119 (sRPE; CR-10Borg's scale) were recorded on a daily basis in order to quantify training load
120 ($TL = sRPE \times \text{training session time in minutes}$) and competitive load ($CL = sRPE \times \text{match time}$),
121 monotony, and strain.¹⁵

122 **Training program during the regular and congested competitive periods**

123 The duration of each training session was 90 min \pm 8 min for all players. Training sessions were
124 performed during the afternoon (3:30 to 5:00PM). **Tables 1a and 1b** summarize the training
125 program during the regular and congested periods of match play.

126

127 **Biochemical analyses**

128 For each soccer player, blood samples were always taken at the same time of day (between
129 08:00-10:00 AM) and assessed for inflammatory parameters (CRP and creatinine) and muscle
130 damage markers (CK). A recovery period of 12 h was scheduled the day before the samples
131 were taken and participants followed an overnight fast. On the test day, blood samples (15 ml)
132 were taken in a seated position via the median antebrachial vein into vacutainer tubes
133 (Vacuette®, Greiner bio One, France) without anti-coagulant and with EDTA (10ml). The
134 blood was immediately centrifuged at 3000 rpm for 10 min and the serum was stored at -80°C
135 until the analyses were carried out. The CRP was determined using the immunoturbidimetry
136 method (CRP Latex/ Beckman Coulter AU 480 system). The CK activity was determined by
137 the UV method (IFCC) using the N-acetyl-cysteine (EC 2.7.3.2/ Beckman Coulter, AU 480
138 system). Creatinine was determined by a kinetic modification of the Jaffe procedure, in which
139 creatinine reacts with picric acid at alkaline pH to form a yellow orange complex
140 (OSR6678/Beckman Coulter, AU 480). All samples were taken and handled within the same
141 laboratory.

142

143 **Quantification of the training and competitive loads**

144 The 10-point Borg scale was used to quantify the perceived effort of the players approximately
145 15 to 20 minutes after each training session and match in response to the question “how hard
146 the training session or match was?”¹⁵ The RPE scores were multiplied by the duration (in
147 minutes) of the session or match to calculate the training load (TL) and competitive load (CL).
148 Total load or global load (GL) was calculated as the sum of training load and competitive load.
149 Monotony was calculated as the weekly average load divided by the standard deviation of the
150 load. Strain was calculated as the total load multiplied by monotony.¹⁶ Data were categorized
151 into two competitive periods of training and games (the regular period and the congested period
152 of match play).

153

154 **Wellness status evaluation (Hopper index)**

155 The Hooper questionnaire, consisting of 4 items (stress, fatigue, sleep, and DOMS)¹¹ was
156 administered every morning before the training session started. The scale ranged from 1 (very,
157 very low) to 7 (very, very high) for stress, fatigue, and DOMS categories. In the specific case
158 of sleep quality, 1= very, very good and 7= very, very bad. The Hooper index was calculated
159 for each day, representing the sum of the 4 rates of the day. The sum of the weekly value for
160 each competitive period (regular and congested period) was calculated and used for further
161 analyses.

162

163 **Physical fitness tests**

164 ***Vertical jumping***

165 Each subject performed two different types of maximal vertical jumps, in the same order (i.e.,
166 SJ followed by CMJ), with hands akimbo and a 60s rest between each trial. The average of the
167 best three trials was taken for further analysis.¹⁷ The SJ was started with a knee angle of
168 approximately 90°. After a short rest (2s) to avoid the storage of elastic energy, players
169 performed the maximal vertical jump. The CMJ started from a standing position allowing for
170 counter movement with the intention of reaching a knee angle of around 90° just before
171 propulsion. Players were asked to jump as high as possible during both tests. Vertical jump
172 height was evaluated using an opto electric system (Opto-Jump Microgate – ITALY). Jump
173 height was calculated according to the following equation: jump height = $1/8 \times g \times t^2$, where g
174 is the acceleration due to gravity and t is the flight time.¹⁸

175

176 ***Yo-Yo intermittent recovery test level I (YYIRT1)***

177 The YYIRT1 was used to assess players' ability to repeat high-intensity exercises.¹⁹ The
178 YYIRT1 consists of repeated 2×20m runs back and forth between the starting, turning, and
179 finishing line at progressively increasing speeds controlled by an audiometronome from a
180 calibrated CD player. The subjects had a 10-s active rest period between each running bout.
181 The test was completed if the subjects failed twice to reach the finish line in time, or decided
182 that he could no longer run at the required pace. The total distance covered (m) was recorded
183 for further analyses.

184

185 ***Repeated shuttle sprint test***

186 Repeated Shuttle Sprint Ability (RSSA) was tested to assess players' ability to repeat sprints.²⁰
187 After a 15- min warm up, players completed 6 × 40-m maximal sprints interspersed with 20 s
188 of passive recovery. Blood lactate was measured immediately after the test using a portable
189 lactate analyzer (Lactate Pro2, Matsport, France).

190 RSSA testing was conducted using an infrared photoelectric cell (Cell Kit Speed Brower,
191 United States) with an accuracy of 0.01 s.

192 The best sprint time was recorded and used for further analysis (RSSA_{best}). In addition, the
193 dependent variables included the mean time obtained over six sprints and the decrease in
194 performance ($RSSA_{decrement} = ([RSSA_{mean}]/[RSSA_{best}] \times 100) - 100$).

195

196 **Statistical analyses**

197 Data are expressed as means and standard deviations (SD). Normal distribution of data was
198 tested and confirmed using the Kolmogorov-Smirnov test. Paired t-tests for dependent samples
199 were used to identify differences in workload parameters (TL, Monotony, Strain), and Hopper
200 index between the congested and the regular periods of match play. We computed a one-way
201 analysis of variance (ANOVA) for repeated measures to determine differences between
202 biochemical and physical fitness parameters measured during the congested vs. regular period
203 of match play. Pearson's product-moment correlation coefficients (r) were computed to identify
204 relations between all parameters. The magnitude of correlation coefficients was considered as
205 trivial ($r < 0.1$), small ($0.1 < r < 0.3$), moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large
206 ($0.7 < r < 0.9$), nearly perfect ($0.9 < r < 1.0$), and perfect ($r = 1.0$).²¹ Statistical significance was
207 set at $p < 0.05$ for all analyses. The 95% confidence intervals (CI) and effects sizes (ES) were
208 calculated to compare differences in mean values for all analyzed parameters. When calculating
209 ES, pooled standard deviations (SD) were used since no control group was available (Cohen's
210 $d = [M1 - M2] / SD_{\text{pooled}}$). ES with values of 0.2, 0.5, and 0.8 were considered to represent small,
211 medium and large differences, respectively.²² All statistical tests were processed using a
212 statistical software (SigmaStat3.5, Systat, Inc, USA).

213

214

215 **Results**

216 Over the 12-week experimental period, 14 players were able to complete the study requirements
217 according to the previously described study design and methodology. Overall, 16 matches were
218 played. Six games were played during the 6 weeks of the non-congested period, and 10 games
219 were played during the 6 weeks of congested match play. The 14 players included in this study
220 performed all matches. The Mean ICC (Intraclass Correlation Coefficients) for the different
221 parameters was presented in **Table 2**.

222

223 **Workload parameters**

224 **Table 3** presents data of global load parameters and accumulated match time during the 12
225 weeks training period (regular vs. congested period of match play). All training parameters were
226 significantly different between comparisons.

227 **Figures 2 and 3** present individual and team measures of total, training and competitive loads
228 during regular and congested periods. Total load was significantly lower in the regular periods
229 as compared to congested period. In contrast, training load parameters were higher in the regular

230 period as compared to congested period. The competitive load and accumulated match time
231 were significantly higher in the congested period as compared to regular period. The weekly
232 total and competitive loads were significantly higher during congested weeks as compared to
233 regular weeks. The weekly training load parameters were higher in the regular period as
234 compared to congested period (**Figures 4 and 5**).

235

236 **Physical fitness data**

237 Results from the performance tests are provided in **Table 4**. There were no significant changes
238 in CMJ Jump height, $RSSA_{\text{decrement}}$, and blood lactate between the three testing sessions.
239 However, The YYIR1, $RSSA_{\text{mean}}$, $RSSA_{\text{best}}$ performance and SJ height differed significantly in
240 T3 after the congested period. Also, the YYIR1 performance increased significantly from T1 to
241 T2 after the regular period. The $RSSA_{\text{best}}$ was significantly lower in T2 as compared to T1.

242

243 **Biochemical data**

244 Parameters connected to inflammation and muscular damage markers showed a number of
245 changes during the competitive periods, the CRP was significantly higher in T3 as compared to
246 T2 and T1. The CK was significantly higher in T3 as compared to T2 and T1. However, CRP
247 and CK did not change significantly between T1 and T2. In addition, there were no significant
248 differences in creatinine during the observation period (**Table 5**).

249

250 **Wellness status**

251 Changes in wellness scores at different competitive periods are presented in **Table 6**. The stress
252 score was significantly higher after the congested period than in the regular period ($p=0.001$,
253 $ES=0.75$). Moreover, the fatigue score increased after the congested period as compared to the
254 regular period ($p=0.008$, $ES=1.54$). The DOMS score increased during the congested period
255 compared with the regular period ($p < 0.001$, $ES=3.43$). However, there was no alteration in the
256 sleep score during the different competitive periods. Finally, the Hopper index increased
257 significantly after the congested period compared with the regular period ($p < 0.001$, $ES=1.92$)
258 (Figure 6).

259

260 **Relation between biochemical parameters and physical fitness**

261 No significant correlation between biochemical parameters and physical fitness performance
262 was found during the congested period of match play. Only a significant relation was observed
263 between $\Delta\%$ CRP and $\Delta\%$ SJ ($r=0.55$, $p=0.03$) during the regular period (**Table 7**).

264 **Relation between biochemical parameters and wellness status**

265 No significant relation was found between inflammatory and muscle damage parameters with
266 wellness scores during the regular period (**Table 8**). Of note, we showed that the $\Delta\%$ of CRP
267 was correlated with the Hopper Index ($r=0.53$, $p=0.04$) and the fatigue score during the
268 congested period ($r=0.64$, $p=0.01$). In addition, the $\Delta\%$ of CK was correlated with the Hopper
269 index ($r=0.63$, $p=0.01$) (**Figure 7**).

270

271 **Relation between wellness status and physical fitness**

272 Several significant correlations were found between wellness scores and physical fitness during
273 the congested period of match play (**Table 9**). The $\Delta\%$ of fatigue score correlated with
274 $\Delta\%$ YYIR1($r=-0.49$, $p=0.04$). In addition, the $\Delta\%$ of DOMS score correlated with $\Delta\%$ RSSA_{best}
275 ($r=0.51$, $p=0.04$). Moreover, we observed a positive correlation between $\Delta\%$ RSSA_{mean} and $\Delta\%$
276 Hooper index ($r=0.54$, $p=0.04$).

277

278 **Relation between percentage of biochemical parameters, physical fitness, Hopper index, 279 and workload parameters**

280 **Table 10** summarizes relationships between percentage changes of biochemical parameters,
281 physical fitness, wellness score and workload parameters during the study. We observed that
282 the Hopper Index were related to workload parameters during the regular period (GL: $r=0.56$,
283 $p=0.03$; monotony: $r=0.54$, $p=0.04$; strain: $r=0.56$, $p=0.03$) and the congested period of match
284 play (GL: $r=0.66$, $p=0.009$; CL: $r=0.69$, $p=0.006$, strain: $r=0.69$, $p=0.006$). The DOMS score
285 was related to workload parameters during the congested period of match play GL: $r=0.70$,
286 $p=0.004$, strain: $r=0.71$, $p=0.004$). The fatigue score was related to workload parameters (CL:
287 $r=0.52$, $p=0.02$). In addition, the increase in CK level was related to monotony ($r=0.69$, $p=$
288 0.005) during the congested period. The increase in CRP was correlated to workload parameters
289 (GL: $r= 0.52$, $p=0.04$, monotony: $r=0.50$, $p=0.04$; strain: $r=0.51$, $p=0.03$). Also, the change in
290 RSSA_{mean} were related to workload parameters during the congested period (CL: $r=0.59$,
291 $p=0.06$), monotony: $r=0.63$, $p=0.01$; strain: $r=0.65$, $p=0.01$) and the change of YYIR1were
292 related to strain ($r=0.52$, $p=0.04$).

293 **Discussion**

294 The aim of this study was to examine fluctuations in inflammatory and muscle damage markers,
295 wellness status and physical fitness during a competitive soccer season, which included a
296 congested period of match play. In addition, the inter-relations among these parameters were
297 assessed. Our findings suggest a significant increase in inflammatory and muscle damage
298 markers as well as wellness measures (stress, fatigue, DOMS, and Hopper index) after the
299 congested period of match play. Additionally, we observed a significant decrease in physical
300 fitness performance. The change in wellness status was related to physical fitness performance
301 decline.

302

303 In this study, CRP and CK concentrations were unchanged after the 6-week regular fixture
304 period. However, increases were observed after the six-week congested fixture period as the
305 players were exposed to a competitive load. The absence of a significant change in resting CRP
306 and CK, in conjunction with physical performance test improvements (YYIR1 and $RSSA_{mean}$,
307 and $RSSA_{best}$) observed in regular period, would confirm an optimal training stimulus. On the
308 contrary, the accumulated fatigue potentially caused by the increase in total load and
309 competitive load seems to lead to an increment in CRP and CK after the congested competitive
310 schedule. Nevertheless, the observed performance decline together with altered inflammatory
311 and muscle damage markers after the congested periods can also be explained by the chronic
312 effects of the 12-week competitive period which is note quadlto the acute effect of the last
313 congested week of match play. In this regard, potential differences in the number of high-
314 intensity actions (e.g., decelerations) during matches between periods should not be disregarded
315 as it would be expected a major role of these actions on muscle damage markers and
316 inflammation. Activities during soccer matches and training sessions include high amounts of
317 jumping, sprinting, and eccentric muscle actions.²³ These results are in agreement with data
318 reported by Walker et al.⁸ who showed a significant increase in CK concentrations after 16
319 competitive weeks in female professional soccer players. The authors of this latter study
320 demonstrated that the increase of CK could be caused by the elevated training and competitive
321 loads. In addition, Pimenta et al.²⁴ showed an increase for CK concentration after the pre-season
322 period when the players were exposed to higher volume and intensity of training. Therefore,
323 investigators have speculated that intensified training or match exposure could influence the
324 increase of inflammation and muscle damage markers.²⁵⁻²⁶ These type of muscle activities will
325 result in micro tear of structural and contractile components in the muscle fibers ²⁷ leading to
326 increased serum CK levels.²⁸

327 Additionally, the current results suggest that the higher number of matches played in the last 6
328 weeks (10 matches, accumulated match times = 644.2 ± 125.9 minutes) potentially affect
329 resting CRP and CK concentrations. Conversely, the current results disagree with the results of
330 Coppalle et al.⁶ who showed anon-significant change in CRP and CK levels after the pre-season
331 period. Differences in study methodology between studies could partially explain these
332 different outcomes. For example, the study of Coppalle et al.⁶ and the current study analyzed
333 the soccer season at different time points (e.g., pre-period and 12 weeks competitive periods).
334 Moreover, Coppalle et al.⁶ for technical reasons, analyzed their blood samples one week after
335 the end of the pre-season which may be sufficient time to allow the recovery of players. Of
336 note, we observed no alterations in the creatinine concentrations during the 12-week
337 competitive period. This result is in agreement with Heisterberg et al.²⁹ who showed that the
338 creatinine concentration was unaltered over the course of a soccer season. Thus, creatinine
339 appears to be of minor interest for blood monitoring of elite soccer players.⁷

340

341 Concerning the wellness status, we noted that the Hopper index, stress, fatigue, and DOMS
342 scores were higher in the congested period as compared to the regular period of match play.
343 Moreover, we also noticed that Hopper index increases were strongly related to workload
344 parameters (total load, training load, competitive load, monotony and strain). This result agrees
345 with data reported by Fessi et al.¹⁴ who showed significant associations between training load
346 parameters and wellness measures (sleep, stress, fatigue, and muscle soreness) in professional
347 soccer players. Accordingly, our data corroborate the outcomes presented by Buchheit et al.³⁰
348 and Thorpe et al.¹⁰ who demonstrated that the daily variations in training load affected wellness
349 measures the following day in Australian Rules football and elite soccer players, respectively.
350 These observations provide evidence supporting the sensitivity of these simples' measures to
351 change in training load. It was hypothesized that fatigue and DOMS scores were affected by
352 the greater total load and competitive load along with the higher number of matches played
353 during the period of congested match play. Moreover, the congested period involved lower
354 training loads compared with the regular period (3853.21 ± 2429.48 vs. 5581.28 ± 1074.25
355 A.U.). These differences are usually observed due to the different number of matches between
356 conditions. These differences may be the results of the fact that there is less time to train (5 vs.
357 4 training days, on average) and that coaches intentionally reduced training volume in congested
358 weeks to control the players training in periods overloaded with matches. However, despite the
359 training load reduction, global load (training and competitions) during congested periods
360 involved a higher disruption of wellness status for players than regular periods. The

361 accumulation of the matches significantly increased players' stress after the congested period
362 of match play. Further, the increase in stress scores can be explained because this period
363 included the most important matches, that is, the play-offs. In contrast, we observed no
364 difference in sleep score between regular and congested periods. These findings might be
365 indicative of sleep perception not being sensitive to the workload. Indeed, for elite soccer, it
366 was suggested that in a competitive period (with different training programs and level of
367 competition), the perception of sleep quality may only provide information on potential
368 recovery status rather than any association with workload.³¹The same tendency was found in
369 the study of Nédélec, et al.³² who showed that training load affects fatigue and muscle soreness
370 parameters to a larger extent compared with sleep. However, more in-depth research including
371 physiological data on sleep, fatigue and muscle soreness are required.

372 In this study, no significant correlations were observed between biochemical parameters and
373 players' performance in the YYIR1, RSSA, SJ and CMJ tests. Thus, we observed that the
374 decline in the applied physical fitness tests during the congested period was associated with
375 increases in CRP and CK concentrations. These findings are in agreement with previous reports
376 who showed negative associations between changes in biochemical markers and performance
377 declines with maintenance of higher CRP and CK values.³³ In the current study, the decline in
378 performance measures of physical fitness after the congested period of match play may be
379 reflected in the observed increase in inflammation (CRP) and muscular damage (CK). Hence,
380 Walker et al.⁸ showed that the increased CK level was associated with the decline in vertical
381 jump performance. This may suggest that the alteration in CK concentration might affect
382 explosive-type performance such as jumping. Moreover, increases in CK level without
383 impairments in muscle performance have been reported.²⁴ Therefore, some caution should be
384 taken when trying to predict muscle recovery and impairments from changes in serum CK
385 concentrations. In the current study, we showed that the increase in CRP concentrations was
386 correlated to the SJ height increases in the regular periods. Based on these contradictory results,
387 we postulate that the observed significant correlations between inflammatory markers and
388 physical fitness test performance should be interpreted with caution. Further, the reported
389 relationships among these parameters do not reflect cause and effect relations and may be
390 spurious. Our results further suggest that the increase in CRP and CK noted in T3 after the
391 congested period of match play may reflect tiredness or a state of fatigue caused by
392 inflammation and muscular damage. To determine more precisely the influence of fatigue on
393 these biological variations, it is necessary to address these two variables. To do this, we
394 examined potential correlations between the wellness scores and biochemical data.

395 In our study, the CRP and CK concentrations were significantly correlated with wellness scores
396 (Hopper index) during the congested period suggesting the involvement of wellness changes
397 on CRP and CK responsiveness. Moreover, we found a significant positive correlation between
398 the CRP and the fatigue score. This suggests that it is useful to follow variations in CRP and
399 CK level to determine the degree of tiredness and change in wellness or vice versa. In addition,
400 there appears a negative relation between physical fitness and wellness measures during a
401 congested period of match play. Thus, the increase in wellness measures was associated with
402 physical fitness declines. More precisely, we noticed that the $\Delta\%$ of fatigue score correlated
403 with $\Delta\%$ YYIR1. In addition, the $\Delta\%$ of DOMS score correlated with $\Delta\%$ RSSA_{best} and we
404 observed a positive correlation between $\Delta\%$ RSSA_{mean_} and $\Delta\%$ Hooper Index. These results
405 cannot, however, be used to indicate whether changes in physical test performance are affected
406 by changes in wellness scores (fatigue, DOMS, and stress) increased or vice-versa. Our results
407 confirm that physical performance depends simultaneously on physical and wellness status.
408 Thus, it seems that the participation in a high number of matches during the congested period
409 induced psychological stress on the soccer players.

410 In this study, we showed a positive correlation between monotony and CK during the congested
411 period of match play. In addition, changes in CRP level showed significant relations with
412 workload parameters. These results confirm that some biochemical markers are sensitive to
413 competitive periods and soccer training which is in agreement with the study of Walker et al.⁸
414 who showed that the CK increases are associated with training load increases and higher
415 number of practices during 16 weeks of soccer training and competition. Thus, these
416 biochemical levels can be used as a potential marker for variation in intensity of training and/or
417 competition.

418

419 **Limitations**

420 It is important to highlight some limitations inherent to this work. This study did not have a
421 control group which does not allow to identify the specific effects of a congested period on the
422 examined markers. We acknowledge that the use of the Hopper questionnaire is limited in team
423 sports. Different motivational levels of the team players may have affected the outcomes of the
424 Hopper questionnaire. Internal training load was only measured by session-RPE and this may
425 have constrained the real quantification of the physiological impact of the training. External
426 load was not used in this study, nevertheless the physical quantification of training impact may
427 be an interesting set of variables to justify the perception of DOMS and fatigue. Moreover,
428 using jump height as a global measure may not sufficiently reflect fatigue in soccer players.

429 Fatigued soccer players might still be able to alter their movement patterns which allow them
430 to achieve similar performances as in unfatigued condition.

431

432 **Strength of the study and implications for future research**

433 Although data on inflammation and muscle damage, and performance are already available in
434 the literature, our study is the first that combines all these markers together with total load
435 (training and competitive load) during a soccer season including a congested period of match
436 play. Our results suggest that the congested period of match play alters various parameters (e.g.,
437 C-reactive protein, CK, Hopper index) in elite soccer players. While the observed changes in
438 inflammatory and muscle damage markers were unrelated with physical fitness declines,
439 measures of wellness were. Future studies should analyze whether the accumulation of matches
440 influences the players' physical and physiological responses during the match played (e.g.,
441 number of sprints, accelerations and decelerations, etc.) In addition, inflammatory and muscle
442 damage markers could be analyzed immediately after the match during both, regular and
443 congested periods.

444

445 **Practical applications**

446 The results of this study confirm the usefulness of wellness measures to provide information
447 about the impact of training and competition on elite soccer players. In addition, our findings
448 support the use of the Hopper questionnaire as a simple monitoring tool for identifying
449 performance decrements in players exposed to highly competitive loads during the congested
450 period.

451

452 **Conclusions**

453 The results of this study showed significant changes in selected biochemical markers, wellness
454 status and physical fitness parameters during the final competitive period of the season
455 (congested match play). The main findings were that elite soccer players' wellness status
456 reflects declines in physical fitness during a congested period of match play while biochemical
457 changes do not. Furthermore, CRP and CK changes correlate with wellness status (fatigue,
458 DOMS, Hopper index), suggesting that it is useful to follow variations in CRP and CK levels
459 to determine the degree of change in wellness status or vice versa.

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463 **References**

- 464 1. Carling C, Le Gall F, Dupont G. Are physical performance and injury risk in a professional
465 soccer team in match-play affected over a prolonged period of fixture congestion? *Int J Sports*
466 *Med.* 2012;33(01):36-42.doi: 10.1055/s-0031-1283190
- 467 2. Dellal A, Lago-Peñas C, Rey E, Chamari K, Orhant E. The effects of a congested fixture
468 period on physical performance, technical activity and injury rate during matches in a
469 professional soccer team. *Br J Sports Med.* 2015;49(6):390-394.doi: 10.1136/bjsports-2012-
470 091290.
- 471 3. Saidi K, Abderrahman AB, Boullosa D, Dupont G, Hackney AC, Bideau B, Zouhal H. The
472 Interplay Between Plasma Hormonal Concentrations, Physical Fitness, Workload and Mood
473 State Changes to Periods of Congested Match Play in Professional Soccer Players. *Front*
474 *Physiol.* 2020;11.
- 475 4. Rowsell GJ, Coutts AJ, Reaburn P, Hill-Haas S. Effects of cold-water immersion on physical
476 performance between successive matches in high-performance junior male soccer players. *J*
477 *Sports Sci.* 2009;27(6):565-573.
- 478 5. Pascoal EHF, Borges JH, Franciscan CA, Spigolon LMP, Borin JP. Pre-Season training
479 affects negatively the immunological parameters and creatine kinase but not power
480 performance in young soccer players. *Arch Sports Med.*2018;2:94-102.
481
- 482 6. Coppalle S, Rave G, Ben Abderrahman AB, Ali A, Salhi I, Zouita S, Zouhal H. Relationship
483 of pre-season training load with in-season biochemical markers, injuries and performance in
484 professional soccer players. *Front Physiol.* 2019;10:409.
485
- 486 7. Djaoui L, Haddad M, Chamari K, Dellal A. Monitoring training load and fatigue in soccer
487 players with physiological markers. *Physiol Behav.* 2017;18:86-94.
488
- 489 8. Walker AJ, McFadden BA, Sanders DJ, Rabideau MM, Hofacker ML, Arent SM. Biomarker
490 response to a competitive season in Division I female soccer players. *J Strength Cond Res.*
491 2019;33(10):2622-2628.
492
- 493 9. Haddad M, Chaouachi A, Wong del P, Castagna C, Hambli M, Hue O, Chamari K. Influence
494 of fatigue, stress, muscle soreness and sleep on perceived exertion during submaximal effort.
495 *Physiol Behav.*2013;119:185–189.
496
- 497 10. Thorpe RT, Strudwick AJ, Buchheit M, Atkinson G, Drust B, Gregson W. Monitoring
498 fatigue during the in-season competitive phase in elite soccer players. *Int J Sports Physiol Perf.*
499 2015;10:958-964.
500
- 501 11. Hooper SL, Mackinnon LT. Monitoring overtraining in athletes recommendations. *Sports*
502 *Med;*1995;20:321-327.
503
- 504 12. Coutts AJ, Reaburn P. Monitoring changes in rugby league players' perceived stress and
505 recovery during intensified training. *Perceptual and Motor Skills.* 2008;106(3):904-916.
506
- 507 13. Chamari K, Haddad M, Wong del P, Dellal A, Chaouachi A. Injury rates in professional
508 soccer players during Ramadan. *J Sci Med Sport.* 2012;30:93-102.

- 509
510 14. Fessi MS, Nourira S, Dellal A, Owen A, Elloumi M, Moalla W. (2016a). Changes of the
511 psychophysical state and feeling of wellness of professional soccer players during pre-season
512 and in-season periods. *Res Sports Med*.doi:10.1080/15438627.2016.1222278
513
- 514 15. Foster C. Monitoring training in athletes with reference to overtraining syndrome. *Med sci.*
515 *sports and exerc.* 1998;30:1164-1168.doi: 10.1097/00005768-199807000-00023.
516
- 517 16. Foster C, Florhaug JA, Franklin J, Gottschall L, Hrovatin LA, Parker S, Dodge C. A new
518 approach to monitoring exercise training. *J.Strength Cond Res.* 2001;15(1):109-115.
519
- 520 17. Claudino JG, Cronin J, Mezêncio B, McMaster DT, McGuigan M, Tricoli V, Serrão JC.
521 The countermovement jump to monitor neuromuscular status: A meta-analysis. *J Sci Med*
522 *Sport.* 2017;20(4):397-402.doi: 10.1016/j.jsams.2016.08.011.
- 523 18. Prieske O, Muehlbauer T, Mueller S, Krueger T, Kibele A, Behm DG, Granacher U. Effects
524 of surface instability on neuromuscular performance during drop jumps and landings. *Eur J*
525 *Appl Physiol.* 2013;113:2943-2951. doi: 10.1007/s00421-013-2724-6
526
- 527 19. Bangsbo J, Iaia FM, Krstrup P. The Yo-Yo intermittent recovery test. *Sports med.*2008;
528 38(1):37-51.doi:10.2165/00007256-200838010-00004.
- 529 20. Impellizzeri FM, Rampinini E, Castagna C, Bishop D, Bravo DF, Tibaudi A, Wisloff U.
530 Validity of a repeated-sprint test for football. *Int. J. Sports Med.*2008;29(11): 899-905. doi:
531 10.1055/s-2008-1038491
532
- 533 21. Hopkins, R. L. II, and Burr, B. M. Modeling freshwater fish distributions using multiscale
534 landscape data: a case study of six narrow range endemics. *Ecol. Model.* 2009;220:2024-2034.
535 doi: 10.1016/j.ecolmodel.2009. 04.027
536
- 537 22. Cohen J. *Statistical power analysis for the behavioural sciences.*1988. 2nd edn. Hillsdale,
538 NJ: Lawrence Erlbaum.
- 539 23. Mohr M, Krstrup P, Bangsbo J. Match performance of high standard soccer players with
540 special reference to development of fatigue. *J Sports Sci.* 2009;21:519-528.
541
- 542 24. Pimenta E, Coelho DB, Capettini L, Gomes T, Pussieldi G, Ribeiro J, Silami Garcia E.
543 Analysis of creatine kinase and alpha-actin concentrations in soccer pre-season. *Rev Brasil de*
544 *Ciência e Mov.*2015;23(4):5-14.
545
- 546 25. Nédélec M, McCall A, Carling C, Legall F, Berthoin S, Dupont G. Recovery in soccer.
547 *Sports Med.* 2012;42:997-1015.
548
- 549 26. Souglis A, Bogdanis GC, Chryssanthopoulos C, Apostolidis N, Geladas ND. Time course
550 of oxidative stress, inflammation, and muscle damage markers for 5 days after a soccer match:
551 effects of sex and playing position. *J Strength Cond Res.* 2018;32:2045-2054. doi:
552 10.1519/JSC.0000000000002436
553

554 27. Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. *Br*
555 *Med Bull.* 2007;81–82:209-230.
556
557 28. Hartmann U and Mester J. Training and overtraining markers in selected sport events. *Med*
558 *Sci Sports Exerc.* 2000;32:209- 215.
559
560 29. Heisterberg MF, Fahrenkrug J, Krstrup P, Storskov A, Kjær M, Andersen JL. Extensive
561 monitoring through multiple blood samples in professional soccer players. *J Strength Cond Res.*
562 2013;27(5):1260-1271.
563
564 30. Buchheit M, Racinais S, Bilsborough JC, Bourdon PC, Voss SC, Hocking J, Coutts AJ.
565 Monitoring fitness, fatigue and running performance during a pre-season training camp in elite
566 football players. *J Sci Med Sport.* 2013;16(6):550-555.
567
568 31. Clemente FM, Mendes B, Nikolaidis PT, Calvete F, Carriço S, Owen AL. Internal training
569 load and its longitudinal relationship with seasonal player wellness in elite professional soccer.
570 *Physiology and Behavior.* 2017;179. doi:10.1016/j.physbeh.2017.06.021
571
572 32. Nédélec M, Halson S, Abaidia AE, Ahmaidi S, Dupont G. Stress, sleep and recovery in elite
573 soccer: A critical review of the literature. *Sports Medicine.* 2015;45:1387-1400.
574
575 33. Silva, J. R., Rebelo, A., Marques, F., Pereira, L., Seabra, A., Ascensão, A., and Magalhães,
576 J. (2014). Biochemical impact of soccer: an analysis of hormonal, muscle damage, and redox
577 markers during the season. *Applied Physiology, Nutrition, and Metabolism*, 39(4), 432-
578 438. 10.1139/apnm-2013-D180.
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588 **Figure legends**

589 **Figure 1** Experimental design.

590 Abbreviations: **RSSA**, repeated sprint shuttle ability; **SJ**, squat jump; **CMJ**, countermovement
591 jump; **YYIR1**, Yo-Yo intermittent recovery test-level 1; **Sun**, Sunday; **Mon**, Monday; **Tue**,
592 Tuesday; **Wed**, Wednesday; **Thu**, Thursday; **Fri**, Friday; **Sat**, Saturday. **RPE**, Rating of
593 Perceived exertion.

594 **Figure 2** Individual and team measures of total load and training load during the regular and
595 congested periods of the season.

596 **Figure 3** Individual and team measures of competitive load and accumulated match time during
597 the regular and congested periods of the season.

598 **Figure 4** Weekly total load and training load during the regular and congested periods of the
599 season.

600 **Figure 5** Weekly competitive load and accumulated match time during the regular and
601 congested periods of the season.

602 **Figure 6** Individual and team measures of the Hopper index during the regular and congested
603 periods of the season.

604 Abbreviations: **A.U**, Arbitrary units; **P**, player; **R**, regular period; **C**, congested period.

605 **Figure 7** Relation between biochemical parameters and wellness status during the congested
606 period.

607 (A) Correlation between $\Delta\%$ CRP and Hopper Index. (B): correlation between $\Delta\%$ CK and
608 DOMS.

609 Abbreviations: **CRP**, C-reactive protein; **DOMS**, delayed onset muscle soreness.

610 **Figure 8** Summary of the main findings of this study.

611 Abbreviations: **GL**, global load; **CRP**, C-reactive protein; **CK**, creatine kinase. **YYIR1**, Yo-
612 Yo intermittent recovery test level 1; **SJ**, squat jump; **HI**, Hopper index; **DOMS**, delayed onset
613 muscle soreness.

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615

616 **Table 1a:** Training characteristics in the regular and congested periods of match play.

Types of training	Duration of a session (min)	Weekly frequency	
		T1-T2 Regular period	T2-T3 Congested period
Low-to-moderate intensity aerobic exercise	25-30	2	2
High-intensity interval running	15-20	2	1
Speed (over short and long distances)	18-20	3	3
Muscle strength	20-25	1	1
Small sided games	15-20	2	3
Specific soccer training	20-30	2	2
Technical	25-35	3	3
Tactical	30-35	3	2

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619 **Table 1b:** Training programs during regular and congested weeks of match play.

Day	Weekly program during the uncongested period and during the period of congested match play (when playing one match per week) Weeks 1 to 6 and weeks 7-10.	Weekly program during the congested period of match play (when playing two matches per week). Weeks 8-9-11-12.
Monday	Full recovery	Warm up, Low-to-moderate intensity aerobic exercise, Technical training
Tuesday	Warm up, Technical training, Low-to-moderate intensity aerobic exercise, Small-sided games,	Full recovery
Wednesday	Warm up, Strength training, Tactical training, Reduced game	Match
Thursday	Warm up, Tactical training, Speed training over short distances, Small-sided games	Warm up, Tactical training, Speed training over short distances, Small-sided games
Friday	Warm up, Technical training, Speed training over long distances, Soccer specific training	Warm up, Technical training, Speed training over long distances, Soccer specific training
Saturday	Warm up /technical training, Speed training over short distances, Soccer specific training	Warm up /technical training, Speed training over short distances, Soccer specific training
Sunday	Match	Match

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621 **Table 2:** Intraclass correlation coefficient.

Parameters	Mean Intraclass correlation coefficient (ICC)	95% confidence interval	
		Lower bound	Upper bound
CRP	0.31	0.30	0.42
Creatinine	0.51	0.43	0.57
CK	0.69	0.58	1.25
YYIR1	0.48	0.51	0.78
RSSA_{mean}	0.61	0.15	0.84
RSSA_{best}	0.69	0.34	0.89
RSSA_{decrement}	0.34	-1.26	0.58
SJ	0.95	0.90	0.98
CMJ	0.98	0.77	0.95
Monotony	0.42	0.32	0.55
Strain	0.89	0.83	0.98
Hopper index	1.33	1.13	1.53
Sleep	0.44	0.33	0.56
Stress	0.43	0.35	0.52
Fatigue	0.68	0.65	0.72
DOMS	0.81	0.72	0.90

622 Abbreviations: **YYIR1**, Yo-Yo intermittent recovery test level 1; **RSSA**, repeated shuttle sprint
623 ability; **SJ**, squat jump; **CMJ**, countermovement jump; **DOMS**, delayed onset muscle soreness;
624 **CRP**, C- reactive protein; **CK**, creatine kinase.

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634 **Table 3:** Global load parameters and accumulated match time during the 12 weeks training
 635 period (regular vs. congested period of match play).

Training periods	From T1 to T2 Regular period	From T2 to T3 Congested period	p-value	Effect size (ES)
Total load (A.U.)	12154.8 ± 929.4	13853.3 ± 2022.2	0.01*	1.3
Training load (A.U.)	9253.3 ± 702.0	6843.7 ± 194.5	< 0.001*	-1.3
Competitive load (A.U.)	2940.0 ± 624.1	6809.6 ± 2029.3	< 0.001*	3.9
Monotony (A.U.)	0.4 ± 0.7	1.8 ± 0.1	< 0.001*	3.7
Strain(A.U.)	817.7 ± 255.2	19843.9 ± 5973.4	< 0.001*	6.3
Accumulated match time (min)	388.2 ± 55.1	644.2 ± 125.9	< 0.001*	3.3

636 Abbreviations: **A.U.**, Arbitrary units. *significant difference between regular and congested
 637 period with $p < 0.001$.

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654 **Table 4:** Changes in physical fitness test performance.

Training periods	T1	T2	T3	p-value	Effect size (ES) ES ₁₋₂ -ES ₂₋₃
YYIR1 (m)	2308.5 ± 363.5	2495.7 ± 339.0	1594.2 ± 295.1	< 0.001* £	0.7; -4
RSSA_{mean}(s)	8.2 ± 0.2	8.0 ± 0.1	8.5 ± 0.4	0.001*	-1.7; 0.4
RSSA_{best} (s)	7.8 ± 0.3	7.5 ± 0.2	8.2 ± 0.2	< 0.001* £	-1.6; 4.4
RSSA_{decrement}(%)	7.0 ± 3.1	7.2 ± 3.8	5.9 ± 3.0	0.93	0.08; -0.5
[Lac]_{RSSA}(mM/L)	18.4 ± 3.2	18.2 ± 2.8	20.2 ± 2.3	0.07	-0.09;-1.1
SJ (cm)	37.9 ± 3.8	37.4 ± 3.2	34.2 ± 3.2	0.01*	-0.2; -1.4
CMJ (cm)	38.7 ± 2.5	39.1 ± 2.6	38.8 ± 2.5	0.85	0.2; -0.1

655 Abbreviations: **YYIR1**, Yo-Yo intermittent recovery test level 1; **RSSA**, repeated shuttle sprint
656 ability; **[Lac]_{RSSA}**, Lactate concentration measured after repeated shuttle sprint ability- test; **SJ**:
657 squat jump, **CMJ**, countermovement jump; *significant difference between T3-T2 and T3-T1
658 with p < 0.05; **£** Significant differences between T2 and T1 with p < 0.05.

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660 **Table 5:** Changes in inflammation and muscle damage markers.

Parameters	T1	T2	T3	p-value	ES ES ₁₋₂ - ES ₂₋₃
CRP (mg/L)	25.5 ± 7.3	32.1 ± 6.9	39 ± 5.6	0.001*£	1.2; 1.5
CK(U/L)	268.5 ± 113.2	278.0 ± 133.4	417.6 ± 237.8	0.002 *£	0.1; 1
Creatinine (mg/L)	10.0 ± 0.8	10 ± 0.7	10.3 ± 1.0	0.2	0.1; 0.3

661 Abbreviations: **CRP**, C reactive protein; **CK**, creatine kinase; *significant differences between
 662 T3 and T2 with $p < 0.05$; £ significant difference between T3 and T1 with $p < 0.05$.

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664 **Table 6:** Changes in wellness status (Hopper index).

Training periods	From T1 to T2 Regular period	From T2 to T3 Congested period	p-value	Effect size (ES)
Sleep	6.6 ± 0.5	7.2 ± 0.9	0.06	1.1
Stress	7.5 ± 0.5	9.0 ± 0.9	0.001*	0.7
Fatigue	10.2 ± 1.4	11.4 ± 1.5	0.008*	1.5
DOMS	7.0 ± 1.2	10.3 ± 1.5	0.001*	3.4
Hopper index	33.5 ± 2.2	37.0 ± 2.9	0.002*	1.9

665 Abbreviations: **DOMS**, delayed onset of muscular soreness; *significant difference between
 666 the congested and the regular period with $p < 0.01$.

667

668 **Table 7:** Relation between biochemical parameters and physical fitness test performance.

		Regular period (T1-T2)					Congested period (T2- T3)				
		YYIR1	RSSA mean	RSSA best	SJ	CMJ	YYIR1	RSSA mean	RSSA best	SJ	CMJ
CRP (mg/L)	r	0.07	0.07	0.09	0.55	0.14	0.04	-0.19	0.10	-0.29	0.26
	p	0.80	0.8	0.75	0.03*	0.62	0.86	0.51	0.71	0.31	0.37
CK (UI/L)	r	-0.05	-0.06	0.16	-0.32	-0.33	0.46	0.23	0.02	-0.02	-0.3
	p	0.85	0.81	0.56	0.25	0.24	0.09	0.41	0.99	0.99	0.15
Creatinine (mg/L)	r	-0.34	-0.12	-0.10	-0.29	0.14	-0.08	-0.13	0.07	-0.29	0.05
	p	0.23	0.68	0.72	0.30	0.62	0.77	0.64	0.79	0.30	0.85

669 Abbreviations: **YYIR1**, Yo-Yo intermittent recovery test level 1; **RSSA**, repeated shuttle sprint
670 ability. **SJ**, squat jump; **CMJ**, countermovement Jump; **CRP**, C reactive protein; **CK**, creatine
671 kinase; *significant correlation between parameters.

672

673 **Table 8:** Relation between biochemical parameters and wellness scores.

		Regular period (T1- T2)					Congested period (T2- T3)				
		Sleep	Stress	Fatigue	DOMS	HI	Sleep	Stress	Fatigue	DOMS	HI
CRP (mg/L)	r	0.37	-0.52	0.37	0.11	0.16	0.24	-0.07	0.64	0.13	0.53
	p	0.18	0.06	0.18	0.68	0.56	0.40	0.8	0.01*	0.64	0.04*
CK (UI/L)	r	-0.12	0.01	-0.20	-0.14	-0.12	-0.18	0.16	-0.42	0.63	0.22
	p	0.67	0.95	0.91	0.61	0.67	0.52	0.57	0.13	0.01*	0.31
Creatinine (mg/L)	r	-0.12	0.01	-0.20	0.06	-0.12	-0.53	0.13	0.02	-0.33	-0.2
	p	0.67	0.95	0.47	0.81	0.67	0.06	0.65	0.93	0.23	0.2

674 Abbreviations: **DOMS**, delayed onset muscle soreness; **CRP**, C-reactive protein; **CK**, creatine

675 kinase; **HI**, Hopper index; *significant correlation between parameters.

676

677 **Table 9:** Relation between wellness scores and physical fitness test performance.

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		Regular period (T1- T2)					Congested period (T2- T3)				
		Sleep	Stress	Fatigue	DOMS	HI	Sleep	Stress	Fatigue	DOMS	HI
YYIR1 (m)	r	0.41	-0.15	0.20	0.14	0.15	-0.03	0.7	-0.49	0.48	0.25
	p	0.14	0.60	0.47	0.61	0.58	0.90	0.78	0.04*	0.07	0.38
RSSA_{mean} (s)	r	0.17	-0.32	0.30	0.40	0.21	0.09	0.25	-0.42	0.02	0.68
	p	0.54	0.25	0.29	0.15	0.46	0.72	0.37	0.13	0.91	0.007*
RSSA_{best} (s)	r	0.27	-0.02	-0.27	-0.08	0.08	-0.45	0.25	-0.25	0.51	0.20
	p	0.33	0.92	0.33	0.76	0.76	0.10	0.37	0.37	0.04*	0.47
SJ (cm)	r	0.10	0.41	0.12	0.08	0.21	0.37	0.08	0.11	0.07	0.32
	p	0.73	0.13	0.68	0.76	0.45	0.18	0.77	0.68	0.80	0.25
CMJ (cm)	r	-0.08	0.11	-0.23	-0.21	-0.21	-0.34	0.28	0.45	-0.27	0.10
	p	0.78	0.69	0.41	0.47	0.46	0.22	0.32	0.10	0.34	0.73

679 Abbreviations: **YYIR1**, Yo-Yo intermittent recovery test level 1; **RSSA**, repeated shuttle sprint
 680 ability; **SJ**, squat jump; **CMJ**, countermovement jump; **HI**, Hopper index; * significant
 681 correlation between parameters.

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685 **Table 10:** Relation between percentage of biochemical parameters, physical fitness test
 686 performance, wellness and workload parameters.

		From T1 to T2 Regular period					From T2 to T3 Congested Period				
		GL	TL	CL	Monotony	Strain	GL	TL	CL	Monotony	Strain
CRP (mg/L)	r	0.30	0.07	0.25	0.38	0.40	0.52	0.07	0.18	0.50	0.51
	p	0.29	0.97	0.33	0.17	0.15	0.04*	0.78	0.53	0.04*	0.03*
CK (UI/L)	r	-0.47	0.42	-0.07	-0.5	-0.45	0.35	0.36	-0.10	0.69	0.30
	p	0.08	0.12	0.81	0.06	0.79	0.22	0.19	0.73	0.005*	0.29
Creatinine (mg/L)	r	-0.49	-0.51	-0.16	0.30	-0.35	-0.25	-0.01	-0.01	-0.15	0.16
	p	0.07	0.06	0.56	0.22	0.51	0.21	0.96	-0.96	0.59	0.72
Sleep	r	0.06	0.12	0.13	0.17	0.13	0.43	-0.16	-0.16	0.03	0.36
	p	0.81	0.66	0.64	0.55	0.63	0.11	0.57	0.57	0.91	0.20
Stress	r	-0.32	-0.27	-0.09	-0.31	-0.36	-0.09	0.24	0.24	0.40	-0.08
	p	0.25	0.34	0.75	0.28	0.19	0.74	0.40	0.40	0.15	0.77
Fatigue	r	0.40	0.23	-0.42	0.40	0.41	-0.15	0.23	0.52	-0.14	-0.04
	p	0.15	0.44	0.13	0.14	0.14	0.59	0.41	0.02*	0.63	0.88
DOMS	r	0.40	0.35	0.08	0.37	0.42	0.70	-0.04	-0.04	0.30	0.71
	p	0.15	0.21	0.77	0.19	0.12	0.004*	0.88	0.88	0.28	0.004*
Hopper index	r	0.56	0.44	0.23	0.54	0.56	0.66	0.07	0.69	0.33	0.69
	p	0.03*	0.21	0.34	0.04*	0.03*	0.009*	0.79	0.006*	0.18	0.006*

YYIR1 (m)	r	0.20	0.29	0.49	0.19	0.20	0.10	-0.27	0.13	0.12	0.52
	p	0.48	0.30	0.07	0.49	0.47	0.72	0.34	0.60	0.67	0.04*
RSSA_{mean} (s)	r	-0.08	0.37	0.05	0.63	0.65	-0.11	-0.18	0.59	-0.17	-0.19
	p	0.77	0.22	0.86	0.01*	0.01*	0.68	0.52	0.06*	0.54	0.50
RSSA_{best} (s)	r	-0.12	-0.03	0.18	0.09	0.02	0.29	-0.41	0.25	-0.10	0.24
	p	0.67	0.89	0.53	0.73	0.93	0.31	0.13	0.35	0.72	0.39
SJ (cm)	r	-0.04	0.06	0.32	0.05	0.01	-0.04	-0.11	-0.24	0.13	-0.01
	p	0.88	0.81	0.25	0.84	0.96	0.88	0.70	0.36	0.63	0.95
CMJ (cm)	r	0.01	0.07	-0.18	-0.003	-0.009*	-0.25	0.18	0.14	0.29	-0.11
	p	0.95	0.79	0.53	0.99	0.97	0.37	0.53	0.58	0.31	0.68

687 Abbreviations: **YYIR1**, Yo-Yo intermittent recovery test level 1; **RSSA**: repeated shuttle sprint
688 ability; **SJ**: squat jump; **CMJ**, countermovement jump; **CRP**, C-reactive protein; **CK**, creatine
689 kinase; **DOMS**, delayed onset muscle soreness; *significant correlation between
690 parameters. **GL**: global load; **TL**, training load; **CL**, competitive load.

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