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Published in:
Scandinavian Journal of Medicine and Science in Sports

DOI:
10.1111/sms.13878

Publication date:
2022

Document version:
Accepted manuscript

Citation for pulished version (APA):
Póvoas, S., Ascensão, A., Magalhães, J., Silva, P., Wiig, H., Raastad, T., Castagna, C., & Andersson, H. (2022). Technical match actions and plasma stress markers in elite female football players during an official FIFA Tournament. *Scandinavian Journal of Medicine and Science in Sports*, *32*(Suppl. 1), 127-139. https://doi.org/10.1111/sms.13878

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Article type : Supplement Article

Submission Type: Original Research

Title of the Article:

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Preferred Running Head: Tournament demands in Elite Female football

Abstract Word Count: 247 words

Text-Only Word Count: 5067 words

Number of Figures and Tables: 2 figures and 4 tables

References number: 52 citations

ABSTRACT

This study analysed the impact of performing four consecutive football matches separated by 48-72h during a FIFA tournament on physical load, technical performance and plasma markers of redox state, muscle damage and inflammation in elite female players.

Forty-eight players from three national teams were evaluated at seven time points: before (baseline) and throughout the tournament (after each match and before two training sessions). Only data from players who played all matches was included in the analyses (N=13). The players were divided into high-rank (n=7) and low-rank (n=6) team players according to FIFA standards. Plasma creatine kinase (CK), C-reactive protein (CRP), total antioxidant status (TAS) and uric acid (UA) were analysed at the selected time points. Technical performance and physical load were also quantified according to team rank.

Players from low-rank teams played significantly more time than high-rank players (85±10 vs. 67±15 min; p=0.02; *d*=1.51). Low-rank team players presented higher scores in technical performance actions than the high-rank team players, but most of the differences were explained by the longer match time played. UA content differed across the matches, increasing from baseline ($F_{(4,40)}$=3.90; p=0.01) and more in the high-rank team players ($F_{(1,10)}$=20.46; p=0.001), while CRP only differed across the matches ($F_{(4,36)}$=2.66;

p=0.05), also increasing from baseline. A large time effect was shown for UA only in the high-rank players (η^2_p=0.50; p=0.02). Four consecutive matches did not result in considerable alterations in plasma stress markers, physical load and technical performance in elite female football players from distinct rank levels.

Key words: Performance, Oxidative Damage, Congested Fixtures, Soccer.

INTRODUCTION

Top-level soccer players' competitive season frequently entails congested fixtures with consecutive matches separated by only 2-3 days of recovery[1]. The players may also be involved in additional commitments, such as national cups and other knockout matches, or representing their countries in international championships. In these tournaments or even in training camps, matches are interspersed by only 1 to 2 days of recovery and the players usually play 4-6 games within the 8 to 10 days of competition[2]. These competitive demands impose strains to various physiological systems, including the musculoskeletal, nervous, immune and metabolic systems[3]. Moreover, these congested competitive and training periods impact physical and tactical performance[4], predispose players to increased injury risk[5] and consequently, require additional recovery strategies between the matches[6].

Early studies with football players from distinct competitive levels, reported post-match decrements of functional performance and alterations in muscle[7] and blood metabolites[7-10]. These alterations accompanying decrements in physical performance include skeletal muscle glycogen depletion, an important hallmark of fatigue and recovery processes during and after intermittent exercise such as football[11,7]. Moreover, football is associated with intense eccentric muscle actions; therefore these match-induced physical impairments were accompanied by significant alterations in biochemical markers of physiological stress such as muscle damage, enhanced oxidative stress, and a pro-inflammatory status[8-10,12-14,15-20]. As referred, one of the consequences of playing consecutive football matches is the perturbation of the redox homeostasis, which has been consistently related to the modulation of several physiological functions in the cells[21]. In fact, depending on the exuberance of the redox stimulus, it can significantly determine signalling pathways associated with either positive adaptations to exercise training and competition loads, or, on the other hand, with dysfunction, causing diminished performance and physical fatigue by affecting membrane lipids, proteins and DNA structure[22-24].

During the last years, a plethora of studies has been devoted to analysing the activity pattern of football matches and its consequences in neuromuscular fatigue and performance[25]. In addition to the alterations observed in muscle and blood biomarkers during and after the match[7,26], decrements in physical performance markers during the match, particularly after high-intensity exercise periods during the first and second halves (pointed to the so-called temporary fatigue) and after the match were described[25,27].

In addition, Andersson and co-workers analysed elite female football players' movement pattern during two consecutive matches in an attempt to ascertain whether performance was affected by fatigue. They concluded that the movement pattern was not affected in the match played 72 hours after the first match, despite changes in redox and physical fatigue markers after the first match[10,14,28].

The redox-related alterations, inflammation and muscle damage induced by several consecutive competitive football matches during an elite level tournament have not been explored so far. Whether successive football matches induce cumulative alterations in these markers, thus being associated to possible compromises in performance during the match or may function as a possible powerful signalling stimulus for muscle performance, repair and recovery between matches, is unknown. Therefore, given the relationship between alterations in redox balance and inflammation with physical and possibility, technical performance, this study aimed to analyse the impact of playing four consecutive football matches (separated by 1-2 days of recovery) within an 8-day Fédération Internationale de Football Association (FIFA) elite female tournament. More specifically, physical and technical performance as well as biological stress markers were assessed after the four tournament matches, that were separated by 2-3 days of recovery, and before two of the training sessions. This study also aimed to ascertain whether the possible variation of these markers throughout consecutive matches, as well as the physical load markers and the number of the selected technical performance actions differed according to the teams' competitive level.

As work hypothesis, we assumed a cumulative effect of match fixtures (i.e., tournament congestion) and training sessions on biological stress markers and physical and technical performance in elite female football players.

METHODS

Participants

Forty-eight elite female football players from three national teams that played the official FIFA tournament – "Algarve Women's Football Cup", participated in this study. Within eight days, the teams played four official matches separated by 48 to 72h of recovery. Team level was based on team´s position in the competition groups, which was determined by FIFA. Thus, players from teams competing in group A were considered as high-rank players, while players from teams competing in group C were considered as low-rank players. The mean age, height and body mass of the players were 26±4 (range 18-34) years, 170±4 (162–179) cm, 63.4±4.8 (53.0–75.8) kg, respectively (Tanita Inner Scan digital BC532, Amsterdam, The Netherlands). Participants had at least five years of experience at top national football competition. At the time of the evaluations, the high-rank team players were in the preseason and the low-rank team players were in the midseason, performing 6-10 training sessions per week, comprising 4-5 technical-tactical and physical fitness training exercises and 2-3 strength training sessions. The evaluated players played in the same playing position during all matches.

Since physical and physiological football demands imposed on goalkeepers considerably differ from those of the outfield players[29], players from this playing position were not included in the data analysis. Due to the large rotation of the players in the matches held during the tournament, only 13 players (3 central backs, 2 full backs, 2 forwards, 4 wingers and 2 midfielders) out of the 48 evaluated players, were involved in all the matches, with playing match time ranging from 20 to 96 min (mean: 77±15 min). Thus, only data from the players who participated in all four matches were included in the final analysis, in order to meet the purpose of the study, which was to analyse the impact of consecutive matches performed in a short period of time.

The present study was approved by the local institutional board as well as by the club officials, and followed the Declaration of Helsinki of the World Medical Association for research with humans. Participants were informed of the aims of the research project and were aware of the procedures including any risks and benefits before giving written informed consent.

Study design

This tournament comprised a total of eight days of competition in which the teams performed four matches interspersed by 48-72h of recovery. The experimental design comprised blood sampling at i) baseline, ii) before two of the training sessions held 24 to 48 h after the preceding match, and iii) after all the matches held during the tournament. Baseline evaluations were performed in the morning of the day before the beginning of the tournament (i.e., first match) (Figure 1). All players were familiarized with all testing procedures, before the commencement of the study. The matches were held under neutral temperatures (~21-22ºC) and humidity conditions (50–70%) (Extech Digital Hygrometer 445715; Grainger, New York, NY, USA).

Figure 1 near here

To monitor physical load and technical performance actions performed during all matches, match time played, perceived muscle soreness (PMS) and rate of perceived exertion (RPE) were accessed, and a technical performance analysis via a video-based monitoring was performed.

Biological stress markers of redox status [total antioxidant status (TAS) and uric acid (UA)], muscle damage (creatine-kinase, CK) and inflammation (C-reactive protein, CRP) were determined via venous blood samples. Blood samples were obtained after the end of all the matches and before two of the training sessions held 24 to 48 h after the preceding match, i.e., at different recovery stages. Blood samples were taken immediately after the player had been substituted or within 15 min after the end of the matches.

Moreover, the training sessions' physical load between the matches was monitored using RPE[30] and PMS[31]. The training session performed on the day before the beginning of the tournament consisted of short high-intensity and sprint training exercises. The recovery sessions held in-between the matches consisted of light-to-moderate technical, physical and tactical exercises.

To avoid possible confounding effects regarding redox status, participants were instructed not to change their regular diet and supplementation intake during the tournament.

Testing procedures

Physical load markers

Match and training time were registered. PMS was assessed using a seven-point visual Likert scale[31] designed to measure the level of muscle soreness in the lower limbs, without muscle palpation. The scale consisted of the following verbal anchors: 1 = very, very good; 2 = very good; 3 = good; 4 = tender but not sore; 5 = sore; 6 = very sore; and 7 = very, very sore. RPE was recorded after the end of the matches or after the player being substituted, using Borg's rating of perceived exertion category ratio 10-scale (CR10) modified by Foster et al.[30] According to the same authors, session-RPE was determined by multiplying the RPE rating of the whole match/training session by its duration.

Technical performance assessment

Technical performance actions were analysed using data provided from *InStat* (*InStat Footbal, Moscow, Russia*). This computerized tracking system provides match analytical information, such as technical performance variables. The following technical performance variables were selected for this study purpose: absolute (n) and relative (%) average and cumulative (all 4 tournament matches) total match actions, successful actions, successful defensive and attacking actions, successful tackles, recoveries, accurate passes, challenges and challenges won. The definitions of the technical variables are as follows: Defensive action successful - action of a team in ball possession with no passage to the opponent's half-field; Attacking action successful – action of a team in ball possession with passage to the opponent's half-field; Challenges won – all types of challenges won on a field (e.g. duels, air duels, dribbles), it is a summary parameter, which includes struggle for neutral ball, air challenges, dribbles, tackles, losses of a ball when the opponent takes possession of it; Tackles successful – active action of a player who tries to tackle a ball from the player who possesses it (the opponent in this case has the loss from tackle registered); Recoveries - player's actions (an interception, picking up, a duel won) from which the opponent's ball possession is over and a chance on counter attack development for his team start, and Passes accurate – a pass that reaches a teammate and is not intercepted by an opponent.

Biological stress markers

Blood sampling and preparations

Venous blood samples were taken by conventional clinical procedures as previously described[8]. Samples were collected from the antecubital arm vein into a 10-mL (EDTA-K3, Iberlab, ref. GV0414) containing tubes immediately after the player left the match. Whole freshly withdrawn blood was centrifuged (10 min x 3000rpm) and aliquots of plasma were obtained, stored and frozen at –80°C and further used to determine biochemical markers of muscle damage (CK), inflammation (CRP) and redox-based alterations (TAS and UA).

Biochemical assays

Plasma CK activity was spectrophotometrically determined according to the manufacturer procedures using a commercial kit (ABX A11A01632, Mompelier, FR). TAS was spectrophotometrically measured using a commercial kit (Randox NX2332 Crumlin, UK) and following the manufacturer instructions. UA was determined by an enzymatic method using a commercial kit (Horiba ABX A11A01670, Montpellier, France) and following the manufacturer instructions. CRP was measured using an enzyme-linked immune sorbent assay system (ELISA-PENTRA 400, Horiba ABX, Montpellier, FR) and following the manufacturer instructions. Samples were analyzed in duplicate and the mean of the two values was used for statistical analysis. The coefficients of variation were 3.7, 3.8, 2.7 and 5.2%, respectively.

Statistical analyses

Results are presented as mean ± SD. None of the parameters showed significant deviations from a normal distribution (Shapiro–Wilk test). Changes in the biological stress markers and in physical load markers (except for average match time played) at different time points during the tournament and differences between both team levels were examined using a two-way analysis of covariance (ANCOVA) with repeated measures with Bonferroni post-hoc multiple comparison tests, adjusting for the effect of individual match time played. Changes in average match time played and in technical performance actions across the tournament per min of played time, were analysed using a two-way analysis of variance (ANOVA) with repeated measures with Bonferroni post-hoc multiple comparison tests. Effect size was calculated using partial eta-squared (η^2_p) and interpreted as follows: $\eta^2_p \geq 0.14$ large effect, $0.14 > \eta^2_p \geq 0.06$ medium effect, $0.06 > \eta^2_p \geq 0.01$ small effect and $\eta^2_p < 0.01$ trivial effect [32] for the plasma stress markers, and also by calculating Cohen's *d* effect size and interpreted as suggested by Batterham and Hopkins[33] (*d*, ≤0.2 trivial, >0.2–0.6 small, >0.6–1.2 moderate, >1.2–2.0 large, and >2.0–4.0 very large) for the physical load and technical performance markers. Differences between high- and low-rank players in physical load markers and technical performance actions were assessed by Students' independent samples t-test. A significance level of 0.05 was chosen. Statistical Package for the Social Sciences (version 22.0; SPSS Inc., IBM, Armonk, New York, USA) was used for all the analyses.

RESULTS

Physical load markers

Table 1 presents the physical load markers evaluated during the matches, including average and cumulative match time played as well as average and cumulative reported match RPE and PMS values. Players in the low-rank teams played on average significantly more match minutes (85±10 vs. 67±15 min; p=0.02; *d*=1.51) and had higher cumulative match session-RPE (2271±328 vs. 1549±613; p=0.02, *d*=1.53) than players in the high-rank teams, respectively. Nevertheless, no differences between the groups were observed regarding the reported post-matches PMS. Moreover, there were no differences across the 4 tournament matches in both team rank players in playing match time and in session-RPE, as well as in PMS (data not shown).

Table 1 near here

Technical performance assessment

Table 2 presents the technical performance actions expressed as mean absolute and relative values to individual match time played during the tournament matches of players of high- and low-rank teams. When considering absolute values, low-rank team players presented higher scores regarding the selected technical performance actions compared to the high-rank team players, with some of the differences achieving statistical significance (p≤0.05; Table 2). However, when these indicators were matched for individual played time (relative values), almost all of the referred differences between high- and low-rank teams were no longer statistically evident.

Table 2 near here

When normalized for match time played, a time x group interaction was shown in average challenges ($F=7.10_{(3,30)}$; $\eta^2_p=0.42$; $p<0.01$) and average successful tackles ($F=6.32_{(3,30)}$; $p<0.01$). A time effect was shown in average actions ($F=2.90_{(3,30)}$; $p=0.05$), average successful actions ($F=3.17_{(3,30)}$; $\eta^2_p=0.24$; $p=0.04$) and average successful attacking actions ($F=4.08_{(3,30)}$; $\eta^2_p=0.29$; $p=0.02$). A group effect was show in average actions ($F=6.15_{(1,10)}$; η^2_p =0.38; p=0.03) and average successful attacking actions ($F=4.92_{(1,10)}$; η^2_p=0.33; p=0.05).

Differences between the 4 matches in technical performance actions were shown in high-rank team players in average successful actions in match 2 (1.04±0.34) vs. 3 (0.72±0.20; p=0.01), average successful defensive actions in match 1 (0.09±0.11) vs. 3 (0.19±0.14; p=0.04), average successful attacking actions in match 3 (0.22±0.06) vs. 4 (0.42±0.05; p=0.03), average challenges in match 3 (0.31±0.14) vs. 1 (0.08±0.08), 2 (0.12±0.04) and 4 (0.15±0.04; $p\leq0.03$). Thus, in 3 out of the 4 markers there was an increase in technical performance actions as the tournament progressed, while one out of the 4 showed a decrease.

In low-rank team players, differences between the 4 matches were shown in average successful tackles with significant differences being shown between match 2 (0.04±0.02) vs. 3 (0.02±0.02) and 4 (0.01±0.01; p=0.02).

High-rank teams showed lower ($p\leq0.05$) number of average actions in match 3 (0.74±0.15 vs. 0.93±0.14) and 4 (0.78±0.15 vs. 1.03±0.22), average successful actions in match 3 (0.40±0.18 vs. 0.64±0.13), average successful attacking actions in match 3 (0.22±0.06 vs. 0.48±0.08) and average successful tackles in match 2 (0.00±0.01 vs. 0.04±0.22), than low-rank team players.

Biological stress markers

As seen in Table 3 and in Figures 2A-2D, UA was elevated from baseline in the high-rank players after matches 3 and 4 and only after match 1 for low-rank teams ($F_{(4,40)}$=3.90; p=0.01). A large time effect was only shown for high-rank players in UA (η^2_p=0.50; p=0.02). When considering the teams together, CRP values after the matches were higher than baseline ($F_{(4,36)}$=2.66; p=0.05). After match 3, the high-rank teams showed lower CK, but higher CRP and TAS values ($p\leq0.05$) than the low-rank players, and higher UA values after all the four tournament matches and at baseline ($F_{(1,10)}$=20.46; p=0.001).

Table 3 near here

Figures 2A, 2B, 2C and 2D near here

In the days between the matches, a significant decrease was shown in UA 48h after the preceding match (match 2) (3.29±0.79 vs. 4.30 ± 0.56; p=0.03) and in TAS 24h after the preceding match (match 3) (1.58±0.15 vs. 1.83 ± 0.08; p=0.01), respectively, though only in low-rank team players. In the recovery days, none of the stress markers was significantly different from baseline conditions (Table 4).

DISCUSSION

The present study assessed for the first time, physical load markers, technical performance actions and biological stress markers during four international games separated by 2-3 days of recovery.

The main findings were that i) physical load markers remained unaltered and technical actions showed slight fluctuations throughout the consecutive matches, ii) physical load markers showed that players in the low-rank teams played significantly more match minutes compared to players from the high-rank teams and, accordingly, they also reported higher cumulative match session-RPE values, iii) technical assessment showed no significant differences between the high-rank and low-rank players in most of the selected actions, when the results were matched for individual playing time, and iv) for the biological stress markers, UA was elevated from baseline in players from the high-rank teams after matches 3 and 4, but only after match 1 for low-rank teams. CRP values were higher than baseline after the matches (time effect). Regarding team level, the low-rank team players showed higher CK compared to the high-rank group. After all the four tournament matches, the players from the high-rank teams showed higher UA values than the low-rank team players, with higher CRP and TAS values shown after match 3 ($p \leq 0.05$).

Four consecutive matches did not result in considerable alterations in plasma stress markers in elite female football players, with the high-rank team players apparently showing enhanced ability to deal with the stress imposed by this congested fixture than the low-rank team players (Table 3). These results are in contrast with those reported by Silva and colleagues [26] regarding the impact of an official male soccer match in plasma redox status and muscle damage markers. In fact, the authors showed that a match from a Portuguese Male Professional Soccer League resulted in significant marked changes in these biomarkers until 48h of the recovery period, although without major impact on performance. Although the activity pattern of the players was not monitored in the present study, another possible explanation for the distinct results between high- and low-rank players regarding muscle damage, inflammation and recovery following the matches, could be associated with the number of accelerations, decelerations and sprints during the match as proposed by previous data[34].

The players in the low-rank teams played, on average, more match minutes compared to the high-rank players (large effect), because the low-rank team used fewer substitutions during the matches and less players' rotation during the tournament compared to the high-rank teams. The high-rank teams may have had a more even players' standard allowing the coaches to rotate more the players during the tournament. Consequently, the high-rank teams had the necessary recourses to optimise the total quantity of match minutes played in the tournament for each player in order to avoid overload. Furthermore, since the "Algarve Cup" is a friendly tournament, the high-rank teams may have been more willing to test new, young players, in international games, whereas the low-rank team may have not had that possibility due to higher variation in players' standard.

Cumulative match session-RPE was also significantly higher for the low-rank players (large effect) than for the high-rank players. Since session-RPE is calculated using the CR10 scale multiplied by match time and no significant differences were observed in average match RPE, though a moderate effect was detected, the differences between the low- and high-rank teams seem to have been closely linked to match time played, with the low-rank teams playing more time and thus, reporting an higher internal load[35].

The perceived muscle soreness did not differ between teams. This is somewhat surprising, since one could expect that players with more match time played would report higher muscle soreness[25]. No difference in perceived soreness may indicate that match intensity for the low-rank players was lower compared to the high-rank players, but this should be interpreted with caution because of possible differences in the preferences for the subjective rating. Data from this tournament characterized by a congested match fixture seem to be in conflict with results from male soccer players engaged in the Portuguese Male Professional Soccer League during the entire season[36]. In that study, the individual match played time throughout the entire season positively impacted physical fitness parameters, namely knee flexor and extensor muscle strength and sprint ability, which suggests that, at least until a certain degree of engagement, the systematic participation of the players in soccer matches throughout the season favours the increase and maintenance of, at least, some soccer player's physical fitness-related features.

In order to analyse whether the quality and quantity of the players' technical performance actions differed according to the teams' ranks, and if it was affected by accumulated matches played during the time course of the tournament, technical analysis was used. Low-rank team players tended to show higher absolute scores in the selected technical performance actions than the high-rank team players, resulting in statistically significant differences in most of them. Importantly, most of these differences were no longer statistically significant when the indicators were matched for individual time played. This indicates an influence of match time played on the number of technical actions performed. However, no marked alterations in technical performance actions in the time-course of the tournament were observed.

Biological stress markers

Regarding the analysed biological stress markers, data showed that UA was elevated from baseline in the high-rank players after matches 3 and 4 and only after math 1 for low-rank teams ($F_{(4,40)}$=3.90; p=0.01), with high-rank players showing a large time effect (η^2_p =0.50; p=0.02). Similar elevations in UA have been shown after male and female football matches[8,14]. Being UA the end product of purine nucleotide metabolism, increased UA suggests an enhanced nucleotide catabolism[37]. Moreover, UA can also function as antioxidant due to its free radical quenching actions, being its contribution to the total plasma antioxidant capacity rather high (~ 35- 65 %) [38,39]. Higher UA and TAS values in the high-rank teams suggests that there was a higher anaerobic metabolic demand and also a stronger antioxidant response (TAS) for the high-rank players. Thus, despite shorter match time, it seems that for the high-rank players the match led to a more pronounced activation of the antioxidant system compared to the low-rank players. In contrast, the possible increased level of the muscle damage-induced oxidative stress condition observed in the low-rank players may had overwhelmed the antioxidant capacity of the athletes with consequences in the reduction of TAS levels.

CK was significantly higher in the low-rank players compared to the high-rank players after the third match. Also, CK absolute values for the low-rank players were much higher (366-657 $U·L^{-1}$) compared to the high-rank players (230-344 $U·L^{-1}$). This suggests that, for the low-rank players, a higher muscular damaged occurred, whilst minor in the high-rank team players. Even though, it is interesting to note that the absolute CK values, found in the present study in the high-rank team players are lower (230-344 $U·L^{-1}$) compared to those obtained in previous studies (~450 $U·L^{-1}$) after a match[10] or in some periods of the season[40]. So, despite the low number of recovery days between matches, limited levels of muscle damage were observed in the high-rank team players after the matches. The rather low CK values observed in the high-rank team players could probably be due to the shorter match time played by these players when compared to low-rank players[41,42], which is also consistent with observations from previous studies[10]. It may indicate that the high-rank team players were able to handle the match load without developing noticeable muscle damage or excessive oxidative stress. Thus, it may be so that the players seem to be well adapted to play on average 77 min without developing excessive accumulated stress. Therefore, if the players' load (e.g., match time) is well managed, a tournament with several consecutive matches with short number of recovery days in-between (2-4) does not seem to negatively affect the players regarding the analysed variables. However, one should take into account that there is a large individual variation in CK values (Fig. 2A) and that, despite these values are within the reference ranges defined for elite soccer players[43], the magnitude of the values is considerably low when compared to others obtained after different muscle damage stimuli such as eccentric resistance exercise[44]. Therefore, this suggestion should be made with caution. Moreover, CK release into the bloodstream might not always reflect the degree of muscle damage; it is possible that the efflux of CK results from either damage or transient changes in membrane permeability[45]. In addition, other mechanisms (e.g., AMP-activated protein kinase) might explain the appearance of CK in plasma after physical exercise, as opposed to structural damage arising from muscle trauma[46].

This is valuable information for national teams, who thus can use these important international tournaments to test new players and to develop their style of play without the players developing excessive fatigue or decrement in technical parameters. Nonetheless, the recovery period between the matches did not significantly affect the stress markers recovery, with UA (48h recovery) and TAS (24h recovery) values being significantly lower than the preceding match. Therefore, despite the differences between team ranks observed in match time played and some stress markers, the overall message of the present study is that the consecutive played matches did not impose considerable increases in levels of the measured serum-based biological stress markers. It is, however, possible that the players used adaptive pacing strategies to regulate the efforts during each match and throughout the tournament without compromising technical performance[47].

Performance in the matches and the recovery after an on-off football match involve a variety of variables and strategies, aiming at optimizing performance to play the next match in 3-4 or 6-7 days in the best possible conditions. However, during some tournaments, particularly those involving female players, the matches are interspersed by only 1 or 2 days of recovery and the players usually play 4-6 matches within the 8-10 days of the competition. These consecutive played matches, necessarily impose a considerable amount of physical and physiological strain, namely glycogen depletion, clearly exacerbated by the amount of eccentric contractions characterizing football (a phenomenon not so evident in other sports in which glycogen decreases do not pair with muscle damage levels)[48,49], possibly resulting in biological stress conditions for these players. The present work was designed to better understand, potential differences in physical load markers and in technical performance actions, as well as possible variations in plasma makers of biological stress during a congested match fixture tournament in elite female football players of different competitive level[40].

Rowsell et al.[50] analyzed alterations in physical performance indices and plasma muscle damage and inflammatory markers between successive matches in high-performance junior male football players. In general, no daily differences were observed in the measured parameters, which suggests some performance and physiological stability in these players throughout the 4-day tournament, which is generally in accordance with the data of the present study.

Also, the absence of hormone fluctuations related to competition performance points out that top-level professional football players training systematically and regularly seem to be very well adapted to competition stress effect[51].

One of the limitations of the present study regards the lack of a control group and of more objective measures of established indicators of physical and physiological performance, such as locomotor activity profile using Global Position Systems units and heart rate data using heart rate monitors, during the different matches throughout the tournament. These tools, eventually used in combination, would possibly allow a more sensitive detection of performance variations[41,52]. Unfortunately, constrains imposed by FIFA did not allow the collection of these data during this tournament.

As some of the athletes only arrived at Algarve very close to the beginning of the tournament, the control of diet intake was not possible, which can also be considered a limitation of the study as certain nutrients and supplements may affect redox status responses. Nevertheless, as referred in the Methods section, during the tournament the athletes were instructed not to change their diet regular intake. The participants medication intake and the period in the menstruation cycle were not registered.

From the obtained data, it can be concluded that four consecutive matches interspersed by 2-3 days of recovery did not induce considerable alterations in physical load and performance indicators as well as in plasma biochemical markers of oxidative stress, inflammation and muscle damage in female football players from distinct rank levels.

PERSPECTIVES

Congested competitive schedules and tournaments played during short time periods are very frequent in football and have been increasing during the last years. Aiming at uncovering possible consequences and better understand the impact of these stimuli, technical and physical load, and plasma biochemical stress response markers were addressed. The present results suggest that tournaments comprising matches separated by 2-3 days result in slight alterations in purine metabolism, redox and inflammatory response that are similar to those obtained in the recovery time between matches of female players from distinct levels. However, it is also of note that these biochemical alterations are more pronounced in players from lower competitive level when compared to high-rank participants. Our data may also be useful when dietary, medical and therapeutic programs including massive prescriptions of anti-inflammatory and/or antioxidant molecules are designed, as these physiological processes are fundamental in the mediation/signalling of tissue functioning, repair and regeneration during and after exercise.

ACKNOWLEDGEMENTS

This study was supported by Swedish Football Association, University Institute of Maia (ISMAI) and Research Centre in Physical Activity Health and Leisure (CIAFEL) (FCT/UID/DTP/00617/2019) from Faculty of Sport, University of Porto. The authors are especially thankful to Institute Jean Piaget of Sines and Albubeira AquaLab for kindly providing all the facilities regarding blood sampling and storage, especially to Ana Almeida, João Grade, Amara Almeida and all involved staff, nurses and to Teresa Bota. The authors also acknowledge the collaboration of Júlio Costa in the organization of the database. The elevated commitment demonstrated by all players and the teams' staff involved throughout the overall tournament is also of note.

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FIGURES AND TABLES LEGENDS

Figure 1 - Schematic description of the experimental protocol, illustrating the time points for the blood sampling: baseline conditions (before the beginning of the tournament, i.e., first match), before two training sessions held 48h and 24h after the preceding match and after all the matches of the official FIFA tournament.

Figures 2A, 2B, 2C and 2D - Plasma stress markers of high- and low-rank team players at baseline conditions, after all the matches and 48h and 24h after the preceding matches (i.e., matches 2 and 3, respectively) of the international official elite female football tournament (high-rank: N = 6; low-rank: N = 7): CK - creatine kinase (1A), CRP - C-reactive protein (1B), TAS - total antioxidant status (1C); UA - uric acid (1D).

Table 1 - Physical load markers of high- and low-team rank players during all the matches and training sessions of the international official elite female football tournament. Values are means ± SD (high-rank: N = 6; low-rank: N = 7).

Table 2 – Technical performance actions of high- and low-team rank team players during all the matches of the international official elite female football tournament. Values are means ± SD (high-rank: N = 6; low-rank: N = 7) and expressed as absolute and relative to individual match time played (actions per min played).

Table 3 – Plasma stress markers (mean ± SD and confidence interval) of high- and low-rank team players at baseline conditions and after all the matches of the international official elite female football tournament. Values are *F*-test and *p* value for analysis of covariance (ANCOVA) model according to time and team rank and controlled for individual match time played (high-rank: N = 6; low-rank: N = 7). Eta squared results refer to time effect adjusted for individual match time played.

Table 4 – Plasma stress markers (mean ± SD and confidence interval) of high- and low-rank team players at baseline conditions and 48h and 24h after the preceding matches (i.e., matches 2 and 3, respectively) during an international official elite female football tournament. Values are *F*-test and *p* value for analysis of covariance (ANCOVA) model according to time and team rank and controlled for individual match time played (high-rank: N = 6; low-rank: N = 7). Eta squared results refer to time effect adjusted for individual match time played.

Table 1 - Physical load markers of high- and low-team rank players during all the matches and training sessions of the international official elite female football tournament. Values are means ± SD (high-rank: N = 6; low-rank: N = 7).

Physical load markers	**Team rank**	**Mean ± SD**	***p***	***d***
Average match time played (min)	High	67 ± 15	0.02	-1.51
	Low	85 ± 10		(large)
Cumulative match time played (min)	High	261 ± 74	0.03	-1.42
	Low	341 ± 39		(large)
Average match RPE (AU; 0-10)	High	7.0 ± 1.0	0.32	0,67
	Low	6.5 ± 0.5		(moderate)
Cumulative match RPE (AU; 0-10)	High	22.3 ± 7.4	0.22	-0.79
	Low	26.1 ± 2.2		(moderate)
Average match session-RPE (AU; 0-10)	High	474 ± 93	0.08	-1.08
	Low	568 ± 82		(moderate)
Cumulative match session-RPE (AU; 0-10)	High	1549 ± 613	0.02	-1.53
	Low	2271 ± 328		(large)
Average match and training time (min)	High	71 ± 4	<0.01	-2.14
	Low	78 ± 4		(very large)
Cumulative match and training time (min)	High	762 ± 99	0.58	0.32
	Low	736 ± 65		(small)
Average match and training sessions-RPE (AU; 0-10)	High	4.9 ± 0.5	0.25	0,67
	Low	4.5 ± 0.7		(moderate)
Cumulative match and training sessions-RPE (AU; 0-10)	High	42.3 ± 15.1	0.59	0.34
	Low	39.0 ± 4.3		(small)
Average day PMS (AU; 1-7)	High	3.8 ± 0.5	0.13	0.80
	Low	3.4 ± 0.5		(moderate)
Cumulative day PMS (AU; 1-7)	High	31.2 ± 4.5	0.53	0.37
	Low	29.3 ± 5.6		(small)

RPE - rating of perceived exertion; PMS - perceived muscle soreness; AU – arbitrary units.

Table 2 – Technical performance actions of high- and low-team rank players during all the matches of the international official elite female football tournament. Values are means ± SD (high-rank: N = 6; low-rank: N = 7) and expressed as absolute and relative to individual match time played (actions per min played).

Technical performance markers	Team rank	Absolute values Mean ± SD	*p*	*d*	Relative values Mean ± SD	*p*	*d*
Average actions (n)	High	50.4 ± 13.1	0.01	-1.72	0.72 ± 0.20	0.06	-1.14
	Low	80.1 ± 21.5		(large)	0.93 ± 0.17		(moderate)
Cumulative actions (n)	High	180.7 ± 69.5	0.01	-1.80	2.71 ± 0.68	0.02	-1.51
	Low	320.6 ± 86.1		(large)	3.74 ± 0.68		(large)
Average successful actions (n)	High	35.4 ± 14.9	0.05	-1.26	0.50 ± 0.20	0.17	-0.82
	Low	56.9 ± 19.3		(large)	0.66 ± 0.19		(moderate)
Cumulative successful actions (n)	High	128.7 ± 69.6	0.04	-1.35	1.91 ± 0.79	0.11	-0.97
	Low	227.7 ± 77.3		(large)	2.66 ± 0.76		(moderate)
Average successful actions (%)	High	68.1 ± 11.3	0.77	-0.17	1.05 ± 0.40	0.63	0.29
	Low	69.9 ± 10.5		(trivial)	0.94 ± 0.37		(small)
Average successful defensive actions (n)	High	10.4 ± 7.4	0.48	-0.41	0.14 ± 0.09	0.85	-0.13
	Low	13.3 ± 6.7		(small)	0.15 ± 0.07		(trivial)
Cumulative successful defensive actions (n)	High	37.7 ± 29.5	0.34	-0.55	0.55 ± 0.37	0.75	-0.18
	Low	53.1 ± 26.8		(small)	0.61 ± 0.29		(trivial)
Average successful attacking actions (n)	High	25.0 ± 9.2	0.02	-1.65	0.36 ± 0.13	0.06	-1.15
	Low	43.6 ± 13.3		(large)	0.51 ± 0.13		(moderate)
Cumulative successful attacking actions (n)	High	91.0 ± 45.1	0.01	-1.70	1.35 ± 0.51	0.03	-1.37
	Low	174.6 ± 53.3		(large)	2.05 ± 0.51		(large)
Average successful tackles (n)	High	1.0 ± 0.5	0.07	-1.22	0.02 ± 0.01	0.29	0.00
	Low	2.1 ± 1.3		(large)	0.02 ± 0.01		(trivial)
Cumulative successful tackles (n)	High	3.5 ± 1.6	0.04	-1.48	0.06 ± 0.03	0.19	-0.75
	Low	8.4 ± 5.0		(large)	0.09 ± 0.05		(moderate)
Average recoveries (n)	High	5.8 ± 4.2	0.76	-0.18	0.08 ± 0.06	0.79	0.00
	Low	6.5 ± 3.4		(moderate)	0.08 ± 0.04		(trivial)
Cumulative recoveries (n)	High	21.2 ± 17.3	0.58	-0.31	0.31 ± 0.22	0.92	0.05
	Low	26.0 ± 13.5		(small)	0.30 ± 0.15		(small)
Average accurate passes (%)	High	74.5 ± 8.7	0.60	-0.32	1.14 ± 0.39	0.69	0.25
	Low	77.1 ± 7.6		(small)	1.04 ± 0.41		(small)
Average challenges (n)	High	12.7 ± 3.4	0.02	-1.64	0.18 ± 0.04	0.05	-1.33

	Low	20.4 ± 6.0		(large)	0.24 ± 0.05		(large)
Cumulative challenges (n)	High	44.0 ± 10.3	0.01	-2.19	0.66 ± 0.14	0.02	-1.61
	Low	81.6 ± 24.0		(very large)	0.95 ± 0.22		(large)
Average challenges won (%)	High	54.5 ± 9.1	0.10	-0.01	0.86 ± 0.39	0.45	0.45
	Low	54.6 ± 15.4		(trivial)	0.72 ± 0.23		(small)

Table 3 – Plasma stress markers (mean ± SD and confidence interval) of high- and low-team rank players at baseline conditions and after all the matches of the international official elite female football tournament. Values are *F*-test and *p* value for analysis of covariance (ANCOVA) model according to time and team rank and controlled for individual match time played (high-rank: N = 6; low-rank: N = 7). Eta squared results refer to time effect adjusted for individual match time played.

Plasma stress marker	**Team rank**	**Baseline**	**Match 1**	**Match 2**	**Match 3**	**Match 4**	η^2_p	***p***	**Two-way ANCOVA** Results of analysis of variance model **Time**	**Team rank**	**Interaction**
									$F_{(df, error)}$ p	$F_{(df, error)}$ p	$F_{(df, error)}$ p
CK (U·l^{-1})	High	230.8 ± 74.2 (152.9-308.7)	324.3 ± 124.7 (193.5-455.2)	344.0 ± 103.9 (235.0-453.0)	241.00 ± 81.2* (155.8-326.2)	274.5 ± 102.7 (166.8-382.2)	0.83	0.58	$0.34_{(4,40)}$	$1.47_{(1,10)}$	$0.96_{(4,40)}$
	Low	366.4 ± 319.3 (71.2-661.7)	563.0 ± 356.4 (233.4-892.6)	657.7 ± 463.4 (229.1-1086.3)	608.6 ± 272.3 (356.7-860.4)	449.9 ± 213.4 (252.6-647.2)	0.08	0.39	0.85	0.25	0.44
CRP (mg·L^{-1})	High	0.75 ± 4.46 (0.18-1.31)	1.28 ± 1.08 (-0.07-2.62)	1.80 ± 0.86 (0.73-2.87)	1.32 ± 0.67* (0.49-2.15)	1.74 ± 0.80 (0.74-2.73)	0.40	0.15	$2.66_{(4,36)}$	$2.51_{(1,9)}$	$0.58_{(4,36)}$
	Low	0.96 ± 1.03 (0.00-1.91)	0.78 ± 0.64 (0.19-1.37)	1.08 ± 0.80 (0.35-1.82)	0.86 ± 0.73 (0.19-1.54)	1.16 ± 1.15 (0.09-2.22)	0.15	0.49	0.05	0.15	0.68
TAS (mmol·L^{-1})	High	1.89 ± 0.08 (1.81-1.97)	1.91 ± 0.09 (1.81-2.00)	1.98 ± 0.20 (1.77-2.18)	1.96 ± 0.09* (1.86-2.05)	1.83 ± 0.11 (1.72-1.94)	0.12	0.72	$0.86_{(4,40)}$	$2.83_{(1,10)}$	$0.66_{(4,40)}$
	Low	1.77 ± 0.11	1.89 ± 0.07	1.95 ± 0.23	1.83 ± 0.08	1.80 ± 0.10	0.14	0.53	0.50	0.12	0.62

		(1.67-1.88)	(1.83-1.96)	(1.74-2.17)	(1.76-1.91)	(1.71-1.89)					
UA (mg·L^{-1})	High	4.57 ± 0.64* (3.89-5.24)	5.00 ± 0.80* (4.16-5.84)	5.77 ± 0.98* (4.74-6.80)	5.35 ± 0.70*# (4.61-6.08)	5.30 ± 1.04*# (4.21-6.39)	0.50	0.02	$3.90_{(4,40)}$	$20.46_{(1,10)}$	$1.07_{(4,40)}$
	Low	3.73 ± 0.51 (3.25-4.20)	4.64 ± 0.62# (4.07-5.21)	4.30 ± 0.56 (3.79-4.81)	4.31 ± 0.73 (3.64-4.99)	4.51 ± 0.78 (3.79-5.24)	0.07	0.84	0.01	0.001	0.38

*Significantly different from low-rank players (p≤0.05).

#Significantly different from baseline values (p≤0.04).

CK - creatine kinase; CRP - C-reactive protein; TAS - total antioxidant status; UA - uric acid; df - degrees of freedom.

Table 4 – Plasma stress markers (mean ± SD and confidence interval) of high- and low-team rank players at baseline conditions and 48h and 24h after the preceding matches (i.e., matches 2 and 3, respectively) during an international official elite female football tournament. Values are *F*-test and *p* value for analysis of covariance (ANCOVA) model according to time and team rank and controlled for individual match time played (high-rank: N = 6; low-rank: N = 7). Eta squared results refer to time effect adjusted for individual match time played.

Plasma stress marker	**Team rank**	**Baseline**	**Match 2**	**48h recovery**	**Match 3**	**24h recovery**	η^2_p	***p***	**Two-way ANCOVA** Results of analysis of variance model **Time**	**Team rank**	**Interaction**
									$F_{(df, error)}$ p	$F_{(df, error)}$ p	$F_{(df, error)}$ p
CK (U·l^{-1})	High	230.8 ± 74.2 (152.9-308.7)	344.0 ± 103.9 (235.0-453.0)	207.2±51.5* (153.2-261.2)	241.00 ± 81.2* (155.8-326.2)	300.5±148.9 (144.2-456.8)	0.50	0.20	$0.61_{(4,40)}$	$2.11_{(1,10)}$	$1.74_{(4,40)}$
	Low	366.4 ± 319.3 (71.2-661.7)	657.7 ± 463.4 (229.1-1086.3)	475.1±313.8 (185.0-765.3)	608.6 ± 272.3# (356.7-860.4)	703.9±327.0 (401.5-1006.3)	0.28	0.15	0.66	0.18	0.16
CRP (mg·L^{-1})	High	0.75 ± 4.46 (0.18-1.31)	1.80 ± 0.86 (0.73-2.87)	1.50±0.75* (0.56-2.43)	1.32 ± 0.67* (0.49-2.15)	1.43±0.45 (0.88-1.99)	0.47	0.08	$2.81_{(4,36)}$	$2.43_{(1,9)}$	$0.94_{(4,36)}$
	Low	0.96 ± 1.03 (0.00-1.91)	1.08 ± 0.80 (0.35-1.82)	1.20±0.96 (0.32-2.09)	0.86 ± 0.73 (0.19-1.54)	1.52±1.38 (0.25-2.79)	0.14	0.55	0.04	0.16	0.55
TAS (mmol·L^{-1})	High	1.89 ± 0.08 (1.81-1.97)	1.98 ± 0.20 (1.77-2.18)	2.05±0.26* (1.78-2.32)	1.96 ± 0.09* (1.86-2.05)	1.77±0.10*# (1.66-1.87)	0.06	0.90	$1.03_{(4,40)}$	$13.86_{(1,10)}$	$3.12_{(4,40)}$
	Low	1.77 ± 0.11 (1.67-1.88)	1.95 ± 0.23 (1.74-2.17)	1.68±0.08 (1.61-1.75)	1.83 ± 0.08 (1.76-1.91)	1.58±0.15$ (1.44-1.71)	0.16	0.47	0.41	0.004	0.03
UA (mg·L^{-1})	High	4.57 ± 0.64* (3.89-5.24)	5.77 ± 0.98* (4.74-6.80)	4.73±0.78* (3.91-5.55)	5.35 ± 0.70*§ (4.61-6.08)	4.98±0.90* (4.04-5.92)	0.38	0.09	$4.53_{(4,40)}$	$26.13_{(1,10)}$	$1.02_{(4,40)}$

	Low	3.73 ± 0.51 (3.25-4.20)	4.30 ± 0.56 (3.79-4.81)	3.29±0.79$ (2.56-4.01)	4.31 ± 0.73# (3.64-4.99)	3.75±0.65 (3.16-4.36)	0.22	0.27	0.004	<0.001	0.41

*Significantly different from low-rank players (p≤0.05).

#Significantly different from the 48h recovery (p≤0.05).

$Significantly different from the preceding match (p≤0.05).

§Significantly different from baseline (p≤0.05).

CK - creatine kinase; CRP - C-reactive protein; TAS - total antioxidant status; UA - uric acid; df - degrees of freedom.

Figure 1 - Schematic description of the experimental protocol, illustrating the time points for the blood sampling: baseline conditions (before the beginning of the tournament, i.e., first match), before two training sessions held 48h and 24h after the preceding match and after all the matches of the official FIFA tournament.

Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
Baseline conditions	Match 1		Match 2		Training session 48h	Match 3	Training session 24h	Match 4
	Immediately after player’s substitution or the end of the match		Immediately after player’s substitution or the end of the match		Before the training session	Immediately after player’s substitution or the end of the match	Before the training session	Immediately after player’s substitution or the end of the match

Figures 2A, 2B, 2C and 2D - Plasma stress markers of high- and low-team rank players at baseline conditions, after all the matches and 48h and 24h after the preceding matches (i.e., matches 2 and 3, respectively) of the international official elite female football tournament (high-rank: N = 6; low-rank: N = 7): CK - creatine kinase (1A), CRP - C-reactive protein (1B), TAS - total antioxidant status (1C); UA - uric acid (1D).

Fig. 2A

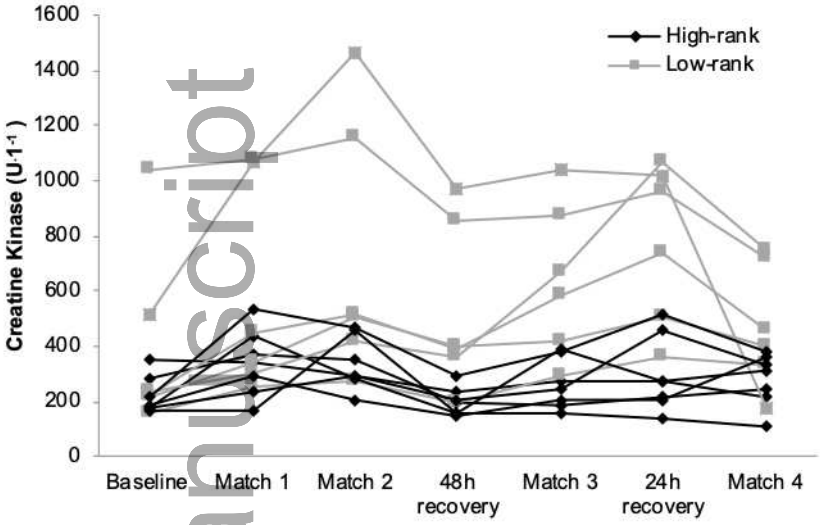

Fig. 2B

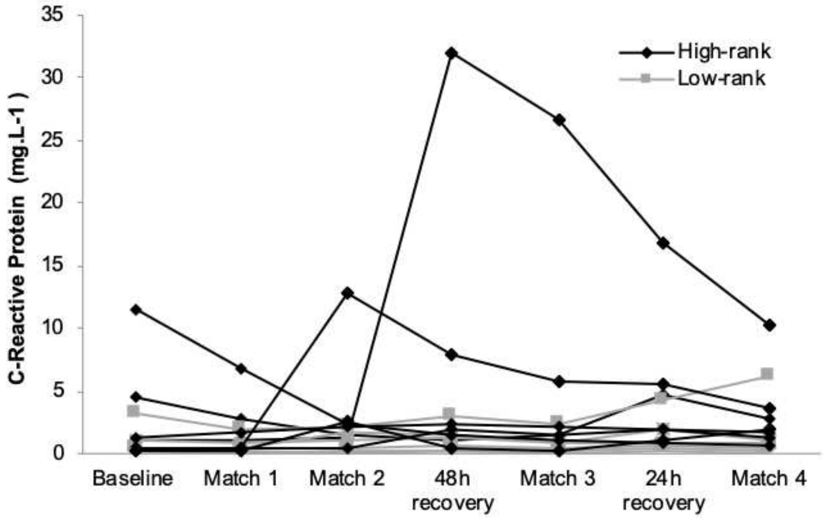

Fig. 2C

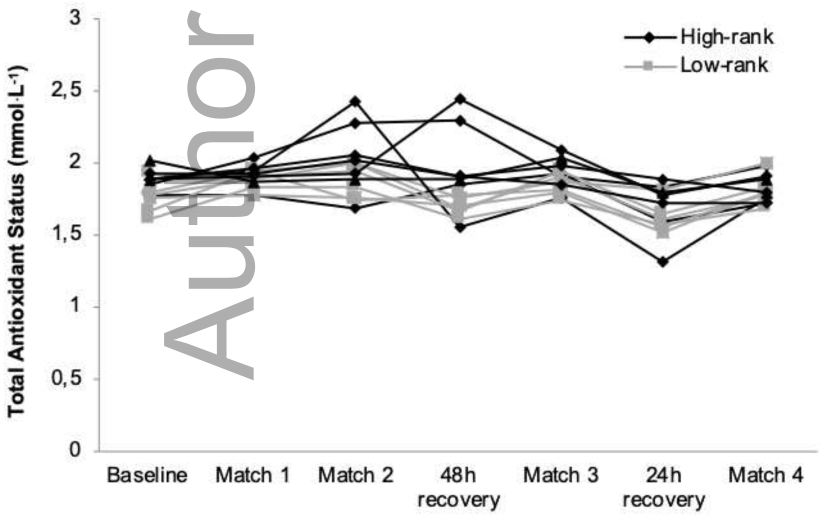

Fig. 2D