Journal of Physical Education and Sport ® (JPES), Vol. 21 (4), Art 235, pp. 1860 - 1870, June 2021
online ISSN: 2247 - 806X; p-ISSN: 2247 – 8051; ISSN - L = 2247 - 8051 © JPES

Original Article

Effects of small-sided games on the haematological profile of soccer players

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Published online: June 30, 2021
(Accepted for publication June 15, 2021)
DOI:10.7752/jpes.2021.04235

Abstract

The aim of this study was to examine the changes in blood parameters caused during Small-Sides Games (SSGs) in U20 elite players in professional league. The sample consisted of eight U20 Greek Super League elite level soccer players, aged 18.3 ± 1 years old, who participated in six SSG's (4 vs 4 + 2 GK), each lasting 4 min with 3 min rest. The size of the pitch was 30m in length and 20m wide and the SSGs took place in a pitch with artificial turf. Blood samples were collected before and immediately after the six SSGs to examine any potential changes of the haematological profile and the levels of cell damage. The Wilcoxon Test (Z) and the Effect Size (r) were used to compare the repeated measures of the variables (pre-post), while the Pearson correlation index (r) was used to test the correlation between the variables. The results showed a significant decrease in the second measurement in Rbc ($p < .05$), Hgb ($p < .05$), Mcv ($p < .01$), Mch ($p < .05$) and Mchc ($p < .05$). On the contrary, Hct, Rdw and iron concentration did not show a significant change ($p > .05$). Regarding the elements of the leukocyte series a significant increase was observed in Wbc ($p < .01$), Neutrophils ($p < .05$), Plt ($p < .05$), and Pct ($p < .01$). On the contrary, a significant decrease was observed in Lymphocytes ($p < .05$) and Pdw ($p < .05$). Finally, Monocytes, Eosinophils and Pct did not show any statistically significant change. Overall, the results showed that exercising through high intensity SSGs can cause specific metabolic effects on the haematological profile of soccer players. Soccer teams need to incorporate the monitoring of haematological parameters of players while planning their training program, as this information can influence the performance and overall wellbeing of players.

Keywords: soccer, small-sided games, haematological parameters, elite level

Introduction

Soccer is a multidimensional dynamic sport involving a variety of technical, tactical, physiological, and psychological elements the interaction of which determines team performance (Souglis et al., 2015). Physical condition combined with accurate execution of specific soccer skills consist a fundamental component of the optimal performance for a player (Portillo et al., 2020). During a typical soccer game, players cover a distance from 10 to 14km with an average peak heart rate and a VO2max above 80% (Mendez-Villanueva et al., 2013; Sporiš et al., 2009). Additionally, they perform approximately 1330 activities, including high speed 220 displacements (Rampinini et al., 2007). Players perform high-intensity and short-duration (<10sec) sprints as well as several eccentric activities such as one-on-one duels, changes of directions, jumps, acceleration, deceleration, and turns (Gravina et al., 2011). The ability of players to perform such rapid activities is essential for optimizing the possibilities of winning a game (Little and Williams, 2005). It is well documented that soccer movements involving eccentric muscle actions are responsible for inducing muscle damages and functional changes in skeletal muscle cells that reduce performance (Choi, 2016; Hughes et al., 2018). Furthermore, eccentric strength exercise induces better stretch-shortening cycle performance and greater concentric muscle power which affects muscle functionality (Douglas et al., 2017; Elmer et al., 2012). In addition, modern elite-level soccer requires players to compete in approximately 40-50 games per season every 3-4 days over a 10-month period with insufficient time available for recovery (Strudwick, 2013). As such, the high-intensity training accumulated over an extended period of intensive daily training sessions and competitive games imposes significant pressure to a variety of physiological systems, which might also be reflected on alterations in haematological values (Owen et al., 2018).

Several studies assessing the relationship between exercise and physiological outcomes have shown that vigorous training causes various changes in blood parameters and muscle tissues (Heisterberg et al., 2014; Owen et al., 2018). However, although players face large changes in physiological biomarkers during the competitive period, these values return to normal ranges during the transition period (Silva et al., 2018). More specifically,

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high intensity exercise can cause short- or long-term fatigue which has been linked to muscle (e.g., depolarization of the muscle membrane) and immune system changes (e.g., perturbations in blood immune cell counts) lasting from 3 to 72 hours (Fransson et al., 2018; Simpson et al., 2020). It has also been indicated that high intensity training results in leakage of cell content and muscle cell breakdown (Anđelković et al., 2015). Furthermore, it has been found that this type of training due to exercise induced plasma expansion causes a reduction in erythrocytes, haemoglobin, haematocrit, iron and ferritin concentration, whereas it elevates white blood cell counts (Bussollaro et al., 2018; Joksimović et al., 2009; Schumacher et al., 2002). Specifically, it is suggested that variations of haematocrit and haemoglobin values were related to the amount of anaerobic or aerobic training, strength training, and the number of competitive games per week (Heisterberg et al., 2013). Thus, contradicting findings showing an increase in the blood cells and haematocrit level following a soccer match can be explained since different types of training may stimulate red blood production (Younesian et al., 2004).

It has been identified that players require great amounts of oxygen within different body systems as well as the optimal level of transported haemospherine within muscles to effectively function (Sporiš et al., 2016). In particular, red blood cells (erythrocytes) obtain the crucial role of transporting oxygen from the lungs to the tissues. Furthermore, haematocrit which represents the amount of red blood cells within blood is affected by the number of red blood cells and the size of these cells (Hu et al., 2008). Haematocrit increment is usually associated with increased oxygen transport capacity of the blood. As a result, the increased haematocrit may lead to increased viscosity of the blood (Hu et al., 2008; Joksimović et al., 2009). Furthermore, physical exercise causes changes in the haemostasis of athletes (Boyadjiev, 2004), while the formation of a stable haemostatic white thrombus is one of the most important functions of the blood platelets. They play a significant role in the blood-clotting procedure through adhesion to the site of injured vessel immediately after its damage (El-Sayed et al., 2004). Furthermore, research findings have shown that physical exercise alters both platelet count and functionality (e.g., Hilberg et al., 2003). Interestingly, findings are contradicting with some studies showing that exercise causes reduction of platelets (Heber & Volf, 2015), increase of the platelet aggregation (Singh et al., 2006) or no effects on the platelets (Alis et al., 2015). Regarding Mean Platetet Volume (MPV) is considered, along with other parameters, to be a marker of platelet activation and reactivity and its reduction is associated with lower fitness level of soccer players. Previous studies have exhibited higher values of MPV during the competitive period which can be explained by the intensity and volume of exercise during this period (Alis et al., 2015).

An essential factor for optimal oxygen uptake as well for transport from respiratory organs to muscles as a component of haemoglobin is iron (Fe) (Dunn et al., 2007). Iron plays a crucial role in performance because it is responsible for haeme and acts as a transporter and storehouse of oxygen. Moreover, players have several risk factors for anaemia and iron depletion which can be developed by poor nutritional intakes, haemolysis caused by repeated foot strikes, blood and iron loss through gastrointestinal, urinary tract and sweat (Dubnov & Constantini, 2004). Iron concentration has various regulatory functions for oxidative protein and enzymes involved in cellular energy production (Beard, 2001). Previous literature has shown that participation in intensive training may induce iron deficiency that may be associated with performance reduction (Koehler et al., 2012; Oliveira et al., 2017; Reinke et al., 2012). On the other hand, high oxygen transport capacity and oxygen uptake, which are prerequisite for success in sports, can be improved by increasing the red cell mass and consequently the haemoglobin concentration within the blood. Thus, the requirement for practitioners to effectively use management and monitoring strategies for their players through training is of paramount importance (Dupont et al., 2010). Consequently, monitoring tools which better explain physiological outputs such as routine screening of collected blood variables seems to be crucial (Meister et al., 2013; Owen et al., 2018). Thus, it is clear that soccer training causes increment in maximum cardiac output which is related to hematological peripheral adaptations targeting oxygen transport and utilization such as improved mitochondria density and reactions which also explain VO_{2max} responses (Gibala et al., 2012; Montero et al., 2015).

To reproduce physical, technical, and tactical soccer requirements of modern soccer training methods have evolved from conditioning exercises without ball to new methods which simultaneously develop a variety of performance capacities (Sarmento et al., 2018). One of the most common training methods that soccer practitioners extensively include into their training sessions are Small-Sided Games (SSGs), also referred to in literature as game-based, skill-based, or small-sided conditioning games (Davids et al., 2013; Gabbett et al., 2009). Due to the beneficial and ecological nature of SSGs, nowadays there is a tendency to replace traditional running conditional exercises with several forms of SSGs (Moran et al., 2019). Soccer practitioners can modify the intensity of SSGs according to the targets of the training (Aguiar et al., 2013; Bujalance-Moreno et al., 2019) by changing some of the following parameters: dimensions of the pitch, number of players, game's rules, and duration (Casamichana et al., 2013; Fanchini et al., 2011; Larsen et al., 2018; Mallo & Navarro, 2008; San Román-Quintana et al., 2013). For example, it has been found that the higher the ratio between the area and the players the higher the perceived exhaustion will be (Hill-Haas et al., 2009a) as well as when the aim of the game is to score a goal without goalkeepers and simultaneously protect their own area (Dellal et al., 2008) or when the coaches provide players with verbal encouragement (Rampinini et al., 2007).This type of training method allows

the attainment of over 80% of peak heart rates and maximal oxygen uptake which is equivalent to the observed during games (Kunz et al., 2019). It has been found that during SSGs, heart rate responses are similar or even higher than high-intensity training exercises, which can be explained by the accumulation of technical components, the frequency of directional changes and the motivational role of the presence of the ball (Dellal et al., 2008; Rampinini et al., 2007).

It is well documented that soccer intensive exercise causes significant changes in a variety of biomarkers and the function of immune system. However, the quality of effects on immunological status of players depends on the kind, the intensity, the duration, and the load of exercise (Simpson et al., 2020). Therefore, the aim of the current study was to investigate the changes in haematological parameters caused by SSGs' training to professional soccer players.

Materials and methods

Sample

Eight elite youth male soccer players aged 18.4 ± 1 years old (68.9 ± 2 kg weight; 1.74 ± 3cm height; 9.46 ± 1% body fat), all members of a Greek Super League team, participated in the current study. Participants were informed about the aims, the procedures, and the possible risks of the study before signing a written consent form. An approval was obtained from the Ethics Committee of the University before the beginning of subject recruitment. During the week before the games, participants were advised to follow a balanced diet as outlined by Souglis (2014) with50-60% of the energy intake from carbohydrates, 25-30% from fat, and 15% from protein. Players were asked to replicate their prerecorded diet during the 5 days after the match. On the competition day, players consumed a meal rich in carbohydrates (approximately 60-65% of total energy intake) 3 hours before the game. All athletes were subjected to routine medical examination (cardiogram, blood pressure measurement, chest X-ray) which showed no pathological conditions.

Anthropometric Measurements

Anthropometric measurements were performed during a preliminary visit to the laboratory. Standing height was measured to the nearest 0.5 cm (Stadiometer; Seca, Birmingham, United Kingdom), while nude body weight was measured to the nearest 0.1 kg (Beam balance 710, Seca, United Kingdom). Body fat was estimated using the 7-skinfold method, measured by an appropriate skinfold caliper (Harpenden Skinfold Caliper, Baty International, West Sussex, England). Environmental temperature and relative humidity at this period were between 16-20oC and 40-60%.

Intervention program

Players took part in 5 training sessions and one official game per week. Each training session lasted 60-80 min and included speed, power, strength, agility, technical and tactical development drills, as well as small-sided games. The SSGs included six 4 vs 4 + 2 GK on artificial turf each lasting 4 minutes with 3 minutes rest between them and dimensions of 30x20m. Blood samples were taken by specialized personnel before and immediately after the six SSGs to determine the change of the haematological profile, as well as the levels of cell damage. The day before the experiment the players did not train, whereas for the 3-day pre-experimental period, players trained normally, but avoided the weekly peak that normally includes SSGs with low number of players. Instead, games with 8 vs 8 players in larger spaces were utilized before the experiment, with the HR average ranging between 65% and 75% of maximal heart rate.

Blood Samples and Analyses

Two blood samples were taken and collected in vials (Becton Dickinson Vacutainer K2 EDTA) before their analysis by haematology (Mindray bc-3000+) and immunoassay (Siemens Advia Centaur) analyzer. The first blood sample was taken before the experimental SSGs while the second blood sample was taken exactly after the experimental SSGs. During sampling, participants were at a sitting position. Samples were collected from the basilic and median basilic vein. More specifically, 5 ml were taken at each blood sampling and placed in glass tubes with no anti-coagulant to collect the serum. In addition, collected blood was placed in special full-blood-count-type tubes with EDTA to specify the number of leucocytes and platelets. Sample centrifugation (10 min at 2.500 rpm) was performed within one hour from sampling. Then serum was stored at -70 ºC until the assay day. Furthermore, two measurements of lactic acid were performed, one before the SSGs and the second immediately after each SSG, by the Lactate plus portable analyzer (Nova Biomedica, USA). Finally, Heart rate (HR) was monitored continuously during the games using the Polar Team 2 pro System (Polar Electro Oy, Kempele, Finland), and the average HR (HR mean) as well as the corresponding average percentage of the individual maximal HR (%HRmax) was calculated for each player.

Data analysis

Descriptive statistics were calculated for each variable including means (M) and standard deviations (±SD). The assumption of normality was verified using the Kolmogorov–Smirnov test ($p > .05$). The statistical analyses for repeated measures comparisons (pre and post) were performed using the Wilcoxon test (Z), while the effect magnitude was measured with Effect Size (r). According to Cohen (1988) and Carson (2012) the effect size was interpreted as small (<0.2), medium (0.5) and large (≥ 0.8). The Pearson correlation coefficient (r) was applied to calculate the relationship between the variables. In all cases, level of significance was preset at 5% ($p < .05$).

Results

The descriptive data of the physiological intensities during the six SSGs assessed by Heart Rate and Lactic Acid indexes are summarized to the following table (Table 1). The values showed that SSGs demands were similar to an official competitive game.

Table 1. *Physiological intensities of SSGs.*

Variables	Rest	SSGs 1st	2nd	3rd	4th	5th	6th	Mean
Heart Rate		175.67±9.29	181.67±12.01	184.33±8.01	180.67±6.04	184±6.24	185.67±7.96	182bpm 89% MHR
Lactic Acid	2.81±0.87	6.57±1.17	7.89±1.18	8.28±1.34	8.04±1.28	7.68±1.12	6.85±1.5	6.84

Haematological changes of pre- to post- SSGs intervention were significant for the majority of the variables. Furthermore, the effect sizes of almost all measures demonstrated medium to large magnitude. More specifically, the Wilcoxon tests indicated that post training measurement of the following erythrocyte series, i.e., red blood cells ($Z = -2.03$, $p < .05$), haemoglobin ($Z = -1.98$, $p < .05$), mean corpuscular volume ($Z = -2.53$, $p < .01$), mean corpuscular haemoglobin ($Z = -2.40$, $p < .05$), mean corpuscular haemoglobin concentration ($Z = -2.03$, $p < .05$), were significantly lower than pre training measurement. On the other hand, haematocrit and red cell distribution width did not show any significant change. The Wilcoxon tests also indicated that post training measurement of the following leukocyte series, i.e., lymphocytes ($Z = -2.18$, $p < .05$), and platelet distribution width ($Z = -2.40$, $p < .05$) were significantly lower than pre training measurement. On the contrary, the Wilcoxon tests indicated that post training measurement of the following leukocyte series, i.e., white blood cell ($Z = -2.52$, $p < .01$), neutrophils ($Z = -2.24$, $p < .05$), and platelets ($Z = -2.10$, $p < .05$), were significantly higher than pre training measurement. Moreover, the Wilcoxon tests showed that monocytes, eosinophils, and platelecrit did not show any significant change. Finally, iron did not significantly change (Table 2).

Table 2. *Haematological variables in the soccer players at pre-, post-SSGs (mean ± s, Wilcoxon-test (Z), sig. and Effect size (r)).*

	Variables	Measurements Pre Mean	Pre Sd	Post Mean	Post Sd	Wilcoxon Test (Z)	Exact Sig. p	Effect Size (r)
Erythrocyte series	Rbc (mmol/ml)	5,12	0,33	5,04	0,37	-2,032	0,05*	0,72
	Hgb (gr %)	15,21	1,13	14,98	1,06	-1,975	0,05*	0,70
	Hct (%)	44,19	3,09	43,56	3,00	-1,54	0,15	0,54
	Mcv (fl = 10^{-15} lt)	86,99	2,86	86,48	2,61	-2,527	0,01**	0,89
	Mch (pg)	29,98	0,93	29,46	1,00	-2,395	0,02*	0,85
	Mchc (gr%)	34,53	0,42	34,14	0,55	-2,033	0,05*	0,72
	Rdw (%)	13,06	0,68	13,13	0,67	-0,853	0,42	0,30
	Fe (μg/dl=10-^{5}kg/m^{3})	120.25	41.85	116.50	41.70	-0.339	0.78	0.12
Leukocyte series	Wbc (μl=10^{-6}L)	7,05	1,41	9,59	2,38	-2,521	0,01**	0,89
	Neutrophils (%)	62,88	8,17	71,88	7,68	-2,24	0,02*	0,79
	Lymphocytes (%)	30,75	6,43	22,50	7,27	-2,176	0,03*	0,77
	Monocytes (%)	4,63	1,60	3,88	0,83	-1,372	0,17	0,49
	Eosinophils (%)	1,75	0,71	1,75	0,46	0	1,00	0,00
	Plt (10^{-6}L)	231,00	53,44	244,00	56,76	-2,10	0,04*	0,74
	Mpv (fl = 10^{-15} Lt)	9,03	0,70	8,94	0,65	-1,282	0,28	0,45
	Pdw (fl = 10^{-15} Lt)	15,71	0,20	15,53	0,20	-2,388	0,02*	0,84
	Pct (fl = 10^{-15} Lt)	0,21	0,04	0,22	0,04	-1,933	0,09	0,68

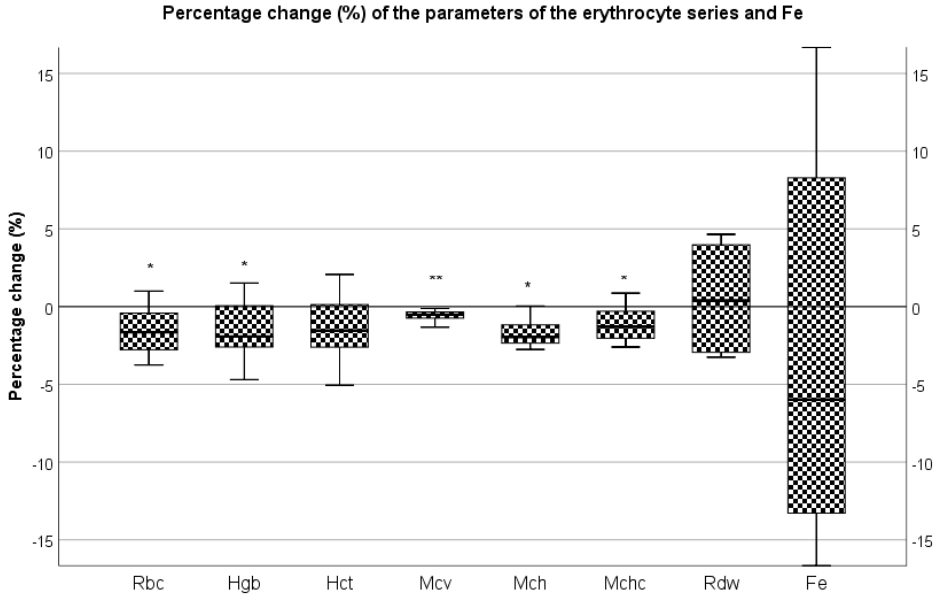
Notes: sig. p<.05 *, p<.01 **, p<.001 ***

Effect size (r): small (<0.2), medium (0.5) and large (≥ 0.8)

Abbreviations: Red blood cells (Rbc), Haemoglobin (Hgb), Haematocrit (Hct), Mean corpuscular volume (Mcv), Mean corpuscular haemoglobin (Mch), Mean corpuscular haemoglobin concentration (Mchc), Red cell distribution width (Rdw), Iron (Fe), White blood cell (Wbc), Neutrophils, Lymphocytes, Monocytes, Eosinophils, Platelets (Plt), Mean platelet volume (Mpv), Platelet distribution width (Pdw), Platelecrit (Pct).

Red blood cells, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration reduced 1.56%, 1.51%, 0.59%, 1.74%, and 1.12% respectively (Figure 1).

Figure 1. Percentage change (mean %) of the parameters of the erythrocyte series in the soccer players at pre-, post-SSGs.

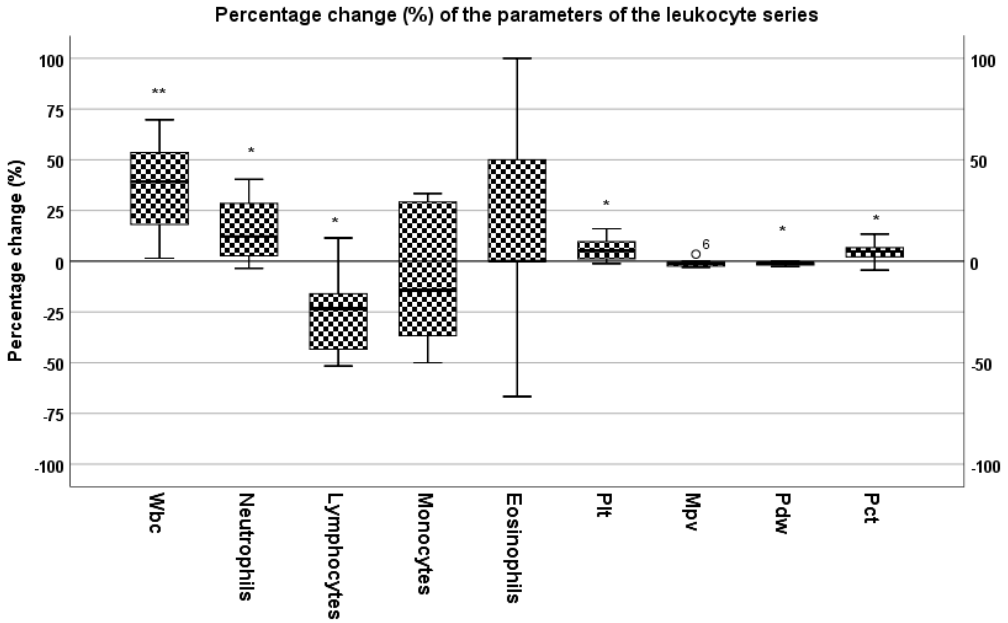


Notes: sig. p<.05 *, p<.01 **, p<.001 ***

Abbreviations: Red blood cells (Rbc), Haemoglobin (Hgb), Haematocrit (Hct), Mean corpuscular volume (Mcv), Mean corpuscular haemoglobin (Mch), Mean corpuscular haemoglobin concentration (Mchc), Red cell distribution width (Rdw), Iron (Fe).

Similarly, lymphocytes and platelet distribution width reduced 26.8% and 1.2% respectively, while white blood cell, neutrophils, and platelets increased 35.9%, 14.3%, and 5.6% respectively (Figure 2).

Figure 2. Percentage change (mean %) of the parameters of the leukocyte series.



Notes: sig. p<.05 *, p<.01 **, p<.001 ***

Abbreviations: White blood cell (Wbc), Neutrophils, Lymphocytes, Monocytes, Eosinophils, Platelets (Plt), Mean platelet volume (Mpv), Platelet distribution width (Pdw), Platelecrit (Pct).

The correlations of erythrocyte series in the second measurements showed that red blood cells exhibited a large positive relationship with haemoglobin ($r = .922$, $p < .01$) and haematocrit ($r= .881$, $p < .01$). Furthermore, the correlations showed a large positive relationship between haemoglobin and haematocrit ($r = .994$, $p < .001$) as well as the relationship between mean corpuscular volume with mean corpuscular haemoglobin ($r = .762$, $p< .05$). On the other hand, it was found that the red cell distribution width had a large negative correlation with mean corpuscular haemoglobin ($r = -.766$, $p < .05$) (Table 3).

Table 3. Statistically significant correlations (Spearman's rho) in the blood parameters of the erythrocyte series immediately after playing 6X4vs4+2Gk SSGs.

Erythrocyte variables		r	p
Rcb	Hgb	.922**	.001
	Hct	.881**	.004
Hgb	Hct	.994***	.000
Mcv	Mch	.762*	.028
Rdw	Mch	-.766*	.027

Notes: sig. p<.05 *, p<.01 **, p<.001 ***
Abbreviations: Red blood cells (Rbc), Haemoglobin (Hgb), Haematocrit (Hct), Mean corpuscular volume (Mcv), Mean corpuscular haemoglobin (Mch), Mean corpuscular haemoglobin concentration (Mchc), Red cell distribution width (Rdw)

Moreover, a large negative relationship between neutrophils and lymphocytes was found ($r = -.970$, $p < .001$), monocytes and platelet distribution width ($r = -.822$, $p < .05$), platelets and Platelet distribution width ($r = -.759$, $p < .05$) as well as between platelet distribution width and platelecrit ($r = -.721$, $p < .05$). On the other hand, a large positive correlation observed between platelecrit and platelets ($r = .922$, $p < .01$), as well as between mean platelet volume and platelet distribution width ($r = .747$, $p < .05$) (Table 4).

Table 4. Statistically significant correlations (Spearman's rho) in the blood parameters of the leukocyte series immediately after playing 6X4vs4+2Gk SSGs.

Leukocyte variables		r	p
Neutrophils	Lymphocytes	-,970***	.000
Monocytes	Pdw	-,822*	.012
Plt	Pdw	-,759*	.029
Pct	Plt	,922**	.001
Mpv	Pdw	,747*	.043
Pdw	Pct	-,721*	.043

Notes: sig. p<.05 *, p<.01 **, p<.001 ***
Abbreviations: Neutrophils, Lymphocytes, Monocytes, Platelets (Plt), Mean platelet volume (Mpv), Platelet distribution width (Pdw), Platelecrit (Pct).

Discussion

The aim of the study was to evaluate the effects of six high intensity 4 vs 4 SSGs + 2 GK on haematological profile of soccer players. The majority of the measured haematological variables indicated significant changes after the SSGs training. The findings suggest that practitioners may use SSGs in order to improve physiological parameters simultaneously with technical and tactical skills of their players.

Similarly to previous findings (Gravina et al., 2011; Sporiš et al., 2016) the red blood cells (or erythrocytes) significantly reduced from 5.12mmol/ml to 5.04mmol/ml after the intervention program. This reduction can be explained by intravascular haemolysis which is caused by mechanical rupture when erythrocytes pass through capillaries in contracting muscles. This process is mainly caused by rapid intense eccentric exercise and the compression of red cells during training (Mairbäurl, 2013). Furthermore, the non-significant haematocrit decrement after exercise can be explained by increased erythrocytes' destruction, number and size, and players' hyperhydration-dilution status (Mairbäurl, 2013; Younesian et al., 2004). However, previous studies have showed that erythrocytes and haematocrit might be increased after a period of training (e.g., Brun et al., 2010). Those contradicting findings can be potentially attributed to different methodological approaches between the studies or varied intensity of the exercises (Brun et al., 2010; Çiçek, 2018). Findings as expected demonstrated that high intensity training and anaerobic exercise may be a possible explanation for decrements in means of corpuscular volume, haemoglobin, and haemoglobin concentration which further reinforces existing literature in this area of research (Heisterberg et al., 2013; Mayr et al., 2006; Silva et al., 2008).

A significant increase from 7.05μl to 9.59μl was observed in the white blood cell (or leukocytes) which reinforces previous findings in the literature (Neves et al., 2015; Souglis & Travlos, 2015; Tsubakihara et al., 2013; Younesian et al., 2004). In detail, a greater increase of leukocytes has been observed during high-intensity exercise compared to low-intensity exercise as well as after an official soccer game (Neves et al., 2015; Souglis & Travlos, 2015). Indeed, leukocyte increase is dependent on the fitness level of the players as well as the intensity and duration of the exertion (Pedersen & Hoffman-Goetz, 2000). Particularly high intensity exercise can cause changes in the mitochondria of leukocytes and DNA damage in lymphocytes (Cury-Boaventura et al., 2008). This can explain the significant decrement in immunological parameters such as the lymphocytes (Malm et al., 2004; Pascoal et al., 2018). Moreover, leukocyte apoptosis plays a crucial role in maintaining lymphoid tissue homeostasis and consequently avoiding immune activation (Worth, Thrasher, & Bobby Gaspar, 2006). In line with this, glutamine metabolism delays apoptosis in neutrophils (Pithon-Curi et al., 2002). However, during intense exercise, the lymph, spleen, and thymus nodes are exposed to glutamine shortage. This occurs because of the higher glutamine demands (from other tissues) compared to the quantity produced by skeletal muscles. Thus, it may affect both the function and the number of leukocytes (Castell, 2003; Neves et al., 2015). Similarly to the findings of the current study, the overall number of leukocytes was increased following a competitive soccer game. This increase can be attributed to the considerable enhancement of neutrophils which are also related to the inflammatory nature of muscle damage (Devrnja & Matković, 2018). A significant increase from 231μl to 244μl of the platelet concentration after the exercise intervention program was observed further reinforcing findings previously presented in the literature, (Souglis & Travlos, 2015; Younesian et al., 2004). Platelet and endothelial function have a vital role in thrombotic events where thrombocytosis (high number of circulating blood platelets) occurs after intensive training. However, platelet count following a soccer match may transiently increase before returning to normal concentration. This mechanism can be explained by either an increased rate of platelet production or the reduced removal of platelets from the blood which is a function of the spleen (Younesian et al., 2004). As a consequence of platelet variations, the platelet distribution width can also be significantly reduced (Kocakulak et al., 2020).

Moreover, the results showed a non-significant reduction of iron after the exercise from 120.25μg/dl to 116.5μg/dl. Nevertheless, literature has shown that iron changes due to exercise probably depend on the type and the duration of the exercise and the intervention (Bussollaro et al., 2018; Jamurtas et al., 2015; Jastrzebska et al., 2017). Therefore, the current intervention programme which included a 4 vs 4 + 2 GK SSGs appears to be effective in avoiding significant reduction of iron concentration due to exercise. Typically, the reduction of iron is explained by myofibres' micro-traumas and intravascular haemolysis which are linked to a shift of iron to the liver. Furthermore, iron is usually reduced due to reticulocytosis and plasma volume expansion as a result of intense exercise. Likewise, iron decrement has been observed due to higher volume of sweat and urine after intense training (Deli et al., 2013). Consequently, iron reduction may reduce haemoglobin and myoglobin synthesis and impair diffusion of oxygen from erythrocytes to muscles' mitochondria. It may also dilute muscle oxidative phosphorylation because of a decline in haem iron-containing electron transport chain proteins, as well as in non-haem iron-containing enzymes and sulphur proteins (Beard, 2001). Although data from some studies have indicated improvements in performance due to iron supplementation (Brownlie IV et al., 2002; Villanueva et al., 2011) other suggest that it may not be required (Jamurtas et al., 2015). The differences in dosage and duration of the treatments probably affect their effectiveness (Hinton & Sinclair 2007; Mougios, 2004). Thus, iron status should be monitored by practitioners during intensive training and competitive periods so as to provide players with iron supplementation in order to maintain balanced stores.

In addition the results showed significant correlations among erythrocyte variables. It was found that the relationship between Rbc and Hgb as well as between Rdc and Hct indicated a large magnitude, finding which was expected as Hgb is a protein in Rbc while Hct is a measurement of the amount of Rbc. Similarly, Hgb showed a large correlation with Hct. In addition, Mcv and Rdw showed a large magnitude with MCV due to the fact that MCH reflects MCV values, which reveals that more hemoglobin when the red blood cells are increased. As far as the leukocyte variables, the correlations showed a large negative relationship between neutrophils and lymphocytes. Specifically, the circulation of neutrophils increases due to exercise while the number of circulating lymphocytes reduces (Ferrer et al., 2009). Furthermore, the large negative correlation between monocytes and Pdw is probably explained by the platelet indirect influence on the behavior of cells such as the monocytes (Seizer et al., 2013). Finally, the results showed a large negative relationship of Plt and Pct with Pdw, whereas a positive large relationship was found between Pct and Plt as well as between Mpv and Pdw. Literature has confirmed these relationships as Mpv and especially Pdw increase due to platelet activation during exercise, while Pdw seems to be a more specific indicator of their activity (Vagdatli et al., 2010).

In modern soccer players participate in a large number of competitive games which increases the stress on their nervous, musculoskeletal, immune, and metabolic systems. Soccer practitioners should adopt a holistic view when defining training variables (e.g., volume, intensity, frequency) and modalities of the exercise intervention (Silva, Brito, Akenhead, & Nassis, 2016). The findings of the current study showed significant alterations in haematological variables suggesting that monitoring the training stress and nutrition needs that affects performance is essential (Owen et al., 2018). In particular, the findings showed reductions in erythrocyte

series (red blood cells, haemoglobin mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration) and in leukocyte series (lymphocytes, platelet distribution width) as well as increases in leukocyte series (neutrophils, platelecrit, white blood cell). These changes negatively affect sport performance through deficiencies in oxygen capacity (i.e., aerobic capacity) and other muscle functions and damages (e.g., soreness, inflammation, range of motion, limb girth, muscle strength, creatine kinase and lactate dehydrogenase) (Ascensão et al., 2008; Khan et al., 2016). Thus, players may benefit from nutritional strategies including sufficient iron, carbohydrate, anti-inflammatory, antioxidant, polyphenol rich foods, and supplementations for an optimal recovery and high performance (Ranchordas et al., 2017; Souglis., et al., 2013).

Conclusion

Practitioners are constantly seeking effective approaches to improve performance and reduce risks of physiological decline and as such considering and evaluating the haematological profile of players particularly during competitive periods with congestive training is essential. For soccer practitioners, the quantification of training and game load it is essential as it can provide them with information about subsequent energy prescription and muscle damages and assist them to schedule appropriate and effective training for players and team development. Adopting SSGs (4 vs 4 + 2 GK) exhibited similar to match-physiological demands which can be used by coaches to achieve peak performance by combining recovering periods and proper nutrition. Therefore, specific periodisation models emphasizing in SSGs exercises and minimising traditional approaches used in individual sports (e.g., running) need to be adopted in soccer.

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