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Original research article

HAEMATOLOGICAL AND IRON STATUS FOLLOWING A SOCCER MATCH

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Abstract. *The aim of the present investigation was to verify the responses of haematological and iron concentrations in Croatian U-21 soccer players following an official soccer match. A group of 19 trained, healthy male soccer players from the U-21 category of the Croatian soccer association took part in this study. An analysis of red blood cell concentration (RBC), haemoglobin (Hb), haematocrit (Htc), mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin corpuscular (MCHC), and Thrombocytes was performed to indicate the haematological status. In addition, iron, total iron binding capacity (TIBC), the unsaturated iron-binding capacity (UIBC) and ferritin analyses were obtained immediately after the soccer match. A significant decrease in Hct (%) was observed immediately after the game. However, there were no significant changes in the haemoglobin levels. Iron concentration increased significantly (p< 0.01) after the soccer match. Ferritin levels were also higher at the end of the match compared to baseline measures. It can be reported that a soccer match may induce an iron increase immediately after the game which was contradictory to recent studies. In summary, this study provided a valuable opportunity to study biochemical parameters that could lead to a possible illness, injuries and severity in match-play.*

Key words: *biochemical analysis, soccer, professional players, fatigue.*

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INTRODUCTION

Soccer is played by over 250 million people in more than 200 countries (Dunning, 1999). Therefore, its popularity leads to a constant effort for improving performance. Male soccer players draw all the attention and the biochemical impact of a soccer match on male players has extensively been researched (Ascensão et al., 2008). However, it is essential to separate age groups based on the potential for differences.

When focusing on physiological demands, several different blood markers within the athletes can be very helpful to not only get an overall better understanding of the sports' demands, but also to identify certain norms and tendencies present in these particular athletes, who unfortunately are often subject to iron and haemoglobin deficiencies (Landahl, Adolfsson, Borjesson, Mannheimer, & Rodjer, 2005). Studies have shown that results of iron status in sport are rather contradictory. Ostojic and Ahmetovic (2009) stated that iron status indicators were stabe and poorly related to the training phase during the sports season. Tan, Dawson, and Peeling (2012) found that iron stores and serum levels were significantly decreased after training sessions. In contrast, some studies have indicated that exercise does not affect the haematology variables (Akgün, 1994; Spiropoulos & Trakada, 2003). Changes in haematological characteristics have been linked to aerobic exercise, such as a decrease in haematocrit and increase in haemoglobin values in young and old individuals (El-Sayed, Sale, Jones, & Chester, 2000). Increase in the leukocytes count was reported in young people following acute resistance exercise (Ramel, Wagner, & Elmadfa, 2003).

Recent studies found reduction in physical performance (Ascensão et al., 2008; Ispirlidis et al., 2008; Magalhães et al., 2010; Rampinini, Coutts, Castagna, Sassi, & Impellizzeri, 2007), increase of muscular markers damage (Andersson, Ekblom, & Krustrup, 2008; Ascensão et al., 2008; Ispirlidis et al., 2008; Magalhães et al., 2010), robust pro- and anti-inflammatory cytokine response (Andersson, Karlsen, Blomhoff, Raastad, & Kadi, 2010; Ispirlidis et al., 2008), increased catabolic (Ispirlidis et al., 2008; Kraemer et al., 2004) and pro oxidant state (Ascensão et al., 2008; Magalhães et al., 2010) are described just after a "friendly" soccer match and during recovery after the match. However, there is evidence that physical intensity during a friendly soccer match (absolute values and percentage of maximum heart rate) can lower than during official matches (Rodrigues et al., 2007) which is probably due to motivational matters. Following a soccer match there is considerable muscle damage and oxidative stress as well as a marked rise in antioxidant status markers for up to 48 h to 72 h post exercise (Andersson et al., 2010; Ascensão et al., 2008; Ispirlidis et al., 2008). Haematological parameters are influenced by several factors, of which training, age, sex, ethnicity, nutrition, and altitude (Ostojic & Ahmetovic, 2009; Schumacher, Jankovits, Bültermann, Schmid, & Berg, 2002) are the most common. Most of these factors can have a positive or negative influence on haematocrit (Hct), haemoglobin (Hb), and red blood cell (RBC) count.

Soccer is an endurance-based sport during which athletes require large amounts of oxygen within the different systems of the body in order for them to function. For this, high iron and haemoglobin values would be very beneficial for these athletes and their performance. Consequently, regular monitoring of selected psychological, biochemical and physiological markers can prevent or avoid overreaching and overtraining throughout a training program (Silva, Santhiago, Papoti, & Gobatto, 2008). Therefore, the main aim of the present investigation was to verify the responses of haematological and iron concentrations in Croatian U-21 soccer players following an official soccer match.

Methods

Participants

A group of 19 trained, healthy male soccer players from the U-21 category of the Croatian soccer association took part in this study. The players were informed about the experimental procedures and possible discomforts associated with the study, and a written informed consent was obtained. The study was approved by the Ethics Committee of the Faculty of Kinesiology, University of Zagreb in accordance with the Helsinki Declaration. The participants were aware that they could withdraw from the study at any time. The participants' characteristics were on average: age 20.26 ± 0.65 years, body mass: 71.3 ± 5.9; body height: 1.77 ± 0.07 and maximal oxygen uptake (VO2max) 64.95± 3.99 $ml \cdot kg^{-1} \cdot min^{-1}$. The selection criteria included: (1) participation at professional (top three division leagues) level of soccer competition for at least 5 years, (2) all players participated in at least 75% training sessions per week and played in at least 16 matches during the season, (3) no consumption of exogenous anabolic-androgenic steroids or other drugs that might have affected their physical performance or hormonal balance during the study (for at least 6 months (5) no recent history of febrile illness, muscle lesions, lower limb trauma, and metabolic diseases.

The soccer players were instructed not to change their normal eating habits during the entire period of data collection. Nutritional supplements were not included in their diets. In addition, players were instructed to refrain from drinking beverages containing caffeine or alcohol and from consuming food 3 h before testing.

Blood collection and analysis

Blood samples were assessed pre-match and immediately after a match in response to a competitive (2×45 min) soccer match. On the day of the game, the players arrived at the laboratory after an overnight fast of between 10 and 12 h. A resting blood sample was taken after the participants had been standing for at least 15 min, after which the participants consumed a light standardized meal and drink and rested for 2 h. The meal consisted of 1.7 g white bread and 0.3 g of a low-fat spread; both values are per kilogram of body mass (Thompson et al., 2003). The participants abstained from alcohol and caffeine consumption for at least 24 h, and did not perform any exercise for the last 72 h before testing.

Whole blood was collected in EDTA tubes then divided for biochemical, antioxidant, and oxidative analyses. EDTA blood samples were sent for immediate analysis of red blood cell concentration (RBC), haemoglobin (Hb), haematocrit (Htc), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and Thrombocytes on automatic analyser (Dimension Xpand Plus Analyzer, Siemens, Munich, Germany). The remaining freshly withdrawn blood was immediately centrifuged at 3000 rpm during 10 min for careful removal of the plasma. The plasma was separated into several aliquots and rapidly frozen at −20 °C for later biochemical analysis. Iron, total iron binding capacity (TIBC), the unsaturated iron-binding capacity (UIBC) and ferritin analyses were automatically performed in Dimension analyser (Dimension Xpand Plus Analyzer, Siemens, Munich, Germany).

Statistical analysis

All statistical analyses were performed using the Statistica v.8.0 software (StatSoft Inc., Tulsa, OK, USA). Descriptive statistics and Kolmogorov–Smirnov tests (normality of the distribution) were calculated for all experimental data before inferential testing. Data were expressed as mean values ± standard deviation. Sample t-test was used where significant variations occurred. The level of significance was set at $p<0.05$.

RESULTS

Table 1 Hematological parameters before and after a soccer match. The values are shown as the mean and Standard Deviation.

	Prematch	Postmatch	p
RBC (mill/mm^3)	5.07 ± 0.27	5.04 ± 0.28	0.46
Hemoglobin (g/L)	150.42 ± 9.06	149.95 ± 9.31	0.54
Thrombocytes (×109/L)	207.79 ± 33.14	242.89 ± 41.32	0.89
HCT (%)	0.45 ± 0.03	0.43 ± 0.02	**0.00**
MCV (fL)	89.68 ± 2.55	84.03 ± 2.29	0.25
MCH (pg)	29.70 ± 0.87	29.72 ± 0.88	0.81
MCHC (g/l)	331.16 ± 6.75	334.89 ± 6.51	**0.00**

(RBC) red blood cell, (HCT) haematocrit, (MCV) mean corpuscular volume, (MCH) mean corpuscular haemoglobin, (MCHC) mean corpuscular haemoglobin concentration; significant difference at $p < 0.05$.

A significant decrease in Hct (%) was observed immediately after the game ($p<0.05$). As shown in Table 1, only MCHC increased significantly ($p < 0.05$) at the end of the soccer match. There were no significant changes in the haemoglobin, red blood cells, thrombocytes, mean corpuscular volume and mean corpuscular haemoglobin following a soccer match.

Table 2 Iron status and ferritin before and after soccer match. The values are shown as the mean and standard deviation.

	Prematch	Postmatch	p
Iron (μmol/L)	19.42 ± 7.14	25.30 ± 6.66	**0.00**
UIBC (μmol/L)	39.96 ± 7.65	38.50 ± 7.70	0.13
TIBC (μmol/l)	57.8 ± 8.3	55.94 ± 6.8	0.24
Feritin (μg/L)	40.63 ± 18.74	49.01 ± 24.34	**0.00**

(TIBC) total iron binding capacity, (UIBC) the unsaturated iron-binding capacity; significant difference at $p < 0.05$.

Iron concentration (μmol/L, Table 2) increased significantly ($p<0.01$) after a soccer match. Ferritin levels were also higher at the end of the match compared to baseline measures (Table 2). However, TIBC ($p=0.24$) and UIBC ($p=0.13$) remained unchanged immediately after a soccer match.

DISCUSSION

Given the fact that soccer match effort implies several acute physiological changes, we tested the hypothesis that an official soccer match induces changes in haematological and iron concentrations in Croatian U-21 soccer players. We found that serum iron concentration demonstrates a significant increase in response to a soccer game. In addition, a significant decrease in Haematocrit values was observed immediately after the soccer match. UIBC and TIBC showed similar values pre to post match testing ($p>0.05$).

Several studies have reported that the decrease in haemoglobin and haematocrit is a sign of physical exertion and heavy participation (Fallon, 2008; Malcovati, Pascutto, & Cazzola, 2003; Rietjens, Kuipers, Hartgens, & Keizer, 2002). Also, Al'Hazzaa, Almuzaini, Al-Refaee, and Sulaiman (2001) stated that decrease in haemoglobin and haematocrit is evident in both endurance oriented sports and in sports that require both aerobic and anaerobic contributions to the energy supply for muscle contraction. We observed that haemoglobin, haematocrit and MCHC diminished in the soccer players after a soccer match. However, only haematocrit and MCHC values showed significant decrease. Despite the decrease in Hb and Hct, we did not find any decrease in the red blood cell count. Accordingly, other studies showed a decrease in Hb and Hct and no changes in RBC (Ostojic & Ahmetovic, 2009; Robach et al., 2014).

Iron, TIBC, and ferritin levels are traditional biomarkers for screening athletes during the training season (Fallon, 2008). We have found a significant increase in Iron and Ferritin values following a 90 min soccer match. Similarly, it was previously reported that eccentric type of exercise induces higher levels of Iron in post compared to pre training measurement (Childs, Jacobs, Kaminski, Halliwell, & Leeuwenburgh, 2001). Iron deficiency may have a negative impact on oxygen transport and immune defense, thus influencing athletic performance (Peeling, Dawson, Goodman, Landers, & Trinder, 2008). Although several studies (Mercer & Densmore, 2005; Ostojic & Ahmetovic, 2009; Resina et al., 1991) have reported a range of 11 to 15% of the players suffering from anemia over a season, none of the players in the present study was iron deficient ($>13.0\ g \bullet dl^{-1}$). This finding suggests that the players were subjected to an appropriate level of training intensity and duration. However, a recent study showed that prolonged intense running may reduce iron levels and increase Ferritin values, as a response to a rise in inflammatory and muscle damage markers (Kłapcińska et al., 2013). Discrepancies in the results may be attributed to the fact that football match-play may represent a lower inflammatory stimulus than long-distance running. Possible increased levels of serum ferritin might be an adaptive response to increased oxidative stress mediated by iron, because ferritin sequesters iron in blood or in cells and attenuates its pro-oxidant activity (Meneghini, 1997; Orino et al., 2001).

CONCLUSION

We can report that a soccer match may induce an iron increase immediately after the game. Ferritin and Haematocrit are also affected by a soccer game. During the competitive season, soccer players are exposed to a variety of physical and psychological stresses from practice, conditioning, and competition. It is essential for players to let their bodies recover from previous practice sessions and games. In summary, this study provided a valuable opportunity to study biochemical parameters that could lead to possible illness, injury and severity in match-play. Accordingly, it is also essential to educate the athletes about the importance of iron for their performance and to make sure they incorporate iron rich foods in their diet to help prevent too low iron stores and haemoglobin presence.

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HEMATOLOŠKI PROFIL I STATUS GVOŽĐA NAKON FUDBALSKOG MEČA

Cilj ovog istraživanja je bio da se proveri hematološki odgovor i koncentracija gvožđa kod hrvatskih U-21 fudbalera nakon zvanične fudbalske utakmice. Grupa od 19 obučenih, zdravih muškaraca fudbalera iz selekcije U-21 Hrvatskog fudbalskog saveza je učestvovalo u ovoj studiji. Analiza koncentracije crvenih krvnih zrnaca (RBC), hemoglobin (Hb), hematokrit (Htc), srednja vrednost zapremine eritrocita (MCV), prosečna količina hemoglobina u eritrocitu (MCH), srednja koncentracija hemoglobina u eritrocitima (MCHC), i trombocita je izvedena u cilju otkrivanja hematološkog statusa. Pored toga, gvožđe, ukupni kapacitet vezivanja gvožđa u serumu (TIBC), nezasićeni kapacitet vezivanja gvožđa (UIBC) i analiza feritina dobijeni su odmah posle fudbalske utakmice. Značajan pad Hct (%) uočen je odmah posle utakmice. Međutim, nije bilo značajnih promena u nivou hemoglobina. Koncentracija gvožđa značajno je povećana (p <0,01) posle fudbalske utakmice. Nivoi Feritina su takođe veći na kraju utakmice u odnosu na polazne mere. Može se zaključiti da je fudbalski meč izazvao povećanje gvožđa odmah nakon utakmice što je u suprotnosti sa nedavnim studijama. Ukratko, ova studija pruža dragocenu priliku za izučavanje biohemijskih parametara koji bi mogli dovesti do nekih bolesti, ili povreda tokom i nakon igre.

Ključne reči: *biohemijske analize, fudbal, profesionalni igrači, zamor*