HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN ELITE SOCCER PLAYERS DURING A COMPETITIVE HALF SEASON

HEMATOLOŠKI I BIOHEMIJSKI PARAMETRI KOD ELITNIH FUDBALERA TOKOM POLOVINE SEZONE

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Summary

Background: The purpose of the present study was to report and discuss the hematological and biochemical behavior of elite soccer players, in order to get more insight in the physiological characteristics of these sportsmen and to provide trainers and sports doctors with useful indicators.
Methods: Nineteen male soccer players volunteered to participate in this study. We followed the young elite soccer players during a competitive half season. Venous blood samples were collected between 9:00 and 10:00 a.m. after an overnight fast (10 h) at baseline, after 45 and 90 days and hematological and biochemical parameters were measured.
Results: Hemoglobin and hematocrit levels were significantly reduced over the observational period (p<0.05), but erythrocyte count and iron levels remained unchanged. Bilirubin and ferritin levels significantly increased in response to regular soccer training (p<0.05). We observed a significant decrease in muscle enzyme plasma activity during the 90 days study period. ANOVA analysis revealed a significant increase in the leukocyte and neutrophil counts (p<0.05), in parallel with a significant decrease in the lymphocyte count (p<0.05) after the observational period of 90 days.
Conclusions: Elite soccer players are characterized by significant changes in biochemical and hematological param-

Kratak sadržaj

Uvod: Cilj ove studije je bio da se odrede i analiziraju hematološki i biohemijski parametri kod elitnih fudbalera, kako bi se dobio bolji uvid u fiziološke karakteristike ovih sportista i pružile korisne informacije trenerima i doktorima sportske medicine.
Metode: Devetnaest fudbalera je dobrovoljno učestvovalo u ovoj studiji. Mlade elitne fudbalere smo pratili tokom druge polovine sezone. Uzorci venske krvi su sakupljeni natašte, između 9.00 i 10.00 časova, na početku studije i nakon 45 i 90 dana redovnih treninga, i određeni su osnovni biohemijski i hematološki parametri.
Rezultati: Nivo hemoglobina i hematokrit se značajno smanjuju u toku posmatranog perioda (p<0,05), dok su broj eritrocita i koncentracija serumskog gvožđa ostali nepromenjeni. Nivo bilirubina i feritina se značajno povećava kao posledica redovnih treninga (p<0,05). Zapazili smo značajno smanjenje nivoa mišićnih enzima u serumu tokom 90 dana. ANOVA je otkrila značajno povećanje broja leukocita i neutrofila (p<0,05), kao i značajno smanjenje broja limfocita (p<0,05) posle 90 dana redovnih treninga.
Zaključak: Elitni mladi fudbaleri pokazuju značajne promene u vrednostima biohemijskih i hematoloških parametara tokom druge polovine sezone, koje su posledica treninga, ali i adaptacije na fizičku aktivnost. Iako su vred-

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eters over the half season, which are linked to training workload, as well as adaptation induced by the soccer training. Although the values of the measured parameters fell within the reference range, regular monitoring of the biochemical and hematological parameters is fundamental for the identification of a healthy status and related optimal performances by sport doctors and trainers and selection of a correct workload by trainers.

Keywords: soccer, hematology, biochemistry

nosti ovih parametara bile u granicama referentnih vrednosti, praćenje biohemijskih i hematoloških parametara je osnova za procenu zdravstvenog statusa sportista i optimalnih sportskih performansi, ali i korisno sredstvo za određivanje optimalnog intenziteta i učestalosti treninga.

Ključne reči: fudbal, hematologija, biohemija

Introduction

Soccer is a multiple-sprint sport that requires high-intensity, intermittent activity to be undertaken over an extended period of time. In addition to intensive daily training sessions, players are involved in additional commitments such as national cups and other matches. These competitive demands may impose strains to various physiological systems, including the musculoskeletal, nervous, immune and metabolic, which might be reflected in changes in the biochemical and hematological parameters (1). Despite its diffusion and popularity, very few studies have been performed concerning the biochemistry and hematology of soccer players. Surprisingly, hematological parameters and biochemical characteristics which can be crucial for predicting optimal physical performance have been scarcely examined in elite soccer players, who are involved in very demanding competitive seasons. We followed the young elite soccer players during a competitive half season. In the present study, we report and discuss the hematological and biochemical behavior of the athletes, in order to get more insight in the physiological characteristics of these sportsmen and to provide trainers and sports doctors with useful indicators.

Materials and Methods

Subjects

Nineteen male soccer players, members of the young selection of the soccer club »Partizan«, Belgrade, Serbia, volunteered to participate in this study. One month prior to entering the study and during the study, the participants were instructed to abstain from vitamin and mineral supplementation, ergogenic aid or any medications in general. Subjects were also advised not to make any drastic changes in their diet during the study period. The physicians of the outpatient clinic »Vita Maxima«, Belgrade, Serbia evaluated the physical performance of all participants, and sport injury rates and incidence were recorded.

The study was undertaken in compliance with the Helsinki Declaration and approved by the Ethical Committee of the Sports Medicine Association of Serbia. The soccer players and parents gave written informed consent after having been explained the procedures, benefits and possible risks of participation in the study.

Baseline measurements and screening for aerobic capacity

Subjects reported to the laboratory and their height, weight and total body fat were measured using a portable stadiometer and body composition analyzer (BC-418, Tanita, Japan), respectively. Then the subjects completed a maximal oxygen consumption test (VO_2max). VO_2max was measured on a motor-driven treadmill (Run race, Techno gym, Italy), using an indirect calorimetry system (Quark b2, Cosmed, Italy) with an incremental exercise test to volitional fatigue. To estimate average energy and nutritional intake, soccer players recorded their dietary intake during 4 consecutive days. The energy, macronutrient and micronutrient intakes were calculated using CRON-O-Meter v0.9.6. software.

Study design

We followed the young elite soccer players during a competitive half season, over a three-month period (March, April and May). During the study, the soccer players were engaged in a controlled training programme, that consisted of 7–8 training sessions per week (~720 min), comprised of exercise on the soccer field (5–6 times a week, ~600 min) and weight training (2 times a week, 120 min) sessions, in addition to a weekly match. Over this period, the training and preparatory programme took place at the sport centre »Partizan-Teleoptik« in Belgrade. Venous blood samples were collected between 9:00 and 10:00 a.m. after an overnight fast (10 h) at baseline, after 45 and 90 days.

Blood collection and analysis

All blood samples were collected into heparin vacutainer tubes, K-EDTA treated vacutainer tubes and nonadditive serum vacutainer tubes (Greiner Bio-one, Kremsmünster, Austria). Plasma and serum were separated by centrifugation and multiple aliquots of each sample were stored at –80 °C until analysis.

The hematological parameters [leukocytes (WBC), erythrocytes (RBC), hemoglobin (Hb), hematocrit (Ht), platelets (PLT), mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin corpuscular (MCHC), lymphocytes, neu-

trophils, basophiles, eosinophils, monocytes] were obtained from CellDyn 3700 (Abbott, Chicago, IL, USA). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), uric acid, creatinine (Cre), billirubin, glucose, total proteins, urea, hs-CRP, along with iron, ferritin and transferrin were measured using an ILab 300 Plus autoanalyser employing reagents purchased from Biosystems S.A. (Barcelona, Spain) and Bioanalytica (Belgrade, Serbia). Percentage changes in plasma volume during the study period were assesed by the method described by Dill and Costill (2).

Statistical analysis

Data were inspected for normal distribution using the Kolmogrov–Smirnov test. Results are expressed as mean ± SEM for normally distributed variables. When the distribution differed from normal, geometric means and 95% confidence intervals were given. Hematological and biochemical parameters were analyzed using the ANOVA repeated-measures general linear model. Non-normally distributed data were transformed logarithmically, before statistical comparisons were made. Spearman's non-parametric correlation analysis was used to assess univariate associations between studied variables. P values ≤ 0.05 were considered statistically significant. Statistical analyses were performed using the PASW Statistics version 18.0 and MedCalc (version 11.4 Software, Belgium) software.

Results

Table I presents the anthropometric characteristics of the athletes and the data obtained in the maximal oxygen consumption test.

The mean daily energy intakes, as well as the mean daily intake of protein, fat, carbohydrates, vitamins and minerals, are presented in *Table II*.

Markers of iron metabolism are shown in *Table III*. Hemoglobin, hematocrit levels, MCV and transferrin were significantly reduced over the observational period ($p=0.045$, $p=0.040$, $p=0.036$, $p=0.025$, respectively). RBC count, platelet counts, MCH, MCHC and iron levels remained unchanged, while ferritin levels significantly increased throughout the study ($p=0.048$). All the values of measured parameters fell within the reference range. Plasma volume increased by 9% after 45 days of regular training and then decreased by 1.5% by the end of the observational period.

We observed significant decrease in AST, CK and LDH plasma activity during the 90 days study period ($p=0.038$, $p=0.049$ and $p=0.003$ respectively), in parallel with the decrease in uric acid, Cre

and total proteins levels ($p=0.009$, $p=0.024$ and $p=0.012$, respectively). Bilirubin levels significantly increased in response to regular soccer training ($p=0.028$). We observed an increase in hs-CRP after the 90 days of regular soccer training, although marginally significant ($p=0.08$). No significant changes were found regarding ALT, glucose and urea levels over the study period. CK, LDH and Cre levels were above normal values during the entire observational period, while the other biochemical parameters were within the reference range.

Table I Anthropometric characteristics of the soccer players.

Age (years)	17.62±0.14
Weight (kg)	72.3±1.8
Height (cm)	180.2±1.4
Body mass index (kg/m^2)	22.24±0.41
Fat (%)	9.72±0.81
VO$_{2max}$ (mL/min/kg)	52.9±0.7
Training experience (years)	9.88±0.21

Values are expressed as mean ± SEM.

Table II Estimated daily energy and nutrient intake of the soccer players.

Energy (kcal)	2932±147
Protein (g)	125±6.3
Carbohydrates (g)	366±23
Monosaccharides (g)	123±18
Fiber (g)	12.6±1.5
Fat (g)	101±7
Saturated fat (g)	29.9±3.2
Cholesterol (mg)	344±38
Vitamin A (IU)	2120±389
Vitamin C (mg)	135±30
Vitamin E (mg)	6.1±1.5
Copper (mg)	1.92±0.5
Iron (mg)	15.1±1.6
Manganese (mg)	4.0±0.9
Selenium (µg)	164±8
Zinc (mg)	13.5±1.8

Values are expressed as mean ± SEM.

Table III Mean values of the hematological parameters and plasma volume changes in elite soccer players at baseline, after 45 and after 90 days of regular soccer training over the competitive half season.

Parameter	Baseline	After 45 days	After 90 days
Hb (g/L)	157±3.9	151±2.0*	152±2.1
Hct (L/L)	0.482±0.010	0.457±0.006**	0.462±0.005
RBC (×10^{12}/L)	5.4±0.10	5.2±0.07	5.3±0.05
MCV (fL)	88.64±0.89	87.58±0.40	86.81±0.73*
MCH (pg)	28.9±0.31	28.9±0.33	28.5±0.31
MCHC (g/L)	326±2.3	330±3.0	329±2.4
PLT (×10^9/L)	253±12.7	247±10.6	249±9.5
Iron (μmol/L)	10.8±0.70	9.6±0.92	10.3±0.84
Ferritin (μg/L) "	49(32.1–77.8)	63(49.6–80.7)	69(51.7–91.8)*
Transferrin (g/L)	2.87±0.09	2.66±0.08*	2.72±0.08
Plasma volume changes (%)		+9%	−1.5%

'Mean±SEM: "Geometric mean values (95th confidence interval).

The difference in relation to baseline was significant at p<0.05 (*) and p<0.01(**).

Table IV Biochemical profile of the soccer players at baseline, after 45 and after 90 days of regular soccer training over the competitive half season.

Parameter	Baseline	After 45 days	After 90 days
ALT (U/L)"	20(18.6–22.5)	18(16.7–20.1)	19(16.1–22.6)
AST (U/L)"	41(35.7–47.0)	32.2(28.6–36.4)*	28.7(24.5–33.6)*
CK (U/L)"	421(204–870)	477(348–654)	342(215–544)#
LDH (U/L)	441±17.5	395±10.5	359±15.8**#
Uric acid (μmol/L)	355±16.6	368±23.5	314±13.7* #
Creatinine (μmol/L)	133±3	135±3	131±3#
Bilirubin (μmol/L)	12.6±1.80	16.8±1.89*	19.6±2.18*
Glucose (mmol/L)	6.1±0.17	6.0±1.9	5.8±0.13
Total proteins (g/L)	77±0.9	75±1.6	73±1.2*#
Urea (mmol/L)	6.6±0.36	6.5±0.38	7.0±0.41
hs-CRP (mg/L)"	1.26(0.89–1.78)	1.58(0.81–2.3)	1.98(1.24–3.17)

'Mean±SE: "Geometric mean values (95th confidence interval).

The difference in relation to baseline was significant at p<0.05 (*) and p<0.01(**). The difference in relation to day 45 was significant at p<0.05 (#).

Table V Total blood leukocyte count, lymphocyte, neutrophil, basophil, eosinophil and monocyte counts in soccer players at baseline, after 45 and after 90 days of regular soccer training.

Parameter	Baseline	After 45 days	After 90 days
WBC (x10^9/L)	6.0±0.2	5.7±0.2	6.7±0.4#
Lymphocytes (%)	39.7±2.1	38.3±1.8	33.9±2.5*
Neutrophils (%)	49.0±2.2	50.1±1.9	55.1±2.1*
Basophils (%)	1.2±0.2	1.1±0.1	1.0±0.15
Eosinophils (%)	2.1±0.2	2.1±0.3	1.9±0.3
Monocytes (%)	8.0±0.6	8.4±0.5	8.1±0.5

Values are expressed as mean ± SEM.

The difference in relation to baseline was significant at p<0.05 (*). The difference in relation to day 45 was significant at p<0.05 (#).

ANOVA analysis revealed a significant increase in the WBC (p=0.05) count in parallel with a significant decrease in the lymphocyte count (p=0.027) and increase in the neutrophil count after 90 days of observation compared to baseline values (p=0.045). There were no significant changes in the basophil, neutrophil or monocyte counts. Total and differential blood leukocyte counts were in the reference range.

BMI was positively correlated with Cre levels at the beginning of the study (r=0.460, p<0.01). We found positive correlation between bilirubin and ferritin levels at baseline and after 90 days of regular soccer training (r=0.531, p<0.05; r=0.571, p<0.05, respectively).

Discussion

The physiologic stress induced by prolonged and intensive physical activity is reflected in transient yet significant changes in biochemical and hematological parameters. Regular monitoring of these parameters in elite athletes during a competitive season might be useful for detecting possible iron deficiency, anemia or other health problems, in addition to exercise planning and training programming.

The stability of hematologic status indices is one of the key determinants of optimal exercise performance, particularly in endurance sports such as soccer. Several investigators suggested that the value of hematologic indices and iron status measures in elite athletes varies during the season as a consequence of different training regimes (3–5). It was shown that hematological parameters were higher at the beginning of the competition season and then declined in well-trained athletes (6). Although low values of hematological variables have been observed in athletes during intensive training periods compared with clinical norms (7), other investigations have verified normal concentrations of hematological indices throughout training programmes (5, 8). We observed that hemoglobin, hematocrit, MCV and transferrin diminished significantly in the soccer players during the first 45 days of regular training. The decrease in Hb and Ht during a competitive season might be an indicator of heavy effort, as showed in aerobic heavy sports such as cycling and triathlon (9, 10), endurance and ultra endurance events (11), but also in football (6). However, despite the decrease in Hb and Hct, we did not detect any decrease in the red blood cell count. Accordingly, other studies showed a decrease in Hb and Hct and no changes in RBC or total red blood cell volume (12, 13). Generally, training induces hemodilution due to plasma volume expansion, which is a compensatory mechanism that increases cardiac output and reduces blood viscosity, thereby optimizing microcirculation and improving oxygen delivery to the working muscle (14). In fact, we observed a plasma volume expansion from baseline to day 45 (*Table III*), which reinforces the premise that the decrement in Hb and Hct during competitive half season occurs because of the plasma volume expansion.

Bilirubin is the end-product of hemoglobin catabolism; therefore, its increased concentration in serum probably indicates a certain degree of hemolysis, owing to mechanical trauma, but also oxidative injuries of the erythrocytes (15, 16). In the present study, bilirubin levels in soccer players increased significantly over the 3 months of regular training. However, we did not detect any impairment at the level of the circulating erythrocyte pool. In addition, we could observe neither an increase in serum iron levels potentially reflecting hemolysis, nor an increase in transferrin levels possibly showing accelerated erythropoiesis secondary to hemolysis. On the other hand, it may be that increased myoglobin levels, due to muscle damage, and subsequent myoglobin degradation, also contribute to the increased bilirubin production.

Ferritin values presented a trend different from that observed in a wide sample of elite football players, in which the parameter peaked at the middle of the season (6). Other studies showed decrease in ferritin during the season in runners and swimmers (17), as well as in subjects performing a 12-week period of heavy training (18). The results of the present study are similar to those reported in the study of Banfi et al. (5), with a significant increase in ferritin levels at the end of the observational period. In addition, we observed a positive correlation between ferritin and bilirubin levels in soccer players. Increased levels of bilirubin detected in the present study could suggest hemolysis and release of free iron in the blood. Iron is essential for normal cell growth and proliferation. However, excess iron might be potentially harmful, as it can catalyze the formation of toxic reactive oxygen species via Fenton chemistry. Therefore, the increased levels of serum ferritin might be an adaptive response to increased oxidative stress mediated by iron, because ferritin sequester iron in blood or in cells and attenuate its pro-oxidant activity. The results of our study are in accordance with several results supporting a role of ferritin in the protection against oxygen free radical-mediated damage (19, 20).

The kind of sport and the related different anthropometric characteristics of athletes might affect Cre levels. Previous studies reported lower serum Cre in cyclists, Nordic skiers and swimmers (21, 22) and higher serum Cre levels in soccer and rugby players than those observed in controls (23). Values of serum Cre in young soccer players exceeded the reference intervals for the general population (62–115 μmol/L) over the entire observational period. This difference may be linked to the generally higher muscle mass in athletes, because total muscle mass is the most important determinant of the creatine pool size and

creatinine production (24). As previously demonstrated, we found significant correlation between the Cre concentration and BMI (21, 25). Serum Cre levels in soccer players decreased significantly throughout the study, which might be associated with the increase in training and competition workloads during competitive season. Similar results were obtained in other sport disciplines, such as alpine skiing and rugby (25).

During high intensity exercise, the purine nucleotide system is extremely active and the elimination of adenosine monophosphate (AMP) causes a buildup of hypoxanthine, which is converted to UA (26). The involvement of purine nucleotide metabolism in soccer has been reported, demonstrating a marked ATP reduction and increased blood UA following soccer activity (27). Regular soccer training induced decrease in plasma UA in soccer players, which might be related to lower release of hypoxanthine from the muscle. Enhanced capacity of muscles to regenerate ATP might be one of the reasons for this (28). Therefore, diminished accumulation of UA might be a part of the adaptive response induced by the soccer training programme.

Soccer is aerobic-anaerobic sport and involves eccentric muscle contractions resulting in muscle cell breakdown and leakage of cell content (29, 30). We observed increased plasma muscle enzymes in soccer players above normal values during the entire competitive season, which might indicate extensive cell damage and an impaired functional status of the muscle tissue. Exercise-induced trauma to the musculature has been shown to lead to an inflammatory response and migration of phagocytic cells into the affected area. We noticed increased neutrophil counts over the observational period, which was accompanied with increase in hs-CRP levels. Such changes implicate that regular soccer training might be associated with minor inflammatory events. Increase in CRP, if observed chronically, is associated with increased risk of coronary vascular disease (31). On the other hand, repair of muscle injury is dependent on inflammatory mediators and it is not clear whether this inflammatory response needs to be curbed for better recovery and improved function. Despite the observed increase in neutrophil count and hsCRP levels, the magnitude of muscle damage in soccer players was reduced significantly over the observational period, as a result of adaptation and conditioning of the muscle through regular training and was not compromised by proinflammatory changes (32).

It is widely accepted that acute and chronic exercise alters the number and function of circulating cells of the innate and acquired immune system (33). In the present study, we detected certain changes: an increase in leukocytes and neutrophil number, hs-CRP, along with decrease in lymphocytes number. We did not find pathological trends in the studied athletes and the fluctuations of the number of total leukocytes and subpopulations seemed to be physiological.

Our study showed that elite soccer players are characterized by physiological values of the biochemical, hematological parameters and the parameters of iron metabolism over the half season. In spite of significant decrease in Hb and Hct levels in soccer players over the competitive half season, erythrocyte pool was found to be well preserved. In addition, the results of our study do not imply significant hemolysis in soccer players. It is therefore proposed that the exercise induced decrease in Hb and Hct is entirely due to plasma volume expansion, without any contribution of red blood cell loss. Also, the observed inflammation might contribute to plasma volume expansion (13). The variability of the parameters during the half season is linked to training and competition workload, as well as the adaptation induced by soccer training. The follow up of the athletes by means of biochemical and hematological parameters is fundamental for sport doctors and trainers to identify a healthy status and the related optimal performances and for trainers to select a correct workload.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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