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ORIGINAL STUDIES

Influence of antioxidants supplementation on creatine kinase (CK) level and modifications of hematological and biochemical parameters in elite soccer players

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Abstract

Background. In elite soccer, intense physical effort during competitive periods often leads to physiological stress, characterized by fluctuations in creatine kinase (CK) levels and alterations in hematological and biochemical parameters, highlighting the need for effective recovery strategies such as antioxidant supplementation.

Aims. The aim of this retrospective clinical research is to explore how individualized antioxidant administration impacts CK optimization and induces changes in hematological and biochemical parameters in elite soccer players across two competitive periods.

Methods. The participants in the study were 30 elite athletes aged 19 to 34 years from a soccer team in League Two. There were measured 14 hematological and 6 biochemical parameters during two competitive periods in C2 (pre-competitive period of training 1) and C5 (pre-competitive period of training 2) stages. An individualized treatment with antioxidant substances was administered throughout both periods for the regulation of CK concentration.

Results. The main findings highlight: (1) the influence of the training effort on the interval of normal values for blood count parameters across two competitive periods in elite soccer players, with interval size showing increases of 88.49% and decreases of 11.51%. Percentage modifications average 56.13% in stage C2 and 58.57% in stage C5 while interval weight ranges from 16.04% to 72.52% in stage C2 and 13.43% to 73.67% in stage C5; (2) improvements in hematological parameters through an 88.49% increase and 11.51% decrease in normal values; (3) improvements in biochemical parameters with a 10.44% increase and 89.56% decrease in normal values; (4) significant correlations (6.55%) between hematological and biochemical parameters, with 9.52% in stage C2 and 3.57% in stage C5; (5) a 64.71% reduction in CK concentration within normal limits through antioxidant administration.

Conclusions. This study proved the influence of the training effort on the changes in the hematological and biochemical parameters of the elite soccer players over two competitive periods. The individualized administration of antioxidants depending on CK concentration contributed to maintaining and improving the sports results.

Keywords: antioxidant supplementation, CK, hematological parameters, biochemical parameters, elite soccer players.

Introduction

Performance in most sports is determined by the technical, tactical, physiological and psychological/social characteristics of the athletes (Bangsbo, 2015). Most athletes rest before competitions or even before the training sessions; they manifest an increased functional level of the main systems of the body (cardiovascular, respiratory, nervous, endocrine system etc.). This state of the body is called the*"starting state"*, specific to the precompetitive period in which all functional parameters have high values. The training state and the personal experience of the athletes influence the physiological changes of the starting

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state (Nikulin & Rodionova, 2011; Anderson, 2017). Each time players are exposed to heavy workouts; their bodies try to adjust in order to regain the balance. However, when the players are in a state of physical demand, such as constant high intensity training and a program of difficult games, it is often possible that their ability to adjust and restore the balance state is overwhelmed. Professional athletes, for example the soccer players, undergo an annual training and competition routine, with recovery periods that are not always correctly adjusted to their workload. The main problem is that, in general, the current program of the championship does not allow the teams to take the minimum time required for proper physical training, because the different stages (competitions and physical, technical and tactical training sessions) overlap. As a result, athletes may experience an imbalance between the exerted effort and the recovery time during the competitive season, which increases the likelihood of excessive workload for some players. The failure to recover and regenerate leads to fatigue. If a high fatigue is maintained for a long time, it will result in overtraining (Balint, 2008).

In order to scientifically conduct the training, an efficient monitoring is required. This one consists of a process of objective and timely evaluation of the athlete's biological, biomechanical and psychological response to the stimuli used in training and competition. The biomechanical monitoring of the training will be made through a complex of means of effective effort management with the help of the information provided by the biochemical analyses (Talaban, 2006; Brancaccio et al., 2008; Nikulin, Rodionova, 2011). The knowledge of the biochemical investigations that take place during the physical effort is highly important in the process of scientific practice of physical education and sport (Rinderu, Ilinca, 2005). Any physical effort is accompanied by changes in the body metabolic processes and by the occurrence of biochemical changes in muscles (Brancaccio et al. 2010), internal organs and blood (Volkov et al., 2000; Mohan et al. 2001; Adami et al., 2010). All the biochemical changes taking place during the physical effort are based on the modification of the metabolism direction (Gleeson, 2002; Brancaccio et al. 2008; Brancaccio, 2010; Banfi et al., 2012). The mineral ions metabolism plays an important role in body activity. It participates in the regulation of the acid-basic balance (Busa, Nuccitelli, 1984; Stringer et al., 1992; Poole et al., 2015); it intervenes in the contraction of muscles and in the activity of enzymes; it influences the neuromuscular excitability and permeability (Vâjială, 2002; Westerblad, et al., 2002 Robergs et al., 2004). During exercise, the body increases the speed of the catabolic processes accompanied by the energy release and ATP synthesis, with simultaneous reduction of anabolism rate and consumption of a significant amount of ATP for ensuring various syntheses (Mihajlov, 2004; Pop et al. 2009; Nikulin, Rodionova, 2011; Isaev, 2014).

In the present-time performance training, the fastest recovery possible of the effort capacity before making the next effort is of major importance. Among the methods used to increase the efficiency of the training, the pharmacological means have an important contribution. Their main task is the directed regulation of the metabolic processes development which is modified most during the maximum efforts period (Vâjială, 2002). The biological functions of the vitamins and minerals were studied and the biochemical measures specific to the micronutrients nutritional status were identified (Lukaski, 2004). Finally, because the liquids consumed during exercise can also be a source of other ingredients (for example, carbohydrates, electrolytes or caffeine) or characteristics (e.g., temperature), they can increase the performance (Garth, Burke, 2013).

The longitudinal evaluation of some blood tests can reveal markers of previously modified situations, which could prevent the amplification of the response before performance is diminished. A large range of studies using different types of exercising protocols have already demonstrated that biomarkers like creatine kinase, urea, creatinine, uric acid and hematologic parameters undergo a certain modulation through exercise (Sawka et al. 2000; Pattwell et al. 2004; Lac, Maso, 2004; Finaud et al. 2006; Peake et al. 2007; Lippi et al. 2008; Lazarim et al. 2009). However, there is no current consensus on the applicability of these biomarkers as markers of the training effects that contribute efficiently to achieving and maintaining better performances. We intended to present in this study the absolute and individual changes in the values of the hematological and biochemical parameters compared to the reference interval. The aim was to assess the training level and the effects that the training/competition and also the treatment with antioxidants had on the elite soccer players.

Hypothesis

The hypothesis of the study is that individualized antioxidants administration, based on training-induced changes in creatine kinase (CK) levels, will optimize CK concentration, stabilize hematological and biochemical parameters and mitigate deviations from the reference intervals during two competitive periods in elite soccer players.

The study aims to:

1) Analyze the intervals of measured values, differences in interval size, percentage changes and the relative weight of measured interval values (%) during two competitive periods in elite soccer players.

2) Examine the impact of training effort on normal values and modified values outside the reference interval, both below and above the ranges of hematological and biochemical parameters.

3) Investigate the regulation of CK concentration through the individualized administration of antioxidant substances.

Materials and methods

Research protocol

a) Period and place of the research

The retrospective clinical study was conducted in accordance with the Romanian soccer national competition calendar and based on information available on the official website of the *Academica Clinceni* Club in Bucharest.

The study was conducted from January 14 to July 30, 2019 and consisted of 6 stages (C): C1– 6 training weeks (4 players were transferred), C2 (n=16) – 14 weeks of

competitive training 1, second part of the League Two Championship; C3 –3 training weeks; C4 – 10 days of camp training, Bulgaria; C5 (n=26) – 10 training days (6 transfers of players), C6 –2 weeks of competitive training 2 in League One. The small number of players (only 12) who participated in the study from the beginning to the end is explained by the fact that some players were transferred from other sports clubs (and their medical records were kept in different medical centers), while other players did not attend the C5 stage.

The study aimed to highlight the influence of effort physiological parameters and hematological and biochemical markers on the elite soccer players before two competitive periods. Dividing the training into parts corresponding to the official game is one of the most real possibilities of modelling the effort under the conditions of competitive games. A number of 28 games (official and friendlies) were played during the study, with 5.54±1.84 days of pause/recovery between games (mean ± SD), 5 training sessions / week, duration of session 90-120 minutes.

b) Subjects and groups

The study involved 30 elite athletes aged 19 to 34 years (mean ± SD, 24.37 ± 4.38 years), members of the soccer team of *Academica Clinceni* Club, Romania. The sports performances of the team were: the 2nd place in the Ranking of the Romanian League Two in the competition season 2018-2019 with 38 games played (28 victories, 3 draws and 7 defeats), 77 goals scored and 24 received and a total score of 87 points. In May the team was promoted to League One.

Parental consent was obtained and signed in accordance with the Helsinki Declaration before starting the research. The study received approval of the Scientific Research Ethics Committee of the University of Pitești (Nr: 3915/05.04.2022) (currently National University of Science and Technology *Politehnica* Bucharest, University Center of Pitești, Romania) and confidentiality and data protection belong to ”Chiajna Medical Center”.

c) Applied tests (Used instruments)

The medical-sports evaluation of the soccer players was carried out within the ”Chiajna Medical Center”. The blood samples were collected under standardized conditions: 2.0 mL of total venous blood was collected in vacuum tubes containing EDTA/K3 to determine the hematological parameters, and 8.0 mL of venous blood was collected in tubes with a VACUETTE (Greiner Bio-one) gel separator to obtain serum for the biochemical measurements. The blood samples were collected in the morning, after 12 hours of fasting, in sitting position, and they were transported at 4ºC, within 30 minutes, to the laboratory of the Medical Bio-Medica International SRL Centre (according to a collaboration agreement). Samples were centrifuged under refrigeration at 1.800 x g for 10 minutes, they were immediately separated and protected from light. According to the Romanian competition regulations, the medical visa of athletes has a validity of 6 months. In this respect, investigations were carried out during the stage C2 – pre-competitive period of training 1 and stage C5–pre-competitive period of training 2. In order to reduce, as much as possible, the analytical variations, all samples were tested by the same technicians, without changing the batches of reagent, standards or control materials.

The Mythic22AL blood count (BC) was performed using the automatic method (CBC with differential) from venous blood collected on the EDTA/K3 anticoagulant. The following parameters were selected out of the 22 parameters of the complete CBC: RBC (109/L), Hb (g/dL), WBC (10^9/L), Neutrophils (10^9/L), Lymphocytes (10^9/L), Monocytes (10^9/L), Eosinophils (10^9/L), Basophils (10^9/L), Neutrophils (%), Lymphocytes (%), Monocytes (%), Eosinophils (%), Basophils (%).

Erythrocyte sedimentation rate (ESR) was determined by means of the Westergren (mm/1h) method. The ESR is the rate at which sediment the erythrocytes from an anticoagulant blood sample within one hour. The faster the erythrocytes are sedimenting, the higher is the ESR as this one is an indicator of acute phase response. An increase of ESR occurs at least 24 hours after the initiation of the inflammatory response; after the completion of the acute phase response, it decreases with a half-life time of 96-144 hours.

Clinical chemistry examination (Dimension EXL, spectrophotometry) of serum sample: Glucose (mg/dL), ALT (U/L), Calcium (mg/dL), Magnesium (mg/dL), Iron (mg/dL) and creatine kinase (CK, U/L). The values of the reference intervals of the hematological and biochemical parameters were compared with the data listed in the medical records of the Medical Bio-Medica International SRL analytical laboratory.

Antioxidant treatment intervention

For the modified values of the creatine kinase (CK), an individualized treatment with antioxidant supplements was recommended: a) when CK ≤ 700 u/l: Tonotil – N(10 ml) – 1 ampoule / day / x and CoQ10 (90-200 mmg) – 1 cmp /day/xxx; b) when CK ≤ 1200 u/l: Tonotil - N (10 ml) - 1 ampoule / day / x, CoQ10 – 1 cmp / day / xxx and natural sodium (5 ml) – 1 ampoule / day / x; c) when CK ≥ 1300 u/l: Tonotil – N (10 ml)– 1 ampoule / day / xxx, CoQ10 (90-200 mmg) – 1 cmp / day / xxx and natural sodium (5 ml) - 1 ampoule / day / xxx.

d) Statistical processing

The values of the Reference Interval of the hematological and biochemical parameters for physically active subjects were determined (Nunes et al. 2010).

The Interval of Measured Values (IMV) was calculated from the normal values at each parameter of the blood count (BC), highlighting the number of subjects and the size of the interval:

$$IMV = X_{min} – X_{max}, (n, \Delta IMV); \quad (1)$$

X_{min} – represents the minimum value; X_{max} – represents the maximum value.

n – number of subjects with normal measured values

Δ – difference of the size of the measured values interval ($X_{max} – X_{min}$)

The percentage changes of IMV size at each tested stage were calculated through the ratio of the reference interval size to the measured interval size:

- $PM = \Delta IMV/\Delta RI \times 100\%$; (2)
- PM – percentage modification of IMV size
- ΔIMV –IMV size

- ΔRI –RI size

The weight of IMV values of the BC parameters was calculated by dividing the reference interval (RI) into number of units and by reporting the minimum and maximum values of IMV to each stage:

- IMV (%) = X_{min}(%) – X_{max}(%), where (3)
- X_{min} (%) = X_{min} ± NRI (%);
- Xmax (%) = X_{max} ± NRI (%);
- X_{min} – minimum value of IMV
- X_{max} –maximum value of IMV
- NRI (%) – percentage value of RI unit
- X_{min} ± NRI (%) – calculation of the percentage value of X_{min} of IMV by reporting the appropriate NRI (%).

The statistical analysis was carried out using KyPlot 6.0 software (KyensLab Inc). The descriptive data - mean ± SD (standard deviation) and the coefficient of variation (CV%) - of the hematological and biochemical parameters were presented at stage C2 (n=16) and stage C5 (n=26) of the study. There were presented the modified values above (≥) and below (≤) the reference limit. The differences of the means between tests of the investigated parameters were analyzed using the t - parametric test, Paired Comparison for Means, (n=12). The analysis of the relationships between the hematologic and biochemical parameters was performed by means of Pearson linear parametric coefficient. The level of confidence was set to 95%; it was considered statistically significant at p value ≤ 0.05.

Results

Table I shows the modifications of the hematological and biochemical parameters in soccer players, regarding the reference interval and its size compared to the values of the measured interval during the pre-competitive period of training 1 (C2, n=16) and the pre-competitive period of training 2 (C5, n=26), the percentage modifications of the measured values and the weight of the measured interval in the normal values.

The reference interval for 20 measured parameters of CBC had values from 0.7 to 269. The normal values of the measured interval (IVM) evaluated in both stages were as follows: at stage C2 (n=16) an average of 14.15 athletes (88.4%) with a measured interval between 0.6 – 121 and at stage C5 (n=26) – 22.9 athletes (88.07%) with a measured interval between 0.7-174. The value of the measured interval size had an average of 15.75 at stage C2 and an increase by 3.61 at stage C5 (19.36). The absolute difference of the measured interval size had increases by 88.49% in 10 parameters (Σ of the differences of the intervals between tests) and decreases by 11.51% in the other 10 parameters. On average, the percentage modifications of IVM were of 56.13% in stage C2 and 58.57% in stage C5. The percentage ratio of IVM was between 16.04% - 72.52% in stage C2 and between 13.43% - 73.67% in stage C5.

Table II presents the results of the hematologic parameters modifications in terms of means and standard deviation (±SD) of the normal values, outside the interval of reference in the stages C2 and C5, the differences of the normal values, the coefficient of variation (CV%) and the statistical significance between tests of the athletes who participated in both evaluations.

Table I

Modifications of the hematological and biochemical parameters, difference and ratio of the soccer players' measured values to the reference interval for physically active subjects.

Parameters	Reference Interval (size of interval, Δ)	Interval Measured Values (n; Δ_{IVM})		Percentage change (%)		Percentage of Measured Values Interval (%)	
		C2	C5	C2	C5	C2	C5
RBC (10^{12}/L)	4.5 – 6.1 (1.6)	4.54 – 5.31 (15; 0.77)	4.52 – 5.53 (24; 1.01)	48.10	63.10	0.64–50.31	0.32 – 62.98
Hgb (g/dL)	13.5– 17.5 (4.0)	14.4 – 17.4 (15; 3.0)	13.9 – 17.0 (26; 3.1)	75.00	77.50	22.50–97.50	10.00 – 87.50
WBC (10^9/L)	4.0 – 10.5 (6.5)	5.0 – 9.7 (16; 4.7)	4.2 – 8.5 (25; 4.3)	72.20	66.15	15.20–87.40	3.80 – 68.40
Neutrophils (10^9/L)	2.0 – 8.0 (6.0)	2.2 – 6.0 (16; 3.8)	2.2 – 4.8 (25; 2.6)	63.30	43.30	3.32–66.64	3.32 – 46.45
Lymphocytes (10^9/L)	1.0 – 5.0 (4.0)	1.5 – 3.7 (16; 2.2)	1.6– 3.7 (26; 2.1)	55.00	52.50	12.50–67.50	15.00 – 67.50
Monocytes (10^9/L)	2.0 – 10.0 (8.0)	5.3 – 9.2 (16; 3.8)	6.3 – 9.4 (25; 3.1)	48.75	38.75	43.43–87.82	68.43 – 93.59
Eosinophils (10^9/L)	0.0 – 5.0 (5.0)	0.5 – 3.5 (16; 3.0)	0.2 – 2.8 (24; 2.6)	60.00	52.00	10.0–70.00	4.00 – 56.00
Basophils (10^9/L)	0.0 – 2.0 (2.0)	0.5 – 1.1 (16; 0.6)	0.1 – 1.2 (26; 1.1)	30.00	55.00	22.0–52.00	2.00 – 60.00
Neutrophils (%)	45.0 – 80.0 (35.0)	46.5 – 63.9 (14; 17.4)	45.5 – 61.4 (23; 15.9)	49.71	45.43	4.28-53.97	1.43-46.83
Lymphocytes (%)	25.0 – 40.0 (15.0)	28.4 – 37.9 (10; 9.5)	27.9 – 39.7 (18; 11.8)	63.33	78.67	22.65-85.96	19.32-97.97
Monocytes (%)	2.0 – 10.0 (8.0)	5.3 – 9.2 (9; 3.9)	6.3 – 9.4 (20; 3.1)	48.75	38.75	31.25-90.00	53.75-92.50
Eosinophils (%)	0.0 – 5.0 (5.0)	0.5 – 3.5 (16; 3.0)	0.2 - 2.8 (24; 2.6)	60.00	52.00	10.0-70.00	4.00-56.00
Basophils (%)	0.0 – 2.0 (2.0)	0.5 – 1.1 (16; 0.6)	0.1 – 1.2 (26; 1.1)	30.00	55.00	25.0-55.0	5.00-60.00
ESR (mm/1h)	3.0 – 20.0 (17.0)	3.0 – 10.0 (15; 7.0)	4.0 – 10.0 (26; 6.0)	41.16	35.29	0.00 – 41.16	5.88 – 41.16
Glucose (mg/dL)	74.0 – 106.0 (32.0)	74.0 – 80.0 (7; 6.0)	74.0 – 96.0 (13; 22.0)	18.75	22.00	0.00-18.75	0.00-68.75
ALT (U/L)	5.0 – 65.0 (60.0)	24.0 – 60.0 (16; 36.0)	21.0 – 53.0 (25; 32.0)	60.00	53.33	31.66-91.63	26.65-79.95
Calcium (mg/dL)	8.5 – 10.0 (1.5)	8.7 – 9.9 (16; 1.2)	8.8 – 10.0 (24; 1.2)	80.00	80.00	13.32-93.24	19.98-100
Magnesium (mg/dL)	1.7 – 2.4 (0.7)	1.7 – 2.3 (15; 0.6)	1.7 – 2.4 (25; 0.7)	85.71	100.0	0.00-85.68	0.00-100
Iron (mg/dL)	59.0 – 158.0 (99.0)	67.0 – 154.0 (14; 87.0)	59.0 – 156.0 (17; 97.0)	87.87	97.97	8.08-95.95	0.00-97.97
CK (U/L)	39.0 – 308.0 (269.0)	160.0 – 281.0 (9; 121)	108.0 – 282.0 (16; 174)	44.98	64.68	44.96-89.92	25.64-90.29

Notes. C2(n)= 16; C5(n) = 26; n –number of subjects; RI - reference interval for physically active subjects; (Δ) – size of interval through the difference of max – min values.

Table II

Results of the hematological parameters, differences of the measured intervals, coefficient of variation and significance between the training stages C2 and C5.

Parameters	Mean ± SD				Normal Values (CV%)		t-Test, n=12	p value (two-sided)
	C2, n=16		C5, n=26					
	Normal Values	Out of Interval	Normal Values	Out of Interval	C2	C5		
RBC (10^{12}/L)	4.94 ± 0.28 (n=15)	4.16 (n=1) ≤	5.02 ± 0,26 (n=24)	4.17; 4.49 (n=2) ≤	5.71	5.28	-1.033	0.323
Hb (g/dL)	15.61±0.94 (n=15)	17.60; (n=1) ≥	15.47±0.78 (n=26)	-	6.07	5.05	0.804	0.438
WBC (10^9/L)	6.84±2.00 (n=16)	–	6.42±1.00 (n=25)	12.4 (n=1) ≥	23.3	20.76	-0.805	0.437
Neutrophils (10^9/L)	3.85±1.17 (n=16)	–	3.28±0.70 (n=25)	1.90 (n=1) ≤	30.4	21.4	0.928	0.373
Lymphocytes (10^9/L)	2.34±0.55 (n=16)	–	2.35±0.55 (n=26)	–	23.7	23.4	-1.363	0.200
Monocytes (10^9/L)	0.57±0.13 (n=16)	–	0.54±0.17 (n=25)	1.1 (n=1) ≥	22.4	31.8	-1.483	0.166
Eosinophils (10^9/L)	0.12±0.09 (n=16)	–	0.09±0.07 (n=24)	0.5 (n=2) ≥	82.6	78.3	-0.247	0.808
Basophils (10^9/L)	0.07±0.05 (n=16)	–	0.02±0.04 (n=26)	–	69.6	120.0	3.924	0.002**
Neutrophils (%)	55.73±5.17 (n=14)	40.3; 43.0 (n=2) ≤	52.03±4.05 (n=23)	41.8±4.94 (n=3) ≤	9.27	7.8	1.378	0.195
Lymphocytes (%)	33.03±3.07 (n=10)	23.8; 24.6 (n=2) ≤ 43.5±3.41 (n=4) ≥	35.31±3.23 (n=18)	– 42.5±2.06 (n=8) ≥	15.9	9.16	-1.098	0.295
Monocytes (%)	7.58±1.41 (n=9)	11.27±1.31 (n=7) ≥	8.06±0.81 (n=20)	11.5±1.43 (n=6) ≥	18.6	10.03	0.808	0.435
Eosinophils (%)	1.78±1.06 (n=16)	–	1.67±0.88 (n=24)	6.2; 7.30 (n=2) ≥	73.8	52.9	-1.328	0.211
Basophils (%)	0.82±0.15 (n=16)	–	0.6±0.28 (n=26)	–	18.5	47.6	3.957	0.002**
ESR (mm/1h)	5.33±1.59 (n=15)	21.00 (n=1) ≥	6.23±1.92 (n=26)	–	29.8	30.9	-0.16	0.875

Note. n – number of athletes; C2 (n=16) - pre-competitive period of training 1; C5 (n=26) - pre-competitive period of training 2; Δ - absolute difference between C2 (n=16) and C5 (n=26) analyses; above (≥) and below (≤) reference limit; Descriptive statistics: Data are presented as mean ± SD and CV (%) – coefficient variation; Parametric test, t-Test, Paired Comparison for Means, df=11; statistically significant when $p \leq 0.05$.

Table III

Results of the biochemical parameters and differences of the absolute values measured in soccer players during stages C2 and C5.

Parameters	Mean ± SD				Normal Values (CV%)		t-Test, n=12	p value (two-sided)
	C2, n=16		C5, n=26					
	Normal Interval	Out of Interval	Normal Interval	Out of Interval	C2	C5		
Glucose (mg/dL)	77.28±2.14 (n=7)	66.78± (n=9) ≤	83.54±6.81 (n=13)	62.69±5.18 (n=13) ≤	2.77	8.16	0.750	0.468
ALT (U/L)	37.12±10.45 (n=16)	–	33.38±8.66 (n=25)	96.00 (n=1) ≥	28.14	25.57	-0.637	0.536
Calcium (mg/dL)	9.32±0.29 (n=16)	–	9.40±0.39 (n=24)	10.1; 10.4 (n=2) ≥	3.19	4.17	-1.538	0.152
Magnesium (mg/dL)	1.99±0.18 (n=15)	1.6 (n=1) ≤	1.96±0.18 (n=25)	2.9 (n=1) ≥	9.18	9.05	-1.076	0.304
Iron(mg/dL)	116.9±24.9 (n=14)	159; 181 (n=2) ≥	97.17±24.45 (n=17)	182.9±17.5 (n=9) ≥	21.4	25.2	-1.703	0.116
CK (U/L)	216.7±40.3 (n=9)	370 - 129600 (n=7) ≥	184.3±52.3 (n=16)	422.9±140.9 (n=10) ≥	18.6	29.1	1.007	0.335

Note. n – number of athletes; C2 (n=16) - pre-competitive period of training 1; C5 (n=26) - pre-competitive period of training 2; Δ - absolute difference between C2 and C5 analyses; above (≥) and below (≤) outside the reference interval; Descriptive statistics: Data are presented as mean ± SD; CV% - coefficient of variation; Parametric test, t-Test, Paired Comparison for Means, df = 11; statistically significant when $p \leq 0.05$.

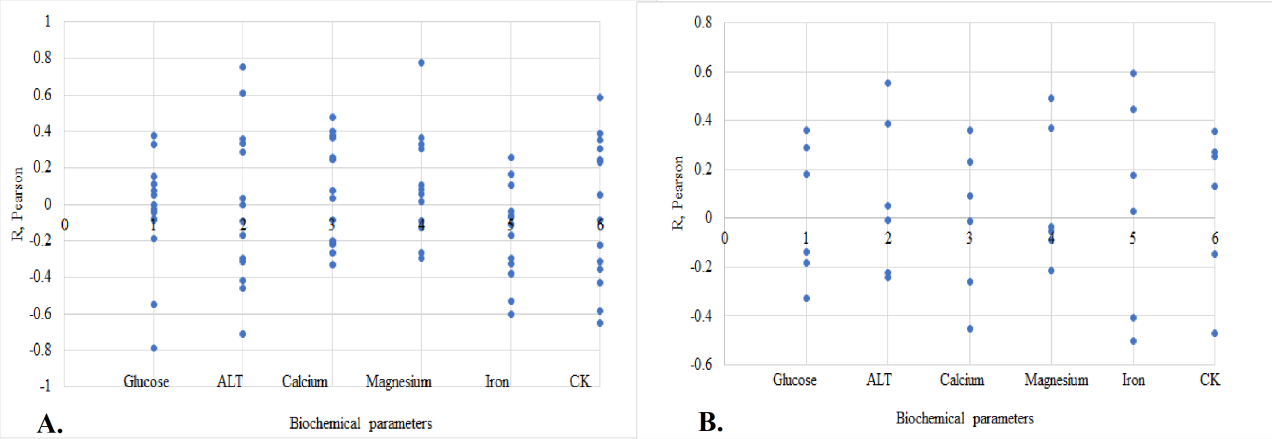

Fig. 1 – Correlation between the hematological and biochemical parameters of blood cell count in the training stages C2 and C5
Note. A. C2 - competitive period of training 1; Hematological parameters (14) – table 2; B. C5 - competitive period of training 2; Biochemical parameters (6) – table 3; R - Pearson linear parametric coefficient; $p \leq 0.05$.

There were noticed differences of the measured interval values (Δ) by increases of 88.49 % and decreases of 11.51% of the normal values. There were also values below (≤) and above (≥) RI out of interval (Kjeldsberg, 1992; Nordin et al. 2004) indicating no training effects on these parameters. CV% was good at RBC and Hb in both stages C2 and C5 (5% - 6%), Neutrophils (%) – CV (7% - 9%) and it was moderate and weak between tests at the other parameters, with significant differences in Basophils (10^9/L; %) at $p < 0.01$ and insignificant at $p > 0.05$ between the other parameters.

Table no III shows the results of the modifications in biochemical parameters regarding the mean and the ±SD of the normal values (NV), out of the reference interval in stages C2 and C5, the differences of normal values, CV% and the statistical significance between tests of the athletes who participated in both evaluations.

Also, compared to the hematological parameters, there were observed differences of the normal values (Δ) through increases of 10.44% and decreases of 89.56% of normal values and also values ≤; ≥ RI out of interval. CV% was good for Glucose, Calcium and Magnesium in C2 and C5 (2% - 9%), while it was moderate and weak for the other parameters between tests, with insignificant differences at $p > 0.05$.

The results of the correlation analysis between the hematological and biochemical parameters in stages C2 and C5 are shown in Figure no 1 (A and B). The description of the hematological parameters (14) is made in table no 2; the biochemical parameters (6) are presented in table no 3.

A number of 168 correlations were analyzed (84 in C2 and 84 in C5) with the following results: 75 positive, 87 negative and 6 inexistent; 11 significant correlations (at $p < 0.01$ and $p < 0.05$). The coefficient of significant correlation ($p < 0.01$) in the pre-competitive period of training 1 (C2) was $R = 0.78$ between RBC (10^{12}/L) and Magnesium (mg/dl). It was R= -0.79 between Hb (g/dL) and Glucose (mg/dl); R = -0.71 between Neutrophils (10^9/L) and ALT (U/L); $R = 0.75$ between Monocytes (%) and ALT (U/L). Also, at $p < 0.05$ between RBC (10^{12}/L) and CK (U/L), it was found out $R = 0.65$; between Neutrophils (10^9/L) and Iron (mg/dl), R = -0.60; between Neutrophils (%) and CK (U/L), $R = -0.59$; between Eosinophils (%) and CK (U/L), R = 0.59; between Basophils (%) and ALT (U/L), $R = 0.61$. The coefficient of significant correlation ($p < 0.05$) in the pre-competitive period of training 2 (C5) was R= -0.62 between Neutrophils (10^9/L) and Glucose (mg/dl); between Basophils (%) and Magnesium (mg/dl), $R = 0.59$.

Discussion

This study is the first to present the absolute and individual differences of the measured values compared to the standard reference interval for the antioxidant substances treatment which was administered to the subjects. The hematological and biochemical analyses highlighted the effects of the training sessions / competitions on the elite soccer players.

The players submitted to this study were included in the category of seniors, aged 20 to 33 years. The training was carried out from January 14 to July 30, 2019, in 6 stages, in which 28 matches (official and friendlies) were played, with about 5 days of pause/recovery between matches, 5 training sessions /week, the training session duration of 120 minutes.

The main findings referred to the blood count parameters (BC): (1) influence of the training effort parameters on the normal values interval between two competitive periods of the elite soccer players, depending on the number of athletes and the size of the interval; differences of the absolute size of the interval measured by increases of 88.49% and decreases of 11.51%; percentage modifications of the intervals measured in stage C2 on average of 56.13% and in stage C5 of 58.57%; weight of the measured interval values in stage C2 (16.04% - 72.52%) and in stage C5 (13.43% - 73.67%); (2) improvement of the normal values of the hematological parameters through increases by 88.49% and decreases by 11.51% of the normal values interval; (3) improvement of the normal

values of the biochemical parameters through increases by 10.44% and decreases by 89.56% of the normal values interval; (4) significant correlation connections of 6.55 % between the hematological and biochemical parameters, with 9.52 % in stage C2, pre-competitive period of training 1 and 3.57% in stage C5, pre-competitive period of training 2; (5) diminution of CK concentration by 64.71% at normal limits by administration of antioxidant substances. These findings confirm the influence of effort training on the modifications of the hematologic and biochemical parameters; the administration of antioxidant substances depending on CK concentration contributed to maintaining and improving the sports performances of the soccer players as demonstrated by the result obtained at the end of the competitive ranking of League Two (2018/2019) and the promotion to League One in the high-performance competitive activity of the next season.

The general table of the Collection of blood samples (CBC) in the elite soccer players revealed modified values of 11.56% in stage C2 (18 modified values at 6 hematological parameters and 19 modified values at 4 biochemical parameters) and 11.92% at stage C5 (26 modified values at 9 hematological parameters and 36 values modified in all biochemical parameters).

Regarding the individual values modified at the hematological and biochemical parameters in both tests, we present the following example: the athlete PPO (33 years old), player in the position of striker, with the most values modified in the hematological parameters - 4 parameters (C2) and 7 parameters (C5) – where it increases at RBC as follows: in stage C5 by 0.01 ≤ RI (C2 – 4.16x10^{12}/L); Monocytes ≥ RI de 1.10x10^{9}/L in stage C5, Eosinophils ≥ RI of 0.50 x10^{9}/L in stage C5, Neutrophils decrease by 4.20% ≤ RI (C2 – 40.30%), Lymphocytes increase in stage C5 by 1.20% ≥ RI (C2 – 42.90%), Monocytes increase by 0.70 % in stage C5 ≥ RI (C2 – 12.40%), Eosinophils ≥ RI de 6.20% and ESR ≥ RI of 21.00 mm/1h . As for the biochemical parameters - 2 parameters (C2) and 1 parameter (C5), where Magnesium ≤ RI of 1.60 mg/dL at stage C2 and CK ≥ RI 129600 U/L at stage C2 and ≥ RI 354.00 U/L in stage C5. We mention that the decrease of CK concentration in stage C5 is due to the administration of antioxidant substances treatment.

The changes of the complete blood count of the athletes in the study reveal the diminution of the number of erythrocytes which causes anemia (Glader, 2004). The intense physical effort can cause the increase of Hb and the critical value >20 g/dL can lead to the blockage of capillaries as a result of the hemoconcentration (Fischbach, 2009a). Marked leukocytosis can be caused, usually on account of the segmented neutrophils, but lymphocytosis may also be present (Skubitz, 2004). The most numerous leukocytes type plays a major role in the primary anti-infectious defense of the body by phagocytosis and digestion of the microorganisms. Their improper activation can lead to damage of the body normal tissues by the release of enzymes and pyogenic agents (Skubitz 2004). Monocytes and macrophages produce numerous bioactive factors: enzymes, complement factors, coagulation factors, reactive species of oxygen and azote, angiogenetic factors, binding proteins, bioactive lipids, chemotactic factors, cytokines and growth factors (Fischbach, 2009b). Eosinophil generates and stores up to 29 known mediators, cytokines, chemokines and growth factors, important in the inflammatory reactions in which this cell is involved (DeMott, Tilzer, 1994). Neutropenia is a very rare blood condition that causes a small number or a complete lack of neutrophils. A decrease in the level of neutrophils usually occurs when the body uses the immune cells faster than it produces them or when the bone marrow does not produce them correctly (Dale & Welte, 2001). Basophils are cells that have the kinetics and natural history of a granulocytes, which mature in the marrow, circulate in the blood and retain certain characteristic ultrastructural features after migration into tissues during the inflammatory and immunological processes (Befus, Denburg, 2004). Compared with CRP and serum amyloid A, the VSH is also elevated in the situations in which the concentration of immunoglobulins, immune complexes and other proteins increases. Erythrocyte sedimentation occurs when the erythrocytes aggregate in the form of a column (Thomas, 1998).

One of the most common causes of low blood sugar is the too intense physical activity. In fact, even moderate exercise can cause blood sugar dropping in the 24 hours after the training session. This post exercise hypoglycemia is a kind of "delayed effect" of the sport (Wallach, 2001). The administration of 1-2 servings of bananas before the competitive activity contributes to the increase of the glucose concentration after competition (Miller et al., 2012). The increased urinary calcium results from the increase of intestinal absorption, the decrease of tubular reabsorption and the resorption or loss of calcium in the bones. It almost always accompanies high levels of calcium in the blood. When calcium is excreted in high amounts, it favors the production of nephrolithiasis and nephrocalcinosis, especially if associated with increased protein intake (Fischbach, 2009b). The actions of calcium and magnesium are closely linked, thus the deficiency of one of these elements influence significantly the metabolism of the other one (magnesium is required for both intestinal absorption and calcium metabolism). In the muscle cell, magnesium acts as calcium antagonist (Thomas, 1998). All micronutrients are important in the diets of growing athletes, but two micronutrients – calcium and iron - are most likely to be low in the diets of young elite athletes (e.g. Wright 2009). Iron deficiency anemia is associated with decreased exercise performance and tissue iron deficiency may impair adaptation (Brownlie et al. 2002; 2004). The additional amount of iron that can be linked to transferrin is called unsaturated or latent iron binding capacity; the sum between the value of serum iron and UIBC is the total iron binding capacity (Fischbach, 2009b). The effects of the cryo-compression on performance and recovery parameters following heavy resistance exercise were studied (DuPont, 2017). The total serum creatine kinase (CK) concentrations were used as an indirect biochemical marker of muscle fatigue and damage (Brancaccio et al. 2010).

There are studies and concerns on the hematological and biochemical behavior of the elite soccer players in order to obtain more information on the physiological characteristics of these athletes and to provide the coaches

and sports medicine physicians with useful indices (Anđelković et al. 2015). The management of effort parameters in soccer activity must be made so that, by using the intensities of the training sessions, the exerted effort be consistent with the objectives of each segment of the partial or general periodization (Cristian, 2013). The effort content is modelled for 90 minutes of game (Mănescu, 2010): 45 minutes of training / endurance game (heart rate (HR): 120 -170 pulses / min); 15 minutes of anaerobic effort – sprint (maximum effort, HR: 160 – 200 pulses / min); 30 minutes of effort of the fixed moments (HR: 100 – 130 pulses/min).

Other studies examined the effects of competition performance on selected biochemical and hematological parameters of the elite male soccer players, highlighting significant differences between the biochemical and hematological parameters (Ayhan et al., 2017). There was also analyzed the importance of control, monitoring and evaluation of different biochemical markers of recovery in soccer players (Marqués-Jiménez et al., 2016). Other specialists studied the effect of training program, in terms of parameters associated with cardiovascular risk indicators, aerobic ability and anthropometric characteristics in professional soccer players (Alexiou et al., 2018). The oxidative stress modifications, noninvasively analyzed in the urine and saliva of the athletes, recommend the use of these biological liquids for the biochemical exploration of stress in exercise and training monitoring (Boroş-Balint et al., 2010). It was evaluated the relationship between high-acceleration head impact (HHI) and serum biomarkers for traumatic brain injury (TBI) in high-school soccer athletes (Joseph et al., 2019).

The changes in the chemical composition of blood reflect the biochemical changes that occur in the muscle activity of different internal organs, skeletal muscles and myocardium. Therefore, based on the analysis of blood chemical composition, it is possible to evaluate the biochemical processes that occur during physical effort (Butova, Masalov, 2011). Also, significant changes occur during physical effort in the chemical composition of the urine too, considerably affecting the physical-chemical properties of this one. The following pathological components are identified in the athletes, after intense exercise: protein elimination, glucose excretion, ketone bodies formation, lactic acid synthesis, density increase, acidity increase (Poleacova, Ciochină, 2018). The soccer game is a mixed sport in terms of contribution of the energy systems to exercise supporting. Medium lactacidemia specific to soccer game varies between 8 and 12 mmol/l. A value of the blood lactate concentration above 6 mmol/l influences the exercise capacity (Hancăş, Gozu, 2012).

Depending on CK concentration, individualized administration of antioxidant substances was performed during the study (table no 3). It was noticed that 6 athletes in the stage C2 and 10 athletes in stage C5 had the concentration CK ≤ 700 u/l. The comparative values between tests of 2 athletes showed that they received treatment at CK ≤ 700 u/l in stage C2, reaching normal values in stage C5. Only one athlete with CK concentration ≥ 1300 u/l (n21) with values of 129600 u/l in stage C2 received treatment at CK ≥ 1300 u/l and in stage C5 of 354.00 U/L he continued the treatment at CK ≤ 700 u/l (Bojana, 2018; Koch et al. 2014). In order to improve sports performance, the supplementation of zinc and magnesium had benefic effects on the oxidants – antioxidants balance in athletes (Popovici et al., 2009). The administration of substances with antioxidant effects could favorably influence the capacity of physical effort by diminishing the oxidative stress induced by the effort (Chiş et al. 2015). In this sense, the coenzyme Q10 or ubiquinone is a vitamin-like compound, included in the category of natural non-nutritional antioxidants. Some studies show that there are increases of the CoQ10 plasma levels in the athletes during aerobic exercise. The supplementation of CoQ10 increases the aerobic maximal power of the young soccer players trained for effort (Ciocoi-Pop et al., 2009).

Soccer is a sport characterized by the rapid activation of both aerobic and anaerobic metabolisms. Energy generation through aerobic metabolism is found to be at around 90 % of total energy that is consumed during the match. Levels of lactic acid before a soccer match are 3-9 mmol/L and at many instances they may reach over 10 mmol/L (Bangsbo, 1994b), which means that the effort of players is intense.

Conclusions

1. The measurement of the complete blood count parameters revealed the biochemical changes in the competitive effort of the elite soccer players in terms of IVM and modified values (outside the RI) and the significance degree between stages.

2. The biochemical changes highlighted in the blood count (CBC) parameters of the elite soccer players occurred as a result of the training effort influence during two competitive periods.

3. The correlation analysis of CBC parameters highlighted the existence of significant links between the general, absolute and individual values of the hematological and biochemical parameters in the first and also the second stage of the research.

4. The administration of the individualized treatment with antioxidant substances to the soccer players contributed to the decrease of CK concentration, to the improvement of the normal values of CBC parameters and to the continuity of their training process.

Conflict of interests

The authors declare no conflict of interest.

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