THE ASSESSMENT OF BLOOD CORTISOL AND CREATINE KINASE CONCENTRATION IN PERSONALIZING THE FOOTBALL PLAYERS TRAINING

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Abstract

Background: Blood tests are a common health assessment for diagnosing diseases and bodily disorders.
Aims: The aim of the work was to analyze the suitability of sampling and assessing athletes' blood-borne biomarkers in a search for ways to optimize their football training.
Methods: In the prospective study, the results of 14 football players from a youth football team competing at the highest national level 'blood tests were evaluated before (P1), immediately after (P2) and 24 hours after (P3) playing a football match. The all players were divided into two groups based on the length of time they were on the field during the game: FMP - (8 players) participation in the match for 90 minutes, and PMP - (6 players) participation in the match for 30-75 minutes.
Results: In the FMP group, at P2, the following statistically significant ($p<0.001$) differences were observed: an increase in the leukocyte count; decreases in erythrocytes (RBC), hemoglobin (HGB), and hematocrit (HCT), and increases in creatine kinase (CK), cortisol and lactates. At P3 when comparing the FMP and PMP group results, we observed an increase in CK ($p=0.03$) and decrease in cortisol ($p=0.02$) in the former group. The concentration of cortisol<356.04nm/L at P3 was the most sensitive and specific (PPV=0.83; NPV=0.88) difference characterizing the group of FMP players compared with the players of the PMP group. In the FMP group compared with PMP at P3 we showed a significantly increased levels of CK ($p=0.002$).
Conclusions: The blood cortisol levels below 356.04nm/L and CK concentrations below 500UI/L taken 24 hours after the football game in the players from a youth football team competing at the highest national level are characteristic of players with a greater potential for physical exercise.

Keywords*: football players; training optimization; blood biomarkers*

Introduction

Physical activity is one of the most important human needs. During physical exercise there are many physiological changes, such as stimulation of muscle protein biosynthesis, stimulation of erythropoiesis, improved endocrine system functions, improved capacity of the cardiovascular system, and even improved central nervous system functions and associated increases in self-esteem and motivation for further effort. It is also believed that regular exercise has anti-inflammatory benefits (Kenney et al., 2015). Participating in competitive sports requires thoughtful and skilful planning of the physical activities involved, to ensure the body learn and adapt to the often-extreme physiological conditions. Performance scales are used in planning, but we can also use the medical possibilities offered through the analysis of athletes' bodily fluids. Medical practitioners' blood analyses can lead to conclusions that inform diagnostic and therapeutic planning. Analysis of blood biomarkers can help training staff fine-tune training plans that help the body better adapt to the effort and extreme conditions encountered in competitive sports and help athletes achieve sporting success.

The aim of the present study was to assess the usefulness of blood counts, cortisol, lactate and creatine kinase levels in analysing how players' bodies are functioning while playing football, and to interpret the results in order to apply the conclusions to the personalized training plans of the athletes.

Methods

The research group consisted of SMS Resovia Sport Masters School players participating in the U-19 Youth League 2017/18 and playing at the highest national level. The eighteen adolescent soccer players who participated in this study (age=17.4±0.3 years; body mass=70.38±3.8 kg; height=178.8±4.3 cm; BMI=22.09±1.3 kg/m2) were non-smoking, healthy, and were qualified for competitive sport based on a current medical examination report. The research presents the results of the players who had been taking part in the game (n=14), the remaining players (n=4) did not participate in the game – they remained in the reserve. It is depicted in Table 5 description. It should also be noted that the homogeneous group of players had the same living conditions, as they shared the same dormitory and had the same diet without any nutritional supplements. Prior to the research, if any of the players were suffering any pain that could have interfered with their psycho-physical capabilities, they did not report it. All players, and their parents, were fully informed about the experimental procedures before giving their written consent to participate.

The research commenced with the players taking part in a football league match. Some of the players (n=6, 42.8%) played in the match for 30-75min. The remaining 8 players participated in the match for the full 90 minutes.

The match started at 13:00. The mean ambient temperature during the match was 10 degrees C, atmospheric pressure was 988.46 hPa, there was a westerly wind of 11 km/h, and it was sunny with no rain. The altitude of the pitch where the match was played is 212 m above sea level. The match in question took place at the end of the first round of the U-19 league. During the match, the players supplemented their fluid loss with clean drinking water of up to 1500 ml/match/person. During the match, the players did not supplement carbohydrates.

The blood for testing was collected from the superficial veins in the right or left forearm. The blood tests and laboratory analyses were performed by the professional company DIAGNOSTYKA, accredited for a medical laboratory Nr/No AM 003.

Blood samples were taken before warming up of the league match (P1), up to 30 minutes after the end of a match (P2) and 24 hours after the match, on the following day (P3). Blood samples were collected at time point P1 between 12:00 and 12:45, time point P2 between 14:30 and 15:00, and time point P3 between 14:30 and 15:00 on the day after the game. Figure 1 diagrammatically shows the timing of competitors' blood samples and the intervals between each, in relation to the timing of the football match. Blood counts were determined from the whole blood samples, and from the plasma collected in a syringe with the anticoagulant EDTA, the cortisol levels were determined. WMA DECLARATION OF HELSINKI – ETHICAL PRINCIPLES FOR MEDICAL RESEARCH INVOLVING HUMAN SUBJECTS 2013, has been applied in this study.

The data used to support the findings of this study are available from the first author upon request.

In **Table 1** we present the biomarkers that were tested for in the venous blood of competitors, the range of reference values, and the assay methodologies and apparatuses used.

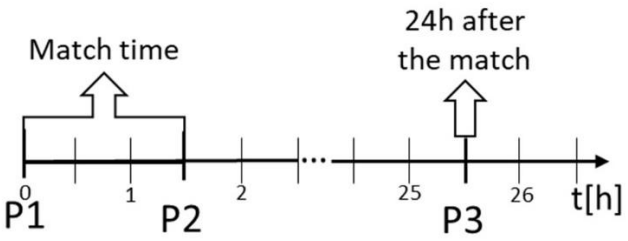

Figure 1. The timing of, and intervals between, the blood samples taken from the football league players: P1 – a blood sample collected immediately before the match; P2 - a sample of blood collected immediately after the match; and P3 - a blood sample taken 24 hours after the match

The normality of distributions was assessed using the Shapiro-Wilk test. The variables has a non-normal distribution therefore the non-parametric tests were used. Data were presented using the statistical measures of arithmetic mean and standard deviation. Differences between individual measurements (P1, P2, P3) were evaluated by the non-parametric Wilcoxon signed-rank test for paired goup and ANOVA Friedman test. Assessment of statistically significant differences between the results of the subgroups of the study group (i.e., groups based on the two playing times: 30-75 min and 90 min) was conducted using the Mann-Whitney U test. In order to estimate the diagnostic values of the tests for biomarkers, ROC (receiver operating characteristic) curve analyses were performed with the determination of cut-off points. Results were deemed to be statistically significant at

p<0.05. Statistica 13.0 (StatSoft, Tulsa, USA) and R software (R Foundation for Statistical Computing, Vienna, Austria) were used for the study's statistical analysis.

Table 1. Venous blood biomarkers, range of reference values and assay methodologies

Biomarker	Unit	Range of reference values	The assay method and the measuring apparatus used
Cortisol	µg/dl nmol/l	5-25 144.9-690	Immuno-chemical Architect Ci4100
Lactate	mmol/l	0.5-2.2	colorimetric, Architect Ci4100
CK	IU/l	30-200	Kinetics, Architect Ci4100
Blood morphology			
RBC	10^{12}/l	4.5–6.5	Automatic hematology analysis, 5DIFF, Sysmex XE-2100D
HGB	g/l	121.0-166.0	
HCT	%	35.0–49.0	
PLT	10^9/l	180.0–430.0	
WBC	10^9/l	3.8–10.0	
MCV	fl	77-92	
MCH	pg	26.5-32.5	
MCHC	g/l	322.0-364.0	

CK – Creatine kinase; RBC – red blood cells; HGB – hemoglobin; HCT – hematocrit; PLT – platelets; WBC – white blood cells; MCHC – mean corpuscular hemoglobin concentration; MCV – mean corpuscular volume; and MCH – mean corpuscular hemoglobin.

Results

The results of the tests carried out in the whole group of competitors are presented in **Table 2**. The conducted analysis using Friedman's ANOVA showed that most parameters showed significant variation. The exceptions are the parameters MCH, Cortisol and Lactate. Before the match, the CK value was higher than normal (below 200IU/l) in more than half of the players (n=8). Some players (n=4) also had higher lactate levels (above 2.2 mmol/l) before the game. Immediately after the match, in all players, we observed a significant increase in leucocytosis; a decrease in the red blood cell parameters RBC, HGB and HCT; a decrease in MCV with simultaneous increase in MCHC; and increases in CK activity. Lactate concentrations, PK and MCH did not change due to the physical effort of the match. However, testing the blood samples taken 24 hours after exercise, we observed in the entire group of athletes: leucocytosis had returned to its baseline value; there was a further, and significant decreases in RBC, HGB and HCT; and a decrease in the number of PK below the baseline value. MCH, cortisol and lactate had not changed; however, there was a significant increase in CK

Table 2. Changes in the blood test indices in the whole study group of players

All players playing between 30 and 90 minutes (N=16)										
Var.	Meas.	$\bar{x}$	sd	min	max	V	p (P1vsP2)	p (P1vsP3)	p (P2vsP3)	P (P1vsP2vsP3)
WBC	P1	5.61	1.30	3.84	7.95	23.22	0.0002 ***	0.4996	0.0032 **	0,0001 ***
	P2	12.98	3.91	7.91	21.56	30.13				
	P3	5.75	1.67	4.00	10.30	29.12				
RBC	P1	5.31	0.29	5.02	5.94	5.54	0.0021 **	0.0198 *	0.1329	0,0008 ***
	P2	5.15	0.26	4.74	5.59	4.95				
	P3	5.03	0.23	4.62	5.48	4.48				
HGB	P1	158.1	7.9	148.0	177.0	50.0			0.1182	0,0001

	P2	153.3	6.8	144.0	168.0	44.6	0.0027 **	0.0147 *		***
	P3	149.7	6.8	139.0	166.0	45.7				
HCT	P1	46.96	2.39	44.20	52.20	5.09	0.0002 ***	0.0222 *	0.4204	0,0001 ***
	P2	45.11	2.14	41.40	48.90	4.74				
	P3	44.88	2.08	40.90	49.30	4.64				
MCV	P1	88.44	1.68	86.20	92.30	1.90	0.0001 ***	0.0002 ***	0.0001 ***	0,0001 ***
	P2	87.61	1.41	85.50	90.20	1.61				
	P3	89.21	1.46	87.20	92.30	1.64				
MCH	P1	29.78	0.66	28.80	31.30	2.22	0.5049	0.5701	0.7583	0,8214
	P2	29.79	0.74	28.70	31.30	2.47				
	P3	33.97	15.59	28.60	88.10	45.90				
MCHC	P1	33.69	0.61	32.50	34.80	1.81	0.0013 *	0.0522	0.0033 **	0,0001 ***
	P2	33.99	0.54	33.30	34.90	1.58				
	P3	33.36	0.50	32.40	34.00	1.51				
PLT	P1	235.6	30.81	186.0	287.0	13.08	0.0187 *	0.0013**	0.0001 ***	0,0001 ***
	P2	245.4	38.20	179.0	313.0	15.57				
	P3	221.2	35.16	166.0	274.0	15.89				
CK	P1	346.4	468.6	124.0	1953	135.3	0.0001 ***	0.0021**	0.1989	0,0001 ***
	P2	642.7	704.6	282.0	2989	109.6				
	P3	788.2	605.0	289.0	2356	76.75				
Cortisol	P1	288.1	107.4	151.8	460.9	1029.2	0.1119	0.1626	0.3812	0,2906
	P2	401.3	133.9	234.6	748.0	920.2				
	P3	324.3	74.8	171.1	447.1	636.5				
Lactate	P1	1.89	0.57	1.19	3.22	30.01	0.8788	0.2868	0.2184	0,2298
	P2	2.23	0.55	1.42	3.18	24.60				
	P3	1.44	0.72	0.85	3.68	49.54				

* - position uncertainty α=0.05; ** - α=0.01; *** - α=0.001.

Analysing the results of the tests taken immediately after and 24 hours after the match (P2 vs P3) we observed that the results of the RBC, HGB, HCT, MCH, CK, lactate levels and cortisol tests did not change. At the same time, WBC, MCHC, PLT decreased significantly.

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Table 3. Results of blood tests carried out in the group of FMP players (played the game for 90 minutes) and PMP (played in the match for 30-75min)

Variable	Measurement	PMP (n=6)		FMP (n=8)		d	p
		$\bar{x}$	sd	$\bar{x}$	sd		
WBC	P1	5.85	1.25	5.43	1.40	-0.42	0,8285
	P2	12.72	4.60	13.17	3.63	0.46	0,1457
	P3	6.38	2.20	5.28	1.08	-1.11	0,2742
RBC	P1	5.40	0.28	5.25	0.30	-0.16	0,9999
	P2	5.25	0.24	5.08	0.26	-0.17	0,6964
	P3	5.13	0.22	4.96	0.21	-0.18	0,2369
HGB	P1	160.5	8.4	156.4	7.5	-0.41	0,7618
	P2	156.0	6.6	151.3	6.7	-0.48	0,8285
	P3	152.8	7.2	147.4	6.0	-0.55	0,1219
HCT	P1	48.10	2.13	46.11	2.33	-1.99	0,6334
	P2	46.15	1.64	44.34	2.23	-1.81	0,6334
	P3	46.07	1.77	43.99	1.92	-2.08	0,0676
MCV	P1	89.10	2.09	87.94	1.21	-1.16	0,1011
	P2	88.03	1.64	87.30	1.23	-0.73	0,2369
	P3	89.80	1.68	88.78	1.20	-1.02	0,2031
MCH	P1	29.72	0.64	29.83	0.72	0.11	0,4597
	P2	29.75	0.75	29.81	0.78	0.06	0,5726
	P3	29.78	0.75	37.11	20.61	7.33	0,7618
MCHC	P1	33.38	0.50	33.93	0.61	0.54	0,0831
	P2	33.82	0.37	34.13	0.63	0.31	0,3599
	P3	33.18	0.51	33.50	0.48	0.32	0,3154
PLT	P1	241.33	30.19	231.25	32.58	-10.08	0,3154
	P2	251.00	27.23	241.13	46.18	-9.88	0,4082
	P3	229.17	33.96	215.25	37.11	-13.92	0,3599
CK	P1	200.17	38.74	456.00	612.17	255.83	0,6334
	P2	374.83	123.71	843.63	896.32	468.79	0,0342*
	P3	499.83	360.69	1004.50	679.69	504.67	0,0020**
Cortisol	P1	320.16	128.62	263.86	89.7	-2.04	0,2369
	P2	421.73	166.70	385.85	112.608	-1.31	0,3599
	P3	367.08	57.13	292.28	72.864	-2.71	0,0025**
Lactates	P1	2.00	0.40	1.80	0.68	-0.20	0,3154
	P2	2.04	0.49	2.37	0.58	0.33	0,0343*
	P3	1.36	0.30	1.50	0.94	0.14	0,2765

* - statistical significance at the level of 0.05; ** - statistical significance at the level of 0.01; d - difference between the two groups (FMP and PMP); p - significance of differences between the two groups (FMP and PMP)

In addition, we observed that the differences in RBC, HGB and HCT values at individual points in our study (P1, P2 and P3) were 5.2%; 5.3% and 3.9% respectively, while the PLT count decreased by 9.89% 24 hours after the match compared with the pre-match count. For the needs of the current study, the group of players was divided into two subgroups: FMP - players playing for the whole match, i.e., for 90min (n=8); and PMP – athletes who played in the match for between 30 and 75 minutes (n=6). **Table 3** presents two sets of blood test results, differentiating between the two sup-groups of players (FMP and PMP). In the group of players who played in the match for 90 minutes, compared with those players who participated for shorter periods, in the blood samples taken immediately after the match (P2) we observed a significantly increased levels of CK and lactates. The significance values are presented in **Figure 2.**

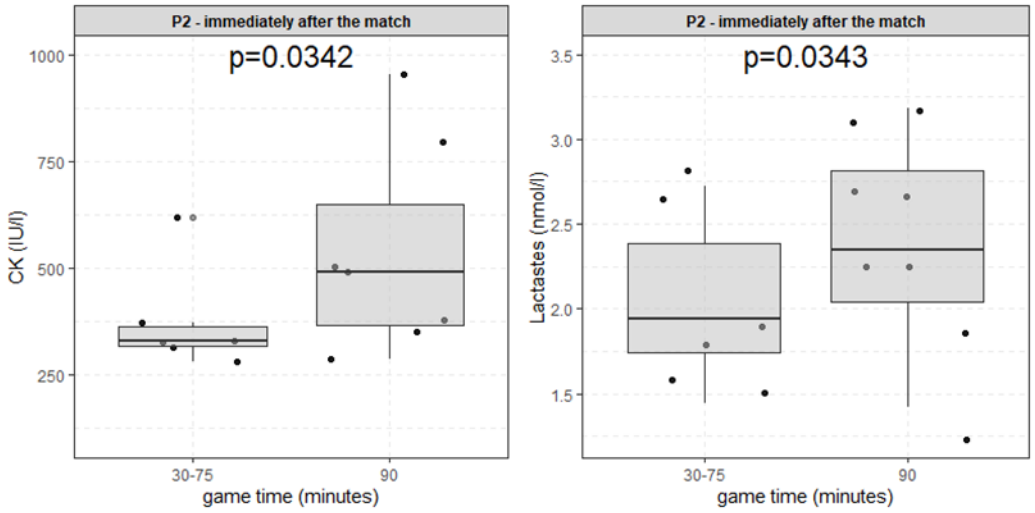

Figure 2. Boxplot for CK (IU l) and lactates (mmol/l) in the blood samples taken immediately after the match, comparing the different game times of the players

In the group of players who played in the match for 90 minutes, compared with those players who participated for shorter periods, the blood samples taken 24 hours after the match (P3) showed a significantly increased levels of CK and decrease levels of cortisol.

The significance values are presented in **Figure 3.**

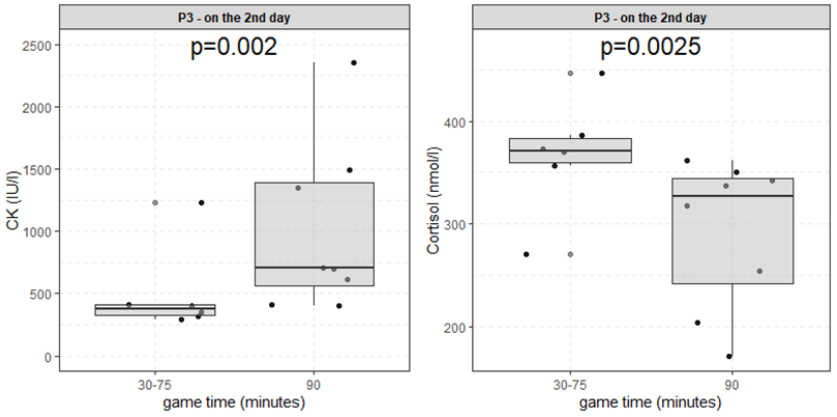

Figure 3. Boxplot for CK (IU l) and Cortisol (nmol/l) at 24 hours after the match, comparing the different game times of the players

In addition, our analyses showed that serum cortisol levels above 356.04 nm/L, tested 24 hours after the match, is the most sensitive and specific indicator that differentiates the group of players playing for 30-75min compared with the group of players who played the entire match, in which the cortisol value was less than 356.04 nm/l **(Table 4, Figure 5).**

Table 4. The diagnostic value of CK (IU/l) and Cortisol (µg/dl) when differentiating between players' game time.

Time point	AUC	Cut-off value	Sensitivity	Specificity	PPV	NPV
CK						
P1	0.323 (0.004, 0642)	140.0	1.00	0.25	0.50	1.00
P2	0.208 (-0.052, 0.469)	282.0	1.00	0.00	0.428	--
P3	0.146 (-0.080, 0.372)	289.0	1.00	0.00	0.429	--
Cortisol						
P1	0.625 (0.282, 0.968)	408.5	0.50	0.88	0.75	0.70
P2	0.521 (0.185, 0.857)	331.2	0.83	0.38	0.50	0.075
P3	0.875 (0.659, 1.091)	356.0	0.83	0.88	0.83	0.88

Our analyses included the cortisol and CK levels in the blood samples of each of the players taken at time P3. The results for each of the biomarkers we tested for are presented in **Table 5**. Based on the previous analyses, we determined that cortisol levels below 356.04nm/L and CK concentrations below 500UI/L were characteristic of players with a greater potential for physical exercise. The results for those players are shown in bold in **Table 5.**

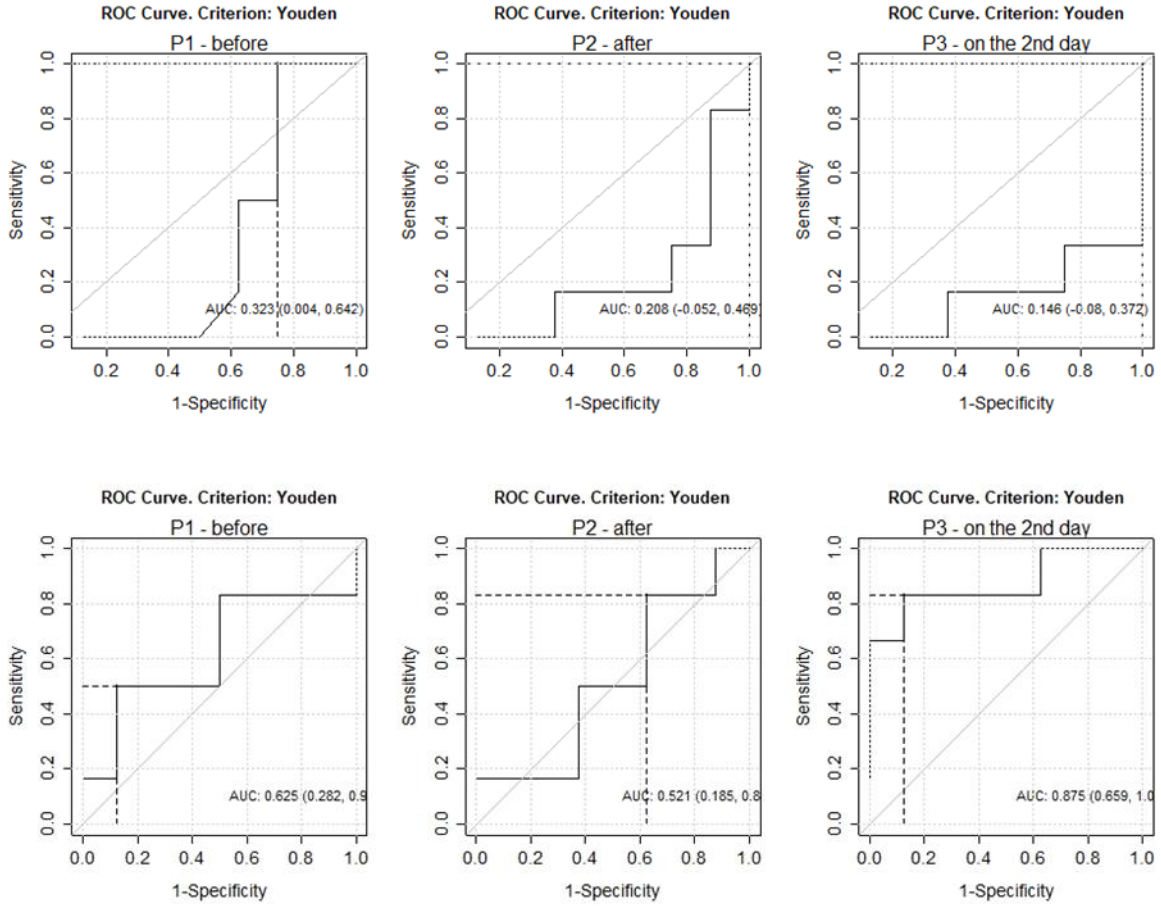

Figure 5. ROC curves presenting the diagnostic value of a) CK (U/l), and b) Cortisol (nmol/l) when differentiating between players' game time

Table 5. The CK (IU/l) concentrations and Cortisol (µg/dl) levels in the blood samples of each of the players taken at time P3; with results for players we determined as having a greater potential for physical exercise bolded

FMP	CK (UI/l)	Cortisol (nmol/l)	PMP	CK(UI/l)	Cortisol (nmol/l)
C3	1352	350.52	C1	289	372.6
C4	709	31.4	C2	1230	447.12
C6	406	336.72	C5	351	369.84
C7	2356	361.56	C11	319	386.4
C8	699	171.12	C12	400	356.04
C9	408	342.24	C14	1493	204.24
C10	613	253.92			
C13	1492	204.24			

C1-C14 - identification numbers assigned to the individual players taking part in the match; FMP - players playing in the match for 90 minutes. PMP - players playing in the match for 30-75min

Discussion

Blood tests are a common health assessment for diagnosing diseases and bodily disorders. The physical effort of sports training is a challenge for the body, and it engages a variety of physiological mechanisms, which may result in health disorder. Therefore, it is important to observe the athlete's performance and health during a variety of fitness tests, analysing the composition of the athlete's blood.

During training sessions, coach can assess the athletes' physical fitness using performance exercises and tests that gradually increase the effort required in order to assess, among other things, the lactate threshold (LT), alternatively called the anaerobic threshold (AT).). The LT indicates the intensity level of the physical effort beyond which the blood lactate concentration exceeds the resting level and systematically increases. During a football match, the player's dynamic physical exercise is of a medium duration, which can cause eccentric, concentric and isometric muscle cramps. During the initial phase of exercise, the blood flow kinetics are faster in the working skeletal muscles than the oxygen uptake kinetics. During periods of maximum effort, the muscular blood flow accounts for 80-90% of the cardiac output. This is accompanied by decreased relative and absolute blood flow through the kidneys and viscera and an intensification of anaerobic glycolysis, which is the main energy source for working muscles in oxygen deficient conditions. Both anaerobic glycolysis and anemization of the kidneys are responsible for the accumulation of lactates during physical exertion. The anaerobic threshold is the point during dynamic physical exercise using the large muscle groups, involving a high workload, oxygen consumption, or heart rate, where the body achieves a balance between the production and elimination of lactate (Hoff, 2005). There are several concepts for determining the AT. One of them, proposed by Sjodin and Jacobs, is a 4-millimolar lactate threshold (Brooks, 1985). In our study, there was no significant increase in the athletes' lactate levels immediately after the match (P1), and none of the competitors would reach the 4-millimolar lactate threshold. Currently, the lactate threshold is more often determined individually based on the dynamics of blood lactate changes (Veale, Pearce, 2009). Therefore, it seems that to successfully determine the AT, a modern training strategy should be based on an individualized approach to testing for lactate concentrations during exercise. However, we did observe significant differences in the lactate concentrations across the whole study group of players when testing 24 hours after the match. These observations may encourage trainers to change their ideas about training with the aim of improving player performance during a football match.

The enzyme creatine kinase is released into the bloodstream in large quantities by damaged skeletal muscle tissue. In our study, we observed that while CK was elevated in most of the players prior to the match, in all the players, both directly after the match and 24 hours after the match, the CK activity had increased significantly. This symptom is typically associated with physical exertion. Hunkin et al. observed in their study of football players, that increases in creatine kinase from an elevated pre-match baseline is likely an indication of residual muscle damage. The authors also observed that CK monitoring may be most appropriately used with younger inexperienced players to help them optimize their training and to better prepare for match play (Hunkin et al., 2014).

In our study, we also observed that the CK value did not change significantly in the 24 hours immediately following the match when compared with the CK levels recorded at the time point immediately after the match. In the Russel et al. study, a significant increase in CK levels was also observed in the 24 hours after the football match, but this was not observed 48 hours after the match (Russell et al., 2016). Therefore, based on our research, the CK value increases significantly after football matches and does not change significantly over the next 24 hours. Considering the constant metabolism of healthy footballers tested in our work, it is possible form a practical conclusion that the CK assessment of football players in order to optimize training is justified and that it is possible at any time within 24 hours of the end of the match. However, based on other studies, it is known that the level of post-exercise CK activity depends on the competitor's level of training and on the intensity of exercise, and that CK increases significantly in cases of acute physically over-exertion (Halson et al., 2003; Hecksteden, et al., 2016). In people who are better-trained, the creatine kinase values are lower. In our study, those players whose physical exertion had to be sustained over the longer period (playing in the match for 90 minutes) were characterized by higher CK values than their other team-mates. If a player's CK levels are assessed periodically during the training cycle, this can help the coach to individualize the training program.

In our study we also observed significant changes in some hematological indices under the influence of the athlete's exercises. Across the entire group of athletes, we observed an increase in the leukocyte count immediately after exercise. To a large extent, the increase in the leukocyte count is associated with increased blood flow and is caused by the release of neutrophils into the blood (Hecksteden et al., 2016; Simpson, 2013). It is also believed that the sympathetic system's increased activity and the resulting catecholamine secretion, coupled with cortisol's indirect role, are responsible for leukocyte mobilization during exercise (Simpson et al., 2015). In our study we also observed that each athlete's WBC count changed in proportion to changes in their cortisol levels, and this was observed across the whole group, and at each time point that we tested for the biomarkers. It is known that exercise increases the activity of the hypothalamic-pituitary-adrenal axis, causing the release of the hormones corticotropin and adrenocorticotropic, and elevated cortisol levels; and this is known to affect leucocytosis and increase the percentage of neutrophils within a few hours after exercise is

discontinued (Simpson et al., 2015; Okutsu, et al. , 2008; Okutsu et al., 2005). It is believed that this mechanism influences the regeneration of tissues that can be damaged during physical exertion (Okutsu et al., 2008).

Our results showed that the body's compensatory mechanisms are functioning correctly in the observation that at each time point when blood samples were taken, the players had low erythrocytes, hemoglobin and hematocrit. Similar observations were made in the players, riders and athletes (Malcovati, et al., 2003; Rietjens et al., 2002; Schumacher et al., 2002 ; Lippi et al., 2014). In our study, the variation in the RBC, HGB and HCT were 'plus' 5.2%; 5.3% and 3.9% at each time point (P1, P2 and P3, respectively) and was a statistical significance. However, it is worth recalling that under normal physiological conditions, the variation in RBC, HGB and HCT values is only ± 3% (Ricós et al., 1999). Hence, when considering the results of RBC, HGB and HCT analysis in the blood cell morphology that we have discussed here, it is important to note that the range and variability in athletes after exercise will be greater than what will be found in the general population. In the work of Montero et al., it was observed that an initial decrease of HCT precedes an increase in endogenous erythropoietin, and in repeated and longer-duration training sessions this results in RBC increasing linearly to cortisol increases (Montero et al., 2017). The significantly statistically significant decrease in HCT after the match observed in our study (at points P2 and P3) compared to the pre-match HCT values (P1) may indirectly indicate the loss of fluid from the intravascular vessels of players due to dehydration during the match. These observations should be confirmed in future studies using more accurate methods to assess player hydration (e.g. body weight assessment based on bioimpedance).

Moreover, in our study we observed that PLTs increased significantly at the end of the match, while they decreased significantly 24 hours after the match. A high PLT count immediately after physical activity of a similar level to participating in a half-marathon was also observed by Lippi et al. (Lippi, et al., 2014). It is now known that haemostasis has a direct impact on the health benefits of regular exercise and physical activity. Acute and strenuous exercise is associated with transient hypercoagulability. On the other hand, regular physical exercise, especially in trained people, reduces the body's ability to aggregate platelets, lowers clotting factors and strengthens fibrinolysis, which in general have inhibitory effects on coagulation (Lippi, Maffulli, 2009). Probably this mechanism explains the statistically significant decrease in the PLT count after physical exercise that we observed in our study.

An interesting observation resulting from our study is the significant decrease in cortisol 24 hours after the match in the group of players playing for the longest duration (i.e., for 90 minutes of the match). In addition, cortisol levels were lower for all the athletes who played for the longest time period, across all three test points of the study. Other researchers have observed reduced blood cortisol levels in athletes who were diagnosed with acute physically over-exertion (Grandys, et al., 2016; Lucía et al.2001; Arazi et al., 2013)

On the other hand, a decrease in cortisol after physical exercise was also described as an adaptive mechanism, demonstrating an increased tolerance to the stress associated with greater physical effort, and was observed to be a long-term preventive of cardiovascular diseases. In our study, in the group with 90 minutes of game time, a significant decrease in cortisol below 356nmol/l was observed at 24 hours after the match and was accompanied by a significant increase in CK, perhaps indicating acute physically over-exertion in that group of players. In the group of four athletes who played for shorter periods (30-75min), analysis cortisol levels and CK at P3 (24 hours after the match), we observed training potential.

The presented paper has potential limitations. Study limitations are related to the lack of individual player work done during the match (e.g. intensity of the run, total distance). Another limitation of our study results from a small number of the subjects, which may affect the results of the statistical analysis. Respectively, the results of our research should be validated in a larger group of football players.

Conclusions

Most of the blood-borne biomarkers assessed changed significantly in relation to the football match. Based on the range of results in the RBC and HGB counts, football players should consider the higher variability of their results when compared with those of the general population (±3% vs ±5.2%).

Due to the significant increase in CK immediately after the match and no significant difference in CK activity between the time point immediately after the football match and that 24 hours following, determining the CK levels of the players in order to individualize and optimize their training is justified, and is possible at any time within 24 hours of the match. An increase in CK activity above 500UI/L and a reduction in cortisol below 356.04nm/L in the blood of athletes 24 hours after a football match appears to be related to optimal strength, conditioning and sports medicine programs.

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