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A Practical Approach to Monitoring Biomarkers of Inflammation and Muscle Damage in Youth Soccer Players During a 6-Month Training Cycle

by

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The aim of the study was to determine the effects of a 6-month training cycle on muscle damage and inflammatory markers in youth male soccer players. Twenty-one soccer players were tested four times: at the beginning (T1) and immediately after the pre-season period (T2), in the middle (T3) and at the end of the competitive period (T4). Muscle damage and inflammatory markers were determined in blood taken 36 hours after the match. Throughout the training cycle significant increases ($p < 0.05$) of creatine kinase (T1: 254.4 $U \cdot L^{-1}$; T4: 304.2 $U \cdot L^{-1}$) and lactate dehydrogenase (T1: 382.8 $U \cdot L^{-1}$; T4: 453.2 $U \cdot L^{-1}$) activities were observed. Significant changes ($p < 0.05$) in platelet count (T1: 210.5·$10^9 \cdot L^{-1}$; T4: 234.2·$10^9 \cdot L^{-1}$), percentage of lymphocyte (T1: 39.80%; T4: 42.97%), monocyte (T1: 6.88%; T4: 9.99%) and granulocyte (T1: 53.32%; T4: 47.05%) as well as in granulocyte-to-lymphocyte (T1: 1.41; T4: 1.17) and lymphocyte-to-monocyte (T1: 6.21; T4: 4.46) ratios were noted. The correlation analysis revealed statistically significant relationships ($p < 0.05$) between: myoglobin and the percentage of leukocyte subpopulations and the granulocyte to lymphocyte ratio; lactate dehydrogenase and the percentage of monocyte; lactate and leukocyte count. In conclusion, the reported muscle damage and inflammatory markers in T3 and T4 indicate the need for fatigue status monitoring in youth soccer players, especially in the competitive period. Moreover granulocyte to lymphocyte and lymphocyte to monocyte ratios proved to be sensitive to fatigue changes and therefore can provide coaches and sport scientists with a broader perspective on the biochemical monitoring of training status in soccer players.

***Key words**: fatigue, soccer, training loads, creatine kinase, myoglobin, leukocyte count.*

Introduction

Physical training involves functional and structural adaptations aimed at improving performance of the body. Irrespective of a training type (strength, endurance, etc.), these adaptations are global and concern, among others, the muscular, skeletal, nervous, cardiovascular, respiratory, and hormonal systems. The underlying causes of the adaptations are homeostasis disorders resulting from physical activity (Coffey and Hawley, 2007). Both single and repeated physical exercises are physiological stress factors, which are responded to by tissue damage and, in turn, may initiate inflammation (Romagnoli et al., 2016). Exercise-induced inflammation, especially in the skeletal muscles, is one of the main factors that stimulate the body to recover and adapt to exercise (Toumi and Best,

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2003). For the desired training adaptations to appear, it is necessary to include appropriate recovery periods in training. Disproportions between exercise and rest can lead to chronic fatigue and even to the overreaching/overtraining syndrome (Meeusen et al., 2013).

Soccer is one of many sports in which both training and competitive loads have increased significantly in recent years. Greater volume of loads are applied to soccer players of different competitive levels, in both youth and senior age categories. Excessive training can not only lead to biochemical disturbances, muscle damage and inflammation, thus causing a decrease in performance (Heisterberg et al., 2013), but also to developmental and health issues such as increased susceptibility to injury or immune deficiencies (Djaoui et al., 2017). On the other hand, the use of a specific type of soccer training periodization does not have to increase the activity of markers of muscle damage and decrease the level of physical performance throughout the season (Aquino et al., 2016). Adverse changes in the body can be detected by systematic observation of various hematological and biochemical markers. The former are muscle damage markers, i.e. creatine kinase (CK), lactate dehydrogenase (LDH), or myoglobin (MGB). In sports practice they are mainly used as markers of post-match acute fatigue, or less frequently, as chronic fatigue markers after cumulative training loads (Fransson et al., 2018; Heisterberg et al., 2013; Meyer and Meister, 2011; Silva et al., 2014, 2018; Varley et al., 2017). Acute soccer match- or training-related fatigue is also determined on the basis of levels of metabolites associated with physical activity, such as lactate (La), urea, bilirubin, creatinine, and inflammatory response markers: C-reactive protein (CRP), interleukin-1⊚⊚⊚ interleukin-6, TNF-⊚⊚ and anti-inflammatory proteins, e.g. interleukin-10 (Brancaccio et al., 2010). There have been few reports on the use of these markers for chronic fatigue monitoring in female and male soccer (Anđelković et al., 2015; Becker et al., 2020; Coppalle et al., 2019; Heisterberg et al., 2013; Walker et al., 2019). There is thus a large research gap to be addressed with respect to health monitoring and reducing the risk of developing chronic fatigue. Moreover, the white blood cell (WBC) count and the percentages of respective leucocyte subpopulations, in particular, granulocytes (GRA) and lymphocytes (LYM), reflect the current state of the athlete's body. An elevated WBC count most often indicates an existing infection or inflammation, while a shift between different leukocyte fractions may be due to physical exercise (Anđelković et al., 2015), or may be a symptom of insufficient post-exercise recovery (Shek et al., 1995). In soccer, the WBC count is mainly used to identify acute fatigue and post-exercise recovery (Farjallah et al., 2020; Mohr et al., 2016). Granulocyte to lymphocyte (GLR), lymphocyte to monocyte (LMR) and platelet to lymphocyte (PLR) ratios are sometimes calculated to determine the degree of development of inflammation in the body. They are mainly used in cancer (Sato et al., 2012) and liver disease (Yang et al., 2018) clinical trials, and less frequently in physically active subjects (Cadegiani and Kater, 2019; Nieman, 1998; Roczniok ert al., 2013; Svendsen et al., 2016).

Inflammation and muscle damage are known to be correlated (Romagnoli et al., 2016; Souglis et al., 2015). However, to the best of our knowledge, there have been no attempts to assess the relationship between muscle damage and inflammatory markers in the context of fatigue status in youth soccer players. Furthermore, researchers have not used computational indices such as GLR, MLR or PLR to assess soccer players' fatigue status. Thus, seeking correlations between muscle damage and inflammatory markers as well as identifying changes in the mentioned computational indices may be significant for selecting appropriate markers to monitor fatigue of youth soccer players.

Therefore, the aim of the present study was to investigate the effects of a 6-month training program on muscle damage and inflammatory markers as well as to seek relationships between them, which can be used as indicators of fatigue in youth soccer players.

Methods

Participants

Twenty-nine youth male soccer players from an academy team of a second division professional club in Poland took part in the study. Due to some players' injuries during training and soccer matches, results of only 21 players (18.1 ± 1.3 years old, body height 179.6 ± 6.5 cm), who completed the training program without

sustaining serious injuries were analyzed. The inclusion criteria were that players participated in 90% of all training sessions, and trained at the club for a full 6-month period. Players had significant soccer-training experience (10.0 ± 1.9 years) and competed at the highest level in their under-19 age category. They trained five times per week (~400 min/week), and their training program involved specific exercises and playing one competitive match each week. Goalkeepers were excluded from the study as they did not participate in the same training sessions as the outfield players.

During the season all the players practiced in the soccer center. The players' nutrition and hydration habits were well controlled following the recommendations of the nutritional staff. Hence, all players followed the same nutritional and hydration protocol during the soccer season.

All participants were provided with a detailed explanation of the proposed investigation and its requirements. They were informed of the potential risks and given consent forms to sign. Parental or guardian consent was obtained for participants under 18 years of age. Participants were free to withdraw from the study at any time, without any consequences. The study protocol was fully approved by the Bioethics Committee at Poznan University of Medical Sciences prior to the study commencement (no. 937/17). The investigation conformed to the Declaration of Helsinki principles, and all health and safety procedures were complied with during the investigation.

Experimental approach

Changes in muscle damage and inflammatory markers in youth soccer players were examined for one half of the competitive season, i.e. over a six-month training period, from January to June. Chronic fatigue induced by accumulative training loads during the season was assessed. Players participated in thirteen under-19 championship matches, and four friendly matches during the pre-season period. Blood samples were collected at the beginning of the pre-season period (T1; 10th January), immediately after the pre-season period (T2; 26th February), in the middle of the competitive period (T3; 24th April), and at the end of the season (T4; 4th June). Fasting blood was taken from participants 36 hours after different matches, between 7 and 8 a.m., by laboratory staff. The day after each match was intended for passive recovery. The evaluation time points were determined with the attempt to assess the players' fatigue status at the beginning of the following training microcycle, not with the aim to assess acute fatigue after a soccer match.

Anthropometric measurements

Body mass of players (barefoot and in light clothing) was measured to 0.1 kg using a portable digital scale (WAGI Wielkopolska®, Poland). Standing body height was measured to the nearest millimeter (0.1 cm) using a stadiometer (GPM, Switzerland). Based on the players' body height and mass, the body mass index (BMI; kg·m^{-2}) was calculated.

Bioelectric impedance analysis (BIA, Tanita BC 418 analyzer, Tanita Corp., Tokyo, Japan) was used to calculate the body fat percentage (BF%) following the manufacturer's directions and procedures. This is a commonly used method in field surveys and also as a supplement to conventional anthropometry. It provides each participant with a profile including %BF, fat-free mass (FFM, kg), and total body water (TBW, kg). Evaluations were conducted between 7 and 8 a.m. Before the measurements, participants were subjected to a 12-hour fasting period and instructed to avoid heavy physical activity, alcohol ingestion, and diuretic intake. During measurements players stood erect holding the hand electrodes, with their bare feet on the BIA unit contact electrodes.

Blood collection, biochemical and hematological evaluations

Capillary blood samples were drawn from the fingertip of the non-dominant hand using a disposable Medlance® Red lancet-spike (HTL-Zone, Germany) with a 1.5 mm blade and 2.0 mm penetration depth. 65 µl of blood was collected to a heparinized capillary tube where lactate concentration (La) was determined with a blood gas analyzer (ABL90 FLEX, Radiometer, Denmark). Furthermore, 300 µl of capillary blood was collected into a Microvette® CB 300 Z tube (Sarstedt, Germany) with a clotting activator, and the separated serum was used to measure creatine kinase (CK) and lactate dehydrogenase (LDH) levels in the fresh serum. Collected samples were frozen and stored at -80°C until analyses for myoglobin (MGB) concentration were performed.

Moreover, 300 µl of capillary blood was collected into a Microvette® CB 300 tube (Sarstedt, Germany) containing K2-EDTA (EDTA dipotassium salt) as an anticoagulant in hematological measurement with a 20-parametric automated Mythic® 18 hematology analyzer (Orphée, Switzerland). Platelet (PLT) and leucocyte (WBC) counts and leucocyte subpopulations: granulocytes (GRA), lymphocytes (LYM) and monocytes (MON), were determined. The granulocyte-to-lymphocyte (GLR), lymphocyte-to-monocyte (LMR), and platelet-to-lymphocyte (PLR) ratios were calculated. Myoglobin concentration (MGB sensitivity test: 0.1 mg·L^{-1}; Cat No. EIA-3955) was determined using commercially available ELISA kits (DRG MedTek, Poland). Spectrophotometric measurements with ELISA tests were made using a multi-mode microplate reader (Synergy 2 SIAFRT, BioTek, USA). The activity of creatine kinase (CK, sensitivity test: 7.4 U·L^{-1}; Cat No. 7-220) and lactate dehydrogenase (LDH, sensitivity test: 6.6 U·L^{-1}; Cat No. 7-239) was measured using an Accent 220S automatic biochemical analyzer (Cormay, Poland).

Structure of the training program

During the winter pre-season period (8 weeks) coaches attempted to improve physical performance of youth soccer players. Players also took part in four friendly matches. Table 1 presents the general characteristics of the training cycle in terms of players' physical preparation carried out during the pre-season. A general program for the training period is presented in Table 2. Until the 13th week the team played 13 championship matches.

Statistical analysis

All data are presented as means and standard deviation (SD). The normality distribution was checked with the Shapiro-Wilk test. The differences between measurement terms for normally distributed variables were analyzed using a one-way repeated-measures analysis of variance (ANOVA) to reveal changes in the mean values of biochemical and hematological variables (MGB, LDH, La, WBC, %LYM, %GRA, GLR, PLR). When a significant effect was found, a post-hoc Tukey's HSD test was performed.

When, after the measurement, at least one variable failed to conform to the normality distribution, the non-parametric one-way repeated-measures analysis of variance Friedman test was used to examine changes in the mean values of biochemical and hematological variables (CK, %MON, PLT, LMR). When a significant effect was found, a post-hoc ANOVA Friedman test was performed. The Pearson correlation coefficient was used to check the significance of correlation between two variables with normal distribution. The Spearman's rank correlation coefficient was then applied when at least one variable did not conform to the normality of distribution. The level of statistical significance was set at $p < 0.05$. Using Cohen's criteria an effect size (ES) of ≥ 0.20 and < 0.50 was considered small, ≥ 0.50 and < 0.80 – medium, and ≥ 0.80 – large (Cohen, 1992). The obtained results were subject to statistical analysis using the 2016 Dell Statistica 13 software package (Dell Inc., Tulsa, Oklahoma, USA).

Results

No significant differences were revealed in terms of estimated body composition indicators. Table 3 presents the mean values of these variables.

Statistical analysis showed significant alterations in the WBC count and WBC subpopulations in relation to subsequent measurements (T1, T2, T3, T4). The largest effect was observed between the evaluations in the competitive period (T3 and T4) and those in the pre-season period (T1 and T2). The WBC count decreased in T4 in relation to T2 ($p = 0.006$; ES = 0.63) and T3 ($p = 0.017$; ES = 0.53). %LYM increased in T4 in relation to T2 ($p = 0.036$; ES = 0.57) and T3 ($p \leq 0.001$; ES = 0.85). A significant increase and a large effect size appeared in %MON between the measurements at T4 and T1 ($p \leq 0.001$; ES = 1.71), T2 ($p \leq 0.001$; EF = 2.16), T3 ($p \leq 0.001$; ES = 1.25), and between T3 and T1 ($p = 0.019$; ES = 0.81) and T2 ($p = 0.004$; ES = 1.33). In relation to %GRA a large effect size and a significant decrease between T4 and other evaluation points (T1 $p = 0.001$; ES = 0.83, T2 $p \leq 0.001$; EF = 1.10 and T3 $p \leq 0.001$; ES = 1.14) were observed. The PLT count differed only between T4 and T3, but the effect size was small ($p = 0.028$; EF = 0.47). A significant decrease and large effect size also occurred in LMR between T4 and T1 ($p \leq 0.001$; ES = 0.92) and T2 ($p = 0.002$; ES = 0.99) and between T3 and T1 ($p \leq 0.001$; ES = 0.97) and T2

(p= 0.003; ES = 1.09). Similar changes were observed
in GLR, but only between T4 and T2 (p = 0.010; ES = 0.76) and T3 ($p \leq 0.001$; ES = 0.95). Among inflammatory markers only in terms of PLR (p = 0.214) no significant effects were found.

A significant increase and medium effect size characterized CK activity changes between T4 and T2 (p = 0.038; ES = 0.52). As far as the LDH activity was concerned, a significant increase was also observed throughout the training period, but the effect was large (T4 and T1 $p \leq 0.001$; ES = 1.07, T4 and T2 $p \leq 0.001$; ES = 1.05, T3 and T1 $p \leq 0.001$; ES = 1.03, T3 and T2 $p \leq 0.001$; ES = 0.99). No significant alterations were found in MGB (p = 0.282) and La (p = 0.555) concentrations. All data are presented in detail in Table 4.

Table 1

Characteristics of training loads carried out during the pre-season period.

Training characteristics and objective		Week	Intensity	Volume (Duration)	Duration of effort	Repetitions	Set	Rest period	Sessions per week
Aerobic capacity/power	Aerobic capacity	1, 2, 3	70-85% MHR	15-60 min	10-15 min	3-6	2-4	Active Return to HR of 120	3-4
	Aerobic power	4, 5, 6	85-95% MHR	10-40 min	2-5 min	2-6	2-4	Active-semi-active 1:1 to 1:2 (W:R)	2-3
Anaerobic capacity/power	Glycolytic capacity	6, 7	95-100% MHR	8-15 min	30 s - 2 min	3-5	1-2	Passive-semi-active 1:1 to 1:3 (W:R) Between sets: 10 min	1-2
	Glycolytic power	7, 8	95-100% MHR of max speed	600-1000 m	8-30 s	3-4	2-3	Active-semi-active 1:3 to 1:6 (W:R) Between sets: 8-10 min	1
Speed	Glycolytic power	1, 2	80-90% max speed	-	-	-	-	-	1
	Speed – power	3, 4, 5	100% max speed	200-400 m	less than 6 s 10-40 m	4-6	3-5	Passive-semi-active 1:10 to 1:20 (1' to 3') Between sets: 4-6 min	1-2
	Speed – power	6, 7, 8	100% max speed	200-300 m	less than 5 s 10-30 m	4-5	2-4	Passive-semi-active 1:10 to 1:15 (1' to 2') Between sets: 2-4 min	1-2
Strength	Strength endurance	1,2	40-50% max strength	30-45 min	20-30 s	15-20	3-4	Passive-semi-active Between sets: 1-2 min	2-3
	Strength endurance	3,4	70-85% max strength	30-40 min	-	6-10	3-4	Passive-semi-active Between sets: 2-3 min	1-2
	Strength maximum	4,5,6	80-100% max strength	30-40 min	-	1-5	4-6	Passive-semi-active Between sets: 3-5 min	2-3
	Speed – strength (power)	7,8	50-70% max strength	30 min	-	2-5	3-5	Passive-semi-active Between sets: 2-3 min	2

MHR = maximum heart rate; W:R = work to recovery time ratio.

Table 2

Training program implemented in a standard microcycle during the competitive period.

Training day	Training characteristics	Intensity	Duration of training
Monday	Active recovery training + aerobic capacity	70–85% MHR	60–75 min
Tuesday	Power and technical and tactical training	50–70% max strength, 85–95% MHR	90 min
Wednesday	Strength/functional training + speed endurance	95–100% MHR of max speed	90 min
Thursday	Speed and power	100% max speed	70 min
Friday	Speed reaction and tactical training	70–85% MHR	70 min
Saturday	Match	85–95% MHR	90 min
Sunday	Free/passive recovery	-	-

MHR = maximum heart rate.

Table 3

Anthropometric indices of studied soccer players at four measurement points (mean ± SD).

Variables	T1	T2	T3	T4
Body mass (kg)	71.87 ± 7.87	72.40 ± 7.00	72.45 ± 6.92	72.14 ± 6.81
Body mass index (kg·m^{-2})	22.20 ± 1.14	22.38 ± 0.87	22.40 ± 0.85	22.31 ± 0.84
Body fat (%)	15.17 ± 2.37	16.21 ± 2.13	15.03 ± 2.31	13.76 ± 2.38
Fat-free mass (kg)	60.92 ± 6.74	60.67 ± 5.99	61.56 ± 6.14	62.20 ± 5.88
Total body water (kg)	44.87 ± 4.83	44.61 ± 4.24	45.41 ± 4.42	45.92 ± 4.13

Table 4

Inflammatory and muscle damage markers in studied soccer players at four measurement points (mean ± SD); n = 21.

	MARKER	T1	T2	T3	T4	*p value*
Inflammatory	WBC ($10^9 \cdot L^{-1}$)[A]	5.72 ± 1.36	6.30 ± 1.32	6.20 ± 1.50	5.42 ± 1.47	***0.003***
	LYM (%)[B]	39.80 ± 7.10	38.76 ± 6.60	36.78 ± 6.47	42.97 ± 7.98	***0.001***
	MON (%)[C]	6.88 ± 1.70	6.69 ± 0.94	8.03 ± 1.07	9.99 ± 1.94	***≤0.001***
	GRA (%)[D]	53.32 ± 7.81	54.55 ± 6.27	55.19 ± 6.95	47.05 ± 7.31	***≤0.001***
	PLT ($10^9 \cdot L^{-1}$) [E]	210.48 ± 61.42	215.62 ± 50.82	207.05 ±5 8.08	234.19 ± 57.90	***0.026***
	GLR [F]	1.41 ± 0.42	1.47 ± 0.40	1.58 ± 0.47	1.17 ± 0.39	***≤0.001***
	LMR [G]	6.21 ± 2.37	5.98 ± 1.76	4.52 ± 0.70	4.46 ± 1.28	***≤0.001***
	PLR	95.79 ± 24.33	92.93 ± 27.64	97.36 ± 34.23	105.38 ± 22.66	*0.214*
Muscle damage	MGB ($mg \cdot L^{-1}$)	73.01 ± 20.64	72.51 ± 27.13	78.91 ± 20.27	79.72 ± 19.74	*0.282*
	CK ($U \cdot L^{-1}$)[H]	254.41 ± 73.78	240.00 ± 87.09	301.57 ± 129.41	304.24 ± 149.93	***0.013***
	LDH ($U \cdot L^{-1}$) [I]	382.81 ± 49.65	382.05 ± 55.22	440.52 ± 62.23	453.24 ± 78.88	***≤0.001***
	La ($mmol \cdot L^{-1}$)	1.32 ± 0.29	1.30 ± 0.39	1.38 ± 0.24	1.26 ± 0.28	*0.555*

CK – creatine kinase; GLR – granulocytes to lymphocytes ratio; GRA – granulocytes; La – lactate; LDH – lactate dehydrogenase; LMR – lymphocyte to monocyte ratio; LYM – lymphocytes; MGB – myoglobin; MON – monocytes; PLR – platelet to lymphocyte ratio; PLT – platelets; WBC – white blood cells.

Effect size (ES)

Significant differences ($p < 0.05$) between:

A *– T4<T2 (ES = 0.63); T4<T3 (ES = 0.53)*

B *– T2<T4 (ES = 0.57); T3<T4 (ES = 0.85)*

C *– T1<T4 (ES = 1.71); T2<T4 (ES = 2.16); T3<T4 (ES = 1.25); T1<T3 (ES = 0.81); T2<T3 (ES = 1.33)*

D *– T4<T1 (ES = 0.83); T4<T2 (ES = 1.10); T4<T3 (ES = 1.14)*

E *– T3<T4 (ES = 0.47)*

F *– T4<T2 (ES = 0.76); T4<T3 (ES = 0.95)*

G *– T3<T1 (ES = 0.97); T3<T2 (ES = 1.09); T4<T1 (d = 0.92); T4<T2 (ES = 0.99)*

H *– T2<T4 (ES = 0.52)*

I *– T1<T3 (ES = 1.03); T2<T3 (ES = 0.99); T1<T4 (ES = 1.07); T2<T4 (ES = 1.05)*

Table 5

Correlations between inflammatory and muscle damage markers (n = 21).

	MARKER	Muscle damage				Inflammatory							
		MGB[a]	CK[b]	LDH[a]	La[a]	WBC[a]	%LYM[a]	%MON[b]	%GRA[a]	PLT[b]	GLR[a]	LMR[b]	PLR[a]
Muscle damage	MGB[a]		.255#	.115	-.095	-.170	.246#	.287#	-.290#	-.060	-.297#	.042	-.060
	CK[b]			.137	.046	-.140	.116	.213	-.150	.207	-.133	-.104	.163
	LDH[a]				-.042	-.000	.105	.307#	-.180	.000	-.132	-.212	-.090
	La[a]					.259#	.140	-.000	-.129	.189	-.110	.110	-.157
Inflammatory	WBC[a]						-.533#	-.194	.926#	.273#	.544#	.039	-.190
	%LYM[a]							.141	-.967#	-.091	-.971#	.507#	-.266#
	%MON[b]								-.363#	.147	-.255#	-.710#	.267#
	%GRA[a]									.033	.968#	-.301#	.190
	PLT[b]										.072	-.154	.697#
	GLR[a]											-.431#	.246#
	LMR[b]												.229#
	PLR[a]												
Mean		76.04	275.06	414.65	1.31	5.91	39.58	7.90	52.53	216.83	1.41	5.29	97.86
SD		21.00	115.77	69.518	0.31	1.44	7.29	1.96	7.70	57.13	0.44	1.81	27.47

CK – creatine kinase; GLR – granulocytes to lymphocytes ratio; GRA – granulocytes; La – lactate; LDH – lactate dehydrogenase; LMR – lymphocyte to monocyte ratio; LYM – lymphocytes; MGB – myoglobin; MON – monocytes; PLR – platelet to lymphocyte ratio; PLT – platelets; WBC – white blood cells.

[a] Pearson correlation; [b] Spearman's rank correlation; # $p < 0.05$.

Table 5 shows the correlations between muscle damage markers (MGB, CK, LDH, La) and inflammatory response markers (WBC with their percentage subpopulations: PLT, GLR, LMR, PLR). The correlation analysis revealed a few statistically significant relationships ($p < 0.05$) between the indicators of muscle damage and inflammation (MGB and %LYM: $p = 0.024$; r = 0.246, MGB and %MON: $p = 0.008$; r = 0.287, MGB and %GRA: $p = 0.007$; r = -0.290, MGB and GLR: $p = 0.010$; r = -0.297, LDH and %MON: $p = 0.004$; r = 0.307, La and WBC: $p = 0.017$; r = 0.259). No significant correlation between the activity of muscle enzymes (CK, LDH) and measured inflammatory markers was found.

Discussion

The aim of this study was to determine the effects of a 6-month training cycle on muscle damage and inflammatory markers which can be used as indicators of recovery status in youth soccer players. Our results showed significant

alterations in the levels of muscle damage and inflammatory markers throughout the monitored training period. Specifically, these changes were mainly seen in the LDH and CK activity between the evaluation points during the competitive and pre-season periods (LDH activity in T3 and T4 was higher than in T1 and T2, and CK activity was higher in T4 than in T2). With regard to the inflammatory markers, during the monitored training period significant increases in the PLT count and %LYM and %MON as well as a decrease in GLR, LMR and %GRA were found. The correlation analysis showed a few statistically significant relationships between the indicators of muscle damage and inflammation (MGB and %LYM, %MON, %GRA, GLR; LDH and %MON). It can be concluded that the applied training periodization caused biochemical disturbances which can be associated with increasing fatigue in the monitored youth soccer players.

A number of studies have so far focused on investigating acute and residual fatigue markers after a soccer match (Marqués-Jiménez et al., 2017; Rampinini et al., 2011; Romagnoli et al., 2016; Silva et al., 2018) or after a single training session (Thorpe et al., 2017). During congested competition schedules in which matches can be played even every three days, players' complete physical and mental recovery may not be achieved (Marqués-Jiménez et al., 2017; Nédélec et al., 2015). Even optimized recovery strategies do not guarantee chronic fatigue prevention. There have been no studies on the biochemical monitoring of athletes' fatigue over a prolonged period of time with regard to relationships between indicators of muscle damage and inflammation.

The confirmed markers of muscle damage include the CK, MGB and LDH blood levels, whereas inflammation is linked to an increase in blood concentration of intracellular proteins, leukocyte count, cytokines, CRP, uric acid or urea (Romagnoli et al., 2016; Souglis et al., 2015). The increase in the MGB, LDH and CK blood levels results most often from myocyte damage during intense prolonged training, incomplete recovery, and increased oxidative stress (Becatti et al., 2017). In the present study this was observed during the competitive period, when physical stress associated with championship matches was added to standard training loads. At T3 and T4, there was a statistically significant increase in LDH and CK activity as compared with the evaluations at T1 and T2 (Table 4). Becatti et al. (2017) also observed a significant increase in LDH and CK activity in the competitive period in relation to the pre-season period. Silva et al. (2014) noted significantly higher values of CK in the midseason and their decline due to reduced training loads off-season. On the other hand, training periodization with an emphasis on technical-tactical ability can reduce the plasmatic activity of CK and LDH with a simultaneous increase in youth soccer players' on-field performance (Aquino et al., 2016). Similarly to Becatti et al. (2017) and Silva et al. (2014), the present study also revealed an increase in MGB concentration in the competitive period (T3, T4). The relatively small variation in MGB concentration at subsequent evaluation points (8-10%), and the lack of statistical significance of the differences in comparison with other muscle damage indicators (CK and LDH), may in this case be explained by our assumed sample taking time, which was 36 hours after the match (significant time after the peak in MGB concentration), i.e. at the beginning of the next training microcycle.

Mechanical muscle damage results in the development of inflammation. Inflammatory response after soccer matches has been well documented (Mohr et al., 2016; Romagnoli et al., 2016; Souglis et al., 2018). Many authors reported an increase in WBC and the granulocytes count after a match, which could be sustained even from 24 to 48 hours (Mohr et al., 2016; Romagnoli et al., 2016; Tsubakihara et al., 2013). Mohr et al. (2016) associated the post-match higher inflammatory response with a shorter recovery period. In their experiment, the WBC count was higher immediately after the second match and 24 hours after its end, than after the third match when the recovery period was 3 and 4 days, respectively. Additionally, Tsubakihara et al. (2013) stated that despite the increase in the WBC and neutrophils count, immunosuppression occurred as indicated by a reduced neutrophil phagocytic activity and lymphocyte function. As with muscle damage, monitoring inflammatory markers seems to be significant for fatigue status indication. Research on the immune status of soccer players during training periodization revealed decreases in their

leucocyte levels during the preparation and the final periods of the season as well as decreases in the lymphocyte count and neutrophil function throughout the entire season (Heisterberg et al., 2013). The present study reported an increase in the WBC count in T2 and T3, with no significant changes in %GRA. At the end of the competitive period, a considerable decrease in the WBC and %GRA was observed, with a significant increase in the %LYM and %MON. Additionally, the highest number of PLTs was observed at T4 (Table 4). Changes in the above markers may suggest a decrease in immunological potential and an increase in inflammatory response. Becatti et al. (2017) in their research conducted over a similar period as the present study did not observe any significant changes in the WBC count, percentage of mono- and lymphocytes nor the PLT count. Contrary to our study results, Anđelković et al. (2015) and Becatti et al. (2017) showed a significant increase in the WBC count and percentage of neutrophils, and a decrease in the lymphocyte count after 90 days of regular soccer training in a competitive season. It is worth noting that the values of all tested variables in the above studies were within the reference range. The post-exercise increase in the circulating neutrophil count is, on the one hand, a result of demargination of cells from endothelial tissues and/or bone marrow, and on the other hand, is part of the phagocytic response to exercise-induced muscle tissue damage (Simpson et al., 2015). Nieman (1998) suggested that the GLR could be a useful indicator of both the intensity of exercise and recovery capacity. In the later studies, it was found that, among athletes, GLR could be one of the markers of fatigue status and even overreaching/overtraining syndrome (Cadegiani and Kater, 2019; Svendsen et al., 2016). GLR usually drops to reference values within 6 to 9 hours after exercise, whereas in situations where physical activity (training) was particularly prolonged and intensive, GLR can be elevated even after 24 hours post-exercise (Gleeson, 2002). The rise of GLR at T3 in the present study may indicate prolonged post-exercise muscle damage and inflammation. On the other hand, a significant decrease in GLR at T4 may indicate an impairment of the immune response system caused by residual fatigue after the entire soccer season (Silva et al., 2018). The LMR is often used as a blood inflammatory biomarker under clinical conditions (Kumarasamy et al., 2019). The present study revealed a systematic decrease in this ratio following the six-month training cycle of soccer players. Despite the lack of reports on the application of this ratio among athletes, the present study indicates that LMR may be used for post-training evaluation of the immune response and, consequently, of athletes' fatigue status.

It is well known that both single and cumulative training loads affect muscle damage and inflammatory markers (Romagnoli et al., 2016; Silva et al., 2018; Souglis et al., 2015). However, the literature to date has not addressed the issue of seeking relationships between these indicators. Some authors have investigated relationships between fatigue status indicators and match performance or physical performance (Aquino et al., 2016; Heisterberg et al., 2013; Walker et al., 2019), or between subjective and objective indicators of fatigue, e.g. RPE or REST-Q SPORT, and biochemical markers (Coppalle et al., 2019). Our correlation analysis was aimed at determining the relationships between muscle damage and inflammatory biomarkers in order to identify groups of variables that could be marked together, in order to make a more complete analysis of the fatigue status in youth soccer players. Our data revealed significant positive correlations between %LYM and MGB, as well as between %MON, MGB and LDH. In addition, significant negative correlations between MGB and %GRA, as well as between MGB and GLR were found. It can be thus suggested that in the research model adopted in the present study, the markings of the above mentioned indicators can complement each other. In addition, it is believed that further search for the optimal sample taking time in order to optimize the study results is necessary.

Monitoring training loads in sport is aimed, on the one hand, at determining the response of the athlete's body to single training loads (acute fatigue) and, on the other hand, at determining the impact of cumulative training loads on chronic fatigue in order to optimize the training process, maximize sport performance, and prevent injuries and the overreaching/overtraining syndrome. The choice of the means and methods of monitoring the training process depends on whether acute or

chronic fatigue is to be assessed. This is crucial because achieving a specific goal requires the selection of specific indicators that are not entirely in line with both types of fatigue. The choice of biomarkers, on the other hand, determines the optimal time of evaluation, which can range from a few minutes (e.g. La) to several hours (CK, LDH). Under experimental conditions, muscle damage and inflammatory markers can be measured multiple times up to 72 hours after specific exercise in order to determine a player's fatigue or recovery status.

In a standard training microcycle lasting usually 7 days, 2-3-day rest periods cannot be afforded. From a practical point of view markers with shorter peaks of post-exercise blood secretion (max 24-36 h) should be used in soccer training conditions. It is worth noting that in the analysis of biochemical markers in accordance with the accepted standards of 48 or even 72 hours after the match, the de facto measurements are made after loads of the next training microcycle are already implemented. In practice, our study is more beneficial because it allows an assessment of players' fatigue status before the start of the next training microcycle.

Limitations of the study

There are certain limitations of the present study. The authors did not take into account specific internal and external training loads during the study period. It is recommended that further research be performed with various training load measures including HR, sRPE, interview questionnaires, or the GPS system. Correlating data such as sRPE or match running performance with biochemical fatigue status markers may shed a new light on the control of training loads and the health condition of soccer players during the training program.

Conclusions

Long-term training, even with the use of objective measurements of external training loads, affects the level of chronic fatigue in youth soccer players. It therefore seems appropriate to supplement the monitoring of players' training loads with the evaluation of internal training loads. The results of the present study show that such indicators certainly include muscle damage markers: CK and LDH activity, as well as hematological markers, i.e. WBC, their subpopulations and PLT. Importantly, computational indices such as GLR and LMR, previously used mainly as inflammatory markers under clinical conditions, proved to be sensitive to fatigue changes in the studied soccer players. Our data also indicate that during the whole training periodization, but especially in the competitive period, optimal fatigue status monitoring in youth soccer players should be carried out. In the competitive period, which involves accumulated training and match exposure, maximum fatigue response is recorded as manifested in the levels of muscle damage (CK, LDH) and inflammatory markers (WBC, %LYM, %MON, %GRA, GLR and LMR).

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