

# Hematological and muscle damage values in amateur players during football season

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## Abstract

**Aim:** Acute intensive physical exercise induce the hematological changes in circulation. However, the effect of football training on hematological and biochemical parameters during the football season is still limited. The present study aimed to investigate the effects of routine football training on physiological parameters namely: hematological, biochemical and iron levels during the pre and competitive match season.

**Materials and Methods:** Amateur football players (n=15) participated in this study. The blood samples were collected and physical performance parameters assessed twice during pre and end of the first league between August and December. The players also performed Cooper run test, 30 m sprint tests. In addition, the hematological and biochemical parameters were analyzed by taking blood samples during pre and end of the first league.

**Results:** Football training resulted in significant decreased erythrocyte (RBC) concentration ( $p<0.001$ ), hemoglobin level (Hb) ( $p<0.01$ ), mean corpuscular hemoglobin concentration (MCHC) ( $p<0.01$ ), lymphocyte (Lymph), ( $p<0.05$ ) unlike the level of neutrophil (Neut), ( $p<0.001$ ), mean corpuscular volume (MCV) ( $p<0.05$ ), mean platelet volume MPV ( $p<0.001$ ) values were increased at the end of the league. The biochemistry parameters of the lactate dehydrogenase (LDH) ( $p<0.001$ ) and urea ( $p<0.01$ ) concentrations were increased whereas the level of alanine aminotransferase (ALT), ( $p<0.05$ ) was decreased at end of the season. The creatine kinase (CK) and paraoxonase (PON1) activity was unaltered during the football training season. The level of the unsaturated iron binding capacity (UIBC) increased ( $p<0.05$ ) and iron (Fe) level decreased end of the season ( $p<0.001$ ). The level of the Leukocyte (WBC), serum ferritin were unaltered during the pre and at the end of the season ( $p>0.05$ ). Moreover, football training had no effect on physical performance tests results ( $p>0.05$ ).

**Conclusion:** Football season mostly had effects on hematological and iron parameters with LDH, urea; this may be related the football training intensity.

**Keywords:** Amateur players; damage parameters; football training; football season

## INTRODUCTION

Football is an aerobic and anaerobic characteristic sport, it contains moderate or short distances with high intensity sprinting with jumping (1,2). Moreover, football training requires a high number of explosive movements such as accelerations, decelerations, changes of direction (3), jumps as well as having a powerful eccentric action, which may result in muscle damage (4). Professional football players have a long competitive season including training, recovery and competition period. For instance, football players may participate in >70 matches/season, 3 to 6-day training a week with 10 month in one season. This intensity may increase in injuries and decline performance due to an increase inflammation (5). Usually, muscle metabolism parameters as a CK, LDH and myoglobin are increased after the strenuous physical activity, these

variables may indicate an index of the cellular and tissue damage (6).

In football training session, muscle damage also has been linked to increased inflammation and depleted glycogen stores of the players (7). This is supported by the reported elevation of plasma creatine kinase activity (CK), and various inflammatory markers (8,9), including AST, ALT, creatinine and urea (6). Moreover, it has been reported that the upper limit for the plasma CK activity is approximately about 1.338 U/L of the football players throughout the championship (10). So, CK activity has been suggested as an early indicator of player fatigue and (10) a potential marker of the player recovery status (11). Also, it has been reported that the plasma CK level and myoglobin level increased 18-20 hours after the ninth game of the season, which returned to baseline level 40-

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42 hours after competition in National Collegiate Athletic Association (NCAA) Division I football team players (12).

Furthermore, it has been shown that leucocyte count increased during competition season compared to the demanding preseason, this highlights the physiological stress of multiple matches played in football (9). However, the majority of studies have investigated the recovery process from football matches, and have only followed the response following a single game (13,14).

Iron is also another important parameter used for detection of physical performance. The prevention of iron deficiency plays a key role in determining fitness performance level because iron is an essential component of hemoglobin. The deficiency of hemoglobin iron causes a decrease in oxygen transport to exercising muscles, thereby reducing physical work performance. Previous studies compared Hb, blood morphology, blood volume (15), as well as iron metabolism between endurance and power based sport disciplines. Related studies have indicated that exercise type, and intensive training can lead to decreased iron stores in endurance sports, due to the 'traumatic' side-effect of contact sports (15). Also, athletic training induced iron deficiency is commonly detected in athletes, it has been observed that erythrocyte numbers, hemoglobin levels and hematocrit values were significantly decreased after intensive training period (16, 17), particularly in endurance sports. However, evidence for these relationships in team sports player is still limited (18). Limited studies have shown that the red blood cells did not change significantly during the football training season (pre-season, competitive season and post season) of players (19,20).

The Paraoxonase 1 (PON1) is a protein which protects against oxidative modification and it is a part of endogenous antioxidant system which is modulated by physical activity (21). Related studies have not yet confirmed the physical exercise affects PON1 activity so far. Moreover, the effect of the football training on hematological and biochemical parameters is still limited as well as PON1 activity during the season.

Therefore, the aim of this study was to compare the effect of football training on hematological and biochemical damage parameters of the pre and end of the season in amateur football players.

## MATERIALS and METHODS

### Selection of subjects

Fifteen male amateur football players (age 18-35 years, body height  $178.6 \pm 1.4$  cm, body weight  $78.13 \pm 2$  kg) were recruited from Amateur Club volunteered to participate in this study. All players performed physical performance test and resting blood samples were obtained in similar times in pre-season (August) and end of the first league (December). This study was approved by the local research ethics committee (2017/109) and a signed informed consent was obtained from all subjects

in accordance with the Declaration of Helsinki. The participants carried out normal football training program between August and December. The players trained every day of the week during the 8 weeks in pre-season, after that the training frequency was reduced to 5 days a week during competitive season.

### Hematological and biochemistry analysis

The participants were asked to rest from training the day before the sampling and the tests and they were asked to consume the same foods and fluids at the same time from 12 h prior to each subsequent test day. Before blood sampling the athletes rested on a bed for at least 30 min and blood samples were taken from the athlete in supine position from the antecubital vein by a qualified laboratory technician using the Vacuette system and collected in tubes containing EDTA K 3 (3 ml) for hematological and analysis. Hematological analysis was performed using fully automated Blood Cell Counter Gen-S (Beckman Coulter, Coulter Corporation, USA). The following hematological variables were determined: hemoglobin (Hb), hematocrit (Hct), red blood cell count (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin content (MCHC), mean corpuscular volume (MCV), white blood cells count (WBC), neutrophils (Neut), lymphocytes (Lymph), and mean platelet volume (MPV).

For analyses of serum Fe, IBC, and ferritin, blood samples were collected without any additive and after centrifugation sera were stored at  $-20$  °C until analyzed. Iron and IBC were measured spectrophotometrically in an Advia 1800 analyzer (Siemens Healthcare, Erlangen, Germany) and ferritin was measured by immunoturbidimetric assay in an OlympusAU400 analyzer (Beckman Coulter, Brea, CA, USA).

Following centrifugation at 825 g for 10 min, serum was analyzed for ALT, AST, CK, LDH and PON1 activities using commercially available kits in a chemistry autoanalyser (Cobas Integra 800; Roche Diagnostic GmbH; Mannheim, Germany).

### Statistical analysis

Data are presented as mean  $\pm$  SE. A one-sample Kolmogorov–Smirnov test was used to determine data normality (since data normality was verified, a nonparametric test was not necessary). All parameters data were analyzed using a paired sample t-test. Significance was accepted at  $P \leq 0.05$ . The SPSS was used for all analyses (SPSS for Windows, version 15.0, Chicago, IL, USA).

## RESULTS

The football players physical performance characteristics are presented in Table 1. No significant differences in body weight (kg), Cooper test score (m) and 30 m sprint test results were recorded.

The Hematological parameters of the RBC count ( $p < 0.001$ ), hemoglobin level ( $p < 0.01$ ), MCHC ( $p < 0.01$ ), Lymph ( $p < 0.05$ ) concentration were significantly decreased at the end of the league whereas the level Neut, ( $p < 0.001$ ),

MCV ( $p < 0.05$ ) and MPV ( $p < 0.001$ ) values were increased. However, the WBC count were not significantly changed by football training or match season (Table 2).

**Table 1. Physical characteristics of the football players**

	Pre-Season (Mean $\pm$ SE)	End of the Season (Mean $\pm$ SE)	P
Weight (kg)	78.13	78.56	0.45
Cooper test (m)	2600	2650	0.67
30 m sprint test (sn)	4.29	4.33	0.34

Values are expressed as mean (n = 15). P > 0.05

**Table 2. Physical characteristics of the football players**

Parameters	Pre-Season (Mean $\pm$ SE)	End of the Season (Mean $\pm$ SE)	P
WBC $10^3/\mu\text{L}$	7.33 $\pm$ 0.45	8.35 $\pm$ 1.1	0.65
Neut $10^3/\mu\text{L}$	4.17 $\pm$ 1.69	5.65 $\pm$ 3.8**	0.001
Lymph $10^3/\mu\text{L}$	2.32 $\pm$ 0.48	1.89 $\pm$ 0.47*	0.05
RBC $10^3/\mu\text{L}$	5.29 $\pm$ 0.32	4.82 $\pm$ 0.23**	0.001
HGB $10^3/\mu\text{L}$	15.71 $\pm$ 0.88	14.51 $\pm$ 0.80*	0.01
HCT (%)	43.8 $\pm$ 9	43.07 $\pm$ 2.63	0.78
MCHC $10^3/\mu\text{L}$	34.31 $\pm$ 0.45	33.7 $\pm$ 0.66*	0.01
MCV $10^3/\mu\text{L}$	86.42 $\pm$ 2.75	89.26 $\pm$ 3.18*	0.01
MPV $10^3/\mu\text{L}$	8 $\pm$ 0.71	9.48 $\pm$ 1.03**	0.001

Values are expressed as mean  $\pm$  SE (n = 15). \*P  $\leq$  0.05; \*\*P < 0.001. WBC: White blood cells; Lymph: Lymphocytes; Neut: Neutrophils; RBC: Red blood cells; HGB: Hemoglobin; HCT: Haematocrit; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; MPV: Mean platelet volume

**Table 3. Blood chemistry variations of players during pre and end of the season**

Parameters	Pre-Season (Mean $\pm$ SE)	End of the Season (Mean $\pm$ SE)	P
Urea (mg/dl)	26.86 $\pm$ 7.19	32.36 $\pm$ 8.04*	0.01
LDH (U/L)	159.45 $\pm$ 36.73	208.27 $\pm$ 52.75**	0.001
CK (U/L)	151.13 $\pm$ 132.19	215.27 $\pm$ 126.95	0.74
ALT (U/L)	21.90 $\pm$ 11.18	13.90 $\pm$ 3.41*	0.05
PON1 (U/L)	174.6 $\pm$ 29.4	154 $\pm$ 29.9	0.66
<b>Iron related parameters</b>			
Fe (mg/dl)	146.68 $\pm$ 46.82	77.72 $\pm$ 44.93**	0.001
Ferritin (ng/dl)	53.51 $\pm$ 25.8	43.92 $\pm$ 1.1	0.80
UIBC ( $\mu\text{dL}$ )	181.90 $\pm$ 66.04	241.09 $\pm$ 44.13*	0.05

Values are expressed as mean  $\pm$  SE (n = 15). \*P  $\leq$  0.05; \*\*P < 0.001. LDH: Lactate dehydrogenase; CK: Creatin kinase; ALT: Alanine aminotransferase; Fe: Demir; UIBC: Unsaturated iron binding capacity; PON1: Paraonase

The biochemistry parameters of the LDH ( $p < 0.001$ ), urea ( $p < 0.01$ ) concentration were increased whereas the level of ALT ( $p < 0.05$ ) was decreased at the end of the season. The creatine kinase (CK) and PON1 activity were unaltered by the football training. The level of the UIBC were increased ( $p < 0.05$ ) and the iron level decreased end of the season ( $p < 0.001$ ). The serum ferritin level was unaltered end of the season ( $p > 0.05$ ) compared to pre season level (Table 3).

## DISCUSSION

In this study, we found that the levels of Neut and Lymph were increased or decreased, it means these parameters slightly affected by football training whereas no effect on WBC count was observed. In fact, immune function is quantified by measuring WBC in the blood, which includes the five major types of these cells (basophilocytes, eosinophilocytes, lymphocytes, monocytes and neutrophilocytes). For instance, neutrophils fight infections and provide an important defense system against microorganism also play a role in inflammatory response and muscle regeneration (22). Many studies reported that WBC counts nearly doubled after the games (23), and remained elevated post-24 h after the game, but returned to baseline after post- 48 h after the game (9,24). In another study it was reported that WBC and neutrophil numbers had significantly elevated at the end of the football training session when compared to the baseline values (25). In this regard, increased amount of training with number of competitions may be detrimental to the immune functionality of football players due to the variance of exercise intensity, duration and training volume (26). Since, it is possible that the immune function may be impaired in professional footballers during periods with a high match frequency (9).

Our results showed that the red blood cell counts and Hb levels were decreased following the competitive season. Also, Fe levels were decreased with increased UIBC capacity. Basically, the low hemoglobin concentration is related with low level of the iron in the blood. In general, athletic-induced iron deficiency is commonly detected in athletes, particularly those who engage in endurance sports. Moreover, iron is an essential component of hemoglobin, myoglobin, cytochromes, and other iron-containing proteins that participate in oxidative phosphorylation (27,28). However, the mechanism responsible for this reduction has not been clearly defined in elite football players. In a previous study and in accordance with our study, it was reported that the hemoglobin and hematocrit values were higher at the beginning of the competition season, followed by a declined in end of the season in professional football players (19). Moreover, one study in the Italian Rugby National team during the 2004-2005 season was followed and the results showed that the Hb values were decreased at the beginning of the season from 152 g/L to 149 g/L at the end of the season (28). In addition no significant

variations were observed among for MCH, MCHC and RDW values in this study. Related to this, one study followed the serum iron level in elite football players during four years, measured four times a year, and this demonstrated that serum iron levels were significantly lower than preseason values (29). So, these results indicate that the training induces a decline in serum iron and this may be an early sign of iron store depletion.

Serum CK concentration is the most sensitive indicator of muscle damage and its level begins to rise approximately 2–12 h after high intensity exercise, in field team sport during training and competition (30,31). In general, CK, LDH and urea increase in response to endurance exercise and are classified as a qualitative inflammatory markers but their levels, depend on exercise intensity and duration (32, 33). Our data showed that regarding the muscle damage markers (CK) displayed no significantly increase during the competition season. On the other hand, urea and LDH markers were significantly elevated during the competitive season. These results are consistent with Becatti et al. study reported that ALT levels do not change during the football season, whereas AST and LDH levels increased during season 1 and season 2, but returned to basal level at the end of the season 3 (34). Guilhem (2015), study showed that significant increase in CK activity occurred from the preparation to the pre-competitive period, which could be due to higher intensity and specialized exercises during this period, which could have increased the exercise-induced muscle damage (35). In addition, one study reported that AST, ALT levels were higher in the midfielder/defender group of the football players; unlike LDH activity level was lower in the same group players (6). These markers values also can be changed dependently player position.

Also, training induces CK augmentation, with higher values recorded for sedentary subjects than athletes, demonstrating the adaptive behavior of trained muscles (36). It seems CK level is a changeable marker and its levels depend on training load during the season and should be monitored during the football training season. Furthermore, CK levels should also be monitored during and after exercise to evaluate recovery, and determine whether muscle trauma or overtraining is present as these lead to consistently higher levels of sustained CK release. Our results also showed that football training did not alter PON1 activity level, although insignificantly reduction at the end of the season was observed. PON1 protein level is associated with oxidation of lipoproteins and it is a part of intrinsic antioxidant system. Evidently, the reduce activity PON1 level is a predictor of oxidative stress diseases, for example coronary artery disease (37, 38). However, a few studies have reported higher levels in regularly training subjects than sedentary controls (39). On the other hand, others have reported that no influence of regular training on baseline level PON1 (40). It may indicate that regular training resulted in an adaptation of PON1 levels. Therefore, PON1 activity may provide a higher antioxidant defence in physically active subjects.

## CONCLUSION

Our limitation of the study that we couldn't get a blood sample at the end of the season from football players, because of the long football season. Also, we did not under control the nutrition status of the players. Our study results indicated that long-term measurement of biochemical muscle metabolism and hematological and iron parameters, when analyzed together, could constitute a useful set of markers in monitoring the recovery period in whole football season, according to player position. These parameters might be used as a clinical tool for determine to football players health and training status end of the season or beginning of the season. Additionally, PON1 level may be used for determine to oxidant-antioxidant level for athletes as a new cellular damage marker.

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