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CHANGES IN PLASMA CREATINE KINASE AND FREE RADICALS IN PROFESSIONAL SOCCER PLAYERS THROUGHOUT A HALF-SEASON

Z. HANDZISKI, V. MALESKA, B. DEJANOVA, S. NIKOLIK, E. HANDZISKA, M. DALIP*

SUMMARY

Plasma creatine kinase (CK) activity and free radical (FR) levels may indicate some changes following acute and prolonged physical exercise. These parameters have certain activity and concentration characteristics, the changes in which may mean adaptation or non adequate adaptation of the reserves. The aim of this study was to evaluate the changes in plasma CK activity and FR levels in professional soccer players during a competition half-season. A total of 30 professional soccer players from a first league soccer club were the subjects in this study. They were evaluated on three occasions: before the pre-seasonal training period, before the competition season, and at the end of the half-season. Venous blood samples were taken before and following a maximal incremental treadmill test, and serum plasma levels of CK and FR were determined using spectrophotometric and "D-ROMs" methods, respectively. Descriptive statistics and ANOVA were used to analyze the results. Taking into account the three training periods, plasma CK activities decreased and FR levels increased both significantly ($p<0.05$) at the end of the half-season. The reason for the significantly decreased exercise induced plasma CK responses at the end of the season might be caused by muscular adaptation. With supportive data about soccer performance, mood and aerobic/anaerobic capacity alterations, these changes could help in assessing risks of inadequate adaptation, overtraining or stagnation in the training processes.

Key words: Exercise, physiology, soccer, creatine kinase, free radicals

* Institute of Physiology, Medical Faculty, Skopje, Republic of Macedonia

ÖZET

PROFESYONEL FUTBOLCULARDA BİR YARI SEZON BOYUNCA GÖZLENEN PLAZMA KREATİN KİNAZ VE SERBEST RADİKAL DEĞİŞİKLİKLERİ

Akut ve uzun süren egzersizlere plazma kreatin kinaz (CK) aktiviteleri ve serbest radikal (FR) seviyelerindeki yanıtlar bazı değişimlerin göstergesi olarak kabul edilebilir. Bu parametrelerdeki aktivite ve konsantrasyon değişimleri rezervlerde uygun adaptasyonun gerçekleşip gerçekleşmediğinin göstergesi olabilir. Bu araştırmanın amacı plazma CK aktiviteleri ve FR seviyelerindeki değişimlerin bir yarı sezon boyunca profesyonel futbolcularda izlenmesiydi. Araştırma 30 profesyonel 1. lig futbolcusu üzerinde gerçekleştirildi. Ölçümler üç ayrı dönemde yapıldı: hazırlık dönemi antrenman süreci başlamadan önce (P1), hazırlık dönemi sonrası sezon açılmadan önce (P2) ve yarı sezonun bitimiyle birlikte (P3). Basamaklı, maksimal bir koşu bandı testi öncesi ve sonrasında deneklerden alınan venöz kan örneklerinde sırasıyla spektrofotometrik ve "D-ROMs" yöntemleri ile plazma CK aktiviteleri ve FR seviyeleri saptandı. Anova ve tanımlayıcı istatistik yöntemleri ile veriler değerlendirildi. Çalışmanın her üç evresi karşılaştırıldığında yarı sezonun sonunda plazma CK aktivitelerinde anlamlı ($p<0.05$) düşüş ve serbest radikal seviyelerinde anlamlı artış saptandı. Yarı sezonun sonunda plazma CK aktivitelerindeki düşüşler kassal adaptasyondan kaynaklanmış olabilir. Bu parametrelerdeki değişimler; oyun performansı, motivasyon durumu ve aerobik/anaerobik kapasitedeki değişimlerle birlikte değerlendirildiğinde, antrenmana yetersiz adaptasyon, sürantrenman ve antrenman sürecinde duraklaman risklerini açıklamada yardımcı göstergeler olabilecektir.

Anahtar sözcükler: *Egzersiz, fizyoloji, futbol, kreatin kinaz, serbest radikaller*

INTRODUCTION

Intracellular proteins from muscles and other tissues appear in the blood serum following physical activity as a result of partial destruction of muscle structures, especially muscles membranes. Exercise-induced muscle damage is usually limited to the ultrastructural integrity of muscle fibers (12). Among these proteins is the creatine kinase (CK) enzyme.

Some studies suggest that metabolic stress induced by exhausting exercise can not be considered as an important factor for the release of

intracellular proteins (18). However, several studies indicate that the level of CK release is related to the overall tension output of the involved muscles. Namely, intensity and duration thresholds exist for serum enzyme responses (4,12). On the other hand, eccentric exercise causes the release of more CK for a longer period compared with isometric or concentric exercise (7).

The level of serum CK may not be related to the amount of muscle mass utilized in strength exercises. Also muscle enzyme release can not be used to predict the magnitude of the muscle function impairment caused by muscle damage (9).

A study by Ohkuwa and co-workers (11) indicates that when the same exercise was repeated three weeks or several moths later, less soreness was expected and no CK response appeared. Accelerated clearance of CK seems to be a factor contributing to the blunted response of this enzyme after a repeated bout of exercise. The magnitude of enzyme efflux from muscle tissue into the blood maybe depressed by training, and plasma CK activities may be better indicators of physical training and physical performance than peak blood lactates and LDH activities in well-trained sprinters.

A harmful consequence of the action of free radicals results when an imbalance exists between the production of oxidants (free radicals, FR) and the activity of the antioxidant system. In this situation, the so called oxidative stress, or oxidation of cellular components takes place. Oxidative stress is possible when local antioxidant defences are depleted because of the high level of oxidants or when the rate constants of the radical reactions are greater than the rate constants of the antioxidant defence mechanisms (10).

On the other hand, the common assumption that increased mitochondrial oxygen consumption leads per se to increased reactive oxygen species (ROS or free radicals) production is not supported by in vitro and in vivo data. It has been demonstrated that ROS have the capacity to contribute to the development of muscle fatigue in situ. But there is still lack of convincing direct evidence that ROS impair exercise performance in vivo in humans. It remains unclear whether exercise-induced oxidative modifications have little significance, induce harmful oxidative damage, or are an integral part of redox regulation (17).

Experiments on rats indicate that the training effect on glutathione peroxidase (antioxidant) activity is insignificant when the duration of

daily running sets is 30 minutes. The effect appears in daily training for 60 minutes and is highest when daily running duration is 90 minutes (8). Nevertheless, some studies conclude that vigorous acute exercise may result in free radical accumulation, whereas exercise training results in possibilities for inhibition of oxidative stress by increasing the capacity of antioxidant systems (13).

There are no documented studies for changes of CK and FR in professional soccer players during prolonged periods of training and matches. The aim of this study was to evaluate the changes of CK and FR plasma levels in professional soccer players during a half-season.

MATERIAL AND METHODS

A total of 30 professional soccer players from a first league soccer club were the subjects in this study. No antioxidative substances were used by the subjects throughout the study. Evaluations were conducted on three occasions:

1. Before the pre-seasonal training period (within three days of initial preparations, P1),
2. Before the competition season (during a week of tapering in which the intensity and volume were low, P2).
3. At the end of the half-season (three days following completion, P3).

Venous blood samples were taken before and after a maximal exercise test and serum plasma activities of CK and levels of FR were determined. A spectrophotometric method was used for CK determination using the increase in absorbance due to the reaction of NADP to NADPH measured at 340 nm, proportional to its activity. The D-ROMs test (Diacron, Italy) was used for oxidative stress determination, which measures blood hydroperoxides levels, generated by lipid peroxidation of biological membranes. Actually, instead of FR, hydroperoxides are relatively stable and maintain pro-oxidative action in biological fluids. The test is based on the ability of transition metals to enter the catalase reaction in the presence of peroxides, with formation of FR which are trapped by an alchilamine.

The maximal exercise test was a progressively incremented treadmill test. Every third minute the velocity and the angle of the treadmill were increased by 1.0 km/h and one degree. The criteria of

maximum loading for each athlete were established as ≥ 95% maximal heart rate and/or voluntary giving up.

Descriptive statistics, ANOVA and Pearson correlations were used for the analysis of the results. The level of significance was accepted as $p<0.05$.

RESULTS

There were significant differences in plasma FR levels and CK activities for both resting and post-exercise test levels, for each of the three training periods (Table 1). Plasma FR levels increased, whereas plasma CK activities decreased, both significantly at the end of the season.

Table 1. Resting and post-test plasma FR levels and CK activities for the three training periods

Training periods		P1	P2	P3
FR (Ucarr)	Resting	253.5 ± 38.4	281.6 ± 27.1[a]	312.8 ± 32.7[bc]
	Post-test	285.0 ± 34.7	310.4 ± 29.6[a]	353.8 ± 38.1[bc]
CK (U/l)	Resting	266.3 ± 181.8	231.2 ± 147.0	177.8 ± 80.1[b]
	Post-test	352.7 ± 208.5	337.4 ± 111.0	223.9 ± 95.2[bc]

a: $p<0.05$ (btw P1 and P2), b: $p<0.05$ (btw P1 and P3), c: $p<0.05$ (btw P2 and P3)

When all three training periods were together taken into account, plasma FR levels and CK activities were determined to have increased significantly following the maximal exercise test (Table 2).

Table 2. Resting and post-exercise test plasma FR and CK levels for all three training periods

Phase of exercise	Resting	Post-test
FR (Ucarr)	282.6 ± 40.7	316.4 ± 44.3*
CK (U/l)	225.1 ± 145.2	304.7 ± 155.9*

* $p<0.05$ (differences between resting and post-test levels)

Table 3. Differences of exercise induced plasma FR and CK responses for the three training periods

Training periods	P1	P2	P3
FR (Ucarr)	40.9 ± 39.0	34.6 ± 18.2	36.4 ± 15.9
CK (U/l)	100.8 ± 60.9	108.1 ± 90.3	46.5 ± 26.5[bc]

b: $p<0.05$ (btw P1 and P3), c: $p<0.05$ (btw P2 and P3)

Exercise induced FR response changed non-significantly during the course of the study. In revenge, exercise induced CK response decreased significantly at the end of the half-season (Table 3).

The changes in plasma FR levels were not significantly correlated with the changes in plasma CK activities during each period of the study, both before and following the maximal exercise test, taken separately. Incidentally, taking into account all the training periods together, and only for the post-exercise state, a significant negative correlation was established between increasing plasma FR levels and decreasing plasma CK activities ($r = -0.27$, $p<0.05$).

At the three different periods, mean VO_2max levels were determined to be 48.3 ± 4.1, 51.3 ± 4.2 and 49.5 ± 4.8 ml/min/kg respectively, with the difference being significant ($p<0.05$) between the first two periods P1 and P2. Players' speeds at the anaerobic threshold (AnT) were calculated to be 12.3 ± 0.8, 12.7 ± 1.7 and 12.2 ± 1.5 km/h respectively at P1, P2 and P3. Athletes displayed lower blood lactates (4.2 ± 1.7 mmol/l vs. 5.4 ± 1.6 mmol/l, $p<0.05$) at a speed over the AnT in period P2 compared with P1, during the maximal ergometer test. At period P3 though, they could reach lower maximal blood lactate levels compared with period P1 (8.1 ± 2.5 vs. 10.0 ± 2.7 mmol/l, $p<0.05$).

DISCUSSION

Overall results revealed that plasma CK activity levels decreased significantly at the end of the half-season. It is not very clear whether this decrement in plasma CK activities is due to accelerated clearance of CK after a period of prolonged training and too many competitions or a sign of depleted reserves of adaptation (3). Considering the higher AnT levels reached at period P2, muscle cell membrane adaptation to training and competition loads might be an explanation.

On the other hand, regarding the three training periods, plasma FR levels increased significantly at the end of half-season, suggesting that oxidative stress increases following a prolonged training period. A view is that antioxidant defence mechanisms might be depleted at the end of a season (2). Lower aerobic training loads as the season progresses should be considered as an explanation too. In fact, lower aerobic capacity findings at the end of half-season in this study support this view.

The significant increments in plasma FR levels and CK activities following the maximal exercise test in each training period were in accordance with several studies that revealed disruption of the muscle membrane with leakage of muscle cell enzymes into the circulation and increased oxidative stress following acute bouts of exercise (1,5,14). Significantly decreased exercise induced responses at the end of the half-season for the plasma CK activities might be explained by adaptation to training and competition loads. The question here would be that whether significant negative correlations between plasma FR levels and CK activities following the maximal exercise test parallels any signs of fatigue, overreaching or overtraining (15,16) at the end of the half-season?

More research is needed with supportive data about alterations in soccer performance, mood, and aerobic/anaerobic capacity, to explain the above mentioned changes that could be indicators of risks such as non-adaptation, overtraining or stagnation in the training processes.

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Correspondence e-mail: handziski@yahoo.com
metin@mol.com.mk