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The search for new diagnostic markers of metabolic response to aerobic exercise: analysis of creatinine, urea, and uric acid levels in football players

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Abstract

Introduction. A football match as well as long-term football training are known to influence players' cellular metabolism and can lead to muscle damage, oxidative stress as well as biochemical and hormonal changes. Aim of Study. Considering the importance of aerobic training for football players, the aim of this study was to evaluate changes in plasma creatinine, uric acid (UA), and urea levels in response to a semi-long distance outdoor run in aerobic conditions in both female and male football players. Material and Methods. 16 voluntarily recruited football players aged 22.50 (women) and 18.35 (men) years, took part in an outdoor run. The female players covered a distance of 7.4 ± 0.3 km, while the male players covered a distance of 10.7 ± 1.0 km. Plasma levels of the studied biochemical parameters were determined using an appropriate diagnostic assay kit. Results. Aerobic exercise did not influence the creatinine level of football players, whereas a significant decrease in median post-exercise UA level was found only in the group of female players. The outdoor run caused a systematic increase in urea plasma concentration during the experiment in both studied groups. Conclusions. The obtained results confirm that UA and urea blood plasma levels could be effective diagnostic markers for evaluation of metabolic response to aerobic exercise, at least among female athletes. The correlations found in this study point to urea as a potential marker of overtraining syndrome in athletes.

KEYWORDS: aerobic training, creatinine, outdoor run, urea, uric acid.

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What is already known on this topic?
To avoid potential injuries effective training needs to be well planned and executed. It is essential to seek early and effective metabolic markers of biological response to physical exercise. Appropriate reactions to disturbing changes in levels of these parameters may safeguard athletes from harmful overtraining.

Introduction

Physical activity, especially outdoor running, has become a popular form of physical recreation in recent years. A well planned training schedule can lead to improvements in one's fitness and health. On the other hand, physical exercise might also lead to serious injuries resulting from disturbances of biochemical homeostasis. This problem concerns especially amateur and professional athletes. Football is a mixed type of physical exercise (both aerobic and anaerobic), which is associated with different actions of players during a game. The high number of accelerations and decelerations associated with the high dynamics of football places an additional burden on players' muscles [1]. Due to different types of exercises involved, football

players must be adapted to generate energy from both anaerobic and aerobic metabolism. Another aspect of football is the power and strength needed during the game allowing players to use a variety of football-specific skills [1-3].

Aerobic training is designed to enhance the body's oxygen transport system. It is important there is a good supply of oxygen to active muscles which must have the capability of using oxygen provided by the circulatory system during football matches and training sessions [4]. Numerous studies point to exercise-induced differences in biochemical parameters, and the relationships of those changes with fitness level. Therefore, observing biochemical changes in female and male football players during training is crucial, in particular, at the end of each training season [1, 5-7]. Such studies allow assessment of the fitness and endurance of young players, and might help indicate future training directions. It is thus essential to seek effective metabolic markers of biological response to physical exercise since appropriate reactions to disturbing changes in levels of these parameters may safeguard athletes from harmful overtraining [5, 8].

One of the common diagnostic markers is plasma creatinine concentration. It is the most widely used and accepted measure of renal function in clinical medicine [8, 9]. Creatinine is non-enzymatic derivative of creatine, and its blood level depends on the player's fitness level, diet, sex, age, and body mass index (BMI). The differences were found in the creatinine level in physically active individuals in comparison with physically inactive (non-training) individuals [10]. In different professional athletes (triathletes, basketball players, cyclists, football players, sailors, skiers, rugby players) the mean plasma creatinine was heterogeneous. Authors reported changes in creatinine level during training as well as intensive exercise. In amateur runners such changes are accompanied by an increase in urea concentration, which decreases within 24 hours after the end of exercise [11].

Uric acid (UA), as a terminal product of purine metabolism and one of the principal antioxidants in human plasma, seems to be an interesting marker of post-exercise metabolic response, especially in athletes' blood. It is well known that purine metabolism is often elevated in athletes due to the high amount of animal proteins in their diet and increased cell turnover [8]. Plasma UA was shown to be much higher than the level of ascorbic acid, which, in particular in professional athletes, plays a vital role in reactive oxygen species neutralization and decreases the risk of oxidative stress related to cell damage. An increase in UA concentration in relation to secondary controls accompanied with increases in other antioxidants (ascorbic acid, α-tocopherol, superoxide dismutase) was revealed in professional football players [8, 12]. It was also evidenced that changes in UA concentration in elite athletes depend on phases of training and competition [6]. Authors noted that acute exercise might influence the increase in UA concentration directly after exercise [13]. Urhausen and Kindermann [14] suggest the possibility of application of urea and UA plasma levels as measures of training-related stress, although those parameters are more frequently used to assess protein catabolism and purine nucleotides degradation [15, 16]. Interestingly, urea blood level is not a very common biochemical marker in athletes. Some literature data indicate that the urea level is not a tissue and/or organ specific marker [8]. Other data suggest that increases in urea and UA blood levels are strongly related to training, as normalization of these parameters indicates athletes' readiness for next part of strenuous training sessions [13].

Aim of Study

Considering different data on plasma levels of creatinine, UA and urea as well as limited data on the influence of different types of physical exercise on the variation of those parameters in both female and male football players, the aim of the study was to assess changes in plasma creatinine, UA, and urea levels in response to a semi-long distance outdoor run in aerobic conditions in female and male football players at the end of a training season.

Material and Methods

Participants

Participants (n = 16) were football players from the Olimpia Szczecin and Pogoń Szczecin S.A. clubs, divided into two groups according to sex. They had no history of any metabolic and cardiovascular diseases. Participants were non-smokers and refrained from taking any medications or supplements known to affect metabolism. Female participants' median (interquartile range) age, body height, body mass and body mass index amounted to 22.50 (19.90–23.55) years, 1.68 (1.65–1.70) m, 58.5 (57.5–65.5) kg and 21.18 (20.19–23.18), respectively. Male participants' median (interquartile range) age, body height, body mass and body mass index were 18.35 (18.05–18.65) years, 1.8 (1.73–1.84) m, 67.5 (64.5–72.0) kg and 21.04 (20.11–22.60), respectively.

The study was conducted in accordance with the ethical standards as described by Kruk [17]. Participants (and their parents, where appropriate) were informed of the experimental procedures and possible risks before giving their written consent. The local ethics committee approval was received before the beginning of the test, in accordance with the Declaration of Helsinki.

Procedures

Outdoor run test

The exercise test was performed on the last day of training season after the participants had completed an annual training cycle and were shortly before holidays. Participants were instructed to be well rested after a good sleep. The test was performed in the afternoon on a warm and cloudless summer day. The overall conditions of the day, sleep, diet, and hydration were as similar as possible to typical conditions in which the players normally trained. The exercise test consisted of a warm-up routine (10 minutes), the main run outdoors (60 minutes), and a series of stretching and breathing exercises (15 minutes). Before the run, immediately after the run, and at the onset of recovery, blood samples were collected.

The female players covered the distance of 7.4 ± ± 0.3 km with the mean speed of 7.5 ± 0.5 km/h. The aim of the exercise was to develop aerobic capacity below the anaerobic threshold, calculated individually for each participant. Therefore, participants were running to maintain a subliminal heart rate of 158 ± 4 beats·min^{-1}. The male players covered a semi-long distance of 10.7 ± 1.0 km with the mean speed of 10.6 ± 1.7 km·h^{-1}. They were running to maintain a subliminal heart rate of 158 ± 3 beats·min^{-1}. Players' heart rate was analysed using a Garmin Forerunner 305 heart rate monitor (Garmin (Europe) Ltd., Romsey, UK). Additionally, in both studied groups, lactate and albumin levels were determined to ensure aerobic metabolism and to assess the hydration level after the exercise test, respectively.

Biochemical analysis

Blood plasma was obtained according to standard diagnostic procedures. Blood samples were taken using a 4.9 mL S-Monovette tube with ethylenediaminetetraacetic acid (EDTA) and separating gel, and then were centrifuged 500 × g for 15 minutes at room temperature to receive blood plasma. The collected plasma samples were frozen at –86 ^{O}C until further analysis. CK creatine, UA, urea, albumin and lactate plasma levels were determined in samples before the exercise test (pre-exercise), immediately after the run (post-exercise), and 15 minutes after the run (recovery).

Plasma lactate levels were marked using diagnostic colorimetric enzymatic method (Liquick Cor-LACTATE) according to the manufacturer's protocol (PZ Cormay S.A., Łomianki, Poland). Absorption of samples was measured at λ = 520 nm at 37 ^{O}C. Plasma creatinine levels (µmol·L^{-1}) were measured using Jaffe's spectrophotometric method according to the manufacturer's protocol (BioMaxima S.A., Lublin, Poland). Plasma urea levels (mmol·L^{-1}) were determined with the kinetic method according to the manufacturer's protocol (BioMaxima S.A., Lublin, Poland). ΔA of samples were measured at λ = 490 nm and λ = 340 nm at 37 ^{O}C for creatinine and urea, respectively. Plasma UA levels (µmol·L^{-1}) were assessed using an enzymatic assay kit (BioMaxima S.A., Lublin, Poland). Absorption of samples was measured at λ = 530 nm at 37 ^{O}C. Plasma albumin levels (g·L^{-1}) were determined using a colorimetric bromocresol green assay kit according to the manufacturer's protocol (BioMaxima S.A., Lublin, Poland). Absorption of samples was measured at λ = 630 nm at 37 ^{O}C. Plasma CK levels (U·L^{-1}) were assessed using a kinetic assay kit (BioMaxima S.A., Lublin, Poland). ΔA measurements were conducted at λ = 340 nm at 37 ^{O}C.

All analysis procedures were validated with the use of multiparameteric control serum (BIOLABO S.A.S, Maizy, France). Absorption measurements were made using Synergy H1 multi-mode reader (BioTek, Winooski, VT, USA).

Statistical analysis

All data were presented as medians (interquartile range) unless otherwise stated. Statistical analysis was performed using the STATISTICA ver. 12 software package (StatSoft, Inc.). Significant differences between analysed time points were assessed using the non-parametric ANOVA Friedman test. Correlations between the examined variables were assessed using Spearman's rank correlation coefficient. The level of statistical significance was set at $p < 0.05$.

Results

Plasma lactate and albumin levels as well as CK activity were measured three times. Since no significant increase in plasma lactate was found we could assume that the performed exercise was aerobic (data not presented). The CK level in female football players was only slightly changed during the experiment from 244

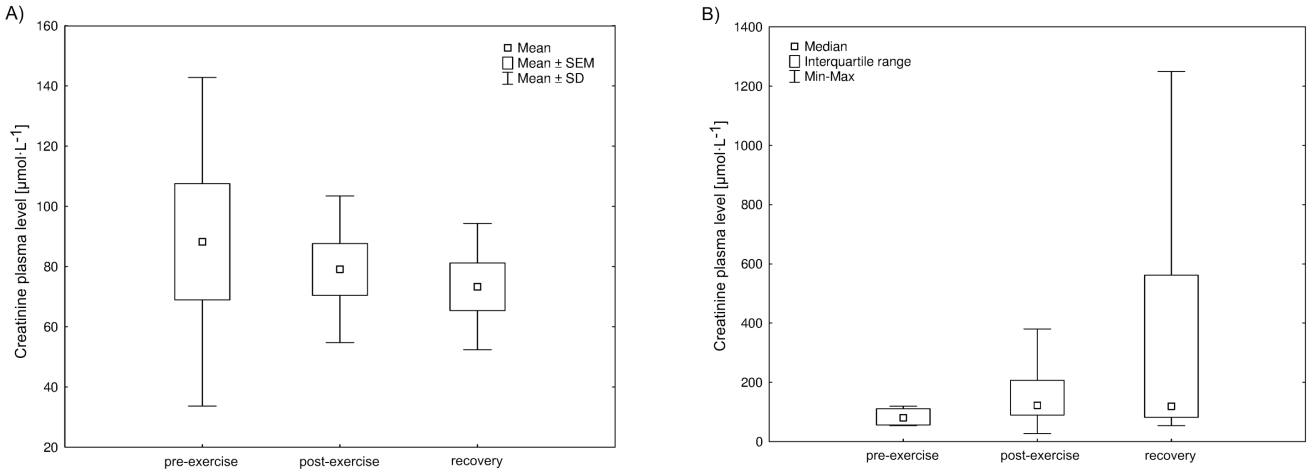

Figure 1. A) Mean blood plasma level of creatinine (µmol·L^{-1}) in female (n = 8) football players before (pre-exercise), immediately after (post-exercise) a 60-min-long outdoor run at a pace ensuring aerobic metabolism (as ensured by individually calculated and monitored heart rate and confirmed by serum lactate levels), and at the beginning of recovery (recovery). The midpoint represents the mean; the box represents standard error of the mean (SEM); the whiskers represent standard deviation (SD). **B)** Mean blood plasma creatinine level (µmol·L^{-1}) in male (n = 8) football players before (pre-exercise), immediately after (post-exercise) a 60-min-long outdoor run at a pace ensuring aerobic metabolism (as ensured by individually calculated and monitored heart rate and confirmed by serum lactate levels) and at the beginning of recovery (recovery). The midpoint represents the median; the box represents the standard interquartile range; whiskers represent the min-max range.

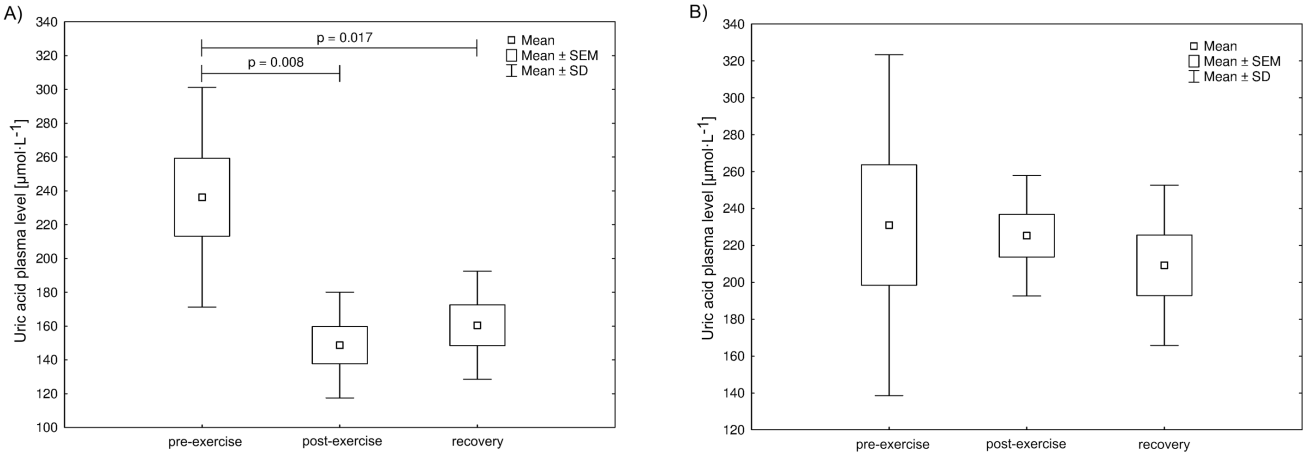

Figure 2. A) Mean blood plasma level of uric acid (UA) (µmol·L^{-1}) in female (n = 8) football players before (pre-exercise), immediately after (post-exercise) a 60-min-long outdoor run at a pace ensuring aerobic metabolism (as ensured by individually calculated and monitored heart rate and confirmed by serum lactate level), and at the beginning of recovery (recovery). The midpoint represents the mean; the box represents the standard error of the mean (SEM); whiskers represent standard deviation (SD). Significance level of differences observed between analysed time points (pre-exercise vs. post-exercise vs. recovery) assessed using analysis of variance (ANOVA) with a repeated measures test followed by contrast analyses. **B)** Mean blood plasma level of uric acid (UA) (µmol·L^{-1}) in male (n = 8) football players before (pre-exercise), immediately after (post-exercise) a 60-min-long outdoor run at a pace ensuring aerobic metabolism (as ensured by individually calculated and monitored heart rate and confirmed by serum lactate level), and at the beginning of recovery (recovery). The midpoint represents the median; the box represents the standard interquartile range; whiskers represent the min-max range.

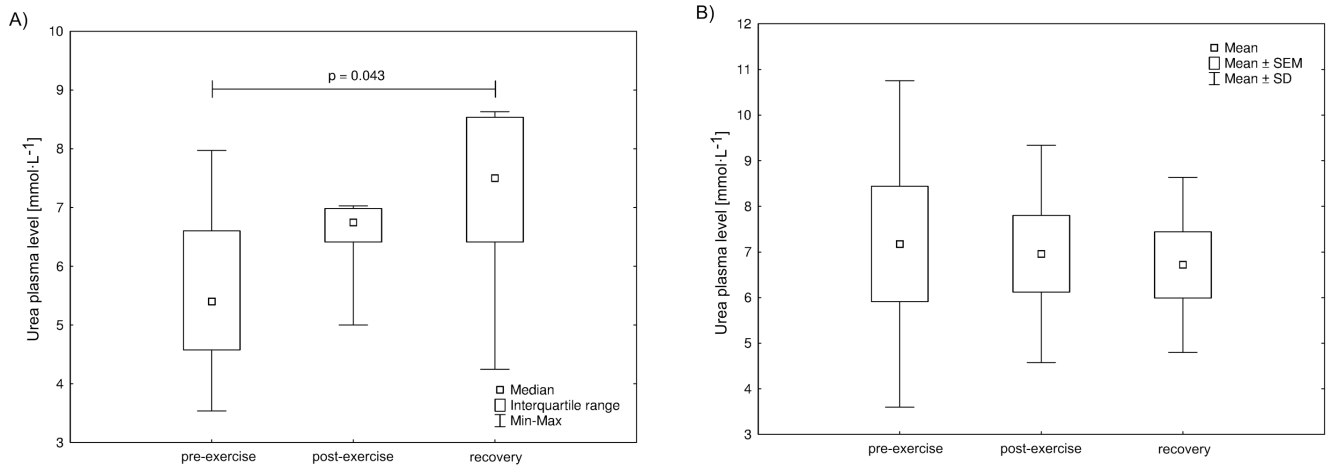

Figure 3. A) Mean blood plasma level of urea (mmol·L^{-1}) in female (n = 8) football players before (pre-exercise), immediately after (post-exercise) a 60-min-long outdoor run at a pace ensuring aerobic metabolism (as ensured by individually calculated and monitored heart rate and confirmed by serum lactate level) and at the beginning of recovery (recovery). The midpoint represents the mean; the box represents standard error of the mean (SEM); whiskers represent standard deviation (SD). Significance level of differences between analysed measurement times (pre-exercise vs. post-exercise vs. recovery) was assessed with ANOVA with a repeated measures test followed by contrast analyses. **B)** Mean blood plasma level of urea (mmol·L^{-1}) in male (n = 8) football players before (pre-exercise), immediately after (post-exercise) a 60-min-long outdoor run at a pace ensuring aerobic metabolism (as ensured by individually calculated and monitored heart rate and confirmed by serum lactate level), and at the beginning of recovery (recovery). The midpoint represents the median; the box represents the standard interquartile range; whiskers represent the min-max range.

(232–252) U·L^{-1} at the beginning, through 255 (246–264) U·L^{-1} after the test, to 233 (220–263) U·L^{-1} during the recovery. All the changes were statistically non-significant. The CK activity in male football players remained practically unchanged during the experiment: 228 (218–252) U·L^{-1}; 234 (219–253) U·L^{-1}; 237 (227–244) U·L^{-1} (before and after the exercise test and at the beginning of the recovery time, respectively). Typically for athletes, the baseline CK activity values before the aerobic exercise were about 1.5-fold and about 1.2-fold higher than the upper reference range values for females (< 167 U·L^{-1}) and males (< 190 U·L^{-1}), respectively.

Creatinine plasma levels are presented in Figure 1. While the widest range of data was noted before the exercise, after completing the run it was narrower, and the values after exercise and at the beginning of recovery time were similar. However, the differences between the creatinine levels at all measurement times were statistically non-significant in both female and male players. Interestingly, in both groups baseline as well as post-exercise and recovery creatinine levels were within the reference range (80-115 µmol·L^{-1} and 53-97 µmol·L^{-1} for women and men, respectively. Figure 2 presents the plasma UA level determined in analysed samples. A statistically significant decrease in post-exercise median UA level was found only in the female players. In both groups under study baseline values as well as post-exercise and recovery values of UA were within the reference range (< 7.5 µmol·L^{-1} and < 7.0 µmol·L^{-1} for women and men, respectively).

These results indicate that a 60-minute-long outdoor run in aerobic conditions caused a systematic increase in urea plasma concentration during the experiment, as noted in both studied groups (Figure 3). The statistical analysis confirmed a significant difference between data obtained before the run and at the beginning of recovery time only among the female football players. It is worth noting that the urea levels at each measurement time were within the reference range (1.67-8.35 mmol·L^{-1}).

Interestingly, Spearman's rank correlation coefficient analysis (Table 1) revealed significant correlations between weekly training frequency and urea levels in both pre-exercise and recovery measurement times as well as between length of training experience and urea level post-exercise in female players, whereas in male players a significant correlation was found between weekly training frequency and urea level during recovery; weekly training frequency and creatine level during recovery; and length of training experience and

Table 1. Coefficients of correlation between examined variables and the length of training experience and weekly training frequency

Correlated variables	Female players		Male players	
	r	p	r	p
Weekly training frequency and urea level in pre-exercise time point	0.76	0.0274*	–0.50	0.2064
Weekly training frequency and urea level at recovery time point	0.80	0.0301*	–0.77	0.0441*
Weekly training frequency and creatinine level in recovery	–0.14	0.7646	–0.90	0.0060
Length of training experience and creatinine level after exercise	0.59	0.1264	0.76	0.0274
Length of training experience and urea level in post-exercise time point	0.79	0.0334*	–0.16	0.7093
Creatinine level before the exercise and creatine kinase activity after exercise	–0.61	0.1436	0.75	0.0305*
Creatinine level in recovery and creatine kinase activity in recovery	0.00	1.00	–0.79	0.0362*

r – Spearman's rank correlation coefficient; p – significance level; * – statistically significant correlation

creatinine level after exercise. There was also a significant correlation between the pre-exercise creatinine level and CK level during recovery in the male players.

Discussion

Physical activity induces changes in metabolism, which can lead to a differentiation of numerous blood biochemical parameters. The levels of these parameters often exceed reference values for the non-training population. This can be associated with the type of training, length of training experience, participation in competitions as well as personal traits [6, 8, 9, 18, 19]. Up to now, different tests analysing physiological indices in athletes (e.g. Yo-Yo test, multistage 20-m shuttle run test, Carminatti's test) have been applied by various authors [20-23]. These studies not only allow assessment of athletes' fitness level, but also reflect team sports-associated movement patterns [6]. It is well known that a football match as well as long-term football training influence athletes' cellular metabolism and can lead to muscle damage, induce oxidative stress as well as biochemical and hormonal changes [8, 12, 24, 25]. It is essential to develop the knowledge of those changes in order to correctly interpret the results of laboratory blood analyses by individuals and sport physicians. Although there are numerous data describing physiological and motoric changes in athletes, there is still the need to learn more about metabolic response to both aerobic and anaerobic exercise. Analysing different biochemical parameters in athletes is a routine procedure in the present-day training process. Although these analyses are routinely conducted, the variance and character of biochemical parameters in athletes need to be studied and described on a regular basis.

Research shows there are differences in the creatinine level between physically active and inactive individuals. It has also been revealed that the type of sport practiced by professional athletes affects the blood concentration of this biochemical parameter [8, 26]. Different training loads, frequency and length of competitions, as well as different periods of training and competition throughout the year can also influence the creatinine plasma level in professional athletes [8, 15]. The results of the present study indicate that an outdoor run did not influence the plasma creatinine level in both female and male football players. However, contrary to these findings, Meyer and Meister [6] noted a significant decrease in the creatinine level during the entire training season. Interestingly, in comparison to data by Banfi and Del Fabbro [26], the plasma creatinine levels in male football players were slightly lower than in elite football players. Our results are in line with the data showing that the creatinine level is not influenced by training or competition [27, 28]. The lack of changes in creatinine concentration was observed in runners after covering the distances of 300 m, 3 × 300 m, 400 m, 2 × 200 m [29], as well as in marathoners [30]. The present study revealed a statistically significant correlation between post-exercise creatinine plasma levels and length of training experience as well as between creatinine plasma levels during recovery

and weekly training frequency in male football players. Moreover, statistically significant correlations between pre-exercise creatinine plasma level and CK activity after exercise as well as between the creatinine plasma level and CK activity during recovery were also found in this group. It can be supposed that these correlations could at least be in part connected with compensation mechanisms occurring during training. However, this hypothesis needs further verification. The obtained results seem to at least partly confirm the hypothesis that variation in plasma creatinine levels could be associated with the period of the training season [31].

Physical exercise is known to induce metabolic changes intensifying catabolic processes. Thus, the UA concentration is one of the most important biochemical parameters indicating changes in purine nucleotides distribution [8, 25]. The present study showed that aerobic exercise affected the UA plasma concentration only in female football players. It decreased significantly after the exercise post-exercise as well as during recovery in comparison to the measurements pre-exercise in this studied group. A significant decrease in the UA level in female football players during the entire training season was also found by Meyer and Meister [6]. On the other hand, Gravina et al. [25] and Ascensão et al. [24] reported an increase in UA concentration in female football players after football matches. The post-exercise decrease in UA combined with literature data indicating an increase in UA plasma concentration during the first 24 hours of recovery could be explained as a possible compensation mechanism of purine nucleotide synthesis and degradation in response to exercise. On the other hand, Cazzola et al. [12] found that pre-exercise, post-exercise and recovery plasma UA concentrations in junior football players were almost twice lower than in professional football players, but comparable with the results by Manna et al. [2] in the same age group. Our data seem to confirm the observation that the UA plasma level is lower in young male football players than in adult professional players. According to literature, no significant increase in UA was detected in the preparatory phase of training, where volume and intensity of training increase gradually, in the same age group of male football players [2]. Moreover, according to literature, UA is one of principal antioxidant compounds in human plasma [8, 15, 32].

It is suggested that also blood albumin is a hydrosoluble antioxidant [12]. It is well known that exercise is a hyperfiltration state, during which the arterial blood pressure and muscle blood flow are markedly increased. It seems therefore that UA blood level is a valuable tool for assessing metabolic response to aerobic exercise, and that it can be used to estimate physical fitness in combination with physiological tests often used by trainers. It is suggested that not only UA, but also the urea plasma level might be useful for the measurement of training-related stress [14], although those parameters are used more frequently to assess protein catabolism and purine nucleotides degradation [15, 16]. The present study shows that the plasma urea level was increasing during the experiment, yet the differences between the measurement times were statistically non-significant in the male football players. Manna et al. found a statistically significant increase in urea plasma level in junior male football players in their preparatory phase of training as well as in the competitive phase of the training cycle [2]. It is worth noting that urea baseline levels in the male football players in the present study were slightly higher than in Manna et al. [2], in the same age-group of football players. Our results may suggest that participants of this study represented a high level of fitness. A statistically significant correlation between the urea plasma level in recovery and weekly training frequency in our study in the male players seems to confirm this suggestion. Moreover, an increase in the post-exercise urea level combined with correlations between the urea level and both length of training experience and weekly training frequency in our study in the female players suggest indicate a possible compensation mechanism of energy sources in response to exercise, which is connected with protein catabolism and/or purine nucleotides metabolism. Based on our observation and literature data, we assume that UA and urea blood levels could be good diagnostic markers for the evaluation of physical fitness and condition of athletes.

Considering different data on the plasma levels of creatinine, UA and urea, as well as limited data describing the influence of different types of physical exercise on those variables in male junior football players, our study attempted to describe the profile of changes of biochemical parameters in this group of athletes.

Conclusion

The obtained results could help explore the problem of metabolic changes on the systemic level based on changes of selected biochemical parameters. It can also facilitate the individualization of the training process as well as assessment of training system effectiveness. Therefore, we suggest that regular monitoring of various biochemical parameters should be used as part

of training courses of both female and male football players. However, more extensive research on those parameters may help correlate metabolic changes with athletes' fitness levels in a more precise manner.

The study confirms that UA and urea blood plasma levels could be efficient diagnostic markers for evaluation of metabolic response to exercise at least among female athletes. It should be emphasised that correlations between urea blood plasma concentration and length of training experience and weekly training frequency found in this study indicate the urea level as a potential marker of athletes' overtraining syndrome.

What this study adds?

Considering the need for effective metabolic markers of biological response to physical exercise to prevent overtraining, the data from the present study indicate uric acid and urea as promising candidate markers. Monitoring their plasma levels can be, in our opinion, recommended for both amateur and professional athletes. The present study shows that especially urea may serve as an early marker of the overtraining syndrome.

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