

POST-EFFORT CHANGES IN ACTIVITY OF TRADITIONAL DIAGNOSTIC ENZYMIC MARKERS IN FOOTBALL PLAYERS' BLOOD

PROMENE U AKTIVNOSTIMA TRADICIONALNIH DIJAGNOSTIČKIH ENZIMSKIH MARKERA U KRVI FUDBALERA POSLE FIZIČKOG NAPREZANJA

Tomasz Chamera¹, Michał Spieszny², Tomasz Klocek², Dorota Kostrzewa-Nowak³, Robert Nowak^{3*}, Milena Lachowicz¹, Rafał Buryta⁴, Krzysztof Ficek⁴, Jerzy Eider⁴, Waldemar Moska¹, Paweł Ciężczyk^{1,4}

¹Faculty of Sport Education, Gdansk University of Physical Education and Sport, Gdansk, Poland

²Institute of Sports, University School of Physical Education in Cracow, Cracow, Poland

³Department of Biochemistry, University of Szczecin, Szczecin, Poland

⁴Faculty of Physical Education and Health Promotion, University of Szczecin, Szczecin, Poland

Summary

Background: Long-term and intensive physical effort causes metabolic and biochemical adaptations for both athletic and non-athletic objectives. Knowing the importance of aerobic training in football players, the aim of this study was to evaluate changes in the activity of: creatinine kinase (CK), creatine kinase MB (CKMB), lactate dehydrogenase (LDH), α -hydroxybutyrate dehydrogenase (HBDH), cholinesterase (ChE) and alkaline phosphatase (ALP) in response to a semi-long distance outdoor run under aerobic conditions among both female and male football players.

Methods: Sixteen participants aged 21.9 ± 2 years (women) and 18.4 ± 0.5 years (men), all of them voluntarily recruited football players, took part in an outdoor run, the women covering a distance of 7.4 ± 0.3 km while men covered a distance of 10.7 ± 1.0 km. Plasma activities of the studied enzymes were determined using an appropriate diagnostic assay kit.

Results: Our results indicate that total LDH activity could be a useful tool in evaluating physical fitness among athletes. We simultaneously established that ChE could not be a marker useful in assessing metabolic response to physical effort in athletes. Moreover, our results suggest that post-effort changes in ALP activity might be used to estimate early symptoms of certain vitamin deficiencies in an athlete's diet.

Conclusions: We confirmed that the assessment of activity of selected traditional diagnostic enzymatic markers provides information about muscle state after physical effort.

Keywords: aerobic training, athletes, enzymatic markers

Address for correspondence:

Robert Nowak
Department of Biochemistry,
3C Felczaka St., 71-412 Szczecin, Poland,
e-mail: robnowak@univ.szczecin.pl

Kratok sadržaj

Uvod: Dugoročno i intenzivno fizičko naprezanje izaziva metaboličke i biohemijske promene radi sportskih ali i nespportskih ciljeva. S obzirom na važnost aerobnog treninga za fudbalere, ova studija imala je za cilj određivanje promena u aktivnostima: kreatinin kinaze (CK), kreatin kinaze MB (CKMB), laktat dehidrogenaze (LDH), α -hidroksibutirat dehidrogenaze (HBDH), holinesteraze (ChE) i alkalne fosfataze (ALP) u okviru odgovora na trčanje napolju, na poludugoj stazi, u aerobnim uslovima, kod igrača fudbala muškog i ženskog pola.

Metode: Šesnaest učesnika starosti $21,9 \pm 2$ godine (žene) i $18,4 \pm 0,5$ godina (muškarci), dobrovoljno regrutovanih fudbalera, učestvovalo je u trci napolju, u kojoj su žene prelazile $7,4 \pm 0,3$ km a muškarci razdaljinu od $10,7 \pm 1,0$ km. Aktivnosti proučavanih enzima u plazmi određene su odgovarajućim dijagnostičkim testovima.

Rezultati: Naši rezultati pokazuju da bi ukupna aktivnost LDH mogla biti korisno sredstvo za procenjivanje fizičke spremnosti kod sportista. Istovremeno smo ustanovili da ChE ne može biti koristan marker za određivanje metaboličkog odgovora na fizičko naprezanje kod sportista. Pored toga, naši rezultati pokazuju da se promene u aktivnosti ALP posle naprezanja mogu koristiti za otkrivanje ranih simptoma deficijencije nekih vitamina u ishrani sportista.

Zaključak: Potvrdili smo da određivanje aktivnosti izabranih tradicionalnih dijagnostičkih enzimskih markera donosi informacije o stanju mišića posle fizičkog naprezanja.

Ključne reči: aerobni trening, sportisti, enzimski markeri

List of abbreviations: ALP, alkaline phosphatase; ChE, cholinesterase; CK, creatinine kinase; CKMB, creatine kinase MB; HBDH, α -hydroxybutyrate dehydrogenase; LDH, lactate dehydrogenase.

Introduction

Long-term and intensive physical effort causes metabolic and biochemical adaptations for both athletic and non-athletic objectives. Physical activity is one of the most important measures to enable an individual to control body weight, delay the occurrence of chronic disorders and prevent various diseases. It is well known that energy turnover is the main factor influencing metabolism intensity. Hence, regular physical activity induces metabolic changes, yet can alter the serum concentrations of numerous laboratory parameters. These modifications, especially in terms of increases, can often lead to results outside the reference value range, leading to additional examinations for the athlete and/or discontinuation of training and competition (1, 2). Various literature data emphasize that long-term training influences athletes' cellular metabolism and can lead to muscle damage, and induce oxidative stress (1, 3, 4) that causes metabolic changes on a biochemical level in diagnostic parameters analysed in athletes' blood (4–7). There are some typical metabolic parameters described as sports markers which are used by trainers and athletes to follow and characterise their strength and efficiency e.g. creatine kinase activity, blood lactate level (8).

Various intracellular enzymes are described in clinical enzymology as tissue or organ markers used as diagnostic markers in identification and differentiation of diseases, e.g. plasma aminotransferases activity (aspartate aminotransferase, AST; alanine aminotransferase, ALT), gamma-glutamyl transferase (GGT) activity belonging to routinely analysed liver diagnostic markers or both mass and activity of creatine kinase isoenzyme MB (CKMB) combined with lactate dehydrogenase activity (LDH) as well as other non-enzymatic markers belonging to routinely analysed heart diagnostic markers (1, 9). However, most abnormalities in elite athletes' biochemical parameters found during routine training monitoring have no clinical significance (2). Therefore, there is still a need to establish the influence of different types of effort (in aerobic, anaerobic and aerobic-anaerobic conditions) on changes in metabolic markers not only belonging to metabolites, but also those belonging to standardized diagnostic enzymatic tissue or organ markers. More importantly, there is a need to find pleiotropic markers of metabolic response to physical effort. Consequently, it seems that changes in blood plasma activity of enzymes characterising different tissues and organs may provide a broader view of the influence of different types of physical effort among high level fitness athletes.

It is well known that aerobic training in football plays an important role and is designed to improve cardiovascular health. It is imperative during soccer matches as well as training sessions that there is a good supply of oxygen to the active muscles, and that

these tissues have the capability to use the oxygen provided by the circulatory system (10). Considering the importance of aerobic training in football players, the aim of this study was to evaluate the post-exertion changes in activity of selected intracellular enzymes belonging to traditional clinical markers of: muscles, liver, heart and bones. To better understand these phenomena, we determined the changes in the activity of: creatine kinase (CK, EC 2.7.3.2), creatine kinase MB (CKMB, EC 2.7.3.2), lactate dehydrogenase (LDH, EC 1.1.1.27), α -hydroxybutyrate dehydrogenase (HBDH, EC 1.1.99.6), cholinesterase (ChE, EC 3.1.1.8) and alkaline phosphatase (ALP, EC 3.1.3.1) in response to a semi-long distance outdoor run under aerobic conditions in both female and male football players.

Materials and Methods

Experimental approach to the problem

The physical effort in football belongs to a mixed type (both aerobic and anaerobic) which is associated with the different activities of a player during the match. The high number of accelerations and decelerations associated with highly dynamic play create an additional burden on the muscles (11–13). Due to the different types of exercise, the players must be adapted to generate energy using both anaerobic and aerobic metabolic pathways. Various data in the literature identify the differences in biochemical parameters as a result of physical effort and the relation of those changes with the subject's fitness level. Moreover, abnormalities in values of biochemical parameters very often have no clinical significance in elite athletes (2). Hence, it is important to establish the influence of different types of effort, especially in aerobic conditions, on changes in metabolic markers and of equal importance is the need to find pleiotropic markers of metabolic response to physical effort in athletes. Therefore, our study aimed to assess post-effort changes in intracellular enzyme activity in football players' blood. We determined CK, CKMB, LDH, HBDH, ChE and ALP activity in response to a semi-long distance outdoor run under aerobic conditions among both female and male football players to explore these biochemical processes.

Participants

Participants ($n=16$) were recruited among football players, belonging to the Olimpia Szczecin and Pogon Szczecin S.A. football clubs, and divided into two groups according to sex. They had no history of any metabolic or cardiovascular diseases, were non-smokers and were cautioned against taking any medications or supplements known to affect metabolism. The baseline characteristics of both studied groups are presented in *Table 1*. The study was conducted in

Table 1 Baseline characteristics of the participants (mean \pm SD).

	Women	Men
n	8	8
Age (years)	21.9 \pm 2	18.4 \pm 0.5
Height (cm)	164 \pm 4	178 \pm 9
Weight (kg)	56.4 \pm 5.0	68.6 \pm 0.93
Length of training experience (years)	8.62 \pm 3.07	9.12 \pm 0.64
The number of weekly training hours	8.25 \pm 1.60	11.56 \pm 0.81

n – number of participants

accordance with the ethical standards as described by Kruk (14). Participants (and their parents, when necessary) were informed of experimental procedures and possible risks of the experiment before giving their written consent to participate. Local ethics committee approval in accordance with the Helsinki Declaration was received before the beginning of the testing.

Procedures

The exercise test was performed on the last day of their training season in the afternoon of a warm, cloudless summer day. The exercise test consisted of a warm-up routine (10 minutes), the main run (60 minutes) and stretching and breathing exercises (15 minutes). The aim of the exercise was to develop aerobic efficiency below the anaerobic threshold, calculated individually for each participant. To this end, participants ran to maintain a subliminal heart rate of 158 \pm 4 beats/min. The female group of participants ran outdoors covering a distance of 7.4 \pm 0.3 km with a mean speed of 7.5 \pm 0.5 km/h. The male group of participants ran a semi-long distance of 10.7 \pm 1.0 km with a mean speed of 10.6 \pm 1.7 km/h. Heart rate was analysed using a Garmin Forerunner 305 heart rate monitor (Garmin (Europe) Ltd., Romsey, UK). Additionally, in both studied groups the level of lactate was determined to ensure aerobic metabolism during the exercise test.

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. Blood samples were centrifuged 500 \times g for 15 minutes at room temperature in order to receive blood plasma. The plasma was obtained and frozen at -80 °C until later performed analyses (15).

Plasma activities of CK, CKMB, LDH, HBDH, ChE and ALP as well as lactate concentration were

determined in samples obtained before exercise (pre-exercise), immediately after the run (post-exercise) and 15 minutes after completion of the exercise test, at the beginning of recovery time (recovery). Plasma lactate level was determined using a diagnostic colorimetric enzymatic method (Liquick Cor-LACTATE) according to the manufacturer's protocol (PZ Cormay S.A., Łomianki, Poland). Absorption was measured at $\lambda = 520$ nm at 37 °C. Plasma activities of ALP, LDH and CK (U/L) were determined using an appropriate kinetic assay kit according to the manufacturer's protocol (BioMaxima S.A., Lublin, Poland). The kinetic assay kits were also used to determine the activity of HBDH and CKMB (U/L) (PZ Cormay S.A., Łomianki, Poland) and ChE (U/L) (Quimica Clinica Aplicada S.A., Amposta, Spain) according to the manufacturers' protocol. All of the reactions were initiated by the addition of plasma to assay kit reaction mixtures and conducted at 37 °C. ΔA measurements were conducted at $\lambda = 340$ nm in the case of CK, CKMB, LDH and HBDH and at $\lambda = 405$ nm in the case of ALP and ChE. All analysis procedures were validated with the use of multiparametric control serum (BIOLABO S.A.S, Maizy, France). Absorption measurements were made on a SEMCO S91E spectrophotometer (EMCO, Warszawa, Poland). The results of the enzyme activity analyses were referred to reference values provided by appropriate kit manufacturers.

Statistical analyses

All data are presented as mean \pm standard deviation in cases of normal data distribution or median (interquartile range) in cases of non-normal data distribution. Statistical analyses were performed using STATISTICA (data analysis software system), version 10 software (StatSoft, Inc. (2011)). We used the Shapiro-Wilk W test to check normal data distribution. Significance level of differences observed between analysed time points (pre-exercise vs. post-exercise vs. recovery) for each participant was calculated according to the results of data distribution analysis. In cases of normal data distribution, we used analysis of variance (ANOVA) with repeated measures test followed by contrast analyses. In cases of non-normal data distribution, we used Friedman's two-way analysis of variance followed by appropriate post-hoc analyses. Each time, $P \leq 0.05$ was considered as a significant difference.

Results

All the participants were recruited from football clubs based on a similar period of training experience and divided into two groups according to sex. Female participants were aged 21.9 \pm 2, while males were aged 18.4 \pm 0.5 years. The baseline characteristics of both studied groups are presented in *Table 1*. It was found that the baseline values of CK activity in both

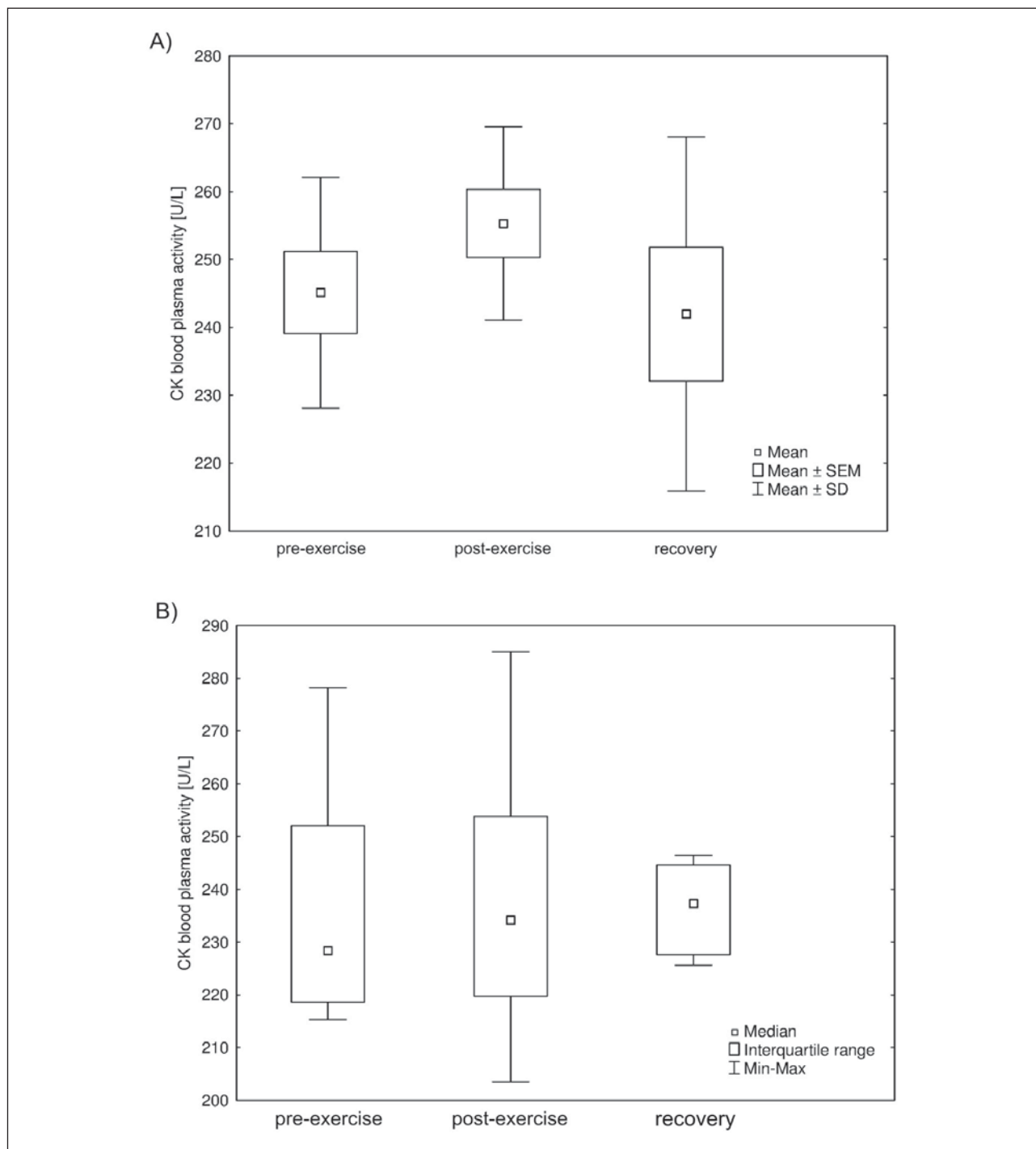


Figure 1 A) Mean blood plasma creatinine kinase (CK) activity (U/L) in female (n=8) football players determined before (pre-exercise), just after (post-exercise) a 60-min-long outdoor run at a pace ensuring aerobic metabolism (as ensured by individually calculated and monitored heart rate and confirmed by serum lactate level determination) and at the beginning of recovery (recovery). The midpoint represents mean; box represents 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) was assessed using analysis of variance (ANOVA) with a repeated measures test followed by contrast analyses.

B) Median blood plasma creatinine kinase (CK) activity (U/L) in male (n=8) football players determined before (pre-exercise), just 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 determination) and at the beginning of recovery (recovery). The midpoint represents median; box represents standard interquartile range; whiskers represent min-max range. Significance level of differences observed between analysed time points (pre-exercise vs. post-exercise vs. recovery) was assessed using Friedman's two-way analysis of variance followed by appropriate post hoc analyses.

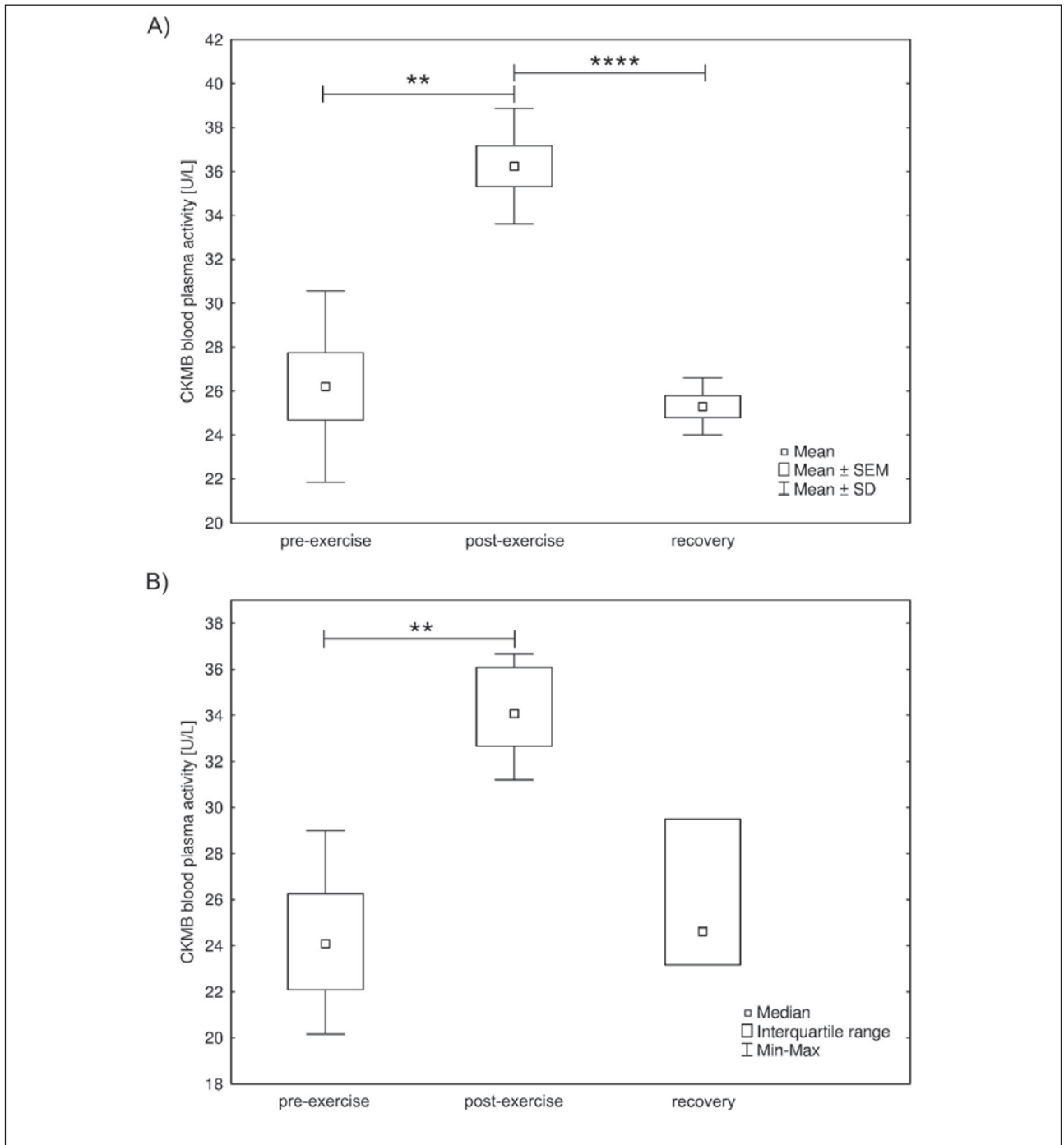


Figure 2 A) Mean blood plasma creatinine kinase MB isoenzyme (CKMB) activity (U/L) in female (n=8) football players determined before (pre-exercise), just after (post-exercise) a 60-min-long outdoor run at a pace ensuring aerobic metabolism (as ensured by individually calculated and monitored heart rate and confirmed by serum lactate level determination) and at the beginning of recovery (recovery). The midpoint represents mean; box represents 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) was assessed using analysis of variance (ANOVA) with a repeated measures test followed by contrast analyses. ** P<0.01; **** P<0.0001.

B) Median blood plasma creatinine kinase MB isoenzyme (CKMB) activity (U/L) in male (n=8) football players determined before (pre-exercise), just 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 determination) and at the beginning of recovery (recovery). The midpoint represents median; box represents standard interquartile range; whiskers represent min-max range. Significance level of differences observed between analysed time points (pre-exercise vs. post-exercise vs. recovery) was assessed using Friedman's two-way analysis of variance followed by appropriate post hoc analyses. ** P<0.01.

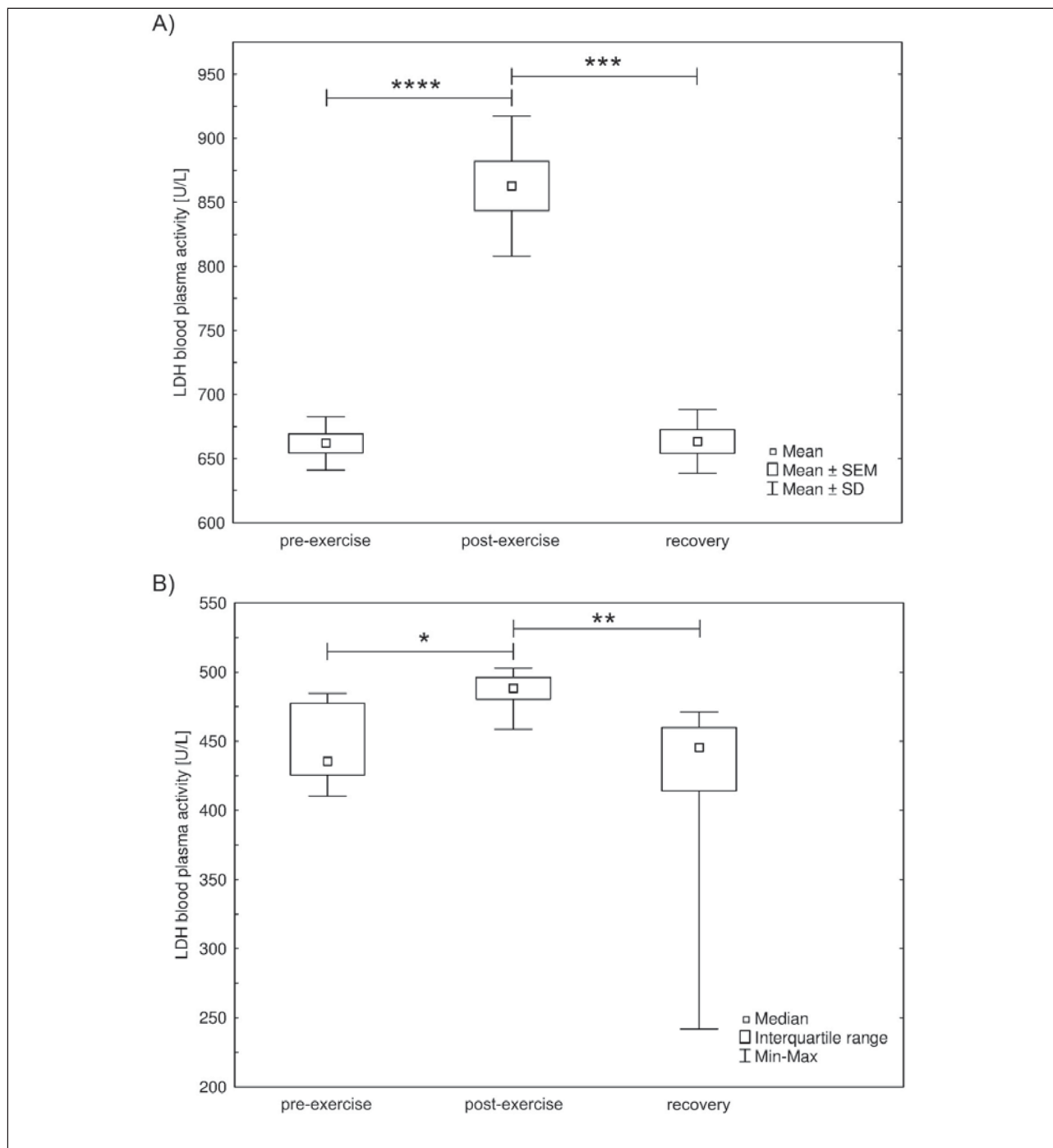


Figure 3 A) Mean blood plasma lactate dehydrogenase (LDH) activity (U/L) in female (n=8) football players determined before (pre-exercise), just after (post-exercise) a 60-min-long outdoor run at a pace ensuring aerobic metabolism (as ensured by individually calculated and monitored heart rate and confirmed by serum lactate level determination) and at the beginning of recovery (recovery). The midpoint represents mean; box represents 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) was assessed using analysis of variance (ANOVA) with a repeated measures test followed by contrast analyses. *** $P \leq 0.001$; **** $P \leq 0.0001$.

B) Median blood plasma lactate dehydrogenase (LDH) activity (U/L) in male (n=8) football players determined before (pre-exercise), just 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 determination) and at the beginning of recovery (recovery). The midpoint represents median; box represents standard interquartile range; whiskers represent min-max range. Significance level of differences observed between analysed time points (pre-exercise vs. post-exercise vs. recovery) was assessed using Friedman's two-way analysis of variance followed by appropriate post hoc analyses. * $P \leq 0.05$; ** $P \leq 0.01$.

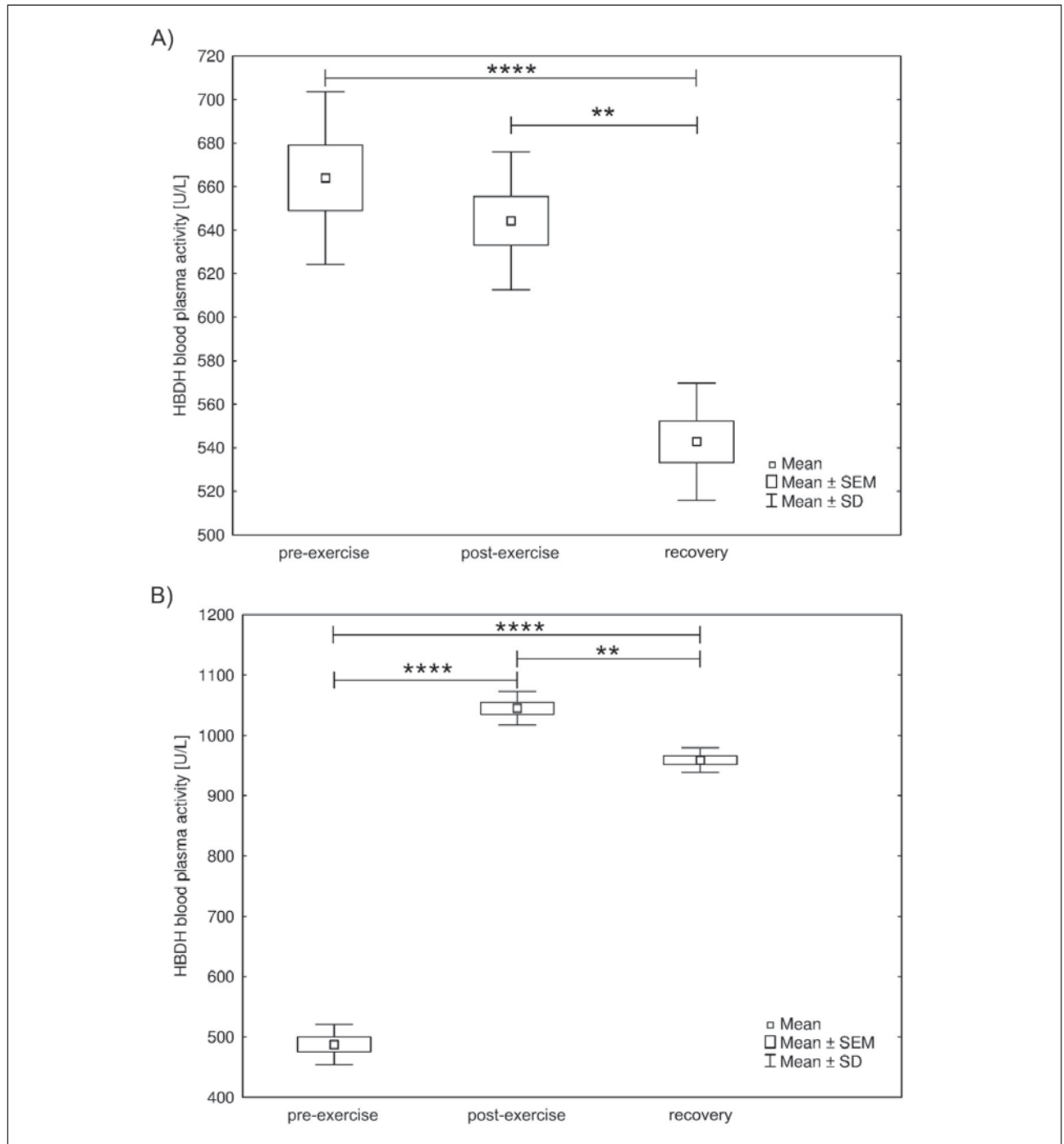


Figure 4 A) Mean blood plasma α -hydroxybutyrate dehydrogenase (HBDH) (U/L) in female (n=8) football players determined before (pre-exercise), just after (post-exercise) a 60-min-long outdoor run at a pace ensuring aerobic metabolism (as ensured by individually calculated and monitored heart rate and confirmed by serum lactate level determination) and at the beginning of recovery (recovery). The midpoint represents mean; box represents 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) was assessed using analysis of variance (ANOVA) with a repeated measures test followed by contrast analyses. ** $P \leq 0.01$; **** $P \leq 0.0001$.

B) Mean blood plasma α -hydroxybutyrate dehydrogenase (HBDH) (U/L) in male (n=8) football players determined before (pre-exercise), just 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 determination) and at the beginning of recovery (recovery). The midpoint represents mean; box represents 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) was assessed using analysis of variance (ANOVA) with a repeated measures test followed by contrast analyses. ** $P \leq 0.01$; **** $P \leq 0.0001$.

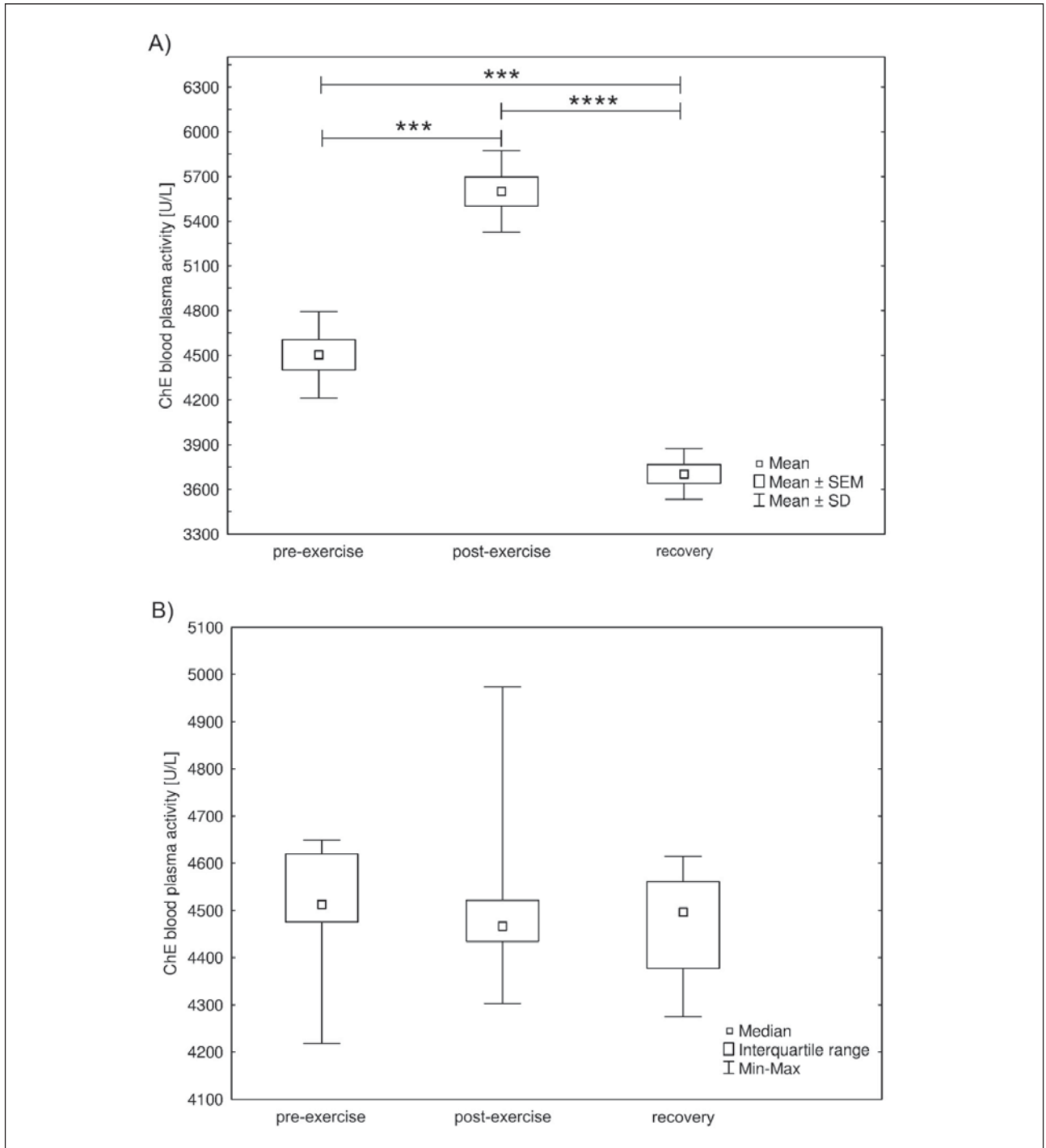


Figure 5 A) Mean blood plasma cholinesterase (ChE) (U/L) in female (n=8) football players determined before (pre-exercise), just after (post-exercise) a 60-min-long outdoor run at a pace ensuring aerobic metabolism (as ensured by individually calculated and monitored heart rate and confirmed by serum lactate level determination) and at the beginning of recovery (recovery). The midpoint represents mean; box represents 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) was assessed using analysis of variance (ANOVA) with a repeated measures test followed by contrast analyses. *** $P \leq 0.001$; **** $P \leq 0.0001$.

B) Median blood plasma cholinesterase (ChE) activity (U/L) in male (n=8) football players determined before (pre-exercise), just 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 determination) and at the beginning of recovery (recovery). The midpoint represents median; box represents standard interquartile range; whiskers represent min-max range. Significance level of differences observed between analysed time points (pre-exercise vs. post-exercise vs. recovery) was assessed using Friedman's two-way analysis of variance followed by appropriate post hoc analyses.

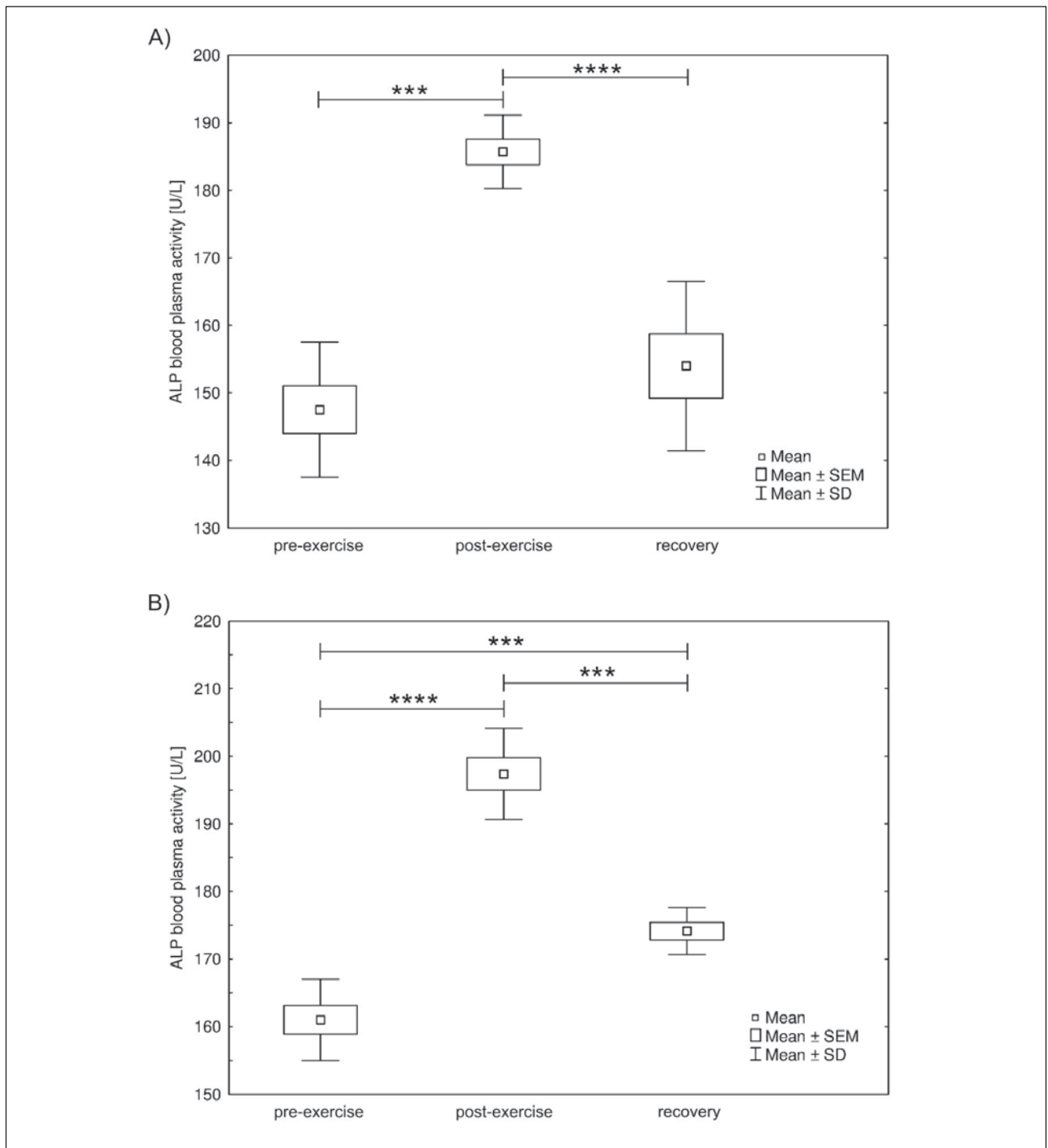


Figure 6 A) Mean blood plasma alkaline phosphatase (ALP) (U/L) in female (n=8) football players determined before (pre-exercise), just after (post-exercise) a 60-min-long outdoor run at a pace ensuring aerobic metabolism (as ensured by individually calculated and monitored heart rate and confirmed by serum lactate level determination) and at the beginning of recovery (recovery). The midpoint represents mean; box represents 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) was assessed using analysis of variance (ANOVA) with a repeated measures test followed by contrast analyses. *** $P \leq 0.001$; **** $P \leq 0.0001$.

B) Mean blood plasma alkaline phosphatase (ALP) (U/L) in male (n=8) football players determined before (pre-exercise), just 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 determination) and at the beginning of recovery (recovery). The midpoint represents mean; box represents 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) was assessed using analysis of variance (ANOVA) with a repeated measures test followed by contrast analyses. *** $P \leq 0.001$; **** $P \leq 0.0001$.

studied groups were about 1.5-fold (women) and 1.2-fold (men) higher than the upper reference limits (<167 and <190 U/L for women and men, respectively). Interestingly, 60 minutes of outdoor running did not influence total CK activity values in the studied athletes' blood (Figure 1). On the other hand, statistically significant changes in CKMB activity and increases in these values in both studied groups immediately after physical effort (Figure 2) were observed, but it was only in women's blood that the increase was also found at the beginning of recovery time. The baseline values of CKMB activity in the studied athletes' blood were almost equal to the upper limit of the reference range (≤ 24 U/L), yet in women's blood these values were slightly higher (26.2 ± 4.4 U/L). Our experiment indicated a statistically significant increase in LDH activity after aerobic effort (Figure 3), which did not persist during recovery time. It is worth noting that activities of LDH and its isoenzyme HBDH before the exercise test in the female athletes were about 1.4 and 3.6-fold higher than the upper reference limit (240–480 U/L and <182 U/L for LDH and HBDH, respectively) but in the male group, 2.6-fold higher values were found only in the case of HBDH. In the male group, as opposed to the female group, a statistically significant increase in HBDH after physical effort was found and it persisted at the beginning of recovery time (Figure 4). Interestingly, only among the female participants were statistically significant changes in ChE activity observed after the run and at the beginning of recovery time (Figure 5). Additionally, in the female group the baseline values of ChE activity were mostly within the range of reference values (4330–11500 U/L), yet in male athletes' blood, the baseline values of ChE were slightly lower than the lower limit of the reference range (5400–13200 U/L). Moreover, we found that the activity of ALP in the examined participants' plasma was statistically significantly higher after aerobic effort as compared to the baseline values (Figure 6) and this increase was also observed at the beginning of recovery time. The ALP activity before the exercise test was about 1.4-fold higher than the upper reference limit (<105 and <115 U/L for women and men, respectively) in both studied groups.

Discussion

Many functional and metabolic changes in the system are yielded with prolonged physical effort and training, which also leads to an increased physical efficiency and tolerance to exertion (1, 4–7). In football and other team sports, energy turnover of the working muscles requires mobilization of aerobic, anaerobic and aerobic-anaerobic metabolic pathways. Furthermore, aerobic training in football plays an important role and is designed mostly to improve the oxygen transport system. It is imperative during soccer matches and training sessions that there is an

adequate supply of oxygen to the active muscles, and that these tissues have the capability to use the oxygen that is provided by the circulatory system (10). Some literature data has evidenced that during a competitive match, e.g. a soccer referee may cover a distance of 9–13 km (16), which is similar to the distance covered by the participants of our exercise experiment. Investigating the impact of physical effort on the activity of intracellular enzyme profiles specific to certain tissues and organs provides additional information not only about the state of muscle, but also its biochemical adaptation to the training process of athletes.

CK activity is an example of a biochemical marker frequently analysed by trainers and sport researchers. In athletes it varies according to the level of training. The investigation of CK activity provides information about muscle state after physical effort (17, 18). Additional information is provided by measurement of the activity of CK isoenzymes: CKMM and CKMB. It was found in our study that a semi-long distance outdoor run among football players did not influence CK activity in either sex. On the other hand, it was found that the aerobic effort yielded a statistically significant increase in CKMB activity in the blood of both studied athlete groups. Brancaccio et al. suggested that exercise may also influence markers of both skeletal and heart muscle damage including CKMB isoenzyme activity, especially when the physical effort is prolonged or strenuous (19). In light of this, it seems that changes in CKMB activity may occur even earlier than those in CK activity, a marker of post-effort metabolic change in football players. Additionally, increases in CKMB isoenzyme activity were found in the blood plasma of ultramarathon runners (20). There is also an opinion that abnormal values of CKMB activity in serum or plasma should be treated with caution, because it may be connected with late cardiomyopathy (19). It must be pointed out that the participants of our study had no history of cardiomyopathy or other heart disorders that rather exclude this phenomenon. The results of our study indicated that an increase in CKMB activity is caused by aerobic effort and, interestingly, this parameter decreases after physical effort to baseline values within 15 minutes. Post-effort increases in CKMB activity were also found by Diaz et al. (21). The higher than reference baseline values in athletes found in our study were also described by Lucia et al. (20) and Lippi et al. (22).

LDH activity is often considered as a marker of muscle cell damage because its blood activity is combined with disruption that occurs in sarcoma (20, 21, 23). According to literature data, the activity of total LDH and its isoenzymes is different in muscle cells and it is combined with the endurance and strength of athletes (19, 21, 24). Interestingly, in muscle biopsies a lower total LDH activity was found at a prevalence of HBDH (LDH1) and LHD2 isoenzyme activity

(24–26). LDH and CK activities measured with the use of needle biopsy were also different before and after the training. It was observed that they changed due to different protocols, intensity and level of training (19, 24). These reports indicated that changes in athletes' blood LDH and HBDH activities are related to the metabolic response to physical effort. In our results, post-effort changes in total LDH activity were found only in women's blood but this increase was observed only immediately after the exercise test. On the other hand, HBDH activity increased only in men's blood and it was observed not only after the run but also at the beginning of recovery time. The increase in total LDH activity after the physical effort in male athletes' blood was also found by other investigators (21, 22, 27). It is worth pointing out that post-effort changes in LDH activity in professional male cyclists were related to the length of distance covered and it was found that this activity increased only in cases of longer distances (27). This may be an explanation of the gender discrepancies in total activity of LDH found in our experiment. We suggested that total LDH activity could be a useful tool to evaluate physical fitness in athletes. It must be emphasised that »abnormally« higher values of total LDH activity is a phenomenon observed very commonly in athletes' blood (2, 21, 22, 27).

ChE, also known as pseudocholinesterase, is a diagnostic marker of liver injuries (28, 29). To the best of our knowledge, there is a lack of information about ChE changes in athletes' blood after physical effort. Moreover, there is no information about baseline values of ChE activity in athletes. Our study indicated that the baseline values of this enzyme in the case of studied participants' were nearly within the range of reference values (4330–11500 and 5400–13200 U/L for women and men, respectively). Interestingly, post-effort values were higher than baseline only in the female football players and these changes were not prolonged, since the activities of ChE at the beginning of recovery time were lower than the post-effort values. Our results indicate that ChE is probably not a useful marker in the assessment of metabolic response to physical effort in athletes.

The activity of blood ALP is a well-known diagnostic marker of mineralization and pathological disorders in bones (2, 30, 31). This traditional diagnostic marker is not very frequently studied in athletes. We found in our study that 60 minutes of outdoor running caused a slight increase in ALP activity immediately after the exercise test in both groups of football players. These data are not in line with Lippi et al. (22) and Diaz et al. (21) who did not observe post-effort changes in ALP activity in studied athletes'

blood. On the other hand, our study was performed on the last day of a whole year of training as well as a competitive season (before the summer holiday break) and this could have influenced the results of ALP activities. In this case, it may reflect the symptoms of bodily fatigue among the participants. Diaz et al. (21) described a correlation between ALP activity and daily vitamin B6 and niacin intake. The participants of our experiment refrained from taking any diet supplements. The results of our study may indicate initial symptoms of vitamin B6 and/or niacin deficiency. It is possible but rather speculative, since the participants themselves reported to have a balanced diet. It would also explain a slightly higher baseline of ALP activity in both women and men. Therefore, our results suggest that post-effort changes in ALP activity might be a useful tool to estimate early symptoms of some vitamin deficiencies in athletes' diets, and help to prevent dangerous metabolic disorders associated with future hypovitaminosis. However, this hypothesis requires further evaluation.

Taking all the data into account, the establishment of the post-effort influence of different types of enzymatic tissues' or organs' metabolic markers would help in the understanding of a pleiotropic response to physical effort in athletes.

Conclusion

To summarize, we confirmed that the assessment of the activity of selected traditional diagnostic enzymatic markers provides information about muscle state after physical effort. Furthermore, our results indicate that total LDH activity could be a useful tool to evaluate physical fitness in athletes. We have also shown that ChE should not really be used as a marker in the assessment of metabolic response to physical effort in athletes. Moreover, our results suggest that post-effort changes in ALP activity might be useful in estimating early symptoms of some vitamin deficiencies in athletes' diets.

Acknowledgments. The authors would like to thank all the individuals who volunteered to participate in the study. The authors declare no conflict of interest. The study was supported by National Science Centre (grant no. 2012/07/B/NZ7/01155).

Conflict of interest statement

The authors stated that have no conflicts of interest regarding the publication of this article.

References

- Banfi G, Colombini A, Lombardi G, Lubkowska A. Metabolic markers in sports medicine. *Adv Clin Chem* 2012; 56: 1–54.
- Fallon KE. The clinical utility of screening of biochemical parameters in elite athletes: analysis of 100 cases. *Br J Sports Med* 2008; 42: 334–7.
- Cazzola R, Russo-Volpe S, Cervato G, Cestaro B. Biochemical assessments of oxidative stress, erythrocyte membrane fluidity and antioxidant status in professional football players and sedentary controls. *Eur J Clin Invest* 2003; 33: 924–30.
- Gravina L, Ruiz F, Lekue JA, Irazusta J, Gil SM. Metabolic impact of a soccer match on female players. *J Sports Sci* 2011; 29: 1345–52.
- Cadefau J, Casademont J, Grau JM, Fernández J, Balaguer A, Vernet M, et al. Biochemical and histochemical adaptation to sprint training in young athletes. *Acta Physiol Scand* 1990; 140: 341–51.
- Meyer T, Meister S. Routine blood parameters in elite soccer players. *Int J Sports Med* 2011; 32: 875–81.
- Wiacek M, Andrzejewski M, Chmura J, Zubrzycki IZ. The changes of the specific physiological parameters in response to 12-week individualized training of young soccer players. *J Strength Cond Res* 2011; 25: 1514–21.
- Mougios V. Reference intervals for plasma creatine kinase in athletes. *Br J Sports Med* 2007; 41: 674–8.
- Sherwi N, Pellicori P, Joseph AC, Buga L. Old and newer biomarkers in heart failure: from pathophysiology to clinical significance. *J Cardiovasc Med (Hagerstown)* 2013; 14: 690–7.
- Buryta R. Significance of controlling the intensity of various types of effort in football. *Centr Eur J Sport Sci Med* 2013; 2: 3–8.
- Hoff J. Training and testing physical capacities for elite soccer players. *J Sports Sci* 2005; 23: 573–82.
- Popadic Gacesa JZ, Barak OF, Grujic NG. Maximal anaerobic power test in athletes of different sport disciplines. *J Strength Cond Res* 2009; 23: 751–5.
- Manna I, Khanna GL, Chandra Dhara P. Effect of training on physiological and biochemical variables of soccer players of different age groups. *Asian J Sports Med* 2010; 1: 5–22.
- Kruk J. Good scientific practice and ethical principles in scientific research and higher education. *Centr Eur J Sport Sci Med* 2013; 1: 25–9.
- Majkić-Singh N, Šumarac Z. Quality indicators of the pre-analytical phase. *J Med Biochem* 2012; 31: 174–83.
- Castagna C, Abt G, D'Ottavio S. Physiological aspects of soccer refereeing performance and training. *Sports Med* 2007; 37: 625–46.
- Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. *Br Med Bull* 2007; 81–82: 209–30.
- Vincent HK, Vincent KR. The effect of training status on the plasma creatine kinase response, soreness and muscle function following resistance exercise. *Int J Sports Med* 1997; 18: 431–7.
- Brancaccio P, Limongelli FM, Maffulli N. Monitoring of serum enzymes in sport. *Br J Sports Med* 2006; 40: 96–7.
- Lucia A, Morán M, Pérez M, Saborido A, Díaz E, Megias A, et al. Short-term effects of marathon running in master runners: no evidence of myocardial injury. *Int J Sports Med* 1999; 20: 482–6.
- Díaz E, Ruiz F, Hoyos I, Zubero J, Gravina L, Gil J, et al. Cell damage, antioxidant status, and cortisol levels related to nutrition in ski mountaineering during a two-day race. *J Sports Sci Med* 2010; 9: 338–46.
- Lippi G, Salvagno GL, Montagnana M, Schena F, Ballestreri F, Guidi GC. Influence of physical exercise and relationship with biochemical variables of NT-pro-brain natriuretic peptide and ischemia modified albumin. *Clin Chim Acta* 2006; 367: 175–80.
- Lippi G, Schena F, Montagnana M, Salvagno GL, Banfi G, Guidi GC. Significant variation of traditional markers of liver injury after a half-marathon run. *Eur J Intern Med* 2011; 22: e36–38.
- Klapcińska B, Iskra J, Poprzecki S, Grzesiok K. The effects of sprint (300 m) running on plasma lactate, uric acid, creatine kinase and lactate dehydrogenase in competitive hurdlers and untrained men. *J Sports Med Phys Fitness* 2001; 41: 306–11.
- Ohkuwa T, Saito M, Miyamura M. Plasma LDH and CK activities after 400 m sprinting by well-trained sprint runners. *Eur J Appl Physiol Occup Physiol* 1984; 52: 296–9.
- Favero TG, Stavrianeas S, Klug GA. Training-induced alterations in lactate dehydrogenase reaction kinetics in rats: a re-examination. *Exp Physiol* 1999; 84: 989–98.
- Mena P, Maynar M, Campillo JE. Changes in plasma enzyme activities in professional racing cyclists. *Br J Sports Med* 1996; 30: 122–4.
- Brown SS, Kalow W, Pilz W, Whittaker M, Woronick CL. The plasma cholinesterases: a new perspective. *Adv Clin Chem* 1981; 22: 1–123.
- Turecky L, Kupcova V, Mojto V, Smutny M, Uhlíkova E, Vozar I. Serum cholinesterase activity and proteosynthetic function of liver in patients with diabetes mellitus. *Bratisl Lek Listy* 2005; 106: 266–9.
- Iida T, Harada T, Ishizaki F, Nitta Y, Aoi S, Ikeda H, et al. Changes in bone mineral density and metabolism in women: evaluation of bodily characteristics, bone metabolic markers and bone mineral density. *Hiroshima J Med Sci* 2013; 62: 49–53.
- Bayhan I, Dogan NU, Ozaksit G, Uygur D, Ugurlu N, Ugur M. Effect of strontium ranelate on serum leptin and bone turnover markers in women with established postmenopausal osteoporosis. *J Reprod Med* 2013; 58: 319–23.

Received: January 27, 2014

Accepted: February 24, 2014