

TRENDS OF HEMATOLOGICAL AND BIOCHEMICAL VALUES IN THE BLOOD OF YOUNG RACE HORSES DURING STANDARDIZED FIELD EXERCISE TESTS

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The aim of this study was to compare the changes in hematological and biochemical parameters in race horses subjected to standardized field exercise tests. The tests were performed two times: in May, at the beginning of the racing season and then four months later. Ten horses were studied in May (5 Thoroughbreds and 5 Purebred Arabian horses). Eight horses were studied in September (4 Thoroughbreds and 4 Arabian horses). For each horse, venous blood samples were collected at rest, after warm-up, just after the end of each gallop, and 30 minutes after the end of the exercise. The concentration of blood lactic acid (LA) hemoglobin (Hb), plasma glucose (Glc), triacylglycerols (TG), glycerol, uric acid (UA), total protein, total thiobarbituric acid-reactive substances (TBARS), the activity of lactate dehydrogenase (LDH), kreatine kinase (CK) and aspartate aminotransferase (AST) and the total antioxidant status (TAS) were determined.

The standardized field exercise test performed in September, in comparison to the first test, evoked the lowest increase in blood LA and plasma Glc and TG levels determined during exercise. The rapid return of elevated levels of LA, Hb and UA to their initial values was also observed in September, as compared to data obtained in May.

Key words: biochemical values, horses, standardized exercise test, training

INTRODUCTION

The breeding value of warm-blood horses can be measured by their utility level. The utility level can be assessed through testing their competitive spirit in racing starts. In addition to the genetic endowment, being in good physical condition is considered to be a prerequisite for positive racing results. Horse fitness is evaluated by the physiological and biochemical parameters determined

while the exercise test is being carried out. Physical exercise can modify the animal's physiologic metabolism. However research on horses is particularly difficult. This is because the different types of training, racing, transport, breed and temperament, as well as season can produce variations in blood constituents levels (Assenza *et al.*, 1994, Podolak *et al.*, 1999, Kedzierski and Bergero, 2006; Kirschvink *et al.*, 2006). Among the many biochemical parameters determined in the blood, only the lactic acid (LA) level is used in practice to evaluate the fitness of racehorses (Lindner, 2000). To compare LA values in horses during training, the velocity at a blood LA concentration of 4 mmol/L (V_4) has generally been used and accepted as an indicator of racing performance (Harkins *et al.*, 1993). The changes in the rate of the LA production by muscles, reflect the degree at which anaerobic glycolysis contributes to the total energy production.

Physical effort also induces changes in the level of other blood born substrates and their metabolites. For example, glucose (Glc) plasma concentration rises after intense exercise as in races and jumping-shows due to activation of the sympathoadrenal system and it decreases during endurance competitions (Assenza *et al.*, 1996; Marlin *et al.*, 2002; Kedzierski and Bergero, 2006).

Another source of energy for working muscles are the oxidizing processes of free fatty acids (FFA), which are released from triacylglycerols (TG). Despite FFA consumption by muscles at this time, TG concentration in the blood of a horse increases (Poso *et al.*, 1989; Podolak *et al.*, 2004; Kedzierski and Bergero, 2006). It is thought that the lipolysis and FFA burning are simultaneously accompanied by the synthesis of TG in the liver (Poso *et al.* 1989). This increase of TG is specific for the exercising horse. It is not recorded in human beings or rodents (Poso *et al.*, 1989). A disorder of lipid metabolism can lead to equine hyperlipaemia and hepatic diseases (Bergero and Nery, 2008).

The plasma glycerol level also increases during physical effort as a result of the intensification of the lipolysis rate occurring in these conditions. In horses, this rise is positively correlated with exercise intensity and fatigue of the examined subjects (Poso *et al.*, 1989).

The other feature characteristic for horses is splenic contraction. Splenic contraction involves the increase of hematological values in the blood depending on the work load (Kedzierski and Podolak, 2002).

Intense effort also leads to adenine nucleotide degradation. A good marker of muscle ATP loss is plasma uric acid (UA) concentration (Castejón *et al.*, 2006).

Exercise induces oxidative stress and damage of several tissues by oxygen free radicals. This may be the reason for increased tissue-enzymes-activity in plasma, which was also observed after exercise (Chiaradia *et al.*, 1998; Marlin *et al.*, 2002).

Physical effort influences many parameters in the horse blood and since training should lead to a more efficient energy metabolism, it seems advisable to determine these changing parameters during a standardized exercise test. Studies of changes in blood born substrates in galloping horses subjected to a standardized exercise test have not been published. It can be assumed that the investigations will lead to a broader knowledge concerning the adaptive

mechanisms of race horses to training. The studies can also lead to an evaluation of the usefulness of the applied tests for determining the horse performance level.

MATERIAL AND METHODS

Horses

This study was undertaken in Poland at the Sluzeviec (Warsaw) racetrack, during two experimental periods. The first experimental period was in May and the second in September. Ten horses (5 Thoroughbreds and 5 Purebred Arabian horses) were studied in May and eight horses (4 Thoroughbreds and 4 Arabian horses) were studied in September. Four of the horses were used in both experimental periods.

All the subjects were clinically healthy. The Thoroughbreds were 2-3 years old and the Arabian horses were 3-4 years old. All the horses were trained on the same race track. All the horses were consistently conditioned with the principles of a trainer preparing the horses for races. The animals were fed with typical fodder used for race horses in Poland. The horses had been in low intensity exercise training prior to the start of testing. After the first standardized exercise test which was performed in May, the intensity of training was increased. The horses were then exercised five days a week. The daily training routine consisted of a warm up, a gallop with a mean speed of 7-10 m/s to a distance of 800-1200 m and cooling down in a horse walker. All the studied horses competed in official races which were held from May until September.

The study was accepted by the Local Ethics Review Committee for Animal Experimentation. The study was conducted according to the European Community regulations concerning the protection of experimental animals.

Exercise Test Protocol

In May, and then again in September, the horses were administered the same effort triangular test, on the race track. The test was composed of three different steps. First the horses had a warm-up of 15 minutes with a rider. Then they were galloped for three steps of 1200 m each, at increasing speeds: 7.50 m/s, 8.33 m/s and 9.17 m/s (450, 500 and 550 m/min, respectively). After every step they had a rest of 1 minute.

For each horse six jugular venous blood samples were collected. The blood was taken at rest, after warm-up, just after the end of each step, and 30 minutes after the end of the last step. Two test tubes were used for each blood drawing: one containing di-potassium EDTA, used for Hb determination, and the second containing ammonium heparin used for the determination of all other parameters.

Laboratory Analysis

Blood LA concentration was determined immediately in the field by Dr Lange's enzymatic cuvette test (Berlin, Germany). The other parameters were determined during the three days following the drawings. The levels of Hb, Glc, TG, UA and total protein, and activity of lactate dehydrogenase (LDH), creatine

kinase (CK) and aspartate aminotransferase (AST) were determined using Dr. Lange diagnostic Kits. The plasma glycerol concentration was measured by Boehringer Mannheim (Darmstadt, Germany) enzymatic test, the total antioxidant status (TAS) by Randox test (Crumlin, United Kingdom). The total thiobarbituric acid-reactive substances (TBARS) were estimated according to the method described by Ledwozyw *et al.* (1986).

Statistical Analysis

Statistical analysis of the results was performed using ANOVA, and Tukey test. The data are shown as mean \pm standard deviation (SD).

RESULTS

During the test performed in May three of the investigated horses ran too fast and they reached a blood LA level of over 10 mmol/L. A similar situation with one of the horses took place during the study in September. The data obtained from these four horses were not considered in this study and, as a result, the number of studied horses was 7 in May as in September.

On the basis of the obtained results of velocity and blood LA concentration the indicator V_4 was calculated. It amounted to 9.3 ± 2.2 m/s in May and 10.5 ± 1.6 m/s in September. This increase was statistically significant ($p \leq 0.10$).

Means and SD for parameters related to energy metabolism like LA, Hb, Glc, TG, glycerol and UA are summarized in Table 1. The amount of exercise which the horses were under caused an increased level of LA, Hb, glycerol and UA. Blood LA concentration increased after the second step, and the following increase after the third step in May was also statistically significant. Blood Hb level was on a higher level than at rest, during warm-up and all steps tested. Only in May was a rise of the plasma glycerol level observed after all the steps of the test. The level of UA was statistically significant only after the third step in May as well as in September. The means of all results of LA, Glc, TG and glycerol obtained in May were statistically higher than those determined in September.

The levels of other determined parameters are shown in Table 2. Plasma LDH, CK and AST activity, as well as levels of total protein, TBARS and TAS did not change significantly. For this reason, they are presented only while at rest, after the last step of the test and after the 30 minute rest.

Table 3 summarizes the significant differences among the differences between values reached after exercise and at rest in May, compared to the results obtained in September. An increase in the levels of LA, TG and glycerol after the third step of the test as compared to the resting values, was higher in May than in September. Similarly, after the 30-minute rest, a post-exercise rise in blood LA, Hb and glycerol levels proved to be higher in May as compared to those found in September.

Table 1. Levels of blood lactic acid (LA), hemoglobin (Hb), and plasma glucose (Glc), triacylglycerides (TG), glycerol and uric acid (UA) in young racehorses during standardized field exercise tests performed twice during the same training season two times: in May and then again in September (mean \pm SD)

Parameter	Month	at rest	after warm-up	after 1 st step	after 2 nd step	after 3 rd step	30' rest	Mean
LA (mmol/L)	May	0.77 \pm 0.11 ^a	1.34 \pm 0.42 ^a	1.31 \pm 0.41 ^a	2.69 \pm 0.82 ^b	5.44 \pm 1.96 ^c	1.57 \pm 0.67 ^{ab}	2.19 \pm 1.81 ^x
	September	0.80 \pm 0.12 ^a	0.98 \pm 0.43 ^a	1.36 \pm 0.39 ^a	2.83 \pm 1.63 ^b	4.10 \pm 1.19 ^{bc}	0.90 \pm 0.52 ^a	1.78 \pm 1.50 ^y
Hb (mmol/L)	May	15.1 \pm 0.74 ^a	18.3 \pm 1.72 ^b	18.1 \pm 1.51 ^b	19.9 \pm 1.82 ^b	20.8 \pm 1.64 ^b	16.1 \pm 1.63 ^a	18.1 \pm 2.42
	September	14.6 \pm 1.35 ^a	18.0 \pm 2.15 ^b	19.1 \pm 1.53 ^b	19.7 \pm 2.15 ^b	20.2 \pm 1.96 ^b	14.3 \pm 1.80 ^a	17.7 \pm 2.94
Glc (mmol/L)	May	6.30 \pm 0.50	6.06 \pm 1.00	5.95 \pm 0.76	6.16 \pm 0.72	6.86 \pm 0.51	6.09 \pm 0.52	6.26 \pm 0.73 ^x
	September	5.67 \pm 0.76	5.00 \pm 0.54	5.04 \pm 0.83	5.20 \pm 1.03	5.87 \pm 0.83	5.54 \pm 0.64	5.39 \pm 0.81 ^y
TG (mmol/L)	May	0.34 \pm 0.10	0.39 \pm 0.20	0.42 \pm 0.21	0.43 \pm 0.11	0.45 \pm 0.16	0.36 \pm 0.14	0.41 \pm 0.16 ^x
	September	0.27 \pm 0.10	0.30 \pm 0.10	0.32 \pm 0.09	0.31 \pm 0.11	0.32 \pm 0.09	0.24 \pm 0.10	0.29 \pm 0.10 ^y
Glycerol (μ mol/L)	May	18.6 \pm 18.0 ^a	63.3 \pm 39.1 ^{ab}	87.1 \pm 44.2 ^b	85.7 \pm 29.4 ^b	93.3 \pm 42.0 ^b	74.9 \pm 64.9 ^{ab}	70.8 \pm 47.1 ^x
	September	42.9 \pm 26.3	50.0 \pm 27.7	65.7 \pm 31.0	67.1 \pm 35.0	67.4 \pm 22.1	50.0 \pm 14.1	57.1 \pm 27.1 ^y
UA (μ mol/L)	May	11.2 \pm 2.66 ^a	15.6 \pm 3.81 ^{ab}	15.5 \pm 3.50 ^{ab}	21.6 \pm 7.31 ^{ab}	26.4 \pm 18.2 ^b	17.5 \pm 5.49 ^{ab}	18.0 \pm 9.47
	September	12.0 \pm 4.18 ^a	13.7 \pm 4.47 ^a	15.1 \pm 3.92 ^{ab}	19.0 \pm 5.69 ^{ab}	22.0 \pm 6.66 ^b	14.9 \pm 9.18 ^{ab}	16.3 \pm 8.70

Data marked with different letters a, b, c are statistically different at $p \geq 0.05$ according to Tukey test (ANOVA). Means obtained in May and September marked x, y are statistically different at $p \geq 0.05$ according to t-student test.

Table 2. Levels of plasma LDH, CK, AST, total protein, TBARs and TAS in young racehorses during standardized field exercise tests performed twice during the same training season two times: in May and then again in September (mean \pm SD)

Exercise test performed:	In May			In September		
	at rest	after 3 rd step	30' rest	at rest	after 3 rd step	30' rest
LDH (U/L)	417 \pm 132	500 \pm 127	454 \pm 114	363 \pm 118	423 \pm 114	396 \pm 110
CK (U/L)	134 \pm 31.3	161 \pm 36.3	166 \pm 34.7	189 \pm 86.8	227 \pm 37.1	229 \pm 51.4
AST (U/L)	294 \pm 55.5	326 \pm 58.8	308 \pm 43.1	290 \pm 131	330 \pm 141	305 \pm 120
Total protein (g/L)	68.5 \pm 6.77	73.7 \pm 6.28	68.0 \pm 6.03	62.7 \pm 5.41	67.7 \pm 3.78	61.5 \pm 3.95
TBARS (μ mol/L)	2.32 \pm 0.43	2.58 \pm 0.65	2.39 \pm 0.57	2.08 \pm 0.45	2.25 \pm 0.39	2.41 \pm 0.50
TAS (mmol/L)	0.55 \pm 0.03	0.48 \pm 0.06	0.50 \pm 0.05	0.61 \pm 0.03	0.65 \pm 0.05	0.62 \pm 0.07

Statistically significant differences were not found according to Tukey test (ANOVA).

Table 3. Comparison of the post-exercise changes in levels of LA, Hb, TG, and glycerol determined during two consecutive standardized field exercise tests (mean \pm SD)

Data evaluated between values obtained:	after 3 rd step and at rest		after 30' post-exercise rest and at rest	
	In May	In September	In May	In September
LA	4.67 \pm 1.92 ^x	3.30 \pm 1.19 ^y	0.80 \pm 0.70 ^x	0.05 \pm 0.41 ^y
Hb	5.65 \pm 1.94	5.54 \pm 1.83	1.03 \pm 2.15 ^x	-0.31 \pm 1.35 ^y
TG	0.11 \pm 0.07 ^x	0.04 \pm 0.07 ^y	0.01 \pm 0.13	-0.03 \pm 0.05
Glycerol	74.8 \pm 34.1 ^x	24.5 \pm 38.2 ^y	56.3 \pm 59.6 ^x	6.93 \pm 35.5 ^y

Data marked with different letters x,y are statistically different at $p \geq 0.05$ according to t-student test.

DISCUSSION

All the horses were healthy and all the parameters measured at rest, fell into the physiological range. The slight increase and/or decrease recorded for the mean values during the trial, stand for a low metabolic engagement in this kind of

effort trial. The individual variability explains the high SD of estimation of some data and decreased the possibility of noticing statistically significant differences.

Blood LA level was used in this study as an indicator of the relative intensity of exercise. LA is a parameter often employed for gauging the intensity of exercise, and training level or exhaustion of horses (Guhl *et al.*, 1996; Hinchcliff *et al.*, 2002). It is claimed that a high, post-exercise LA level is correlated with quite rapid runs (Davie and Evans, 2000). A properly managed training program leads to lowering of LA levels after exercise that enables a higher speed (Geor *et al.*, 2000, Hinchcliff *et al.*, 2002). Moreover, a blood LA concentration after a strenuous treadmill exercise is correlated with the quality of the horse racing performance (Evans *et al.* 1993; Gondim *et al.*, 2007). Thus, it can be ascertained that the four-month race training studied here led to a decrease in the relative intensity of exercise performed with the same speed and duration.

Changes in the blood Hb levels of horses are related to the intensity of exercise. These levels are not related to the fitness of trained subjects (Kedzierski and Podolak, 2002; Stopyra *et al.*, 2004).

The plasma Glc level determined during the test was generally lower in September than in May. The tendency for a decrease in the plasma Glc concentration in consecutive phases of training was reported previously (Malinowski *et al.*, 2002).

Comparison of TG values obtained in the two studied phases of training indicated that TG synthesis rate while exercising decreases with training. Determination of plasma TG level has not been generally accepted for monitoring the post-exercise changes in horses (Lindner, 2000). There are only a few data available in the literature concerning this problem. In these works, it is assumed that the lipid metabolism in horses varies in regard to intensity or duration of exercise, as well as to breed, age and sex of the investigated animals (Poso *et al.* 1989; Podolak *et al.*, 1999; Podolak *et al.*, 2004; Kedzierski and Bergero, 2006). The statement above implies that the horses subjected to training develop a higher efficiency of the mechanism controlling lipid metabolism during the following race seasons (Kedzierski and Podolak, 2002). In the experiment described by Munoz *et al.* (2002) just a two-month training of Arabian horses exerted a significant effect on the regulation of the energetic processes in these animals. For humans, regular exercise is also beneficial as it improves the lipid serum profile (Eisenmann *et al.*, 2001).

Plasma glycerol level was also transiently elevated during the exercise test. Similar changes were described in other exercising horses (Poso *et al.*, 1989; Kedzierski and Bergero, 2006; Kedzierski *et al.*, 2007). From the studies of Poso *et al.* (1989), it appears that the intensity of exercise and the level of fatigue both increase plasma glycerol and TG levels. In the present study, an exercise-involved rise in plasma glycerol and TG levels proved to be lower in September, after the four-month race-training. These observations confirm the effect of progressive development of a mechanism underlying the control of lipid metabolism together with training.

The increase in plasma UA level observed during the tests the horses were put through related to the intensity of the following steps of the test and was typical for exercised horses (Balogh *et al.*, 2001; Marlin *et al.*, 2002; Castejón *et al.*, 2006).

The exercise test that the horses were put through did not influence significantly the activity of plasma LDH, CK and AST, as well as total protein, TBARS and TAS levels. The increase in LDH, CK or AST was observed in exercised race horses (Harris *et al.*, 1998; Kedzierski and Bergero, 2006), after endurance rides (Marlin *et al.*, 2002; Barton *et al.*, 2003) and also after show-jump competitions (Balogh *et al.*, 2001). The total plasma protein also increases after exercise as a result of blood dehydration, but these changes can be lowered by the conditioning process (Hinchcliff *et al.*, 2002). A strenuous race or endurance effort can also increase the plasma TBARS concentration (White *et al.*, 2001; Marlin *et al.*, 2002). Not enough is known about the effect of exercise on plasma TAS (Balogh *et al.*, 2001; White *et al.*, 2001). It can be stated, that the effort of the horses in this study was not intensive enough to change the parameters mentioned in this paragraph.

Looking at all the blood or plasma indices analyzed in this study, the levels of LA, TG and glycerol determined at rest and just after effort can reflect the efficiency of the four-month race-training process.

Fitness or quality of racehorses can be measured also by the rapid return of the elevated levels of biochemical parameters to their initial values during post-exercise rest. A quick return of the measured biochemical parameters to the initial level during recovery after a strenuous exercise indicates the fitness of the examined horses (Szarska, 1994). From this point of view, changes in levels of LA, Hb and glycerol during the 30-minute post-exercise rest also indicated that the four-month race training led to improvement of the physical performance of the studied horses.

CONCLUSIONS

The present study provides the evidence that four months of conventional race training of young horses causes a decrease of LA and TG production during exercise and also an increase in the rapid return of elevated levels of LA, Hb and UA after effort to their initial values.

The studied racehorses seem to tolerate the effort trials very well, as the metabolic engagement can be considered limited. This is really important in practice because the same kind of test must be repeated from time to time during the race seasons.

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TRENDOVI PROMENA VREDNOSTI HEMATOLOŠKIH I BIOHEMIJSKIH PARAMETRA U KRVI MLADIH TRKAČKIH KONJA TOKOM STANDARDIZOVANIH TESTOVA OPTEREĆENJA

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SADRŽAJ

Cilj ovih ispitivanja je bio da se uporede promene u vrednostima hematoloških i biohemijskih parametara trkačkih konja posle podvrgavanja standardizovanim testovima opterećenja. Testiranje je obavljeno dva puta: u maju, na početku trkačke sezone i četiri meseca kasnije. U maju je ispitano deset konja (pet engleskih punokrvnjaka i pet arabera), a u septembru osam (četiri i četiri). Od svakog konja su uzorci krvi uzimani venepunkcijom u mirovanju, posle zagrevanja, na kraju galopa i nakon 30 minuta odmaranja. Određivane su vrednosti sledećih parametara: mlečne kiseline, hemoglobina, glukoze, triglicerida, glicerola, mokraćne kiseline, ukupnih proteina i supstanci koje reaguju sa tio-barbiturnom kiselinom. Osim toga, određivana je i aktivnost laktat-dehidrogenaze, kreatin kinaze (CK) i aspartat aminotransferaze kao i ukupni antioksidativni status.

Standardno opterećenje je u septembru dovelo do manjeg porasta koncentracije mlečne kiseline, glukoze i triglicerida u poređenju sa prvim testom u maju. Povratak registrovanih vrednosti za koncentraciju mlečne kiseline i mokraćne kiseline i hemoglobin na početni nivo je takođe bio brži u septembru.