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Changes in hematological parameters and clotting times in the horse during a long distance running

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Summary

It is well known that athletes undergo simultaneous potentiation of hemostatic pathways that affects both coagulation and fibrinolytic activity. Increased fibrinolytic activity appears to counterbalance exercise--induced increases in clotting. However, data available on horses are not unequivocal. On account of this, we investigated the effect of an endurance competition on some clotting (PT, aPTT, TT and fibrinogen) and haematological (RBC, WBC, Hb, Hct, MCV, MCH, MCHC, PLT) parameters in 6 horses during an official long distance run. Blood samples were collected from all the horses: at rest, immediately after the run, 30' and 60' after the run. Only TT showed statistically significant differences between the different experimental conditions, being significantly prolonged immediately after the run, 30' and 60' after the run compared to rest. The remaining clotting parameters (aPTT, PT, fibrinogen) did not demonstrate statistical significance. This could be due to the type of exercise that implies a submaximal effort for horses.

Keywords: clotting time, haematological parameters, long distance running, horse

The increase in clotting and fibrinolytic activity due to exercise is widely documented in man, for both maximal (10, 16, 19, 33, 35) and near-maximal efforts (1, 14, 24, 34). The simultaneous activation of fibrinolytic activity prevents detrimental effects of hypercoagulation (4, 6). However, studies on haemostatic balance showed that the clotting activity was sustained during a time when fibrinolytic activity declined, suggesting a more favorable situation for clot formation after exercise (14, 19). It was observed a direct correlation between fibrinolysis and exercise intensity (12); it is activated during aerobic efforts in man (8). Several haemostatic changes involving platelets, coagulation and fibrinolysis have been reported after exercise in horses (9, 22). Data available on effects of exercise on clotting parameters in horses are not univocal: some researchers have found an evidence of fibrinogen degradation with physical activity (20), whereas others have found no change in fibrinogen (11, 21). The effects of maximal exercise on haemostasis in horses have been previously studied (2, 3, 18). These investigations have failed to document significant changes in clotting times, with the exception of an increase on platelets count (2). Previous investigations on trotters (25) did not shwed an increased coagulability and, conversely to man (6), no significant changes in clotting times (pT, aPTT and fibrinogen) were showed in trained subjects. Few authors investigated the effects of near-maximal efforts on hemostatic processes of horses, showing an increase in fibrinolytic activity and a faster platelet aggregability, without significant changes in clotting times (22). This activity observed in horses in response to endurance exercise (11) and also documented in the rat (26), could be due to the activation of fibrinolysis. Other studies investigated changes of some hematological parameters during physical activity; increases in PCV, platelet count, RBC and WBC count (2, 7, 28) were showed after running exercise in the horse.

Because the lack of data on changes in haemostasis of horses during near-maximal efforts, the aim of our research is to investigate the influence of a long distance running on clotting (PT, aPTT, TT and fibrinogen) and haematological (RBC, WBC, Hb, Hct, MCV, MCH, MCHC and PLT) parameters in this species.

Material and methods

Six endurance Arab horses (average age 9 ± 1 years, average body weight 430 ± 35 kg) clinically healthy and traditionally trained, were used for our study. Subjects were fed three times a day: at 07:00 on hay, at 13.00 on concentrates, and at 19:00 both on hay and concentrates.

Horses took part to an official long distance running, consisting of a 16-km mainly hill course, to do at the given speed of 8 km per hour. Race occoured the last week of october, at an environmental temperature of 28°C and with a Rh of 70%; heart index (HI), calculated with the formula: Temperature [(°C × 1.8) + 32] + Rh% (5), was 152. On all the horses blood samples were collected through jugular venipuncture: at rest, immediately after the trial, 30' and 60' after the trial. Two type of vacutainer tubes (Terumo Corporation, Japan) were used: with EDTA to evaluate, by means of a double-capillary automatic cell counter (Hemat 8 SEAC) red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscolar volume (MCV), mean corpuscolar haemoglobin (MCH), mean corpuscolar haemoglobin concentration (MCHC) and platelets (PLT); tubes containing 3.8% sodium citrate were immediately centrifuged at 2600 rpm for 10 min and on obtained plasma, by means of a coagulometer (SEAC Clot 2), Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), Thrombin Time (TT) and fibrinogen were assessed.

Statistical analysis. Since the intragroup variance was not significant, the statistical elaboration of data was carried out on mean values of the clotting and haematological parameters studied. Analysis of variance (one-way and repeated measures ANOVA) was applied in order to evaluate the statistical significant differences between the different experimental conditions (at rest *vs* immediately after the trial, at rest *vs* 30' and *vs* 60' after the trial, immediately after the trial *vs* 30' and *vs* 60' after the trial and 30' after the trial *vs* 60' after the trial). If ANOVA showed an acceptable level of significance ($P < 0.05$), Bonferroni's test was applied for post hoc comparison.

Results and discussion

Table 1 shows the mean values of the clotting parameters (PT, aPTT, TT and fibrinogen) together with the relative standard deviations, standard errors and statistical significances obtained on the different experimental conditions (at rest, immediately after the trial, 30' and 60' after the trial) in 6 long distance running horses. Table 2 shows the mean values of the hematological parameters (RBC, WBC, Hb, Hct, MCV, MCH, MCHC and PLT), together with the relative standard deviations, standard errors and statistical significances obtained on the different experimental conditions (at rest, immediately after the trial, 30' and 60' after the trial) in 6 long distance running horses. Figure 1 shows the variations of the clotting parameters (PT, aPTT and TT) observed on the different experimental conditions (at rest, immediately after the trial, 30' and 60' after the trial) in 6 long distance running horses. Figure 2 shows the scatter graph of fibrinogen mean value obtained on the different experimental conditions (at rest, immediately after the trial, 30' and 60' after the trial) in 6 long distance running horses.

Among clotting parameters, only TT showed statistical significant differences between the different experimental conditions and, applying repeated mea-

Tab. 1. Mean values of the clotting parameters, together with the relative standard deviations, standard errors and statistical significances obtained on the different experimental conditions, in 6 long distance running horses ($\bar{x}$ ± SD)

Parameter	Experimental conditions											
	Rest			Trial			After 30'			After 60'		
PT (sec)	12.57	0.68	0.28	11.92	0.81	0.33	12.13	0.93	0.38	11.70	0.64	0.26
aPTT (sec)	43.57	2.00	0.81	44.08	5.26	2.14	41.15	3.82	1.56	40.45	5.00	2.04
TT (sec)	8.78	0.61	0.25	24.03*	3.42	1.40	25.81*	3.09	1.26	25.97*	2.74	1.12
Fibrinogen (mg/dl)	148.70	22.73	9.28	159.30	22.90	9.35	152.80	19.64	8.02	130.30	12.47	5.09

Explanation: * – *vs* rest: $P < 0.001$

Tab. 2. Mean values of the hematological parameters, together with the relative standard deviations, standard errors and statistical significances obtained on the different experimental conditions, in 6 long distance running horses ($\bar{x}$ ± SD)

Parameter	Experimental conditions											
	Rest			Trial			After 30'			After 60'		
RBC (M/μL)	6.03	0.29	0.12	9.13^{a}	0.91	0.37	$7.00^{c,d}$	0.62	0.25	$7.00^{c,d}$	0.58	0.23
WBC (K/μL)	7.18	1.59	0.65	10.07^{b}	1.46	0.59	8.70	1.24	0.51	9.05	0.98	0.40
Hb (g/dL)	9.13	0.37	0.15	13.10^{a}	1.03	0.42	$10.18^{c,d}$	0.82	0.33	10.07^{d}	0.60	0.25
Hct (%)	25.48	1.36	0.55	39.60^{a}	3.30	1.35	$30.15^{b,d}$	2.45	1.00	$30.15^{b,d}$	1.73	0.71
MCV (fL)	42.50	1.76	0.72	43.17	1.72	0.70	42.83	1.60	0.65	43.33	1.37	0.56
MCH (pg)	15.18	0.33	0.13	14.38	0.50	0.20	14.57	0.44	0.18	14.45	0.64	0.26
MCHC (g/dL)	35.88	0.66	0.27	33.10^{a}	0.33	0.13	33.80^{a}	0.33	0.13	33.48^{a}	0.43	0.17
PLT (K/μL)	133.70	14.35	5.86	176.20^{a}	13.59	5.55	158.70^{c}	9.91	4.05	149.30^{e}	16.72	6.82

Explanation: a – *vs* rest: $P < 0.001$; b – *vs* rest: $P < 0.01$; c – *vs* rest: $P < 0.05$; d – *vs* trial: $P < 0.001$; e – *vs* trial: $P < 0.05$

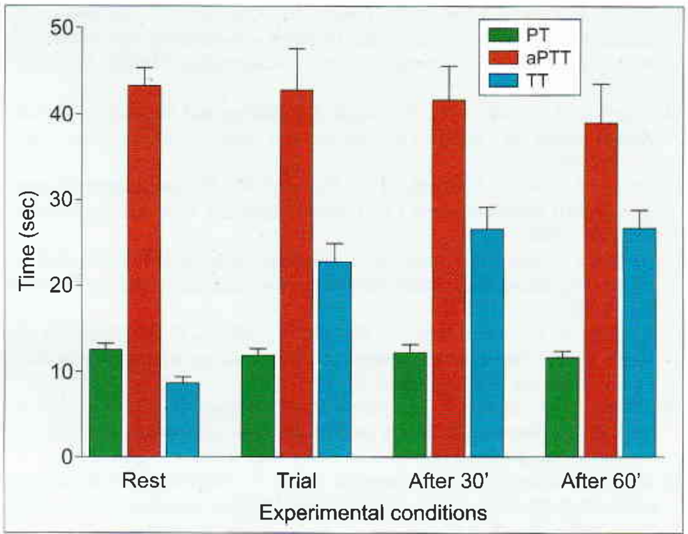

Fig. 1. Variations of the clotting parameters (PT, aPTT and TT) observed on the different experimental conditions in 6 long distance running horses

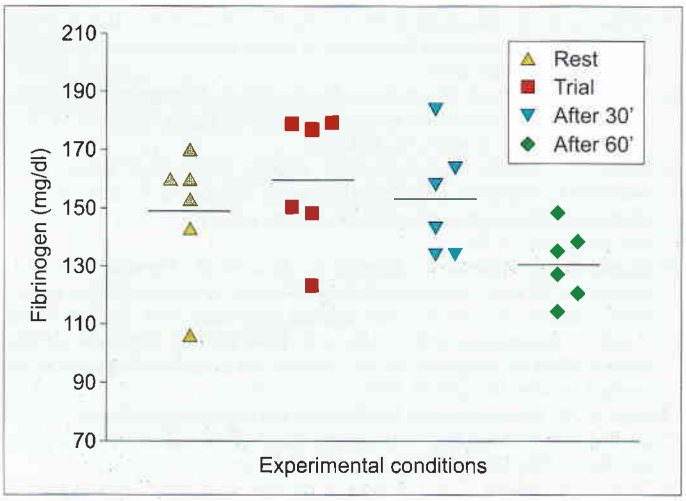

Fig. 2. Scatter graph of fibrinogen mean value obtained on the different experimental conditions in 6 long distance running horses

sures ANOVA, P value was P < 0.0001, with a $F_{(3,15)}$ = 51.63. By applying Bonferroni's multiple comparison test, TT showed a statistical significant increase immediately after the trial, 30' and 60' after the trial compared to rest (P < 0.001). Regarding hematological parameters, by applying repeated measures ANOVA, P value for RBC was P < 0.0001, with a $F_{(3, 15)}$ = 35.89, for WBC was P < 0.006, with a $F_{(3, 15)}$ = 6.12, for Hb was P < 0.0001, with a $F_{(3, 15)}$ = 53.43, for Hct was P < 0.0001, with a $F_{(3, 15)}$ = 62.67, for MCHC was P < 0.0001, with a $F_{(3, 15)}$ = 49.63 and for PLT was P < 0.001, with a $F_{(3, 15)}$ = 9.58. By applying Bonferroni's multiple comparison test, RBC showed a statistical significant increase immediately after the trial (P < 0.001), 30' and 60' after the trial (P < 0.05) compared to rest and a statistical significant decrease 30' and 60' after the trial (P < 0.001) compared to immediately after the trial. WBC showed a statistically significant increase immediately after the trial (P < 0.01) compared to rest. Hb showed a statistical significant increase immediately after the trial (P < 0.001) and 30' after the trial (P < 0.05) compared to rest and a statistical significant decrease 30' and 60' after the trial (P < 0.001) compared to immediately after the trial. Hct showed a statistical significant increase immediately after the trial (P < 0.001), 30' and 60' after the trial (P < 0.01) compared to rest and a statistical significant decrease 30' and 60' after the trial (P < 0.001) compared to immediately after the trial. MCHC showed a statistical significant decrease immediately after the trial, 30' and 60' after the trial compared to rest (P < 0.001). PLT showed a statistical significant increase immediately after the trial (P < 0.001) and 30' after the trial (P < 0.05) compared to rest and a statistical significant decrease 60' after the trial compared to immediately after the trial (P < 0.05).

From the analysis of the obtained results, among the clotting parameters considered, we observed statistical significant differences only for thrombin time (TT): TT was significantly prolonged immediately after the trial, 30' and 60' after the trial, according to other authors, which have obtained a slight but significant prolongation of aPTT (23). It is possible that these previous researches did not detect exercise-induced hypercoagulability because blood samples were not collected during exercise, when clotting times were shortened, but when values may have returned to baseline (22). In man, several studies have shown that blood is hypercoagulable when it is checked immediately after strenuous physical exercise or after a marathon run (13, 15). Previous researches showed that thrombin formation increases during exercise as a function of exercise intensity. Our prolonged TT implies a decrease of thrombin, which could be fully inactivated by AT-III, as postulated in other studies, avoiding fibrin formation (23). However, another study showed an increase in AT-III levels in men performing anaerobic exercise (12); the role of this clotting inhibitor needs to be detailed. In our study, the remaining clotting parameters (aPTT, PT, fibrinogen) did not showed statistical significant differences between the different experimental conditions. This could be due to the type of exercise and the duration of strenuousness: horses which are exercised under the threshold of maximal oxygen consumption did not showed changes in clotting times, as observed in men after maximal exercise and after long duration activities (4). Horses in the present study were subjected to a near-maximal effort which did not induce changes in clotting activity. As for haematological parameters (RBC, WBC, Hb, Hct, MCV, MCH, MCHC and PLT), acute maximal and near-maximal exercise is associated with marked shifts of intravascular plasma water into the interstitium without a proporzional loss of plasma proteins (12, 32). This is reflected in the statistical significant increase in haematocrit observed after exercise compared to rest. The increased erythrocytes number, as well as the increased haemoglobin level, guarantee more efficient distribution of oxygen in the organism (27, 29). Horses may store up to 60% of erythrocytes in spleen (29). During effort spleen contracts and erythrocytes get into the circulatory system, which improves oxygen transport. The statistical significant increase observed in Hct and Hb after exercise compared to rest testifies that the increase of the haematological indices is due not only by post-exercise blood dehydration but also by activation of erythrocyte reserves in spleen and proper reaction of a trained subject to effort (30). The increase in haematocrit value due to physical activity bas been described in horse (17) and in man (32). Exercise also induces an activation of platelets since platelets count and aggregability show a marked rise, in man, after acute exercise or marathon runs (1, 15, 31). In horses, Bayly et al. (3) obtained similar results after maximal efforts. Furthermore, it was reported that there is an increased proportion of circulating platelet-neutrophil aggregates in horses in response to near-maximal exercise (36).

Conclusions

Study of platelet aggregability, together with other investigations on clotting and haematologic parameters, leads us to better understand the haemostatic processes in the horse, to monitoring this important mechanism particularly during athletic performance and, from a clinical point of view, to prevent clotting disorders.

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