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MODIFICATIONS OF PLATELET AGGREGATION DURING TREADMILL SECTION AND OBSTACLE COURSE IN ATHLETIC HORSE

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Eight clinically healthy Sella Italiana horses were used in order to assess the variations of platelet aggregation in response to different workload. Blood samples were collected from each horse at rest, immediately after exercise and 30 min after the end of the exercise. By means of an aggregometer platelet aggregation was quantitated as maximum degree of platelet aggregation and initial velocity platelet aggregation. Two-way repeated measures analysis of variance (ANOVA) was applied to determine significant differences ($p<0.05$) due to the intensity of exercise and different experimental condition. Our results showed a similar trend of platelet aggregation between treadmill and obstacle course, but statistically significant differences emerged on studied parameters in relation to different workload. These results showed an inhibition of platelet response that probably represents a protective endothelial mechanism through the production of nitric oxide during different workloads in athletic horses.

Key words: different workload, horse, physical exercise, platelet aggregation

INTRODUCTION

The platelet cohesion, more commonly referred as platelet aggregation, was quickly identified as the most important event for haemostatic plug formation (Jackson, 2007; Ruggeri, 1997), that may be considered as an indirect index of the platelet functionality. The role of platelets in haemostasis involve their adherence to sites of vessel injury, aggregation to form haemostatic plugs or thrombi, and acceleration of the coagulation cascade leading to the formation of thrombin (Rand *et al*., 2003). The efficiency with which platelets adhere and aggregate at the sites of vessel wall injury is dependent on the synergistic action of various adhesive and soluble agonist receptors, with the contribution of each of the individual receptors dependent on the prevailing blood flow conditions (Jackson *et al*., 2003). The activation of platelets is regulated and modulated by numerous relatively well-characterized factors, including adenosine diphosphate

(ADP) (Pelagalli *et al*., 2002). Moreover, since it is known that exercise has variable effects on equine blood parameters (Piccione *et al*., 2008), it also causes modifications of platelet function (Sakita *et al*., 1997; El-Sayed *et al*., 2000), although the exact mechanisms and the regulatory pathways involved in the effects of exercise on platelet function are not completely understood. Also in humans the effect of physical exercise on platelet aggregation and its functions produced conflicting results, and the exact effects of exercise remain yet undetermined (El-Sayed, 2002). Some of them reported that strenuous exercise results in increased platelet aggregation, other reported unchanged or decreased platelet aggregation in response to exercise (El-Sayed, 2002). Some of the benefits of physical activity may result from effects on haemostasis. However, the increased burden of cardiovascular complications and sudden death occurring during and immediately after exercise prompts investigations to elucidate the biological relationship between physical exercise and haemostatic function in humans (Lippi and Maffulli, 2009). Previously, in horses changes in clotting times were measured during long distance running (Piccione *et al*., 2004b), during official trot races (Piccione *et al*., 2005a) and during show jumps (Piccione *et al*., 2004a), and following different storage conditions (Casella *et al*., 2009b). Moreover, circadian variation of blood clotting times in horse during competition (Piccione *et al*., 2005b) and training (Casella *et al.*, 2009a) were evaluated, but the effect of different workload on clotting parameters and on platelet aggregation in athletic horses was not still studied. On this purpose, the aim of this study was to evaluate the platelet response to different workload in athletic horses. The maximum degree of platelet aggregation and the slope of platelet aggregation values were measured in response to treadmill and obstacle course in jumper horses.

MATERIALS AND METHODS

Eight Sella Italiana jumpers horses, aged between 7 and 9 years, mean body weight 475 ± 25 kg from the same Horse Training Centre, were used. Before the start of the study, all subjects underwent a heart exam, respiratory auscultations, and routine haematology and plasma biochemistry. Only clinically healthy animals were used. Horses were traditionally fed with hay and a mix of cereals (oats and barley), three times a day (08:00, 12:00 and 20:00) and received water *ad libitum*. Each horse was tested on two exercises interrupted by three days of rest. The three-day interruption between the first and the second exercise was included in the protocol to avoid the possibility of habituation and fatigue. Exercise 1 was a standardized treadmill test (Horsegym 2000, GMBH) preceded by warm-up at zero slope (5-min walk; 75 m/m) and consisted of 20 min of walking at 140 m/m all at a 5% incline. Exercise 2 was a standardized obstacle course preceded by warm-up consisting of walking, trotting, and galloping, including three jumps of 0.8-1.20 m height and 1 m width. The exercise consisted of a 400 m long trail with ten 1.25 high jumps (5 vertical jumps, 4 long jumps, and one double vertical and long jump).

On all horses, at rest and after exercise, blood samples were taken by jugular vein puncture with heparinized vacutainer (Terumo Corporation, Japan) for determination of blood lactate concentration by commercially available meters (Accusport, Boehringer, Mannheim, Germany).

On each subject, blood samples were collected at rest, immediately after exercise and 30 min after the end of exercise in both experimental conditions. Blood samples, were collected by means of jugular venipuncture using vacutainer tubes (Terumo Corporation, Japan) containing 3.8% sodium citrate (1 part citrate: 9 parts blood). On blood samples platelet-rich and platelet-poor plasma were prepared by centrifugation. To prepare platelet-rich plasma (PRP), samples were centrifuged, within 15 minutes following collection, at 300 g x 20 min and PRP obtained was removed, using a plastic transfer pipette, and was transferred into plastic containers. To prepare platelet-poor plasma (PPP), the original blood sample tubes were recentrifuged at 3000 g x 10 min and PPP obtained was removed and transferred into plastic containers, also. Platelet aggregation was measured by adding ADP as agonist that promotes platelet activation and using an aggregometer (Clot 2, SEAC-Radim, Company, Florence, Italy). The final concentrations of the aggregating agent were ADP 1 and 0.5 μM. Platelet aggregation was recorded for at least 4 min.

Platelet aggregation responses were quantitated using two parameters: the maximum degree of aggregation and the initial velocity of aggregation. The maximum degree of aggregation was determined by measuring the maximum height of the aggregation wave over a 4 min period beginning at the onset of platelet aggregation. The maximum degree of aggregation was expressed as a percent of the maximum possible change in light transmission. The initial velocity of aggregation was determined by drawing a line tangent through the steepest linear part of the aggregation tracing, and determining the slope from 1 point along the curve. The slope of this tangent was expressed in %/min.

All results were expressed as means ± standard deviation of the means (SD). Data were normally distributed ($p<0.05$, Kolmogorov-Smirnov test). Two-way repeated measures analysis of variance (ANOVA) was applied to determine the effects of exercise and of different experimental conditions. Bonferroni's test was applied for post hoc comparison and $p \leq 0.05$ was considered statistically significant. Data were analyzed using STATISTICA 5.5 (Stat Soft Inc.) software package.

RESULTS

The mean values ± standard deviations of blood lactate at rest were 1.25 ± 0.16 mmol/L for treadmill and 1.22 ± 0.20 mmol/L for the obstacle course. After exercise lactate concentration was: 1.99 ± 0.30 mmol/L for treadmill and 5.90 ± 1.16 mmol/L for obstacle course group.

Table 1 shows the mean values ± SD of maximum degree of platelet aggregation and slope of platelet aggregation during the different experimental conditions, expressed in their conventional units, with the relative statistical significances.

ANOVA showed a significant effect of exercise ($p<0.04$) and of different experimental conditions ($p<0.05$) on studied parameters. At final ADP concentrations of 1 and 0.5 μM exercise 1 showed a statistically significant decrease of maximum degree of platelet aggregation and slope of platelet aggregation after exercise *vs* at rest and a statistically significant increase of maximum degree of platelet aggregation after 30 min *vs* after exercise, and a statistically significant increase of slope of platelet aggregation after 30 min *vs* at rest. At final ADP concentrations of 1 and 0.5 μM exercise 2 showed a statistically significant decrease of maximum degree of platelet aggregation and slope of platelet aggregation after exercise *vs* at rest and a statistically significant increase of maximum degree of platelet aggregation after 30 min *vs* at rest and after exercise, and a statistically significant increase of slope of platelet aggregation after 30 min *vs* at rest.

Table 1. Mean values ± SD of maximum degree of platelet aggregation and slope of platelet aggregation during the different experimental conditions, expressed in conventional units, with the relative statistical significance

Parameter	ADP	Exercise	Experimental conditions		
			Rest	After exercise	After 30 min
Aggregation (%)	0.5 μM	Treadmill*	39.50±5.03	34.33±4.49	42.67±4.68
		Obstacle course	40.90±3.91	27.40±5.14	35.00±5.21
	1 μM	Treadmill*	39.50±6.36	33.05±4.37	45.83±7.12
		Obstacle course	40.25±3.53	26.50±3.79	49.80±6.44
Slope (%/min)	0.5 μM	Treadmill*	4.13±0.97	2.33±1.57	2.81±1.35
		Obstacle course	4.64±0.84	3.41±0.56	4.20±0.98
	1 μM	Treadmill*	4.27±1.17	2.83±1.35	3.33±0.87
		Obstacle course	4.17±1.05	2.00±0.92	2.28±0.35

Significance: **vs* Obstacle course $p<0.04$

Figure 1. The pattern of mean values (±SD) of maximum degree of platelet aggregation (%) – a, and slope of platelet aggregation (%/min) – b, with ADP 1 μM (n=8)

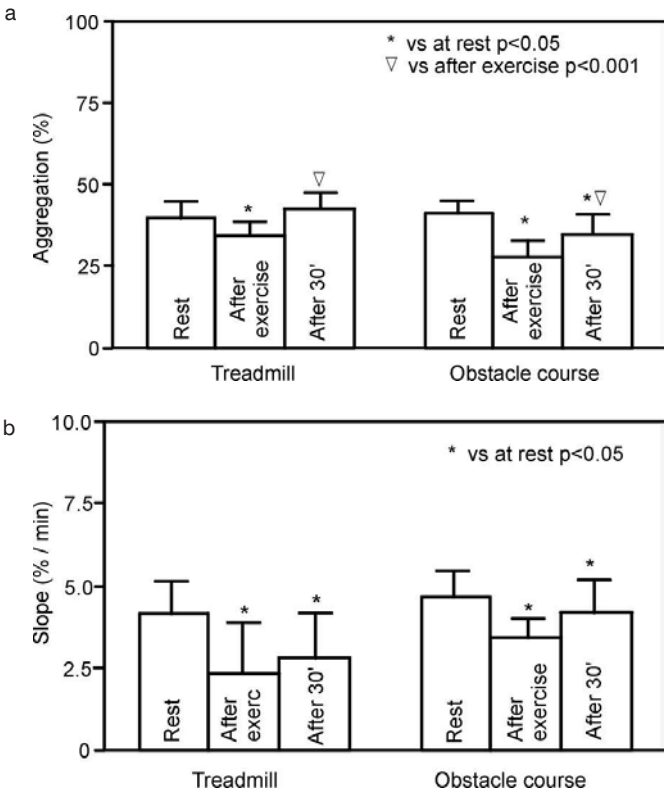

Figure 2. The pattern of mean values (±SD) of maximum degree of platelet aggregation (%) – a, and slope of platelet aggregation (%/min) – b, with ADP 0.5 μM (n=8)

The pattern of mean values (±SD) of maximum degree of aggregation and slope, at final concentrations of ADP 1 μM and ADP 0.5 μM, together relative significances observed in athletic horses during exercise of different workload is shown in Figures 1 (a, b) and 2 (a, b).

DISCUSSION

Platelet aggregation is a complex process that may be influenced by many endogenous and exogenous factors. Particularly, platelet reactivity seems to be affected by a variety of stressors as exercise. Platelet responses to exercise depend on several factors, such as exercise intensity, duration and training condition (Petidis *et al*., 2008). Our results, in fact, showed a similar trend between treadmill and obstacle course but statistically significant differences emerged on studied parameters in relation to different workload that can be underlined by the lactate values. Lactate results observed after treadmill section indicated that it was a submaximal exercise, and lactate results observed after obstacle course showed that it was a supramaximal exercise (Hodgson and Rose, 1994). As recent reports suggested moderate exercise intensity is followed by activation of blood fibrinolysis without concomitant hypercoagulability, while intensive exercise is associated with concurrent activation of blood coagulation and fibrinolysis (El-Sayed *et al*., 2004). This justifies the obtained differences in relation to the type of exercise. In both exercises it is evident a decrease on studied parameters after exercise, that might be explained by two hypotheses (Petidis *et al*., 2008): either noradrenaline that increases much more than adrenaline during exercise exerts different effects on platelet aggregation or other factors induced by physical exercise counter-regulate the negative effects of adrenaline on platelet aggregation. In the first case increased adrenaline levels result in decreased aggregation to adrenaline, probably due to adrenergic receptor downregulation (Kjeldsen *et al*., 1995). In the second case, noradrenaline is able to stimulate endothelial cells to release prostacyclin and nitric oxide, both of which are known to be potent inhibitors of platelet aggregation (Jones *et al*., 1993); in fact the increased prostacyclin production and plasma nitric oxide metabolites may suppress platelet reactivity (de Graaf *et al*., 1992). 30 min after exercise the studied parameters statistically increase because equine platelet reactivity is altered by the end of exercise.

In conclusion, the modifications observed represent a reaction of the organism to exercise intensity that it is due to an inhibition that probably represents a protective endothelial mechanism through the production of nitric oxide in response to physical exercise.

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PROMENE U AGREGACIJI TROMBOCITA TOKOM RADA KONJA NA POKRETNOJ TRACI I PRESKAKANJA PREPONA

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SADRŽAJ

U ovoj studiji su vršena ispitivanja u cilju utvrđivanja varijacija u stepenu agregacije trombocita u zavisnosti od nivoa opterećenja na osam konja rase Sella Italiana. Uzorci krvi su prikupljani od svih grla u mirovanju, odmah nakon opterećenja i 30 minuta po prestanku rada. Agregacija trombocita je procenjivana pomoću agregometra i to kao maksimum agregacije i kao inicijalna brzina agregacije. Stepen značajnosti utvrđenih razlika u srednjim vrednostima je određivan analizom varijanse (ANOVA) na nivou od 95%. Naši rezultati ukazuju na sličan trend promena pri primeni oba tipa opterećenja a dokazene su i statistički značajne razlike u zavisnosti od njihovog stepena. Zapažene promene ukazuju na inhibiciju "odgovora" trombocita koji verovatno predstavlja odbrambeni endotelijalni mehanizam realizovan produkcijom azot oksida.