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Original article

Different training schedules influence platelet aggregation in show jumping horses

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Abstract

Depending on the intensity, duration and type of physical exercise, equine metabolism has to adapt to nervous, cardiovascular, endocrine and respiratory system requirements. In horses, exercise and training are known to have considerable effects on the mechanisms of hemostatic system involving platelet activity. The aim of the present study was to evaluate the effect of different training schedules on platelet aggregation in 15 Italian Saddle jumping horses. Animals were divided into three equal groups: Group A was subjected to a high intensity-training program; group B to a light training program, group C included sedentary horses. From each animal, blood samples were collected by jugular venipuncture at rest on the 1st, 3rd and 5th days, and afterwards, once a week, for a total of 5 weeks data recording, in order to assess the maximum degree of platelet aggregation and the initial velocity of aggregation (slope) platelet aggregation. Two-way analysis of variance (ANOVA) showed a significant effect of the different training schedules on studied parameters.

The results revealed a different degree of platelet aggregation and a different initial velocity of platelet aggregation that changes during the different training schedules in horses that could represent a different protective endothelial mechanism. These findings could have an important role for a clearer knowledge of the physiological reference values of platelet aggregation and for a better interpretation of these variations during the training.

Key words: horse, platelet aggregation, training, workload

Introduction

In horses, has been shown that exercise affects haemostatic process associated to an acute and transient increase in blood coagulability seems to be counterbalanced by a simultaneous increase in fibrinolytic activity depending on both the intensity and the duration of exercise (Piccione et al. 2014a). Some authors reported that strenuous exercise results in increased platelet aggregation (Giordano et al. 2010,

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Table 1. Training program performed by each group. Group A was subjected to an intense training program, Group B was subjected to a light training program and Group C included sedentary horses.

Group A (n=5)			
Days of week	Gait	Duration (min)	Obstacle height (cm)
First and third day	Walk Trot Canter (400 m/min) Obstacle Walk	5 30 20 1 5	90 (n=8)
Second, fourth and sixth day	Walk Trot Canter (400 m/min) Walk	5 25 25 5	
Fifth day	Walk Trot Canter (400 m/min) Obstacle Walk	5 30 20 1 5	1.20 (n=13)
Seventh day	Rest		
Group B (n=5)			
Days of week	Gait	Duration (min)	Obstacle height (cm)
First and third day	Walk Trot Canter (300 m/min) Walk	5 30 20 5	
Fifth day	Walk Trot Canter (300 m/min) Obstacle Walk	5 30 20 1 5	0.80 (n=6)
Second, fourth, sixth and seventh day	Rest		
Group C (n=5)			
No activity during experimental period			

Assenza et al. 2013), whereas others reported unchanged or decreased platelet aggregation in response to exercise (El-sayed et al. 2005, Piccione et al. 2014b). Regular training sections promote changes in the structure and function of body. The repetitive exercise results in a multitude of changes involving cells, tissue, organ, and whole organism. It is well known that the best strategy to evaluate the training adaptation is the regular monitoring of selected psychological, biochemical and physiological markers. The efficiency of platelets to adhere at 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. Although it has long been known that platelet aggregation typically occurs at sites of perturbed flow after vascular injury, it has been assumed that this process is directly caused by the accumulation of soluble platelet agonists at sites of flow disturbance (Nesbitt et al. 2009). Since the aggregation has been quickly identified as the most important indirect index of the platelet functionality (Piccione et al. 2008). Since the frequency of haemorrhagic problems in athletic horses, we want to investigate the effect of different training program workloads on the platelet response (maximum degree of platelet aggregation and slope of platelet aggregation values) in jumping horses subjected to specific training programs.

Materials and Methods

The study involved a laboratory component and a veterinary clinic component, both conducted at the Department of Veterinary Sciences, University of Messina. (Italy). All treatments, housing, and animal care reported previously were carried out in accordance with the standards recommended by the European Directive 2010/63/EU for animal experiments.

The study was carried out on 15 clinically healthy horses (Italian Saddle) from the same training centre located in Sicily (Italy), in spring (Min. Temp. 18°C, Max Temp 24°C, Relative Humidity about 65%). Before the start of the study, all subjects underwent to a cardiorespiratory examination and to the evaluation of haematological and biochemical parameters at rest. All blood values were within the reference range. All horses, 9 ± 1 years old, with an average body weight of 420 ± 40 Kg, were geldings and sedentary. During the experimental period, the animals were divided into three groups according to the intensity of training program as reported in Table 1. Group A was subjected to an intense training program, Group B was subjected to a light training program and Group C included sedentary horses.

The horses were fed three time a day (7:00 AM, 1:00 PM and 7:00 PM) with a standard rations, composed of hay (first cut meadow hay, sun cured, late cut, 8 kg/horse/day, 6.9% crude protein on average) and a mixture of cereals (oats and barley, 50% each, about 3.5 kg/horse/day): dry matter 86.36% (9.11% horse's digestible protein, 13.05% crude protein, 20.7% crude fiber and 3.42% crude lipid) and moisture 13.63%. Water was available *ad libitum*.

From each animal, blood samples were collected by jugular venipuncture into 3.6 ml vacutainer tubes containing 3.8% sodium citrate (Terumo Corporation, Tokyo, Japan), when the horses were at rest before feeding (at 6.00 AM). During the first week, blood samples were collected on the 1st, 3rd and 5th days. Afterwards, blood samples were collected once a week, for a total of 5 weeks data recording. All horses showed no stress reaction during blood sampling that was performed in less than 30 sec, excluding an excitement-induced spleen contraction (Satué et al. 2012).

On all blood samples, stored at room temperature (22°C) pending analyses (Sweeny et al. 2002), platelet-rich and platelet-poor plasma were obtained by centrifugation within 2 hours after collection. To prepare platelet-rich plasma (PRP), samples were centrifuged, within 15 minutes following collection, at 300g 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, too. Platelet count has been standardized; PPP has been checked by a coulter counter and was free from platelet particles. 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,M. 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 (slope). 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 slope 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 (Piccione et al. 2010a).

All results were expressed as mean ± standard deviation (SD). Data were normally distributed ($P>0.05$, Kolmogorov-Smirnov test). Two-way repeated measures analysis of variance (ANOVA) was applied to determine the effect of different training schedules and of day of sampling on studied parameters throughout the experimental period. Bonferroni's test was applied for post hoc comparison and $P<0.05$ was considered statistically significant. Data were analyzed using STATISTICA 7.0 (Stat Soft Inc.) software package.

Results

The application of two-way ANOVA showed no significant differences due to the day of sampling on studied parameters, whereas a statistical effect of the different training schedules was found on the maximum degree of platelet aggregation (Fig. 1) and slope (Fig. 2). In particular, the percentage of aggregation and slope, evaluated after the platelet activation with ADP 1 µM and 0.5 µM, decreased in statistically significant way in groups A and B respect to group C.

Discussion

The results of this study confirmed that the platelet aggregation is affected by exercise and in particular by different training schedules. It is well known that physical exercise activates blood coagulation and enhances blood fibrinolytic activity (El-Sayed et al. 2005). Available information suggest that intensive exercise induces hypercoagulability with simultaneous enhancement of blood fibrinolysis. Whereas moderate exercise maintains the delicate balance between the

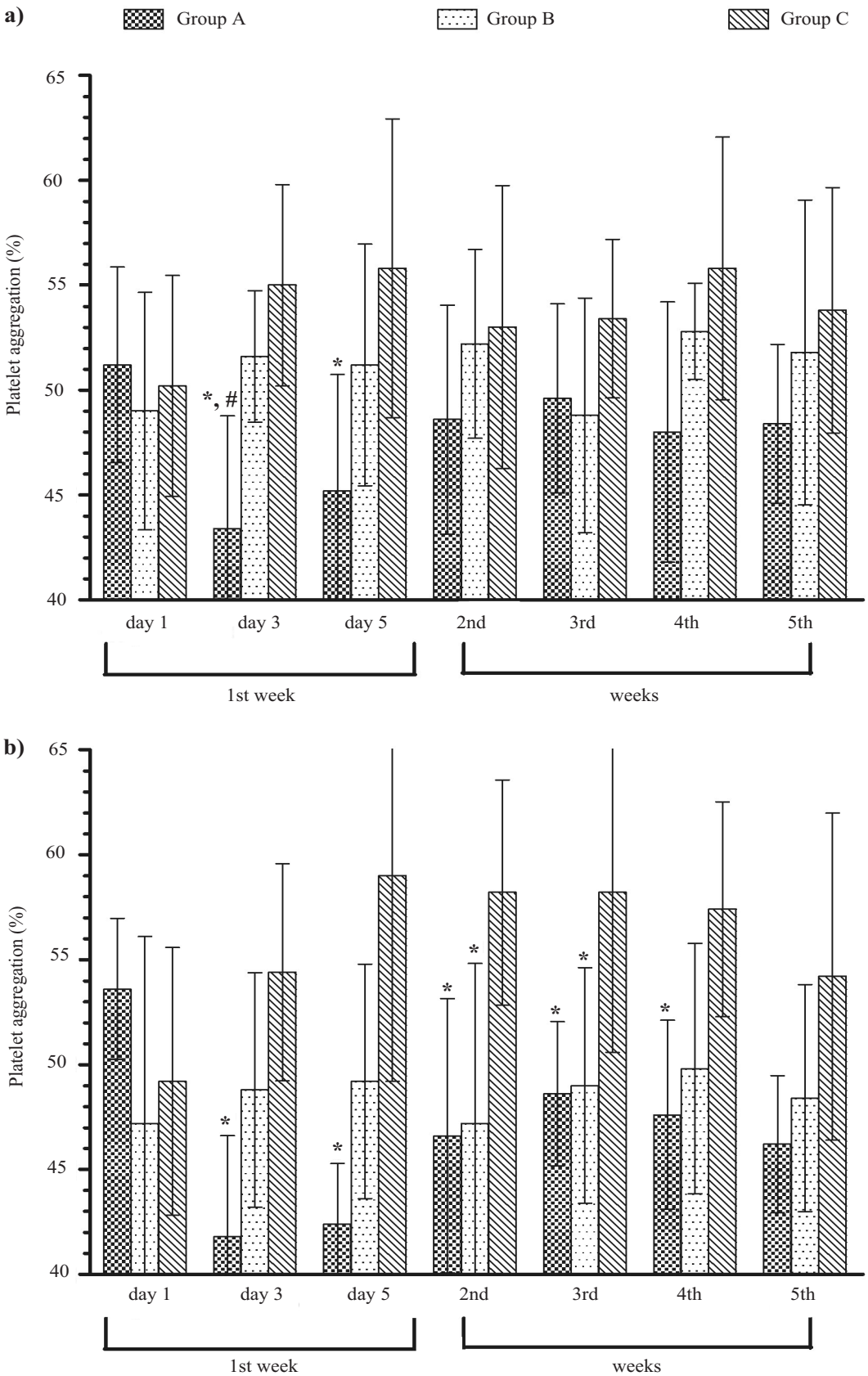

Fig. 1. The pattern of mean values (± SD) of maximum degree of platelet aggregation together with statistical significances obtained in groups A (horses that participated an intense training program), B (horses that participated in a light training program) and C (sedentary horses) after platelet activation with ADP 0.5 μM (a) and 1 μM (b). # indicates significance compared to Group B ($p<0.05$); * indicates significance compared to Group C ($p<0.05$)

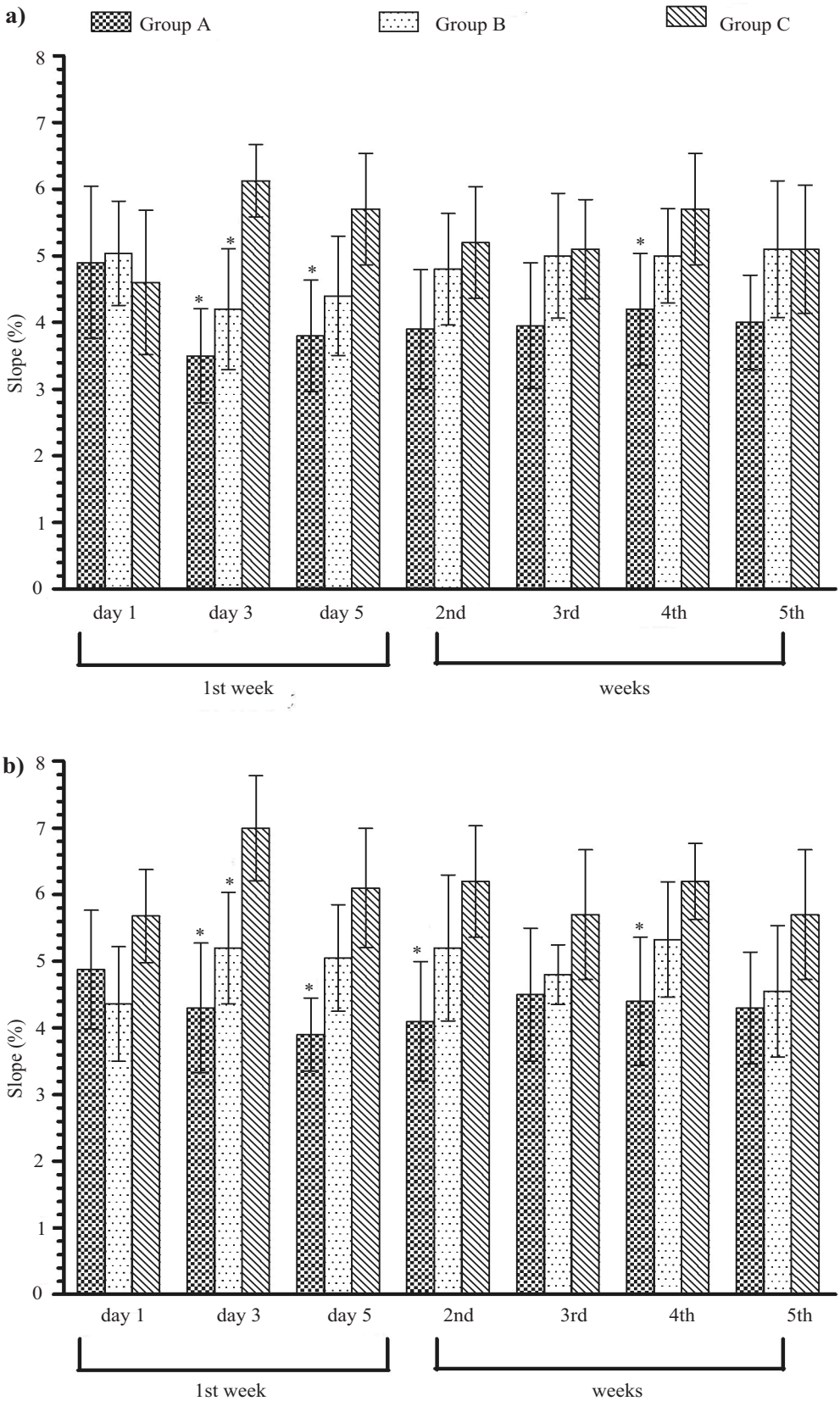

Fig. 2. The pattern of mean values (± SD) of slope, determined by drawing a line tangent through the steepest linear part of the aggregation tracing, and starting from 1 point along the curve. The slope of this tangent was expressed in %/min together with statistical significances obtained in groups A (horses that participated an intense training program), B (horses that participated in a light training program) and C (sedentary horses) after platelet activation with ADP 0.5 μM (a) and 1 μM (b). * indicates significance compared to Group C ($p<0.05$)

clot formation and the clot dissolution (El-Sayed et al. 2005). Our results showed a greater inhibition of platelet response obtained in horses of group A respect to horses of group B. These findings agree with the results of previous studies carried out on athletic horse (Piccione et al. 2010b, Piccione et al. 2014b). It is likely that increases in sodium citrate concentration associated with blood hemoconcentration during exercise resulted in reduced ionized calcium concentration and platelet aggregation (Hinchcliff et al. 2004). However, the study of exercise effects on platelets aggregation are conflicting, due to the analytical methods used (El-Sayed et al. 2005). As observed in human, in which moderate intensity exercise was followed by activation of blood fibrinolysis without concomitant hypercoagulability, whereas the intensive exercise was associated with concurrent activation of blood coagulation and fibrinolysis (El-Sayed et al. 2005). We have observed a different decrease of studied parameters in horses of group A and B. It might be explained by two hypotheses: 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 down regulation (El-Sayed et al. 2005). There is also evidence that β2-adrenergic receptor stimulation causes inhibition of adhesion and aggregation of platelets through increased nitric oxide synthase activity in platelets. In the second case, noradrenaline be able to stimulate endothelial cells to release prostacyclin and nitric oxide, both of which are known to be potent inhibitors of platelet aggregation; in fact, the increased prostacyclin production and plasma nitric oxide metabolites may suppress platelet reactivity (Casella et al. 2010). The initial velocity of platelet aggregation also showed significant decreases in relation to training program showing a significant decrease in horses that completed an intensive training program.

It is well known that there is alteration of coagulation in any exercising horses, platelet adhesiveness may decrease more in exercise-induced pulmonary hemorrhage-positive horses than in horses following exercise (Hodgson and Rose 1994). In conclusion, the results revealed a different degree of platelet aggregation due to different training schedules that probably represents a different protective endothelial mechanism and consequently a different initial velocity of platelet aggregation that changes during the different training schedules in horses. These findings could have an important role for a clearer knowledge of the physiological reference values of platelet aggregation and for a better interpretation of these variations during the training.

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