

## HAEMATOLOGICAL RESPONSE TO DIFFERENT WORKLOAD IN JUMPER HORSES

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### Summary

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Five clinically healthy Sella Italiana horses were used in order to assess the haematological response to different workload. Blood samples were collected on each horse at rest, immediately after the exercise and 30 min after the end of the exercise. An automated haematology analyzer was used to assess red blood cells counts, haemoglobin concentration, haematocrit (Hct) and white blood cells counts. One-way for repeated measures analysis of variance (ANOVA) was used to determine the statistical differences and it showed a significant effect of the exercise on sand track on Hct. Our results confirm that exercise has variable effects on the studied parameters depending on work intensity. Based on our results it is possible to affirm that the indices studied could be useful in the assessment of the fitness of sport horses and aerobic working capacity, correlated to blood fluidity.

**Key words:** haematological parameters, horse, performance, physical exercise, workload

### INTRODUCTION

The performance or 'output' of an equine athlete is determined by many complicated interdependent biological and physiological processes. Understanding how these process function and relate to each other is mandatory if the horse is to be effectively trained and managed during its working or competitive life (Warwic, 2004). Certain cardiovascular and haematological adaptations are necessary to guarantee the correct oxygen and blood-borne substrates supply to active muscles during exercise and the release of metabolites. These systems could act as limiting factors to the aerobic potential and thereby, could limit the physical performance (Persson, 1983; Sewell *et al.*, 1992; King

*et al.*, 1994; Munoz *et al.*, 1997). During strenuous exercise, horses undergo a variety of physiological changes including an increase in haematocrit (Hct), cardiac output, increase in mean pulmonary arterial blood pressure, and in the arterial hypoxemia (Wagner *et al.*, 1989; Hopper *et al.*, 1991; Olsen, 1991). Exercise-induced changes in blood rheology have been reported to be related to the rating of perceived exertion. The factor correlated with exertion was Hct (Brun *et al.*, 1990), which was hypothesized to represent a signal among the other well-characterized ones (e.g. heart rate, lactate, blood glucose) that are integrated at a conscious level to generate the feeling of exertion

(Brun, 2002). Plasma viscosity is the best statistical determinant of the maximal oxygen consumption ( $VO_{2max}$ ) and of the aerobic working capacity ( $W_{170}$ ), which are both indices of aerobic exercising capacity (Brun *et al.*, 1989). There is a strong relationship between red blood cell volume (RCV) and aerobic performance; however, while RCV and Hct are usually looked at independently, they are interdependent in the optimization of blood flow during exercise (McKeever, 2004). The red cell pool is under the direct influence of catecholamine concentrations, so exercise has a variable effect on red cell indices, depending on the speed and duration of the exercise bout (Persson & Lydin, 1973). The horse is somewhat unique compared to most other mammalian species in that the spleen is very capacious and capricious organ, storing between 6 and 12 L of red-cell-rich blood at rest (Persson, 1967; Carlson, 1987; McKeever *et al.*, 1993a; McKeever & Hinchcliff, 1995). Exercise is an accepted way to cause splenic contraction and a viable way to estimate the splenic reserve contribution to the total circulating blood volume. The resulting Hct value is skewed upward by the dynamic fluid shifts caused by the changes in flow and hydrostatic pressure induced by exercise. Acute reductions in Hct caused by exercise-induced fluid shifts are linked to exercise intensity (McKeever *et al.*, 1993b; McKeever & Hinchcliff, 1995; Greenleaf & Morimoto, 1996). While most of Hct increase is related to splenic erythrocyte release, there are also substantial fluid shifts out of the plasma during exercise, and therefore, some of the increase in Hct is due to fluid movement (Carlson, 1983). On the basis of such considerations, the purpose of this study was to investigate changes of some haematological parameters: red blood

cells (RBC), haemoglobin concentration (Hb), haematocrit (Hct); white blood cells (WBC), in athletic horses trained at two different work loads. We were interested to know whether the magnitude of changes in the blood was the same in the two exercises of different degrees of intensity and if the indices studied could be useful in assessing the fitness of sport horses.

## MATERIALS AND METHODS

Five ten-years-old Sella Italiana jumpers horses (mean body weight  $420 \pm 30$  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 at rest. 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*. Training exercises consisted in a one hour of walking in the morning (10:30-11:30) in a horse-walker (Tecno exerciser, Pessa Studio, Italy) at 80 m/min, electronically controlled, every day. The daily routine was carried out in the afternoon (17:00-18:00) 5 days per week: 10 minutes of walking, 20 minutes of trotting, 20 minutes of galloping, 8 obstacles 80 cm high jump and 5 minutes of walking on a sandy track. On each subject, blood samples were collected at rest, immediately after the exercise and 30 min after the end of the exercise in both experimental conditions. Blood samples, were collected by means of jugular venipuncture using vacutainer tubes with  $K_3$ -EDTA (Terumo Corporation, Japan). An automated haematology analyzer (Hemat 8, SEAC, Italy) was used to assess the following parameters: RBC counts, Hb, Hct; WBC counts.

One-way repeated measures analysis of variance (ANOVA) was used to determine the statistical differences between mean values of the studied parameters. All the results were expressed as means  $\pm$  standard errors of the means (SEM). Probabilities  $<0.05$  were considered statistically significant.

## RESULTS

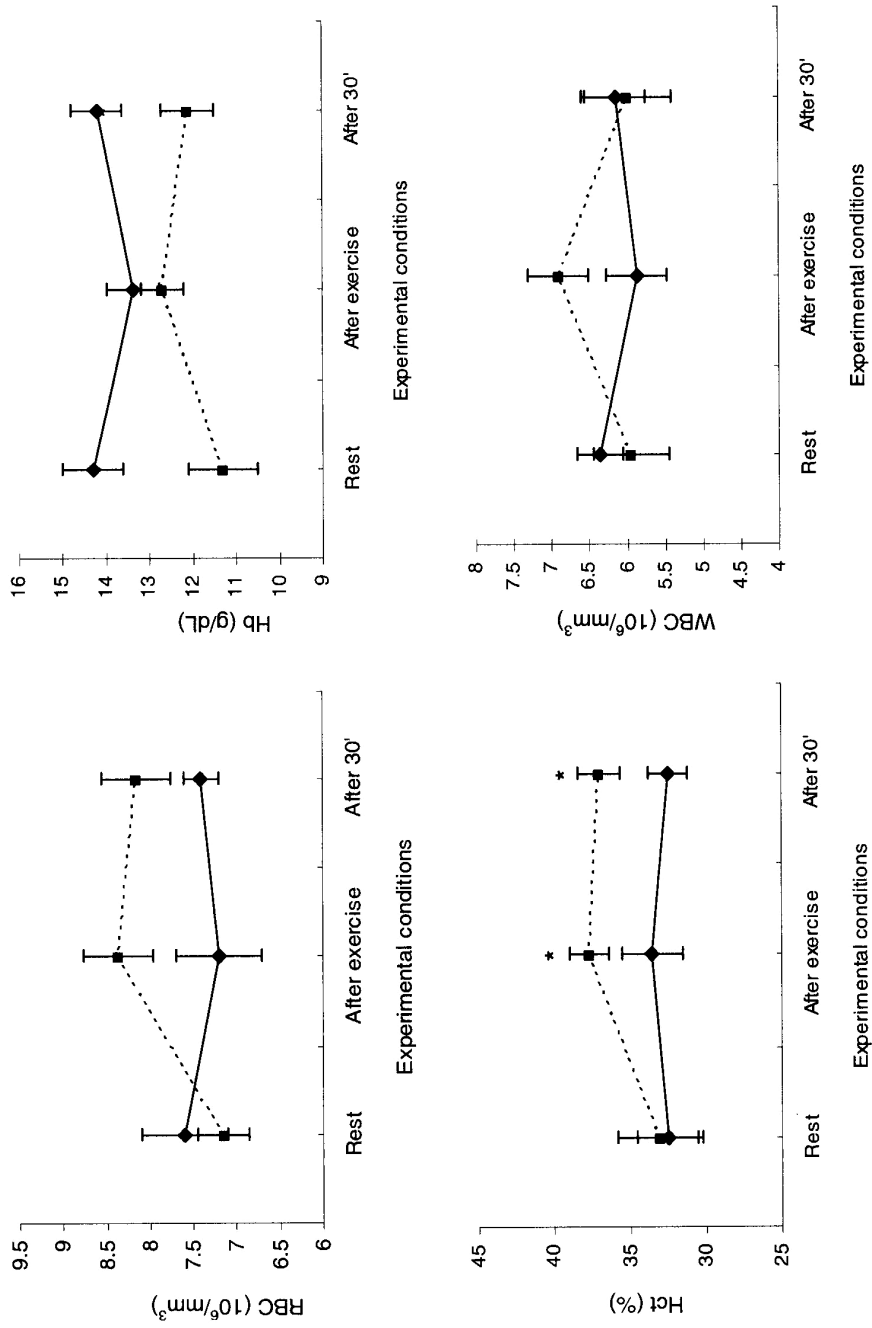
The ANOVA for repeated measures showed a significant effect of the exercise on sand track on Hct,  $F_{(2,8)}=8.64$ ,  $P<0.01$ . No statistical significant differences were observed for the other parameters in both the experimental conditions.

Figure 1 shows how exercises of different intensity may influence the haematological parameters in a opposite way: all parameters decreased after the exercise in the horse-walker and return at approximately resting value 30 minutes after the end of the exercise. On the contrary, all the parameters increased after the exercise on the sand track and only WBC returned at approximately resting value 30 minutes after the end of the exercise.

## DISCUSSION

Our results confirm that exercise has variable effects on the studied blood parameters depending on work intensity (McKeever *et al.*, 1993a). In fact, exercise generally results in mobilization of splenic erythrocytes, responsible for the elevation in Hct, Hb and RBC (Persson 1967; 1969; Persson & Lydin, 1973; Delgado *et al.*, 1975), that are under the influence of catecholamines (Wade & Freud, 1990; Rose & Hodgson, 1994); both the intensity and duration of exercise are important in determining the magnitude of the cate-

cholamine response (Convertino *et al.*, 1981). The increase of the studied parameters after the exercise in the horse-walker was not as statistical significant as in endurance horse exercise, maybe because exercise intensity had not fully mobilized the splenic erythrocyte reserve (Carlson, 1987). In fact, this occurs during high intensity exercise with high heart rate (at least 100 beats/min within 1 min after termination of work) (Carlson, 1987). The response at short distance, high intensity racing differs markedly from the response at endurance exercise. The rate of energy expenditure of the racing horse is high. Readily available muscle energy stores are rapidly depleted, oxidative metabolism is pushed to the limit and ultimately, energy must be derived from anaerobic metabolism. The mobilization of erythrocytes from the splenic reservoir, resulting from a greater demand for oxygen carriage (McKeever, 2004), provides an auto transfusion which plays a critical role in increasing oxygen carrying capacity during this brief period of maximal demand (Rose & Allen 1985). This can explain the statistically significant differences in Hct showed after the sand track exercise: after exercise vs rest and 30 minutes after the end of the exercise vs rest,  $P<0.01$ . A relatively small change in Hct represents a much larger change in plasma volume (McKeever, 2004). The magnitude of the increase in post exercise Hct being a function of age and training (Persson, 1967; Persson & Ulberg 1981), is a function of exercise intensity, with a linear relationship between Hct and speed (Harrison, 1985; McKeever *et al.*, 1993c). Furthermore, it was shown that exercise resulted in a more extensive change of fluidity in well-trained horses compared to untrained animals (Stoiber *et al.*, 2005). The high Hct value, recorded 30 minutes after the



**Fig. 1.** The pattern of mean value and the standard errors of the means together with the relative statistical significance of RBC, Hb, Hct and WBC recorded at rest, immediately after the end of exercise and after 30 min of post exercise rest in 5 Sella Italiana jumper horses carried out on horse-walker (solid line) and on sand track (dotted line), \*  $P < 0.01$  vs rest.

end of the exercise similar to that recorded immediately after exercise, was probably due to thermal stress during recovery from exercise that impeded the restoration of Hct (Lindinger *et al.*, 1995). Moreover high intensity exercise induced a moderate leukocytosis (Archer & Clabby, 1965; Skurai *et al.*, 1967; Allen & Powell, 1983; Snow *et al.*, 1983; Allen *et al.*, 1984). There are significant differences in the response of leukocytes at exercise of different intensities and durations (Rose & Hodgson, 1994; McKeever, 2004). In horse, the leukocytosis associated with maximal exercise is slight to moderate and is due primarily to an increase in lymphocytes with variable increase in the neutrophil count (Archer & Clabby, 1965; Snow *et al.*, 1983; Allen *et al.*, 1984). Mechanisms of this response have not been documented in the horse. Immediately after the gallop, there is a change in the neutrophil/lymphocyte ratio, but a little variation in the total leukocyte count (Sejesterd, 1992; McKeever *et al.*, 1993a). The increase in lymphocytes probably reflects the release of large numbers of lymphocytes into the circulation in association with mobilization of the splenic erythrocyte reservoir (Carlson, 1987; Rose & Hodgson, 1994). After 30 min of post exercise rest RBC, Hb and WBC values were the same as that recorded at rest and this is probably due to the good training degree of the studied subjects (Paladino *et al.*, 2005); the training programme did not exceed the ability of horses.

Based on our results it is possible to affirm that the indices studied could be useful in the assessment of the fitness of sport horses and aerobic working capacity, correlated to blood fluidity (Brun, 2002). Plasma viscosity is the best statistical determinant of the maximal oxygen

consumption ( $VO_{2max}$ ). Also, it may well be that overtraining is a largely psychological syndrome, where there is fatigue and poor performance despite little change in  $VO_{2max}$ , and a detraining period was simply described as some weeks of relative inactivity where horses were walked for 20 minutes daily, which have been enough exercise to maintain  $VO_{2max}$  (Tyler *et al.*, 1996). Thus the alternation of aerobic/aerobic-anaerobic activity, distinctive in jumper horse metabolism, aids the good aerobic ability keeping, without keeping the subjects in stressing conditions that may induce an overtraining. Furthermore, this alternation leads to a good degree of adaptation useful in the metabolic balance so that the haematological parameters may reach basal values few minutes after the end of the exercise.

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