

Effect of Exercise and Training on Erythrocyte Content of 2,3-DPG and Oxygen Content of Blood in the Horse

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Summary

Included among the major factors determining oxygen delivery to the tissues are: hemoglobin (Hb); oxygen content of blood and erythrocyte concentration of diphosphoglycerate (2,3-DPG). The use of the human oxyhemoglobin dissociation curve to calculate the oxygen content in the horse may not be accurate. In this study, we evaluated the effect of exercise and training on blood Hb levels and erythrocyte concentration of 2,3-DPG in five mature normal horses. We also compared oxygen content of blood determined from a nomogram with that determined directly.

Arterial and mixed venous blood samples were drawn at rest and during standardized submaximal exercise on a treadmill (speed 100 m/min; 12% incline; heart rate 120) at 2, 5, 10 and 15 min after the onset of exercise, before and after a one-month training period. Arterial and mixed venous oxygen content were calculated with a blood gas analyzer and measured directly with a Lex-O₂-Con analyzer. The 2,3-DPG and Hb were measured by spectrophotometry. The 2,3-DPG increased from 1.65 mmoles/l of blood at rest to 2.73 after 5 min of exercise, and then remained constant. This increase was associated with an increase in Hb from 113 g/l at rest to 140 g/l at 2 min of exercise. At the start of exercise, the 2,3-DPG increased from 14.5 to 15.4 μ moles/g Hb and continued to increase gradually to 16.2 μ moles/g Hb at 15 min of exercise.

Training had no effect on the erythrocyte concentration of 2,3-DPG. There was a correlation of 0.88 between the two techniques used to evaluate oxygen content of blood.

Index terms: Hemoglobin dissociation; erythrocyte.

Introduction

Oxygen delivery to the tissues is dependent upon many factors including cardiac output, hemoglobin concentration (Hb), oxygen content of blood, and erythrocyte concentration of diphosphoglycerate (2,3-DPG). The 2,3-DPG is a product of glycolysis in the red blood cells. As with PaCO₂, body temperature, and blood pH, its concentration can change Hb affinity (Benesch and Benesch, 1969; Bunn and Kitchen, 1973). There will be a shift of the oxyhemoglobin dissociation curve to the left or right depending on the

variations of these factors (Shapiro *et al.*, 1982). An increase in 2,3-DPG shifts the oxyhemoglobin dissociation curve to the right and enhances unloading of oxygen. At the erythrocyte's level, 2,3-DPG will alter the allosteric configuration of Hb and thereby decrease its affinity for oxygen.

The effect of exercise on cardiac output, blood gas levels, acid-base balance, and on hematologic parameters have been extensively studied during the past fifteen years (Lindholm and Saltin, 1974; Thomas and Fregin, 1981; Rose *et al.*, 1983; Bayly *et al.*, 1983). Although the effect of exercise and training on erythrocyte 2,3-DPG concentration has been well described in humans, it has not been fully described in the horse. In the human, the results of the different studies on 2,3-DPG concentration variations during exercise are conflicting. Dempsey *et al.* (1971) and Shappell *et al.* (1971) reported that there was no change in erythrocyte 2,3-DPG concentration with exercise. Austin *et al.* (1973) described an increase in 2,3-DPG following exhaustive effort and Bonner *et al.* (1975) did not find any significant increase in 2,3-DPG after a maximal exercise of short duration. Few authors have studied the effect of training on erythrocyte 2,3-DPG in humans and the results differ from one study to another. Shappell *et al.* (1971) and Böning *et al.* (1975) described a significant increase in 2,3-DPG after an 8 week training regime, whereas Rand *et al.* (1973) showed that training did not change the 2,3-DPG concentration.

In the horse, Lewis and McClean (1975) and Lykkeboe *et al.* (1977) evaluated the effect of exercise and training on 2,3-DPG. These authors showed that there was no significant change in erythrocyte 2,3-DPG concentration after maximal exercise. However they described a significant decrease in 2,3-DPG associated with an increase in Hb following training.

Most of the studies in humans and horses did not evaluate erythrocyte 2,3-DPG concentration and hemoglobin levels during exercise. One purpose of this study was to evaluate the effects of submaximal, standardized exercise and training on 2,3-DPG concentration.

Many authors have evaluated the oxygen content of blood of horses at rest and during exercise (Bayly *et al.*, 1983; Littlejohn *et al.*, 1983; Thornton *et al.*, 1983). The oxygen content was either measured using a direct technique (Thornton *et al.*, 1983; Pan *et al.*, 1984; Parks and Manohar, 1984) or was calculated by using the oxyhemoglobin dissociation curve described for human blood analysis (Severinghaus, 1966). The oxyhemoglobin dissociation curve of the horse has been calculated in part (Schmidt-Nielsen and Larimer, 1958). However, some parts of the curve are not known. Calculation of the oxygen content of equine blood using the human oxyhemoglobin dissociation curve may give rise to false values of oxygen content. To determine if this method did accurately reflect blood oxygen content in the horse, we compared the results obtained by a direct method for measuring blood oxygen content with those obtained by using the equations described by Severinghaus (1966).

Materials and Methods

Five healthy Standardbred horses, aged 5 ± 1.5 years, (mean \pm standard error of the mean, $\bar{x} \pm$ SEM) and weighing 425 ± 35 kg were included in this study.

A subcutaneous transposition of the right common carotid artery was performed under general anesthesia to facilitate arterial blood sampling during exercise. The surgery was

followed by a five week rest period during which the horses were accustomed to running on a 12% inclined motordriven treadmill (Safe-T-Mill^R, Talbot Carlson, Audubon, Iowa, USA).

Each horse was studied four times on separate days both before and after a four week training period. The aerobic training consisted of periods of lunging and treadmill exercise of increasing duration, starting with 30 minutes of lunging at the trot and 15 minutes of exercise on the treadmill (speed 100 m/min; 12% incline) and increasing by 2 minutes every other day. Arterial and mixed venous blood samples were drawn from catheters inserted in the carotid and pulmonary arteries, respectively. The sampling was made at rest and during steady state exercise on the treadmill (Fig. 1). The speed of exercise varied between 90 and 110 m/min in order to obtain a three-fold increase in heart rate compared to the resting value as described for a submaximal effort in the horse by Ascheim *et al.* (1970).

The arterial blood samples were collected through a 16 gauge, 8 cm indwelling catheter, introduced percutaneously into the carotid artery. The mixed venous blood samples were obtained by using a 140 cm polyethylene catheter (PE-205, Clay-Adams, Parsippany, New-Jersey) introduced into the left jugular vein and threaded successively into the right atrium, the right ventricle and the pulmonary artery.

The blood samples were obtained in heparinized plastic syringes (Becton-Dickinson & Co., Rutherford, NJ, USA) at rest and at 2, 5, 10, and 15 min of exercise. The syringes were then stored on ice and the blood samples were analyzed within 2 hours of collection for blood gases, Hb saturation, and oxygen content using a blood gas analyzer (ABL-3, Radiometer, Copenhagen, Denmark). A portion of each sample was then analyzed with a Lex-O₂-Con analyzer (Lexington Instruments, Waltham, MA, USA) to measure arterial and mixed venous oxygen content. Unlike other oximeters that measure light absorption of blood, the Lex-O₂-Con measures the released oxygen from the red blood cells after hemolysis of the blood. The oxygen passes through a galvanometric cell which gives an electrical signal proportional to the number of oxygen molecules (Sykes *et al.*, 1981), therefore it measures bound oxygen. Selman *et al.* (1975) and

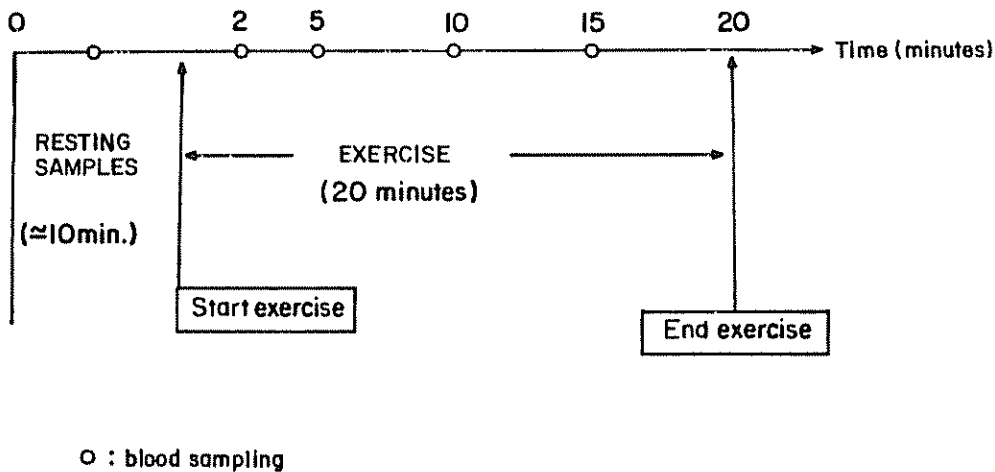


FIGURE 1. Sampling protocol during the exercise study

Cole and Williams (1976) have reported that the accuracy of this technique is ± 0.3 vol percent. The remaining portion of each blood sample was used to determine erythrocyte 2,3-DPG concentration by spectrophotometry using the technique described by Bergmeyer (1978).

Statistical analysis. A two-way analysis of variance (ANOVA) was performed to evaluate the exercise-induced change in 2,3-DPG and the effect of training on the changes. When an F value was significant at the 0.05 probability level, a planned comparison was performed to determine the differences in 2,3-DPG at the different times of measurement. A correlation study between the results of oxygen content obtained by the two techniques was done to assess the reliability of the calculated values of oxygen content in the horse.

Results

The values ($\bar{x} \pm \text{SEM}$) of erythrocyte concentration of 2,3-DPG and hemoglobin at rest and during submaximal exercise are presented in Fig. 2. The 2,3-DPG concentration increased from 1.65 mmol/l of blood at rest to 2.06 mmol/l after 2 min of exercise ($P < 0.05$). As exercise progressed, the 2,3-DPG concentration continued to increase gradually to 2.73 mmol/l at 5 min of exercise and remained constant during the rest of the exercise ($P < 0.05$). After 15 min of exercise, the 2,3-DPG had increased by 64.2% ($P < 0.05$). However, the gradual increase in 2,3-DPG between 2 and 15 min was not significant.

The increase in 2,3-DPG concentration at the onset of exercise was associated with a simultaneous increase in blood hemoglobin ($P < 0.05$). From a resting value of 113 g/l the hemoglobin increased to 140 g/l after 2 min of exercise. During the rest of the exercise, it remained stable ($P < 0.05$). The 2,3-DPG concentration expressed as a function of hemoglobin increased during the exercise ($P < 0.05$). From a resting value of 14.5 ± 0.35 $\mu\text{mol/g Hb}$ it increased to 15.4 ± 0.25 $\mu\text{mol/g Hb}$ at 2 min. It then increased gradually to 16.2 $\mu\text{mol/gHb}$ at 15 min of exercise.

A correlation of 0.87 for the arterial samples and 0.88 for the mixed venous samples was found between the results of the two techniques (Fig. 3 and 4).

Training had no significant effect on erythrocyte 2,3-DPG concentration or oxygen content of arterial and mixed venous blood.

Discussion

In our study, we observed a significant increase in the 2,3-DPG concentration associated with a significant increase of Hb during a submaximal exercise. These results correspond to those of Stull and Lawrence (1983) who demonstrated an increase in 2,3-DPG concentration during standardized exercise. Our results also agree with some studies done on humans (Faulkner *et al.*, 1970; Austin *et al.*, 1973; Bonner *et al.*, 1975) but do not agree with previous studies performed in the horse (Lewis and McClean, 1975; Lykkeboe *et al.*, 1977) and in humans (Dempsey *et al.*, 1971; Shappell *et al.*, 1971).

The differences in the results may be attributed to the time of sampling. Our study and that of Stull and Lawrence (1983) are the only two that have described the variations of 2,3-DPG during a standardized exercise test. The absence of variations of 2,3-DPG concentration with exercise reported by Lewis and McLean (1975) and Lykkeboe *et al.*

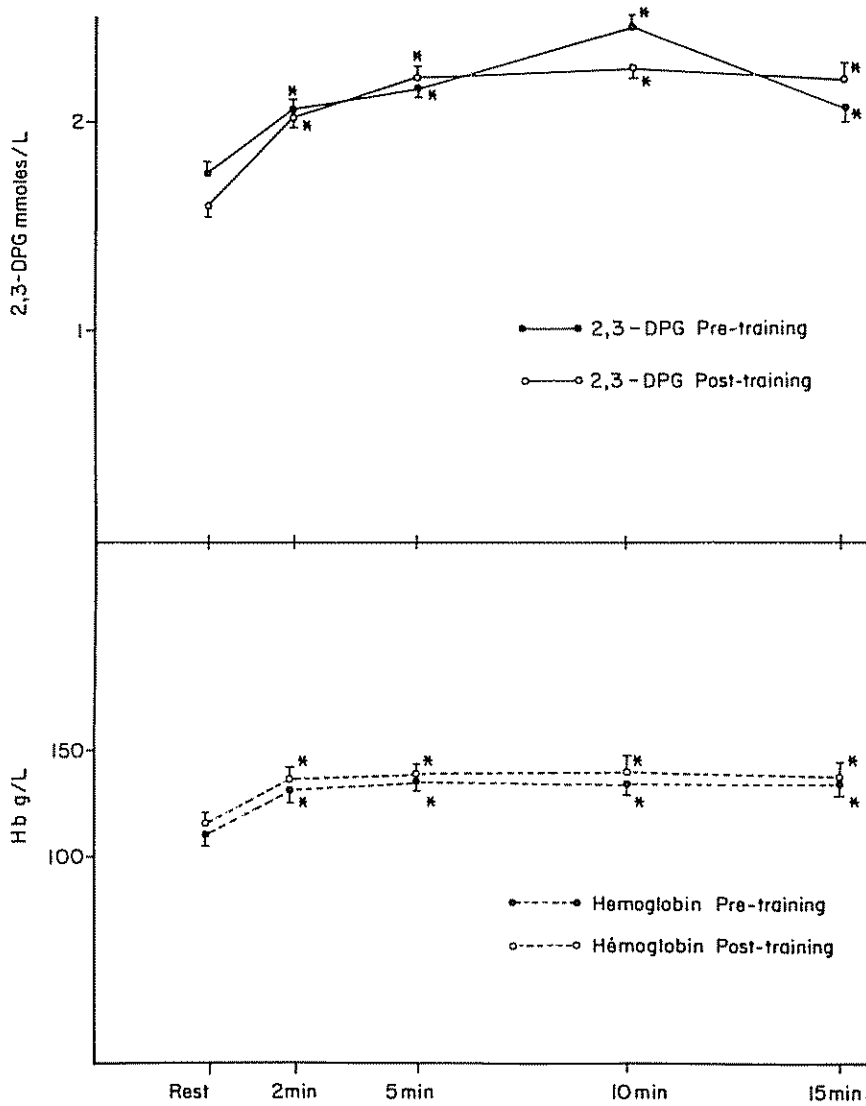


FIGURE 2. Effect of exercise on erythrocyte 2,3-DPG and blood hemoglobin in the untrained horse.

* significantly different from resting value.

(1977) could be due to a rapid return of 2,3-DPG concentration to resting values before sampling occurred. Evidence for this hypothesis could be the absence of increase in blood hemoglobin after exercise described in the study of Lykkeboe *et al.* (1977).

The increase in blood Hb has been explained as a physiological adaptive change to exercise to ensure a better oxygen delivery to the tissues (Persson *et al.*, 1973). In our study, the increase in the ratio (2,3-DPG:Hb) observed during exercise demonstrated that the increase in the 2,3-DPG concentration was not only due to the increase in Hb.

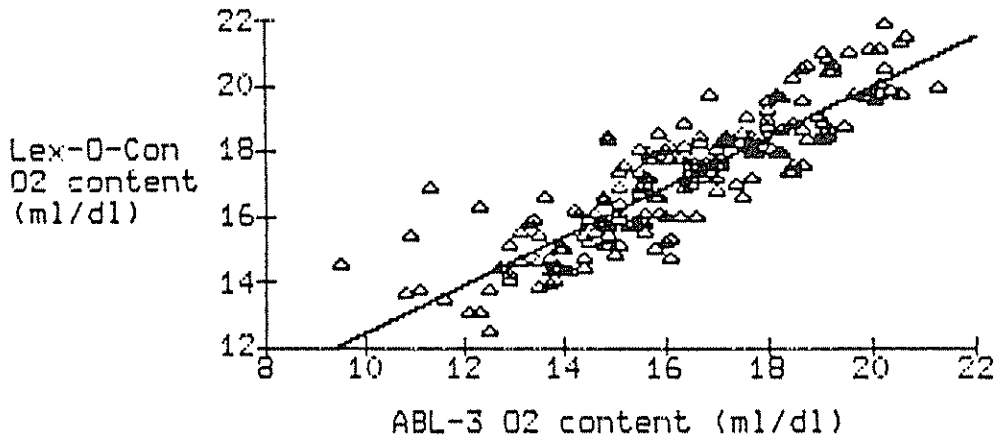


FIGURE 3. Correlation between the two methods to evaluate oxygen content in arterial blood. The regression equation is $Y = 0.748 \cdot X + 4.995$. ABL-3 oxygen content is determined from a nomogram.

There could be a biochemical adaptation of the red blood cells to improve the liberation of oxygen to the tissues during exercise. These results do not agree with those obtained by Stull and Lawrence (1983) who found no variation in the ratio (2,3-DPG:Hb) during exercise. However, we cannot explain the reason for this discrepancy. In the present study, we assessed 2,3-DPG on pulmonary arterial blood while other authors made the analysis on venous blood from the jugular vein (Lewis and McLean, 1975; Lykkeboe *et al.*, 1977; Stull and Lawrence, 1983). The assay used in the present study was the same as in the work reported by these previous studies. Therefore, the 2,3-DPG assay could not explain the discrepancy in the results as Bergmeyer reports a small variability of the technique (Bergmeyer, 1978).

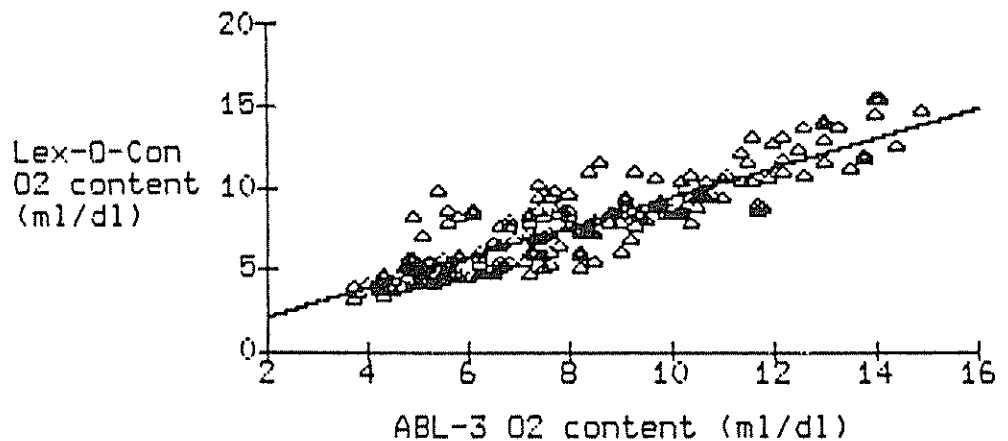


FIGURE 4. Correlation between the two methods to evaluate oxygen content in mixed venous blood. The regression equation is $Y = 0.904 \cdot X + 0.346$. ABL-3 oxygen content is determined from a nomogram.

TABLE 1. Effect of exercise and training on heart rate (HR).

	Resting	Exercise			
		2 min	5 min	10 min	15 min
Pre training	40.0 ± 2.11	120.0 ± 3.8	122.0 ± 4.0	115.0 ± 4.5	115.0 ± 3.8
Post training	31.0 ± 1.13*	95.0 ± 5.0	100.0 ± 2.3	96.0 ± 3.5	98.0 ± 3.8

Values expressed as mean ± SEM

We observed no effect of training on the erythrocyte 2,3-DPG concentration. These findings do not agree with studies of Lewis and McLean (1975), Lykkeboe *et al.* (1977) and Stull and Lawrence (1983). This could be explained by the intensity and duration of our training program. Even if we had a significant training effect as shown by the decrease of heart rate after training (Table 1), the intensity and duration of training were less than previously reported by Lykkeboe *et al.* (1977). Sampling differences may have led to the discrepancy of our results with other studies.

The correlation between the results obtained by the two methods used to evaluate oxygen content of blood is in agreement with the results of Clerbaux *et al.* (1986). These authors calculated the P_{50} of horses and determined the oxyhemoglobin saturation curve for equine blood. They found that the oxyphoric constant of hemoglobin, the volume of oxygen transported by 1 g of Hb, of equine blood was very close to the reported values for humans (Severinghaus, 1966). The oxyhemoglobin dissociation curve of the horse is well correlated to the human curve and is slightly shifted to the left. Thus, correlation between the two methods of evaluating the oxygen content was to be expected. Although, there is a good correlation between the two techniques used to measure the oxygen content, the calculation of oxygen content from the human oxyhemoglobin dissociation curve (Severinghaus, 1966) is not accurate enough for physiological studies related to ventilation, gas exchange and oxygen carrying capacity of equine blood. If Severinghaus equations are used to calculate O_2 content, the results should be corrected using the regression equations described in this paper (Fig. 3 and 4).

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Field Observations on Selenium Status, Whole Blood Glutathione Peroxidase and Plasma Gamma-Glutamyl Transferase Activities in Thoroughbred Racehorses

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Summary

The aim of this investigation was to examine the effect of selenium supplementation on whole blood (WB) glutathione peroxidase (GSH-Px) in racehorses and to determine if any relationship existed between selenium status and plasma gamma-glutamyl transferase (GGT) activity. Investigations throughout the training/racing period were carried out in two large stables with additional single measurements in other stables. Selenium supplementation resulted in gradual increases in both WB GSH-Px activity and plasma selenium concentrations. From a knowledge of the horses' training programs and performances, it was concluded that 1) the requirements for Se in the racehorse may be lower than previously recommended; 2) there is no causal relationship between elevations in plasma GGT activity and low Se status; and 3) the monitoring of plasma GGT activity may provide a sensitive indicator of the horse's response to training and its possible racing performance.

Index terms: Supplementation; training; stress.

Introduction

In 1979, Blackmore *et al.* suggested that elevated plasma gamma-glutamyl transferase (GGT) activities found in horses from a stable with a history of unexpectedly poor racing performance may be associated with a low selenium (Se) status. However, a further study by Blackmore *et al.* (1982) concluded that no relationship between Se status and plasma GGT activity existed.

The present study was undertaken in an attempt to explain why a leading thoroughbred racing stable in Newmarket had a proportion of horses with plasma GGT activities above the normally accepted range for our laboratory (Blackmore and Brobst, 1981). Despite elevations in activity of this enzyme, clinically the animals were normal and had no other hematological or biochemical abnormalities as detected from routine blood