

# EFFECTS OF A 1600m LONG TROTTING RACE ON SERUM PROTEINS, LIPOPROTEINS AND LIPIDS IN STANDARD BRED HORSES

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*The evaluation of total concentrations of proteins, lipoproteins and lipids together with the relative contribution of their fractions in serum was performed in six healthy trotters before and immediately after a 1600 metres long race.*

*The results obtained demonstrated a significant increase of the total protein level in the post-race serum samples. The relative contribution of the separated protein fractions i. e. albumin, alpha, beta and gamma globulins was similar in the resting and in the post-race samples.*

*The levels of total lipoproteins, cholesterol and phospholipids increased significantly after the race. Agarose gel electrophoresis separated serum lipoproteins into four fractions: pre-alpha, alpha, pre-beta and beta. The alpha lipoproteins were the major fraction in the horse sera. The relative amount of the pre-alpha lipoproteins was increased while beta lipoproteins decreased in the serum samples obtained after the race.*

*Key words: protein, lipoprotein, cholesterol, phospholipids, Standardbred horse.*

## INTRODUCTION

There has been a great increase in information about the physiological effects of endurance exercise on horses, reflecting the increasing popularity of the sport during the last 20 years.

Exercise induces changes in several physiological parameters, particularly those of the cardio-vascular and respiratory function, inducing tachycardia, hypertension and redistribution of blood to the skeletal muscles. Increased respiratory rate and depth, together with biochemical changes including hyperglycaemia, increased lipolysis and lactate production occur due to an increase in the carbon dioxide content of the circulating blood. The studies of Carlson and Mansmann (1974) showed that electrolyte disturbances also developed during endurance rides. Although various biochemical parameters have been estimated in exercising horses (Sreter, 1959; Cardinet et al, 1963;

Lindholm and Saltin, 1974; Milne et al, 1976) there has been little work done on biochemical changes in horse blood during endurance rides. Andersen (1975) reported that the observed increase in serum activity of creatinekinase, lactic dehydrogenase and aldolase originated primarily from skeletal muscles. Rose et al., (1977, 1982) and Jabionska et al (1991) reported that total protein, albumin and cholesterol content in horse blood were significantly different in samples taken at rest and after a race.

The present study aimed to evaluate changes in serum proteins, lipoproteins and lipids in trotters before and immediately after a race.

#### MATERIAL AND METHODS

A total of six healthy trotters, 4 to 7 years old and of both sexes, were observed during a 1600 m long race. The winning speed was 1 kilometre 1 minute 25 seconds. Double blood samples were collected by puncture of the jugular vein at rest, before the race and immediately after the race. Serum obtained by spontaneous blood clotting was frozen and processed, in the laboratory.

The level of total serum proteins was determined by a colorimetric method (Cartier and Picard, 1957). Serum protein fractions were separated by paper electrophoresis and serum lipoproteins on 1 g/dl agarose gel (Dyerberg and Hjerne, 1970).

The concentration of total serum lipoproteins was determined by a turbidimetric method with phenol-NaCl according to Kunkel (Polonovski et al., 1957) and beta lipoprotein level by a procedure described by Dangerfield and Faulkner (1964). Cholesterol and phospholipid levels in the serum, were estimated by the method described by King (1951).

The results obtained were statistically analysed by Student's "t" test.

#### RESULTS AND DISCUSSION

Total serum protein concentration, and serum content of the different protein fractions at rest and in postrace samples are shown in table 1.

Table 1. The concentration of total proteins and the relative contribution of protein fractions separated by paper electrophoresis in the blood serum before and immediately after a race.

n = 6	Before race	Immediately after the race
	$(\bar{X} \pm 1SD)$	
Total proteins (g/l)	47,18±7,90	59,35±8,49*
Albumin (%)	52,60±1,28	50,13±3,43
$\alpha$ 1 globulins (%)	4,78±0,48	4,80±0,38
$\alpha$ 2 globulins (%)	12,96±2,33	11,63±1,65
$\beta$ globulins (%)	11,93±1,06	13,24±1,76
$\gamma$ globulins (%)	17,73±2,29	20,20±3,18

\* p < 0,5



Total serum protein concentration was significantly different ( $p < 0.05$ ) in samples obtained in resting animals ( $47.18 \pm 7.90$  g/l) and after the race ( $59.35 \pm 8.49$  g/l). A significant increase of the total protein concentration in post race horse serum was previously reported by Rose et al. (1977, 1982), Poso (1983) and Jablonska et al. (1991), who concluded that the degree of the change depended upon the duration and the intensity of the exercise. The observed hyperproteinaemia may simply reflect increased sweating associated with the training. Sweating is certainly the most important mechanism for heat dissipation in horses during endurance exercise, resulting in mild to severe dehydration due to the large volume of fluid lost (Carlson, 1974; Rose et al., 1977, 1982; Luke, 1980). A slight decrease of circulatory volume occurs during exercise due to the shift of fluids out of the vascular compartment, resulting in a decrease of the plasma volume and hyperproteinaemia.

Albumin, alpha 1, alpha 2, beta and gamma globulins in the horse serum were separated by paper electrophoresis. The relative contribution of the separate protein fractions was similar in the samples taken at rest and after the race. Severe training is designed to produce maximal development of the muscular tissue, so that alterations of the serum albumin level were expected, since albumin presents the dominant protein fraction in the serum and the largest part of the reservoir of amino acids available to the body (Putnam, 1960). Although the procedure of taking blood samples immediately after the race may not always reflect the effects of exercise on albumin content, the results obtained were similar to those obtained by Rose et al. (1977), Jablonska et al. (1991) and Poso (1983).

Total serum protein level as an indicator of the state of hydration of the organism revealed that a shift of the fluids out of the vascular compartment occurred during the exercise. The maintenance of normal blood volume is an essential prerequisite for the transport function of blood, also depending on a rapidly moving blood stream, adjustable to the needs of the organism and each organ, separately.

The concentration of the total serum lipoproteins, beta lipoproteins, cholesterol and phospholipids in pre race and postrace samples, and the relative contribution of the separate fractions to the lipoprotein pool are presented in table 2.

According to the results obtained total lipoprotein, cholesterol and phospholipid concentrations were normal in blood serum of the trotters, but the difference between the resting and the postrace samples was significant.

Four fractions: pre-alpha, alpha, pre-beta and beta lipoproteins were separated by agarose-gel electrophoresis (figure 1).

According to the results obtained by electrophoresis the major lipoprotein fraction in horse serum was alpha lipoprotein (67.46 - 69.16%). The relative contribution of the pre-alpha and beta lipoproteins to the total lipoprotein pool was significantly altered during the race, although the established values were normal. All values obtained in the evaluation were within the physiological limits for horses (Alexander and Day, 1973; Chapman, 1980; Vitić and Stevanović, 1993).

Table 2. The concentrations of total lipoproteins, beta lipoproteins, cholesterol and phospholipids in blood serum, and relative contributions of the lipoprotein fractions separated by agarose gel electrophoresis

n = 6	Before race	Immediately after the race
	$(\bar{X} \pm 1SD)$	
Total lipoproteins (dl)	5,72 $\pm$ 0,20	6,31 $\pm$ 0,16***
$\beta$ lipoproteins (dl)	2,06 $\pm$ 0,20	2,09 $\pm$ 0,09
Cholesterol (mmol)	1,61 $\pm$ 0,35	2,49 $\pm$ 0,43**
Phospholipidis (mmol)	2,04 $\pm$ 0,23	2,39 $\pm$ 0,35*
Lipoprotein fractions (%)		
Pre- $\alpha$ lipoproteins	9,41 $\pm$ 0,46	10,57 $\pm$ 0,78*
$\alpha$ lipoproteins	58,05 $\pm$ 0,33	58,59 $\pm$ 0,88
Pre- $\beta$ lipoproteins	3,50 $\pm$ 0,97	3,54 $\pm$ 0,88
$\beta$ lipoproteins	29,04 $\pm$ 0,65	27,29 $\pm$ 1,14***

\*p &lt; 0,05

\*\*p &lt; 0,01

\*\*\*p &lt; 0,001

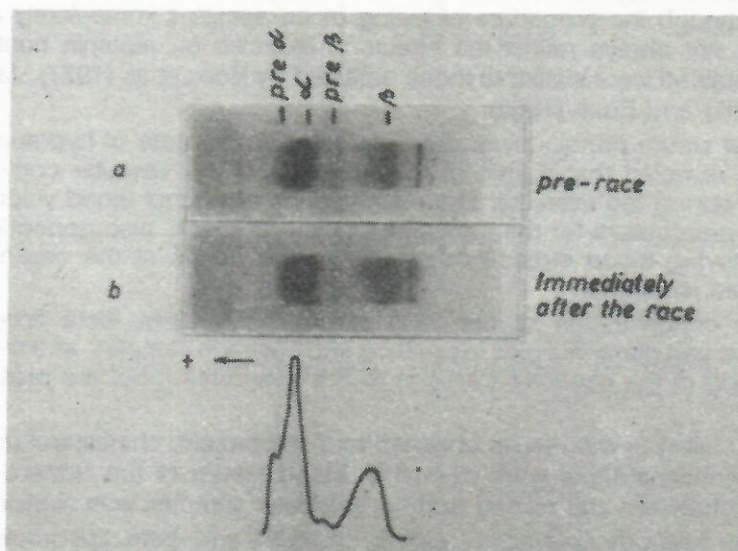


Figure 1. Agarose gel electrophoresis (1 g/dl) of horse lipoproteins and the relative contribution of the lipoprotein fractions.

A significant elevation of the cholesterol content in the post race samples was demonstrated by Rose et al. (1977, 1982) and Andersen (1975), but the results obtained by Poso (1983) were the opposite. The mechanism of the



established physiological increase of the lipoprotein, cholesterol and phospholipid content in trotters has not been determined, yet. Such alterations might be the result of massive mobilisation of body lipides due to the exercise, as was previously described in endurance rides, when the metabolic changed are directed to meet the increasing need for energy by lipolysis and glycogenolysis induced by liberation of insulin, cortisol, epinephrine and norepinephrine (Lucke and Hall, 1980; Grosskopf et al, 1983). On the other hand, a decrease of the total lipoproteins, phospholipids and cholesterol together with the other plasma constituents (proteins, etc.) could reflect the shift of the fluids into the extracellular compartment, as the result of an increased fluid loss in racing animals.

Beside the elevated concentration of total lipoproteins, exercise induced alterations in the relative amounts of the separate lipoprotein fractions. An increase in the relative amount of the alpha lipoproteins together with a decrease of the beta fraction was established in trotters. The results obtained may reflect the increasing need of the muscular tissue for energy, when the basic source of energy for muscles are fatty acids. The necessary fatty acids are donated to the cells from the blood by beta lipoproteins which are simultaneously transformed into alpha lipoproteins.

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## EFEKTI TRKE NA 1600m NA PROTEINE, LIPOPROTEINE I LIPIDE KRVNOG SERUMA KASAČA

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### SADRŽAJ

Koncentracija ukupnih proteina, lipoproteina i lipida kao i relativna zastupljenost njihovih frakcija u krvnom serumu šest kasača ispitivana je pre trke i neposredno nakon trke duge 1600 metara.

Koncentracija ukupnih proteina u krvnom serumu je značajno povećana nakon trke. Relativno učešće pojedinih proteinskih frakcija: albumina, alfa, beta i gama globulina u uzorcima uzetim u mirovanju i nakon trke bilo je približno isto.

Koncentracije ukupnih lipoproteina, holesterola i fosfolipida su se značajno povećale nakon trke. Elektroforezom na gelu agaroze lipoproteini krvnog seruma su razdvojeni na 4 frakcije: pre-alfa, alfa, pre-beta i beta lipoproteine. Relativna zastupljenost alfa lipoproteinske frakcije je, u serumu kasača, bila najveća. Relativni udeo pre-alfa lipoproteina se povećao u uzorcima uzetim nakon trke, pri čemu je udeo beta lipoproteina bio smanjen.