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SOME PROTEIN, LIPOPROTEIN AND LIPID ALTERATIONS IN SERUM OF SHOW JUMPING HORSES DURING DIFFERENT PHASES OF TRAINING

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The serum total protein, lipoprotein and lipid content, together with the relative contribution of their separate fractions was evaluated in six healthy show jumping horses. The blood was sampled in the pre-jumping period, after the first (100cm) and second (120cm) jump, and again in the rest period 60 minutes after the end of the event. A significant increase of total protein level was found in the post-jumping serum sampls. The relative contribution of the protein fractions separated by paper electrophoresis was changed during the exercise. An increas in the relative amount of albumins was accompanied by a decrease in the relative amount of alpha globulins in post-jumping borse sera.

The total lipoprotein, beta lipoprotein and cholesterol levels increased significantly in the samples obtained 60 minute after the jumpings. Serum lipoproteins were separated into four fractions: pre-alpha, pre-beta and beta lipoproteins, by agarose gel electrophoresis. In the rest period and during the event the alpha lipoproteins were the major fraction although in the serum samples obtained 60 minutes after the jumping, their relative amount significantly decreased, while the beta lipoprotein increased, due to the increasing needs of muscle tissue for energy.

Key words: protein, lipoprotein, cholesterol, phospholipids, jumping horse.

INTRODUCTION

Over the last twenty years, there has been a great increase in information on the physiological effects of endurance exercise in horses reflecting the increase in popularity of endurance riding throughout the world.

Endurance exercise provides a most demanding physiological and biochemical challenge for horses competing in such events. Exercise induced modifications of thermoregulatory, cardiovascular and respiratory system responses in severely stressed and dehydrated animals due to the training, are probably very important, considering the extensive fluid and electrolyte losses that occur during sweating. The substrates and metabolic pathways occurring in skeletal muscles competition are regulated to a large extent by training induced muscular adaptation.

During recent years normal values of blood constituents for various breeds and types of horses have been recorded (Sreter, 1959; Cardinet et al., 1963; Lindholm and Saltin, 1974; Milne et al; 1976). Vitić and Nikolić, 1981; Vitić, 1984; Vitić and Stevanović, 1993. Many authors such as Carlson and Mansman (1974), Show and Mc Kenzie (1977), Lucke and Holl (1980a, b), Rose (1982), Stevanović et al., (1994) reported an increase in total protein level in horse sera during, and immediately after the endurance rides. Rose et al., (1977; 1982), Jablonska et al., (1991), Stevanović et al., (1994) reporter that total serum protein, albumin and cholesterol contents in horse blood were significantly different in the rest and post race samples.

The present study aimed to evaluate the changes of proteins, lipoproteins and lipids in serum samples of jumping horses obtained at rest, after the first jump (100cm), after the second jump (120cm), and 60 minutes after the event.

MATERIAL AND METHODS

Blood samples from a total of six show jumping horses (7-12 years old) of both sexes were obtained by jugular vein punction in the different phases of training. The strength of the exercise was 20% diminated compared to a real competition. The first blood samples were obtained in the rest period, then after the first jump (100 cm), after the second jump (120 cm), and again in the rest period, 60 minutes after the second jump. Serum obtained by spontaneous blood cloting was frozen, and processed the next day at the laboratory.

The total serum protein level was determined colorimetrically (Cartier and Picard, 1957). Serum protein fractions were separated by paper electrophoresis, and lipoproteins by electrophoresis on 1g/dl agarose gel (Dyerborg and Hjorne, 1970).

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

The results obtained were statistically analyzed by students "t" test.

corticosterol) which are involved in the control of many metabolic pathways included in energy supply during exercise, such alterations of the serum albumin level could be expected.

The concentrations of the total serum lipoproteins, beta lipoproteins, cholesterol and phospholipids in the pre-jumping period, after the first and the second jump, and 60 minutes after the second jump together with the relative contribution of the separate fractions of the lipoprotein pool are presented in table 2.

Table 2. The concentration of total lipoproteins, β - lipoproteins, cholesterol and phospholipids in horse blood sera and the relative contribution of lipoprotein fractions separated by agarose gel electrophoresis

n = 6	Rssting horses	First jump	p	Second jump	p	60 mon. after second jump	p
Total lipoproteins (g/L)	5.96 ± 0.16	6.11 ± 0.03	NS	6.07 ± 0.06	NS	7.37 ± 0.25	***
β - lipiproteins (g/L)	3.38 ± 0.28	3.64 ± 0.37	NS	3.12 ± 0.15	NS	4.54 ± 0.31	*
Cholesterol (mmol/L)	1.03 ± 0.04	1.20 ± 0.12	NS	1.19 ± 0.13	NS	1.87 ± 0.17	***
Phospholipids (mmol/L)	1.91 ± 0.15	1.87 ± 0.16	NS	2.06 ± 0.09	NS	2.22 ± 0.16	NS
	LIPOPROTEIN FRACTIONS (%)						
pre - α lipoproteins	8.68 ± 0.74	8.73 ± 0.68	NS	8.86 ± 0.46	NS	8.70 ± 0.70	NS
α lipoproteins	51.10 ± 1.16	51.43 ± 1.63	NS	51.46 ± 1.30	NS	40.69 ± 2.14	***
pre - β lipoproteins	3.83 ± 0.55	3.66 ± 0.59	NS	3.51 ± 0.21	NS	3.74 ± 0.49	NS
β lipoproteins	36.39 ± 1.06	36.18 ± 0.98	NS	36.19 ± 1.00	NS	46.87 ± 1.98	***

NS (non significant); * ($p<0.05$); *** ($p<0.001$)

All obtained lipoprotein, cholesterol and phospholipid values in jumpers were within the physiological limits for horses (Alexander and Day, 1973; Chapman, 1980; Vitić and Stevanović, 1993). Although the total lipoprotein, beta lipoprotein, cholesterol and phospholipid concentrations in the horse sera were within the physiological limits, the difference between the pre and the post-jumping samples was significant. The concentration of total lipoproteins ($p<0.001$), beta lipoproteins ($p<0.05$) and cholesterol ($p<0.001$) were significantly higher in the post-jumping samples. A significant elevation of the cholesterol content in the post-jumping samples was also found by Rose et al., (1977; 1982) and Andersen (1975a, b), but the results obtained by Poso (1983) were the opposite. The mechanism of the established physiological increase of the lipoprotein, beta lipoprotein and cholesterol content in exercising horses has not been determined yet. It is well known that the basic metabolic changes during training are lipolysis and glycogenolysis, induced by liberation of insulin, cortisol, epinephrine and norepinephrine (Lucke and Hall, 1980; Grosskopt et al., 1983), resulting in massive oxidation of the fats and reduction of glycogen utilization rate, leading to an increase work capacity of skeletal muscles. Thus the alterations in

RESULTS AND DISCUSSION

Total serum protein concentration and the content of separate protein fractions, in resting animals, after the first and the second jump, and 60 minutes after second jump are shown in table 1.

Table 1. The concentration of total proteins and relative contribution of protein fractions separated by paper electrophoresis in horse blood sera

n = 6	Resting horses	First jump	p	Second jump	p	60 min. after second jump	p
Tatal proteins (g/L)	44.41 ± 2.23	43.99 ± 2.01	NS	48.73 ± 1.65	NS	55.32 ± 0.94	***
Albumins (%)	47.72 ± 1.31	49.11 ± 1.39	NS	51.77 ± 1.65	NS	54.21 ± 0.68	***
α_1 - globulins (%)	7.84 ± 0.35	5.84 ± 0.38	***	6.31 ± 0.42	*	5.18 ± 0.16	***
α_2 - globulins (%)	11.27 ± 0.66	13.91 ± 0.35	NS	10.19 ± 0.49	NS	9.13 ± 0.47	*
β - globulins (%)	13.61 ± 0.49	13.08 ± 0.70	NS	12.47 ± 0.53	NS	12.29 ± 0.48	NS
γ - globulins (%)	19.74 ± 0.91	18.05 ± 1.13	NS	19.25 ± 1.43	NS	19.19 ± 1.54	NS

NS (non significant); * ($p < 0.05$); *** ($p < 0.001$)

The concentration of total protein in pre and post jumping samples were similar. A highly significant ($p<0.001$) elevation of the protein content was observed only in samples obtained 60 minutes after the event. Such an increase of the total protein concentration in post-exercise horse serum samples was previously reported by Rose et al., (1977; 1982), Poso (1983), Jablonska et. al., (1991), Stevanović et la., (1994), who concluded that the degree of the change depended upon the duration and intensity of exercise. It is possible that the increase found in proteinemia may simply reflect hemoconcentration and reduced circulatory volume due increased sweating, which is the most important mechanism for heart dissipation and thermoregulation in horses during endurance exercise. This is the way the total protein level indicates the hydration state of the organism. The maintenance of the normal blood volume is essential for insuring the transporting function of blood depending on the rapidly moving blood stream, adjustable to the needs of the organism and each organ separately.

Horse blood serum proteins were separated into five fractions: albumins, alpha$_1$, alpha$_2$, beta and gamma globulins, by paper electrophoresis. Among the separated fractions the albumins were dominant, and their relative amount significantly increased ($p<-0.001$) in serum sampled 60 minutes after the jumping. An increase in the relative amount of albumins was accompanied by a decrease of alpha$_1$ and alpha$_2$ globulins. Since albumins represent the largest part of the amino acid resource available to the body (Putnam, 1960), and they are known as transporters for fatty acids and steroid hormones (cortisol and

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NEKE PROMENE PROTEINA, LIPOPROTEINA I LIPIDA U KRVNOM SERUMU PREPONSKIH KONJA U RAZLIČITIM FAZAMA TRENINGA

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SADRŽAJ

Koncentracija ukupnih proteina, lipoproteina i lipida, kao i relativna zastupljenost njihovih frakcija u krvnom serumu šest preponskih konja ispitana je pre skoka, posle prvog (100cm) i drugog (120cm) skoka i 60 minuta nakon skakanja.

Koncentracija ukupnih proteina u krvnom serumu se značajno povećala 60 minuta nakon skakanja. Relativno učešće pojedinačnih proteinskih frakcija se posle skakanja promenilo tako što je porast relativne zastupljenosti albuminske frakcije bio praćen padom relativne zastupljenosti alfa globulinske frakcije.

Koncentracija ukupnih lipoproteina, beta lipoproteina i holesterola se povećala u krvnom serumu 60 minuta nakon preskakanja prepona. Elektroforezom na gelu agaroze lipoproteini krvnog seruma konja su razdvojeni na četiri frakcije: pre alfa, alfa, pre beta i beta lipoproteine. Među lipoproteinima u vreme mirovanja i neposredno posle skakanja prepona dominirala je alfa frakcija, ali se zbog povećanih potreba mišića za energijom, njena relativna zastupljenost 60 minuta nakon preskakanja prepona smanjila u korist beta lipoproteinske frakcije.

lipoprotein and cholesterol content estimated in this work, probably reflect the massive mobilization of body lipids, due to the exercise.

The horse serum lipoproteins were separated into four fractions: pre-alpha, alpha, pre-beta and beta lipoproteins, by agarose-gel electrophoresis. The relative contributions alpha and beta lipoproteins to the total lipoprotein pool altered significantly ($p<0.001$) during the exercise. As the alpha lipoproteins have the electrophoretic motility of serum alpha globulins, a decrease in their relative amount after the event could be the explanation for the established decrease in relative amount of alpha globulins (protein fraction) in the same samples. The increase of the relative amount of beta lipoprotein 60 minutes after second jump was accompanied by a decrease in the alpha lipoprotein content, due to the increasing needs of the muscular tissue for the energy provided by transformation of beta lipoproteins (known as donors of fatty acids for the cells) in the alpha ipoprotein fraction.

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