Acta vet. scand. 1983, 24, 170-184.

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# THE EFFECT OF EXERCISE ON BLOOD PARAMETERS IN STANDARDBRED AND FINNISH-BRED HORSES

By

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PÖSÖ, A. R., T. SOVERI and H. E. OKSANEN: The effect of exercise on blood parameters in standardbred and Finnish-bred horses. Acta vet. scand. 1983, 24, 170—184. — Serum enzyme activities, albumin, protein, urea, cholesterol, triglyceride, free fatty acid, glucose and lactate concentrations as well as hematocrit values were measured in standardbred and Finnish-bred horses at rest and after (i) a short controlled exercise and (ii) a trotting competition. There were no breed differences in the enzyme activities at rest and the 2 breeds responded in the same manner to the exercise. Only after the race proper significant increases in the enzyme activities were found. The activities rose more in the standardbred horses than in the Finnishbred horses. Urea and cholesterol concentrations as well as hematocrit values increased significantly after the exercise. At rest hematocrit values were significantly higher in the standardbred horses and the difference persisted throughout the exercise. After the race proper also albumin and protein concentrations were higher in the standardbred than in the Finnish-bred horses. Free fatty acid and triglyceride concentrations increased significantly during the exercise. Although glucose and lactate concentrations increased in both breeds, the behaviour of these parameters differed. Glucose concentrations remained increased for a longer period and the recovery from the increased lactate level was faster in the standardbred than in the Finnish-bred horses. The observed differences suggest that the standardbred horses have higher anaerobic capacity than the Finnish-bred horses.

exercise; standardbred horses; Finnish-bred horses; serum enzymes; protein; hematocrit; triglycerides; free fatty acids; glucose; lactate.

Several studies on enzyme activities and other biochemical and physiological parameters in the blood of horses at rest and after exercise have been carried out during recent years (*Engel*hardt et al. 1973, Lindholm & Saltin 1974, Anderson 1975, Milne et al. 1976, Snow & MacKenzie 1977, Rose et al. 1977, Rose & Hodgson 1982). Almost all studies mentioned above deal with warm-blooded horses and only very little is known about biochemical differences between warm-blooded and cold-blooded horses and their response to exercise.

Our purpose with this study was twofold: (i) to compare cold-blooded Finnish-bred horses with standardbred horses at rest, and (ii) to test the effect of short intense exercise on blood biochemical parameters of both breeds. It should be mentioned in this connection that the speed of standardbred horses during maximal trotting effort is significantly higher that that of Finnish-bred horses and thus the relative intensity of the exercise is equal but the absolute work load is larger for standardbred horses. Therefore special interest was paid to such parameters as glucose, lactate, free fatty acids and triglycerides which have been reported to change according to the intensity of the exercise (Engelhardt et al. 1973, Krzywanek 1973, Milne et al. 1976, Snow & MacKenzie 1977, Rose & Sampson 1982). To evaluate the difference in the maximal work load blood samples were taken at 2 different occasions; (i) during and after a short controlled exercise, and (ii) after a real race when both the speed and apprehension of the horses were presumably at the highest level.

## MATERIAL AND METHODS

Seven Finnish-bred (1 stallion, 1 gelding and 5 mares) and 9 standardbred (4 stallions and 5 mares) horses from 3 to 8 years of age and apparently healthy were used. During the experimental period all horses were under training for trotting and 6 Finnish-bred and 5 standardbred horses were considered to be in such a condition as to compete in an actual race.

Experiments were carried out at Vermon Ravirata (Vermo Trotting Track, Espoo, Finland). The test procedure was as follows. While the horses were still in the stables a blood sample was drawn from the jugular vein. Then the horses were taken to the track and warmed up by light trotting for about 2500 m with speeds ranging from 6 to 7.5 m/s. This was followed by a 30 min recovery period in the stables, during which 2 blood samples were taken 5 and 30 min after the termination of the warm-up period. After returning to the track, trotting of about 1000 m at low speed was immediately followed by maximal trotting for 1600 m. The maximal speeds were (Finnish-bred horses)

over 9 m/s and (standardbred horses) over 10 m/s. After the sprint the horses were returned to the stables and blood samples were taken, 5, 15 and 30 min post sprint.

Blood samples were also taken from 105 standardbred (45 stallions, 20 geldings and 40 mares) and 41 Finnish-bred (19 stallions, 7 geldings and 15 mares) horses after trotting competitions of 1600, 2100, 2200 or 2400 m. These samples were taken 10 to 20 min after the race and the exercise was considered to be maximal in every case. Samples at rest from some of the horses (n = 13 in both breeds) which participated in the race were taken within 2 weeks.

Blood samples were drawn from the jugular vein to evacuated tubes. The blood was thoroughly mixed and divided into 3 as follows: (i) For lactate and glucose determinations a portion of blood was immediately precipitated with 0.6 mol/l perchlorid acid, stored on ice for 30 min and centrifuged. The supernatant was neutralized with KOH and stored in a freezer. (ii) From the second portion hematocrit values were immediately measured. (iii) The rest of the blood was centrifuged to separate the serum which was stored in a freezer until the measurement of metabolites and enzyme activities.

Enzyme activities were, with the exception of gamma glutamyltransferase, measured according to the recommendations of the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974, 1979). The enzymes measured in this way were alanine aminotransferase (ALAT, EC 2.6.1.12), aspartate aminotransferase (ASAT, EC 2.6.1.1.), alkaline phosphatase (AFOS, EC 3.1.3.1), lactate dehydrogenase (LD, EC 1.1.1.27) and creatine phosphokinase (CK, EC 2.7.3.2.). Gamma glutamyltransferase (GGT, EC 2.3.2.2.) was measured as described by Persijn & Slik (1976). All the enzyme activities were measured at 37°C with a computer directed analyzer (Gilford 3500, Oberlin, Ohio, U.S.A.) and the activities are expressed as  $\mu$ mol  $\times$  l<sup>-1</sup>  $\times$  min<sup>-1</sup>. Serum urea nitrogen (Talke & Schubert 1965), cholesterol (Allain et al. 1974) and triglycerides (Bucolo & David 1973) were measured with enzymatic methods. Plasma free fatty acids were measured according to Ho & Meng (1969). Blood glucose was determined with glucose oxidase method (Trinder 1969), total protein with biuret method (Weichselbaum 1946) and serum albumin with bromcresol green (Doumas et al. 1971). Lactate was measured from the neutralized perchloric acid supernatant with lactate dehydrogenase (*Gutmann & Wahlefeld* 1974). Hematocrit was determined by using the microhematocrit technique.

Test kits from Medix (Kauniainen, Finland) were used for determination of ALAT, ASAT and LD. Test kits for AFOS, CK, GGT, glucose and total protein were purchased from Boehringer Gmbh (Mannheim, FRG). Albumin was determined with the test kit from Worthington (Freehold, New Jersey, U.S.A.). Urea nitrogen, cholesterol and triglycerides were measured with test kits from Calbiochem (Lucerne, Switzerland). Lactate dehydrogenase for determination of lactate was the product of Miles Seravac (Maidenhead, Berkshire, U.K.).

Values are given as means  $\pm$  s.e.m. Comparisons between means were made with unpaired Student's t-test.

## RESULTS

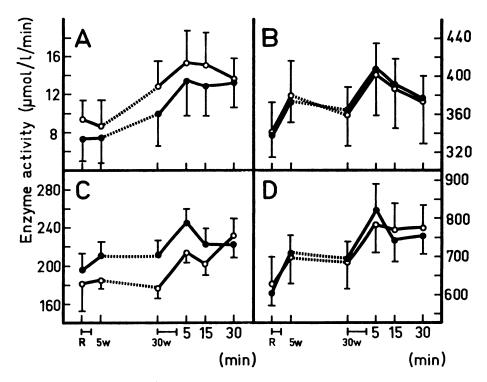
The basal activities of alanine aminotransferase, aspartate transferase, alkaline phosphatase, creatine phosphokinase, gamma glutamyltransferase and lactate dehydrogenase were similar both in the serum of Finnish-bred and standardbred horses

Enzyme	R/E	Standardbred	Finnish-bred	Difference between the	
	R	n = 13	n = 13		
	Е	n = 98	n = 33	breeds	
AFOS	R	$341 \pm 13$	$410 \pm 29$	P < 0.05	
	Е	$375 \pm 10^*$	$399 \pm 16$	ns	
ALAT	R	$16 \pm 2$	$11 \pm 1$	P < 0.05	
	Е	$22 \pm 1$	$18 \pm 1^{***}$	ns	
ASAT	R	$363 \pm 33$	$325 \pm 24$	ns	
	Е	$495 \pm 17^{***}$	$440 \pm 27$	ns	
CK	R	$184 \pm 16$	$166 \pm 13$	ns	
	Е	$261 \pm 11$ ***	$199 \pm 11$	ns	
GGT	R	$26 \pm 2$	$23 \pm 2$	ns	
	E	$28 \pm 2$	$21 \pm 2$	ns	
LD	R	$603 \pm 39$	$637 \pm 28$	ns	
	Е	$725 \pm 24$ ***	$632 \pm 26$	P < 0.01	

Table 1. Effect of a race proper on serum enzyme activities (U/l) in standardbred and Finnish-bred horses.

R = values at rest; E = values after the race. Significant increase from R to E:

\* = P < 0.05; \*\* = P < 0.01; \*\*\* = P < 0.001.



F i g u r e 1. Effect of exercise on serum enzyme activities. Blood samples were drawn from the jugular vein at rest (R), 5 and 30 min after the warm-up trotting (5w and 30w) and 5, 15, and 30 min after maximal trotting of 1600 m (5, 15 and 30). Standardbred horses  $\bigcirc$ — $\bigcirc$ ; Finnish-bred horses  $\bigcirc$ — $\bigcirc$ . A. ALAT B. ASAT C. CK D. LD.

(Fig. 1, Table 1). The controlled exercise did not cause any significant differences between the 2 breeds. The activities of alkaline phosphatase and gamma glutamyltransferase remained constant during the whole experiment. The rest values were for AFOS  $559 \pm 66$  and  $584 \pm 39$  U/l, and for GGT  $40 \pm 6$  and  $56 \pm 13$  U/l in standardbred and Finnish-bred horses, respectively. Small increases were found in the activities of ALAT (Fig. 1A), ASAT (Fig. 1B), CK (Fig. 1C) and LD (Fig. 1D) after the short controlled exercise although these changes did not reach the level of statistical significance.

The increases in the enzyme activities after the race proper were similar to those after the controlled exercise although the magnitude was larger (Table 1). In accordance with the results

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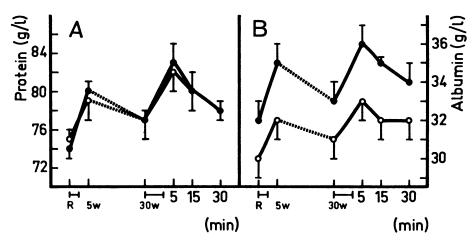


Figure 2. Effect of exercise on serum total protein and albumin concentrations.

Sampling and groups as in Fig. 1. A. total protein. In both breeds the concentrations at 5 (P < 0.01) and 15 (P < 0.05) min were significantly higher than the at rest value. B. Albumin. In the standardbred horses the concentrations at 5w (P < 0.05), 5 (P < 0.05) and 15 (P < 0.05) min were significantly higher than the at rest value.

from the controlled exercise the activity of ALAT increased significantly in both breeds. In the standardbred, but not in the Finnish-bred horses, the increases in the activities of ASAT, CK and LD were also statistically significant. For recording the changes in the activities of muscular enzymes in serum a longer

	R/E	$\frac{\text{Standardbred}}{n = 13}$	Finnish-bred n = 13	Difference
g/l of serum	R			between the
	E	n = 68	n = 25	breeds
Albumin	R	$33.9\pm0.6$	$30.8 \pm 0.6$	P < 0.05
	Ε	$39.6 \pm 0.3^{*}$	$36.5\pm0.6$ *	P < 0.001
Total protein	R	$62.4 \pm 1.2$	$60.9\pm0.9$	ns
-	Ε	$78.3 \pm 0.6$ *	$75.4 \pm 1.1^{*}$	P < 0.05

T a ble 2. Effect of a race proper on the concentrations of albumin and total protein in the serum of standardbred and Finnish-bred horses.

R = values at rest; E = values after the race.

Significant increase from R to E:

ns = not significant.

<sup>\*</sup> P < 0.001.

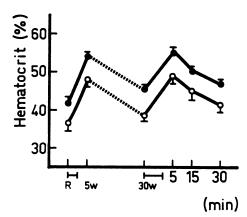


Figure 3. Effect of exercise on blood hematocrit values.

Sampling and groups as in Fig. 1. Statistical differences from the at rest value were in the standardbred horses: 5w (P < 0.001), 5 (P < 0.001), 15 (P < 0.001),  $30 \min$ (P < 0.01) and in the Finnish-bred horses: 5w (P < 0.01), 5 (P < 0.01),  $15 \min (P < 0.05)$ .

Table 3. Hematocrit values at rest of well trained and less trained standardbred and Finnish-bred horses.

Breed	n	Training status	Hematocrit (%)	Р
Standardbred	5	less trained	$40.8\pm1.2$	
	4	well trained	$44.0 \pm 1.5$	ns
Finnish-bred	<b>2</b>	less trained	$30.5\pm0.5$	
	5	well trained	$39.2 \pm 1.9$	< 0.001

ns = not significant.

follow-up period, which was not possible to arrange in the present study, would be needed, since, for example, the activity of CK in serum reaches its maximum 5 h after exercise (*Aitken et al.* 1975).

Exercise increased protein and albumin concentrations in the serum (Fig. 2). In the standardbred horses the increase in albumin was larger than that in the Finnish-bred horses and resulted in a significant difference between the 2 breeds, both after light trotting and sprinting, as well as after the race itself (Table 2). A similar difference in total protein concentration was seen after the race (Table 2) but not after the controlled exercise (Fig. 2). The concentration of urea nitrogen, which at rest was  $7.2 \pm 0.4$  and  $6.8 \pm 0.6$  mmol/l in standardbred and Finnish-bred horses, respectively, was not affected by the exercise.

The increased protein concentration was accompanied with an increase in the hematocrit value (Fig. 3). The values at

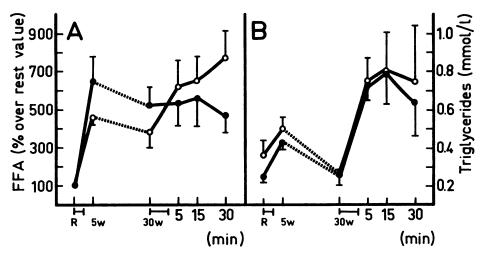


Figure 4. Effect of exercise on serum free fatty acids and triglyceride concentrations.

Sampling and groups as in Fig. 1. A. FFA. In both breeds all the values after the warm-up and maximal trotting effort were significantly higher than the respective at rest value. B. Triglycerides. Statistical differences from the at rest value were in the standardbred horses: 5 (P < 0.001), 15 (P < 0.01), 30 min (P < 0.05) and in the Finnishbred horses: 5 min (P < 0.01).

rest for the standardbred horses were significantly (P < 0.05) higher than in the Finnish-bred horses and the exercise further strengthened this difference. When hematocrit values from horses which were fit enough to compete in a race were compared with those in horses of the same breed which were still under training it was found that in both breeds the horses with longer training had higher hematocrit values (Table 3). In the Finnish-bred horses the training-induced increase was statistically significant but not so in the standardbred horses.

The at rest values of serum free fatty acids (FFA) were similar in both breeds. Their concentration increased after the warm-up period of trotting and stayed high during the whole period of the experiment (Fig. 4A). In the standardbred horses the concentration of FFA leveled off after the initial increase while in the Finnish-bred horses their concentration continued to increase during the whole experiment. Thus the response of the 2 breeds seemed to differ even though the differences did not reach the level of statistical significance.

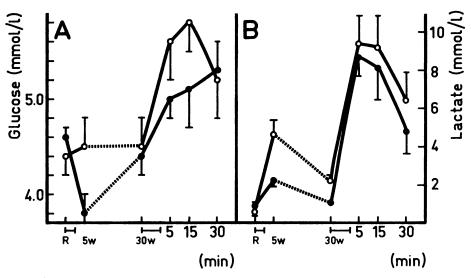


Figure 5. Effect of exercise on blood glucose and lactate concentrations.

Sampling and groups as in Fig. 1. A. Glucose. Statistical differences from the at rest value were in the standardbred horses: 5w (P < 0.01),  $30 \min (P < 0.05)$  and in the Finnish-bred horses: 5 (P < 0.05),  $15 \min (P < 0.01)$ . B. Lactate. Statistical differences from the at rest value were in the standardbred horses: 5w (P < 0.01), 5 (P < 0.001), 15 (P < 0.001),  $30 \min (P < 0.01)$  and in the Finnish-bred horses: 5w (P < 0.001), 5 (P < 0.001), 15 (P < 0.001),  $30 \min (P < 0.01)$ , 5 (P < 0.001), 15 (P < 0.001),  $30 \min (P < 0.01)$ , 5 (P < 0.001), 15 (P < 0.001),  $30 \min (P < 0.01)$ .

Basal triglyceride concentration in the blood was similar in the 2 breeds and the controlled exercise did not cause any differences between the breeds (Fig. 4B). Both the warm-up trotting and sprinting increased triglyceride concentration. After the race triglyceride concentrations were  $1.50 \pm 0.06$  and  $0.78 \pm 0.07$ mmol/l in the standardbred and the Finnish-bred horses, respectively. The effect of the intensity of the exercise was thus seen only in the standardbred horses.

No effect of exercise or differences between the breeds was found in the cholesterol concentration; the basal values were  $2.3 \pm 0.1$  and  $2.5 \pm 0.1$  mmol/l in standardbred and Finnish-bred horses, respectively.

No breed differences were found in the basal blood glucose values. Glucose concentration tended to increase after the exercise and the increase tended to be higher in the Finnish-bred

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horses (Fig. 5A). When the intensity of the exercise was increased, as happened under real competition conditions, the blood glucose rose highly significantly in both breeds. The increase was from  $4.0 \pm 0.1$  to  $4.7 \pm 0.1$  mmol/l (P < 0.001) in the standardbred horses and from  $4.4 \pm 0.2$  to  $5.5 \pm 0.1$  mmol/l (P < 0.001) in the Finnish-bred horses. The larger increase of blood glucose values in the Finnish-bred horses also resulted in a significant difference (P < 0.001) between the 2 breeds.

The warm-up trotting significantly elevated the blood lactate concentration in the Finnish-bred horses only (Fig. 5B). During the following recovery period of 30 min the lactate level returned to the basal level in the standardbred but not in the Finnish-bred horses. Maximal controlled exercise increased lactate concentrations equally in both breeds, but again the recovery tended to be slower in the Finnish-bred horses. After the race the standardbred horses had higher blood lactate concentrations than the Finnish-bred horses; the maximal values after the race were  $12.8 \pm 1.0$  and  $8.3 \pm 0.6$  mmol/l (P < 0.001) in standardbred and Finnish-bred horses, respectively.

### DISCUSSION

Hematocrit, total protein and albumin concentrations in the blood increased during the exercise indicating slight hemoconcentration and release of red cells from the splenic reservoir (*Persson* 1968). In keeping with earlier literature (*Snow & MacKenzie* 1977, *Rose & Hodgson* 1982) the trained horses of both breeds tended to have higher hematocrit values. The standardbred horses always had significantly higher hematocrit values than the Finnish-bred horses. Since the difference which was found in the at-rest-values persisted throughout the whole period of exercise and recovery, it is not likely to be as a result of the different apprehension before and during the exercise period (*Rose & Hodgson* 1982) but rather represents a true difference between the 2 breeds.

During exercise, the 2 main energy sources for muscle are glucose and free fatty acids (*Carlson et al.* 1965, *Anderson* 1975). Lipids are especially important during long distance exercise and training has been shown to increase lipid utilization during exercise (*Goodman et al.* 1973, *Snow & MacKenzie* 1977, *Lucke & Hall* 1978, *Rose et al.* 1980). According to *Rose & Samp*son (1982) the mobilization of FFA is related to the duration of the exercise rather than its intensity, but we found that lipolysis accelerates very rapidly and thus FFA may also represent important energy source during short intense periods of exercise. It appears that standardbred horses mobilize their FFA faster and they may also utilize FFA more effectively than Finnishbred horses.

Triglyceride concentration in the blood has been reported to increase after prolonged exercise in the horse (*Rose et al.* 1980) and it was thought to reflect accelerated lipid metabolism in general. However it was found that triglycerides were elevated after short exercise periods. The reason for this may be the rapid increase of FFA concentration in the blood. The liver of the horse has a great capacity to synthesize triglycerides from FFA (*Bartley* 1980) and thus the rate of triglyceride synthesis is regulated by the availability of FFA.

Serum triglyceride values in samples taken after the competition were significantly higher in the standardbred than in the Finnish-bred horses. This difference could be a consequence of more rapid lipolysis in the standardbred horses or it could represent a breed difference in the capacity of the liver to synthesize triglycerides. However, further studies are needed to clarify the observed changes and differences between the 2 breeds.

After prolonged exercise blood glucose usually decreases (Carlson et al. 1965, Rose et al. 1977, Snow & MacKenzie 1977, Lucke & Hall 1978, 1980) but both decreased and increased values have been reported after short exercises (Engelhardt et al. 1973, Lindholm & Saltin 1974, Anderson 1975). In untrained horses blood glucose decreases after short exercise (Hambleton et al. 1980) while in trained animals or when the intensity of short exercise is increased (Engelhardt et al. 1973, Lindholm & Saltin 1974, Snow & MacKenzie 1977, Hambleton et al. 1980) glucose concentration increases. Snow & MacKenzie showed that in trained horses the increased level of blood glucose persisted longer after the exercise than in untrained horses. In our study the Finnish-bred and the standardbred horses responded differently to the exercise. The light warm-up trotting lowered blood glucose in the standardbred but not in the Finnish-bred horses. After the sprinting blood glucose concentration was still increasing at the end of the 30 min follow-up period while in the Finnish-bred horses it was declining. Also in the samples

which were taken after the race proper blood glucose was significantly higher in the Finnish-bred horses.

The increase in blood lactate concentration during short periods of exercise depends both on the speed and the condition of the horse. Marked increases in blood lactate concentration are not observed until the speed approaches the maximal, usually over 9-11 m/s (Engelhardt et al. 1973, Lindholm & Saltin 1974). Snow & Mackenzie (1977) showed that with the same relative work load (maximal exercise) lactate increases more in the blood of trained horses than in the same horses before training. On the other hand smaller increases in blood lactate occur in trained horses when the absolute work load is kept constant (Krzywanek 1973, Milne et al. 1977, Seren et al. 1977). In the present study the warm-up period of trotting seemed to be harder exercise for the Finnish-bred horses manifested by a higher lactate level. Also the recovery was not as complete before the following sprint as in the standardbred horses. The larger material which in our study was taken after the competition shows that standardbred horses had higher blood lactate values than Finnish-bred horses.

In summary, only minor differences seem to exist between the 2 breeds of horses tested by several biochemical parameters at rest or after exercise. However, standardbred horses responded to short intense exercise like well trained horses while the Finnish-bred horses, despite their training, resembled untrained horses. Higher blood lactate values after the competition, slower decrease in blood glucose concentration, possibly greater capacity to use fats and higher hematocrit values already at rest support this. Whether this difference is due to different training programmes or whether it is a real breed difference remains to be seen.

#### **ACKNOWLEDGEMENTS**

We thank Ms. Pirjo Puroranta for her excellent technical assistance and the owners and trainers of the horses for cooperation.

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### SAMMANFATTNING

#### Ansträngningens inverkan på blodparametrar hos amerikanska travare och finska hästar.

Halten av serum enzymaktiviteter, albumin, protein, urea, kolesterol, triglycerider, fria fettsyror, glukos och laktat samt hämatokritvärden bestämdes hos amerikanska travare samt finska hästar i vila och (i) efter kort kontrollerad ansträngning och (ii) efter travtävlingen. Ingen rasskillnad i enzymaktiviteter vid vila konstaterades och de två raserna svarade likadant på ansträngningen. Först efter traven påvisades en reell stegring av enzymaktiviteter. Aktiviteterna steg mera hos amerikanska travare än hos finska hästar. Urea- och kolesterolkoncentrationerna förblev oförändrade efter ansträngningarna. Förhöjningen av protein- och albuminkoncentrationerna samt hämatokritvärderna var markant efter ansträngningen. Vid vila var hämatokritvärdena markant högre hos amerikanska travare och skillnaden kvarstod alltigenom ansträngningen. Efter traven var också albumin- och proteinkoncentrationerna högre hos amerikanska travare än hos finska hästar. Halten av fria fettsyror och triglycerider steg markant under ansträngningen. Ehuru koncentrationen av glukos och laktat steg i bägge raser avvek beteendet av dessa parametrar. Halten av glukos förblev förhöjd för en längre period och återställandet av det förhöjda laktatvärdet försiggick fortare hos amerikanska travare än hos finska hästar. De observerade skillnaderna tyda på, att amerikanska hästar besitta en högre anaerobisk kapacitet än finska hästar.

(Received March 15, 1983).

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