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Biochemical Changes in Thoroughbred Racehorses Following Submaximal and Maximal Exercise

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Summary

Sixteen plasma biochemical variables and blood lactate were measured in Thoroughbred racehorses prior to and during the first hour after exercise. When horses were exercised at a canter, changes occurred in a number of blood constituents. With the exception of creatinine, phosphorus and potassium, all variables returned to their pre-exercise values within an hour. Phosphorus and potassium, however, were below their pre-exercise value after an hour. Exercise at a gallop resulted in changes in blood lactate and in all plasma constituents assayed, except in chloride and conjugated bilirubin. Of the constituents altered, only sodium, potassium, cholesterol and alkaline phosphatase returned to their respective pre-exercise values after one hour. At this time, bicarbonate, phosphorus and total bilirubin were below their resting values, whereas glucose, urea, creatinine, urate, calcium, lactate dehydrogenase, creatine kinase and blood lactate were above their pre-exercise values.

Introduction

The purpose of the present study was to compare and contrast the changes in blood constituents in mature Thoroughbred racehorses. Changes in blood constituents with increasing fitness and maturity in Thoroughbred horses have been documented (Judson *et al.*, 1983). Therefore, evaluation of changes due to different exercise levels is best achieved with horses at comparable levels of fitness. By using horses in racing condition we minimized the changes which have been reported with the progression of training programmes.

Materials and Methods

Studies were carried out on 16 clinically healthy, mature Thoroughbred horses in racing condition. The horses were housed in loose-boxes and maintained on a diet consisting of wheaten or oaten chaff, oats, bran and lucerne hay.

The horses were exercised on a racetrack between 06.00 h and 08.00 h. Submaximal exercise was a canter for two minutes over 1.2 km (10 m/s) and maximal exercise was a gallop for one minute over 1 km (16.6 m/s).

Blood was collected from the jugular vein into lithium heparinized 'vacutainers' at rest, just prior to exercise and at 1, 15, 30 and 60 minutes after exercise. An aliquot of the blood was immediately deproteinized with ice-cold perchloric acid for lactate estimation. The protein-free supernatant of blood was neutralized with potassium carbonate before the assay for lactate was carried out using the method of Hohorst (1974).

The blood samples were centrifuged within two hours of collection and the plasma retained for biochemical analyses. Plasma samples from four horses given submaximal exercise and from four horses given maximal exercise were assayed using a 'SMAC' instrument (Sequential Multiple Analyzer plus Computer, Technicon Instrument Corporation, Tarrytown, NY, USA). The constituents assayed were sodium, potassium, chloride, bicarbonate, glucose, urea, creatinine, urate, phosphate, calcium, cholesterol, conjugated bilirubin, total bilirubin, alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase and creatine kinase. The analytical methods have been described by Schwartz *et al.* (1974), except for the conjugated bilirubin method which was based on the procedure of Gambino and Schreiber (1965). Enzyme assays were performed at 37°C. Albumin and total protein were measured on all plasma samples by the procedures of Ingwersen and Raabo (1978) and Kachmar (1970), respectively. The plasma anion gap was calculated by subtracting the sum of the chloride and bicarbonate concentrations from the sum of the sodium and potassium concentrations and expressed in mmol/l.

Statistical analysis of the data was by two-way analysis of variance and Duncan's multiple-range test (Duncan 1955), except for data given in Table 3 which was by one-way analysis of variance.

Results

Submaximal exercise induced a change from resting values for bicarbonate, anion gap, calcium, cholesterol and aspartate aminotransferase at $p < 0.05$, and for potassium, glucose, blood lactate, creatinine, inorganic phosphate, total protein and alkaline phosphatase at $p < 0.001$ (Table 1). As shown in Table 1, all constituents returned to pre-exercise values within 60 minutes, except for creatinine, inorganic phosphate and potassium. At 60 minutes, the latter two constituents were significantly lower than their resting concentrations ($p < 0.05$).

Maximal exercise induced a change from resting values for potassium and creatine kinase at $p < 0.05$, for cholesterol and total bilirubin at $p < 0.01$ and for the other constituents measured at $p < 0.001$, except for chloride and conjugated bilirubin which were not affected by maximal exercise. Of the constituents altered by maximal exercise, only sodium, albumin, total protein, cholesterol and alkaline phosphatase returned to their respective resting values within 60 minutes of exercise (Table 2).

To facilitate interpretation of the immediate effects of maximal and submaximal exercise on the various constituents, the percentage changes of the pre-exercise values at one minute post-exercise were calculated for all constituents altered by exercise (Tables 1 and 2). Table 3 gives the per cent changes in constituents which were found to differ significantly from the per cent changes in total protein.

TABLE 1. Plasma biochemical constituents in horses before and after submaximal exercise*

Constituent	Pre-exercise	Minutes post-exercise			
		1	15	30	60
Sodium (mmol/l)	139	138	140	140	140
Potassium** (mmol/l)	3.8 ^a	4.4 ^b	4.0 ^a	3.7 ^a	3.3 ^c
Chloride (mmol/l)	100	99	101	101	101
Bicarbonate** (mmol/l)	27 ^a	23 ^b	26 ^a	27 ^a	27 ^a
Anion gap** (mmol/l)	15.8 ^a	20.7 ^b	17.2 ^a	15.4 ^a	14.5 ^a
Glucose** (mmol/l)	6.1 ^a	7.6 ^b	7.0 ^c	6.3 ^a	6.6 ^{ac}
Blood lactate** (mmol/l)	0.5 ^a	1.7 ^b	0.8 ^c	0.6 ^{ac}	0.6 ^{ac}
Urea (mmol/l)	4.5	4.4	4.5	4.5	4.5
Creatinine** (mmol/l)	0.12 ^a	0.14 ^b	0.14 ^b	0.14 ^b	0.14 ^b
Urate (mmol/l)	0.02	0.02	0.03	0.02	0.02
Phosphate** (mmol/l)	1.13 ^a	1.20 ^b	1.09 ^a	1.01 ^c	0.95 ^d
Calcium** (mmol/l)	3.03 ^{ab}	3.08 ^a	2.98 ^b	2.98 ^b	3.03 ^{ab}
Total protein** (g/l)	74 ^a	80 ^b	76 ^a	75 ^a	74 ^a
Cholesterol** (mmol/l)	3.1 ^a	3.5 ^b	3.2 ^a	3.5 ^b	3.2 ^a
Conjugated bilirubin (μ mol/l)	4	5	4	4	4
Total bilirubin (μ mol/l)	45	46	47	47	48
Alkaline phosphatase** (U/l)	217 ^a	243 ^b	219 ^a	219 ^a	215 ^a
Lactate dehydrogenase (U/l)	306	325	314	298	300
Aspartate aminotransferase** (U/l)	351 ^a	369 ^b	355 ^a	351 ^a	353 ^a
Creatine kinase (U/l)	296	240	355	377	276

*Mean values for 4 horses, except blood lactate and total protein values which are for 8 horses.

**Constituents altered by exercise. For each of these constituents, means not having the same superscript differed ($p < 0.05$).

Discussion

Total plasma protein concentration increased after submaximal and maximal exercise, although the increase after maximal exercise was slightly greater (14% vs 9%). There was also a slower return to pre-exercise values after maximal exercise. Increases in total protein after a short-duration exercise are considered to be the result of a redistribution of fluid and electrolytes from the vascular compartment to the tissue extracellular fluid spaces, whereas the net loss from the body via evaporation from the respiratory tract and sweat is of significance in horses competing in long-distance endurance events (Carlson 1975; Rose *et al.*, 1980a). This seems to be confirmed by the rapid increases in plasma protein concentrations in the present study and by the return to pre-exercise values within 15 minutes after submaximal exercise and 30 minutes after maximal exercise. The plasma protein concentrations remained well above pre-exercise values 30 minutes after completion of long-distance endurance events (Rose *et al.*, 1980a, 1980b).

The changes in plasma sodium and chloride concentrations after exercise are similar to those previously reported in Thoroughbreds after maximal exercise (Keenan 1979). However, the changes in plasma potassium concentrations present an enigma. The initial

TABLE 2. Plasma biochemical constituents in horses before and after maximal exercise*.

Constituent**	Pre-exercise	Minutes post-exercise			
		1	15	30	60
Sodium (mmol/l)	139 ^a	150 ^b	144 ^c		138 ^a
Potassium (mmol/l)	3.8 ^a	4.0 ^a	4.0 ^a		3.5 ^b
Chloride (mmol/l)	104 ^a	103 ^a	102 ^a		103 ^a
Bicarbonate (mmol/l)	29 ^a	7 ^b	6 ^b		24 ^c
Anion gap (mmol/l)	10.6 ^a	44.0 ^b	40.3 ^c		15.0 ^d
Glucose (mmol/l)	6.6 ^a	10.4 ^b	9.6 ^b		8.4 ^c
Blood lactate (mmol/l)	0.6 ^a	16.5 ^b	16.2 ^b	8.9 ^c	3.3 ^d
Urea (mmol/l)	4.2 ^a	4.2 ^a	4.3 ^a		4.6 ^b
Creatinine (mmol/l)	0.17 ^a	0.21 ^b	0.21 ^b		0.20 ^c
Urate (mmol/l)	0.02 ^a	0.08 ^b	0.15 ^c		0.13 ^c
Phosphate (mmol/l)	0.98 ^a	1.27 ^b	1.06 ^a		0.64 ^c
Calcium (mmol/l)	3.13 ^a	3.56 ^b	3.34 ^c		3.28 ^c
Albumin (g/l)	41 ^a	49 ^b	45 ^c	41 ^a	40 ^a
Total protein (g/l)	67 ^a	77 ^b	73 ^c	68 ^a	66 ^a
Cholesterol (mmol/l)	2.9 ^a	3.4 ^b	3.2 ^b		2.8 ^a
Conjugated bilirubin (μ mol/l)	2 ^a	3 ^a	2 ^a		2 ^a
Total bilirubin (μ mol/l)	31 ^a	23 ^b	24 ^b		24 ^b
Alkaline phosphatase (U/l)	221 ^a	286 ^b	263 ^c		227 ^a
Lactate dehydrogenase (U/l)	231 ^a	311 ^{bc}	336 ^b		280 ^c
Creatine kinase (U/l)	102 ^a	152 ^a	143 ^a		255 ^b

*Mean values for 4 horses, except blood lactate, albumin and total protein which are for 8 horses.

**All constituents except chloride and conjugated bilirubin were affected by exercise (see text). For each constituent, means not having the same superscript differed ($p < 0.05$).

changes in potassium after submaximal exercise (19%) appeared greater than those seen after maximal exercise (6%). This is not adequately explained by the classical theories of the basis for the changes in potassium concentrations. These theories include haemoconcentration, selective movement of potassium ions out of the muscle cells and the efflux of intracellular potassium ions, in exchange for hydrogen ions, during metabolic acidosis (Milne 1974). In both exercise groups, plasma potassium concentrations increased after exercise but then returned to and eventually decreased below the pre-exercise values. The decrease in potassium and phosphate concentrations at 60 minutes post exercise has been reported by others (Codazza *et al.*, 1974; Keenan 1979; Rose *et al.*, 1980b). These authors considered the changes to be due to attempts to re-establish the intracellular high-energy phosphate reserves (Keenan 1979) or to temporary dilution effects following the movement of fluid into the extracellular fluid compartment (Rose *et al.*, 1980b). However, the second explanation is not supported in the study reported here since the changes in plasma protein concentration did not decrease below pre-exercise values.

The decreases in bicarbonate concentration were paralleled by increases in the anion gap. These changes were more marked after maximal exercise and may be explained by

TABLE 3. Per cent changes in pre-exercise values of selected plasma constituents at one minute post-exercise†.

Constituent	Submaximal exercise	Maximal exercise
Potassium	19 ± 11*	
Bicarbonate	-14 ± 10***	-77 ± 5***
Glucose	25 ± 9**	56 ± 13***
Blood lactate	231 ± 137***	2480 ± 287***
Creatinine		21 ± 2*
Urate		463 ± 111***
Phosphate		31 ± 10***
Total bilirubin		-26 ± 10***
Alkaline phosphatase		30 ± 9**
Lactate dehydrogenase		35 ± 4***
Total protein	9 ± 4	14 ± 6

†Mean values ± SD for 4 horses, except values for total protein and blood lactate which are for 8 horses. For each level of exercise, those values with the superscript *, ** or *** differed from the total protein value at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

the difference in the severity of the metabolic acidosis stemming from the differences in blood lactate concentrations observed in these horses. Similar changes in the acid-base balance following maximal exercise in horses have been described by others (Snow and Mackenzie, 1977).

In the present study, a 20-fold to 25-fold increase was seen in blood lactate values after the horses had worked at 16.6 m/s over 1 km. Similar increases have been reported after Thoroughbred races (Keenan 1979). In contrast, only small increases in blood lactate were detected after cantering. These small increases and the lack of continued lactate production (demonstrated by the rapid return to pre-exercise values verify that this submaximal work load is below the anaerobic threshold for skeletal muscle metabolism.

The changes in plasma glucose concentrations after maximal exercise in this study were similar to those previously reported in Thoroughbred horses (Sréter 1959; Takagi and Sakurai, 1971; Snow and Mackenzie, 1977). Circulating glucose concentrations are not considered to be of clinical relevance since glucose is not an important source of energy for muscle metabolism during brief maximal exercise, so long as adequate muscle glycogen reserves exist (Snow and Mackenzie, 1977).

In the present study short-distance submaximal exercise appeared to cause minimal changes in the plasma concentrations of conjugated and total bilirubin. This is in contrast to the increased concentrations of bilirubin noted after submaximal exercise over longer distances (Lucke and Hall, 1980; Rose *et al.*, 1980b). Such increases may be due to increased production of bilirubin and decreased hepatic perfusion. In the study reported here, there was a decrease in the plasma concentration of total bilirubin following maximal exercise, which reflects a decrease in unconjugated bilirubin since there was a minimal change in the conjugated bilirubin concentrations. This has not been previously reported in horses. An increase in the binding of substrates such as free fatty acids to plasma albumin after exercise may have displaced bilirubin from albumin so that there is

a loss of unconjugated bilirubin from the vascular compartment. This fall in bilirubin concentrations is transient since Sréter (1959) observed raised plasma bilirubin concentrations in Thoroughbred horses 20 hours after racing.

Plasma urate concentrations were markedly increased after maximal exercise, especially at 15 minutes. Similar findings have been reported in horses after completion of Thoroughbred races (Keenan 1979). Various explanations have been offered as to the cause of the increase in urate, including inadequate activity of the enzyme uricase which is responsible for the conversion of urate to allantoin (Keenan 1979) and an increased breakdown of nucleotides to urate (Snow *et al.*, 1982). There were negligible changes in urate after submaximal exercise, unlike the marked increases previously reported in horses competing in long-distance endurance events (Lucke and Hall, 1980; Rose *et al.*, 1980b).

Both maximal and submaximal exercise appeared to cause immediate and sustained increases in the plasma creatinine concentration which did not return to pre-exercise levels after 60 minutes. The increases after maximal exercise were of greater magnitude than those seen after submaximal exercise. Similar findings have been reported in human athletes (Hamilton *et al.*, 1972) and in horses, following both maximal exercise (Keenan 1979) and extended submaximal exercise (Codazza *et al.*, 1974; Lucke and Hall, 1980; Rose *et al.*, 1980b). It would appear that the increased creatinine may be the combined result of an increased breakdown of phosphocreatine in muscle cells and haemoconcentration. It is considered to be a temporary change in muscle physiology and not renal pathology (Rose *et al.*, 1980b). Anderson (1975) considers that exercise causes a selective change in the permeability of the muscle cell membrane which persists after completion of the exercise régime. This would appear to be supported by the present study, since plasma creatinine and the relatively muscle-specific enzyme, creatine kinase, remained elevated at 60 minutes. Furthermore, there were greater increases and variation in creatinine concentrations after the more severe exercise. At 60 minutes post exercise, the effect of haemoconcentration had diminished since the total protein had returned to pre-exercise values. Other studies have also shown a continued rise in creatine kinase activities in horses after exercise, peaking five hours after exercise (Anderson 1975; Milne 1974).

The changes in the activities of lactate dehydrogenase and aspartate aminotransferase after submaximal exercise (Table 1) were of similar magnitude to those of the total protein and may be explained by haemoconcentration. Similar short-term changes in aspartate aminotransferase have been recorded in trained human athletes in whom the increases were both proportional to the intensity of work and a consequence of haemoconcentration (Metivier *et al.*, 1980). The changes in plasma enzyme activities after maximal exercise were greater than those reported by Keenan (1979) in horses after racing, but less than those following long-distance endurance events (Anderson 1975; Lucke and Hall, 1980; Rose *et al.*, 1980b).

As expected, maximal exercise caused greater changes in the blood concentrations of the electrolytes and metabolites than did submaximal exercise. However, there were two plasma constituents that responded differently to this general pattern: unconjugated bilirubin decreased after maximal exercise, and the per cent increase in urate after maximal exercise was of much greater magnitude than in any other constituent apart from blood lactate. In general, the rate of return towards pre-exercise values for all

constituents was more rapid and complete 60 minutes after submaximal exercise, but many constituents were significantly altered from the pre-exercise values 60 minutes after completion of maximal exercise. At this time, potassium and phosphate had decreased below their pre-exercise values, following both submaximal and maximal exercise. These changes are of significance when clearance ratios are measured for the investigation of electrolyte or mineral (e.g. calcium and phosphate) imbalance as causes of lameness or poor athletic performance (Traver *et al.*, 1976).

As the present study shows, a training gallop at 16 m/s can cause changes in blood biochemistry similar to those reported after gallop races (Keenan 1979). The extent of change in blood constituents after a training gallop and the time taken to re-establish the pre-exercise values of the constituents may be informative criteria for assessing the fitness of a horse.

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Haematology and Blood Biochemistry of Horses during a 210 km Endurance Ride

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Summary

Various haematological analyses were performed on daily pre-ride and post-ride blood samples collected from 20 randomly sampled horses participating in the South African National Endurance Ride of 1981. The ride is an annual three-day event during which distances of 80 km, 80 km and 50 km are covered over the three days respectively.

The values of the majority of parameters increased during the ride on each of the three days and returned to approximately the pre-ride values overnight. The blood glucose decreased during the ride and also returned to the pre-ride values overnight. An exception was the plasma urea concentration which showed a cumulative increase during the three days. Only minor changes occurred in the concentrations of the plasma cations.

The horses were ridden faster on the first day of the ride and the alterations in the blood constituents were more marked on this day than on the two subsequent days.

The greater alterations in the concentration of blood constituents observed in less fit horses ridden at average speeds exceeding 19 km/h suggest that they were more stressed than the fit or more slowly ridden horses.

Introduction

Endurance riding has gained popularity in South Africa since 1974. Local competitive rides are normally one-day events over distances of 80 km and exceptionally over distances of 160 km. During July each year, a national ride is organised in the central part of the country at a small town called Fauresmith. This is a three-day event over a total distance of 210 km of which 80 km are covered on the first day, a further 80 km on the second day and the final 50 km on the third day. Approximately 70 to 90 horses are entered annually, with approximately 70% succeeding in completing the ride.

Strict veterinary control is exercised by a panel of veterinarians at the various check points and on the route. All competitors are compelled to rest their horses for half an hour at the first check point, 30 km from the start, and for one hour at the second check point (on the first two days), a further 30 km away. Apart from a general examination for habitus, lameness and possible injuries, pulse and respiratory rates are determined for each horse 20 minutes after arrival at each check point and at the end of each day's ride.