

Effects of Acetazolamide on Cardiorespiratory and Metabolic Responses to Submaximal Exercise

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ABSTRACT. This study was designed to determine whether acetazolamide administration would result in 1) a reduction in the exercise time to fatigue, 2) an inhibition of skeletal muscle glycolysis, 3) an increase in the arterial oxygen tension at rest and during exercise, and, 4) hypercapnia during exercise. Six horses received either no treatment or acetazolamide (30 mg kg⁻¹ q12h PO) for 3 days, in a crossover experiment. Exercise requiring $\approx 65\%$ $\dot{V}O_{2\max}$ was performed until fatigue. The oxygen consumption during exercise was not different as a result of acetazolamide. However, the $\dot{V}CO_2$ was lower during the first 4 min of exercise after acetazolamide. During exercise, heart rate (192 vs. 173 bpm) and cardiac output (292 vs. 261 l min⁻¹) were higher following acetazolamide, while there were no differences in stroke volume. The arterial oxygen content and the arteriovenous oxygen content difference were lower during exercise after acetazolamide, despite the arterial oxygen tension being ≈ 9 Torr higher. The pH of arterial blood was lower before and during exercise while the arterial carbon dioxide tension was higher after acetazolamide. Although the mean time to fatigue was not different between treatments (24.8 control vs. 20.8 min acetazolamide) 5 of the 6 horses had a reduced exercise capacity following acetazolamide administration.

Key words: Horses; acidosis; blood gases; fatigue; metabolic regulation.

INTRODUCTION

With moderate to severe exercise intensities, the time to fatigue may be delayed by a pre-existing metabolic alkalosis whereas a prior metabolic acidosis may impair the duration of performance.^{8,17} The mechanism for these effects is unclear, but in human subjects that received ammonium chloride prior to exercise, an inhibition of glycolysis has been demonstrated in the exercising skeletal muscle.¹⁷

In most studies involving induction of metabolic acidosis prior to exercise, either ammonium chloride or the carbonic anhydrase inhibitor acetazolamide have been used as the acidifying agents.^{8,10,16,17} Because carbonic anhydrase has a principal role in CO₂ transport in many tissues, including

erythrocytes,¹¹ administration of the diuretic agent acetazolamide, a carbonic anhydrase inhibitor, results in a complex series of physiological effects. The net results at rest are losses of potassium, bicarbonate, and sodium via the kidney, altered CO₂ transport, metabolic acidosis, and an increase in alveolar ventilation.¹⁸ The metabolic acidosis and delayed CO₂ transport appear to limit exercise capacity in rats¹⁹ and man¹⁶ during submaximal exercise and horses during high intensity exercise.¹⁵

It was anticipated that during exercise requiring 65% of the maximal oxygen con-

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sumption inhibition of carbonic anhydrase would result in hypercapnia, impairment of muscle metabolism and a more rapid induction of fatigue. Therefore, the following questions were asked: 1) does acetazolamide reduce the exercise time to fatigue? 2) does acetazolamide administration result in inhibition of skeletal muscle glycolysis? 3) is the increase in resting arterial oxygen tension (PaO_2) associated with alveolar hyperventilation following acetazolamide administration maintained throughout the course of the exercise? and 4) does acetazolamide administration result in hypercapnia during exercise of moderate intensity?

MATERIALS AND METHODS

Six Thoroughbred horses, ranging in age from 3 to 5 years and weighing 440 to 536 kg (496 ± 14 kg) were used. Three weeks before the experiment the $\dot{V}\text{O}_2\text{max}$ of each horse was measured four times.¹⁴ The mean $\dot{V}\text{O}_2\text{max}$ (\pm SE) was 140 ± 5 ml kg^{-1} min^{-1} . The horses were randomly assigned to either control or acetazolamide treatment before the first experimental day and given the alternate treatment at least 10 days later. Each horse was exercised at the speed determined to produce 65% of the $\dot{V}\text{O}_2\text{max}$ until it could no longer keep pace with the treadmill belt despite verbal encouragement. In horses receiving acetazolamide, 30 mg kg^{-1} of the drug was administered twice daily by nasogastric tube for 3 days prior to exercise. This dose of acetazolamide was selected as lower dose regimens were found unsuitable for the induction of a stable metabolic acidosis. On the morning of the exercise test, pulmonary and systemic arterial catheters were placed via the jugular vein and carotid artery (previously translocated to a subcutaneous position). Prior to exercise, a muscle biopsy was collected from the middle gluteal muscle and immediately frozen in liquid nitrogen for later biochemical analysis. A thermocouple was placed at the level of the right atrium via the jugular vein. Five min after walking the horse onto the treadmill, resting arterial and

mixed venous blood were simultaneously collected into heparinized syringes to measure blood gas and acid base values.¹ The blood temperature was measured to allow for correction of differences in temperature between the electrodes of the blood gas machine and the temperature of the horse. These samples were stored in an ice bath prior to analysis. The arterial and mixed venous oxygen content and hemoglobin (Hb) concentration were measured on these samples using an Instrumentation Laboratories oximeter (IL-282). Arterial blood was also collected into sodium fluoride and potassium oxalate tubes and lithium heparin tubes for measurement of plasma lactate (La) and plasma electrolytes, respectively. After collection samples were placed in an ice bath until centrifuged to separate erythrocytes and plasma. Plasma for subsequent electrolyte analysis was decanted into tubes and stored at -80°C until analysis. Samples of plasma for La determinations were deproteinated with perchloric acid and stored at -80°C until analysis. A cardiometer¹⁴ was applied for the measurement of heart rate (HR) during exercise. Oxygen uptake ($\dot{V}\text{O}_2$) and carbon dioxide production ($\dot{V}\text{CO}_2$) were measured as described previously.¹⁴ Cardiac output (\dot{Q}) was determined using the direct Fick method. The treadmill speed was increased to the level pre-determined to elicit 65% of the $\dot{V}\text{O}_2\text{max}$ (5.7 to 7.5 m s^{-1}). Arterial and mixed venous blood samples were collected after 1, 3, 5, 10, and 15 min of exercise. Samples were analyzed for gases, pH, acid-base values, electrolytes and La. Blood temperature was measured at each blood sample collection time. Expired gas samples were collected at the same time as blood samples. When the horse was unable to maintain its speed, further blood and expired gas samples were collected, the treadmill was stopped and a muscle biopsy obtained from the middle gluteal muscle within 30 seconds of the cessation of exercise.

During recovery, expired gas and blood samples were collected at 1, 3, 5, 10, 15, 30

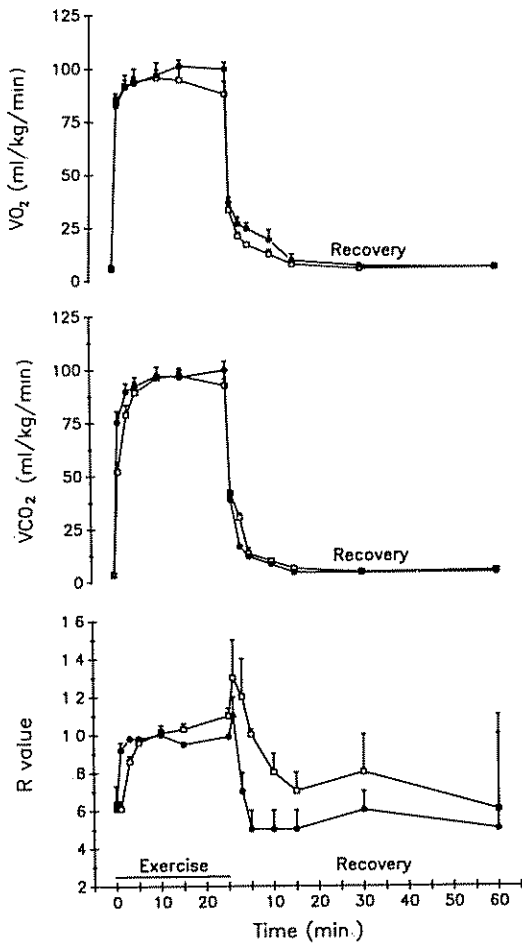


Fig 1 Oxygen uptake ($\dot{V}O_2$), carbon dioxide elimination ($\dot{V}CO_2$), and respiratory exchange ratio (R value) in horses exercising at 65% of the $\dot{V}O_{2max}$ until fatigue with no treatment (\bullet) and after acetazolamide-induced acidosis (\square). In all Figs. values are mean \pm SE. Where no error bar is visible it fell within the symbol depicting the mean. Because the exercise time for the two treatments were different the symbols for the final point in exercise have been normalized in all Figs.

and 60 min post-exercise and analyzed as described above. Muscle biopsies were collected at 30 and 60 min post-exercise.

Muscle metabolites measured included adenosine triphosphate (ATP), adenosine diphosphate (ADP), creatine phosphate (CP), glucose-6-phosphate (G-6-P), La, and glycogen, using methods described by Kelso et al.⁹

Muscle pH and plasma La were measured as described previously.¹⁴ The plasma sodium (Na) and potassium (K) were measured using flame photometry (Instrumentation Laboratories Model 143). A chloride titrator (Radiometer CMT 10) was used to make plasma chloride (Cl) determinations.

The concentration of acetazolamide in the plasma (14 hours after the last dose) was determined using the enzymatic method of Maren et al.¹²

Results were analyzed by a multifactor repeated measures analysis of variance to determine significant differences between acetazolamide and control treatments. All results are reported as mean \pm SE. Differences of $p < 0.05$ were considered as significant.

RESULTS

Plasma acetazolamide concentrations. The mean pre-exercise unbound plasma acetazolamide was $6.1 \pm 3.0 \mu\text{mol l}^{-1}$.

Bodyweight prior to exercise. In the control condition bodyweight prior to exercise was 496 ± 14 kg compared to 493 ± 16 kg for acetazolamide treatment.

Exercise time to fatigue. Exercise times to fatigue were 24.8 ± 1.6 min and 20.8 ± 1.0 min for the control and acetazolamide conditions, respectively.

Metabolic response to exercise. Oxygen consumption rose rapidly in response to exercise and reached peak values of 101.3 ± 2.8 ml $\text{kg}^{-1} \text{min}^{-1}$ for untreated horses and 95.7 ± 7.2 ml $\text{kg}^{-1} \text{min}^{-1}$ following acetazolamide treatment (Fig. 1). Although the difference in these values was not statistically significant, 4 of the 6 horses had lower peak $\dot{V}O_2$ values following acetazolamide administration. Acetazolamide treatment resulted in lower $\dot{V}CO_2$ values during the first 4 min of exercise than in the untreated horses. However, no differences were found during recovery (Fig. 1). Similarly, the values for respiratory exchange ratio (R) during exercise were lower after acetazolamide treatment for the first 3 min of exercise but not during the remainder of exercise or re-

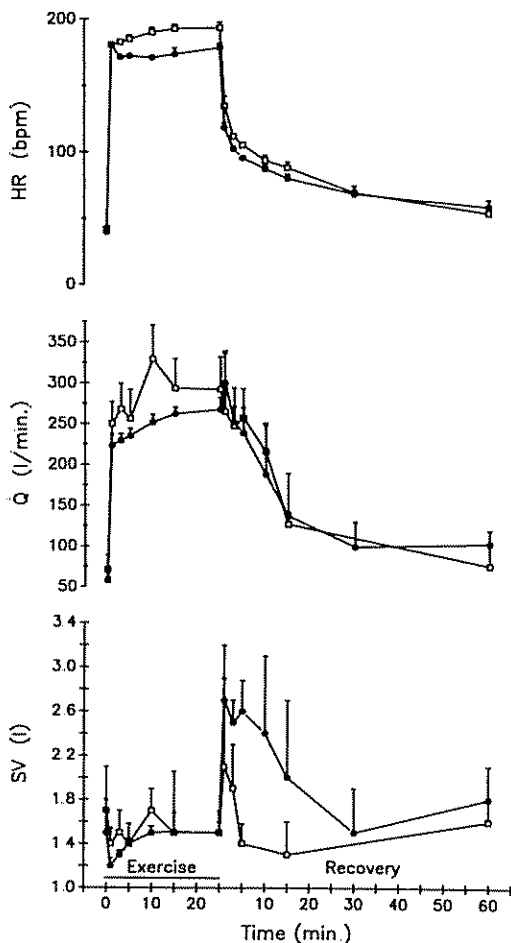


Fig. 2. Changes in heart rate (HR), cardiac output (\dot{Q}), and stroke volume (SV) in horses exercising at 65% of the $\dot{V}O_2$ max until fatigue with no treatment and following acetazolamide induced metabolic acidosis. Symbols as Fig. 1.

recovery (Fig. 1). Heart rate and \dot{Q} were not different between groups at rest but rose rapidly in response to exercise in both groups. However, the peak HR and \dot{Q} were higher during exercise after acetazolamide treatment when compared to the control conditions (192.8 ± 4.3 vs. 178.0 ± 3.4 bpm and 292.4 ± 36.5 vs. 261.4 ± 8.0 l min^{-1} , respectively) (Fig. 2). Despite this difference in \dot{Q} , stroke volume was unchanged as a result of the treatment or exercise (Fig. 2).

Arterial Hb was higher at rest (155 ± 10 g

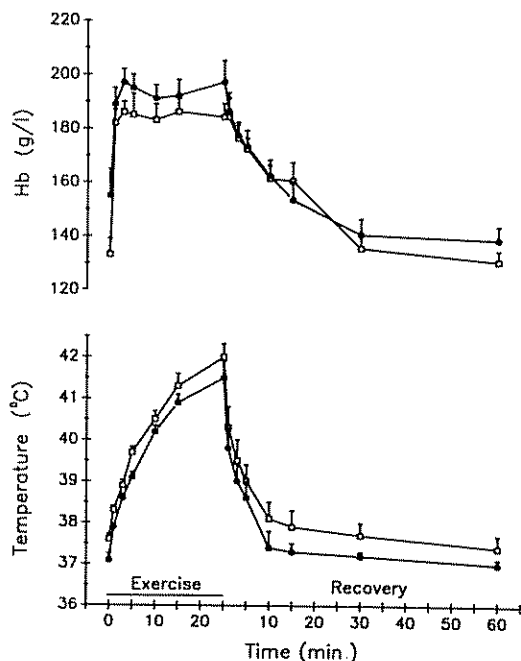


Fig. 3. Values for the temperature of blood at the level of the right atrium and the concentration of hemoglobin (Hb) in arterial blood in horses exercising at 65% of the $\dot{V}O_2$ max until fatigue with no treatment and following acetazolamide induced metabolic acidosis. Symbols as Fig. 1.

l^{-1}) in the control condition when compared to the acetazolamide treated horses (133 ± 6 g l^{-1}) (Fig. 3). Arterial Hb rose rapidly in response to exercise in both conditions and was more than 25% above the resting value by the end of exercise. This had returned to resting values by 60 min post-exercise.

No effect was found as a result of acetazolamide administration on the temperature of blood at the level of the right atrium at rest. However, the blood temperature at fatigue was higher following acetazolamide treatment ($42.0 \pm 0.3^\circ\text{C}$) when compared to the control condition ($41.5 \pm 0.4^\circ\text{C}$) (Fig. 3). Although blood temperature returned toward pre-exercise values in both groups in the 60 min recovery period a temperature differential of $\approx 0.5^\circ\text{C}$ between the two groups persisted throughout recovery.

Muscle and blood metabolites and pH No

Table 1. The concentration of selected muscle metabolites and pH at rest, after exercise, and during recovery in horses exercised at 65 % of the $\dot{V}O_2$ max until fatigue with no treatment and after acetazolamide induced acidosis

Muscle metabolites are expressed as mmol mol⁻¹ TCr. Values are \pm SE; *n*=6 horses. The 0 min post-exercise sample was collected within 30 seconds of the horses stopping exercise. Acet. = acetazolamide treatment

	Before exercise	After exercise		
		0 min	30 min	60 min
Lactate				
Control	147 \pm 53	347 \pm 84 ^a	327 \pm 82	257 \pm 130
Acet.	170 \pm 53	219 \pm 62 ^b	230 \pm 81	218 \pm 76
ATP				
Control	152 \pm 25	151 \pm 24	152 \pm 21	149 \pm 18
Acet.	153 \pm 16	139 \pm 18	143 \pm 12	150 \pm 30
CP				
Control	662 \pm 98	567 \pm 101	622 \pm 85	624 \pm 90
Acet.	621 \pm 51 ^b	508 \pm 88 ^{ab,b}	553 \pm 77 ^b	587 \pm 80 ^b
G-6-P				
Control	3.4 \pm 1.4	5.5 \pm 1.8 ^a	3.6 \pm 1.0	3.6 \pm 1.4
Acet.	4.0 \pm 0.6	5.4 \pm 1.4 ^a	3.7 \pm 1.0	3.6 \pm 1.2
Glycogen				
Control	3 732 \pm 648	3 234 \pm 612	3 292 \pm 528	3 432 \pm 822
Acet.	3 268 \pm 686	2 569 \pm 511	2 508 \pm 473	2 732 \pm 317
pH (units)				
Control	6.90 \pm 0.07	6.72 \pm 0.10 ^a	6.83 \pm 0.04	6.90 \pm 0.10
Acet.	6.79 \pm 0.06 ^b	6.63 \pm 0.10 ^{ab,b}	6.75 \pm 0.08 ^b	6.78 \pm 0.04 ^b

^a Mean different from rest value for that treatment (*p*<0.05).

^b Mean different from mean value of control group at the same sample time (*p*<0.05).

differences were found between the treatment groups at any sampling time for muscle ATP, ADP, glycogen or G-6-P (Table 1). Muscle CP and pH were lower at all sampling times following acetazolamide administration. Muscle pH fell by 0.16 units in the control and 0.18 units in the acetazolamide treated groups in response to the exercise (Table 1). Although not different at rest, plasma La was lower during and after exercise, and muscle La was lower on cessation of exercise following acetazolamide administration when compared to control (Fig. 4).

Blood gases and acid-base measurements. Prior to exercise, arterial blood pH (pHa) and the calculated arterial blood bicarbonate concentration were 7.42 ± 0.01 units and

29.8 ± 2.0 mmol l⁻¹ respectively, for the control situation. These values were lower (7.37 ± 0.02 units and 16.9 ± 2.9 mmol l⁻¹ respectively) following acetazolamide treatment. The pHa was unaffected by exercise in the control condition, but was below the resting value throughout exercise following acetazolamide administration. There was no difference between groups during recovery (Fig. 4). The arterial oxygen tension (PaO₂) was higher at all measurement points following acetazolamide administration when compared to the control condition. This difference was ≈ 15 Torr at rest, decreased to around 9 Torr by the end of exercise and increased to greater than 20 Torr by the third min of recovery (Fig. 5). The PaO₂ was lower

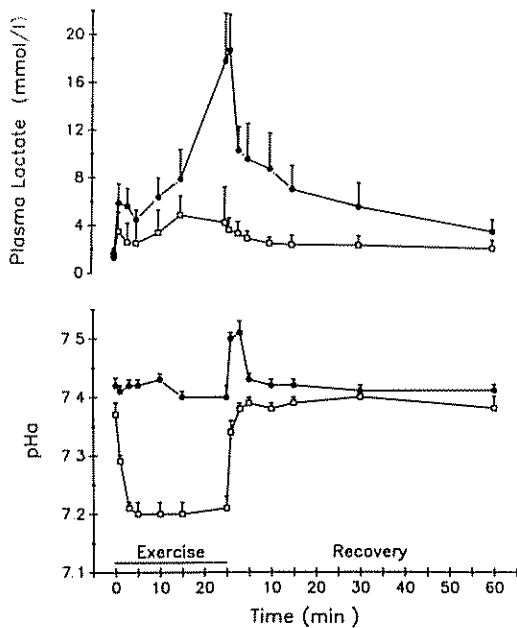


Fig 4. The concentration of lactate in plasma and pH of arterial blood (pHa) in horses exercising at 65% of the $\dot{V}O_2$ max until fatigue with no treatment and following acetazolamide induced metabolic acidosis. Symbols as Fig 1.

than rest after 1 min of exercise in both treatment groups, however, values were not different from rest by the third min of exercise. Values for PaO_2 were higher after 1 min of recovery when compared to the respective values at rest for both treatments. The arterial carbon dioxide tension ($PaCO_2$) was lower at rest but higher throughout exercise in horses receiving acetazolamide when compared to the control situation. This difference decreased during the first 3 min of recovery after which the values for the two groups were similar. By 15 min of recovery the $PaCO_2$ was lower in the acetazolamide treated horses when compared to the control animals. Arterial oxygen content (CaO_2) was lower at rest and during exercise following treatment with acetazolamide. The oxygen content of mixed venous blood ($C\bar{v}O_2$) was lower at rest but not during exercise or recovery following acetazolamide treatment. The arteriovenous oxygen content differ-

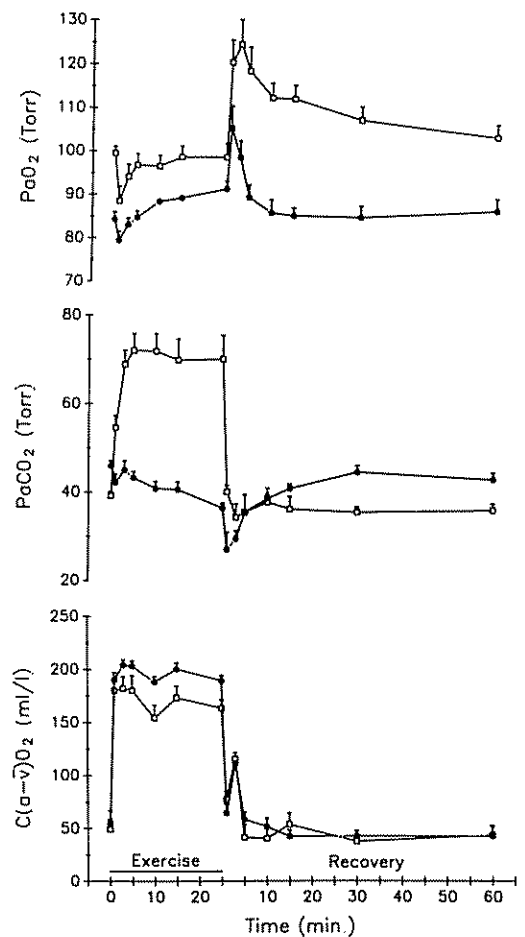


Fig 5. The tensions of oxygen (PaO_2) and carbon dioxide ($P\bar{v}CO_2$) and the arteriovenous oxygen content difference $C(a-\bar{v})O_2$ in horses exercising at 65% of the $\dot{V}O_2$ max until fatigue with no treatment and following acetazolamide induced metabolic acidosis. Symbols as Fig 1.

ence, $C(a-\bar{v})O_2$, was lower during exercise following treatment with acetazolamide (Fig. 5).

Plasma electrolytes At rest, plasma Na was not affected whereas plasma K was lower and plasma Cl higher following induction of acidosis by acetazolamide (Fig. 6). Plasma K increased by approximately 70% and 50% during exercise in the control and acetazolamide groups, respectively. The increase in plasma K in the control group was greater

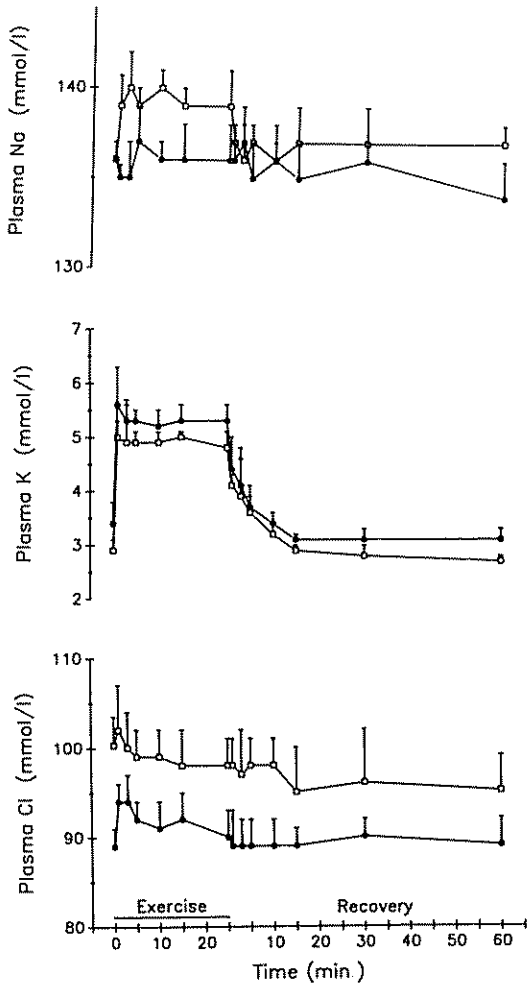


Fig. 6. The concentrations of sodium (Na), potassium (K) and chloride (Cl) in arterial blood plasma of horses exercising at 65% of the $\dot{V}O_2$ max until fatigue with no treatment and following acetazolamide induced metabolic acidosis. Symbols as Fig. 1.

than that in the acetazolamide treated horses. These differences were not present in the post-exercise samples. Plasma Na and Cl were unchanged from their rest values during exercise or recovery in both groups. However, despite there being no change in response to exercise, plasma Cl was higher at all measurement times following acetazolamide administration when compared to the control condition.

DISCUSSION

Acetazolamide related effects Acetazolamide produced a non-progressive metabolic acidosis as evidenced by an average pre-exercise base deficit of 13 mmol l^{-1} in the acetazolamide treated horses. A hyperventilatory response to this acidosis was evident from the lower pH_a and PaCO₂ and higher PaO₂ values in the acetazolamide treated horses at rest. These findings support a substantial inhibition of carbonic anhydrase and are similar to effects observed in human subjects following ingestion of dichlorophenamide⁴ and horses following acetazolamide.¹⁵ Further support comes from the report of Swenson and Maren¹⁹ who demonstrated a 98.8% inhibition of carbonic anhydrase in human subjects when the plasma acetazolamide concentration was $7.5 \mu\text{mol l}^{-1}$, a value only slightly higher than that occurring in this study. Although acetazolamide is a diuretic, the horses were able to compensate for the initial fluid losses resulting from administration of this agent prior to the exercise. This is reflected by the bodyweight of the horses in the two groups being similar immediately prior to the exercise.

Metabolic effects Although the mean difference in exercise time was not different for the two groups 5 of the 6 horses in the present study had a shorter exercise time to fatigue (4 min) following acetazolamide treatment. In contrast, the sixth horse ran for approximately 3 min longer following administration of acetazolamide. The results from this animal contributed to the relatively large variation in exercise times in this study and probably explain why the mean exercise times between the groups were not statistically different. We have no explanation why this horse responded in this fashion. However, the reduction in mean exercise time to fatigue following acetazolamide administration was equivalent to these horses exercising $\approx 1600 \text{ m}$ less than when in the control condition. A reduction in exercise capacity after induction of metabolic acidosis prior to exercise is similar to several

reports for man^{8,10,16,17} and horses,¹⁵ but in contrast to the study of Swenson and Maren¹⁹ who investigated the effects of acetazolamide in low intensity exercise in man. In several of these studies dosing with ammonium chloride, an agent which does not alter CO₂ kinetics, was used to acidify the subjects. Based on this property we attempted to utilize this agent in these studies; it did not prove to be an effective acidifying agent in the horse.

The changes in HR, muscle metabolites, blood and muscle pH and blood temperature were similar to those expected during exercise requiring 65% of the $\dot{V}O_2$ max to fatigue.⁷ Administration of acetazolamide produced few changes in skeletal muscle metabolism in response to the exercise. The muscle CP was lower in the acetazolamide treated horses at all measurement times. We have no explanation for this finding. The most notable differences between the treatments was the lower muscle and plasma La in the acetazolamide treated horses during exercise. This reduction in La production has been ascribed to a pH-induced depression in glycolysis.¹⁷ Although the muscle pH was lower on cessation of exercise in the acetazolamide group the lower La accumulation occurred without apparent detrimental effects on muscle glycolysis as there was no reduction in the rate of glycogen depletion in these horses during exercise when compared to the control condition. Therefore, although such inhibitory effects of acidosis are possible, recent evidence indicates that the extent of the proposed adverse effects of acidosis on glycolysis are less than previously suggested.⁶ A much more likely cause of the lower La production following acetazolamide administration is shorter exercise time to fatigue in this group. As most La accumulated in both groups after 15 min of exercise, the shorter time to fatigue (20.8 vs 24.8 min) would likely have resulted in a substantial reduction in La accumulation in the acidotic group.

Despite no difference in mean values, the peak $\dot{V}O_2$ during exercise was lower in 4 of the 6 horses following acetazolamide admin-

istration. This was likely to be the result of a reduced extraction of oxygen as the arteriovenous oxygen content difference was lower in the acetazolamide treated horses during exercise. This occurred despite the HR and \dot{Q} being higher. The lower $\dot{V}O_2$ in these 4 horses could be associated with a decreased pH which reduces muscle metabolism, a shift in the oxyhemoglobin dissociation curve to the right, or the retention of CO₂ within the tissues as a result of carbonic anhydrase inhibition. Similar effects occur in the horse during high intensity exercise.¹⁵ Although acetazolamide administration has been associated with a lower $\dot{V}O_2$ max in human subjects such effects may only occur at high $\dot{V}O_2$ requirements as Davies et al.⁵ did not find any differences in $\dot{V}O_2$ at submaximal workloads in man.

Blood gases. The PaO₂ underwent a modest reduction in both treatment groups during the first min of exercise after which PaO₂ increased. This is consistent with previous findings in this species and the increase in PaO₂ with increasing exercise duration is likely related to increase in ventilation.^{2,3,7,15} Acetazolamide administration produced a resting PaO₂ which was ≈ 15 Torr greater than the control condition. This resting alveolar hyperventilation (confirmed by an increase in the PaO₂ as calculated from the ideal gas equation), and the chemoreceptor stimulation resulting from the metabolic acidosis, were likely to have resulted in the higher PaO₂ values at all measurement times following acetazolamide administration. This was reflected by the PaO₂ being ≈ 9 Torr higher in these horses than in the control group throughout the course of the exercise. Thus, the difference in the alveolar-arterial O₂ tension which occurred at rest in response to acetazolamide treatment was maintained throughout the exercise.

The hypercapnia which occurred in the horses following acetazolamide administration is consistent with an inhibition of carbonic anhydrase. Similar effects have been reported in man and horses in response to high intensity exercise.^{15,16} Inhibition of carbonic anhydrase within the lung may affect

CO₂ transport.¹⁸ Although this effect is secondary to that of carbonic anhydrase in the erythrocytes it may explain why the $\dot{V}CO_2$ was lower during first 4 min of exercise following acetazolamide administration. This combined with the high PaCO₂ and $P\bar{V}CO_2$ values (data not shown) would reflect an impairment in unloading CO₂ from the lung despite the large gradient of CO₂ from pulmonary capillary blood across the alveoli. This may have contributed to the decrease in exercise capacity following acetazolamide administration.

Plasma electrolytes. The changes observed were similar to those previously reported in response to acetazolamide administration.^{13,15} Bicarbonate, K and to a lesser extent Na would have been excreted in the urine whereas Cl was conserved following acetazolamide administration. This would explain the lower resting plasma K and higher plasma Cl in the acetazolamide treated horses. The elevations in plasma K in response to the exercise were anticipated.^{7,15} However, the smaller increase in plasma K following acetazolamide administration may be the result of the greater urinary K loss and a higher extracellular [H⁺] in the acetazolamide treated horses.

In conclusion, acetazolamide administration resulted in increased PaO₂ values at all measurement points throughout the experimental period, hypercapnia during exercise, and a reduced exercise time to fatigue in all but one of the horses studied. The reasons for this reduction in performance capacity are not clear but are not likely to be the result of impaired muscle glycolysis. Other effects of acetazolamide administration such as hypercapnia, mild acidosis, and alterations in electrolyte balance and a more rapid increase in body temperature probably contributed to the shorter time to fatigue in these horses.

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