

Effects of training on potassium homeostasis during exercise and skeletal muscle Na⁺,K⁺-ATPase concentration in young adult and middle-aged Dutch Warmblood horses

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Objective—To investigate the effects of moderate short-term training on K⁺ regulation in plasma and erythrocytes during exercise and on skeletal muscle Na⁺,K⁺-ATPase concentration in young adult and middle-aged horses.

Animals—Four 4- to 6-year-old and four 10- to 16-year-old Dutch Warmblood horses.

Procedure—The horses underwent a 6-minute exercise trial before and after 12 days of training. Skeletal muscle Na⁺,K⁺-ATPase concentration was analyzed in gluteus medius and semitendinosus muscle specimens before and after the 12-day training period. Blood samples were collected before and immediately after the trials and at 3, 5, 7, and 10 minutes after cessation of exercise for assessment of several hematologic variables and analysis of plasma and whole-blood K⁺ concentrations.

Results—After training, Na⁺,K⁺-ATPase concentration in the gluteus medius, but not semitendinosus, muscle of middle-aged horses increased (32%), compared with pretraining values; this did not affect the degree of hyperkalemia that developed during exercise. The development of hyperkalemia during exercise in young adult horses was blunted (albeit not significantly) without any change in the concentration of Na⁺,K⁺-ATPase in either of the muscles. After training, the erythrocyte K⁺ concentration increased (7% to 10%) significantly in both groups of horses but did not change during the exercise trials.

Conclusions and Clinical Relevance—In horses, the activation of skeletal muscle Na⁺,K⁺-ATPase during exercise is likely to decrease with age. Training appears to result in an increase in Na⁺,K⁺-ATPase activity in skeletal muscle with subsequent upregulation of Na⁺,K⁺-ATPase concentration if the existing Na⁺,K⁺-ATPase capacity cannot meet requirements. (*Am J Vet Res* 2005;66:1252–1258)

Contraction of skeletal muscle is associated with an influx of Na⁺ and an efflux of K⁺ across the plasma membrane of muscle cells. A net gain of Na⁺ and net

loss of K⁺ result in reduction of the Na⁺ and K⁺ gradients, which are critically important for generation of the action potential that elicits contraction.¹⁻⁴ The Na⁺ and K⁺ gradients across the plasma membrane are ultimately regulated via the Na⁺,K⁺-ATPase (ie, the Na⁺,K⁺ pump). It has been shown that high extracellular K⁺ concentration,⁵ inhibition of Na⁺,K⁺-ATPase activity, or reducing the pump concentration^{6,7} decreases muscle force by increasing the rate of fatigue development and reducing the rate of recovery. Thus, changes in the K⁺ handling mechanism over the plasma membrane in skeletal muscle may influence excitability, fatigability, and force production and thereby affect the working performance. Also, activation of the Na⁺,K⁺ pump in tissues other than the working muscles may contribute to the maintenance of an appropriate plasma K⁺ concentration.⁸

Training has been shown to influence K⁺ homeostasis by producing an increase in Na⁺,K⁺-ATPase activity^{9,10} as well as an increase in the Na⁺,K⁺ pump concentration in skeletal muscle in several animal species including rats,¹¹ cattle,¹² and young^{13,14} and old humans.¹⁵ As a consequence, the development of hyperkalemia during exercise was blunted in trained dogs,¹⁰ calves,¹⁶ and humans.¹⁴ Moreover, the increase in Na⁺,K⁺ pump concentration after training seems to correlate with improvement of performance.¹³

In horses, the required increase in circulatory capacity during maximal exercise is partly achieved by mobilization of splenic erythrocytes into the systemic circulation, resulting in an increase in the quantity of circulating RBCs of 50%.¹⁷ In addition, exercise is usually associated with an increase in plasma concentrations of catecholamines,^{18,19} which is known to be an important activator of the Na⁺,K⁺ pump.^{1,20} The question of whether activation of the Na⁺,K⁺ pump in erythrocytes results in increased uptake of K⁺ in the RBCs that contributes considerably to K⁺ homeostasis during exercise in horses has been addressed in a previous study.²¹ However, recent studies in young^{22,23} and young

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adult horses²¹ have revealed that the Na⁺,K⁺ pump concentration in skeletal muscle increases after training of moderate intensity. In a high-intensity exercise trial in horses,²¹ this increase of Na⁺,K⁺ pump concentration in skeletal muscle was indeed associated with a lower peak of the plasma K⁺ concentration, compared with pretraining values. In many species, the Na⁺,K⁺ pump concentration in skeletal muscle decreases with age.^{20,24,25} To the authors' knowledge, this has not been evaluated in horses of different ages within any 1 study. The purpose of the study reported here was to investigate the effects of moderate short-term training on K⁺ regulation in plasma and RBCs during exercise and the skeletal muscle Na⁺,K⁺-ATPase concentration in young adult and middle-aged horses.

Materials and Methods

The Animal Ethical Committee of the Faculty of Veterinary Medicine, Utrecht University, approved the experimental protocol. During the experiment, horses were examined daily by a veterinarian.

Horses—Eight Dutch Warmblood horses were used in the study; there were 4 young adult (4 to 6 years old; mean \pm SE weight, 557.5 \pm 1.3 kg) and 4 middle-aged (10 to 16 years old; mean weight, 585.0 \pm 1.3 kg) horses. The horses were housed in the stables of the Department of Equine Sciences, Utrecht University. All horses were owned by the university and used for the training of veterinary medical students. The horses all received the same diet consisting of commercial pellets and forage; they had not been in intensive work for > 1 year and had never had any form of exertional rhabdomyolysis. They had been accustomed to a treadmill[†] during the 2 weeks prior to the study (approx 2 to 3 min/d of walking and slow trotting on the treadmill). Food, water, and a mineral-salt block were available ad libitum to the horses throughout the experimental period.

Exercise trial and sampling schedule—All horses underwent an exercise trial before (pretraining trial) and after (post-training trial) a 12-day period of training for determination of the effect of training on plasma and erythrocyte K⁺ concentrations and several hematologic variables, including the number of RBCs, blood hemoglobin concentration, Hct, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The exercise trial consisted of walking and slow trotting (warm-up) for 2 minutes. Then the speed of the treadmill was increased so that within 1 minute, the horses began to canter (7 m/s), which was maintained for 3 minutes before cessation. These exercise trials were performed with the treadmill set at 0% inclination. Blood samples (10 mL) were collected from a jugular vein of each horse into evacuated collecting tubes containing lithium heparin at rest and at 0, 3, 5, 7, and 10 minutes after cessation of each exercise trial. These samples were stored on ice until analyzed. Muscle biopsy specimens were collected (at rest) 1 week before the training program started and after the 12-day training period to determine the effect of training on the skeletal muscle Na⁺,K⁺-ATPase concentration.

Training regimen—Each horse was trained on the treadmill for 60 min/d. The training program started with walking (1.7 m/s) and trotting (4 m/s) alternately every 5 minutes. Then, trotting time was gradually increased from days 1 through 5 so that the horses were only allowed a 5-minute walk between 0 and 5, 25 and 30, and 55 and 60 minutes of the exercise period. From days 1 to 5, the treadmill was set at 1% inclination. Thereafter, the treadmill inclination was

gradually increased until it reached 5% on the last day of training. From days 6 to 12, the horses were allowed an extra 2-minute walk between 48 and 50 minutes of the exercise period. After 60 minutes of treadmill training, the horses were walked in a horse-walker for 30 minutes before being returned to their stables.

Muscle biopsy procedures—Muscle samples were taken from the gluteus medius and semitendinosus muscles percutaneously by use of a needle biopsy technique according to the method described by Snow and Guy.²⁶ The site of the biopsy was locally anesthetized with lidocaine hydrochloride^b (2 mL) administered SC by use of a 23-gauge, 1-inch needle. To minimize variation in the Na⁺,K⁺-ATPase concentration due to the nonhomogeneous distribution of fiber types in skeletal muscle, the biopsy locations were consistently identified by use of anatomical landmarks. Samples of the gluteus medius muscle were obtained from a site two thirds of the distance along an imaginary line extending from the tuber coxae to the tuber sacrale; samples of the semitendinosus muscle were obtained from a site one third of the distance from the ischial tuber to the caudal side of the femorotibial joint. Samples were taken in a direction perpendicular to the skin. The sampling depth was approximately 8 cm for the gluteus medius muscle and 4 cm for semitendinosus muscle. Biopsy specimens (approx 70 to 100 mg) were immediately frozen in liquid nitrogen and stored at -80°C until analyzed.

Measurement of Na⁺,K⁺-ATPase in muscle—The Na⁺,K⁺-ATPase concentration was quantified by measuring the tritiated (³H) ouabain-binding capacity of muscle samples in the presence of vanadate, as described by Nørgaard et al.²⁷ This method allows the quantification of the total concentration of Na⁺,K⁺-ATPase in small samples of muscle; furthermore, results of studies^{1,20} in rat and human skeletal muscle have indicated that the values obtained correspond to the total population of functional Na⁺,K⁺ pumps. There was not enough tissue to make a complete saturation curve with ouabain concentrations in the range of 10⁻⁸ to 10⁻⁶M, so a ouabain concentration of 10⁻⁶M was used because this has been shown to be above that required for saturation in rat²⁷ and Dutch Warmblood foal²² muscle tissue. In brief, frozen biopsy specimens were gently thawed, cut into small pieces (5 to 10 mg), and incubated in baskets that had their bottom surfaces attached to a gas inlet to allow continuous gassing with air and ensure agitation. The specimens were prewashed twice for 10 minutes at 37°C in unlabeled buffer solution to remove any Na⁺ and K⁺ that could interfere with the binding of ouabain or vanadate. The unlabeled buffer solution contained Tris (24mM), MgSO₄ (3mM), vanadate (1mM), and sucrose (250mM); the final pH was adjusted to 7.3 with hydrochloric acid. Incubation took place at 37°C in buffer containing ³H-ouabain (0.6 $\mu\text{Ci}/\text{mL}$), and unlabeled ouabain was added to a final concentration of 10⁻⁶M for 120 minutes under continuous gassing with air. One set of specimens was incubated with an ouabain concentration of 10⁻³M to allow correction for the unspecific uptake of ³H-ouabain. After incubation, unbound ³H-ouabain was removed via 4 rinses (30 minute/rinse) with unlabeled buffer solution on ice. After the last washout period, specimens were gently pressed between 2 pieces of filter paper. Each specimen was put into a counting vial and weighed. Then, 0.5 mL of 5% trichloroacetic acid containing 0.1mM ouabain was added to each specimen. Finally, after 16 hours of extraction in the refrigerator, 3 mL of scintillation cocktail^c was added and the ³H activity was measured by use of a liquid scintillation counter.^d On the basis of the specific activity of ³H-ouabain in the incubation medium, the amount of ³H-ouabain (pmol/g of wet wt) taken up and retained in the muscle samples was

calculated after correction for unspecific uptake and isotopic purity.

Blood sample analyses—Blood analyses were performed at the Laboratory Clinic of the Department of Farm Animal Health at Utrecht University. Whole-blood samples were analyzed by use of an automated quantitative hematology analyzer.^c Data collected included RBC count, hemoglobin concentration, Hct, MCV, MCH, and MCHC. Potassium ion concentrations in plasma samples (containing heparin) were determined via an ion-selective membrane electrode technique available in an automatic chemistry analyzer.^f Potassium ion concentrations in whole blood samples were measured in the same manner after hemolysis and dilution. Hemolysis was achieved by mixing 2 mL of the blood sample with 2 mL of distilled water; when hemolysis was completed, a 40X dilution with saline (0.9% NaCl) solution was necessary to allow measurement with the automatic chemistry analyzer. Results were corrected for the applied dilutions. Erythrocyte K⁺ concentration was calculated by use of the following equation²⁸:

$$\text{Erythrocyte K}^+ \text{ concentration} = (100/\text{Hct})(\text{WBK}^+ - \text{PK}^+) + \text{PK}^+$$

where erythrocyte K⁺ concentration is the K⁺ concentration in the erythrocyte water (mmol/L of RBCs), WBK⁺ is the measured whole blood K⁺ concentration (mmol/L), PK⁺ is the measured plasma K⁺ concentration, and Hct is expressed as either a percentage or a fraction (L/L), with no correction for trapped plasma.

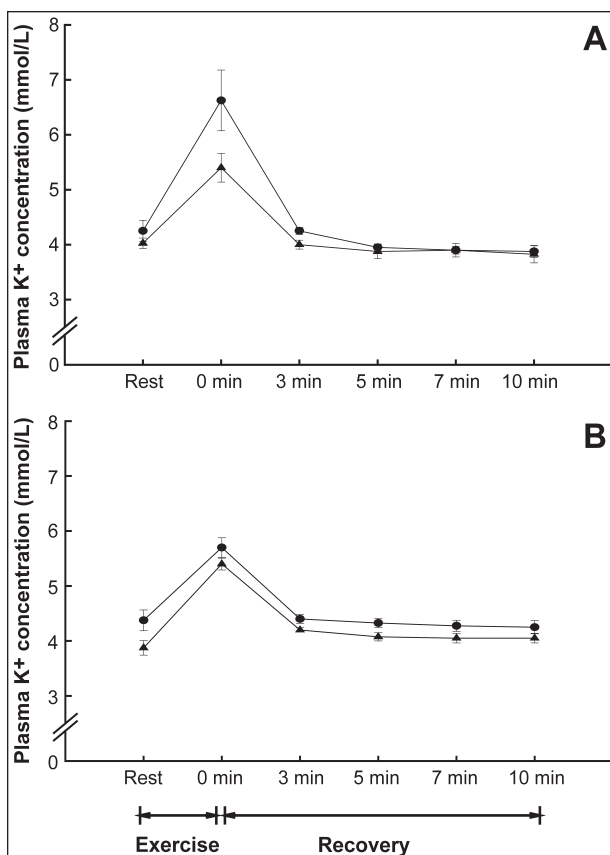


Figure 1—Plasma K⁺ concentration (mean ± SE) in 4 young adult (A) and 4 middle-aged (B) horses at rest, after 6 minutes of exercise, and during the initial 10 minutes of recovery after cessation of exercise before (circles) and after (triangles) a 12-day period of training. Please note that the timescale on the x-axis is not linear.

Statistical analyses—All data are given as mean values ± SE. A general linear model for a repeated-measure design was used to determine the effect of exercise time and training. The differences among group means were subjected to an ANOVA, and the least-significant difference post hoc test was performed if a significance of difference was indicated. The significance of differences between paired data was assessed by use of the paired sample *t* test. Differences were considered to be significant at values of *P* < 0.05.

Results

Effect of exercise trial—Compared with the values at rest, the pretraining and post-training exercise trials induced increases (*P* ≤ 0.05) of 23% to 36% in plasma K⁺ concentration and 20% to 32% in whole-blood K⁺ concentration of the young adult and middle-aged horses (Figures 1 and 2). In addition, compared with values at rest, the hemoglobin concentration, number of erythrocytes, and Hct value were significantly (*P* ≤ 0.05) increased by 18% to 25%, 19% to 25%, and 20% to 27%, respectively, in the horses (Table 1; Figure 3). After the pretraining and post-training trials in both young adult and middle-aged horses, the plasma K⁺ concentration returned to resting values within 3 to 5 minutes of recovery. In comparison, the whole-blood K⁺ concentration, Hct, hemoglo-

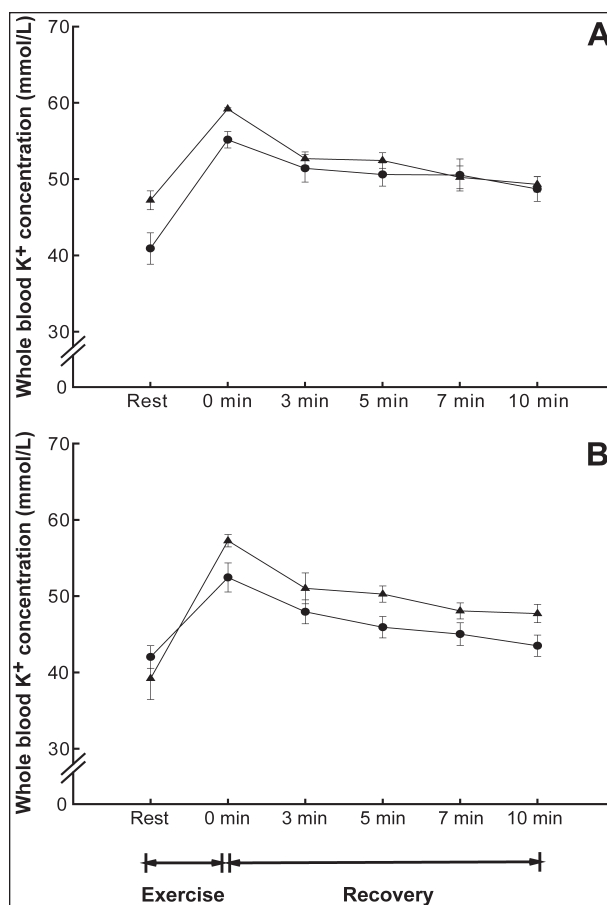


Figure 2—Whole blood K⁺ concentration (mean ± SE) in 4 young adult (A) and 4 middle-aged (B) horses at rest, after 6 minutes of exercise, and during the initial 10 minutes of recovery after cessation of exercise before (circles) and after (triangles) a 12-day period of training. Please note that the timescale on the x-axis is not linear.

Table 1—Blood hemoglobin (Hb) concentration, RBC number, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in 4 young adult and 4 middle-aged horses at rest, after 60 minutes of exercise (0 min), and during the initial 10 minutes of recovery after cessation of exercise before and after a 12-day period of training.

Group Variable	Before training						After training					
	Rest	0 min	3 min	5 min	7 min	10 min	Rest	0 min	3 min	5 min	7 min	10 min
Young adult horses												
Hb (mmol/L)	8.4 ± 0.5	11.2 ± 0.5 ^a	10.6 ± 0.6 ^a	10.4 ± 0.5 ^a	10.2 ± 0.6 ^a	9.8 ± 0.5	9.1 ± 0.3	11.4 ± 0.3 ^b	10.5 ± 0.3 ^b	10.1 ± 0.4 ^b	10.0 ± 0.3	9.5 ± 0.3
RBCs (T/L)*	8.2 ± 0.6	10.9 ± 0.6	10.4 ± 0.6 ^a	10.1 ± 0.6 ^a	9.9 ± 0.7	9.5 ± 0.6	8.7 ± 0.5	10.9 ± 0.4 ^b	10.1 ± 0.4 ^b	9.7 ± 0.4	9.4 ± 0.4	9.0 ± 0.3
MCH (mmol)	1,022 ± 15	1,025 ± 16	1,025 ± 18	1,031 ± 18	1,028 ± 19	1,032 ± 24	1,046 ± 20	1,042 ± 21	1,041 ± 20	1,048 ± 17	1,062 ± 24	1,051 ± 22
MCHC (mmol/L)	21.2 ± 0.3	20.6 ± 0.1	20.7 ± 0.1	20.9 ± 0.2	20.8 ± 0.1	20.9 ± 0.1	21.8 ± 0.2	21.6 ± 0.1	21.6 ± 0.2	21.6 ± 0.2	21.9 ± 0.1	21.7 ± 0.2
Middle-aged horses												
Hb (mmol/L)	9.0 ± 0.2	11.0 ± 0.4 ^a	10.3 ± 0.3 ^a	9.8 ± 0.3	9.6 ± 0.3	9.2 ± 0.3	8.3 ± 0.5	10.7 ± 0.5 ^d	10.3 ± 0.2 ^d	9.9 ± 0.1 ^d	9.6 ± 0.1 ^d	9.2 ± 0.1 ^d
RBCs (T/L)	8.3 ± 0.3	10.2 ± 0.5 ^a	9.4 ± 0.4	9.0 ± 0.4	8.7 ± 0.4	8.5 ± 0.3	7.5 ± 0.5	9.8 ± 0.5 ^d	9.4 ± 0.3 ^d	9.0 ± 0.2 ^d	8.7 ± 0.2 ^d	8.4 ± 0.2
MCV (fL)	50.1 ± 0.7	50.4 ± 0.8	50.4 ± 0.9	50.3 ± 0.8	50.3 ± 0.8	50.3 ± 0.8	50.0 ± 0.6	50.1 ± 0.6	50.0 ± 0.7	50.0 ± 0.6	50.1 ± 0.6	50.0 ± 0.6
MCH (mmol)	1,086 ± 15	1,079 ± 9	1,093 ± 13	1,083 ± 13	1,099 ± 15	1,085 ± 15	1,102 ± 13	1,096 ± 12	1,096 ± 7	1,101 ± 11	1,103 ± 12	1,091 ± 11
MCHC (mmol/L)	21.7 ± 0.1	21.4 ± 0.2	21.7 ± 0.1	21.5 ± 0.1	21.9 ± 0.2	21.6 ± 0.1	22.1 ± 0.2	21.9 ± 0.1	22.0 ± 0.2	22.1 ± 0.1	22.0 ± 0.2	21.8 ± 0.1

Resting values before and after training were not significantly different.
 *RBC count assessed as Tera (T) or 10¹² cells/L.
^aValue significantly ($P < 0.05$) different from the value at rest before training in this group of horses. ^{b,d}Value significantly ($P < 0.05$) different from the value at rest after training in this group of horses.

bin concentration, and number of erythrocytes returned to the resting values at a somewhat lower rate and remained increased for as long as 10 minutes after cessation of exercise. Erythrocyte K⁺ concentration and MCV, MCH, and MCHC values were not changed as a result of exercise (Figure 4; Table 1).

Effect of training—Training was associated with a significant increase (32%) in the concentration of Na⁺,K⁺-ATPase in gluteus medius muscle of the middle-aged, but not young adult, horses (Figure 5). The Na⁺,K⁺-ATPase concentration in the semitendinosus muscle was not changed in either group of horses after a 12-day period of training. The erythrocyte K⁺ concentration was significantly ($P < 0.01$) increased following training in both the young adult and middle-aged horses (Figure 4). Overall mean values of the plasma K⁺ concentration after training were lower than the pretraining values, but these differences were not significant ($P = 0.08$ for the young adult and $P = 0.056$ for the middle-aged horses; Figure 1). The increase in plasma K⁺ concentration during exercise detected in the young adult horses decreased (albeit not significantly [$P = 0.06$]) by 19% after 12 days of training, whereas the change in peak plasma K⁺ concentration during exercise after 12 days of training in the middle-aged horses was smaller. Training did not have any significant effect on the blood hemoglobin concentration; Hct, MCV, MCH, MCHC values; erythrocyte number; and the whole blood K⁺ concentration (Table 1; Figure 2).

Effect of age—There was no significant difference in the Na⁺,K⁺-ATPase concentration in gluteus medius or semitendinosus muscle between young adult and middle-aged horses before training (Figure 5). Also, the post-training value of the Na⁺,K⁺-ATPase concentration in gluteus medius muscle of young adult horses was not significantly ($P > 0.4$) different from that of the middle-aged horses. When pre- and posttraining data were

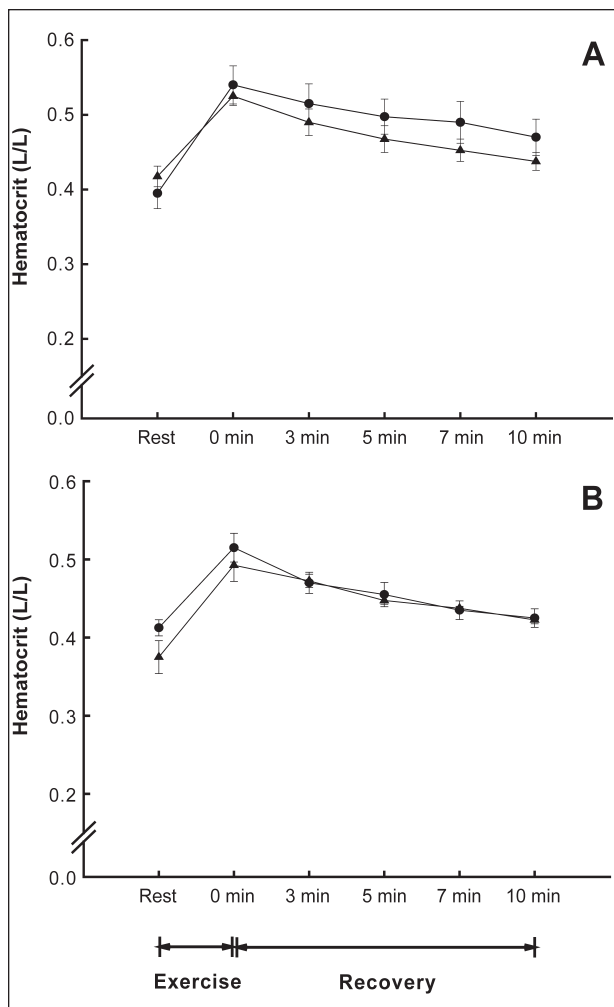


Figure 3—Hematocrit values (mean ± SE) in 4 young adult (A) and 4 middle-aged (B) horses at rest, after 6 minutes of exercise, and during the initial 10 minutes of recovery after cessation of exercise before (circles) and after (triangles) a 12-day period of training. Please note that the timescale on the x-axis is not linear.

pooled, the young adult horses had greater values ($P \leq 0.05$) of Hct, erythrocyte number, and whole

blood K^+ and erythrocyte K^+ concentrations and lower plasma K^+ concentration and MCH and MCHC values, compared with findings in the middle-aged horses (Figures 1–4; Table 1).

Discussion

There were 2 major findings of the present study in horses. First, the skeletal muscle Na^+,K^+ -pump appears to respond differently, depending on the age of the horses, to short-term (12 days' duration) moderate training for 1 hour/d. Second, an increase in Na^+,K^+ -ATPase concentration (32%) in the gluteus medius muscle of middle-aged horses after training was not associated with any effect on the magnitude of hyperkalemia during exercise; in young adult horses, the magnitude of hyperkalemia during post-training exercise was decreased (albeit not significantly) by 19% (compared with the pretraining exercise values) without any significant change in the muscle Na^+,K^+ -ATPase concentration.

Values of Na^+,K^+ -ATPase concentration in the gluteus medius muscle of young adult horses in the present study were in the range (100 to 150 pmol/g of muscle wet wt) determined in previous studies^{21,29} that involved the same type of measurement, but 50% lower than values in foals.^{22,23} Indeed, it has been well established that the Na^+,K^+ -ATPase concentration in mammalian skeletal muscle decreases with age.^{20,24,25}

The development of hyperkalemia during exercise is blunted after training in humans,¹⁴ Hereford calves,¹⁶ and dogs¹⁰ as well as in young adult horses.²¹ This is thought to be the result of an adaptation to improve K^+ homeostasis.¹⁴ Indeed, training-induced upregulation of Na^+,K^+ -ATPase concentration in skeletal muscle has been identified in humans^{11,13-15,30} and Thoroughbreds.²¹ In our previous studies^{22,23} of young Dutch Warmblood foals, we identified an effect of a 5-month period of sprint training on the Na^+,K^+ -ATPase concentration (ie, 20% increase, compared with untrained control horses) in both the gluteus medius and semitendinosus muscles.

In the present study, an upregulation of the Na^+,K^+ -ATPase concentration was detected only in the gluteus medius muscle of middle-aged horses. This discrepancy with findings of previous studies of foals^{22,23} and young adult horses²¹ is probably related to differences in the exercise challenge and the strain imposed on the Na^+,K^+ -exchange in muscle fibers. The intensity of exercise training (trotting at a speed of 4 m/s) in our study may have been too low to induce a considerable increase in extracellular K^+ concentration (through the loss of K^+ from contracting muscle), compared with that induced by sprinting. Sprint training may involve a larger recruitment of fast fibers, compared with the training given in this study. Because the semitendinosus muscle of horses contains a higher percentage of fast-twitch and a lower percentage of slow-twitch fibers than the gluteus medius muscle,^{31,32} this may explain the lack of response of the semitendinosus muscle in both groups in the present study. The fact that the Na^+,K^+ -ATPase concentration in the young adult horses did not increase in our study, contrary to findings of a study by McCutcheon et al,²¹ may be related to the

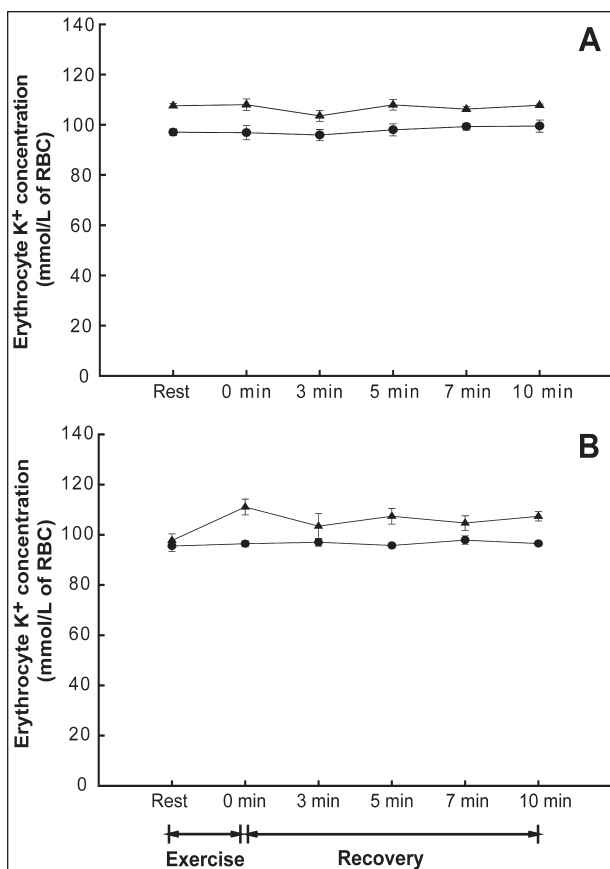


Figure 4—Erythrocyte K^+ concentration (mean \pm SE) in 4 young adult (A) and 4 middle-aged (B) horses at rest, after 6 minutes of exercise, and during the initial 10 minutes of recovery after cessation of exercise before (circles) and after (triangles) a 12-day period of training. Please note that the timescale on the x-axis is not linear.

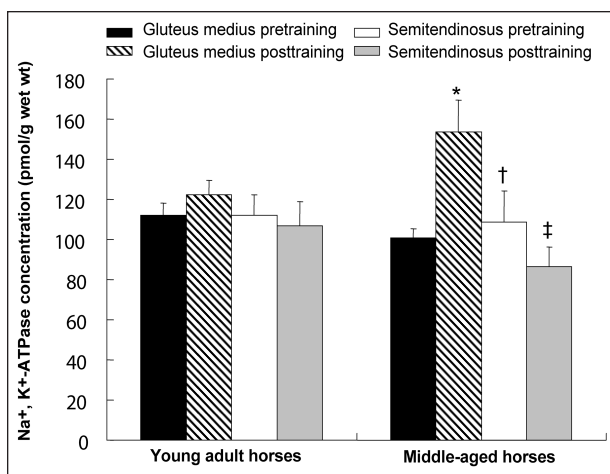


Figure 5— Na^+,K^+ -ATPase concentration (mean \pm SE) in specimens of the gluteus medius and semitendinosus muscles of 4 young adult and 4 middle-aged horses before (pretraining) and after (post-training) a 12-day period of training. *Value significantly ($P < 0.02$) different from that of gluteus medius muscle before training. †Value significantly ($P < 0.01$) different from that of gluteus medius muscle after training. ‡Value significantly ($P < 0.001$) different from that of gluteus medius muscle after training.

much smaller degree of treadmill inclination used in our study (1% to 5% vs 10%).

The difference in the response to training between the gluteus medius muscles of young adult and middle-aged horses is interesting; this may be related to the findings in the vastus lateralis muscle of humans, which indicated that exercise-induced hyperkalemia was decreased without an increase in skeletal muscle Na⁺,K⁺-ATPase concentration after a 10-week period of training,⁹ compared with pretraining values, whereas more intense training resulted in an upregulation of Na⁺,K⁺-ATPase concentration in the same muscle.¹³⁻¹⁵ Similarly, the decrease in peak plasma K⁺ concentration of young adult horses may be the result of an increase in activity of the pump without an increase in the skeletal muscle Na⁺,K⁺-ATPase concentration. In the middle-aged horses, the peak plasma K⁺ concentration was not affected by training, whereas the Na⁺,K⁺-ATPase concentration increased. This suggests that the Na⁺,K⁺ pump in the middle-aged horses can be activated to a smaller extent than that of the younger horses, forcing the middle-aged horses to compensate for the required increase in Na⁺,K⁺ pump activity by increasing the Na⁺,K⁺ pump concentration. Indeed, alterations in Na⁺,K⁺-ATPase activity and patterns of expression of α - and β -subunit isoforms are known to be associated with advancing age in rats.³³

The difference in degree to which the Na⁺,K⁺ pump can be activated between young adult and middle-aged horses may be related to the difference in the stage of development in association with either their ability to exercise or their hormonal response to training. It is well known that exercise performance decreases with age. In horses, aging appears to be associated with a decline in maximal aerobic capacity.³⁴ Thus, the intensity of the same training regimen imposed on young adult and middle-aged horses may vary according to their age. It is also possible that differences in circulating hormone concentrations (eg, thyroid hormones, insulin-like growth factor-I, insulin, catecholamines, glucocorticoids, and aldosterone) and the changes in these hormone concentrations in response to exercise influence the changes in skeletal muscle Na⁺,K⁺-ATPase activity in young adult and middle-aged horses. In a study by Malinowski et al,³⁵ plasma thyroid hormones and insulin-like growth factor-I concentrations were lower in old horses, compared with values in young adult horses. In addition, during exercise, plasma aldosterone concentration increases much more in old horses than it does in young adult horses.³⁶

The exercise-associated increase in plasma K⁺ concentration in horses detected in our study is consistent with findings of previous studies^{21,37,38} in horses. This can theoretically be a result of either a release of K⁺ from the working muscles or a decrease in plasma volume. As a short bout of treadmill exercise has been shown to have only a minor effect on the plasma volume in horses,^{17,37} the increase (approx 30%) in plasma K⁺ concentration during exercise in the present study suggests that there a considerable release of K⁺ from contracting muscles. The lack of a significant decrease in peak plasma K⁺ concentration during exercise in the middle-aged trained horses may be

due to the aforementioned apparent incapability to increase the activity of the Na⁺,K⁺ pump. This is different from the situation in humans; Klitgaard and Clausen¹⁵ determined that not only trained young but also trained elderly people were capable of such a significant decrease in peak plasma K⁺ concentration during exercise.

In horses, Hct, hemoglobin concentration, and oxygen-carrying capacity can be increased to a considerable extent by mobilization of erythrocytes from the spleen in response to the sympathetic stimulation induced by exercise.^{17,39} In our study, Hct increased by 20% to 27% and erythrocyte number and total hemoglobin concentration were increased to a similar extent. The increase in circulating erythrocytes may increase aerobic metabolism in response to exercise in horses more than twice as much as that achieved in either humans or dogs.¹⁷

During exercise, the plasma concentration of catecholamines increases considerably.⁴⁰ This may induce an increase in Na⁺,K⁺-pump activity within seconds.^{1,20} Because the erythrocyte is one of the classic targets for catecholamine action, it has been suggested that circulating erythrocytes might also be important for the clearance of extracellular K⁺ from the plasma during exercise in horses. This suggestion does not appear to be valid because in our study, the intraerythrocyte K⁺ concentration did not change significantly throughout the exercising period in horses before or after training, suggesting that the contribution of circulating erythrocytes to K⁺ homeostasis during exercise is relatively small.

Our data indicate that 12 days of training induced a significant increase of 7% to 10% in erythrocyte K⁺ concentration in both young adult and middle-aged horses. Because values of MCV, MCH, and MCHC were not different between pretrained and post-trained horses, this indicates that there was no change in erythrocyte water content. This is consistent with the fact that the intraerythrocyte K⁺ concentration, but not the erythrocyte membrane Na⁺,K⁺-ATPase activity, is higher in human athletes than in sedentary individuals.⁴¹ Also, a high intraerythrocyte K⁺ concentration has been reported⁴² to be related to high exercise performance in race horses. Thus, the increase in erythrocyte K⁺ concentration may be an indication of training-induced adaptations in the cellular handling of cations.

Findings of the present study indicate that the skeletal muscle Na⁺,K⁺-pump responds to short-term moderate training, depending on the age of the horses. Our data suggest that the activation of the Na⁺,K⁺-pump of skeletal muscle during exercise decreases with age. It seems that training first induces changes in the acute regulation (ie, augmented activity) of the existing Na⁺,K⁺-pumps; upregulation of the Na⁺,K⁺-pump concentration occurs later if the capacity of the existing pumps cannot keep pace. Therefore, an increase in the concentration of Na⁺,K⁺-ATPase may not be the only causative factor in the reduction of the degree of exercise-induced hyperkalemia after training, but the increase in activity of the Na⁺,K⁺-ATPase may be just as important for improvement of K⁺ regulation.

- a. Mustang 2000, Kagra AG, Fahrwangen, Switzerland.
- b. Alfacaïne 2% + adrenaline, Alfasan Nederland BV, Woerden, The Netherlands.
- c. Optifluor, Packard BioScience BV, Groningen, The Netherlands.
- d. Minaxi tri-carb 4000 series, Packard Bioscience BV, Groningen, The Netherlands.
- e. Sismex K-1000, Diamond Diagnostics, Holliston, Mass.
- f. Beckman CX-5 delta clinical system, Global Medical Instrumentation Inc, Ramsey, Minn.

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