Changes of Serum Mineral Concentrations in Horses during Exercise

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ABSTRACT : We investigated the exercise-induced changes in the serum concentration of several minerals in horses. Four well-trained Thoroughbred horses performed exercise for 5 d. The blood hemoglobin (Hb) concentration increased during exercise, recovered to the pre-exercise level immediately after cooling down and did not change again up till the end of experiment. The changes in serum zinc (Zn) and copper (Cu) concentrations were similar to those of blood Hb during the experiment. The serum magnesium (Mg), inorganic phosphorus (Pi) and iron (Fe) concentrations also increased during exercise. Though the serum Pi concentration recovered to the pre-exercise level immediately after the cooling down, it decreased further before the end of the experiment. The serum Mg concentration was lower immediately after cooling down than its pre-exercise level but gradually recovered from the temporal reduction. The recovery of the serum Fe concentration was delayed compared to that of other minerals and recovered 2 h after cooling down. The serum calcium (Ca) concentration did not change during exercise but rapidly decreased after cooling down. As a result, it was lower immediately after cooling down than its pre-exercise level. It recovered, however, to the pre-exercise level 2 h after cooling down. The temporal increase in the serum concentrations of all minerals except Ca is considered to result from hemoconcentration induced by exercise and the stable concentration of the serum Ca during exercise is possibly due to its strict regulation of homeostasis. These results indicate that the serum concentration of each mineral responds differently to exercise in horses, which may be due to the difference in metabolism among these minerals. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 4 : 531-536)***

Key Words : Horse, Exercise, Serum-mineral, Hemoconcentration, Metabolism

INTRODUCTION

Studies have shown that exercise affects circulating mineral levels in humans. Van Beaumont et al. (1973) and Aloia et al. (1985) reported that the plasma concentration of potassium (K) but not sodium (Na) was increased by exercise in humans. Exercise also increased plasma calcium (Ca) and inorganic phosphorus (Pi) concentrations in humans (Aloia et al., 1985). Although there have been reports that exercise did not affect the plasma magnesium (Mg) concentration (Cunningham et al., 1985; Deuster et al., 1987), Aloia et al. (1985) demonstrated a temporal increase in plasma Mg during exercise in humans. The plasma zinc (Zn) concentration increased during exercise and fell below the pre-exercise level 1 h after exercise in humans (Van Rij et al., 1986). Plasma copper (Cu) and iron (Fe) concentrations temporally increased during exercise in humans (Ohno et al., 1984; Kasugai et al., 1992). These changes in the serum levels of minerals may affect mineral nutrition. For example, Yamada et al. (1996) reported that exercise induced an increase in Na and P retention due to a decrease in urinary excretion, but the K, Ca and Mg balance was changed in humans.

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Studies in horses have indicated that exercise affects the serum concentrations of not only K but Na (Rose et al., 1980; Snow et al., 1982; Harris et al., 1988), unlike in humans (Aloia et al., 1985). The response of circulating mineral concentrations to exercise may therefore differ between horses and humans. However, few reports have examined the changes in circulating levels of minerals other than Na and K during exercise in horses. The present study was conducted to investigate the effect of exercise on serum concentration of several minerals in horses.

MATERIALS AND METHODS

Animals and diet

We used four well-trained Thoroughbred horses, familiar with a high-speed treadmill (Mustang 2200, Kagra, Switzerland). These animals were 3 yr old and their average weight was 440 kg (range, 420 to 459 kg). The horses were individually housed in box stalls with free access to water. They were fed an equal amount of experimental diet (table 1) twice a day. The diet was expected to maintain or exceed the current recommendations (NRC, 1989) and to maintain a body condition score of approximately five (Henneke et al., 1983).

Standard exercise test (SET)

The horses performed SET on the high-speed treadmill for 5 d in a room with controlled environmental temperature (between 17.2°C and 18.2°C) and relative humidity (between 42.6% and 56.2%). They cantered at 6.0 m/s, 8.0 m/s and 10.0 m/s for 3 min each (3% incline) after a

Table 1. Experimental diet (g/day[a])

Diet	
Redient	
Timothy hay	10.000
Alfalfa hay	1.000
Oat grain	3.000
Vitamin-mineral mixture[b]	25
Chemical analysis	
Calcium	24.6
Magnesium	13.4
Phosphorus	25.9
Zinc	0.47
Copper	0.10
Iron	0.81

[a] Daily intake.
[b] Yamako Siryo Inc., Ibaragi, Japan.

warm up consisting of walking at 1.7 m/s for 5 min (0% incline) and trotting at 3.5 m/s for 5 min (0% incline). Then they were cooled down by trotting at 3.5 m/s for 5 min (0% incline) and by walking at 1.7 m/s for 10 min (0% incline). Heart rate was recorded during the last 15 s at each speed using a heart rate monitor (Bandage-XL, Pollar, Finland).

Blood collection

A catheter was inserted into the left jugular vein under local anesthesia and fixed to the skin with adhesive tape before exercise. Extension lines were attached to the catheter to allow blood collection. Blood samples were collected before SET, at the end of cantering at 10 m/s, immediately after cooling down, and 1, 2, 4 and 8 h after the end of cooling down. The catheter was flushed with heparinized saline between sampling times to suppress blood clotting. Before blood samples were collected, 5 ml of blood and saline were drawn and discarded. Each sample was taken into a plain tube for serum mineral analysis and heparinized tube for blood hemoglobin (Hb) analysis and placed into ice. Then serum was separated by centrifugation (3,000 rpm, 4°C, 10 min) and immediately placed in a deep-freezer and stored at -80°C before analyses.

Analyses

Serum Ca, Mg, Cu, Zn and Fe were determined by an atomic absorption spectrophotometer (AA-6600F, Shimadzu, Japan) after digestion with nitric acid and perchloric acid. Serum was deproteinized by trichloroacetic acid for the determination of Pi. The concentration of Pi in deproteinized serum was determined by a colorimetric method (Gomori, 1942) using a spectrophotometer (U-2000A, Hitachi, Japan). Blood Hb was determined with a commercial kit (Hemoglobin Test Wako, Wako Chemicals, Japan).

Statistical analysis

First each parameter was analyzed by the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the time before and after exercise as repeated measurements (Time), and the day of exercise (Day) and the interaction. Because the effect of Time was significant and the interaction was not significant for each mineral and heart rate, these data were pooled and the changes within a day were re-analyzed by paired t-test in the second analysis. Significant levels for all statistical tests were set at $p<0.05$.

RESULTS

Heart rate

The heart rate response to SET is shown in figure 1. The effect of Time was significant ($p<0.001$) but that of Day and the interaction were not. The rate increased to 182±3 beats/min during exercise at the maximum speed.

Serum mineral concentration

The effect of Time was significant ($p<0.001$) for serum Ca concentration but that of Day and the interaction were not (table 2). The serum Ca concentration did not change during exercise but rapidly decreased ($p<0.05$) after the cooling down. It then recovered to the pre-exercise level 1h after the cooling down.

The effect of Time was significant ($p<0.001$) for serum Mg concentration but that of Day and the interaction were not. The serum Mg concentration significantly ($p<0.05$)

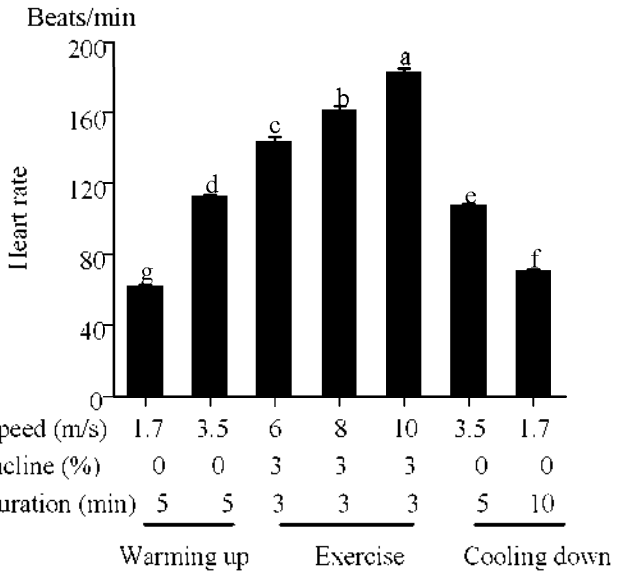

Figure 1. Effect of exercise on heart rate of horses on a treadmill
Values are shown as the mean±s.e.
Values with different letters are significantly different ($p<0.05$).

Table 2. Changes in serum mineral and blood hemoglobin concentrations in horses during standard exercise test

	Pre	Exercise	Time after cooling down (h)					Effects		
			0	1	2	4	8	Time	Day	Interaction
Serum calcium (mg/dl)										
Mean	11.25	11.34	10.79	11.22	11.41	11.29	11.14	**	NS	NS
s.e.	0.09^{a}	0.08^{a}	0.10^{b}	0.18^{ab}	0.07^{a}	0.09^{a}	0.09^{a}			
Serum magnesium (mg/dl)										
Mean	1.65	1.76	1.54	1.60	1.63	1.66	1.68	**	NS	NS
s.e.	0.03^{b}	0.03^{a}	0.03^{c}	0.04^{bc}	0.04^{bc}	0.04^{ab}	0.03^{ab}			
Serum inorganic phosphorus (mg/dl)										
Mean	3.92	4.56	3.98	3.49	3.43	3.49	3.50	**	**	NS
s.e.	0.12^{bc}	0.12^{a}	0.11^{b}	0.09^{cd}	0.10^{d}	0.08^{d}	0.10^{cd}			
Serum zinc (μg/dl)										
Mean	63.4	69.7	60.4	62.9	62.5	60.7	65.6	**	NS	NS
s.e.	1.6^{b}	1.9^{a}	1.6^{b}	1.7^{b}	1.6^{b}	1.9^{b}	2.1^{ab}			
Serum copper (μg/dl)										
Mean	97.4	107.9	96.0	98.9	98.6	100.3	98.7	**	NS	NS
s.e.	2.6^{b}	3.6^{a}	2.7^{b}	3.5^{ab}	3.2^{ab}	2.6^{ab}	2.8^{ab}			
Serum iron (μg/dl)										
Mean	172	237	206	198	193	182	181	**	NS	NS
s.e.	7^{e}	13^{a}	8^{b}	9^{bd}	9^{bc}	8^{cd}	13^{bc}			
Blood hemoglobin (g/dl)										
Mean	11.9	21.1	12.3	12.4	11.5	12.7	12.8	**	NS	NS
s.e.	0.5^{b}	0.8^{a}	0.7^{b}	0.5^{b}	0.5^{b}	0.5^{b}	0.5^{b}			

** $p<0.001$; NS, not significant

Values with different letters are significantly different ($p<0.05$).

Pre means the value before warming up.

Exercise means the value at the end of cantering.

increased during exercise and decreased ($p<0.05$) immediately after the end of cooling down. As a result, it was significantly ($p<0.05$) lower at the end of cooling down than in the pre-exercise period. Thereafter, the serum Mg concentration gradually recovered to the pre-exercise level.

The effect of Time or Day was significant ($p<0.001$) for serum Pi concentration but the interaction was not. The serum Pi concentration significantly ($p<0.05$) increased during exercise and recovered to the pre-exercise level 1 h after the cooling down. Then it significantly decreased ($p<0.05$) compared with the pre-exercise level ($p<0.05$). The hypophosphatemia continued to the end of the experiment. The mean value of serum Pi concentration was decreased gradually with the day of exercise, i.e., it decreased from 4.26 mg/dl on the first day, to 4.15, 3.69, 3.22 and 3.51 mg/dl on the second, third, fourth and fifth day of exercise, respectively.

The effect of Time was significant ($p<0.001$) for serum Zn and Cu concentrations but that of Day and the interaction were not. Serum Zn and Cu concentrations temporally increased ($p<0.05$) during exercise and recovered to the pre-exercise levels immediately after the cooling down.

The effect of Time was significant ($p<0.001$) for serum Fe concentration but that of Day and the interaction were not. The serum Fe concentration significantly ($p<0.05$) increased during exercise, and then gradually decreased. It recovered to the pre-exercise level 2 h after cooling down.

The effect of Time was significant ($p<0.001$) for blood Hb concentration but that of Day and the interaction were not. The blood Hb concentration changed similarly to serum Zn and Cu concentrations, i.e., a temporal increase ($p<0.05$) during exercise and a rapid recovery after the cooling down.

DISCUSSION

Heart rate increased to 182±3 beats/min at the maximum speed of exercise in this experiment. This response to the SET suggested that the exercise was moderate because the maximum heart rate was approximately 220-230 beats/min in horses exercised heavily (Hodgson et al., 1990).

Blood Hb increased during the exercise and recovered immediately after the cooling down. Blood plasma volume is known to decrease during exercise through the movement of blood fluid to tissues and splenic red blood cells are transferred to the circulation, resulting in the increase in blood Hb concentration in horses (Kohn et al., 1978). This

phenomenon is called hemoconcentration. Ohira et al. (1995) suggested that hemoconcentration ceased within 30 min after exercise in humans. A study in horses (Smith et al., 1989) also suggested that hemoconcentration was induced by a similar exercise to that performed in the present experiment. Thus, we considered that the temporal increase in blood Hb concentration resulted from hemoconcentration in this experiment.

Though serum concentrations of all minerals except Ca showed a temporal increase during exercise, the serum Ca concentration was stable during exercise but decreased after cooling down. On the other hand, Ljunghall et al. (1984, 1985) have indicated a temporal increase in the circulating Ca concentration during exercise in humans. Grimston et al. (1993) indicated that exercise increased the circulating calcitonin level and suggested that this increase did not result from hemoconcentration but from a stimulation of secretion by hypercalcemia in humans. Additionally, Aloia et al. (1985) suggested that the increase in calcitonin level partly suppresses hypercalcemia induced by hemoconcentration in humans. We did not find an increase in the Ca concentration in serum collected at the end of exercise. Therefore, the serum Ca concentration might be temporally increased before the end of exercise, which increased calcitonin secretion, and the rise in calcitonin prevented the hypercalcemia induced by hemoconcentration. The serum Ca concentration was decreased after cooling down. The action of calcitonin possibly persisted, which might decrease the serum Ca concentration in this period. There may be an alternative factor that affects serum Ca concentration in exercising horses. Schryver et al. (1978) indicated that approximately 350 to 500 mg/h of Ca was lost through sweat during exercise in horses and suggested that these losses were quite large. The blood plasma volume in resting horse ranges from 16 to 20 l (Mckeever et al., 1987) and the plasma Ca concentration from 10.5 to 13.5 mg/dl in resting horses (Lewis, 1995). Therefore, total plasma Ca is estimated to range from 1,680 to 2,700 mg. It is likely that the loss of Ca through sweat is not negligible in exercising horses and possibly counteracts the increase in serum Ca induced by hemoconcentration during exercise. Additionally, the loss of Ca through sweat might decrease the serum Ca concentration after exercise because hemoconcentration rapidly ceased.

The serum Mg concentration increased during exercise in this experiment, which was consistent with a study in humans (Aloia et al., 1985). We consider that the temporal increase in serum Mg is due to hemoconcentration during exercise. The serum Mg concentration decreased at the end of the cooling down. As a result, it was lower at the end of cooling down than in the pre-exercise period. The concentration gradually recovered thereafter. As mentioned above, calcitonin secretion might be increased by exercise. Calcitonin was reported to decrease the serum concentration of Mg in humans (Dreosti, 1995). Therefore, the high level of calcitonin might reduce the serum Mg concentration after the cooling down. Golf et al. (1984) suggested that exercise enhanced the movement of Mg from extracellular fluid to muscular tissue in humans. Additionally, Vermann et al. (1983) postulated that exercise-induced lipolysis increased the flux of Mg into adipocytes in humans. The decrease in serum Mg is possibly masked by the hemoconcentration during exercise and the recovery from hemoconcentration reveals the hypomagnesemic effects of exercise.

The serum Pi concentration rapidly increased during exercise, probably as a result of hemoconcentration during exercise. It gradually decreased after exercise, however, and at 2 and 4 h after the cooling down was lower than the pre-exercise level. Cunningham et al. (1985) showed that the plasma Pi concentration was lower in the post-exercise than the pre-exercise period in humans. Their report consists with the result of this experiment. Because the loss of P through sweat is known to be slight in horses (Schryver et al., 1978), it did not explain the reduction in the serum concentration of Pi after exercise. Yamada et al. (1996) suggested that Pi was taken up by muscular tissue after exercise in humans. In addition, calcitonin is known to reduce the serum Pi concentration by increasing urinary P excretion and decreasing bone resorption in humans (Arnaud, 1988; Suki and Rouse, 1991). Exercise may increase circulating calcitonin levels. Therefore, the hypophosphatemia after exercise may result from the movement of Pi into muscles and/or the action of calcitonin in horses. The mean value of the serum Pi concentration decreased with day of exercise though P intake satisfied the requirement for horses. The exercise-induced increase in the serum calcitonin level may increase urinary P excretion, which, in turn, reduces the serum Pi concentration throughout the experiment. An experiment in humans showed that the serum Pi concentration decreased during 4-day exercise and urinary excretion of P was stimulated by exercise (Yamada et al., 1996). Additionally, some researchers reported that exercise suppressed bone resorption in humans. Hiney et al. (2000) also reported that exercise suppressed bone resorption in horses. The suppression of bone resorption may disturb Pi homeostasis in horses. Further study is needed to clarify the effect of exercise on the changes in serum levels of Ca-regulating hormones and bone metabolism in horses.

The serum Zn concentration temporally increased during exercise. This change was similar to that in Hb. Hemoconcentration is reported to raise the serum Zn concentration in humans (Van Rij et al., 1986). Aruoma et al. (1988) reported that exercise temporally increased the plasma Zn concentration in some subjects but not in others. Lukasuki et al. (1984) reported that the nutritional status of

Zn affected the response of the plasma Zn concentration to exercise in humans, i.e., the temporal increase in serum Zn was more remarkable in subjects given a diet satisfying the Zn requirement than in those given a Zn-deficient diet. Because horses were given a diet containing enough Zn in the present experiment, the exercise increased the serum Zn concentration. Ohno et al. (1985) indicated that Zn in erythrocytes was decreased by exercise in humans given a conventional diet and suggested that the efflux of Zn from erythrocytes increased the plasma Zn concentration during exercise. This movement of Zn possibly results in an increase in the serum Zn concentration during exercise in horses.

The serum Cu concentration temporally increased during exercise. This change was similar to that in the blood Hb concentration. Exercise was also reported to increase serum Cu levels in humans (Ohno et al., 1984). Hemoconcentration probably results in a temporal increase in serum the Cu concentration during exercise.

The serum Fe concentration increased during exercise then gradually recovered and reached the pre-exercise level 2 h after cooling down. Kasugai et al. (1992) indicated that the serum Fe concentration increased at the end of exercise in humans and suggested that this increase resulted from hemoconcentration induced by exercise. These results were consistent with the increase in serum Fe concentration during exercise shown in the present experiment. On the other hand, serum Fe recovered more slowly from the increase than blood hemoglobin and other minerals such as serum Cu and Zn. As a result, the serum Fe concentration was still higher 2 h after exercise than its pre-exercise level. Broun (1922) reported that exercise induced hemolysis in humans. Ohira et al. (1995) indicated that the serum Fe concentration was increased 30 min after exercise, and so was not related to hemoconcentration in humans. They suggested that the increase was due to serum Fe originating from ruptured erythrocytes. Hanzawa et al. (1998) also reported that exercise induced hemolysis in horses. Exercise probably induced hemolysis in the present experiment. Therefore, we consider that the slow recovery of serum Fe is, at least partly, induced by hemolysis during exercise. Suzuki et al. (1999) reported that serum creatine kinase activity and the myoglobin level rose after exercise, which resulted from muscle damage in humans. Snow et al. (1982) also indicated that exercise increased serum creatine kinase activity in horses. Myoglobin is probably released from muscle after exercise in horses because of muscle damage. The increase in serum myoglobin concentration may contribute to the slow recovery of serum Fe concentration after exercise in horses.

In conclusion, exercise-induced hemoconcentration strongly affected the serum mineral concentrations determined in the present experiment. The changes serum Cu and Zn concentrations can be explained by the hemoconcentration. The hemoconcentration also temporally increased in serum Mg, Pi and Fe concentrations. However, the serum Ca concentration was stable during and decreased after exercise. The rise in serum Ca by hemoconcentration was possibly suppressed by its strictly homeostatic mechanism increasing circulating calcitonin level. The post-exercise reduction of serum Pi and Mg concentrations may be related to the changes in their distribution in the body and/or the action of calcitonin. The slow recovery of serum Fe from the temporal increase probably resulted from Fe influx from ruptured erythrocytes and/or damaged muscular tissue. Thus, it is likely that exercise affects each the metabolism of each mineral differently. Further studies are needed to evaluate the mechanisms by which exercise affects mineral metabolism in horses.

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