

# Effects of sweat loss induced by treadmill exercise on magnesium and calcium homeostasis in Franches-Montagnes horses

D. Weiss<sup>1</sup>, M. A. Weishaupt<sup>2</sup>, R. Forrer<sup>3</sup>, A. Fakler<sup>4</sup>, U.E. Spichiger<sup>4</sup>, D. Burger<sup>5</sup>, M. Wanner<sup>1</sup> and J.-L. Riond<sup>1</sup>

<sup>1</sup>Institute of Animal Nutrition; <sup>2</sup>Department of Veterinary Surgery; <sup>3</sup>Clinical Laboratory, Department of Veterinary Internal Medicine, University of Zurich;

<sup>4</sup>Centre for Chemical Sensors, Biosensors and (Bio-)Analytical Chemistry, Department of Pharmacy, Swiss Institute of Technology, Zurich;

<sup>5</sup>Swiss National Stud, Avenches, Switzerland

## Summary

The physiological consequences of magnesium and calcium loss via sweat during a prolonged exercise test was investigated in eleven stallions. The test on a high-speed treadmill inclined at 3% consisted of 6 trot-intervals of 20 min each at 3.0 m/s. Blood samples were collected 1 hour before the exercise test, after each trot-interval and after 1 and 2 hours of recovery. The body weight loss ranged between 19 and 25 kg with a mean reduction of the body mass of  $3.71\% \pm 0.45$ . Mean total magnesium concentration decreased continuously during the exercise and the first hour of the recovery period. Mean erythrocyte magnesium concentration decreased during the last two trot intervals and was significantly lower during the second hour of recovery. The mean total calcium concentration decreased during the exercise test and increased during the recovery period. Mean parathyroid hormone serum concentrations at the end of the test were significantly greater. Serum 1,25-dihydroxyvitamin D concentrations did not change with time. According to the results of the present study, additional magnesium and calcium supply are superfluous in the case of a single prolonged exercise. However, high dietary supply may be beneficial in hard working and heavily sweating horses to prevent the occurrence of critically low ionised magnesium and calcium values.

**Keywords:** horse, magnesium, calcium, treadmill, sweat, nutrition

## Auswirkung des durch Laufbandarbeit induzierten Schweißverlusts auf die Magnesium- und Kalzium-Homöostase bei Freiburger Pferden

Die physiologischen Konsequenzen des mit dem Schweiß ausgeschiedenen Magnesiums und Calciums im Verlauf einer standardisierten Dauerbelastung wurden bei elf Hengsten untersucht. Der Test auf einem Hochgeschwindigkeitslaufband bestand aus 6 Trabintervallen von je 20 Minuten Dauer bei einer Geschwindigkeit von 3.0 m/s und einer Steigung von 3%. Blutproben wurden 1 Stunde vor der Belastung, nach jedem Trabintervall und je 1 und 2 Stunden nach Beendigung der Belastung entnommen. Die Abnahme des Körpergewichtes lag zwischen 19 und 25 kg, was einer Reduktion der Körpermasse von  $3.71\% \pm 0.45$  entsprach. Die Mittelwerte der totalen Plasmakonzentrationen von Magnesium nahmen kontinuierlich im Verlauf des Testes bis zur erster Stunde der Erholungsperiode ab. Die Mittelwerte der Magnesiumkonzentrationen in den Erythrozyten nahmen während der letzten beiden Trabintervalle ab und waren signifikant niedriger in der zweiten Stunde der Erholungsperiode. Die Mittelwerte der totalen Calciumkonzentrationen nahmen im Verlauf der Belastung ab und normalisierten sich während der Erholungsperiode. Die Mittelwerte der Serumkonzentrationen des Parathormons am Ende der Belastung waren signifikant höher. Es wurden keine Veränderungen der Serumkonzentrationen des 1,25-Dihydroxy-Vitamins D über die Zeit beobachtet. Auf der Basis der Ergebnisse der vorliegenden Untersuchung wird geschlossen, dass eine zusätzliche Magnesium- und Calciumgabe für den Fall einer einzigen Dauerbelastung überflüssig ist. Jedoch dürften hohe orale Gaben bei schwer arbeitenden Pferden, die profus schwitzen, vorteilhaft sein, um dem Vorkommen von kritisch niedrigen ionisierten Magnesium- und Calciumkonzentrationen im Plasma vorzubeugen.

**Schlüsselwörter:** Pferd, Magnesium, Calcium, Laufband, Schweiß, Ernährung

## Introduction

Prolonged exercise in horses is usually associated with a major loss of water and electrolytes (Maughan and Lindinger, 1995; McCutcheon and Geor, 1996; Flaminio and Rush, 1998). The heat produced by muscle activity is primarily eliminated in horses through the evaporation of sweat and to a lesser extent (20–30%) via expiration (Thiel et al., 1987; Guthrie and Lund, 1998). Other types of heat dissipation which play a minor role are convection, conduction and radiation. During a 160 km endurance ride, horses may lose up to 10% of their body weight, approximately 90% of which is water (Carlson, 1983; Lindiger and Ecker, 1995; White, 1998). In contrast to humans, the sweat of horses is hypertonic in comparison to plasma (Carlson and Ocen, 1979; Rose et al., 1980; Kerr and Snow, 1983). Numerous reports are available on the quantities and the factors influencing the losses of sodium, potassium, chloride, calcium (Ca) and magnesium (Mg) in sweat (Kerr and Snow, 1983;

McCutcheon et al., 1995; McCutcheon and Geor, 1996, 1998; McConaghy et al., 1995).

The importance of the Mg and Ca loss on endurance performance has only been partially addressed (Harris et al., 1995; Flaminio and Rush, 1998). These two ions may be involved in the pathogenesis of the exhausted horse syndrome and of the slow-onset rhabdomyolysis. For Mg, the sweat-plasma ratio ranges from 1:1 to 8:1 and from 1:1 to 2:1 for Ca (Carlson and Ocen, 1979; Kerr and Snow, 1983; Meyer, 1990; McCutcheon and Geor, 1996; Frappe, 1998). In many reports, the total Ca ( $Ca_{tot}$ ) and ionised Ca ( $Ca_{ion}$ ) serum concentration were observed to decrease or remain unchanged in the course of endurance rides (Ecker and Lindinger, 1995; Andrews et al., 1995a,b; Hinchcliff et al., 1995). Similarly, the serum concentrations of total Mg ( $Mg_{tot}$ ) decreased or remained unchanged (Carlson and Mansmann, 1974; Snow et al., 1982; Ecker and Lindinger, 1995).

Because Mg is mainly an intracellular cation and only less than 1% is present in the extracellular fluid, serum or plasma total  $Mg_{tot}$  are not a reliable indicator of the Mg body status (Elin, 1994). Measurements of serum ionized Mg ( $Mg_{ion}$ ) are presently being evaluated for their clinical usefulness (Huijgen *et al.*, 1999). Determinations of intracellular Mg such of that of erythrocytes ( $Mg_{ery}$ ) have also been advocated (Durlach and Bara, 2000). However, none of these methods is satisfactory in order to make an assessment of Mg body status.

In this study, the response in the plasma of  $Mg_{tot}$ ,  $Mg_{ion}$ ,  $Ca_{tot}$  and  $Ca_{ion}$  and  $Mg_{ery}$  to a long-term effort was investigated under standardized conditions on a treadmill in horses which did not receive supplemental water and electrolytes before and during the test.

## Animals, materials and methods

### Horses and conditioning programme

Eleven clinically healthy Franches-Montagnes stallions with a mean age of  $8.6 \pm 1.3$  (SD) years and a mean body weight of  $590.9 \pm 19.8$  kg were used. The experimental protocol was approved by the germane veterinary office according to the text of the Swiss law on animal protection. The horses were regularly trained for carriage and English riding. Additionally, each horse was conditioned during 25 minutes on a high-speed treadmill (Mustang 2200, Kagra AG, Fahrwangen, Switzerland) 5 times during the last 3 weeks before the experiment. On the other days, the horses were ridden or driven for at least 45 minutes except on Sundays. Horses were otherwise stabled in box stalls with free access to water.

### Diet

The horses daily received 12 kg hay in two portions, 4 kg entire oats in three portions and once daily 30 g of a mineral mixture (Totalin<sup>®</sup>, Werner Stricker AG, Zollikofen-Bern, Switzerland). Straw used as bedding was ingested ad libitum. Free access to a trace-mineralized salt was allowed.

### Exercise test

Horses were warmed-up during 5 minutes at the walk (1.7 m/s). Subsequently each horse underwent a low intensity prolonged exercise test consisting of 6 trot-intervals at 3.0 m/s on a high-speed treadmill inclined at 3%. Each trot-interval lasted 20 minutes and was followed by 3 minutes standing and 3 minutes walking at 1.5 m/s. Horses were cooled down after the last trot-interval during 10 minutes walking on the treadmill (1.5 m/s) and during 10 minutes handwalking. The room temperature and relative humidity were monitored by a custom built hygro-thermometer (Haenni Instruments AG, Jegenstorf, Switzerland). The horses performed the exercise in a room with temperatures ranging between 21 and 29°C and relative humidity ranging between 58 and 80%. Fresh air was provided by a hanging ventilator (Isler Bioengineering AG, Zurich, Switzerland) in front of the horse with a maximal air supply of 4500 m<sup>3</sup>/h. The ventilating system was working with 50 to 70% of its maximal capacity. After the handwalking the horses were returned to individual box stalls with free access to water.

### Body weight, heart rate and rectal temperature

Heart rate was measured during exercise with a monitor (Polar Horse Trainer Advanced<sup>®</sup>, Polar Electro Oy, Kempele, Finland) and in the box stalls with a stethoscope. The rectal temperature was measured in the box stalls and during the standing phase between trot-intervals with an electronic thermometer (Maximum Thermometer CE 0120, Wanner-Technik, Wertheim, Germany). The horses were weighed directly before and after the exercise test on a custom built large animal scale (accuracy  $\pm 0.5$  kg; Ammann-Waagen, Ermatingen, Switzerland). The body mass was corrected for the manure excreted and collected from the floor during exercise. None of the horses urinated during the test.

### Blood sampling and analyses

Blood samples were collected 1 hour before the exercise test (PE), during the standing phase after each trot-interval (T1 - T6) and 1 and 2 hours after the last trot-interval (R1h, R2h) via a catheter (13 gauge, 105 mm; Intranule, Vygon, Ecouen, France) placed in the right jugular vein. The first sample which was taken through a needle (18 gauge, 3.8 cm) from the left jugular vein. The catheter was sutured to the skin and connected with a 15 cm extension tube (B. Braun Medical AG, Emmenbrücke, Switzerland) and a multidirectional-stopcock-system (Discofix<sup>®</sup>, B. Braun Medical AG). The blood was collected with a blood-collecting system (S-Monovetten<sup>®</sup>, Sarstedt, Sevelen, Switzerland). For the analysis of  $Mg_{tot}$ ,  $Mg_{ion}$ ,  $Ca_{tot}$ ,  $Ca_{ion}$ , inorganic phosphorus (P<sub>i</sub>) and  $Mg_{ery}$ , 9-ml lithium-heparin tubes were used. A 9-ml tube without any ingredient was used to collect serum for parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D) analysis. Hematological analysis was carried out on a sample collected with a 2-ml K-EDTA tube. Plasma and serum were separated within 5 min after collection by centrifugation (1580 g for 10 min at 20°C). Plasma for electrolyte determination was stored in a deep-freezer at  $-18^{\circ}\text{C}$ , whereas serum for the determination of PTH and 1,25(OH)<sub>2</sub>D was kept at  $-80^{\circ}\text{C}$ . Plasma for the determination of  $Ca_{ion}$  and  $Mg_{ion}$  was stored at  $+4^{\circ}\text{C}$  in a refrigerator and analysed with ion-selective membranes within 24 hours. These measurements were performed at ambient temperature. The 8-channel electrode monitor was equipped with FET operational amplifiers AD515 KH (input impedance 1013W/2pF; bias current  $< 150$  pA; capacity neutralisation; Analog Devices, Norwood MA, USA), an active low pass filter in each channel for noise rejection and a latchable CMOS Multiplexer DG 529 (Siliconix GmbH, Filderstadt, Germany) controlled by a Macintosh IIfx (Apple Computer, Cupertino CA, USA). A Solartron-Schlumberger 7150 Digital Multimeter (resolution: 1 mV; full scale  $\pm 2\text{V}$ ; Solartron Instrumentation Group, Farnborough, Hampshire, England; a division of Schlumberger Electronics Ltd, UK) was used with remote control through a National Instruments IEEE 488 interface by the Macintosh IIfx, programmed in LabView5. The results for  $Mg_{ion}$  and  $Ca_{ion}$  obtained in mmol/kg solvent (= plasma) were expressed in mmol/L after division with the specific gravity. The plasma gravity was determined with a refractometer (Uricon-N<sup>®</sup>, ATAGO Co., LTD, Japan). The whole blood for erythrocyte count and for the determination of packed cell volume (PCV) was stored at room temperature and analysed with an electronic cell-counter (Cell-Dyn<sup>®</sup> 3500, Abbott-Diagnostics, Abbott Park IL, USA) within 24 hours.

$Ca_{tot}$ ,  $P_i$  and  $Mg_{tot}$  were determined by colorimetric methods with an automatic analyzer (Cobas Mira, F. Hoffmann-La Roche, Basle, Switzerland). An enzymatic test (Cobas Mira) was used for the determination of lactate in the plasma. After plasma and buffy coat were aspirated, the erythrocytes were washed three times with physiologic NaCl-solution and centrifuged each time (1580 g for 10 min at 20° C). Then, 0.5 ml erythrocytes were diluted with 2 ml distilled water for destruction and stored in a deep-freeze.  $Mg_{ery}$  measurement was performed with atomic-absorption-spectroscopy (SpectrAA-20, Varian, Zug, Switzerland).

PTH and  $1,25(OH)_2D$  were quantitated at PE, T1, T3, T6 and R1h by use of an immunoradiometric assay for the quantitative determination of human intact PTH (Intact PTH T Kit, Nichols Institute Diagnostics, San Juan Capistrano CA, USA) and a radioimmunoassay for  $1,25-(OH)_2D$  (Radioimmunoassay Kit, Nichols Institute Diagnostics).

### Statistics

All results in graphs are presented as mean and 95% confidence limits of the mean. For each measured variable, the mean, the standard deviation and the 95% confidence limits of the mean were calculated. Significant time effects were determined by repeated measures ANOVA. With a probability from ANOVA equal or less than 0.05 a paired t-test adjusted with Bonferroni was conducted to determine whether there were significant differences among means. The level of significance was fixed at 0.05. The statistics were carried out with SYSTAT<sup>®</sup> 7.0 for Windows<sup>®</sup> (1997).

## Results

### Body weight, heart rate and rectal temperature

Ten of the eleven horses completed the exercise program. At the end of the test, they showed signs of fatigue like stumbling and reluctance to maintain speed. The sweat production of all horses became obvious as drops and the treadmill surface became moist and wet. The data of one horse eliminated because of lameness was not included. The body weight loss ranged between 19 and 25 kg with an average of 21.9 kg, which implies an mean reduction of body mass of  $3.71\% \pm 0.45$ . The mean heart rate during the time period extending from minute 3 to 19 of each trot interval progressively diminished from 133 beats per minute at T1 to 117 beats per minute at T6. The mean rectal temperature increased at the beginning of exercise and reached a plateau between 39.3° C and 39.9° C during the test. None of the horses had a rectal temperature higher than 41° C.

### Blood chemical analyses

PCV increased significantly ( $p = 0.001$ ) during the exercise from 46.6% at T1 to 49.6% at T6 which was accompanied by an increase of the number of erythrocytes by 760'000. None of the individual lactate values exceeded 2 mmol/L (range: 0.21 – 1.98 mmol/L). A progressive increase of lactate concentrations in plasma could be seen between PE and R1h and T6 and R1h were significantly higher than the pre-exercise value.

### Magnesium, calcium and phosphorus

$Mg_{tot}$  decreased continuously during exercise until R1h (Figure 1A). Using the PE value for comparison, the decline was significant during the second part of the test (T4 – T6) and the first hour of recovery (R1h). The mean  $Mg_{tot}$  concentrations of T6, R1h and R2h were below the lower limit of the normal range ( $< 0.7$  mmol/L) during the last trot-interval and the first two hours of recovery. Plasma  $Mg_{ion}$  concentrations tended to decrease parallel to  $Mg_{tot}$  during the test (Figure 1B). However, this downwards trend was not significant because of the large standard deviations related to differences between animals and irregularities in the individual concentration profiles. The proportion of mean  $Mg_{ion}$  to mean  $Mg_{tot}$  ranged between 74.05 and 89.34%.  $Mg_{ery}$  tended to increase up to T4, from then on it decreased continuously (Figure 1C).  $Mg_{ery}$  at R2h was significantly lower than  $Mg_{ery}$  of T3 to T6.

$Ca_{tot}$  decreased during the exercise test and increased during the recovery period (Figure 1D). The  $Ca_{tot}$  concentration was significantly lower in the second part of the test (T4 – T6) in comparison to the pre-exercise level, but even then it was never under the lower limit of the normal range (2.6 mmol/L). Similarly to plasma  $Mg_{ion}$  concentrations,  $Ca_{ion}$  concentrations tended to decrease parallel to  $Ca_{tot}$  concentrations during the test (Figure 1D). After two hours of recovery, the mean pre-exercise  $Ca_{tot}$  concentrations were still not reached. The trend was not significant for the same reason given for  $Mg_{ion}$ . Using the mean values for comparisons, the proportion of mean  $Ca_{ion}$  concentrations to mean  $Ca_{tot}$  concentration was maintained in a small range between 41.53 and 44.58%

The mean  $P_i$  significantly increased from PE to T1 and then it regularly decreased until R1h. The mean  $P_i$  value at R1h was slightly below the lower limit of the physiological range (0.9 mmol/L) and was significantly lower than the mean values at T6 and R2h. In one horse,  $Ca_{ion}$  and  $Mg_{ion}$  decreased at T6 to values of 0.49 mmol/L of 0.21 mmol/L, respectively whereas  $Ca_{tot}$  and  $Mg_{tot}$  were within the normal range. This horse showed muscle tremors and elevated increased respiratory rate during the recovery period.

### Hormones

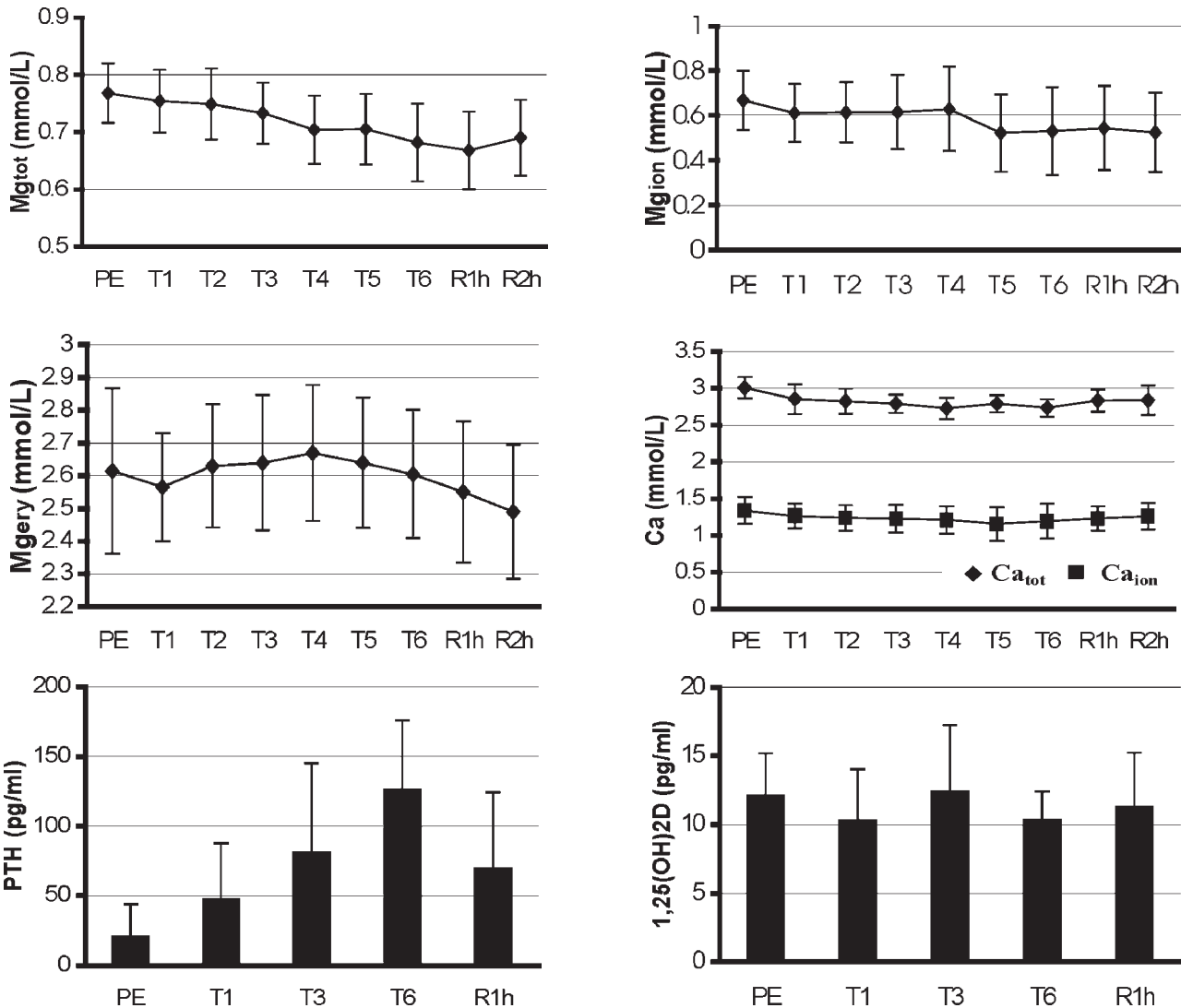
The mean PTH serum concentration at the end of the test was significantly higher than that at PE and T1 (Figure 1E). A trend for a decrease was observed in the recovery period. No significant change of serum  $1,25(OH)_2D$  concentrations over time could be detected (Figure 1F).

## Discussion

The loss of water and electrolytes associated with the loss of body weight can knowingly lead to a considerable reduction in the effective circulating blood volume decreasing the cardiovascular and thermoregulatory capacities (McCutcheon and Geor, 1996; Flaminio and Rush, 1998). The present study focused on the effect of sweat loss on Mg and Ca homeostasis. It should be noticed that the environmental conditions of treadmill room were not controlled and not maintained constant. Under treadmill conditions there is a lack of adequate air movement, even with fans, resulting in less effective dissipation of exercise generated heat than under field conditions (Sosa

Leon et al., 1995). Indeed, quite a few horses of this study showed signs of fatigue and difficulties to complete the exercise test although the intensity and duration was low compared to common endurance rides. Interestingly, the heart rate decreased during the trot intervals which may be related to an adjustment to the test and the environment.

exercising horses (Meyer et al., 1991) and thus does probably not contribute to the decrease in plasma Mg. In a horse of 600 kg, approximately 2 to 3 g Mg and 8 to 10 g Ca are present in the extracellular fluid. Because the decrease of the plasma concentrations were not pronounced, the two ions are most likely mobilised from body stores which include the gastrointestinal



**Fig. 1:** Mean and 95% confidence interval of blood parameters during a treadmill test in 10 horses. PE = pre-exercise value, T1 to T6 = 20-min trot intervals, R1h = 1 hour in recovery, R2h = 2 hours in recovery. A: total magnesium plasma concentrations; B: ionised magnesium plasma concentrations; C: erythrocyte magnesium concentrations; D: total and ionised calcium concentrations; E: parathyroid serum concentrations; F: 1,25-dihydroxyvitamin D serum concentrations

*Mittelwerte und 95% Vertrauensintervalle von Blutparametern im Verlauf einer Dauerbelastung auf einem Laufband bei 10 Pferden. PE = Wert vor der Belastung, T1 bis T6 = Trabintervalle von 20 Min., R1h = eine Stunde nach Beginn der Erholung, R2h = 2 Stunden nach Beginn der Erholung. A: Totale Plasmakonzentration des Magnesiums; B: Ionisierte Plasmakonzentration des Magnesiums; C: Magnesiumkonzentration in den Erythrozyten; D: Totale und ionisierte Plasmakonzentration des Calciums; E: Serumkonzentration des Parathormons; F: Serumkonzentration des 1,25-Dihydroxy-Vitamins D*

Assuming that 90% of the body weight loss is due to water losses and that approximately 20–30% of the water is lost via expiration, it may be assumed that with the mean body weight loss of 21.9 kg observed in this study, 14 to 16 L of sweat were produced. Assuming a concentration in the sweat of 2–6 mmol/L for Ca and 1–6 mmol/L for Mg, an approximate amount of 1–4 g of Ca and 0.3–2 g of Mg may have been excreted with the sweat. The loss was reflected in the significant decrease of the plasma concentrations of  $Ca_{tot}$  and  $Mg_{tot}$ . In contrast to the situation in human athletes, urinary Mg loss is not increased in

tract and bone for Ca and bone, the gastrointestinal tract and soft tissues for Mg. During Mg deficiency in humans, the bone and skeletal muscle Mg pools can provide a Mg amount equivalent to 15% of total body Mg (Wallach, 1988). In contrast to humans, the rat skeletal muscle does not provide a substantial amount of Mg during Mg deficiency. Plasma  $Mg_{ion}$  and  $Ca_{ion}$  concentrations are not sensitive indicators of Mg and Ca body status, respectively. Knowingly, the blood pH influences the values of  $Mg_{ion}$  and  $Ca_{ion}$  because the extent of binding increases with increasing pH (Ecker and Lin-

dinger, 1995). No measurements of blood pH were made in this study and  $Mg_{ion}$  and  $Ca_{ion}$  concentrations were not corrected for the actual pH. The  $Mg_{ion}$  to  $Mg_{tot}$  ratio is relatively high in comparison to other species. Normally around 70 to 75% of the plasma Mg is ultrafiltrable, of which the main portion is ionised (Rude, 1993; Riond et al., 1995). The ultrafiltrable fraction consists of ionised Mg (65%) and Mg complexed with anions (8%; Sanders et al., 1999). The high  $Mg_{ion}$  to  $Mg_{tot}$  ratio observed in this study may be a particularity of horses or it may be related to interferences with the selectivity of the electrodes which is supported by the observed irregularities in the individual profiles of  $Mg_{ion}$ . The interferences could be caused by ions such as Ca or by lipids. Indeed, the lipid content of horse plasma is known to be high and through almost irreversible binding of the plasma lipid components to the membranes, the potential in the channels of the electrodes may be altered and thus spurious values may be obtained.

The factors that influence the plasma Mg concentrations have been documented in laboratory animals and humans (Durlach and Bara, 2000). Some of these factors may be relevant for exercising horses. For example, the concentrations of catecholamines are increased during exercise. High adrenaline concentrations are associated with a decrease of the influx of Mg into cells which results in a net efflux of Mg from the intracellular compartment (Durlach, 1980; Romani et al., 1993). Moreover, increased serum free fatty acids concentrations as a consequence of increased lipolysis which occurs during exercise induce a decrease in the Mg concentrations (Flink et al., 1979). The presumed mechanism is Mg chelation by the free fatty acids and/or fixation in the adipocytes. Also, the concentration of insulin is decreased during exercise. Insulin and insulin-like growth factor-1 (IGF-1) are known to translocate Mg from the extracellular space to the intracellular space (Durlach, 1980; Takaya et al., 1998). Thus, with low insulin concentration a net Mg efflux from the intracellular compartment may be expected. During exercise, high adrenalin and low insulin may contribute to a Mg mobilisation from the intracellular compartment.

The  $Mg_{ery}$  values of the present study are of the same order of magnitude of those already reported for horses (Walser, 1967; Büttner et al., 1998). Because of physical exercise the PCV of horses may increase tremendously, mainly as a consequence of splenic contraction and somewhat by dehydration. The increase of the PCV can be 25% (Persson et al., 1973; McKeever et al., 1993) so that more than half of the blood volume can be occupied by erythrocytes. With Mg deficiency,  $Mg_{ery}$  is decreased (Durlach and Bara, 2000). However, this decrease is more moderate than that in plasma of the animals tested and it is slow presumably because it is related to Mg concentrations in the medullary cavity of bones during hematopoiesis. In fact, it is generally accepted that there is no Mg exchange between erythrocytes and plasma although this dogma has been questioned. In the horses of this study, the significant decrease of  $Mg_{ery}$  at the end of the test suggests that Mg is released from the erythrocytes in response to the decreased  $Mg_{tot}$  or possibly as a consequence of the increased adrenaline and decreased insulin concentrations. This may be a particularity of equine erythrocytes because species differences exist in the physiology of these cells (Wheatley et al., 1994).

As demonstrated in horses by Estepa et al. (1998), the Ca decrease induced an increase in the serum concentration of PTH in the horses of this study. The increase of PTH concentration could partly be explained by dehydration. The lower  $P_i$  values especially at the end of test may possibly be explained by

higher  $P_i$  urinary excretion. The profile of  $P_i$  before, after and at the beginning of the exertion is similar to the findings of Snow et al. (1982); but they found the highest concentrations at the end of the exercise. The fact that serum  $1,25(OH)_2D$  concentrations remained unchanged can be explained by the fact that not enough time had elapsed (Goff et al., 1986; Holick, 1996) and by the low insulin and Mg concentrations which inhibit  $1,25(OH)_2D$  synthesis in the renal tubules (Durlach and Bara, 2000).

## Conclusion

The recommendations for the Mg requirement in horses are not sufficiently well defined (Pagan, 1998). It is thus not easy to make assessments on the adequacy of giving supplements for endurance rides. According to the results of the present study, additional Mg and Ca supply is superfluous in the case of a single prolonged exercise. Indeed, the body stores are sufficient to replace the Mg and Ca losses via sweat. These body stores have to be replenished during recovery. However, serum concentrations are influenced by the dietary supply. Higher serum concentrations are observed with a higher dietary supply which in turn influence the body stores (Riond et al., 2000). Therefore, a high dietary supply in hard working and heavily sweating sports horses may be beneficial to prevent the occurrence of critically low  $Mg_{ion}$  and  $Ca_{ion}$  values, which may have clinical consequences. It has to be noted that in the case of marginal supply for regular prolonged efforts, the efficiency of the mechanism for retention of Mg and Ca are activated.

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#### Acknowledgements

The authors gratefully thank Ms. Carmen Dettling, Ms. Brigitte Schmucki and Ms. Barbara Schneider for their skillful technical assistance and the Swiss National Stud for providing us the horses and the facility.

Dr. Daniel Weiss  
Prof. Dr. Marcel Wanner  
Dr. Jean-Luc Riond

Institute of Animal Nutrition  
University of Zurich  
Winterthurerstrasse 260  
CH-8057 Zurich

Tel.: 0041-1-6358812 (direct), 6358801 (secretary)  
Fax: 0041-1-6358932;  
e-mail: jriond@vetphys.unizh.ch