Potassium-free and potassium-containing electrolytes affect plasma ions and acid-base status of endurance horses

By

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(Abstract)

Effects of potassium supplementation were evaluated in four studies in endurance horses during races and treadmill exercise. In the first and second studies a potassium-free experimental formula was compared to potassium rich commercial formulas. The first study showed that supplementation increased plasma [K^+], and that the extra sodium in the potassium-free experimental formulas helped to attenuate acidosis at the end of the ride. In the second study supplementation also increased plasma [K^+], however speeds were lower and no increases were observed in plasma concentrations during the race. Supplementation of potassium during recovery helped to restore plasma [K^+]. Higher plasma [Ca^{++}] was found in horses supplied with experimental feeds, due to a lower dietary cation anion balance (DCAB). Three eliminated horses had heart rate arrhythmias and labile heart rates accompanied with higher plasma [K^+] and lower [Ca^{++}] than finishers. Also horses supplied with the experimental sodium-rich formula were less dehydrated than the ones receiving commercial formulas. The third study involved an 80 km endurance exercise test on the treadmill, and plasma [K^+] was affected by potassium supplementation during exercise and recovery. The supply of potassium caused higher plasma [K^+] helping to restore body stores. Also chloride supply in the electrolyte formulas maintained plasma [Cl^-] levels during exercise and affected plasma

concentrations during recovery. The fourth study showed that potassium supply affects plasma concentration, but also increases lactate production and glucose during sub-maximal exercise. A potassium-free electrolyte supply caused higher plasma [Ca^{++}] during exercise. Higher sodium supply in the potassium-free electrolytes improved hydration during exercise. These studies show that potassium should supplemented after exercise and but not be done during exercise because of the risk of increased neuromuscular excitability.

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Introduction

Endurance exercise is one of the most challenging activities performed by horses. It is a competition against the clock in which the horse exercises for hours under adverse conditions of weather and trail. Main recognized problems in this sport are dehydration, lameness, heart rate arrhythmias, myopathies, colic, thumps, and exhaustion. It is a challenge for riders to guide the horses in the most efficient way sparing energy. "To finish is to win."

Veterinarians have a determinant role in the recognition of latent clinical problems. They are the responsible for the well being of the horses. Riders also have their share in the maintenance of the horse's well being. The rider has to closely know about his horse in order to get the best performance from it and recognize any problems. Management of nutrition is generally in the hands of owners and riders, without scientific basis. Electrolyte supplements on the marked for example, but not many guidelines are given for their use. Products are on the market but not many guidelines are given for its use. Riders usually give these products in an empirical way. In recent years a greater interest has been given to clinical problems in endurance horses and how to prevent them. The wellbeing of horses became a goal for equestrian federations involved in national and international competitions. This dissertation mainly concentrates on the prevention of clinical problems related to increased neuromuscular hyperexcitability and dehydration.

Plasma [K^+] increases proportionally to exercise intensity despite sweat losses. With increasing extra-cellular [K^+] increases in the resting membrane potential occur and these can lead to increased neuromuscular excitability and related clinical signs. Therefore potassium supplementation should only be left until after exercise to replace sweat losses.

Introduction

The specific objectives of this study were:

1) to determine the effects of supplementation with a K-free electrolyte mixture on plasma [K^+] and acid-base status during endurance exercise, compared to K-containing mixtures. The results will test the hypothesis that K-supplementation increases the risk of neuromuscular hyperexcitability.
2) to determine the effects of chronic adaptation to different feed energy sources, fat and fiber versus starch and sugar, and any interactions with K and Na supplementation on acid-base status, strong ion difference, plasma [K^+] and [H^+], and the risk of neuromuscular hyperexcitability.

Literature Review

Endurance Races

History

Ancient times and selection of horses for endurance (Hyland, 2003)

One cannot talk about horse endurance history without making a brief overview about the horse in human life and conquests. Early equine usage falls into several categories. Initially the horse was hunted for meat. The first records of riding horses are still not clear. Once domesticated the horse had immense influence on human life, the most significant being its use in war. Other uses pertained to society's highest classes. The horse was a partner for hunt, and hunt was training for war. Egypt, Syria, the Bible lands, Asia Minor, near Eastern Mesopotamian tracts, and Armenia made military use of the horse between 1600 and 600 BC. Another use for the horse was the long distance trade in horses from the Maylan.

Horses also were used to drive chariots. Chariot horses performed mainly on level plains. Training of chariot horses can be likened to modern endurance training, and in some sections resemble interval training.

There are records of Mongol armies moving through the Carpathian Mountains in the invasion of Europe covering 60 miles per day (1241 BC), and Russian record shows a Kazkh horse that covered 66 miles in 4.5 hours traveling over desert terrain.

Initial training of the Assyrian and Kikkuli's horses was thought to select horses that had the toughest physical attributes. Incremental stress was part of the training, and only the toughest horses would have entered full training. Training was about 6 months long with hard work days and some rest periods. Day 178 of training was the toughest with five sections

totaling 75.5 km, with 46 km done at a fast speed with sections of 19 km. Such distances were covered without the use of shoes.

Persian kings and their armies obtained horses from many sources, mainly battles. It was remarkable how Persians became horsemen without a tradition of early horsemanship. The courier's mount was vital for speedy communications in the Persian Empire. Herodotus describes 1600 miles of Royal Post Road from Susa to Sardis. It was equipped with 111 post stations, each 15 miles apart. It is estimated that a galloping courier could cover the distance in a week. In Persia the horse was also an important war implement.

In the Macedonian Empire (359 BC-323BC) under Philip II and Alexander III, a consistent, extensive and successful hard use of the cavalry was made. It was remarkable how the Macedonian cavalry totaling about 4500 won a battle over the Persians that had 30000 soldiers. Macedonians by horse and foot were better disciplined than Persians and won the battle of Issus and other. A clutch of endurance rides, covered considerable distances and were run at high speeds. In one ride for a battle, Alexander covered 200 miles in eleven days, with five days of rest in between through uninhabited waterless desert. The speeds were not high, but without water, adequate food, with unshod hooves on abrasive sand it was truly a great achievement. Alexander died very early and his command was filled by the Romans. Later the German horsemen destroyed the Roman army (year 378).

The development of stirrups made war with horses easier and more effective (Kust, 1983). For agriculture the development of the collar was the tool needed for the horses work. Both developed in the middle ages. The horse continued to be important in war, conquests and agriculture until the beginning of the 19th century.

Horses in America

Literature Review

In the 16th century when Europeans discovered and conquered the Americas, the rulers of men were dependent upon the horse. Spanish conquered Central and South America with horses. Many horses were captured by Indians and it is believed to be the origin of the mustang and other wild horses of the west. Apaches and Navahos later possessed troops of mounted warriors. The conquistadores brought war horses to America, while the North Europeans brought a work horse. Farming and transport were uppermost in their minds. A lighter type of horse was brought to Virginia from Britain.

In the 20th Century the motor vehicles and tractors displaced the horse on the roads and farms. Cavalry was still important in the American Civil War. The Russians were still able to use cavalry effectively against Germans in the 2nd World War.

The pony express

Postal services begun as royal messenger organizations (Dent, 1974). The mail system of the Empire of Augustus was a vehicular one, a two horse carriage. Some services had to be done by riders. Mongol couriers described by Marco Polo were also horse driven. The Royal Mail in England was carried on horseback until 1784. In the USA the service described by Marc Twain in fact only ran for 2 years, 1860 and 1861. The route extended from St Joseph to Sacramento. Most riders were armed with a carbine and two revolvers. Nineteen hundred miles were covered in eight days. The mailmen rode 50 miles without stopping, keeping the horse at maximum speed for 10 miles. Coming to the station the horse was changed to another, and the rider continued. The horses were stripped from all unnecessary weight, wore a little saddle, and no visible blanket. The shoes were light or none at all. The little mail pockets were strapped under the rider's tights. There were about 80 pony riders in the saddle all the time, night and day, stretching from Missouri to California. The pony express was the inspiration for the

modern endurance races, although other races were held before. In the year 1893 a 1000 mile race was organized between Chadron, Nebraska and Chicago, Illnois. At the time some rules were established and riders had to take care of their horses (Coldsmith, 1979). In 1913 a group of breeders organized an endurance ride sponsored by the Morgan Horse Club in Vermont (Johnson, 1979). The purpose of the ride was to stimulate interest in the breeding and use of good saddle horses, and to learn and demonstrate the best methods of caring for horses during and after long severe work without artificial methods or stimulants. No horse was allowed to travel faster than 6 mph over the 154 miles. The horses were weighed at the beginning and end of the ride and the weight loss considered in the judging. The rides continued and by 1922 the distance to be ridden was 300 miles, with 60 miles per day. The 300 miles were covered in 5 days (5.5 mph). Participants included the US Cavalry, the National Steeple Chase and Hunting Association of the US, the Department of Agriculture, and the Thoroughbred Endurance Club. Morgan and Arabian horses predominated in these races. By 1923 pulse temperature and respiration were used as judging criteria. Annual rides were hold in Virginia and Colorado, but by 1927 enthusiasm decreased and the races stopped.

Wendell Robie is considered to be the founder of the first official endurance race in the World, The Tevis Cup, in 1955. The competition also was named the Western States Trail Ride and was inspired in the pony express (The XP Rides, 2004).

Present status and governing bodies

In the US most rides are supervised by the American Endurance Ride Conference (AERC, 2001). There are currently about 20 races a month, from March to December. The

other months have races in warmer regions. The races vary between 25 and 100 miles for one day rides. To be an official race, the minimal distance is 50 miles in one day.

The AERC was founded in 1972 and is considered to be one of the national organizations of endurance races in the US (AERC, 2001), the other is the United States Team. The International Equestrian Federation (FEI) is responsible for overseeing all international competitions (FEI, 2003). Among its functions it assures the wellbeing of horses, develops competent officials and assures open and fair competitions. The National Federations represent the nation within the FEI, promote FEI international competitions, assure that the competitions within the nations adhere to FEI rules and regulations, and approve the national teams and competitions from the nation.

An endurance ride is a competition to test the speed and endurance ability of a horse (FEI, 2003). It is a competition against the clock. The horse that finishes the course in the shortest time will in general be classified the winner of the competition. To be successful, the competitor will have to have knowledge of pace and proper use of his horse across country. The term “first examination” is used to denote a clinical examination carried out by a veterinarian to establish the general health status of a horse. The term “inspection” denotes the procedure used to decide whether horses are fit to participate and/or continue in an event or competition. The competition consists of a number of phases that are sections of the competition. At the end of each phase, at least every 40 km, there will be a compulsory halt for veterinary inspection. A 160 km competition must have at least 4 veterinary gates plus the final inspection, or four vet gates and a compulsory trot by. Veterinary gates are the places where veterinary inspections are undertaken.

The first examination takes place as soon as possible after the arrival of the horses at the stables of the ride. In FEI competitions it is performed by an official veterinarian. The aim is to establish the horses' identity, and to establish their sanitary condition. This examination can be coupled with the first inspection.

The fist inspection should whenever possible, take place on the day preceding the start of the competition and is performed by the veterinary commission. Criteria consist of:

- Heart rates: horses with abnormally high pulse rates will be eliminated. Any abnormal heart sounds must be recorded.
- Respiratory System: abnormalities in rate or character of the breathing deemed by the veterinary commission that endangers the welfare of the horse, will be cause of elimination.
- General condition: temperatures may be recorded and mucous membranes will be examined. Horses in a generally poor condition or with high temperature will be eliminated.
- Irregularity of gates: a horse with an irregularity of gait consistently observable at walk or trot under all conditions and thought to cause pain or threaten the athletic future of the horse will be eliminated at the first or final inspection or any inspection during the course.
- Soreness, laceration and wounds: any evidence of soreness, laceration and wounds in the mouth, on he limbs and on the body, such as girth and saddle galls must be recorded. If participation of continuation in the competition can aggravate such soreness, the horse will be eliminated.

Inspections at Compulsory Halts (Veterinary Checks):

Further inspections take place during the compulsory rest periods, after no longer than 30 minutes recovery following arrival at the veterinary gates. The inspection will determine the fitness of the horse to continue the competition.

- Heart rates: horses with a pulse rate above the maximum rate set in the schedule of the competition, after no more than 30 minutes recovery, or showing any abnormality of the heart or lungs, which could endanger its health, should be eliminated. The schedule of the competition should set limits of 64 beats per minute.
- General conditions: horses showing excessive fatigue, heat stroke, colic, myopathies, and severe dehydration, or abnormally high temperatures (40^{o}C or 104.8 ^{o}F) must be eliminated.
- Lameness: horses showing irregularity of gates must be eliminated.
- Soreness, lacerations and wounds: if these were noted at the first inspection and have been aggravated, or any new soreness, wound or laceration that can be aggravated by further participation, the horse must be eliminated.

Final inspection:

The pulse must be taken and registered on the vet card within 30 minutes of arrival at the finish line. The inspection is to determine whether the horse is still fit to continue and it will include the same control as the inspections during the course.

In FEI competitions three members of the veterinary commission must examine together all horses that completed the course within 2 hours and determine if any of them needs to be administered any invasive medication immediately. The horses that need treatment must be eliminated.

Literature Review

Veterinary Gate:

1. Veterinary checks during an endurance ride

The veterinary gate into a timed hold has proven to be the best veterinary check and is now internationally established.

2. How does a veterinary gate function?

A rider comes to a Vet Gate and a timer writes down the time of the arrival. The rider and his crew have no more than 30 minutes from that time to take care of the horse. The riding time is not stopped until the rider presents his horse at the gate to the vet area (in time) to have it checked by the veterinarians. The pulse of the horse must not exceed 64, otherwise he is sent back. Horses which do not meet pulse criteria within 30 minutes are eliminated. The hold begins when the rider asks to see the veterinarian, providing his pulse is down to 64 or less. In principle holds are between 15 and 40 minutes.

Veterinary clinical signs:

Pulse:

Pulse recovery with rest has become the main objective measure of fitness to continue (AERC, 1999). The pulse will indicate the degree of fatigue of a horse. In previous studies (Rose et al, 1977; Rose, 1986) showed that during endurance races the pulse has a direct relation with the degree of stress and biochemical parameters. Horses with 60 to 65 or higher beats per minute 30 minutes after arrival at the vet checks were more dehydrated, had more altered renal, muscle and hepatic functions, and had lower muscle glycogen stores. Irregular pulses and arrhythmias are signs of electrolyte disturbances (Flaminio and Rush, 1998).

Literature Review

Cardiac recovery index:

The ability of the horse's circulatory system to accommodate the level of exertion experienced at the event is monitored by use of the Cardiac Recovery Index (CRI) (AERC, 1999). A first heart rate is taken, and the horse is trotted 40 m back and forth. A second heart rate is taken exactly one minute after the first one was taken. A horse in good fitness will have a lower second heart rate. A tired or fatigued animal will have the second heat rate elevated by 5 to 20 beats per minutes (Ridgeway, 1993, 1999). The best interpretation will be made if the first heart rate is between 60 and 80 beats per minute. When this test is done under hot and humid conditions, the CRI will be higher compared to less severe climatic conditions (Harris et al, 1995).

Respiration:

Respiratory recovery depends on the weather conditions. Respiratory frequency will be elevated during hyperthermia, dehydration and fatigue. Panting under hot and humid conditions may be consistent with optimal performance (AERC, 1999). If pulse and other signs of recovery are prompt and progressive, panters will have a core temperature below 103.5^{o}F. Any horse with the temperature above 103.5^{o}F should have their signs closely monitored for other signs of fatigue. The respiration of animals with an oxygen debt will have deep breathing moving large amounts of air, different from panters, which move small amounts.

Body temperature:

Most of the energy produced during exercise is converted to heat. Rectal temperatures of 101-103^{o}F are tolerable, temperature above 103^{o}F for longer periods can be dangerous (AERC, 1999). If temperature stays elevated for more then 30 minutes it might be deleterious for the central nervous system.

Dehydration:

The persistency of a skin fold pinched at the point of the shoulder may indicate water loss in excess of 3% of the horses body weight (AERC, 1999). The skin pinch on the side of the neck is less reliable as a hydration marker since it can be easily influenced by the elasticity of the skin and fat content. Increased skin tenting, scat sweat, dry injected mucous membranes, and sinking of the eyeball are all signs of dehydration. When several of these signs are present it could be extreme fatigue or exhaustion.

Capillary refill and mucous membranes:

After blanching a spot on the gum just above an upper tooth with pressure of a thumb or a finger the time to return of full color normally would take about 2 seconds. Poor capillary refill denotes low blood pressure, or volume. Often poor refill and dry tacky mucous membranes denote dehydration (AERC, 1999).

Gut motility:

The diversion of blood from visceral to muscle circulation can cause diminished gut sounds or even complete ileus (AERC, 1999). Reduced gut sounds in an apparently healthy horse are of less concern than a horse that has other metabolic abnormalities. Both should be monitored closely. Hyper-motile gut sounds may be a prelude to an ileus or electrolyte alterations (high $[K^{+}]$ and/or low $[Ca^{++}]$).

Other signs:

Muscle fasciculations and cramps can occur as a result of higher plasma $[K^{+}]$, being a result of increased neuromuscular excitability. Thumps (synchronous diaphragmatic flutter) is

caused by the hyperexcitability of the phrenic nerve. It is generally associated with hypocalcaemia and metabolic alkalosis.

Recognition of the exhausted horse:

Any of the following signs may be present in a horse with metabolic fatigue (AERC, 1999).

- Delayed heart rate recoveries with pulse above 64 or labile pulse
- Poor cardiac recovery index
- Decreased gut sounds
- Mucous coated feces
- Lack of appetite
- No interest in the surroundings
- Depressed posture
- Lack of thirst in the presence of dehydration
- Anxious facial expression, glazed eyes
- Oblivious to external stimuli like insect bites, rider aids
- Loss of impulsion and elasticity of gait, ataxic or weak
- Skin pinch tented
- Mucous membranes showing dark margination, muddy color, dryness
- Poor jugular refill
- Flaccid anal sphincter
- Thumps
- Hyperthermia
- Myoglobinuria
- Myositis, muscle fasciculations, exertional rhabdomyolysis

- Increased digital pulses
- Colic

Myopathies:

Its occurrence during endurance rides may be related to dehydration and electrolyte imbalances, but may be heat related, transport stress related or a polysaccharide storage myopathy (AERC, 1999). The early onset cases (within the first 5-20 miles) are often the most severe and are likely to develop renal compromise.

Neuromuscular excitability

Resting membrane Potential (Ganong, 1991; Guyton and Hall, 2000):

The unequal distribution of ions on either side of the plasma membrane leads to an electrical potential across the membrane, the membrane potential. When nerve or muscle cells are not transmitting signals the membrane potential is in the resting state (resting membrane potential).

The membrane potential varies for different types of cells. The measured resting membrane potential in human skeletal muscle ranges from –65 to –90 mV (Campion, 1974), and it varies with fiber type. The ions that are primarily responsible for the membrane potential are Na^+, K^+, and Cl^-. They have different concentrations across the cell membrane and therefore generate the membrane potential.

Ions enter and leave the cell through "ion channels" located in the cell membrane. Some of these channels are "passive", so that ions may freely move diffusively through the channel. Some are "chemically gated", as in the sodium pumps which pump Na^+ (and some calcium, Ca^{++}) out of the cell, while pumping in potassium (K^+) in the ratio of 2 K^+ for every 3 Na^+

pumped out. This not only compensates for leakage of K^+ through passive channels, but also establishes a concentration gradient; but since K^+ and Na^+ have the same charge, it does not affect the resting potential. Still other channels are "voltage gated", where they remain closed at some potentials and open at others. Typical ion concentrations (mmol / liter) maintained in human motor neurons for the resting potential are:

Interior	Na = 15	K = 150	Cl = 10	large anions = 65
Exterior	Na = 150	K = 5	Cl = 110	large anions = .2

Another factor in determining the membrane potential is the relative permeability of the membrane for each ion. The relative permeabilities of the ions are 1.0 for K^+, 0.45 for Cl^-, and 0.04 for Na^+. K^+ has the greatest contribution to the membrane potential. The membrane potential can be calculated based on the contribution of each ion, based on the Goldman equation.

The Goldman Equation

$$E_m = \frac{RT}{F} ln \frac{P_K\left[K^+\right]_{out} + P_{Na}\left[Na^+\right]_{out} + P_{Cl}\left[Cl^-\right]_{in}}{P_K\left[K^+\right]_{in} + P_{Na}\left[Na^+\right]_{in} + P_{Cl}\left[Cl^-\right]_{out}}$$

P_K= permeability to K+ $[K^+]_{out}$ = K^+ concentration outside cell

P_{Na}= permeability to Na+ $[Na^+]_{out}$ = Na^+ concentration outside cell

P_{Cl}= permeability to Cl- $[Cl^-]_{out}$ = Cl^- concentration outside cell

R= Universal gas constant (8.31 joules/mole/deg. K)

T= temperature in degrees Kelvin (273+deg. C)

F= Faraday constant

P_{Na} is low relative to P_K , therefore Na^+ contributes little to V. Increases in the external concentration of K^+ decrease the resting potential. Since K^+ is the ion with the greatest

permeability and influence on the resting membrane potential the Nernst equation can be used to calculate it. The Nernst equation helps to give the value for the electrical difference necessary for a particular ion to be at equilibrium. Putting in numbers for this equation for body temp, $E_x = 61/z \log_{10} [X_o]/[X_i]$. Hence, the equilibrium potential will be different for each ion because of the equation, e.g., Na^+ and K^+ are present at different [ICF] and [ECF]. Since the membrane is most permeable to K^+, the resting potential for most cells is near to the equilibrium potential for K^+.

$E_K = 61.5 \log [K_O^+]/[K_I^+]$ at 37^oC

E_K being the equilibrium potential for K

In summary, to maintain the ionic concentrations of the resting membrane potential, Na^+ is actively transported out of cells and K^+ into cells. K^+ diffuses out of cells and Na^+ in. Due to the existence of K^+ channels, K^+ permeability is greater than Na^+ permeability. The cell membrane is maintained in a polarized state, with the outside positive in relation to the negative interior.

The Goldman equation takes in account the distribution of Na^+, K^+, and Cl^- and the permeability of the membrane to each of these ions. The Goldman equation describes the influences of the main ions on resting potential. However the main contributor to the resting membrane potential is K^+, therefore the Nernst equation can be used to predict the resting membrane potential.

Action Potential (Ganong, 1991; Guyton and Hall, 2000)

Nerve signals are transmitted by action potentials. An action potential is a rapid change in the membrane potential. The critical level of the cell membrane depolarization at which an action potential is initiated is the threshold potential. The first event of an approaching action

potential is the beginning of a depolarization of the membrane. After an initial 15mV depolarization, the rate of depolarization increases. This point corresponds to the threshold potential. Thereafter the potential reaches and overshoots the zero potential to approximately 35 mV. It then falls rapidly towards the resting level. When repolarization is about 70 % of resting, the rate of depolarization decreases.

Sub-threshold stimuli do not initiate action potentials but they have an effect on membrane potential. These stimuli lead to localized depolarizing potential, which is called electrotonic potential. The one produced at the cathode being called catelectrotonic and the one generated at the anode anelectrotonic. When the cathodal stimuli are great enough to produce about 15 mV depolarization, it reaches the threshold potential. This greater response at the cathode to stimuli of sufficient strength to produce 7-15 mV of depolarization is produced when Na^+ channels begin to open and it is called local response. These initiate a slight active contribution to the depolarizing process. However the potential decays, because the repolarizing forces are still stronger.

Ion movements during action potential

When a slight decrease in the resting potential occurs, an increase K^+ efflux and Cl^- influx occurs and restores the resting potential. When depolarization exceeds about 7mV the voltage-gated Na^+ channels open at an increased rate, and can reach the firing level. The influx of Na^+ is so great that it temporarily overrides repolarizing forces. During the action potential there is a great flux of Na^+ ions into the cell. The membrane potential rises rapidly into the positive direction. This phase is called depolarization. Soon the Na^+ channels enter a closed state called inactivated state and remain closed before returning to the resting state. Potassium voltage gage

channels open and K^+ gets out of the cell (increased K^+ conductance). This is the repolarization phase. A slow return of the K^+ channels to the closed state is called after-hyperpolarization.

Neuromuscular excitability

Neuromuscular excitability is the capacity of a cell to give rise to an action potential when driven by an adequate stimulus. Increases in extra-cellular potassium concentration change the extra-intra-cellular potassium ratio and the cell turns more depolarized and excitable. When K^+ gets too high, the membrane potential is held above threshold and the cell loses its excitability. This can occur during anoxia or hypoxia. The Na^+-K^+ pump needs ATP to pump K^+ back into the cell, which is low in those cases. Exercise also may cause hyperexcitability, since plasma or extra-cellular K^+ increases.

In cases where extra-cellular Ca^{++} becomes low, spontaneous firing occurs. The threshold gets closer to the resting membrane potential. Decreases in extra-cellular Ca^{++} concentration increases the excitability of nerve and muscle cells by decreasing the amount of depolarization necessary o initiate the changes in the Na^+ and K^+ conductance that produce the action potential. Ca^{++} stabilizes the Na^+ channels at normal concentrations, and when in low concentrations, Na^+ flows into the cell. (Neuroguide, 2004). Increases in extra-cellular Ca^{++} concentration stabilize the membrane by decreasing excitability. Decreases in extra-cellular Na^+ concentration decrease the size of the action potential, but has no effect on the resting potential.

Re-establishment of Na and K ionic gradients after action potentials

The Na^+-K^+ pump transports K^+ in and Na^+ outside the cell using energy. To maintain resting potential this pump continuously works, but the activity increases exponentially when Na^+ increases intra-cellularly.

A single action potential causes a brief contraction followed by relaxation. This response is called a muscle twitch. The duration of the twitch depends on the muscle fiber type. Fast fiber twitches have the duration of 7 ms, whereas slow fiber twitch duration can be of 100 ms.

Potassium in the body—amount and distribution

Most of the body potassium is intra-cellular (Patrick, 1977), where it has a similar function as sodium in the extra-cellular compartment in maintaining water content. There is however a great difficulty to assess intra-cellular potassium stores. Potassium is maintained in the cell by the Na^+-K^+ pump. Plasma potassium is a poor index of total potassium stores (Johnson et al, 1991). Erythrocyte potassium concentration has been suggested to be a good (Muyelle et al, 1984) or poor (Juel et al, 1999) indicator of total body potassium stores. Skeletal muscle potassium concentration has been shown to be a good indicator of potassium stores in humans (Carlmark et al, 1982; Ericsson et al, 1983) and in horses (Johnson et al, 1991). However when measuring intra-muscular potassium concentration it has to be noted that even the slightest injury can lead to an overestimation of the potassium concentration (Sejersted and Sjogaard, 2000).

The total potassium content of healthy adult horses has been estimated to be 28000 to 30000 mM (Tasker, 1967). Whole body potassium is depleted by decreased feed intake, diarrhea, nasogastric-reflux, acid-base imbalances, endotoxemia, sweating, lactation, and abnormal renal

function (Rose, 1981; Snow et al, 1982; Spurlock et al, 1985; Tasker, 1967 b; Traver et al, 1976). The amount of potassium stores in the muscle are about 70%, in the skeleton 5%, in the skin 3%, in the blood 3%, in the gastrointestinal content 10% (4-24%) and in other tissues 7% (Meyer and Coenen, 2002). Normal potassium concentrations in the body are 2.4-4.7 mmol/L in plasma (Orsini and Divers, 1998), 83-102 mmol/L in erythrocytes (Muyelle et al, 1984), 91.06 +/- 2.96 mmol/L wet weight (Johnson et al, 1991), and 304 +/- 21 mol/Kg dry weight muscle (Gottlieb-Vedi et al, 1996).

Absorption and excretion of potassium

Potassium is mainly (52-74%) absorbed in the small intestine (Frape, 1999; Hintz and Shriver, 1976). Absorption at the small intestine occurs in the villous epithelium (White, 1990). Potassium is absorbed passively by the small intestine as the volume of intestinal contents decrease. Ions move between gut and blood by transcellular and paracellular pathways. The Na^+K^+ pump on the basolateral membranes of the absorbing epithelial cell maintains low Na^+ intracellular levels and negative membrane potential. High K intracellular concentration is maintained. Diffusion through paracellular pathways in the small intestine is the primary mechanism by which cells absorb K^+, from the diet or from secretion. In the colon the apical and basolateral membranes are permeable to K^+. Because of the high concentration of K^+ in the cells some K^+ leaks passively across the apical membrane of epithelial cells. Factors that elevate intra-cellular K^+, such as aldosterone-stimulated Na^+ absorption, increase K^+ secretion in the large intestine.

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Kidney and gut and K^+ excretion

Potassium excretion is determined by the sum of three processes: 1) The rate of potassium filtration (glomerular filtration rate multiplied by the plasma potassium concentration). 2) The rate of potassium reabsorption by the tubules. 3) The rate of potassium secretion by the tubules (Guyton and Hall, 2000).

The rate of potassium filtration is usually constant because of auto-regulatory mechanisms. Severe decreases in glomerular filtration rates can occur in certain diseases and cause serious potassium accumulation. In normal individuals 65% of the potassium ions are filtered across the glomeruli then reabsorbed by the proximal tubules. Twenty five to 30% of the filtered potassium is reabsorbed in the loop of Henle, where it is co-transported with sodium and chloride.

The most important sites for regulating potassium excretion are the distal tubules, where these segments potassium can be reabsorbed or secreted. With high potassium intakes the extra potassium excretion is achieved almost entirely by increasing the secretion at the distal collecting tubules. When potassium ingestion is reduced, potassium excretion can fall considerably (Meyer et al, 1986). Excretion is proportional to alterations in the intra-cellular $[K^+]$ in the renal tubular cells, and it occurs through diffusion down a favorable electrochemical gradient. When Na^+ is reabsorbed through aldosterone stimulation, the trans-membrane potential will increase further and so will K^+ excretion. An inverse relationship between Na^+ and K^+ excretion in urine is due to the above mechanisms.

Secretion of potassium from the blood into the tubular lumen is a two-step process. First uptake from the interstitium into the cell occurs by the Na^+-K^+ ATPase pump in the basolateral membrane. It moves Na into the interstitium, and K into the cell. The second step is passive

diffusion of K^+ from the interior of the cell into the tubular fluid. The high intracellular [K^+] is the driving force for the diffusion. The luminal membrane of the principal cells is highly permeable to K^+.

The cells in the distal and cortical collecting tubules are called principal cells. The primary factors that control K^+ secretion by the principal cells are the activity of the Na^+-K^+ ATPase pump, the electrochemical gradient for K^+ secretion from the blood to the tubular lumen, and the permeability of the luminal membrane for K^+. Increased extra-cellular [K^+] stimulates Na^+-K^+ ATPase and increased amounts are secreted into the tubule. Increased [K^+] will also lead to an increase in the gradient between the renal interstitium and the interior of the epithelial cell. Finally increased [K^+] stimulates aldosterone, which further stimulates K secretion by stimulating Na^+-K^+-ATPase. Increased tubular flow rate as in volume expansion, high sodium intake, or diuretic drug treatment stimulates K^+ secretion. During high flow rate, secreted K^+ is flushed down the tubule and its concentration in the tubule remains low.

During potassium depletion the intercalated cells can reabsorb K^+ from the collecting tubules. The mechanism thought to be involved is the hydrogen-potassium ATPase pump localized in the luminal membrane. The transporter reabsorbs K^+ in exchange for H^+. The K^+ then diffuses from the basolateral cells to the blood.

During an 80 km simulated endurance ride (Snow et al, 1982), urine K^+ losses have been reported to be 257 mmol/L, in a total of 2.8 L of urine. During this ride, however, horses were not supplemented with electrolytes, which could have changed urine electrolyte concentration.

The equine gut has been thought to be a reservoir for electrolytes (Meyer et al, 1982). Aldosterone also regulates fecal K^+ excretion (Clarke et al, 1992). During a 5-week K-depleting experiment (Meyer et al, 1986) the concentration of K^+ decreased in feces and urine. Horses

were supplied with 3-4 mg/kg BW/day of K during the depleting experiment. Values, however, did not decrease below 25 and 20 mmol/L for feces and urine, respectively. The K^+ concentration was significantly reduced in the gut contents, mainly in the descending colon and there was a direct relationship to plasma [K^+]. During the depletion period exercise tests were undertaken and K^+ excretion increased in feces and urine during exercise. Total body K^+ contents revealed a reduction of 13% during the 5 week depletion period. Skeletal losses were major, whereas heart, skin and gut losses were lower. Muscle potassium losses were about 9%.

Losses in sweat

Measured sweat concentration of K^+ in horses have been reported to be about 53 +/- 3.96 mmol/L during endurance training in ambient temperatures between 30 and 40^{o}C (Carlson and Ocen, 1979). In a controlled study on the treadmill, values of 35 mmol/L (McCutcheon et al, 1995) during exercise at 50% of $VO_{2\ max}$ in hot and dry ambient conditions were measured. Values decreased from 49 +/-5 to 25 +/- 1 mmol/L (Kerr and Snow, 1983) during endurance exercise at 18 km/h for 80 km. In this study authors reported that contamination increased initial values and corrected values were reported separately as 32 +/- 2 mmol/L (Snow et al, 1982). During a five week K^+ depletion experiment (Meyer et al, 1986) sweating rate was significantly reduced during exercise, sweat [K^+] however was not reduced. Equine sweat [K^+] is higher than plasma [K^+]. Estimated losses during a 160 km endurance race, considering a 100 L of sweat loss (Marlin and Nankervis, 2004) would be 3500 mmol of K^+. This would be mainly from the muscles during contraction, but would be available from the gastrointestinal tract reservoir if the animal drank during the race (Meyer et al, 1986). Different measurements are partially due to different collection techniques during the studies.

Effects of potassium intake

Studies in ponies indicate that increases in K^+ intake lead to increases in urinary excretion, however, increased retention and apparent digestibility (Hintz and Schryver, 1976). Fecal excretion and serum K^+ levels were greater when ponies were fed highest levels of K. The kidney is the primary pathway of K excretion. Daily requirements for K were calculated to be 6g/kg DM intake.

The effects of the adaptation of an increased K^+ intake in horses was investigated in regard to exercise and post-exercise K^+ and fluid balances (Jansson et al, 1999). Two levels of K^+ (4.1 and 5.4 mmol/kg BW day) were fed to 4 Standardbred horses during 17 days in a crossover design study. The effects were studied at rest and in response to 29 km of exercise. Adaptation to increased K^+ intake was complete within 24 hours after diets were introduced and consisted of increased urinary and fecal K^+ excretion. There were no differences between the treatments during or after exercise in plasma [K^+], only a higher sweat [Na^+] and a lower Na^+ urinary excretion in the higher level K^+ group.

Currently the daily potassium requirements for maintenance are estimated to be 0.05 g/kg BW, or 1.52 g/Mcal of DE (NRC, 1989). For working animals the requirements are 1.1, 1.4, and 1.8 times maintenance for light, medium and hard work, respectively. In a growing horse 1.5 g of K is needed per kg of gain for skeletal growth in addition to the maintenance requirements.

Forages and oilseed meals generally contain 1-2% of potassium in the dry matter, and cereal grains contain 0.3-0.4%. For a 500 kg horse ingesting 2% of its weight as hay, the daily K ingestion would be 100 g, above the daily requirements for a hard working horse. Assuming a

50% grain: hay diet, the daily K^+ intake would be 65 g. In both cases the requirements would be met. In cases of prolonged exercise, sweat losses would have to be replaced.

Potassium regulatory mechanisms

Potassium regulation involves two control systems: external and internal balance. External balance is determined by the difference between potassium intake and excretion. Internal balance refers to the distribution of potassium between the extra-cellular and intra-cellular fluids (Cox et al, 1978).

Extra-cellular potassium concentration is regulated at approximately 4 mmol/L and a precise regulatory mechanism is necessary because many cell functions are sensitive to $[K^+]$. An increase of plasma $[K^+]$ of 4 mmol/L, caused by supplementation of K or by exercise could lead to cardiac arrest and fibrillation (Guyton and Hall, 2000). Failure to rapidly get rid of the extra potassium could lead to life-threatening hyperkalemia.

On the other side, even small losses of K^+ from extra-cellular fluid could lead to hypokalemia. Maintenance of potassium balance depends mainly on excretion by the kidneys, which adjust potassium excretion rapidly and precisely.

The distribution of potassium between the extra and intracellular compartments also is important for its homeostasis. The intra cellular compartment can serve as an overflow site for extra-cellular K^+ during hyperkalemia, or as a source of K^+ during hypokalemia. The redistribution between extra and intra-cellular compartments is a first line defense against changes in extra-cellular concentration.

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After ingestion of feeds containing potassium, plasma K^+ concentration rises, and the ion moves rapidly into the cells until the kidneys can eliminate the excess. Mechanisms in this process involve: insulin, aldosterone and β-adrenergic stimulation.

Insulin simulates cell potassium uptake after a meal. Increased potassium intake stimulates aldosterone secretion, which increases potassium cellular uptake. Increased secretion of epinephrine can cause the movement of K^+ into cells, by the activation of β_2-adrenergic receptors.

Acid-base abnormalities can cause changes in potassium distribution. Metabolic acidosis can increase the extra-cellular $[K^+]$, by causing potassium loss from the cells. Metabolic alkalosis decreases extra cellular $[K^+]$. An increase in hydrogen concentration reduces the activity of the Na^+-K^+-ATPase pump, reducing extra-cellular K^+ absorption into the cell.

Insulin

High concentrations in potassium stimulate insulin release from the pancreas *in vivo* and *in vitro* (Gomez and Curry, 1973, Hiatt et al, 1972). However potassium increases caused by exercise did not elicit insulin secretion in dogs (Knochel, 1977). The lowest dose of insulin necessary to stimulate K^+ uptake by cells is about 4μU/ml (Andres et al, 1962; Zierler and Rabinowitz, 1964). The effect of insulin on net cellular K^+ uptake depends on serum insulin level. The K uptake by cells is mediated by the Na^+-K^+-ATPase (Gavryck et al, 1975).

Aldosterone

Acute or chronic administration of potassium increases, and potassium depletion decreases aldosterone secretion in humans and animals (Boyd et al, 1973; Himathongkam et al, 1975; McCaa et al, 1975; Williams et al, 1970). Potassium-stimulated aldosterone secretion is

directly related to the medium potassium concentration. Potassium is a potent and directly acting aldosterone secretagogue (Cox et al, 1978).

Aldosterone increases have been reported after high intensity exercise in horses (Masri et al, 1990). This increase may have been stimulated by increases in plasma [K^+], although it was not measured in this study.

Acute intravenous administration of aldosterone in horses (Jansson et al, 2002) induced increased excretion of K in feces but not in urine and sweat. Plasma [K^+] was lower after aldosterone administration. It also caused reduced Na excretion by 99 and 72% in urine and feces, respectively but not in sweat. In humans aldosterone causes a reduction in sweat Na^+ (Guyton and Hall, 2000).

Epinephrine

Epinephrine also influences potassium homeostasis. Physiologically hyperkalemia occurs during exercise. Epinephrine stimulates K^+ uptake by muscles, and this effect is mediated by adrenergic stimulation. One cellular mechanism by which adrenergic agents might affect the uptake of potassium is at level of Na^+-K^+-ATPase (Hays et al, 1974).

Potassium during exercise

Recordings from isolated muscle preparations have shown that each stimulation or action potential is associated with K^+ release from the muscle cells. The amount of K^+ released will depend on the amplitude of the action potential and the fiber depolarization area (Serested and Sjogaard, 2000). Interstitial [K^+] is regarded as the most important factor for muscle cell excitability. Venous [K^+] should be at least as high as interstitial [K^+] in a well perfused muscle.

However differences have been reported to be as high as 3mmol/L (Gerbert et al, 1972; Hirche et at, 1980).

The highest venous [K^+] has been measured in humans after maximum exercise, and values of 8.3 – 9.0 mmol/L have been recorded (Medbo and Sejersted, 1990, 1994). Arterial [K^+] as high as 9 mmol/L have been found in Thoroughbred horses (Harris and Snow, 1992) during high intensity exercise. Microdialysis technique has been used in humans to obtain a more accurate account of the amount of K^+ released during muscle contraction, however, all techniques that involve introduction of needles or hollow fibers into the muscle tissue are prone to biased measurement of true interstitial [K^+] (Serested and Sjorgaard, 2000).

Under basal conditions K^+ release during one action potential ranges from 2-9 μmol/kg wet weight muscle. The major K^+ release occurs through the delayed rectifier K^+ channels. Variations of amounts released are due to fiber surface to volume ratio differences between species. K^+ release is also higher in fast twitch fibers compared to slow twitch fibers. There is a close relation between venous [K^+] concentration and interstitial [K^+] concentration in well perfused muscles (Serested and Sjorgaard, 2000).

The main mechanism for K^+ re-uptake by muscles is the Na^+-K^+ pump. At rest only 2-6% of the pump capacity is utilized. The Na^+-K^+ pump activity is greatly enhanced during exercise. Catecholamines and increases in intra-cellular [Na^+] stimulate the pump activity. During exercise maximum Na^+-K^+ pump capacity can be obtained. However during exercise venous [K^+] increases, and it is thought to be because of inadequate pump capacity (Verburg et al, 1999). Increased plasma [K^+] with exercise has been reported in numerous species (Chutkow, 1973; Hirche et al, 1980; Hnik et al, 1976; Medbo and Sejersted, 1990; Nold et al, 1991; Sejersted et al, 1982; Sjorgaard, 1983). The net loss of K^+ from exercising muscle is the result of

K^+ release from muscle cell surpassing K^+ uptake. Direct quantification can be obtained by measurements of arterial [K^+] and venous [K^+] together with blood flow on the exercising muscle, or from the analysis of cellular [K^+] per dry weight of muscle. Some investigators use the difference between the arterial and venous [K^+]. This calculation would only be correct if there is a fluid steady state, and assuming that [K^+] is equal between lymph and venous blood.

The state of training affects plasma [K^+] during exercise. Training results in blunting of exercise induced rise in [K^+] (Kjeldsen et al, 1990; McKenna et al, 1993; McKenna, 1995; McCutcheon et al, 1999). This appears to result from an increased activity or concentration of Na^+- K^+ pump in skeletal muscle (McKenna et al, 1993; McCutcheon et al, 1999).

Plasma [K^+] increases proportionally to exercise intensity (Castellino et al, 1987; Harris and Snow, 1992; Juel at al, 1990; Medbo and Sejersted, 1990; Wilkerson et al, 1982). The smallest increase in exercise intensity in horses to cause an increase in plasma [K^+] is 4 m/s. During a simulated endurance race horses traveled at 4.16 m/s and increases were reported at 20 and 42 km of the race (Nyman et al, 1996). In a treadmill study where horses exercised at 3.7 m/s, increases in plasma [K^+] were also reported (Marlin et al, 1998). In another treadmill study horses galloping at 8 m/s showed a plasma [K^+] increase of 160% (Harris et al, 1995b). During a 100 km endurance race horses run at an average speed of 4.44 m/s had plasma [K^+] which was higher than rest (Sloet et al, 1991). During a high intensity exercise study (Harris and Snow, 1992) plasma [K^+] was proportional to exercise intensity ($r = 0.92$). Endurance races with speeds slower than 4 m/s or at lower intensities did not show increases in plasma [K^+] (Delar et al, 1982, Lucke and Hall, 1978).

Plasma [K^+], fatigue and neuromuscular hyperexcitability

Moderate increases in interstitial [K^+] may cause force potentiation but at high concentrations force development is suppressed (Boklay et al, 1995, Cairns et al, 1995) or at even higher concentrations contracture develops (Dulhunty, 1980). Increases in interstitial [K^+] may develop in combination with a decrease in cellular [K^+], which can depolarize the sarcolemma beyond -60 mV (Juel, 1986, 1988a, 1988b). In a mouse study when resting membrane potential reached between -60 and -55mV a marked force decline occurred (Cairns et al, 1997). It has also been argued that changes in the ion concentrations are more pronounced in the t tubules (Westerblad et al, 1990). Other studies indicate that extra-cellular [K^+] is a factor for the development of fatigue (Bangsbo et al, 1996, Lindinger and Heigenhauser, 1991). Other factors like acidity and lactic acid have recently been discredited as factors inducing fatigue under certain conditions, as these factors actually protect muscle from fatigue (Allen and Westerblad, 2004; Pedersen et al, 2004). Increases in extra-cellular [K^+] will lead to increases in resting potential therefore excitability will be increased.

Plasma [K^+] decreases rapidly once exercise ceases and encountered values are lower than resting ones (Kowalchuk et al, 1988, Lindinger and Sjogaard, 1991). The instantaneous termination of K^+ release from contracting muscles due to cessation of action potential propagation, while the Na^+-K^+ pump is still very active, leads to a faster K^+ uptake and plasma [K^+] drops rapidly. When interstitial [K^+] reaches 4 mmol/L, pump activity is reduced. Plasma [K^+] values often below rest within 3 minutes of exercise cessation and recover slowly back to resting level (Melbo and Sejersted, 1990, Lindinger and Sjogaard, 1991). Numerous authors reported decreases in equine plasma [K^+] after and during endurance races, incriminating sweat losses for the decreases (Delar et al, 1982, Carlson and Mannsman, 1974, Lucke and Hall, 1978,

Lindinger and Ecker, 1995, Barton et al, 2002). However, authors collected blood between 5 and 30 minutes of exercise cessation, or did not have precise timing for blood collection. Sampling time after exercise ceases is crucial to evaluate plasma [K^+] changes.

Potassium turnover in the body

In a study done in resting horses weighing in average 450 kg input and output of K^+ were measured (Tasker, 1967). Feces and urine daily outputs were 993 and 2196 mmol of K^+, respectively. Daily ingestion of hay amounted 3930 mmol of K^+. Total body stores in a horse are about 29000 mmol. Seventy % or 20300 mmol of K^+ are in muscle, 3% (870 mmol) in blood, 10% (2900 mmol) in the gut, 5% (1450 mmol) in the skeleton, 5% (1450 mmol) in the skin, and 7% (2030 mmol) in other tissues (Meyer and Coenen, 2002). During exercise plasma [K^+] increases proportionally to exercise intensity and glycogen brake down rate. Some of the K^+ will be taken up by non-contracting muscles (Schott et al, 2002). Some of this K^+ will be lost in sweat, which concentration has been measured between 35 and 53 mmol/L (Carlson and Ocen, 1979; McCutcheon et al, 1995; McConaghy et al, 1995). During an endurance race lasting 10 hours, where sweating rate was around 10 L of sweat per hour, losses would be 5300 mmol of K^+ (assuming the maximal measured values). These ions would have to be replaced after exercise. About 8 kg of hay (1% of K) would provide 2051 mmol of K^+. The rest could be replaced in form of K salts. Part of the K lost in sweat would be replaced with the absorption of K^+ from the gut. After exercise ceases plasma [K^+] decreases below resting values. Na^+-K^+ pump is still very active when exercise stops, and leads to a faster K^+ uptake and plasma [K^+] drops rapidly. Plasma [K^+] values often below rest within 3 minutes of exercise cessation and recover slowly back to resting level (Melbo and Sejersted, 1990, Lindinger and Sjogaard, 1991).

Literature Review

Energy

Efficiency- feces, heat, acid, water

Energy is often defined as the capacity of a system to work (Lehninger, 1965). Energy efficiency can be defined biochemically as the relative efficiency of ATP generation. This will affect oxygen consumption, carbon dioxide production, and the speed at which heat is released. During work muscle metabolism transforms chemical energy into kinetic mechanical energy with the production of acid and heat (Kronfeld, 2001a).

FUEL + OXYGEN => WORK + ACID + HEAT

Work in the form of muscle contraction depends on oxidation, which involves transfers of hydrogen ions or electrons. Oxidation refers to the donation of electrons, whereas reduction accepts electrons. Oxidation implies acid formation. Fatigue has been involved with acid formation. More acid formation reflects inefficiency, and acidosis tends to impair metabolic efficiency. Another inefficiency of work is heat formation. Heat accumulation will lead to peripheral fatigue in the muscular system and centrally in the brain. Excessive heat will lead to sweating, which is the main heat loss mechanism (Hodgson et al, 1993) and consequently water losses that lead to dehydration.

The free energy change of a reaction is the energy released in a reaction or process that can be harnessed to something useful (Houston, 2001). Examples from a biological prospective are muscle contraction or moving ions across a membrane. When free energy release is negative the free energy change is exergonic, and when positive endergonic. A reaction with a positive value can not occur by itself. ATP breakdown (hydrolysis), oxidation of fuels, and glycolysis are examples of biological exergonic reactions, whereas protein synthesis, creating ion gradients across membranes, and storing of fuels are examples of endergonic processes.

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In any chemical reaction the change in energy of the reactants when they are turned into products is called enthalpy change. This can be measured as the total heat energy change. When heat energy is given off or released in a reaction the reaction is exothermic, and when energy is gained the reaction is endothermic. The oxidative reactions of the body are exothermic and are linked by energy capture in form of P to endothermic reactions. The efficiency of energy capture during the oxidation of different substrates can be calculated from their heats of combustion and the number of ATP molecules generated per unit of substrate. The yield of ATP from glucose oxidation is 36 ATPs (Houston, 2001).

Energy partition

For athletes the loss of energy as heat, or heat production (H_T) has a great importance. For farm animals H_T is partitioned into maintenance heat (H_m), and production work heat (H_W). H_T is partitioned at fasting heat production (FHP), which is similar to net energy for maintenance (NE_m) (Kronfeld and Harris, 1997).

Metabolic efficiency

The relationship between rate of energy retention and metabolisable energy intake is called efficiency of utilization of metabolisable energy (ME) (Blaxter, 1989). The heat for maintenance (H_m), varies with the diet and nutrients arising from a diet. Dietary protein yields amino acids, dietary fat yields long –chain fatty acids (LCFA) and glycerol, which accounts for 5% of the ME of fat. Dietary carbohydrates are divided into these which are hydrolyzed, yielding glucose, and those which are fermented, yielding short chain fatty acids (SCFA). NE_m is different for each nutrient. SCFA and amino acids are more thermogenic than LCFA and glucose. Heat above maintenance develops from work. The work efficiency (k_W) varies with the

proportion of glucose or LCFA. Glucose oxidation predominating at higher intensities (k_W= 0.228) and LCFA at lower intensities (k_W=0.245).The difference is 7% but leads to a 45% higher heat production per unit of NE_w in glucose oxidation. The use of LCFA is an advantage for exercising horses, which generate a lot of heat.

Exercise and heat production

During exercise the inefficient metabolic conversion of chemical energy into mechanical power, leads to build up of metabolic heat. Only about 20% of the total converted energy is available for muscle contraction (Astrand and Rodahl, 1986), and the rest is converted to heat. As the metabolic heat produced increases, thermoregulatory mechanisms are activated to maintain body temperature in a tight range (Hodgson, 1994). Compared to humans horses have a larger metabolic capacity, however a lower surface area: body mass ratio (1:35-40 for humans and 1:90-100 for horses) (Hodgson et al, 1993). This fact will make thermoregulation more difficult for horses. However, despite these disadvantages, the effects of thermal stress are avoided under most circumstances.

Oxygen consumption provides a direct indication of metabolic rate and heat production. During moderate exercise, for example endurance exercise (2.5-6 m/s), the metabolic rate is about 30-40% of maximal oxygen consumption (VO_{2max}) (40-60 ml/kg/min). During the cross country phase of the 3-day event, exercise intensity can be greater than 75% of VO_{2max}. During Thoroughbred races, intensities may reach above VO_{2max}, 130-190 ml/kg/min (Rose et al, 1988, 1990; Hodgson et al, 1993). From the measurements above, the energy production per minute (Mcal/min) can be calculated.

$M = VO_2$ (l/min) * k * exercise duration (min), where k is the amount of heat liberated per L of oxygen (5 kcal/L)

The theoretical effects of exercise-induced heat increase on the body temperature can be made from the energy production necessary to increase body temperature 1 ^oC. The specific heat capacity for humans is 3.5 kJ/kg/min. Using this value for horses exercising at 5m/s, consuming 25 L O_2/min, it would produce 400kJ/min, and it would mean a gain in body temperature of 0.25-0.30^oC per minute. In a racing Thoroughbred the gain would be 1^oC/ min.

During treadmill exercise at intensities of 40, 65, and 90% of VO_{2max} during 40, 15, and 10 minutes respectively, body temperatures were measured and heat loss evaluated (Hodgson et al, 1993). Exercise at 40% of VO_{2max} resulted in the blood temperature of the pulmonary artery increasing from 38 to 40.4^oC, from rest to the end of exercise. It was calculated that 93% of the energy generated was dissipated. During exercise at 65 and 90% of VO_{2max}, blood temperature increased in the pulmonary artery to 41.7 and 42.8^oC, respectively, meaning 91 and 86% of heat dissipation. The ambient temperatures were 21-23.5^oC, and the air speed blown against the horses 2m/s. During this experiment it was also shown that 5 minutes after exercise started, muscle temperature increased 1.3, 2.9, and 4.2^oC, for exercise at 40, 65, and 90% of VO_{2max}, respectively. These increases represent a lag phase between the time exercise starts and when heat dissipation gets activated.

Moderate temperature increases during exercise have some advantages. Muscular performance will be increased up to 40^oC (Astrand and Rodahl, 1986). Other advantages are increases in the maximal heart rate (Goetz and Manohar, 1990), a shift of the oxyhemoglobin to the right (Reeves, 1980), which could mean increased O_2 delivery to muscles during work.

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Mechanisms of heat dissipation

Four mechanisms for heat dissipation in the exercising horse exist (Carlson, 1983). Radiation is the transfer of heat from the body surface to surrounding surfaces (through vacuum) via electromagnetic waves. Convection is the loss of heat due to the movement of air surrounding the horse. Wind blowing across the horse's skin causes convective heat transfer. At low ambient temperatures convection makes considerable contributions to heat dissipation. However, when ambient temperature rises and approaches the horse's temperature, this mechanism becomes less effective. When a horse is swimming heat transfer by radiation and convection are increased. Conduction is the direct transfer of heat between surfaces in contact, for example from muscle to other body tissues. During exercise conduction of heat through the circulatory system is the main mechanism by which heat is transferred from muscle to skin and respiratory system. Evaporation is the primary mechanism for heat dissipation in exercising horses. Evaporation from the skin and from the respiratory tract contributes to heat loss (Schroter and Watkins, 1989; Heileman et al, 1990; Hodgson et al, 1993). Evaporation of sweat from the body surface consists of an endothermic reaction. The evaporation of one liter of sweat would balance the heat produced during 7-8 min of endurance exercise. The driving force for sweat evaporation is the skin-ambient vapor pressure difference. Effective sweating depends on air movement over the skin and a vapor pressure difference between the surface of the skin and the air. Sweating is most effective in hot dry temperature, and almost ineffective when the air is saturated with water vapor.

The sweating stimulation depends on thermal stimuli and sympathetic nervous stimulation through adrenaline secretion. The latter can be caused by exercise or excitement.

The former can be initiated by an increase in skin temperature (Jenkinson, 1973; Snow, 1977; Kerr and Snow, 1983).

Sweating rates in humans during exercise have been measured to be above 1.5 L/h (Costill, 1977; Gisolfi et al, 1977). In horses a sweating rate of 15 L/h has been reported (Hodgson et al, 1993, Scott et al, 1996). Maximal sweating rate per unit of skin in humans is about 1 $L/m^2/h$ whereas in horses 3 $L/m^2/h$. Horses compensate for having a low body surface area: body mass ratio and a high heat production, sweating three times the rate of humans.

Evaporation of water across the respiratory tract can account for 15% of heat losses. Inspired air becomes saturated when it passes through the respiratory tract. Exhaled air can be 85% saturated (Heileman et al, 1990). Estimates of heat loss through the lungs can be made. Temperature differences of 0.1-0.2 ^oC have been found between the pulmonary and carotid arteries (Hodgson et al, 1993). From direct measurements of water loss through the respiratory tract (Heileman et al, 1990) and the difference in the pulmonary vessel temperatures (Hodgson et al, 1993), respiratory heat loss accounts for 25% of the energy produced during exercise (Hodgson, 1994).

The cardiovascular system has a major role in thermoregulation. Blood flow transfers the heat via conduction. Blood flow can be deviated to the skin, and increase cardiac output (Hodgson, 1994). By increasing blood flow to the skin there is transfer of heat from the core. Blood flow will provide the heat for vaporization and supply fluid for sweat. The vascular system is arranged to supply capillary beds, veins and arteriovenous anastomoses. The arrangement of the vascular bed increases the area of blood flow.

In horses exercising on the treadmill at speeds similar to endurance races, a temperature difference of 2.5^oC was observed between the blood in the carotid artery and superficial thoracic

vein (Hodgson et al, 1993). However when sweating rate is too high it may surpass evaporation and sweat drips on the floor. When sweating leads to dehydration greater than 5% of body weight, heat loss via evaporation may be reduced (Hodgson, 1994) because the organism favors the deviation of blood to working muscle. This will compromise thermoregulation and thermal stress will increase.

Exercise and sweat losses

Different techniques have been utilized to measure or estimate sweat losses in horses. Older studies used collection directly from the skin or gauze pads placed under the saddle blanket (Carlson and Ocen, 1979; Rose et al, 1980). More recent studies used sealed polyethylene pouches on shaved areas of the horse's body (McCutcheon et al, 1995). Dew point hygrometry has also been used with results being similar to sweat collection from the pouches described above (Kingston et al, 1997). A study comparing different methods of sweat loss measurements concluded that the calculation of ion losses from a mean body sweating rate extrapolated from a measured local sweating rate or from change in net exchangeable cation content provide similar results (Kingston et al, 1999). The calculation of ion losses from changes in extra-cellular ion content derived from plasma total solids and ion concentrations results in underestimation of losses. Sweating rates have been estimated from weight losses during exercise. Ninety % of weight losses during exercise are due to sweat losses (Carlson, 1983). Moderate exercise (3.5 m/s) for 6 hours resulted in 5-6% loss of body weight, with one horse losing 9.1% body weight (Carlson, 1983). In another study during cool weather conditions, horses run 80 km at 18 km/h and lost 7.6 ± 0.5% (Kerr and Snow, 1983). During the Tevis ride one horse was reported to lose 10.5% of its body weight (Carlson, 1983).

Thoroughbred horses racing over 1-2 miles may lose up to 10 L (2% body weight) (Carlson, 1987). Sweat losses vary with different ambient conditions. In a treadmill study comparing different ambient conditions of horses, exercising in hot and dry conditions or hot and humid conditions yield the same sweating rates that are higher (2.6% BW losses) than exercise in cold and dry conditions (4.2% BW losses) (McCutcheon et al, 1995).

Fluid losses from sweating can be approximated by weighing the horse before and after work. In order to make accurate measurements urinary and fecal losses would have to be recorded as well. It has been estimated that an endurance horse could lose 100 L of sweat, if sweating 10 L per hour for 10 hours. If a dehydrated endurance horse (6-7% body weight losses) lost 40 kg the horse must have ingested 60 L o water and food during the 10 hour race (Marlin and Nankervis, 2004).

Table 1. Sweat composition in different equine exercise studies

	Rose et al, 1980	Carlson and Ocen, 1979	McCutcheon et al, 1995, in hot dry condition	McConaghy et al, 1995
Conditions of study	100 km race, 16-30^{o}C	20-25 miles training	Treadmill exercise 50% VO_{2max}, 32-34^{o}C	Treadmill exercise 50% VO_{2max}, 21^{o}C, 65% RH
Sodium (mmol/L)	249 ± 9	131.8 ± 8.1	167.3 ± 6.6	159.4 ± 5.8
Potassium (mmol/L)	78 ± 8	53.1 ± 3.9	33.5 ± 2.2	39.6 ± 1.5
Chloride (mmol/L)	301 ± 20	174.4 ± 12.6	181.1 ± 5.4	157 ± 4.6
Total protein (g/L)	----------	----------	0.75 ± 0.12	4.8 ± 0.4
Calcium (mmol/L)	----------	3.1 ± 0.3	1.95 ± 0.27	3.7 ± 0.1
Magnesium (mmol/L)	----------	2.3 ± 0.4	----------	3.3 ± 0.2
Sweat rate (ml/m^2/min)	----------	----------	34 ± 5.1	

The effects of terrain, speed, temperature and distance on water and ion losses have been evaluated during endurance races (Ecker and Lindinger, 1995). Comparison of elite performers

with average competitors did not reveal differences in average weight losses, however there was a higher percentage of average horses (18%) that had high to severe body mass losses (7 % and above) compared to the elite horses (8%). Presence of mud and high temperatures were a predictor for Na^+ and Cl^- losses, and temperature was a predictor for Ca^{++} losses. There was no significant correlation with K^+ losses. Distance was also a predictor for Na^+, Cl^- and K^+ losses when speed was included in the model.

Plasma water and electrolyte changes during endurance exercise

Decreases in plasma water during endurance races have been reported and were indicated by increases in plasma total protein and albumin (Barton et al, 2002; Jahn et al, 1996; Schott et al 1997; Sloet van Oldruithenborgh-Oosterbahn et al, 1991). Plasma [Na^+] has been reported to increase (Barton et al, 2002; Delar et al, 1982; Fregin, 1979, Sloet van Oldruithenborgh-Oosterbahn et al, 1991; Rose et al, 1980), decrease (Carlson and Mannsman, 1974) or not to change (Lindinger and Ecker, 1995; Schott et al, 1997; Jahn et al, 1996). Increases in [Na^+] show a water shift out of the vascular compartment to the working muscles. These increases help to maintain hyperosmolarity and thirst.

Progressive decreases in plasma [Ca^{++}] during prolonged exercise have been reported previously (Aguilera-Tejero et al, 2001; Carlson and Mannsman, 1974; Lucke and Hall, 1978; Schott et al, 1997; Sloet van Oldruithenborgh-Oosterbahn et al, 1991; Rose et al, 1980). Metabolic alkalosis, which is a common finding in endurance horses, increases the binding of Ca^{++} to albumin leading to hypocalcemia(Wijnberg et al, 2002).

Plasma [Cl^-] has consistently been reported to decrease during endurance races (Barton et al, 2002; Delar et al, 1982; Lindinger and Ecker, 1995; Rose et al, 1977). Decreases of 9.8-

16% in plasma [Cl^-] have been reported (Lucke and Hall, 1979; Rose et al, 1979). Since [Cl^-] is double in sweat compared to plasma, decreases are expected. Hypochloremia has been associated with metabolic alkalosis. In the traditional interpretation of acid-base balance, when Cl^- losses become excessive, the kidneys reabsorb bicarbonate to maintain electroneutrality, generating metabolic alkalosis.

Increases (Sloet van Oldruithenborgh-Oosterbahn et al, 1991), decreases (Delar et al, 1982) or no changes (Rose et al, 1980) in plasma [Mg^{++}] have been found. Hypomagnesemia has been found in horses with muscle tetany during strenuous exercise (Flaminio and Rush, 1998).

Plasma lactate increases are generally very mild during endurance exercise, indicating the mainly aerobic nature of this type of exercise (Barton et al, 2002; Jahn et al, 1996; Schott et al, 1997; Sloet van Oldruithenborgh-Oosterbahn et al, 1991). Horses capable of sustaining speeds greater than 5 m/s or those that gallop at the end of a race for 400 m may develop higher levels of lactate (Groskopft and van Rensburg, 1983; Rose et al, 1979). Anaerobic exercise may be necessary to obtain tactical advantage during the races.

Clinical signs associated with electrolyte losses can occur during endurance races. Sodium losses in sweat will lead to a reduction in plasma volume, associated with increased blood viscosity, when hematocrit increases above 58%, and inadequate tissue perfusion leading to inefficient oxygen and substrate transport (Carlson, 1985). This can contribute to decreased renal function or shutdown (Rose, 1977). Severe hyponatremia may lead to muscle cramping probably due to inhibition of Na^+-Ca^{++} ATPase (Fettman, 1986). Other signs of hyponatremia may include fatigue, diarrhea, and neurologic signs, as seen in humans (Knochel, 1980).

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Hypomagnesemia increases the release of acetylcholine at the neuromuscular junctions, promoting muscle spasms and tetany (Carlson, 1983). Hypokalemia leads to decreased membrane potential and may reduce the response of smooth muscle leading to peripheral vasodilatation, reduced gut motility and cardiac arrhythmias. However plasma [K^+] is not a good predictor of total body stores and if the horse is eating grass or hay sweat losses will be replaced. Some similar signs can be observed with hyperkalemia, i.e. cardiac arrhythmias, muscle fasciculations and cramps. Hypochloremia leads to metabolic alkalosis. Metabolic alkalosis will lead to hypocalcaemia and is involved in the occurrence of synchronous diaphragmatic flutter. Alkalosis diminishes the ionized calcium in the blood. Low plasma [Ca^{++}] and high plasma [K^+] will decrease the membrane threshold and increase the resting membrane potential, respectively. The phrenic nerve will get stimulated by the cardiac stimulation and the diaphragm will get stimulated by electrical impulses from the depolarization of the heart leading to synchronous diaphragmatic flutter. This can happen with normal or low plasma [K^+] (Mannsman et al, 1974), which occurs after exercise ceases and K^+ is pumped back into muscle cells and due to some losses to urine (Snow et al, 1982). During exercise the combination of high [K^+] and low [Ca^{++}] can lead to hyperexcitability of the muscles, leading to muscle fasciculations, cramps, cardiac arrhythmias, and altered gut motility. Increases in plasma [K^+] and decreases in plasma [Ca^{++}] will decrease the membrane potential and decrease the threshold potential leading to increased neuromuscular excitability. Since neuromuscular excitability is the capacity of a cell to give rise to an action potential when driven by adequate stimulus, increases in [K^+] and decreases in [Ca^{++}] in plasma will increase neuromuscular excitability leading to clinical signs described above.

Supplementation of water and electrolytes

Body weight losses during exercise give an initial over-estimate of the amount of water lost (Carlson, 1983). Estimates of electrolyte losses can be made from sweat loss studies. Fluid losses can reach 15 L per hour in hot climates, and 6-12 L per hour in colder conditions (Snow et al, 1982). Weight loss averages of 5-7% have been reported during endurance races (Fregin et al, 1979; Hargreaves et al, 2002). In severe cases sweat losses can reach 9-10% (Carlson, 1979; Fowler, 1979).

Horses during 160 km races could lose 100 L of fluid if sweating 10 L per hour. If a horse (500kg) ends the race with 40 kg weight loss, it suggests consumption of about 60 L of water during the race (Marlin and Nankervis, 2004). Water should be available throughout the races, and should be supplied at least at every hour. Ideally an isotonic solution containing electrolytes and water helps to rehydrate faster than water alone or electrolyte pastes (Butudom et al, 2002; Nyman et al, 1996). Horses can be taught to drink water with electrolytes.

The contents of the equine gastrointestinal tract also offer a fluid and electrolyte reservoir that might be used during prolonged exercise (Meyer, 1987). In ponies consuming a hay only diet, the water content was 183 ml/kg BW. In another group with the same diet exercised for one hour at low intensity exercise (3.3 m/s), water content was 138 ml/kg BW. After feed ingestion corrections, it was concluded that exercising ponies absorbed 5 L from the gastrointestinal tract. In ponies fed a completely pelleted diet the water content of the ingesta was 101 ml/kg BW. The sodium, chloride and potassium (to a lesser extent) in ingesta were significantly lower in exercised ponies, indicating absorption from the gastrointestinal tract (Meyer and Coenen, 1989).

Different studies have examined the effects of electrolyte administration during exercise and after furosemide administration in horses, which is regarded as an experimental model of

water and electrolyte losses. During a 62 km simulated endurance race (3 rounds of 20, 22 and 20 km) 13 horses were divided up into 3 electrolyte supplementation groups) (Nyman et al, 1996). Five horses were offered tap water, 4 horses administered a salt paste (30 g of NaCl) and offered tap water for drinking immediately before each round and, and 4 horses a saline solution (0.9%NaCl). The 3 rounds were divided by 20 minutes of rest. Speeds for each round were 13.7, 15.7 and 13.8 km/h. During the ride there were no differences between the three groups in fluid intake. However, until one hour after the ride fluid intake was higher in the saline group compared to the salt paste group and higher than the water group until three hours after the ride. No differences in body weight changes were seen among the groups. Total protein did not change during the ride for the water and salt groups. The saline group total protein increased during the ride, and was lower than resting values 1 and 3 hours after the ride. In the saline and salt paste groups, values were lower than resting in the morning after the ride. Plasma $[Na^+]$ and osmolarity were higher than pre ride throughout the ride and only returned to pre ride values at 16 hours after the ride in the salt paste group. In the water groups no changes in plasma $[Na^+]$ were observed. In the saline group, NaCl intake was higher than in the paste group. The $[Na^+]$ in the saline group was higher than the water only group only at 20 min after the ride. Plasma $[K^+]$ decreased until the end of the ride in all groups. Until 3 hours after the ride plasma $[K^+]$ was lower than pre ride in the salt paste and saline groups. Plasma $[Cl^-]$ did not change during the ride in the water group and increased in the saline and paste groups, post ride and at 42 km, respectively. This study demonstrated that the quantitative higher intake of Na^+ lead to higher $[Na^+]$ plasma concentrations in the saline group. Ingestion of more chloride however, did not show any influence on plasma $[Cl^-]$ concentrations. Saline treatments lead to the highest fluid consumption among all treatments. No side effects were observed for the salt paste treatment.

In horses dehydrated by furosemide no side effects and a better rehydration was observed after salt paste administration than with water alone (Sosa Leon et al, 1998, Schott et al, 2002). In another experiment (Coenen et al, 1995) where NaCl was supplied at different times, one and four hours prior to 2 hours of exercise at 12 km/h, or a control group receiving no extra NaCl. Supplemented groups had higher Na and Cl plasma concentrations during and after exercise, K concentration was also higher in the supplemented groups. Both electrolyte supplementations stimulated water intake, but with the supply 4 hours before exercise water consumption was higher before exercise then supplementation one hour prior to exercise.

Supplementation of electrolytes (Na, Cl, and K) during 60 km of treadmill endurance exercise yielded higher plasma concentrations of Na and Cl during and after exercise and lower weight losses than with water supplementation (Dusterdieck et al, 1999). Despite K supplementation no differences were seen in plasma values between the electrolyte and water treated groups. Similar results were found in another study (Marlin et al, 1998) where water was compared to an oral rehydration solution (Na, K, Cl) both administered before 60 minutes of low intensity exercise (1.7-3.7m/s). Plasma [Na^+] and [Cl^-] were higher in the rehydration treatment compared to water. Plasma [K^+] was not influenced by the treatment. The same happened when water or an oral hydration solution were administered after exercise dehydration of 2% (Marlin et al, 1998b).

In all these studies administration of electrolytes before and during exercise were beneficial to water consumption and consequently hydration of horses.

Although Na, K and Cl are the main electrolytes in sweat many commercial products also contain Ca and Mg. Their effects however have not yet been tested. It is not known if the administration of Ca and Mg during the rides is beneficial.

Literature Review

Supplementation of oral glucose during endurance races has been used to try to improve energy status during exercise. However no studies have shown any benefit of such a supplementation in horses. One study examined the effect of intravenous infusion glucose during treadmill exercise (6 m/s at 2^{o} incline) until fatigue (Farris et al, 1995). During the trial initially 52.5 g of glucose were infused during the first 15 minutes of exercise and then glucose was infused to maintain plasma glucose at 9.0 mmol/L. This was compared to saline infusion during exercise. Glucose infusion prolonged time to fatigue by 14.1%. This study suggests that glucose availability may be a limiting factor for prolonged exercise in the horse. In another study (Geor et al, 2000) glucose was infused during low intensity exercise of horses on the treadmill. A glucose tracer was infused and the appearance of endogenous glucose could be measured. The glucose infusion only partially suppressed hepatic glucose output, the glucose infusion resulted in higher glucose use during exercise, but it did not affect muscle glycogen utilization. Glucose supply during exercise increases carbohydrate utilization during exercise.

Oral glucose and glucose polymers have been tried in muscle glycogen replacement formulas without much success (Davies et al, 1994). Glycogen replacement was not different between water and glucose polymer administration. In another study, glucose in addition to electrolytes was not better than electrolytes in rehydration of horses (Monreal et al, 1999).

Lower protein levels with essential amino acids added in the horse's diet has been proven to be beneficial (less acidogenic) for horses exercising at high intensities, compared to higher protein intakes (Graham-Thiers et al, 2003). This type of diet would be beneficial for endurance horses, since higher protein levels generate more heat and require water for renal excretion (Kronfeld, 1996).

Acid –base balance, traditional and physicochemical approach

The traditional approach to acid-base balance is expressed by the Henderson-Hasselbalch equation in which bicarbonate and carbonic acid are buffers (Guyton and Hall, 2000). The partial pressure of carbon dioxide (PCO_2) and pH are measured directly, and [HCO_3^-] is calculated using the equation; $pH = pK_a + \log [HCO_3^-]/ [PCO_2]$. Arterial or venous PCO_2 is used to indicate the respiratory component of the acid base disturbance and changes in [HCO_3^-] indicate problems with the metabolic component. Small increases in PCO_2 or a decrease in pH can stimulate the pulmonary ventilation. The principal buffer systems in the body are the HCO_3^-, plasma protein, phosphate and hemoglobin. The most important are the HCO_3^- and the hemoglobin buffers. The respiratory system can change the pH of the body fluids altering the alveolar ventilation, changing PCO_2. The kidneys can increase or decrease [HCO_3^-] in the body fluids. The kidneys also can excrete H^+. If H^+ secretion exceeds the HCO_3^- filtration rate, almost all HCO_3^- will be reabsorbed and none will appear in the urine. If the H^+ secretion is less than the HCO_3^- ion filtration rate, some ions will escape reabsorption and appear in the urine. Under acid stress, H^+ secretion rises and all HCO_3^- will be reabsorbed, and the urine will be acidic. The advantage of this approach is that PCO_2 and pH are relatively easy to measure. However when acid-base changes are associated with exercise are explained using the Henderson-Hasselbalch equation, not all alterations are taken in account. The equation is physicochemically correct, but the HCO_3^- buffer is weak in blood because its dissociation constant (pK=6.1) is different from blood (7.4), and because its buffer capacity is small. Changes in [HCO_3^-] also do not cause changes in [H^+] (Stewart, 1981).

A quantitative physicochemical approach to acid-base balance was developed by Stewart (1981, 1983). The acid-base status is determined by three independent variables: the strong ion

difference (SID), which is the difference between $Na^+ + K^+ - (Cl^- + Lac^-)$ ions; the total concentration of weak acids [A_{tot}] (mainly protein in the case of plasma); the partial pressure of CO_2. There also are 6 equations that must be solved simultaneously, and a computer program is best to solve the equations (Watson, 1996). Dependent variables are [HCO_3^-] and [H^+]. The dependent variables change in relation to the independent variable change. Generally when SID decreases, there is a decrease in pH. When SID increases, as in hypochloremia, pH increases. An increase in [A_{tot}] from dehydration can decrease pH. However the whole model needs to be evaluated for a complete interpretation of the acid-basic status.

During repeated sprints in horses approached with Stewart's system it was found that plasma [H^+] was stable up to the lactate threshold, after which [H^+] increased and [HCO_3^-] decreased (Taylor et al, 1995). Concurrent decreases in [Cl^-], increases in [Na^+] and [K^+] offset the increases of [Lac^-]. An increase in PCO_2 was the main cause for an increase in [H^+]. Other high intensity studies also repeated the same results (Ferrante et al, 1994; Taylor et al, 1995).

During endurance races and exercise no changes (Rose et al, 1979), increases (Gillespie et al, 1975; Jahn et al, 1996) were found in plasma pH. No studies have analyzed endurance exercise under Stewarts's model.

Feed energy sources and its effects on acid basic balance

The horse is a monogastric herbivore that evolved digesting and utilizing high fiber diets through fermentation in the cecum and colon.

Dietary energy can be supplied to the horse by different sources:

a) Hydrolysable carbohydrates like starch and sugar

b) Non-hydrolysable carbohydrates like cellulose, hemicellulose, and pectins (components of dietary fiber)

c) Fats

d) Proteins

Energy storage in the horse has been estimated accounting the amount of fuels (not including protein). Fat is the largest store in the horse. Estimates of 1400-1800 g for muscle triglyceride, and 40000g as adipose triglyceride have been made for a 450 kg horse. Liver and muscle glycogen have been estimated to be 90-220 g and 3150-4095 g, respectively (Pagan, 1994).

During sub maximal exercise plasma free fatty acids have been considered to be the most important energy source quantitatively (Lawrence, 1994). Initial work on fat feeding suggested that horses on a 12% fat diet ridden on a 67 km trail had higher blood glucose levels than horses on a control diet (3% fat)(Slade et al, 1975). In another study horses were fed 4, 8, 12 and 16% of fat in the diet and trotted for 6 hours (Hambelton et al, 1980). Horses on the highest fat % had the highest blood glucose suggesting sparing of glucose. The combination of conditioning and supplementation of fat is called fat adaptation (Kronfeld, 1996). Fat feeding has proven to be beneficial not only for low intensity, but also for high intensity exercise in humans and horses (Ferrante et al, 1994; Taylor et al, 1995b; Spriet and Howlett, 1999). Involved mechanisms are accumulation of citrate, which inhibits hexokinase and phosphorilase, sparing glucose and glycogen (Spriet and Howlett, 1999). During high intensity exercise in horses, fat adaptation increased the lactate threshold (Custalow et al, 1993), increased blood lactate by about 35% (Ferrante at al, 1993; Taylor et al, 1993). During aerobic exercise, however, lactate blood lactate was lower (Greiwe et al, 1989). Higher performance of horses was also reported with increases

in running speed (Oldham et al, 1989, 1990; Harkins et al, 1992), and prolonged time to maximal oxygen deficit (Eaton et al, 1995).

Historically carbohydrates have been fed to horses to supply the extra energy needed for a working horse. The substitution of oats for hay improved the power: weight ratio, a decrease in heat production, and a reduction in water requirements (Kronfeld and Harris, 1997). Hydrolysable carbohydrates yield glucose and fermentable carbohydrates SCFA. Higher intakes of hydrolysable carbohydrates will saturate glucose absorption and may predispose to forms of tying–up and developmental orthopedic disease. Overload of hydrolysable carbohydrates will lead to fermentation and can lead to osmotic diarrhea, colic and laminitis.

During high intensity exercise the catabolism of carbohydrates accounts for the majority of energy used (Harris, 1997). The energy comes mainly from the use of muscle and liver glycogen. After the cross-country-phase o a three-day event (Hodgson et al, 1984) and after endurance races glycogen may be depleted as much as 75% of resting stores (Snow et al, 1982). Supplementation of carbohydrates during exercise has been tested. One study examined the effect of intravenous infusion glucose during treadmill exercise (6 m/s at 2^{o} incline) until fatigue (Farris et al, 1995). Glucose infusion prolonged time to fatigue by 14.1%. High carbohydrate feeding after exercise has been shown to hasten glycogen resynthesis after glycogen depleting exercise (Lacombe et al, 2004). However, risks associated with this practice exist.

The equine athlete only needs dietary protein in limited quantity and high in quality (Kronfeld and Harris, 1997). Dietary protein is utilized inefficiently and produces much heat. The oxidation of phosphorus and sulphur from proteins yield acids. Increases in protein intake in horses increase water requirements.

Fermentation of fermentable carbohydrates in the hindgut yields SCFA, in particular acetic, propionic, and butyric acids which are transported and metabolized differently from dietary fat. The SCFA are another energy source for the horse. The percentage of SCFA depends on the diet, with acetate decreasing and propionate increasing as grain replaces hay in the diet (Hintz et al, 1971). Acetate can be used for energy or fatty acid synthesis. Propionate can also be used for energy, or maintenance of blood glucose levels. Values as high as 61% of the available blood glucose may be originated from propionate in resting ponies on a roughage diet (Simmons and Ford, 1991). The efficiency of energy production from the diet via volatile fatty acid pathway will be lower than the energy obtained from hydrolysable carbohydrates (Harris, 1997).

Effects on acid-base balance

Effects on different energy sources on acid-base balance during exercise have been studied. During intense exercise increases lactic acid and CO_2 are common findings. Despite those findings metabolic alkalosis has been observed in some studies (Bayly et al, 1989, Forster et al, 1990). Therefore effects were analyzed with the comprehensive physicochemical approach (Stewart, 1981). A comparison of the effects of a control diet (rich in hydrolysable carbohydrates) versus a high fat diet (14% fat as corn oil) in horses during high intensity exercise was done (Taylor et al, 1995). Blood lactate concentration was 39% higher at fatigue in the fat adapted group compared to the control group ($P < 0.001$). During the last exercise test of the study the change in SID from rest to fatigue was different between the two dietary groups. The SID decreased by 0.9 mEq/L at fatigue in the control group, whereas the fat-adapted group increased by 3.1 mEq/L. This was due to a 7 mEq/L increase in $[Na^+]$ in the fat adapted

compared to a 0.5 mEq/L increase in the control group. The increase in SID decreased [H^+] in the fat adapted group. The higher lactate in the fat adapted horses may confer metabolic advantages during intense exercise in trained horses, because horses seem to tolerate the accumulation of lactate during short high intensity exercise. The higher [Na^+] leading to a greater SID and lower [H^+] in fat adapted horses may also be beneficial metabolically, since acidosis is associated with fatigue.

In another study a lower central venous PCO_2 was associated with fat adaptation (Ferrante et al, 1994). It reflected a lower production of CO_2 for a given rate of oxidation, in keeping a lower respiratory quotient when fat was oxidized. In this study eight Arabian horses were assigned to two different diets (control, rich in hydrolysable carbohydrates, and fat, 15% of fat as corn oil). Horses were submitted to two repeated sprint exercise tests on the treadmill. Half of the horses in each feed group also were administered 300 mg/kg BW of sodium bicarbonate with 3 L of water about two hours before exercise and the others only water. The increase in blood lactate was higher in fat adapted horses than in control horses. A smaller increase in blood lactate was found in horses fed the control diet and given the bicarbonate solution two hours before the exercise test. The extent of the blood lactate response to the combination of fat adaptation and sodium bicarbonate was greater than the sum of separate responses of fat adaptation or bicarbonate supplementation. Fat adaptation may facilitate lactate production in the muscle during anaerobic exercise and sodium bicarbonate may facilitate lactate efflux from muscle cells (Kronfeld et al, 1998). Also plasma [Na^+] was higher in the the horses treated with sodium bicarbonate than in horses treated with water. Higher plasma SID was observed in the bicarbonate group and it lead to lower [H^+].

In conclusion a high fat diet in horses exercising at high intensity exercise will attenuate acidosis via lower CO_2 production and will allow horses to work with a higher lactate production. Also sodium bicarbonate supplementation before high intensity exercise will attenuate acidosis through a higher SID.

Another way to influence acid-basic balance through diet is through manipulation of the amounts of cations and anions in the diet.

Dietary cation-anion balance (DCAB)

Sodium, potassium and chloride are the most important ions involved in regulation of the osmotic pressure in body fluids, as well as the maintenance of acid basic balance (Baker et al, 1993). Highly cationic diets are alkalogenic, and anionic diets acidogenic. Other ions as such as Ca^{++}, Mg^{++}, $S^{-2}$and P^{-3} can be included in the calculation. Studies in horses comparing low and high DCAB diets showed that the anion or cation that was in the highest amounts (Na, K, Cl) was excreted in urine and feces, i.e. its excretion was dependent on its intake (Baker et al, 1993). Low DACB diets based on high Cl^- yielded a higher excretion compared to all other diets (Baker et al, 1991). Higher excretion of N^+ and K^+ in the urine was found in anaerobically exercised horses fed a high DCAB based on high Na^+ or K^+ intake, respectively. Decreasing DCAB resulted in increased urinary chloride excretion in sedentary and exercised horses. As DCAB decreased, urinary calcium excretion increased across all treatments in sedentary horses. In exercising horses urinary calcium excretion was significantly higher in the low DCAB fed horses compared to the high DCAB fed horses (Baker et al, 1993). In another study, decreasing DACB yielded lower blood pH compared to medium high and high DACB diets in resting animals. Results only showed a trend for lower pH in low DACB compared to other diets in anaerobically

exercised horses (Stutz et al, 1992). No effects of low or high DACB were seen in plasma pH after exercise, only at rest before exercise (Popplewell et al, 1993).

Hypocalcemia during endurance exercise and other equestrian sports has not been fully understood. Possible mechanisms involved include Ca^{++} binding to proteins and inorganic or organic anions in the blood, hormonal alterations as well as intracellular shift of Ca ions into muscle cells (Geiser et al, 1995). Hypocalcemia is counter-regulated by parathyroid hormone (PTH), which stimulates reabsorption of Ca^{++} by the kidneys or bones (Fitzpatrick and Bilezikian, 1999). Decreases in plasma [Ca^{++}] and increases in PTH were reported after show jumping competition (Aguilera-Tejero et al, 1998). During endurance exercise however decreases of plasma [Ca^{++}] were accompanied by increases or decreases in PTH (Aguilera-Tejero et al, 2001). Calcium homeostasis and plasma PTH were evaluated during training and exercise in Standardbread horses (Veuvert et al, 2002). Decreases in plasma [Ca^{++}] were found after low intensity and high intensity exercise. Decreases were attenuated after training. A negative correlation between ionized normalized Ca^{++} and pH was found ($r = 0.39$, $P < 0.001$). Decreased binding of Ca^{++} to protein has been shown with decreasing pH (Peoples, 1988), however, during the study a decrease in plasma [Ca^{++}] was found despite decrease in pH (Veuvert et al, 2002). The authors argue that Ca^{++} could bind to lactate or albumin. No response of PTH was found after low intensity exercise, but an increase in selected bone markers was seen (Veuvert et al, 2002b).

Milk fever is a metabolic disorder in which Ca homeostatic mechanisms fail to maintain plasma Ca concentrations at the onset of lactation. Studies have suggested that the response of kidney and bone to PTH is impaired in cows that develop milk fever and that the response of the tissues can be modified by prepartum diet (Abu Damir et al, 1994; Block, 1984; Phillippo et al,

1994). The use of dietary anions to prevent milk fever has suggested that diets rich in cations, especially Na and K increase the susceptibility of cows to milk fever (Beede, 1992; Block, 1984; Dishington, 1975). The effects of the addition of K^+ and Na^+ to prepartum rations, increasing DCAB, on milk fever incidence in dairy cows were analyzed (Goff and Horst, 1997). Cows fed a higher content of K in the diet (2.1 and 3.1%) had a much higher incidence (10 versus 50%) of milk fever and hypocalcaemia than cows fed a 1.1% K diet. Plasma PTH and 1,25 hidoxy vitamin D increased within 24 hours after calving. Plasma PTH concentrations were lower in cows fed he 1.1% K diet that the cows fed the 2.1 and 3.1%K diets. Cows fed the 1.1% K diet had lower urine and plasma pH than did cows fed the higher K diets. Plasma hydroxyproline concentrations of cows fed the 1.1% K diet shortly before calving were increased compared to those fed 2.1 and 3.1%K diets. Cows fed a high Na diet (1.3%) had an incidence of 62.5% of clinical milk fever. Concentrations of PTH of cows fed the high Na diet were higher than cows fed a low Na and K diet. Plasma hydroxyproline concentrations before calving were higher in the cows fed the low Na diet than the ones fed the high Na diet. Urine and plasma pH were higher in the high Na diet compared to the low Na diet. There is growing evidence that diets with a highly positive dietary cation-anion balance cause metabolic alkalosis in cows reducing the responsiveness of bone and kidney to PTH (Gaynor et al, 1989; Goff et al, 1991; Phillippo et al, 1994). Plasma hydroxyproline can be used as an index of the activity of bone osteoclasts and plasma PTH has the control over its activity. In this study despite having higher PTH concentrations, hydroxyproline was lower in the animals fed high Na and K diets, suggesting that the bones of cows on these diets were refractory to PTH stimulation.

Since endurance horses generally become alkalotic, a high DACB caused by a high ingestion of K and Na salts during the race added with a high forage K rich diet, could decrease

blood [H^+] and exacerbate hypocalcemia during endurance exercise. A chronic alkalosis or alkalinemia due to a high DCAB diet may be the reason for an inadequate response to PTH in endurance horses causing hypocalcemia, similar to periparturient cows.

Potassium supplementation

During exercise plasma [K^+] increases the resting membrane potential and therefore neuromuscular excitability. Thus supplementation of K^+ during exercise that increases plasma [K^+] would not be indicated. Increasing K^+ supplementation could contribute to hypocalcemia during exercise, since it increases DCAB contributing alkalosis and hypocalcemia in preparturient cows. After exercise ceases, supplementation is beneficial to replace sweat losses. K^+ also may be involved in the transport of glucose into the cells after exercise (Bo et al, 1999). Insulin secretion can be stimulated with K^+ supplementation, and therefore may help glycogen repletion after exercise. When glycogen is depleted as during endurance races, K^+ supplementation may help to restore muscle glycogen.

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Potassium supplementation affects acid-base status and plasma ion concentrations of horses during endurance exercise

Hess, T. M., D. S. Kronfeld, C. A. Williams, J. N. Waldron, P. M. Graham-Thiers, K. Geiwe-Crandell, M. A. Lopes, P. A. Harris 2005. Potassium supplementation affects acid-base status and plasma ion concentrations of horses during endurance exercise. Am J. Vet. Res.: in press.

ABSTRACT: The objective of this study was to compare effects on plasma ions and acid-base status of supplementing horses during an 80 km race with a K-free electrolyte mixture (EM-K) or K-rich electrolyte mixtures (EM+K). Forty six horses participated, mean age was 11 years, initial weight 421 kg, for 45 Arabians and 1 Thoroughbred horse. Weight was measured at 0, 56 and 80 km, and blood collected 0, at 21, 37, 56 and 80 km, and 30 minutes after the race (REC). Consumed electrolytes were recorded. Gas-syringe blood was analyzed for hematocrit (Hct) and plasma for pH, P_{CO_2}, $[Na^+]$, $[K^+]$, $[Cl^-]$, $[Ca^{++}]$, $[Mg^{++}]$, lactate ($[La^-]$), albumin (alb), phosphate (PO_4^-), and total protein (TP). Plasma $[H^+]$, $[HCO_3^-]$, strong ion difference (SID) and osmolality were calculated. Results of the 34 finishers were evaluated. Weight decreased by 4.74 and 4.50% at 56 and 80 km, respectively. Intake was 33 g less for K and 36 g more for Na in the EM-K group than in the EM+K. Increases were found in plasma $[H^+]$, $[Na^+]$, $[K^+]$, $[Mg^{++}]$, $[PO_4^-]$, $[La^-]$, P_{CO_2}, TP, alb, Hct and osmolarity; decreases in $[HCO_3^-]$, $[Ca^{++}]$ and $[Cl^-]$. Plasma $[H^+]$ was lower in EM-K group than in the EM+K group ($P = 0.024$). Increases in plasma $[K^+]$ at 80 km and REC were less ($P = 0.033$, and $P=0.021$, respectively) in EM-K group than in the EM+K group. Changes in plasma $[H^+]$ were attributable mainly to changes in SID, and these to changes in plasma $[Na^+]$ in both groups. Mean increases in plasma $[H^+]$ and $[K^+]$ in this race were moderate

and unlikely to contribute to signs of muscle fatigue and hyper-excitability. Evaluation of plasma [K^+] effects on membrane potential suggests that a degree of depolarization likely to cause hyper-excitability would be uncommon but reached in more horses given EM+K than in those horses given EM-K by the end of this race.

Key Words: Potassium, endurance, acid-base, horse

Introduction

Electrolyte and water losses occur during prolonged exercise and have been implicated in medical problems (Carlson and Mannsman, 1974; Carlson, 1985). Many clinical signs seen at veterinary checks have been ascribed to increased neuromuscular excitability in striated and smooth muscles (AERC, 1999; Carlson and Mannsman, 1974; Carlson, 1985; Foreman, 1998; Kronfeld, 2001a; Leroux et al, 1995). Such signs may include cardiac arrhythmias, slower post-exercise decrease in heart rate, muscle cramps and twitches, as well as increased or decreased intestinal motility.

Neuromuscular excitability depends on resting membrane potential and threshold potential. The resting membrane potential is mainly determined by the K^+ distribution across the cell membrane according to the Nernst equation (Ganong, 1999), so that when plasma $[K^+]$ increases, resting membrane potential will increase (become less negative) and more closely approach the threshold for an action potential, thereby increasing neuromuscular excitability. Plasma $[K^+]$ increases in line with intensity of exercise at speeds above 4 m/s in horses (Harris and Snow, 1992; Kronfeld, 2001b).

We propose that provision of K-free electrolyte supplementation during endurance exercise may moderate the increase in plasma $[K^+]$ and thereby reduce the risk of increasing neuromuscular excitability. Secondarily, K is largely replaced by Na to maintain the cation equivalents in designing a K-free formula, and this exchange may affect the plasma $[H^+]$ response to exercise. The objectives of this study were to compare the effects of an experimental K-free, Na-abundant electrolyte mixture (EM-K) and commercial K-rich mixtures (EM+K) on changes in acid-base-status and on plasma concentrations of ions and

associated variables during an 80 km race. A companion study on antioxidant status was also conducted in this race, and preliminary reports have been presented.

Material and Methods

This study was undertaken during the Middleburg Research Ride 2001, which was held on April 1 and followed *American Endurance Ride Conference* (AERC, 1999) rules. The race started at 0700 and covered 80 km of rolling hills with altitudes varying from 121 to 442 m. Rest stops were provided at 21, 37, 56 and 72 km with *veterinary checks* (AERC, 1999). The trail between two rest stops is called a loop, and this trail consisted of five loops. Ambient temperature ranged from 4.7 to 10.7 ^{o}C, and humidity from 97 to100%. A 2x2 factorial design compared 2 vitamin treatments (vitamin E alone or with vitamin C) and 2 electrolyte treatments. The protocol was approved by the institutional animal care and use committee.

The 46 horses with an average age of 10.8 ± 0.6 years included 45 Arabians, purebred or crossbred, and 1 Thoroughbred. A pre-ride survey provided a nutritional and performance history of each animal. This survey determined that owners of 22 horses required use of their customary commercial electrolyte supplements containing K (EM+K), and owners of 24 horses volunteered to use the novel K-free mixture (EM-K).

Electrolyte mixtures were administered orally by syringe. Riders using EM-K were oriented to supply two doses per loop. Riders using EM+K followed manufacturer's directions, which were usually one to three doses per loop. One or two doses were also given two hours before the ride started. The formula of EM-K was based on sweat composition studies except for the absence of K and approximates the electrolytes lost in 2.5 L of sweat

Table 1(Kronfeld, 2001b). Each dose contained of 21.8 g of sodium chloride, 1.7 g of calcium chloride, 1.1 g of magnesium chloride, and 0.13 g of monosodium monophosphate. The commercial formulas all contained K (Endura-Max, Kentucky Equine Research, Versailles, KY; Enduralyte, Life Science Products, St. Joseph MO; Lyte-Now, Pro-formula Labs, Fort Lauderdale, FL; Ora-Lyte, The Butler Company, Columbus, OH). Intakes of electrolytes during the race were calculated from the formulas and amounts given, as recorded by the riders. Horses received water but no electrolytes or food between the 80 km and REC samples.

Horses were weighed without tack before the start (PRE), after the 56 km vet check exam, and after the race finish at 80 km before access to water, using an electronic scale (Tyrel Platform, Model TC-105, Allweights Hamilton Scale Corp, Richmond, VA). Horses were submitted to AERC regulation veterinary checks prior to the race, at rest stops and at the finish (AERC, 1999). Heart rate and rectal temperature were recorded immediately before blood sampling.

Jugular blood samples were collected about 0600 (PRE), then 60 to 120 seconds after arrival at 21, 37, 56, and 80 km veterinary checks, and 20 to 30 minutes after finishing (REC). An aliquot of blood was collected into a blood gas syringe (Dryhep Plus Kit, Becton Dickinson, Franklin Lakes, NJ) and another was collected to heparinized Vacutainer tubes (Vacutainer Green, Becton Dickinson, Franklin Lakes, NJ). Blood was kept in ice-water, and the Vacuatiner tube centrifuged 20 to 30 minutes after collection. The gas syringe aliquot was analyzed 20 to 30 minutes after collection for pH, P_{CO_2}, hematocrit (Hct), plasma $[Na^+]$, $[K^+]$, $[Cl^-]$, $[Ca^{++}]$ and $[Mg^{++}]$, using a blood-gas analyzer (Stat Profile M, Nova Biochemical, Waltham, MA). Within sample CV was lower than 2% for all variables

analyzed by the blood gas instrument, except for Hct , which was 5%. In addition, plasma [H^+], [HCO_3^-], [Ca^{++}] and osmolality were calculated (Stat Profile M, Nova Biochemical, Waltham, MA). Plasma was stored at –80°C for further analysis. Spectrophotometric assays were used for plasma concentrations of lactate ([La^-]), albumin ([alb]), phosphate ([PO_4^-]), and total protein ([TP]) within two weeks of the ride (Beckman Instruments, Inc., Brea, CA).

Changes in plasma pH were interpreted initially in the traditional Van Slyke manner based on the Henderson-Hasselbach equation (Van Slyle, 1921), which represents pH as a function of pK plus the log of the ratio of [HCO_3^-] to [H_2CO_3 or 0.0307 x PCO_2]. Metabolic changes are associated with [HCO_3^-], respiratory changes with H_2CO_3 or PCO_2. Despite widespread acceptance of this emphasis on the bicarbonate buffer, it has limitations; for example, the effects of proteins and osmolarity are not represented. Also, pH is not dependent upon [HCO_3^-] but is dependent instead upon concentrations of strong ions, PCO_2 and weak acids that are not represented in the Henderson-Hasselbach equation (Kronfeld, 2001a; Stewart, 1981; Watson, 1996). For these reasons, changes in acid-base status during exercise have been analyzed preferably by the comprehensive physicochemical model of Stewart (Kronfeld, 1998, 1999, 2001a; Lindinger et al, 1992, Stewart, 1981; Watson, 1996).

In this model (Stewart, 1981), concentrations of H^+ and HCO_3^- are dependent upon the strong ion difference (SID), P_{CO_2}, and total weak acids (A_{tot}, mainly proteinates and phosphates). The SID was calculated as the algebraic sum of [Na^+] and [K^+] minus the sum of [Cl^-] and [La^-]; the algebraic sum of other strong ions was assumed to be < 1 mEq/L and to contribute negligibly to changes in SID observed during exercise and recovery. Estimates of weak acids ($A_{tot,}$ mEq/L) were predicted from TP (g/L), multiplying it by 0.211 (Stämpfli et al, 1999).

The partition of changes in plasma [H^+] into contributions from SID, P_{CO_2} and A_{tot} was performed using software designed for the Stewart system (Watson, 1996). Resting values for SID, PCO_2 and A_{tot} were set in the panel for independent variables, and the predicted value of [H^+] was observed in the panel for dependent variables. Then the series of values of SID at each sampling stage were entered one at a time (without changing P_{CO_2} and A_{tot}) to yield corresponding series of changes in [H^+] contributed by the changes in SID (Kronfeld et al, 1999; Watson, 1996). The procedure was repeated for P_{CO_2} (without changing SID and A_{tot}) and for A_{tot} (without changing SID and P_{CO_2}).

Resting membrane potential (E_K, mV) was calculated from extra-cellular [K^+] (K_o) and [K^+] the (K_i) using the Nerst equation (Kronfeld, 2001a).

$$E_K = 61.5 \log [K_o]/[K_i] \text{ at } 37\ ^oC$$

This equation uses the ratio of extracellular/intracellular concentrations of K^+ only, because the permeability constant of K^+ is much higher than those of other ions. Assumptions are needed to test the possible effects of changes in plasma [K^+] on E_K. Measured plasma [K^+] were used for K_o without the approximately equal and opposing adjustments for plasma solids and for lymph. For K_i, a mean equine middle gluteal muscle intracellular [K^+] of 124 mEq/L (K_i mEq/L water) was calculated from data on K (μM/g of wet weight) and water (%) (Johnson et al, 1991). This value was used for rest and exercise.

Data were summarized as least squares means and standard errors. The effects of sampling stage (PRE, 21, 56, 80 km, and REC), treatments (EM-K, EM+K, E, E+C) and their interactions were evaluated by ANOVA with repeated measures in a mixed model and applied to the 34 horses that completed the 80 km (SAS Institute Inc., Cary, NC). Non-significant interactions were dropped from the model, and treatments E and E+C were

dropped because they had no interactions with EM-K and EM+K. Significance of differences between means were tested by least significant differences covered by a significant F-test for the ANOVA (SAS Institute Inc., Cary, NC). Fisher's exact test was used to compare frequencies of non-finishers in the 2 groups. Simple relationships of plasma $[H^+]$ to SID, A_{tot} and PCO_2, and SID to plasma $[Na^+]$, $[K^+]$, $[Cl^-]$ and $[La^-]$ were tested by linear regression (SAS Institute Inc., Cary, NC). Statistical significance was inferred from $P < 0.05$.

Results

Body weight averages were 421.0 ± 4.9 kg before the race for 46 starters and 423.8 ± 5.1 kg for 34 finishers. No differences between groups were found for mean weight losses, which were greatest at 56 km: 4.52% for 17 EM-K finishers, 4.36% for 17 EM+K finishers, and 4.80% for 9 non-finishers.

The 12 non-finishers comprised 7 from the 24 horses in the EM-K group, and 5 from 22 in EM+K ($P = 0.74$). Reasons for elimination were lameness (3 horses), exertional rhabdomyolysis (2), failure to recover heart rate in 30 min (1), slow gut sounds (1), and rider option (5).

Average speed was 3.30 ± 0.08 m/s over 80 km, and 3.87, 3.34, 3.29, 2.84, and 2.72 m/s for the 5 loops. Average heart rates during blood collection were 44 ± 2, 72 ± 2, 66 ± 2, 70 ± 2, 78 ± 2, and 52 ± 2 beats per minute at PRE, 21, 37, 56 and 80 km, and REC, respectively. Rectal temperatures at blood collection were 36.5, 38.7, 38.3, 38.5, and 38.7 ± 1.2 ^oC at PRE, 21, 37, 56 and 80 km, and REC, respectively.

Total amounts of electrolytes consumed are summarized in Table 1. Consumption of K was zero in the EM-K group and 33 g in the EM+K group. Consumption of Na was 26 g higher in the EM-K group than in the EM+K group. Consumption of Ca and Mg was 350% (5.7g) and 450% (3.1g) higher, respectively, in the EM+K than EM-K supplied horses,.

All dependent variables changed during the race, that is, with sampling stage. No differences were found between treatments, except for plasma [H^+], and [Ca^{++}], so the data were combined (Table 2). With increasing distance, increases were found in plasma [Mg^{++}], [PO_4^-], [TP], [alb], Hct and osmolality; decreases in P_{CO_2}, [HCO_3^-] and [Cl^-].

Plasma [K^+] increased ($P < 0.001$) from PRE to 56 km and decreased ($P < 0.001$) from 56 km to REC. Plasma [Na^+] increased ($P < 0.001$) from PRE to 37 and 56 km and decreased ($P < 0.001$) from 56 km to REC. Plasma [Cl^-] decreased ($P < 0.001$) from PRE to 21 km and returned to pre-race values at 37 km and thereafter. Plasma [La^-] increased ($P < 0.001$) 4-fold by 21 km then decreased ($P < 0.001$) by 37 km and remained about twice the PRE value until REC. Plasma [H^+] declined ($P < 0.001$) from PRE to 21 km, then increased ($P < 0.001$) from 21 to 80 km, and changed little ($P < 0.068$) during 30 min recovery.

Plasma [H^+] was lower overall ($P = 0.024$) in the EM-K group than in the EM+K group (Figure 1). Plasma [H^+] was lower specifically at 80 km ($P = 0.022$) and REC ($P = 0.013$) in the EM-K horses compared to the EM+K group.

Plasma [Ca^{++}] was higher ($P = 0.026$) in the EM-K than in the EM+K treatment, despite lower Ca^{++} in the EM-K. Plasma [Ca^{++}] was higher ($P = 0.014$) at 21-km in the EM-K horses than in the EM+K group.

A stage-by-treatment interaction (P = 0.048) was found for plasma [K^+] (Figure 2). Plasma [K^+] was lower at 80 km (P = 0.033) and REC (P = 0.021) in the EM-K horses than in the EM+K group.

Mean changes from PRE to 21, 37, 56 and 80 km, and REC in plasma [H^+] are partitioned among the 3 independent variables for each electrolyte group (Figure 3). In both groups, P_{CO_2} was the dominant independent variable at 21 km, SID thereafter. Actual changes from rest (PRE) in plasma [H^+] were negatively related to changes from rest in SID in both groups (data not shown but are derived from data in Table 2): EM-K (r = 0.836, P = 0.038) and EM+K (r = 0.865, P = 0.026). Calculated contributions of changes from rest in SID to the changes from rest in plasma [H^+] are positively related (Figure 3). Changes from rest in SID were highly correlated with corresponding changes in plasma [Na^+] in both groups (Figure 4): EM+K (r = 0.906, P = 0.013) and EM-K (r = 0.998, P < 0.0001).

In the EM-K group, the initial -3.0 nEq/L change in plasma [H^+] at 21 km was partitioned into –2.4, +0.11 and +0.9 nEq/L contributions from changes in P_{CO_2}, SID and Atot, respectively (Fig 3). Subsequently, the predominant contribution to change in plasma [H^+] was from SID at 37 and 56 km, at 80 km, and at REC (Fig 3). In the EM+K group, the initial –2.6 nEq/L change in [H^+] at 21 km was partitioned into –2.1, +1.6 and + 0.9 nEq/L contributions from changes in PCO_2, SID and Atot, respectively (Fig 3). Subsequently, the predominant contribution to change in plasma [H^+] was from SID at all stages (Fig 3).

Estimated mean E_K before the race were -91.0 and -92.2 mV for the EM-K and EM+K groups, respectively. Assuming no change in K_i during the race, estimated mean changes in E_K from rest to 80 km were –0.392 and +2.52 mV for EM-K and EM+K, respectively.

Discussion

Most of the present results are consistent with previous observations on endurance horses (Carlson and Mannsmann, 1974; Delar et al, 1982; Dybal et al, 1980; Fregin, 1979; Lindinger and Ecker, 1995; Lucke and Hall, 1978; Schott et al, 1997; Sloet et al, 1991). A salient finding is that plasma [K^+] increases during prolonged exercise at only 3.4 m/s over hilly terrain. New findings include a biphasic response of plasma [H^+], which decreased initially then increased during prolonged exercise and increased further during recovery. Also new is the finding that changes in plasma [H^+] and [K^+] in the final stage were moderated by supplementation with EM-K. Traditional acid-base analysis revealed that initial respiratory alkalosis early in the ride was followed by respiratory and metabolic acidosis by the end of the ride, and by only metabolic acidosis during recovery. Moreover, application of the Stewart comprehensive model revealed the major impacts of SID on plasma [H^+], and plasma [Na^+] on SID. Thus the lower plasma [K^+] and plasma [H^+] in the last stage may be attributable to the absence of K and to the higher amount of Na, respectively, in the EM-K formula.

The critical differences between treatments in plasma [K^+] and [H^+] were not evident until the last stage, perhaps because this race was at the start of the season. Riders agreed that our race was less challenging than most in mid-season. Also, weather conditions were mild. Horses were eliminated for reasons similar to those in previous reports (Sloet et al, 1991; Lindinger and Ecker, 1995; Schott et al, 1997). The present elimination rate of 26% was smaller than previous rates of 40% or more (Hargreaves et al, 2002; Williams et al, 2003). Weight losses of only 5% at 56 km and 80 km are similar to a 5% weight loss in

another 80 km ride (Fregin, 1979), and smaller than 7% losses at 80 km during 160 km rides.(Fregin, 1979; Hargreaves et al, 2002). The 5% sweat losses are consistent with mild ambient conditions, footing and trail difficulty in the present study (Ecker and Lindinger, 1995).

Increases in plasma [Na^+] during exercise presumably helped to maintain hyperosmolarity, hence thirst, during the race. Effects of increased plasma [Na^+] at 37 and 56 km would contribute to the decrease in plasma [H^+] via SID and hyperosmolarity. The decrease in plasma [Na^+] seen at REC reflects fluid redistribution after further drinking of water. Plasma osmolality was also increased by 5% in a 62 km race in horses supplemented with salt paste and saline (Nyman et al, 1996).

Progressive decreases in plasma [Ca^{++}] during prolonged exercise were reported previously (Carlson and Mannsmann, 1974; Schott et al, 1997; Lucke and Hall, 1978; Sloet et al, 1991; Rose et al, 1980; Aguilera-Tejero et al, 2001). Decreases from PRE to 21 and 37 km in plasma [Ca^{++}] in the present study were probably due partly to a greater sweating rate during the first two loops, and partly to alkalosis at 21 km. Hypocalcemia can be caused by Ca^{++} loss in sweat, by shifting of ions into red blood cells or muscles, or by increased binding to albumin (Wijnberg et al, 2002). Clinical signs of hypocalcemia may include synchronous diaphragmatic flutter, tachypnea, stiff gait, profuse sweating or ataxia (Carlson and Mansmann, 1974; Leroux et al, 1995; Aguilera-Tejero et al, 2002). Low plasma [Ca^{++}] is not always associated with these typical clinical signs (Wijnberg et al, 2002), however, and none were observed during our study.

A higher Ca concentration in the commercial formula (EM+K) did not lead to higher plasma [Ca^{++}]. Equine sweat studies reveal huge variation in electrolyte composition

(Carlson and Ocen, 1979; McCutcheon et al, 1995; Schott and Hichcliff, 1990). Therefore commercial formulas were so much higher in Ca and Mg compared to EM-K.

Plasma [Ca^{++}] can be influenced by dietary cation-anion balance (DCAB). In resting horses diets high in Na and K will increase DCAB and make horses more alkalotic (Baker et al, 1993). In exercising horses, however, decreased plasma [H^{+}] has not been observed, despite high DCAB diets, and lower plasma [Ca^{++}] (Stutz et al, 1992). In the present study, it was not possible to calculate DCAB, however a high hay diet combined with EM+K could have had the same effect, leading to lower [Ca^{++}] in the beginning of the ride. Chronic feeding of a high DCAB diet could have led to chronic resting alkalosis, leading to inadequate response by PTH in response to exercise causing hypocalcemia (Fitzpatrick and Bilezikian, 1999). Another factor pointing to a higher DCAB, was a higher plasma SID during the race (Figure 5) in EM+K supplied horses. At the end of the ride this difference in [Ca^{++}] was not observed, probably because SID was negative. From 80 km to REC, EM-K horses were less acidic than EM+K, however no difference in [Ca^{++}] was observed because neither group was alkalotic.

The increase in plasma [Mg^{++}] confirms one previous study of endurance horses (Sloet et al, 1991). Other studies, however, found decreases (Rose et al, 1980) or no changes (Delar et al, 1982). Hypomagnesemia has been reported in horses with muscle tetany during strenuous exercise (Flaminio and Rush, 1998). Hypomagnesemia can potentiate the effects of hypocalcemia (Carlson, 1985), because it increases the release of acetylcholine at neuromuscular junctions. Perhaps exercise was not strenuous enough during the present ride to cause a decrease in plasma [Mg^{++}]. Increases may just reflect a water shift from blood to muscles.

Plasma pH increasing initially (Table 3) is consistent with hyperventilation and the 8% decrease in PCO_2, that is, a primary respiratory alkalosis in the traditional Van Slyke assessment of acid-base in terms of the bicarbonate buffer system (Henderson-Hasselbalch equation) (Van Slyke, 1921). The increase in PCO_2 accounted for 99% of the change in plasma pH at 21 km. There was no evidence of compensation by metabolic acidosis.

An increase in plasma $[HCO_3^-]$ was mainly responsible for the metabolic alkalosis at 37 and 56 km. Bicarbonate $[HCO_3^-]$ is reabsorbed by the kidneys compensating for the Cl^- lost in sweat (Flaminio and Rush, 1998). Dehydration increases the reabsorption of Na^+, but H^+ is excreted in exchange, further contributing to alkalosis. PCO_2 was within the pre-race range, so had no effect on pH. At the end of the race, the low plasma pH represented a metabolic acidosis associated with a decrease in plasma $[HCO_3^-]$ leading to a metabolic acidosis. There was no increase in lactate or PCO_2, variables that according to the traditional interpretation would be likely to account for the acidosis.

The biphasic plasma $[H^+]$ response was evaluated by the Stewart model (Stewart, 1981), which showed that P_{CO_2} was the predominant contributing factor to the decrease in plasma $[H^+]$ at 21 km, and that SID was predominant during the subsequent rise in plasma $[H^+]$ (Figure 3). Respiratory alkalosis has been observed previously in horses running at 40% of VO_{2max} on a treadmill (Bayly et al, 1995). In our study, hemoconcentration and a lower plasma $[Cl^-]$ also contributed to the decrease in plasma $[H^+]$ at 21 km. Alkalosis in endurance races has previously been attributed mainly to Cl^- sweat losses (Carlson and Mansmann, 1974; McCutcheon et al, 1995; Jahn et al, 1996; Rose et al, 1980), but the impact of SID on the change in plasma $[H^+]$ at 21 km was slight in the EM+K group and negligible in the EM-K group in the present study.

The Stewart's model (Stewart, 1981) reveals the dominance of the SID contribution to the increasing plasma [H^+] from 21 km to REC (Figure 3). This dominance was also indicated by the overall negative regression of changes in plasma [H^+] on changes in SID, and by the partition specifically at 80 km and REC, where the increased plasma [H^+] mainly reflected a 3.3 mEq/L decrease in SID. This dominance of SID agrees with previous results from human athletes subjected to maximal exercise but not horses during repeated sprints, in which P_{CO_2} predominated (Lindinger et al, 1992; Kronfeld et al, 1999). The progressive increase in plasma [H^+] from 21 km to REC occurred despite hemoconcentration from 37 km on, which tends to increase SID, hence to moderate acidosis (Stewart, 1981).

The only significant difference between treatment groups in ions contributing to SID was the lower plasma [K^+] in the EM-K group at 80 km and REC, which would have opposed the lower plasma [H^+] in the EM-K group. Thus differences in other strong ions that were not statistically significant must have accounted for the greater impact of SID in the EM-K group.

The high correlations of plasma [H^+] with SID, and SID with plasma [Na^+], illustrate how the comprehensive physico-chemical model of Stewart (Stewart, 1981) can extract information that is not evident in routine analysis of variance. The model results suggest that the 26 g higher Na intake, rather than the 33 g lower K intake, in the EM-K group than in the EM+K group, may explain the lower plasma [H^+] in the EM-K group at 80 km and REC.

Plasma [K^+] increased progressively to about 10% above PRE at 56 km when speed was 3.4 m/s over hilly terrain. This result compares with a previously estimated minimal speed of 4 m/s on the flat at which plasma [K^+] increased during prolonged exercise in the horse (Kronfeld, 2001a). An impression may have been given in some previous reports that

plasma [K^+] decreases during endurance exercise in the horse, because samples were taken 3 to 60 minutes after exercise when plasma [K^+] is falling rapidly as K^+ moves into muscle cells and urine (Kronfeld, 2001a; Jansson et al, 1994; Lindinger et al, 1992) compared to 1 to 2 minutes in the present study.

Despite K^+ losses in sweat (Carlson and Ocen, 1979; McCutcheon et al, 1995), plasma [K^+] may increase in proportion to exercise intensity (Harris and Snow, 1992) as K^+ moves out of working muscle cells. Increasing plasma [K^+] initially facilitates exercise by dilating arterioles in muscle, providing adequate oxygen supply. Higher plasma [K^+], however, will exert cat electronic effects on E_K and may exacerbate neuromuscular excitability (Ganong, 1999). Eventually high plasma [K^+] reaches a critical level and inhibits action potentials, so muscles and nerves become unable to respond (Ganong, 1999; Mainwood et al, 1985). To evaluate these possible adverse effects in relation to increases in plasma [K^+] observed in the present study, it is necessary to make reasonable assumptions based on previous research in horses and other species, such as a [K_i] of 124 mEq/L (Johnson et al, 1991). No relevant data has been found in the horse, but a textbook value of about +7mV of depolarization leads to a zone of local responses in which cathodal stimuli are facilitated, that is, in which increases in excitability are greater up to the firing threshold (Ganong, 1999). In the present context, a zone of 7 to 15 mV of depolarization may be considered to represent hyperexcitability, and persistent depolarization greater than about 15 mV to represent prolonged refractory periods and decreased muscle response (Ganong, 1999; Mainwood et al, 1985).

The mean calculated E_K in the present study is –90.5 mV, so estimates of –83.5 and –75.5 mV may be predicted for catelectronic local responses and firing thresholds,

respectively. The Nerst equation yields corresponding estimates of 5.45 and 7.35 mEq/L for $[K_o]$ or plasma $[K^+]$. Such values have been recorded, albeit for faster speeds and briefer periods, without clinical manifestations (Harris and Snow, 1992; Kronfeld, 2001a).

Although the mean increases in plasma $[K^+]$ at 56 and 80 km are well below the predicted mean of 5.45 mEq/L that corresponds to a depolarization of +7mV, an evaluation of the frequency distributions reveals a difference between the EM-K and EM+K group (Figure 5). The SDs are 0.414 and 0.442 mEq/L for the EM-K and EM-+K groups at 80 km respectively. Dividing these SD's into the difference between the respective means of 4.06 and 4.33 mEq/L at 80 km and 5.45 mEq/L (+7mV depolarization) will yield respective estimates of 3.354 and 2.522 for Z, with corresponding probabilities of 1 horse in 171 or 1 in 769 reaching the zone of local catelectronic responses in the EM+K or EM-K groups, respectively (Rothman, 1986).

The sensitivity of these results to the critical assumption of $[K_i]$ of 124 mEq/L may be tested with the alternative assumption of 150 mEq/L, which may be regarded as a population mean for many muscles in many species (Rothman, 1986). It gives corresponding probabilities of 1 horse in 53 or 495 in the EM+K or EM-K groups reaching a zone of hyperexcitability. Regression of the means of the equine samples towards the general population mean suggests that the alternative estimates indicate respective ranges of 1 horse in 53 to 171 given EM+K and 1 horse in 495 to 769 given EM-K. The conclusions drawn from these estimates are that hyperexcitability due to elevated plasma $[K^+]$ in this mild race would be uncommon but, nevertheless, chances would be lower at the end of the race in horses given EM-K than in those given EM+K.

Implications

No clinical effects of treatment were evident in the present race, which was not challenging according to the riders. Thus the potential clinical impact of increasing plasma [K^+] remains in question for faster and harder endurance races. The likelihood of clinical manifestations of neuromuscular excitability should increase with the work intensity of a horse, because increases in [K^+] are proportional to work intensities (Harris and Snow, 1982; Kronfeld, 2001a). Therefore a K-free electrolyte mixture should be more beneficial in faster horses and in more competitive races. More strenuous conditions are also conductive to greater sweat losses and to a greater need to replace K during slower sections of the race and immediately after exercise by the administration of a K-rich electrolyte-glucose mixture, as recommended previously (Kronfeld, 2001a).

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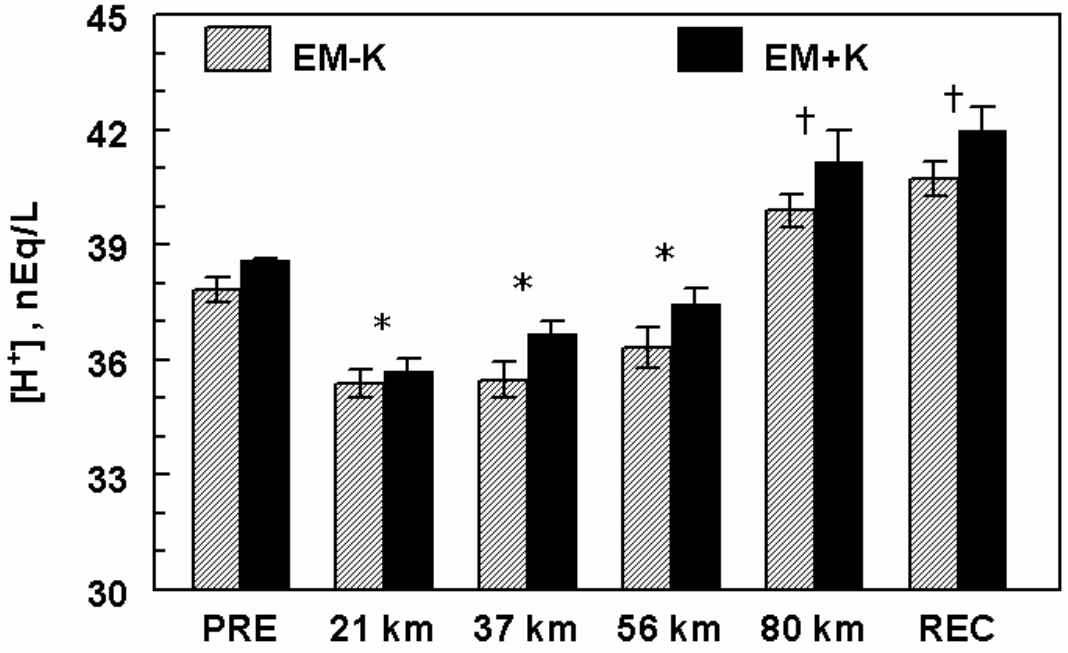

Figure 1. Mean (± SE) plasma [H^+] versus stage of sample collection before (PRE), during (at the 21-, 37-, 56-, and 80-km inspection points), and after (during recovery [REC]) the 80-km endurance ride in horses treated orally with an experimental potassium-free sodium-abundant electrolyte mixture (EM–K; n = 17) or commercial potassium-rich mixtures (EM+K; 17). Notice the overall ($P < 0.05$) differences between EM–K and EM+K treated horses for all stages of sample collection.

*Significant ($P < 0.05$) decrease from PRE values for all (EM–K and EM+K treated) horses.

†Significant ($P < 0.05$) increase from PRE values for all horses.

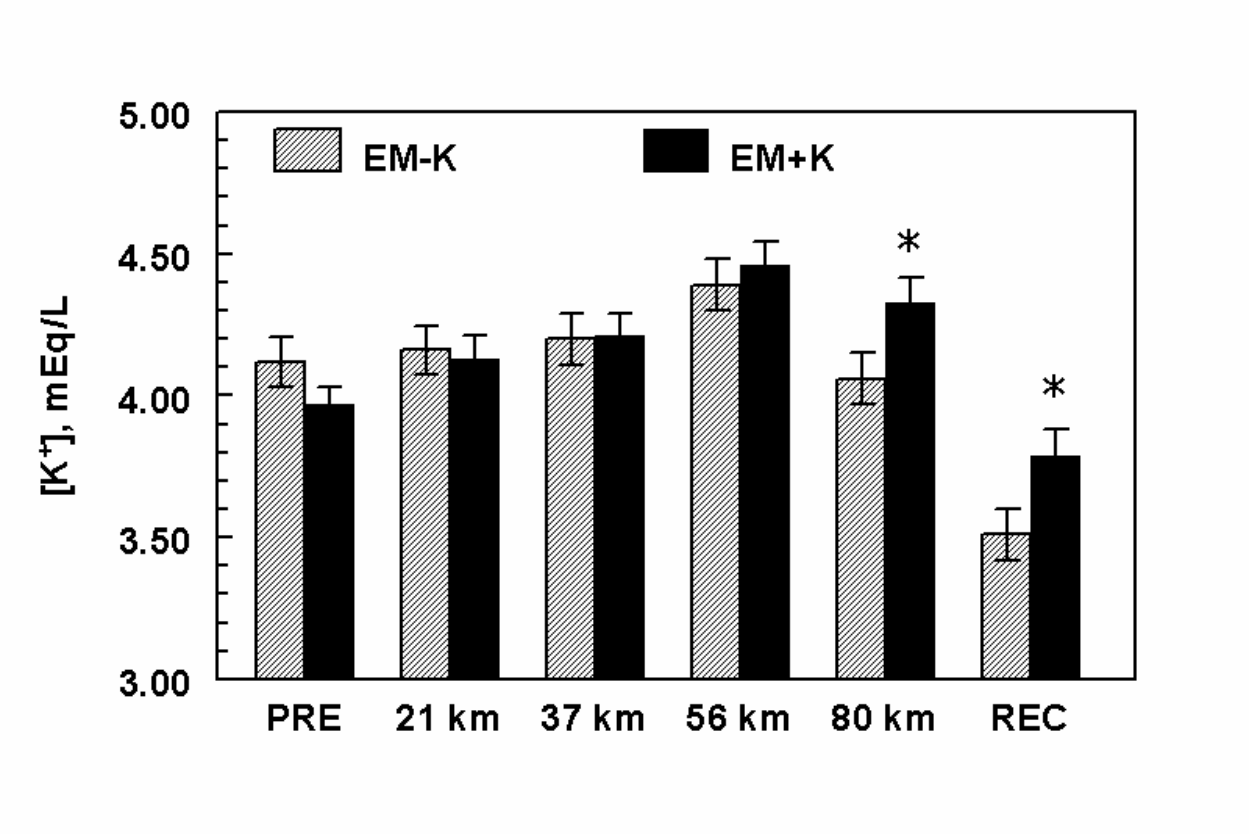

Figure 2. Mean (± SE) plasma [K^+] versus stage of sample collection before (PRE), during, and after (REC) the 80-km endurance ride in EM–K and EM+K treated horses. Notice the increase ($P < 0.05$) with stage from before the ride to the 56-km inspection point and the decrease ($P < 0.05$) from the 80-km inspection point to during recovery for all (EM–K and EM+K treated) horses to levels lower than PRE.

*Significant ($P < 0.05$) differences between EM–K and EM+K treated horses at the 80-km inspection point and also during recovery.

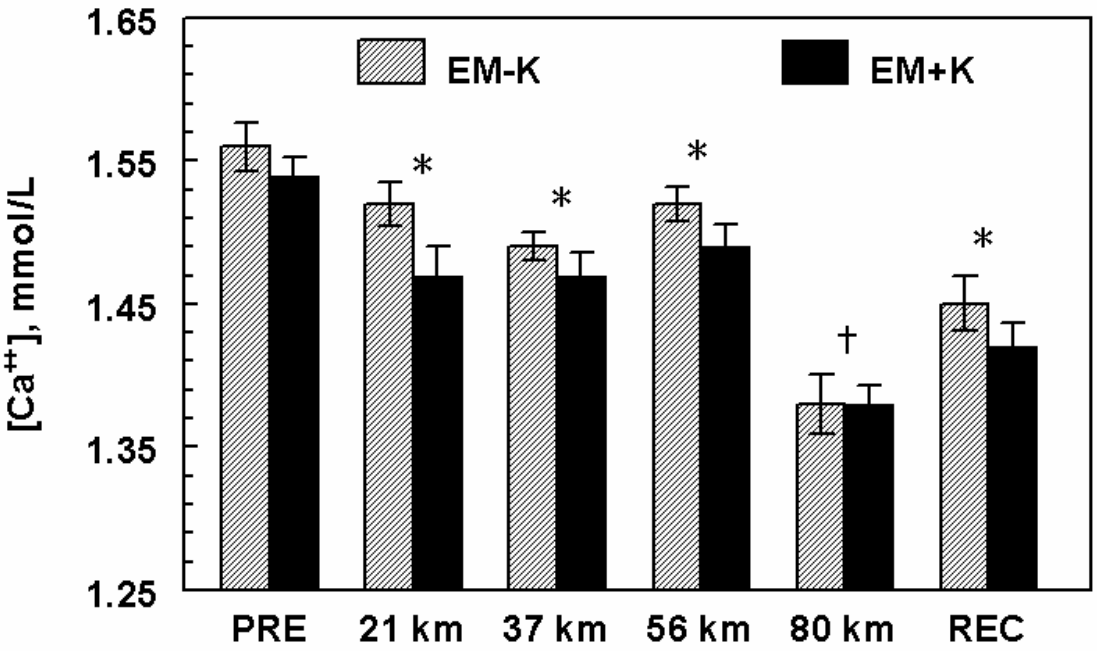

Figure 3. Mean (± SE) plasma [Ca^{++}] versus stage of sample collection before (PRE), during (at the 21-, 37-, 56-, and 80-km inspection points), and after (during recovery [REC]) the 80-km endurance ride in horses treated orally with an experimental potassium-free sodium-abundant electrolyte mixture (EM–K; n = 17) or commercial potassium-rich mixtures (EM+K; 17). Overall ($P < 0.05$) differences between EM–K and EM+K treated horses for all stages of sample collection.

*Significant ($P < 0.05$) decrease from PRE values for all (EM–K and EM+K treated) horses.

†Significant ($P < 0.05$) decrease from 21, 37, 56 km, and lower than PRE and REC values for all horses.

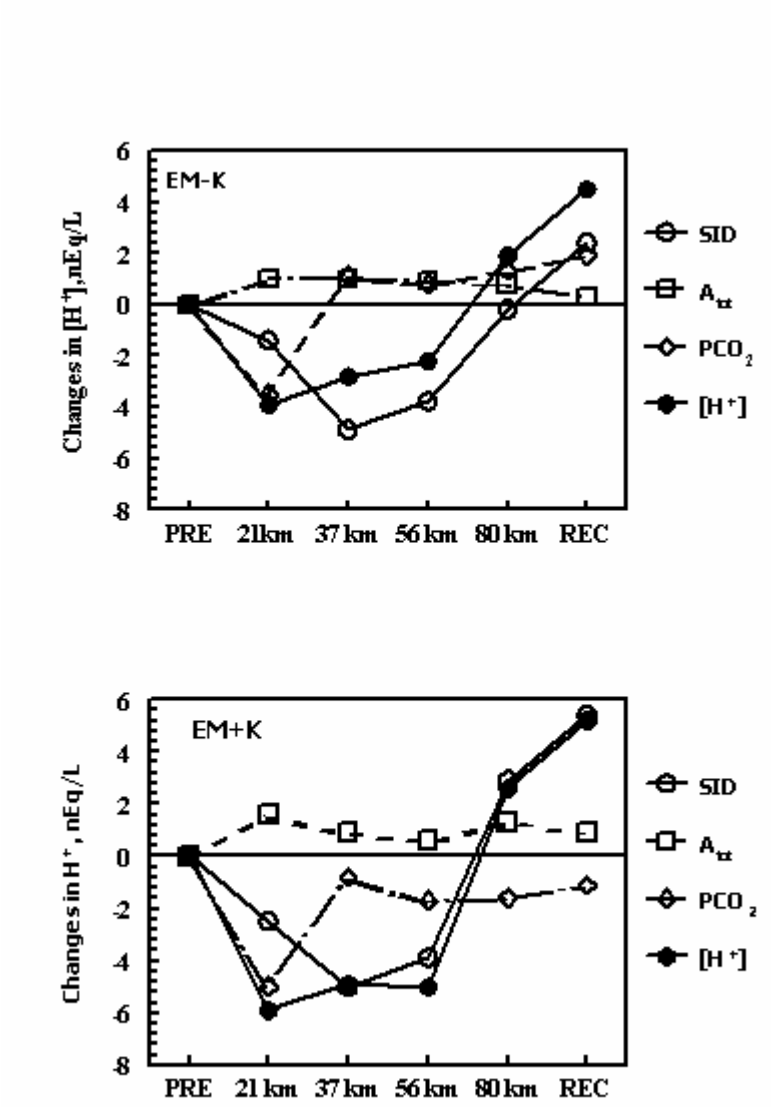

Figure 4. Partitioning of changes in plasma H^+ concentration from resting values (PRE) during the 80-km endurance ride and recovery (REC) into contributions from 3 independent variables, the strong ion difference (SID), total weak acids (A_{tot}), and $PvCO_2$ in EM−K (top panel) and EM+K (bottom panel) treated horses.

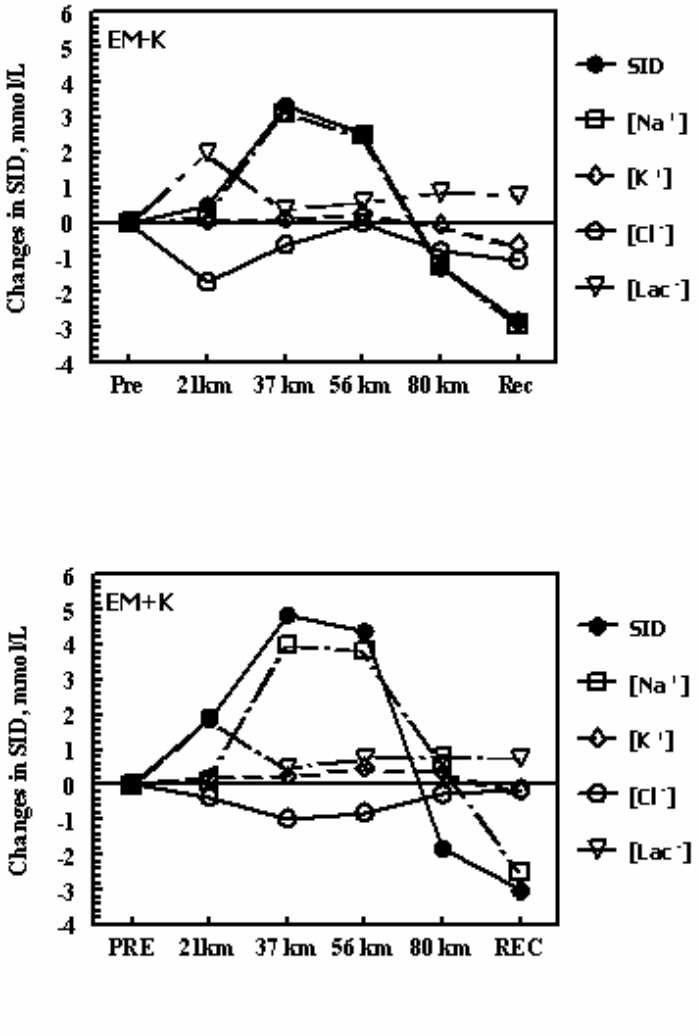

Figure 5. Changes in plasma concentrations of strong ions and their algebraic sum, the SID, from resting values (PRE) during the 80-km endurance ride and recovery (REC) in EM−K (top panel) and EM+K (bottom panel) treated horses.

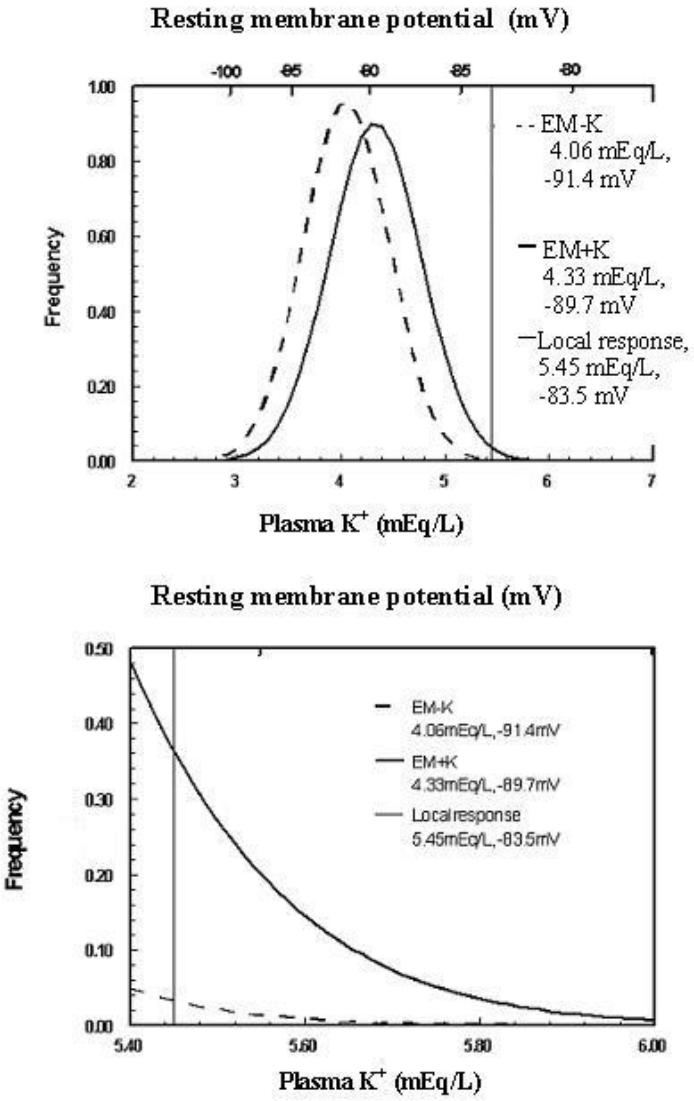

Figure 6. Frequency distributions of plasma K^+ concentration in EM–K (solid curved line) and EM+K treated (dashed curved line) horses are compared with a line for the start of local responses or disproportionately greater increases in excitability (solid vertical line), which corresponds to depolarization of +7mV from the mean E_K of muscle cells at the 80-km inspection point. The +7mV is a textbook value based on other species[7] and assumed here for the horse (upper figure). The cut off areas under the curves (magnified in lower figure) are proportional to the cumulative frequencies, namely, 1 horse in approximately 170 given EM+K and 1 horse in approximately 770 given EM–K.

	AW	Schott and Hichcliff, 1993 (g/L)	Meyer, 1990 (g/L)	McCutcheon et al, 1995 (g/L)
Na^{+}	23	6.97	2.77	**3.08**
K^{+}	39	2.03	1.47	**1.60**
Cl^{-}	35.5	9.51	5.33	**5.54**
Ca^{++}	40	0.44	**0.123**	
Mg^{++}	24	0.055	**0.052**	
P^{-}	31	**0.012**		

Table 1 Formulation of electrolyte supplement based on three studies, values used are in bold

	EM-K		***EM+K***	
	mean	**90% CI**	**mean**	**90% CI**
K^+	0	0	33.3	27.6, 39.1
Na^+	64.3	57.8, 70.7	38.2	31.9, 44.5
Ca^{++}	2.3	2.1, 2.6	8.0	5.8, 10.2
Mg^{++}	0.9	0.9, 1.0	4.0	1.4, 6.7
Cl^-	105.5	94.9, 116	98.9	87.2, 110.5
$P0_4^-$	0.21	0.19, 0.24	0	0

Table 2—Oral intake of electrolytes by EM–K and EM+K treated horses during the 80-km endurance ride.

	PRE	21 km	37 km	56 km	80 km	REC
Hct (%)	39 ± 0.4 [a]	47 ± 0.7[b]	47 ± 0.6[b]	47 ± 0.6[b]	46 ±0.9 [b]	36 ± 1.1 [c]
Tp (g/dl)	6.73 ± 0.06 [a]	7.21 ± 0.09 [b]	7.25 ± 0.10 [b]	7.09 ± 0.08 [bc]	7.02 ± 0.09 [c]	6.95± 0.12 [c]
Alb (g/dl)	3.5 ± 0.04[a]	3.8 ± 0.06[b]	3.8 ± 0.06[b]	3.8 ± 0.05[b]	3.8 ± 0.07 [b]	3.6 ± 0.05[c]
Lac(mmol/L)	0.70 ± 0.09[a]	2.64 ± 0.37[b]	1.13 ± 0.09[c]	1.38 ± 0.14[c]	1.53 ± 0.14[c]	1.46 ± 0.11[c]
Na^+(mmol/L)	145 ± 0.30[ac]	146 ± 0.33[a]	149 ± 0.33[b]	148 ± 0.44[b]	145 ± 0.51[ac]	142 ± 0.57[d]
K^+(mmol/L)	4.01 ± 0.06[a]	4.13 ± 0.06[b]	4.22 ± 0.04 [b]	4.42 ± 0.04 [c]	4.10 ± 0.08[a]	3.61 ± 0.08[d]
Cl^-(mmol/L)	104.4 ± 0.23[a]	102.4 ± 0.40[b]	103.6 ± 0.37[a]	104.0 ± 0.49[a]	103.9 ± 0.69[a]	103.8 ± 0.61 [a]
Ca^{++}(mmol/L)	1.53 ± 0.01[a]	1.44 ± 0.01[b]	1.43 ±0.01[b]	1.46 ± 0.01[b]	1.35 ±0.01[c]	1.43 ± 0.01[bd]
Mg^{++}(mmol/L)	0.35 ± 0.006[ab]	0.34 ± 0.005[a]	0.36 ± 0.007[b]	0.37 ± 0.007[bc]	0.40 ± 0.006[d]	0.40 ± 0.007[d]
PO_4^-(mg/dl)	2.56 ± 0.12[a]	2.31 ± 0.12[b]	2.09 ± 0.12 [c]	2.71 ± 0.14[ad]	3.56 ± 0.12[e]	2.57 ± 0.12[ad]
pH	7.416 ±0.003 [a]	7.450 ± 0.003[b]	7.443 ± 0.004[b]	7.432 ±0.004 [c]	7.396 ±0.006 [d]	7.381 ±0.004 [d]
PCO_2(mmHg)	48.4 ± 0.57[a]	45.4 ± 0.75[b]	49.5 ± 0.42 [a]	48.3 ± 0.40[a]	49.2 ± 0.40[a]	49.6 ± 0.36[a]
HCO_3^-(mmol/L)	31.2 ± 0.26[a]	31.5± 0.53[ab]	33.5 ± 0.24[c]	32.3 ± 0.36[bd]	30.5 ± 0.49[ae]	29.4 ± 0.33[e]
$[H^+]$ (nEq/L)	38.4 ± 0.27[a]	35.6 ± 0.24[b]	36.1 ± 0.30[b]	37.9 ± 0.34[c]	40.6 ± 0.51[d]	41.3 ± 0.38[e]
SID (mEq/L)	44.6 ± 0.60[ac]	45.7 ± 0.60[a]	48.9 ± 0.36[b]	47.7 ± 0.35[b]	43.4 ± 0.58[c]	41.3 ± 0.43[d]
Osm(mosm/kg)	290.6 ± 0.63 [a]	294.8 ± 0.86[b]	299.9 ± 0.73[c]	297.6 ± 0.87 [d]	291.2 ± 0.93 [ae]	288.06 ± 1.07[f]
A_{tot}(mEq/L)	14.2 ± 0.13[a]	15.1 ±0.20[b]	15.4 ± 0.21[b]	14.9 ± 0.16[bc]	14.7 ± 0.18[c]	14.7 ± 0.26[c]

Table 3. Mean and mean SE values of plasma variables measured in 34 horses before, during, and after an 80-km endurance ride. Different superscripts within rows differ ($P < 0.05$).

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Potassium-Free Electrolytes and Calcium Supplementation in an Endurance Race

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ABSTRACT: Proper electrolyte supplementation during endurance races is crucial for the prevention of dehydration and associated complications. Increases in plasma [K^+] and decreases in plasma [Ca^{++}] occur during exercise and can lead to increases in neuromuscular excitability. A K-free Na-rich electrolyte mixture (EM-K) and a recovery formula (EM-REC) were tested against commercial formulas rich in K (EM+K) during an 80 km endurance race. Also, two experimental feeds, one rich in hydrolysable carbohydrates and fat (ES) and one rich in fiber and fat (EF) were supplied to horses during three months before the ride and compared to horses usually fed commercial feeds (CF). Blood samples were taken the day before, within 3 minutes of the arrival at the vet checks at km 27, 48, 80, and three hours of recovery. Plasma samples were analyzed for pH, hematocrit (Hct), [Na^+], [K^+], [Cl^-], [Ca^{++}], [Mg^{++}], total protein(TP), albumin (alb), glycerol (gly), tryglycerides (TG), glucose), insulin, and cortisol. Effects of sampling times, treatments and interactions were evaluated by ANOVA in a mixed model with repeated measures and applied to the 25 horses that completed the 80 km. With increasing distance, increases were found in plasma [pH], [Na^+], [PO_4^-], [TP], [alb], [cortisol], [Gly], [TG], Hct and osmolarity; decreases in [K^+], [Mg^{++}], PCO_2, [HCO_3^-], [insulin], [Ca^{++}] and [Cl^-]. Horses supplied with EM-K had 12.5% lower (P = 0.001) plasma [K^+], 7.8% lower (P = 0.024) TP, and 8.4 % lower (P = 0.004) alb, also, at three hours after the race they had 6.8% lower TP (P = 0.045), compared to the

EM+K supplemented horses. Horses fed ES and EF had higher [Ca^{++}] at 27 (P = 0.027), 56 (P = 0.006) and 80 km (P = 0.022) compared to horses fed CF. The lower [K^{+}] in the EM-K group, and the higher [Ca^{++}] in the ES and EF supplemented horses should help prevent increases in neuromuscular excitability and related clinical signs. The lower TP and albumin indicate less dehydration in the EM-K group and could help prevent related disorders.

Key words: horse, [Ca^{++}], [K^{+}], neuromuscular excitability, electrolytes

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Introduction

During endurance races the horse undergoes losses of water and electrolytes through sweat. The supply of electrolytes during endurance races helps to restore losses and maintain plasma volume (Kronfeld, 2001b; Lindinger and Ecker, 1995; Nyman et al, 1996). During sweating large amounts of Na^+ are lost, which can lead to the reduced water content in the vascular compartment increasing total protein and albumin and leading to clinical signs of dehydration. Reduction of plasma osmolarity can abolish thirst and lead to further dehydration in horses (Carlson, 1983). Sodium supplementation helps to prevent a reduction in plasma osmolarity restores thirst, it tends also to offset the acidogenic effect of hypo-osmolarity.

During endurance exercise many clinical signs seen at veterinary checks are associated with increased neuromuscular excitability in striated and smooth muscles (Carlson and Mannsman, 1974; Carlson, 1985; Foreman, 1998; Leroux et al, 1995, Kronfeld, 2001a). Such signs may include heart rate arrhythmias, slower heart rate recoveries, muscle cramps and twitches, as well as increased or decreased intestinal motility. Neuromuscular excitability depends on resting membrane potential and threshold potential. The resting membrane potential is mainly determined by the K^+ distribution across the cell membrane according to the Nernst equation (Ganong, 1999), so that when plasma $[K^+]$ increases, resting membrane potential will decrease and more closely approach the threshold for an action potential, thereby increasing neuromuscular excitability. We propose that these increases would be exacerbated by K supplementation.

Plasma $[Ca^{++}]$ decreases during prolonged exercise in horses (Lindinger and Ecker, 1995). When extra-cellular $[Ca^{++}]$ is too low, neuromuscular excitability increases

(DiBartola, 2000) because [Ca^{++}] determines the threshold potential (Neuroguide, 2004). Hypocalcemia exacerbates the effects of hyperkalemia on membrane excitability.

Diet affects acid base status of horses at rest through the algebraic sum of strong cations and strong anions, the dietary cation-anion balance (DCAB). Alkalosis increases the binding of Ca^{++} to albumin, reducing its plasma ionized concentrations (Wijnberg et al, 2002). Alkalosis also inhibits PTH receptors, affecting Ca^{++} mobilization and reabsorption (Fitzpatrick and Bilezikian, 1999).

The objectives of this study were to compare the effects of a K-free Na rich electrolyte mixture (EM-K) and commercial, K-rich mixtures (EM+K) on changes in plasma concentrations of ions, and clinical signs associated with neuromuscular hyperexcitability during an 80 km race. Another objective was to compare the effects of electrolytes higher in Na^+ in the EM-K formulas with commercial formulas lower in Na^+ on clinical parameters related to hydration. Furthermore, the effects of the DACB of the feeds will be evaluated together with the different electrolyte DCAB (high in the EM+K horses versus a lower DCAB in the EM-K supplemented horses) on plasma [Ca^{++}] during the race.

Material and Methods

This study was undertaken during the Middleburg Research Ride 2002, which was held on April 14 and followed *American Endurance Ride Conference* (AERC) rules (1999). The race started at 0700 and covered 80 km of rolling hills with altitudes varying from 135 to 450 m. Rest stops were provided at 27, 48 and 72 km with *veterinary checks* (AERC, 1999). The trail between two rest stops is called a loop, and this trail consisted of four loops. Ambient temperature ranged from 25 to 32 ^oC, and humidity from 60 to 100%. A 2x3

factorial design compared two electrolyte treatments (Table 1) and three feed treatments (experimental concentrate rich in starch and sugar, ES, experimental concentrate rich in fat and fiber, EF, and commercial concentrates rich in fat, CF). The protocol was approved by the institutional animal care and use committee.

The 40 horses with an average age of 11 ± 1 years included 37 Arabians, purebred or crossbred, 1 Quarter Horse, 1 Mustang, and 1 Thoroughbred. A pre-ride survey provided a nutritional and performance history of each animal. Owners either required use of their customary commercial electrolyte supplements containing K (EM+K, n = 18) or volunteered to use a K-free mixture (EM-K, n = 22) during the race. The horses were supplied with ES (n = 15) and EF (n=14) three months before the ride. Horses receiving CF (n = 11) were fed their customary feed during the three months preceding the ride. The feeds were isoenergetic (3.3 Mcal/kg DM) and isonitrogenous (12% CP). Starch was 6, 33 and 16% in EF, ES, and CF, respectively; crude fat 17, 8 and 11%, and Ca 2.0, 1.9, and 1.0% respectively. Horses were fed 1.7 to 5.1 kg of feed a day and hay ad libitum.

Electrolyte mixtures were administered orally by syringe. Riders using EM-K were oriented to supply two doses per loop, and riders using EM+K followed manufacturer's directions, usually one to three doses per loop. One or two doses were also given two hours before the ride started. The formula of EM-K was based on sweat composition studies except for the absence of K and approximates the electrolytes lost in 2.5 L of sweat (Kronfeld, 2001b). Each dose consisted of 21.8 g of sodium chloride, 1.7 g of calcium chloride, 1.1 g of magnesium chloride, and 0.13 g of monosodium monophosphate. The commercial formulas all contained K (Endura-Max, Kentucky Equine Research, Versailles, KY; Enduralyte, Life Science Products, St. Joseph MO; Lyte-Now, Pro-formula Labs, Fort

Lauderdale, FL; Ora-Lyte, The Butler Company, Columbus, OH). Composition of EM-K and EM+K, as well as amounts of electrolytes consumed, which were calculated from the amount reported by the riders are in Table 1. Horses received water, electrolytes, and food between the 80 km and REC samples. Horses that were in the EM-K group during the race were also supplied with a recovery formula (EM-R) to be supplied just after their final veterinary inspection. EM-R contained electrolytes lost in 12 L of sweat plus 250 g of glucose. It consisted of 78.4 g of sodium chloride, 30.6 g of potassium chloride, 6.8 g of calcium chloride, 4.4 g of magnesium chloride, 0.53 g of monosodium monophosphate, and 250 g of glucose. Horses in the EM+K group supplied their normal electrolyte formula, 1-3 doses of EM+K.

Horses were weighed without tack before the start (PRE), after the 56 km vet check exam, and after the race finish at 80 km and at recovery (REC) on an electronic scale (Tyrel Platform, Model TC-105, Allweights Hamilton Scale Corp, Richmond, VA, USA). Horses were submitted to AERC regulation veterinary checks prior to the race, at rest stops and at the finish (AERC, 1999).

Jugular blood samples were collected the afternoon before the ride (PRE), within 2 min after arrival at veterinary checks, and 3 hours after finishing (REC). Heart rate and rectal temperature were recorded immediately after every blood sampling. An aliquot of blood was collected into a blood gas syringe (Dryhep Plus Kit, Becton Dickinson, Franklin Lakes, NJ, USA) and another was collected to heparinized Vacutainer tubes (Vacutainer Green, Becton Dickinson, Franklin Lakes, NJ, USA). Blood was kept in ice-water and analyzed or centrifuged within 1 hour of collection. The gas syringe aliquot was analyzed for pH, PCO_2, hematocrit (Hct), plasma $[Na^+]$, $[K^+]$, $[Cl^-]$, $[Ca^{++}]$ and $[Mg^{++}]$, using a blood-gas

analyzer (Stat Profile M, Nova Biochemical, Waltham, MA). Plasma separated from the collected blood was stored at –80^{o}C for further analysis. Spectrophotometric assays were used for plasma concentrations of lactate ([La^-]), albumin ([alb]), phosphate ([PO_4^-]), total protein ([TP]), triglycerides (TG), glycerol (Gly) within two weeks of the ride (Beckman Instruments, Inc., Brea, CA). Plasma insulin and cortisol were analyzed by radiomimmunoassay (Coat-A-Count, Cortisol, Diagnostic Products, Los Angeles, CA).

Changes in plasma pH were interpreted initially in the traditional Van Slyke manner based on the Henderson-Hasselbach equation, which represents pH as a function of pK plus the log of the ratio of [HCO_3^-] to [H_2CO_3 or 0.0307 x PCO_2] (Van Slyke, 1921). Metabolic changes are associated with [HCO_3^-], respiratory changes with H_2CO_3 or PCO_2. Despite widespread acceptance of this emphasis on the bicarbonate buffer, it has limitations; for example, the effects of proteins and osmolarity are not represented. Also, pH is not dependent upon [HCO_3^-] but is dependent instead upon concentrations of strong ions, PCO_2 and weak acids that are not represented in the Henderson-Hasselbach equation (Kronfeld, 2001a; Stewart, 1981; Watson, 1996). For these reasons, changes in acid-base status during exercise have been analyzed preferably by the comprehensive physicochemical model of Stewart (Stewart, 1981).

In this model (Stewart, 1981), concentrations of H^+ and HCO_3^- are dependent upon the strong ion difference (SID), PCO_2, and total weak acids (A_{tot}, mainly proteinates and phosphates). The SID is calculated as the algebraic sum of [Na^+], [K^+] minus the sum of [Cl^-] and [La^-]; the algebraic sum of other strong ions was assumed to be < 1 mEq/L and to contribute negligibly to changes in SID observed during exercise and recovery. Estimates of

weak acids ($A_{tot,}$ mEq/L) were predicted from TP (g/L), multiplying it by 0.211 (Stämpflie et al, 1999).

The partition of changes in plasma [H^+] into contributions from SID, PCO_2 and A_{tot} was performed using software designed for the Stewart system (Watson, 1996). Resting values for SID, PCO_2 and A_{tot} were set in the panel for independent variables, and the predicted value of [H^+] was observed in the panel for dependent variables. Then the series of values of SID at each sampling stage were entered one at a time (without changing PCO_2 and A_{tot}) to yield corresponding series of changes in [H^+] contributed by the changes in SID. The procedure was repeated for PCO_2 (without changing SID and A_{tot}) and for A_{tot} (without changing SID and PCO_2).

Feed and electrolyte DCAB were calculated (Stratton-Phelps et al, 2003) from the reported feed and forage daily ingestions as well as from the electrolytes consumed during the race. Calculations were done based on a daily feed ingestion of 2.5% of the horses' body weight. Feeds were analyzed for its electrolyte and S content, and forage values were obtained from NRC, and from samples analyzed (DHI Forage Testing Laboratory Ithaca, NY) for Cl and S. Commercial electrolyte DCAB calculation was based on the label information. DCAB was adjusted for intake.

Data were summarized as least squares means and standard errors. The effects of sampling stage (PRE, 27, 48, 80 km, and REC), treatments (EM-K, EM+K, ES, EF, CF) and their interactions were evaluated by ANOVA with repeated measures in a mixed model (SAS Institute Inc., Cary, NC, USA) and applied to the 25 horses that completed the 80 km. Non-significant interactions were dropped from the model. Significance of differences between means was tested by least significant differences covered by a significant F-test for the

ANOVA (SAS Institute Inc., Cary, NC, USA). Statistical significance was inferred from $P < 0.05$, a trend from $P < 0.10$ (Rosner, 1995). Empirical relationships of plasma $[H^+]$ on A_{tot}, PCO_2 and plasma $[Na^+]$, $[K^+]$, $[Cl^-]$ and $[La^-]$ were tested with linear regressions (SAS Institute Inc., Cary, NC, USA). Plasma data of eliminated horses and finishers were compared by the t-test.

Results

The 40 horses weighed 433 ± 8 kg before the race, and the average weights for the 25 finishers were 420 ± 8 kg at 27 km, 420 ± 8 kg at 48 km, 411 ± 8 kg at 80 km and 414 ± 8 kg at REC. Mean weight losses in the 25 finishers were 13.6 kg at 27 km, 13.1 kg at 48 km, 22.7 kg at 80 km and 18.9 kg at REC, which correspond to 3.2, 3.1, 5.4 and 4.5% losses, respectively.

Reasons for elimination were lameness (4), exertional rhabdomyolysis (1, EM-K, EF), failure to recover heart rate in 30 min (3, 1 EM+K, ES and 2 EM-K, ES, CS), labile heart rate (2, 1 EM-K, EF; 1 EM+K, ES), arrhythmias (2, 1EM-K, EF; 1 EM+K, CS), slow gut sounds (1EM-K, EF), sore back (1), and rider option (1). In this case the horse was examined by the veterinarians, passed the vet checks, but the rider decided to withdraw. Comparison of non-finishers versus finishers at 27 km, revealed a lower PCO_2 ($P = 0.009$) in eliminated (42.29 ± 1.59 mmHg) versus finishers (45.77 ± 0.49 mmHg). Lower ($P = 0.036$) plasma $[Na^+]$ was found in eliminated (142.44 ± 0.94 mmol/L) than in finishers (144.72 ± 0.47 mmol/L) at 27 km. Higher plasma $[K^+]$ ($P = 0.029$) was found in eliminated (3.67 ± 0.08 mmol/L) versus finishers (3.47 ± 0.038 mmol/L) at 27 km. A trend for lower $[Ca^{++}]$ ($P = 0.073$) was found in the eliminated (1.35 ± 0.046 mmol/L) versus finishers (1.43 ± 0.017

mmol/L) at 27 km. Higher plasma glucose (P = 0.007) was found in the eliminated (167.8 ± 17.5 g/dl) versus finishers (136.3 ± 3.3 g/dl) at 27 km. Higher plasma [La-] (P = 0.007) was found in the eliminated (2.45 ± 0.58 mmol/L) versus finishers (1.38 ± 0.12 mmol/L) at 27 km. Plasma osmolarity was lower (P = 0.044) in the eliminated (288.5 ± 1.9 mosm/L) compared to finishers (292.6 ± 0.9 mosm/L) at 27 km.

Comparison at 48 km revealed a trend (P = 0.078) for eliminated horses to have lower plasma [Cl^-] (98.8 ± 1.7 mmol/L) compared to finishers (101.5 ± 0.6 mmol/L). Plasma insulin was higher (P = 0.001) in the eliminated horses (23.3 ± 5.31 μU/L) compared to finishers (10.7 ± 0.96 μU/L) at 48 km.

At REC comparison of finishers versus eliminated showed higher (P = 0.001) insulin in the eliminated (30.9 ± 5.24 μIU/L) versus finishers (15.92 ± 1.26 μIU/L). Also eliminated horses had higher (P = 0.041) cortisol (114.6 ± 21.3 mg/dl) compared to finishers (76.7 ± 6.2 mg/dl).

Overall comparison between finishers and non-finishers comparing data at all sampling points revealed higher insulin (P < 0.001) in eliminated (21.6 ± 1.97 μU/L) versus finishers (14.1 ± 0.62 μU/L). Eliminated horses also had higher (P = 0.011) glucose (140.3 ± 4.49 mg/dl) compared to finishers (129.1 ± 2.11 mg/dl). Lower Hct (P = 0.019) and TP (0.056) were present in eliminated (43.6 ± 0.9 %, 6.9 ± 0.1 g/dl) compared to finishers (45.9 ± 0.5%, 7.1 ± 0.1 g/dl).

Average speed was 2.77 ± 0.10 m/s over 80 km, and 2.92, 3.00, 2.60, and 2.14 m/s for the 4 loops. Average heart rates during blood collection were 43 ± 2, 65 ± 2, 70 ± 2, 70 ± 2, and 58 ± 2 beats per minute at PRE, 27, 48 and 80 km, and REC, respectively.

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Total amounts of electrolytes consumed from two hours before and during the race are summarized in Table 1. Consumption of K^+ was zero for in the EM-K group and zero for PO_4^- for in the EM+K group. Consumption was higher for Na^+ and lower for Ca^{++} and Mg^{++} in the EM-K group than in the EM+K group. Differences were found between stages for all plasma electrolytes, and stage-by-treatment interactions for TP, albumin and [K^+] (Table 2).

No significant differences were found among the 3 feed DCABs ($P > 0.3$). Calculated DCAB was 326.9 ± 20.9, 318.2 ± 26.2, and 374.7 ± 26.2 mEq/ kg for ES, EF, and CF, respectively. A feed by electrolyte interaction ($P = 0.052$) was found when DCAB was analyzed with the total electrolyte consume included. Horses supplied with CF and EM+K had higher DCABs compared to all other feeds combined with any of both electrolytes, and also higher than CF supplied with EM-K (Table 3). An electrolyte effect was also found ($P = 0.027$), where EM-K supplied horses (299.5 ± 17.7 mEq/Kg) had a lower DCAB than EM+K ones (357.0 ± 16.2 mEq/kg), with all three feeds (Table 3).

With increasing distance, increases were found in plasma [pH], [PO_4^-], [TP], [alb], [cortisol], [Gly], [TG], Hct and osmolarity; decreases in [Mg^{++}], PCO_2, [HCO_3^-], [insulin], [Ca^{++}] and [Cl^-] (Table 2). Plasma [K^+] decreased ($P < 0.030$) from PRE to 27 km, and did not change further until REC. Plasma [Na^+] increased ($P < 0.001$) from PRE to 27 and 48 km and decreased below resting levels at REC ($P < 0.0001$). Plasma [Cl^-] decreased ($P < 0.04$) from PRE to 27 km, furthermore from 48 to 80 km ($P = 0.021$), increased to REC ($P=0.050$), but remained below resting levels ($P = 0.001$). Plasma [La^-] increased ($P = 0.001$) 176% by 27 km, 212% at 48 km, 232% at 80 km, and was still 177% higher than PRE at REC. Plasma glucose did not change during the race, but increased above resting during recovery ($P = 0.006$).

Plasma [H^+] declined ($P < 0.001$) from PRE to 21 km, then increased ($P < 0.001$) from 21 to 80 km, and changed little ($P < 0.068$) to REC, but returned to resting levels.

A stage-by-treatment interaction ($P = 0.011$) was found for plasma [K^+]. Plasma [K^+] was lower at 80-km ($P = 0.001$) in the EM-K horses than in the EM+K group (Figure 1). Sampling stage variation was different between the two electrolyte groups. Plasma [K^+] did not change from PRE to 27 km, decreased from 27 to 80 km ($P = 0.028$), but increased ($P = 0.035$) from 80 km to REC in the EM-K group (Figure 1). Plasma [K^+] decreased form PRE to 27 km ($P = 0.005$), increased from 27 to 80 km ($P = 0.005$), and decreased from 80 km to REC ($P = 0.029$) in the EM+K group (Figure 1).

A feed effect ($P = 0.010$) was found for plasma pH. Horses supplied with ES were more acidic than horses supplied with EF ($P = 0.0053$) or CF ($P = 0.024$) throughout the ride. There was a negative relation between plasma pH and [Ca^{++}] during the ride ($r = 0.47$, $P < 0.001$).

A time-by-electrolyte effect was found for TP ($P = 0.034$). At 80 km and REC TP was lower ($P = 0.024$ and $P = 0.045$) in the EM-K group compared to the EM+K group (figure 2).

A time-by-electrolyte interaction was also found for albumin ($P = 0.025$). At 80 km albumin was lower ($P = 0.004$) in the EM-K group than in the EM+K group (Figure 3).

A time-by-feed interaction ($P = 0.019$) was found for plasma [Ca^{++}]. Plasma [Ca^{++}] was higher at 27 ($P = 0.027$), 48 ($P = 0.006$), in the EF and ES than in the CF treatment; and at 80 km ES was higher than CF ($P = 0.022$), however EF was not different from CF (Figure 4). There was no interaction between feed and electrolyte supplementation ($P = 0.35$), however in analyzing the feed by electrolyte slices, horses supplied with CF on any of both

electrolytes (EM-K = 1.43 ± 0.020 mmol/L; EM+K = 1.43 ± 0.018 mmol/L) plasma [Ca^{++}] was lower than the EF, ES, EM+K, EM-K supplied horses.

Plasma [Na^{+}] and [PO_4^{-}] were not different between EM-K and EM+K groups, despite the difference in the ingested amounts (Tables 1 and 2).

Mean changes in plasma [H^{+}] are partitioned among the 3 independent variables for each feed group (Figure 5). In all groups, SID was the dominant independent variable at 27 km, SID and PCO_2 thereafter.

In the ES group, the initial -5.0 nEq/L change in plasma [H^{+}] at 27 km was partitioned into –5.3, -1.2 and +1.8 nEq/L contributions from changes in SID, PCO_2 and Atot, respectively (Figure 5). Subsequently, the predominant contribution to change in plasma [H^{+}] was from SID and PCO_2 at 56 and 80 km, and at REC (Fig 5*)*. Overall, plasma [H^{+}] was linearly and negatively related to SID (r = 0.93, P = 0.021), and to a lesser extend to plasma [Na^{+}] (r = 0.86, P = 0.064), but it was not related to PCO_2, $A_{tot,}$, [Cl^{-}] and [La^{-}] (all P > 0.16).

In the EF group, the initial –8.8 nEq/L change in at 27 km was partitioned into –6.8, -3.6 and + 1.6 nEq/L contributions from changes in SID, PCO_2, and Atot, respectively (Figure 5). Subsequently, the predominant contribution to change in plasma [H^{+}] was from SID and PCO_2 at all stages (Figure 5). Overall, plasma [H^{+}] was not related to SID, PCO_2, Atot, plasma [Na^{+}] [K^{+}], [Cl^{-}] and [La^{-}] (all P > 0.25).

In the CF group the initial –5.8nEq/L change at 27 km was partitioned into –4.9, -3.6, and +1.3 neq/L contributions from changes in SID, PCO_2, and Atot, respectively. Subsequently, the predominant contribution to change in plasma [H^{+}] was from SID and PCO_2 at 56 and 80 km. At REC contributions of –4.6 and +1.3 nEq/L were from PCO_2 and

Atot, respectively (Fig 5). Overall, plasma [H^+] was linearly and negatively related to SID (r = 0.946, P = 0.015), and to plasma [Na^+] (r = 0.99, P=0.001), but it was not related to PCO_2, A_{tot}, [K^+], [Cl^-] and [La^-] (all P > 0.16).

Discussion

The results confirmed that supplementation of potassium will affect plasma [K^+]. New findings are that plasma [H^+] could be influenced during low intensity exercise by different feed energy source compositions. Furthermore, lower DCAB in the EM-K horses may have attenuated hypocalcemia during exercise in ES and EF supplied horses. Also, a higher sodium amount in the electrolytes attenuated dehydration in EM-K supplied horses.

Elimination reasons were similar to other endurance races (Fregin, 1979; Lucke and Hall, 1978; Sloet et al, 1991). The present elimination rate of 38 % was also similar to previous reports (Heagraves et al, 2002; Williams et al, 2003). Riders found the ride challenging and it was the first warm day after the winter, so horses were not adapted nor had their winter coat clipped. Weight losses increased progressively during the race reaching 5.4 % at 80 km. These losses are similar to other reports (Hargreaves et al, 2002; Lindinger and Ecker, 1995; Scott et al, 1997). Despite the warm weather, weight losses were not higher than in a similar race in the previous year under colder conditions (Hess et al, 2002).

Eliminations at 27 km had some differences from finishing horses. A lower PCO_2 may have been due to more hyperpnea in eliminated horses. This over-breathing could be due to pain or an effect of thermoregulation. Eliminated horses had higher [K^+] and a trend for lower [Ca^{++}], both of which could lead to increased neuromuscular excitability. Horses were eliminated for heart rate arrhythmias (2) and a labile pulse (1), which might reflect

increased neuromuscular excitability (Carlson and Mansmann, 1974; Leroux et al, 1995; Kronfeld, 2001a). Two other horses were eliminated for lameness and one for rhabdomyolysis. Lower [Na^+] and osmolarity are factors that could have led to hypotonic dehydration and absence of thirst (Carlson, 1983). All blood parameters combined could lead to rhabdomyolysis (Harris, 1997).

At 48 km eliminated horses showed a trend to have lower [Cl^-] and had higher insulin. Clinically horses were eliminated for failure to recover heart rate within 30 min to 64 bpm (2), and colic (1). Both clinical signs can be related to dehydration, and low plasma [Cl^-] occurs due to sweat losses. Higher insulin may occur because horses could have been insulin resistant (Kronfeld and Harris, 2003; Kronfeld et al, in press). One horse was on CF and the other receiving a 50:50% mixture of EF and CF diet.

Comparison between eliminated and finishers at recovery showed again higher insulin in eliminated horses at recovery an indication of some degree of insulin resistance (Kronfeld et al, in press). Higher cortisol indicates more stress due to pain or distress in eliminated horses. No differences in plasma cortisol were found between eliminated and finishing horses in another race (Dybal et al, 1980).

Overall higher insulin and glucose in eliminated horses indicate that horses may have been due to insulin resistance (Kronfeld et al, in press). From eliminated horses 4 were on ES diet, 4 on CF and 5 EF diet. However, riders reported that the EF horses had been receiving a mixture of CF and EF in the 15 days before the ride. No difference in insulin was found between eliminated and finishing horses in another race (Dybal et al, 1980). Lower TP and Hct in eliminated horses overall could be due to less exercise (distance) performed by

eliminated horses and indicated that dehydration was not the determining factor contributing to clinical signs of eliminated horses.

Increases from PRE of 9.7-12% for TP and 8% in albumin are similar to other studies (Hargreaves et al, 2002; Jahn et al, 1996; Scott et al, 1997). The decrease of 10% in plasma water was partially responsible for the 20% increase in Hct. Hct as well as albumin had not returned to resting levels at REC. Horses had just eaten, which could have lead to a water shift from the vascular to the digestive compartment (Kerr and Snow, 1982).

Increases in plasma [Na^+] helped to keep a high osmolarity, thus thirst during the race. At 80 km it returned to resting levels and was below PRE at REC. Lower plasma [Na^+] at these times could indicate that drinking diluted its concentration, or that previous higher concentrations induced its elimination by urine, or simply fluid shifts from muscle to the vascular compartment. Increases in plasma [Na^+] at 27 and 48 km contributed to a decrease in plasma [H^+] through an increase in SID. In spite of the increase in [Na^+], plasma osmolarity did not change throughout the race or at REC. In previous reports osmolarity increased (Nyman et al, 1996).

Decreases in plasma [Ca^{++}] during endurance races have been reported before (Carlson and Mansmann, 1974; Rose et al, 1980; Sloet et al, 1991; Aguilera-Tejero et al, 2001). Decreases at 27, 48 and 80 km are due to sweating and alkalosis. Those decreases may be caused by losses in sweat, shifting of ions to red blood cells or muscles and increased binding to albumin during alkalosis (Wijnberg et al, 2002). Synchronous diaphragmatic flutter, tachypnea, stiff gait, profuse sweating and ataxia are clinical observations of hypocalcemia, but were not observed during this race (Aguilera-Tejero et al, 2001; Carlson and Mannsman, 1974; Leroux et al, 1995).

A decrease in plasma [Mg^{++}] in the beginning of the race confirms the same result of another study (Rose et al, 1980). After 27 km [Mg^{++}] returned to resting concentrations. Another study shows no changes (Delar et al, 1982). Despite having been reported to be a tough ride, hypomagnesemia only occurred in the beginning of the race and no clinical signs were associated with it.

Plasma lactate increased 1.8 times at 27 km and progressively increased to 2.4 times the resting levels. Horses were alkalotic at all sample points during the race, and there was no correlation of lactate with plasma [H^+]. As during repeated sprints, lactacidosis did not occur (Kronfeld et al, 1999). Changes in other variables affecting SID, in this case increases in [Na^+] and decreases in [Cl^-] had the greatest impact on plasma [H^+]. Increases in [La^-] also did not have a significant effect on SID. Increases were similar to other races (Jahn et al, 1996; Scott et al, 1997; Sloet et al, 1991), but smaller than a previous race on a similar trail (Hess et al, 2002). Speed was also lower than that previous ride. A lactate increase towards the end of the race may indicate some degree of fatigue or aerobic metabolism serving as a tactical tool in the race.

Plasma pH increased from PRE to 27 km, and was increased to a lesser extend at 80 km, returning to PRE at REC. A 6% decrease in PCO_2, and a 4% increase in plasma [HCO_3^-] initially influenced pH. Hyperpnea caused the decrease in PCO_2. Bicarbonate reabsorption through the kidneys in exchange with [Cl^-] (Flaminio and Rush, 1998) caused the increase in plasma [HCO_3^-]. A decreased PCO_2, or a respiratory alkalosis was the main responsible for the decreased pH until the end of the race, according to Van Slyke's acid base model. Bicarbonate decreased from 27 to 48 km and did not contribute to alkalosis, but may have been a partial metabolic compensation of the respiratory alkalosis. At REC the 8% higher

PCO_2 was compensated by an 8% decrease in plasma [HCO_3^-], and pH returned to resting levels.

Further information is given by Stewart's acid-base model. SID increased by 10, 9.6, and 4.4% at 27, 48, and 80 km, causing [H^+] to decrease. SID increases were caused by increases in [Na^+], and decreases in [Cl^-]. Decreases of 6, 8, and 7% in PCO_2 at 27, 48, and 80 km due to hyperpnea, also contributed at a lower degree to the decreases in [H^+]. At REC SID had returned to resting levels and so had [H^+]. PCO_2 was still lower, but increased A_{tot} partially compensated for it. The Stewarts model explains fully how sweat losses lead to metabolic alkalosis.

Plasma glucose decreased very little during the ride and was higher than PRE at REC. Other studies showed decreases (Dybal et al, 1980; Lucke and Hall, 1978; Snow, 1982) or increases (Dusterick et al, 1999; Lucke and Hall, 1980). Decreases were small and would not affect performance. Independent of the feed supplementation plasma glucose levels were maintained throughout the race. Plasma insulin decreased by 40 to 50% during the ride and similar decreases were reported before (Luck and Hall, 1980, Dybal et al., 1980). At REC insulin was still below resting, even though horses had been fed glucose and their feeds. Decreases in insulin and increases in glycerol and TG indicate that the body was utilizing mainly fat to maintain glycemia. Plasma glycerol increased up to 30 times during the race, and triglycerides increased up to 300 %. After the race these parameters returned to resting values. Other reports found the same results (Lucke and Hall, 1978, 1979; Snow et al, 1982).

Plasma cortisol increased about 100% during the race and returned to PRE after the race. Similar results were seen before (Dybal et al, 1980). No differences were seen in any

feed related parameters, despite different feeds. The amounts fed varied considerably between riders (500 g to 5 kg of grain daily), which would hide the feed effects.

Horses on ES diets were less alkalotic than horses on EF and CF diets. A smaller decrease in SID contributed to a lower decrease in plasma [H^+] (Figure 5). Supply of ES was an advantage to attenuate alkalosis in this race.

A 7 and 6.5% lower TP at 80 km and REC in the EM-K group compared to the EM+K shows that EM-K horses were less dehydrated. These results are similar to previous treadmill endurance exercise studies where horses that were supplied with electrolyte paste or isotonic solutions had lower total protein. (Coenen et al, 1995; Dusterdieck et al, 1999; Marlin et al, 1998). However, during a simulated endurance race (Nyman et al, 1996), no differences were found in horses that had an electrolyte paste compared to horses that received only water. In the present study horses were administered an average of 60 g of Na and 98 g of Cl (Table 1), and in the simulated endurance race the horses received 35g of Na and 55 g of Cl. The higher Na administration in our study was probably responsible for maintaining thirst, promoting the horses to drink more and therefore maintaining TP lower. An 8 % lower alb at 80 km in the EM-K group confirms the results from TP.

A 13% lower plasma [K^+] at 80 km in the EM-K group compared to the EM+K group could help maintain membrane potential and reduce neuromuscular excitability. Clinically, however the difference caused in the resting membrane potential due to mean plasma [K^+] is small and is unlikely to affect the clinical parameters related to it. However, horses eliminated at the first vet check had higher [K^+] and lower [Ca^{++}], factors which combined may have led to the arrhythmias and labile heart rates observed in the eliminated horses. The plasma [K^+] decreased during the race proportional to speed. In races lower than 4 m/s on

the flat, K supplementation could be done and would help to replace sweat losses, however one must be aware of the combined effects of higher [K^+] and lower [Ca^{++}]. Recovery plasma [K^+] was not different between EM-K and EM+K supplied horses, indicating that supplementation after exercise helps reestablish plasma concentrations and replace sweat losses.

A 7-9%, 6-9% and 3-7% higher plasma [Ca^{++}] was found in the ES and EF groups at 27, 48, and 80 km respectively compared to CF horses. The higher plasma [Ca^{++}] should help horses to prevent symptoms related to neuromuscular hyperexcitability. Endurance trainers and riders believe that high dietary calcium predisposes the horses to hypocalcemia (Flaminio and Rush, 1998). Our results show the opposite. Higher dietary calcium helped horses to maintain plasma [Ca^{++}] during exercise. DCAB can influence pH and consequently Ca^{++} balance during rest. In horses a higher DCAB diet cause a greater excretion of Ca^{++} in feces and a lower excretion in urine (Baker et al, 1993) at rest or during exercise. However Ca balance is greater with high DCAB diets (Baker et al, 1993), so high DCAB diets are indicated for high intensity exercising horses. In cattle however, it was shown that diets higher in K^+ and Na^+ (high DCAB) induce chronic alkalosis, an inadequate response to PTH and can increase Ca^{++} excretion, and more cases of periparturient hypocalcemia (Goff and Horst, 1997). Furthermore, it has been shown that animals on high K or Na diets are refractory to PTH stimulation (Block, 1984; Goff et al, 1991). A higher DCAB during endurance exercise caused by EM+K could have led to inadequate PTH response, consequently a higher Ca^{++} excretion and lower bone reabsorption, leading to lower [Ca^{++}]. This fact was observed in the CF horses, even though DCAB was changed during the race with the supply of electrolytes. Furthermore in cattle the addition of extra Ca to the diet

partially protected the cows from hypocalcemia when they were fed a high K diet (high DCAB) (Goff and Horst, 1997). The higher Ca content in the ES and EF diets may also have contributed to higher plasma [Ca^{++}]. EF and ES diets are considered to be medium to high DACB and the CS a high DCAB diet. The high DCAB in the CF supplied horses could have led to chronic alkalosis and a reduced response of PTH during exercise.

Implications

Current commercial formulas should increase Na amounts because higher Na intake in the electrolytes can help to prevent dehydration in endurance horses. Potassium supplementation even at speeds below 4 m/s over hilly terrain contributes to higher plasma values and if combined with hypocalcemia can lead to neuromuscular hyperexcitability. A lower DCAB seems to attenuate hypocalcemia during prolonged exercise. A comparison of feeds with greater differences in DCAB in exercising horses could help confirm present results. Higher plasma [Ca^{++}] and lower [K^{+}] during exercise could prevent the occurrence of signs related to increased neuromuscular excitability during endurance races.

The present results show the advantages of K-free, high Na, high Ca and low DCAB in electrolyte mixtures for supplementation of horses during endurance races. They also suggest that insulin resistance as well as neuromuscular hyperexcitability may contribute to elimination.

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Journal Article 2

Williams C. A., D. S. Kronfeld, T. M. Hess, J. N. Waldron, K. E. Saker, R. M. Hoffman, and P. A. Harris. 2003. Oxidative stress in horses in three 80 km races. Proc. 18th Equine Nutr. Physiol. Symp. 18:47-52.

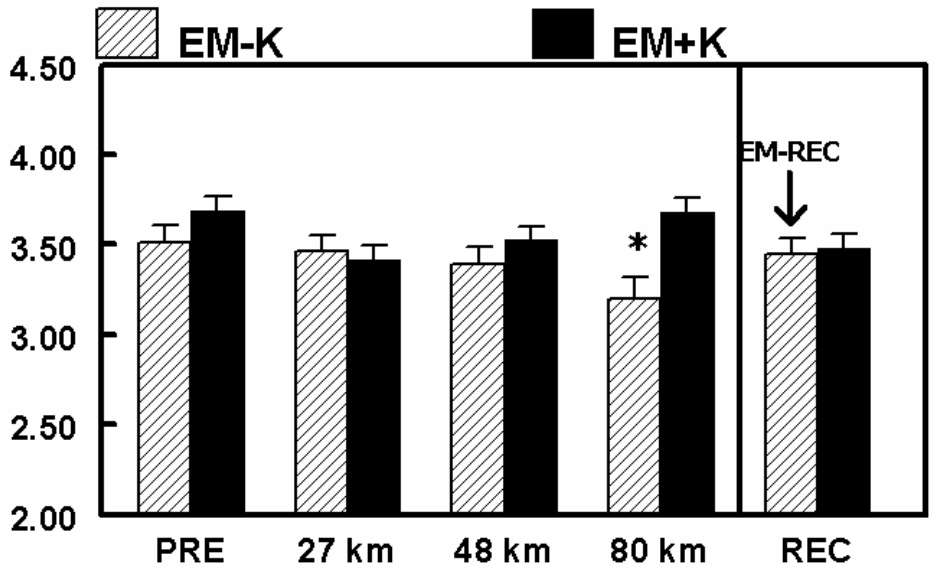

Figure 1. Mean (± SE) plasma [K^+] versus stage of sample collection before, during, and after the 80-km endurance ride in EM–K and EM+K treated horses. Notice plasma [K^+] decreasing from 27 to 80 km (P = 0.028), and an increasing (P = 0.035) from 80km to REC in the EM-K group. A decrease form PRE to 27 km (P=0.005), an increase from 27 to 80 km (P = 0.005), and a decrease from 80 to REC (P=0.029) in the EM+K group.

*Significant (*P* = 0.001) differences between EM–K and EM+K treated horses at the 80-km inspection point.

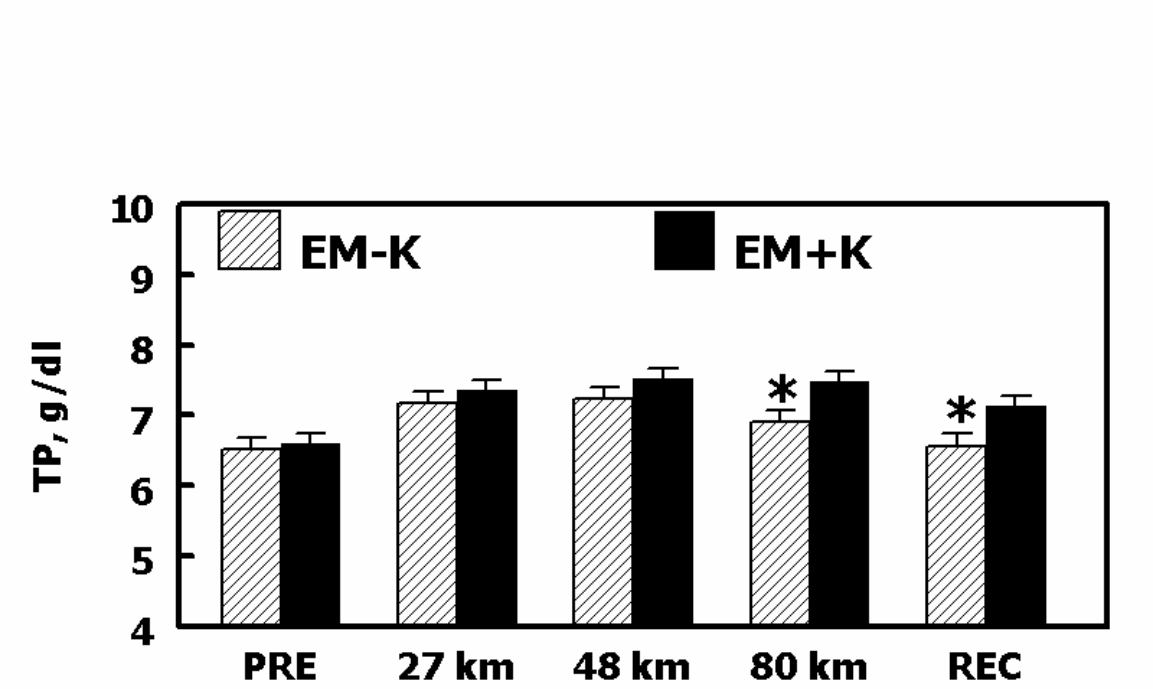

Figure 2. Mean (± SE) plasma TP versus stage of sample collection before, during, and after the 80-km endurance ride in EM–K and EM+K treated horses. Notice an increase ($P < 0.001$) with stage from PRE to 27 km, then a decrease ($P < 0.001$) from 80 km to REC. Significant differences between electrolyte mixtures (EM-K, EM+K) at 80 km (*P=0.024) and REC (*P =0.045), indicating less dehydration with EM-K.

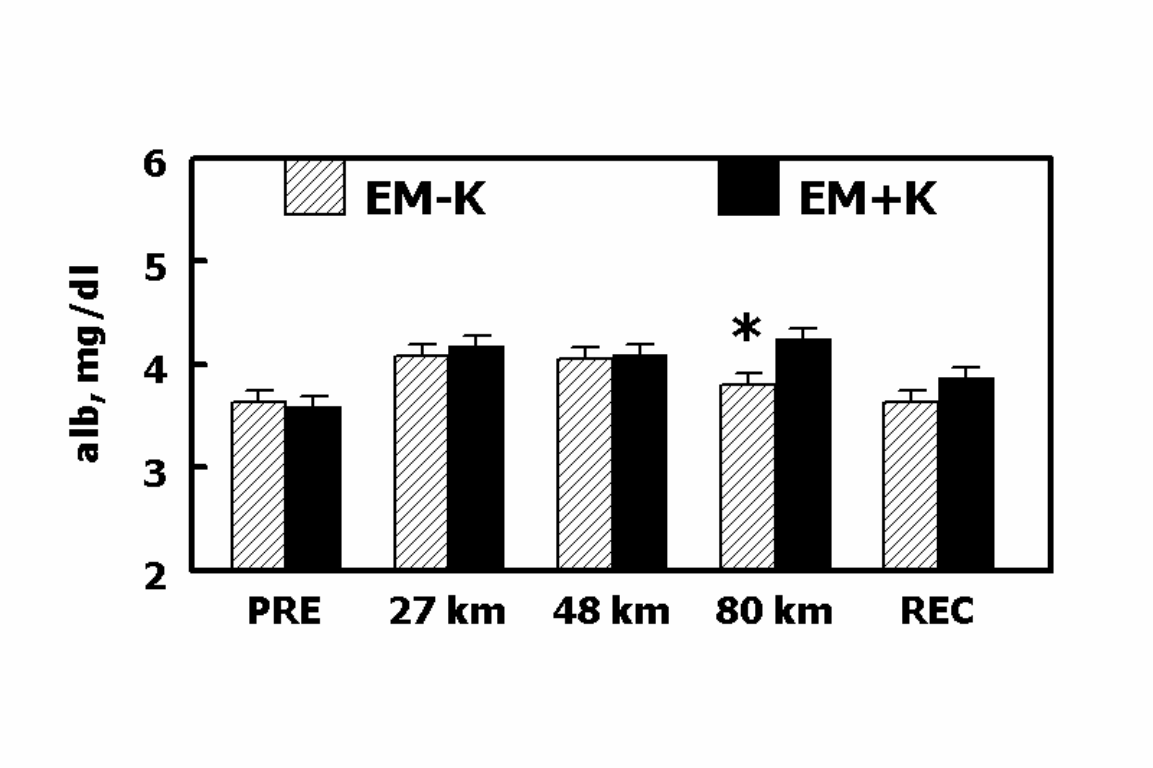

Figure 3. Mean (± SE) plasma albumin versus stage of sample collection before, during, and after the 80-km endurance ride in EM–K and EM+K treated horses. Notice an increase ($P < 0.001$) with stage from PRE to 27 km, then a decrease ($P < 0.001$) from 80 km to REC. Significant differences between electrolyte mixtures (EM-K, EM+K) at 80 km (*$P = 0.004$), indicating less dehydration with EM-K.

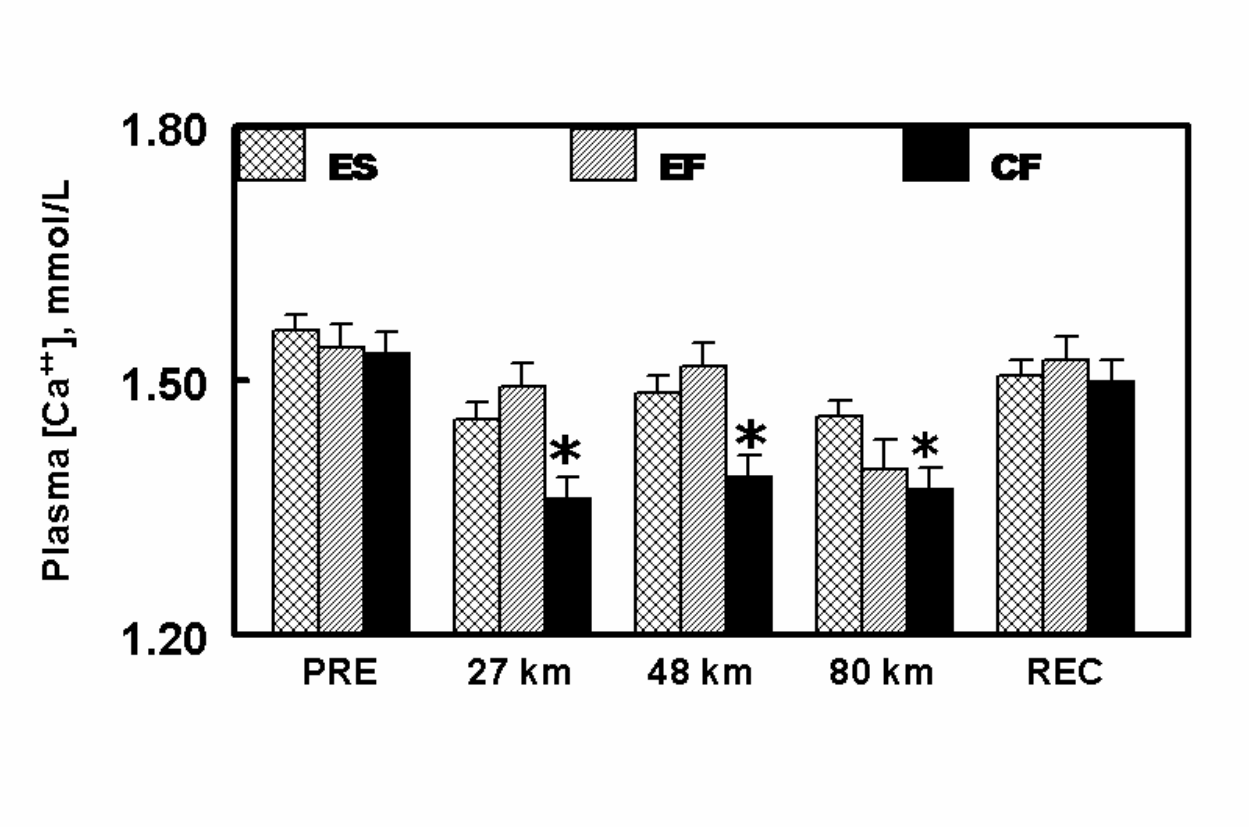

Figure 4. Mean (± SE) plasma [Ca^{++}] versus stage of sample collection before, during, and after the 80-km endurance ride in ES, EF, and CF treated horses. Notice a decrease from PRE to 27 km ($P < 0.001$), a small increase ($P=0.053$) from 27 to 56 km, a further decrease ($P=0.0039$) from 56 to 80 km, and an increase ($P<0.0001$) from 80 km to REC.

* Lower plasma [Ca^{++}] in the CF group at 27, 56 and 80 km ($P < 0.022$).

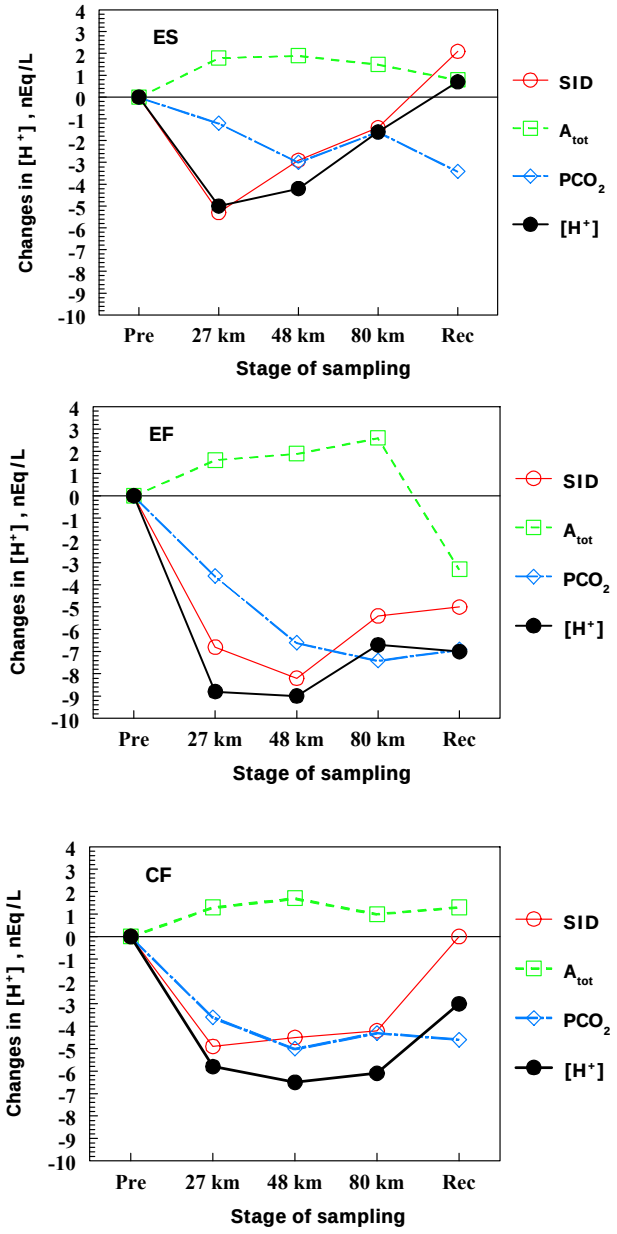

Figure 5. Partitioning of changes in plasma H^+ concentration from resting values (PRE) during the 80-km endurance ride and recovery (REC) into contributions from 3 independent variables, the strong ion difference (SID), total weak acids (A_{tot}), and P_{CO_2} in ES (top panel), EF (middle panel), and CF (bottom panel) treated horses.

	EM-K		***EM+K***	
	mean	**90% CI**	**mean**	**90% CI**
K^+	0	0	21.3	17.7, 25
Na^+	60	54.3, 65.1	31.3	26.4, 36.1
Ca^{++}	2.2	2.0, 2.4	6.2	4.3, 8.1
Mg^{++}	0.9	0.8, 1.0	2.3	0.6, 4.1
Cl^-	98	88.7, 106.3	73.6	61.3, 85.8
$P0_4^-$	0.21	0.17, 0.22	0	0

Table 1. Oral intake of electrolytes in grams by EM−K and EM+K treated horses during the 80-km endurance ride.

	PRE	27 km	48 km	80 km	REC
Hct (%)	39 ± 0.5^{a}	49 ± 0.6^{b}	48 ± 0.6^{b}	50 ± 1.0^{b}	44 ± 0.7^{c}
Tp (g/dl)	6.6 ± 0.07^{a}	7.3 ± 0.08^{b}	7.4 ± 0.11^{b}	7.2 ± 0.12^{b}	6.9 ± 0.10^{c}
Alb (g/dl)	3.5 ± 0.03^{a}	3.8 ± 0.04^{b}	3.8 ± 0.05^{b}	3.8 ± 0.07^{b}	3.8 ± 0.06^{b}
Lac(mmol/L)	0.75 ± 0.03^{a}	1.34 ± 0.16^{b}	1.62 ± 0.12^{bc}	1.77 ± 0.22^{c}	1.35 ± 0.07^{b}
Na^{+}(mmol/L)	141.5 ± 0.27^{a}	145.0 ± 0.44^{b}	144.1 ± 0.56^{b}	140.8 ± 0.67^{ac}	140.1 ± 0.62^{c}
K^{+}(mmol/L0	3.61 ± 0.05^{a}	3.46 ± 0.04^{b}	3.46 ± 0.04^{b}	3.45 ± 0.07^{b}	3.48 ± 0.05^{a}
Cl^{-}(mmol/L)	104.0 ± 0.28^{a}	102.4 ± 0.41^{b}	101.7 ± 0.63^{bc}	99.6 ± 0.88^{c}	101.4 ± 0.60^{b}
Ca^{++}(mmol/L)	1.54 ± 0.007^{a}	1.43 ± 0.017^{b}	1.46 ± 0.017^{c}	1.41 ± 0.015^{b}	1.50 ± 0.008^{a}
Mg^{++}(mmol/L)	0.26 ± 0.018^{a}	0.24 ± 0.004^{b}	0.27 ± 0.007^{ac}	0.26 ± 0.005^{a}	0.28 ± 0.06^{c}
PO_4^{-}(mg/dl)	2.11 ± 0.15^{a}	2.22 ± 0.15^{a}	2.27 ± 0.15^{a}	3.73 ± 0.16^{b}	4.20 ± 0.015^{c}
pH	7.444 ± 0.002^{a}	7.493 ± 0.004^{b}	7.470 ± 0.004^{c}	7.462 ± 0.006^{c}	7.445 ± 0.005^{a}
PCO_2(mmHg)	48.5 ± 0.38^{a}	45.6 ± 0.54^{b}	44.7 ± 0.52^{b}	45.1 ± 0.59^{b}	44.6 ± 0.45^{b}
HCO_3^{-}(mmol/L)	33.49 ± 0.29^{a}	$34.87 \pm .38^{b}$	32.35 ± 0.46^{c}	32.01 ± 0.40^{c}	30.85 ± 0.41^{d}
$[H^{+}]$ (nEq/L)	35.95 ± 0.20^{a}	32.16 ± 0.28^{b}	33.94 ± 0.32^{c}	34.49 ± 0.49^{c}	35.99 ± 0.24^{a}
SID (mEq/L)	40.4 ± 0.31^{a}	44.8 ± 0.32^{b}	44.3 ± 048^{b}	43.0 ± 0.77^{b}	40.9 ± 0.57^{a}
Osm(mosm/L)	286.5 ± 0.54^{a}	292.9 ± 0.82^{a}	291.9 ± 0.92^{a}	280.3 ± 1.43^{a}	287.7 ± 0.98^{a}
A_{tot}(mEq/L)	13.9 ± 0.16^{a}	15.4 ± 0.17^{b}	15.6 ± 0.74^{b}	15.3 ± 0.24^{b}	14.6 ± 0.21^{c}
Glucose (mg/dl)	125.7 ± 2.6^{ab}	135.6 ± 4.7^{ac}	122.5 ± 4.3^{b}	124.1 ± 5.0^{b}	142.8 ± 4.4^{c}
Insulin (μu/ml)	21.5 ± 1.98^{a}	13.5 ± 0.97^{b}	12.3 ± 2.03^{b}	11.4 ± 1.49^{b}	15.9 ± 2.31^{b}
Cortisol (ng/dl)	76.4 ± 3.64^{a}	145.9 ± 6.4^{b}	154.7 ± 7.9^{b}	157.0 ± 11.05^{b}	78.5 ± 8.93^{a}
Glycerol (mg/dl)	1.12 ± 0.18^{a}	13.0 ± 1.16^{b}	22.0 ± 2.82^{c}	33.4 ± 4.17^{d}	4.4 ± 0.52^{a}
Triglyc. (mg/dl)	15.8 ± 1.68^{a}	21.6 ± 1.33^{a}	32.7 ± 3.58^{b}	42.9 ± 4.33^{c}	18.5 ± 2.0^{a}

Table 2. Mean and mean SE values of plasma variables measured in 25 horses before, during, and after an 80-km endurance ride. Different superscripts within rows differ ($P < 0.05$).

FEED → ELECTROLYTE	ES	EF	CS
EM-K[a]	310.62 ± 23.72[a]	307.29 ± 33.54[a]	280.60 ± 33.54[a]
EM+K[b]	326.63 ± 25.98[a]	314.32 ± 29.05[a]	430.17 ± 29.05[b]

Table 3. Feed and electrolyte calculated DCAB, mEq/kg.

Different superscripts differ ($P < 0.05$).

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Potassium supplementation affects plasma [K^+] during a simulated 80 km endurance test on the treadmill

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ABSTRACT: During exercise plasma [K^+] increases and can lead to increased neuromuscular excitability and related clinical signs. Supplementation of K during exercise can further increase plasma [K^+]. A K-free electrolyte mixture (EM-K) was tested and compared to a K-rich mixture (EM+K) during an 80 km simulated endurance exercise test (EET) on a treadmill. Twelve horses were tested in a cross over design performing four bouts (B) of 20 km at 45% of their maximum heart rates with three 30-minutes of rest (R) between bouts. Before the start of EET and during each R horses were supplied with EM-K or EM+K. Blood samples were collected before (PRE), at 10 km of each B, at 20 minutes of every R and 10 minutes after B4 (REC) and analyzed for hematocrit (Hct), and plasma for pH, PCO_2, lactate ([La-]), phosphate ([PO_4^-]), albumin (alb), and electrolytes. Horses were weighed and electrocardiograms done at PRE, every R, and REC; weights were also measured in the morning after (MA) EET. Effects of stage (PRE, PEL, 10 km, 30 km, 50 km, 70 km, R1, R2, R3, REC_{10}, REC_{120}, REC_{300}, and MA) treatment (EM-K vs. EM+K) and interactions were evaluated by ANOVA in a mixed model with repeated measures. Body weight losses during EET increased up to 4.96% at REC_{10}, and were 2.3% below PRE at MA ($P < 0.001$). Hct increased during B, returned to PRE at every R ($P < 0.001$). Plasma alb

increased during EET ($P < 0.001$), returning to PRE values at REC. Plasma pH increased with exercise, decreased with R and REC ($P < 0.001$). Plasma PCO_2 and $[Ca^{++}]$ decreased during exercise and increased during R periods, and $[Ca^{++}]$ was higher than PRE at REC ($P < 0.001$). Plasma $[Mg^{++}]$ decreased with exercise, and remained lower than PRE at REC ($P < 0.001$). Plasma $[Na^{+}]$, [Cl-], [La-], and PO_4 increased progressively during the EET ($P<0.0001$). No abnormalities were observed on the EKG results. A treatment effect was found for plasma $[K^{+}]$ ($P = 0.028$), where horses that received EM-K had lower values than EM+K. Lower plasma $[K^{+}]$ could help maintain resting membrane potential and prevent signs of neuromuscular hyperexcitability.

Key words: potassium, neuromuscular hyperexcitability, electrolytes, endurance, horse

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Introduction

During equine exercise plasma [K^+] increases in proportion to exercise intensity (Harris and Snow, 1992; Kronfeld, 2001). Accumulation of extra-cellular K^+ is a key component in muscle fatigue (Nielsen et al, 2001). Muscle force in isolated muscles declines steeply when extra-cellular [K^+] increases due to changes in neuromuscular excitability. Neuromuscular excitability depends on resting membrane potential and threshold potential. The resting membrane potential is mainly determined by the K^+ distribution across the cell membrane according to the Nernst equation (Ganong, 1999), so that when plasma [K^+] increases, resting membrane potential will increase (become less negative) and more closely approach the threshold for an action potential, thereby increasing neuromuscular excitability.

Potassium is the main intra-cellular cation and its main stores are in the muscles (Meyer and Coenen, 2003). It is in close relation to glycogen molecules in the muscle. During endurance exercise significant breakdown of glycogen occurs with release of K^+ moves outside the muscles during muscle contraction (Bergstrom and Hultman, 1966; Snow et al., 1981). After exercise Na^+-K^+-pump activity helps to restore muscle K^+ (McCutcheon et al, 1999). The provision of glucose has been shown to enhance glycogen replenishment after exercise (Davie et al, 1995). The provision of glucose and K^+ would facilitate both muscle glycogen and K^+ replenishment.

During endurance exercise electrolyte and water losses occur (Sloet et al, 1991, Lindinger and Ecker, 1995). Despite these losses increases in plasma [K^+] will occur at speeds over 4 m/s on the flat (Kronfeld, 2001). Supplementation of K in electrolytes during races where speed exceed 4 m/s may further increase plasma [K^+], thus neuromuscular excitability. The objectives of this study were to compare the effects of an experimental K-

free, electrolyte mixture (EM-K) and a K-rich mixture (EM+K) on changes in acid-base-status and plasma concentrations of ions and associated variables during 80 km of endurance exercise on the treadmill. The resuls tested the hypothesis that K-supplementation increases the risk of neuromuscular hyperexcitability. Also the replenishment of K was tested in a K-rich glucose polymer recovery formula.

Materials and Methods

This study was conducted at the Virginia Tech Middleburg Agricultural Research and Extension Center from the first week of May to the first week of June of 2003, and was approved by the Institutional Animal Care and Use Committee. Twelve Arabian or Arabian–cross geldings were maintained on a mixed grass pasture with orchard grass hay round bales. They were fed two meals of 1 kg of a 10% fat textured feed, which was about 20% of the total ration (2.5% body weight basis). Samples were submitted for nutrient analysis (Table 1).

Horses were trained for 12 weeks for an aerobic foundation, three times a week alternating between lunging, treadmill training (Equi Gym Products LLC, Paris, KY) and riding. Two weeks before the exercise test the horses covered 45 km at 30 to 60% of their maximal heart rate. About three weeks before the endurance exercise test the horses performed an exercise test to determine their maximum heart rate (Eaton et al, 1995; Hodgson and Rose, 1994).

The effects of the two electrolyte formulas one rich in K (EM+K) and one without K (EM-K) were tested in a balanced randomized cross over design. Horses performed an 80 km simulated endurance ride (endurance exercise test, EET) at 51 ± 1 % of HR max. The 80

km endurance exercise test consisted of four loops of 20 km, with 30 min rest stops (R1, R2, R3) between the four loops. Each dose of EM-K consisted of 21.8 g of sodium chloride, 1.7 g of calcium chloride, 1.1 g of magnesium chloride, and 0.13 g of monosodium monophosphate. Each dose of EM+K had the same composition of EM-K added with 6.6 g of potassium chloride. The electrolytes were administered orally 60 to 90 minutes before the EET started, when 1.5 doses of EM-K or 1.5 doses of EM+K were administered to the horses (EM+K). At 25 minutes of each R, horses got 1.5 doses of EM-K or 1.5 doses of EM+K. During each R horses had access to water and hay. Ingested water and hay were calculated by subtracting the initial amount from the final amount left over.

Electrolyte composition was based on previous sweat composition studies (McCutcheon et al, 1995; Meyer, 1990; Schott and Hichcliff, 1993). Each dose of EM-K supplied contained 7.7 g of Na^{+}, 16 g of Cl^{-}, 0.3 g of Ca^{++}, 0.1g of Mg^{++}, and 0.03g of P^{-} , amount equivalent to 2.5 L of sweat. Each dose of EM+K 7.7 g of Na^{+}, 3.5 g of K^{+}, 19g of Cl^{-}, 0.3 g of Ca^{++}, 0.1g of Mg^{++}, and 0.03g of P^{-}, amount equivalent to 2.5 L of sweat.

Ten to 15 minutes after finishing the EET the horses were supplied with recovery formulas trough naso-gastric gavage. Horses that were supplied with EM-K during the EET were supplied with five doses of a glucose polymer (1g/kg BW) added with 10 g of KCl (GP+K). Each dose was administered every hour, for five hours. Horses that had been supplied with EM+K during the EET were supplied only with the glucose polymer (GP) (1 g/kg BW) but no K, every hour for 5 hours starting 10 to 15 minutes after finishing the EET.

Horses were weighed before starting the EET, just after finishing each loop, at 29 minute of every R, one minute after finishing the EET, after getting the last GP or GP+K dose, and in the morning after the EET.

Heart rate was monitored during exercise with a commercial digital heart rate monitor (Polar Pacer, Polar CIC Inc., Port Washington, NY). Electrocardiograms (EKG) were done on the horses 30 minutes before the start, at 20 minutes of every R, and at 15 minutes after finishing the EET (HP defibrillator 43000A, Bothel, WA).

Jugular blood samples were taken before the horses got their initial electrolyte dose (PRE), five minutes before starting the EET after getting the EM (PEL), at 10, 30, 50, and 70 km of exercise on the treadmill, at 25 minutes of every R (R1, R2, R3), after 10 minutes of finishing the EET (REC_{10}), two hours after the first dose of GP (REC_{120}), one minute before fifth dose of GP or GP+K (REC_{300}), and in the morning after (MA) the EET (total 13 samples). Heart rate and rectal temperature were measured every time blood was taken.

An aliquot of blood was sampled into a heparinized 3 cc syringe (Becton Dickinson, Franklin Lakes, NJ) and another was collected with a syringe and transferred to heparinized vacutainer tubes (Vacutainer green, Becton Dickinson, Franklin Lakes, NJ). Blood from the small syringe was analyzed immediately by a blood-gas analyzer (Stat Profile M, Nova Biochemical, Waltham, MA) for pH, PCO_2, hematocrit (Hct), plasma $[Na^+]$, $[K^+]$, $[Cl^-]$, $[Ca^{++}]$, $[Mg^{++}]$, and glucose. The blood in the vacutainer tubes was kept in ice water, and centrifuged within 10 minutes. Plasma was separated, frozen and kept at -80^oC for posterior analysis. Spectophotometric assays were used for determination of plasma concentrations of lactate ([La-]), albumin ([alb]), phosphate ($[PO_4^-]$), triglycerides (TG) within two weeks of the test (Beckman Instruments Inc., Brea, CA). In addition, plasma $[H^+]$ and osmolality were calculated (Stat Profile M, Nova Biochemical, Waltham, MA). Plasma insulin (ins) was analyzed by radioimmunoassay (Coat-A-Count Insulin, Diagnostic Products, Los Angeles, CA).

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Changes in acid-base status during exercise have been analyzed preferably by the comprehensive physicochemical model of Stewart (Kronfeld et al, 1998; Lindinger et al, 1992, Stewart, 1981; Watson, 1996). In this model (Stewart, 1981), concentrations of H^+ and HCO_3^- are dependent upon the strong ion difference (SID), PCO_2, and total weak acids (A_{tot}, mainly proteinates and phosphates). The SID was calculated as the algebraic sum of $[Na^+]$ and $[K^+]$ minus the sum of $[Cl^-]$ and $[La^-]$; the algebraic sum of other strong ions was assumed to be < 1 mEq/L and to contribute negligibly to changes in SID observed during exercise and recovery. Estimates of weak acids ($A_{tot,}$ mEq/L) were predicted from alb (g/L), multiplying it by 4.07 (Rossing et al, 1986). The partition of changes in plasma $[H^+]$ into contributions from SID, PCO_2 and A_{tot} was performed using software designed for the Stewart system (Watson, 1996). Resting values for SID, PCO_2 and A_{tot} were set in the panel for independent variables, and the predicted value of $[H^+]$ was observed in the panel for dependent variables. Then the series of values of SID at each sampling stage were entered one at a time (without changing PCO_2 and A_{tot}) to yield corresponding series of changes in $[H^+]$ contributed by the changes in SID (Kronfeld et al, 1999; Watson, 1996). The procedure was repeated for PCO_2 (without changing SID and A_{tot}) and for A_{tot} (without changing SID and PCO_2).

Resting membrane potential (E_K, mV) was calculated from extra-cellular $[K^+]$ (K_o) and $[K^+]$ the (K_i) using the Nerst equation (Ganong, 1999).

$$E_K = 61.5 \log [K_o]/[K_i] \text{ at } 37\ ^oC$$

This equation uses the ratio of extracellular/intracellular concentrations of K^+ only, because the permeability constant of K^+ is much higher than those of other ions. Assumptions are needed to test the possible effects of changes in plasma $[K^+]$ on E_K.

Measured plasma [K^+] was used for K_o without the approximately equal and opposing adjustments for plasma solids and for lymph. For K_i, a mean equine middle gluteal muscle intracellular [K^+] of 124 mEq/L (K_i mEq/L water) was calculated from data on K (μM/g of wet weight) and water (%) (Johnson et al, 1991).

Data were summarized as least squares means and standard errors. The effects of sampling time (PRE, PEL, 10 km, R1, 30 km, R2, 50 km, R3, 70 km, REC_{10}, REC_{120}, REC_{300}, MA), treatments (EM-K, EM+K) and their interactions were evaluated by ANOVA with repeated measures in a mixed model and applied to the 12 horses (SAS Institute Inc., Cary, NC). Significance of differences between means was tested by least significant differences covered by a significant F-test for the ANOVA. Simple relationships of plasma [H^+] to SID, A_{tot} and PCO_2, and SID to plasma [Na^+], [K^+], [Cl^-] and [La^-] were tested by linear regression. Statistical significance was inferred from $P < 0.05$.

Results

The 12 horses weighed 448 ± 14 kg before the EETs. Mean weight losses were 7.6, 15.2, 18.7, and 18.6 kg at 20, 40, 60, and 80 km of the EET, respectively. After 5 doses of GP the weight loss was of 6.6 kg, but at the morning after the horses still had 10.1 kg less than at rest. These weight losses correspond to 1.7, 3.4, 4.18, and 4.16 % weight losses at 20, 40, 60, and 80 km of EET, respectively. After the 5th GP dose weight losses were 1.47 %, and in the MA the horses had a deficit of 2.25 % BW. No differences were found in weight losses between the treatments.

Speeds during the exercise blood sampling were 4.47, 4.81, 5.00, and 4.86 ± 0.22 m/s for the 10, 30, 50, and 70 km samples, respectively.

Total amounts of electrolytes consumed during the EET were 51 g of Na^+, 1.86 g of Ca^{++}, 0.78 g of Mg^{++}, 85g of Cl^-, and 0.18 g of PO_4^- for the EM-K horses. For the EM+K group the amounts consumed were: 51 g of Na^+, 21 g of K^+, 1.86 g of Ca^{++}, 0.78 g of Mg^{++}, 0.18 g of PO_4^-, and 104 g of Cl^-. Total amount of KCl consumed in the GP+K treatment was 50g (26.2 g of K^+, and 23.8 g of Cl^-).

Total amount of water and hay consumed was 19.2 ± 1.06 kg and 1.6 ± 0.08 kg, respectively. No differences in amounts water or hay consumed were found between the treatments. The amount of K^+ in the hay during the 80 km EET was about 47 g, about the amount present in 18 L of sweat (Kronfeld, 2001b).

Dietary CAB was calculated for the feed and was 71.4 mEq/kg and for the pasture 528.3 mEq/kg. Total DCAB was 447.1 mEq/kg. Electrolyte DCAB was -177 mEq for 136 g of consumed EM-K and -175 mEq/kg for 176 g of consumed EM+K during EET.

Temperature and humidity were tested in the statistical model as covariates, however neither had any effect on measured variables, therefore they were dropped from the model. Daily temperature and RH for each horse during the EET is in Table 3.

No abnormalities in the EKGs were seen in any of the horses and no differences were seen between both groups.

Basal hematocrit was 35.3 ± 0.8%, increased progressively by 25% with exercise until 50 km ($P < 0.001$), staying the same until 70 km. At every resting period and REC_{120} Hct returned to resting levels. At REC_{300} and MA Hct levels were 12.5 and 16.7% higher than PRE ($P < 0.001$), respectively.

Basal albumin was 3.25 ± 0.04 g/dl, it increased by 6.0% at R1 ($P < 0.001$), after that increased a little ore 6.2% with exercise ($P < 0.001$) and decreased a little with Rs ($P < 0.02$) and returned to resting levels at REC, but was 8.3% higher than PRE at MA ($P < 0.001$).

Basal plasma [Na^+] was 137.8 ± 0.4 mmol/L, increased 3% ($P < 0.001$) with electrolyte supplementation (PEL) and than progressively during the EET ($P < 0.001$) up to 7.5%, decreased a little during recovery ($P < 0.001$), and was 2.5% higher than PRE at MA ($P < 0.001$).

Basal plasma osmolarity was 286.5 ± 1.1 mosm/L, it increased progressively up to 4.5% during the EET, remaining higher at REC and getting 1.1% below PRE at MA.

Basal plasma [Cl^-] was 100.7 ± 1.6 mmol/L. It increased 2.7% with the electrolyte supplementation and than progressively up to 8.0% with the EET ($P < 0.001$), at REC decreased a little ($P < 0.001$) and at MA was still 3.9% higher than PRE ($P < 0.001$).

Basal plasma [Ca^{++}] was 1.48 ± 0.06 mmol/L. It increased 4.1% after the electrolyte supply ($P < 0.001$), decreased up to 2.1% with exercise and increased up to 4.1% at rest and REC ($P < 0.05$). It was never below PRE at any sampling time.

Basal plasma [Mg^{++}] was 0.26 ± 0.008 mmol/L. It decreased 15.2% after the electrolyte supply ($P <0.001$), decreased further up to 19% with exercise ($P < 0.001$) and increased a little to PEL at R2, R3, REC_{10}, REC_{120} ($P < 0.001$), decreasing again and not returning to PRE levels at any sample time ($P < 0.001$).

Basal plasma [La^-] was 0.59 ± 0.08 mmol/L. It increased progressively during the EET up to 291 % until 70 km ($P < 0.001$), decreasing at REC from 70 km ($P < 0.001$), at returning o PRE levels at MA.

Basal plasma [PO_4^-] was 2.79 ± 0.09 mmol/L. It increased progressively with the EET ($P < 0.001$) up to 40%, decreasing a little during the rest periods, returning to PRE at REC, getting 36% below PRE at MA ($P < 0.001$).

Basal plasma [H^+] was 38.01 ± 0.61 nEq/L. It decreased 10.1 % during exercise at 10 km ($P < 0.001$) and increased progressively during Rs up to 8.5% ($P < 0.001$), returned to PRE at 70 km, increased to 9.2% during R10, and R120, decreasing at R300, and returned to PRE at MA ($P < 0.001$).

Basal plasma PCO_2 was 47.3 ± 1.3 mmHg. It decreased up to 8.3% during exercise ($P < 0.001$) and increased to PRE during Rs, was similar to PRE at REC and 10.1% below PRE at MA ($P < 0.001$).

Basal plasma glucose was 5.43 ± 0.5 mmol/L. It increased 15% at R2 ($P = 0.056$), and increased 56% above PRE at REC ($P < 0.001$), and returned to PRE at MA.

Basal plasma insulin was 9.3 ± 0.8 μu/ml. It decreased progressively during exercise up to 99% ($P < 0.021$), being higher during R than exercise. Increased 30 % at REC_{120}, and 40% at R_{300} ($P < 0.001$) and returned to PRE at MA.

Basal plasma TG was 30.8 ± 2.8 mg/dl. It decreased up to 44% during Rs and up to 18% during exercise (30, 50 km) ($P < 0.001$), then increased to PRE at 70 km, was 75% and 69% below PRE REC and MA, respectively ($P <0.001$).

An electrolyte by sampling time interaction ($P < 0.001$) was found for plasma [K^+]. At 50 ($P =0.023$) and 80 km ($P = 0.0036$) EM-K supplemented horses had lower plasma [K^+] than EM+K horses (Figure 1). Also at REC 10 EM-K horses had lower [K+]($P = 0.045$) than EM+K horses. After the supply of GP+K solutions to EM-K supplied horses plasma [K^+]

was higher at REC_{300} ($P < 0.001$) and MA ($P = 0.004$) than horses supplied with GP-K solutions (Figure 2).

Plasma $[K^+]$ increased during exercise and decreased below resting levels at Rs, but was equal PRE at R2 and R_{120} in the EM-K group. In the EM+K plasma $[K^+]$ returned to PRE at every R, at REC_{10}, REC_{300} and MA.

An electrolyte by sampling time interaction ($P = 0.032$) was also found for plasma $[Cl^-]$ during recovery. GP+K (EM-K) horses had higher $[Cl^-]$ at REC_{300} ($P = 0.046$) than GP-K (EM+K) horses (Figure 3).

Mean changes in plasma $[H^+]$ were partitioned among the three independent variables (Figure 4). The initial decrease in plasma $[H^+]$ after the first dose electrolytes was administered (PEL) was partitioned in 0.0 for A_{tot}, +1.0 for PCO_2 and -1.9nEq/L for SID. At 10 km plasma $[H^+]$ decreased 5.5 nEq/L and partitioned in +0.17, -2.7 and -3.0 nEq/L for contributions from changes in Atot, PCO_2 and SID, respectively. At R1 a 0.5nEq/L decrease in $[H^+]$ was partitioned into 0.8, 2.4, and -3.4 nEq/L for contributions from changes in A_{tot}, PCO_2, and SID, respectively. At 30 km a 6.1 nEq/L decrease in plasma $[H^+]$ was partitioned into 0.9, -3.1, and -4 nEq/L for contributions from changes in A_{tot}, PCO_2, and SID, respectively. During the loops, decreases in $[H^+]$ were due to increases in SID and decreases in PCO_2. During the rest periods, increases in $[H^+]$ were due to increases in PCO_2, which also compensated for SID increases. Increases in $[H^+]$ due to A_{tot} attenuated the decreases in $[H^+]$ throughout EET. At REC smaller changes were seen and contributions were similar from each of the three independent variables. Plasma $[H^+]$ was linearly and positively related to PCO_2 ($r = 0.42$, $P < 0.001$), negatively to SID ($r = 0.30$, $P < 0.001$), positively to $[Na^+]$ ($r =$

0.17, P = 0.021), $[K^+]$ (r = 0.53, P < 0.001), and $[Cl^-]$ (r = 0.32, P < 0.001), and not related to A_{tot} and [La-].

Estimated mean E_K before the race were -93.99 and -94.06 mV for the EM-K and EM+K groups, respectively. Assuming no change in K_i during the race, estimated mean changes in E_K from rest to 50 km and to 70 km were -5.72mV -4.62mV respectively for EM-K and from rest to 50 km and to 70 km -7.0mV and - 6.21mV respectively for EM+K.

Discussion

This study once again confirms that supplementation of potassium affects plasma $[K^+]$ and leads to E_K levels much closer to neuromuscular excitability. Supplementation after exercise helps restore plasma levels and consequently body stores. This study also confirms that chloride supplementation also will affect plasma $[Cl^-]$, which can be maintained despite sweat losses. New is that this treadmill study simulated the same degree of dehydration that occurs during actual races without the use of diuretic drugs, such as the furosemide model (Tobin et al, 1978).

Increases in albumin of 6.46 % in the present study are lower than during a 100 km endurance race (Rose et al, 1977). A decrease in plasma water was in part responsible for a 25% increase hematocrit seen during this study. About 18% of the increase in Hct was due to splenic contraction. The increase found is higher than previously reported ones during endurance rides (Barton et al, 2003; Fregin, 1979), and also than endurance exercise and furosemide administration on the treadmill (Butodum et al, 2002). But increases were similar to a simulated race (Nyman et al, 1996). In the present study the highest values were found during exercise, which should be higher than after exercise ceases when samples were

taken (Nyman et am, 1996). Therefore values could have been higher than actual races. Compared to the treadmill exercise, conditions during the race were more favorable to horses which led to less dehydration.

Increases in [Na^+] have been seen during endurance races (Sloet et al, 1991), but also decreases (Barton et al, 2003). In studies simulating endurance exercise on the treadmill horses supplied with oral electrolyte solutions or in feeds had higher [Na^+] than water supplied or control animals (Coenen et al, 1995; Dusterdieck et al, 1999). Previously reported increases however were lower than the 7.4% present ones. In one treadmill study ingested Na was of 58 g (Dusterdieck et al, 1999) during 60 km of exercise, compared to 51 g during 80 km in the present one. Horses had lower weight losses so were presumably less dehydrated than in the present experiment (Dusterdieck et al, 1999). Since electrolytes were supplied in the form of a paste in the present study compared to a dilution in 900 ml of water in the previous study, a greater water shift from the blood compartment to the gastrointestinal compartment may have occurred leading to higher plasma [Na^+] in the present study. Also, blood samples were taken at different points of exercise during the two experiments. In the current experiment highest plasma [Na^+] were seen during the highest intensities of exercise, which would correspond to the greatest water shifts to the muscle compartment. In the previous study blood was taken only in the beginning and end of each 15 km bout of exercise. Higher [Na^+] help to maintain osmolarity and thirst, thus helping to prevent dehydration. Increases of 4.3 % in osmolarity were seen in the current study, higher than a previous study (Dusterdieck et al, 1999).

The amount of Na^+ supplied in the current study was the estimated amount lost in 15 L of sweat (McCutcheon and Geor, 1996). Body weight losses of 18 kg were seen at 80 km

of the present study. In addition 20 kg of water had been consumed, horses still had a deficit of 85g of Na at the end of the EET, the amount lost in 25 L of sweat (considering that 100% of body weight losses to be from sweat).

Plasma [Cl^-] increased 8% during the EET. During endurance races decreases (Barton et al, 2003; Sloet et al, 1991) or no changes (Lindinger and Ecker, 1995) have been reported. During treadmill studies increases of 5% (Coenen et al, 1995; Dusterdieck et al, 1999) in plasma [Cl^-] in electrolyte supplied horses have been reported. During a 62 km endurance race (Nyman et al, 1996) increases were about 10% in salt paste supplied horses at 42 km, comparable to the present study. Horses were supplied with 90 g of NaCl (50 g of Cl), less than the present study. However ambient conditions were milder than the current ones (Nyman et al, 1996), therefore weight and presumably sweat losses were lower leading to less Cl^- losses and similar plasma [Cl^-], despite lower Cl^- intake. At recovery plasma [Cl^-] decreased, was higher in the GP+K supplied group at R_{300} and was higher than PRE in the MA in both groups. Supplementation of Cl^- has been shown to affect plasma [Cl^-] during exercise (Coenen, 1999). Maintenance of plasma [Cl^-] levels, tends to maintain pH and alkalosis due to sweat losses may be attenuated. More recent races still report metabolic alkalosis (Jahn et al, 1996). During high speed races, where metabolic acidosis has been reported (Rose et al, 1979), Cl^- supplementation should be done with caution to avoid an increment in plasma acidity.

Plasma [Ca^{++}] was maintained at resting levels during exercise and increased during rest periods. It never reached levels below resting during the entire study. During most endurance races decreases (Lindinger and Ecker, 1995; Lucke and Hall, 1978; Sloet et al, 1991) have been reported. Dietary CAB can affect plasma pH metabolism in horses during

rest and exercise and could affect plasma [Ca^{++}] during exercise. However up to date experiments did not show any differences in plasma [Ca^{++}] among different DCAB diets (Cooper et al, 1998). A positive relation between plasma [H^{+}] and [Ca^{++}] was observed ($r = 0.20$; $P < 0.001$).

In cattle, a high DCAB has been shown to increase the incidence of periparturient hypocalcemia (Goff and Horst, 1997). In this study DCAB is considered to be high (Table 1), but no hypocalcemia has been observed. The high DCAB was attenuated by the supplementary electrolytes, however total DCAB continued to be high with addition of EM-K or EM+K (DCAB = -136 mEq/kg and -176 mEq/kg, respectively) to the daily ration intake (DCAB = 447 mEq/kg). There is a possibility that the immediate effects of the ingestion of the supplementary electrolytes attenuated the potentially high DCAB effect from feed intake.

Plasma [Mg^{++}] decreased during exercise and remained lower than PRE at recovery and MA. During endurance races decreases have been reported (Rose et al, 1980). No clinical signs were associated with decreased plasma [Mg^{++}].

Plasma [H^{+}] decreased with exercise and increased to PRE or higher during Rs. The highest plasma [H^{+}] was observed at REC_{120}. Partition of plasma [H^{+}] revealed that during exercise an increase in SID and decrease in PCO_2 contributed to the decrease in [H^{+}]. During rest decreases in [H^{+}] caused by the increase in SID was attenuated by increases in PCO_2. Decreases in SID during exercise were caused by increases in [Na^{+}] and [K^{+}] and progressive increases in [Cl^{-}], during rest decreases in SID were caused by increases in [Na^{+}] and [Cl^{-}].

During exercise decreases in PCO_2 were caused by hyperpnea, and during rest PCO_2 returned to PRE levels. Alkalosis has been observed during endurance exercise (Jahn et al, 1996; Rose et al, 1979). In those studies alkalosis was attributed to hyperventilation and Cl^{-}

sweat losses. If plasma [Cl^-] had been lower in the present study, alkalosis would have been more severe. Alkalosis was probably attenuated by Cl supplementation.

During recovery a difference between the blood gas machine calculated and the partitioning calculated [H^+] was seen (Figure 4). Some assumptions like the conversion of albumin into A_{tot} may have led to this difference.

Plasma glucose decreased from 50 km to REC_{10}. During endurance rides decreases (Dybal et al, 1980; Lucke and Hall, 1978; Sloet et al, 1991) or no changes have been reported (Lucke and Hall, 1980; Jahn et al, 1996). During the present study horses only had access to hay, and no grain. Plasma insulin also decreased during the study, reaching the lowest level at 70 km (0.16 μIU/ml). Decreases have been reported in another study (Dybal et al, 1980). Triglycerides also decreased at 30 and 50 km of the EET, further decreasing at Rs. Horses were probably metabolizing TG to spare glucose during the EET. TG further decreased at REC. NEFA mobilization was inhibited by the increase in insulin at that time. In a previous study (Hess et al, 2003), TG and glycerol increased and horses did not have decreases in plasma glucose. Increased TG utilization was not effective in maintaining plasma glucose during this study.

Although K^+ losses occur in sweat (Carlson and Ocen, 1979; McCutcheon et al, 1995), plasma [K^+] may increase in proportion to exercise intensity (Harris and Snow, 1992) as K^+ moves out of working muscle cells. Increasing plasma [K^+] initially allows exercise by dilating arterioles in muscle therefore providing enough oxygen and nutrients. Higher plasma [K^+], however, will exert catelectronic effects on E_K and may exacerbate neuromuscular excitability (Ganong, 1999). Eventually high plasma [K^+] reaches a critical level and inhibits action potentials, so muscles and nerves become unable to respond

(Ganong, 1999; Mainwood et al, 1985). To evaluate these possible adverse effects in relation to increases in plasma [K^+] observed in the present study, it is necessary to make assumptions based on previous research in horses and other species, such as a [K_i] of 124 mEq/L (Johnson et al, 1991). A textbook value of about +7mV of depolarization leads to a zone of local responses in which cathodal stimuli are facilitated, that is, in which increases in excitability are greater up to the firing threshold (Ganong, 1999). A zone of 7 to 15 mV of depolarization may be considered to represent hyperexcitability, and persistent depolarization greater than about 15 mV to represent prolonged refractory periods and decreased muscle response (Ganong, 1999; Mainwood et al, 1985).

The mean calculated E_K in the present study is –94.0 mV, so estimates of –87.1 and –79.1 mV may be predicted for catelectronic local responses and firing thresholds, respectively. The Nerst equation yields corresponding estimates of 4.63 and 6.28 mEq/L for [K_o] or plasma [K^+]. Such values have been recorded, albeit for faster speeds and briefer periods, without clinical manifestations (Harris and Snow, 1992; Kronfeld, 2001b).

The mean increases in plasma [K^+] at 50 and 70 km are very close to the predicted mean of 4.63 mEq/L that corresponds to a depolarization of +7mV, an evaluation of the frequency distributions reveals a difference between the EM-K and EM+K group (Figure 5). The SDs are 0.272 and 0.305 mEq/L for the EM+K group at 50 and 70 km respectively, and 0.173 and 0.241 mEq/L for the EM-K at 50 and 70 km, respectively. Dividing these SD's into the difference between the respective means of 4.43 and 4.25 mEq/L at 50 and 70 km for EM-K and 4.62 and 4.50 mEq/L at 50 and 70 km for EM+K and 4.63 mEq/L (+7mV depolarization) will yield respective estimates of 1.156 and 1.58 (EM-K, 50 and 70 km) and 0.037 and 0.42 (EM+K 50 and 70 km) for z (Fisher's normal deviant), with corresponding

probabilities of 1 horse in 17 (50 km) and 1 horse in 33 (70 km) in the EM-K group or 1 in 4 (50 km) and 1 in 6 (70 km) in the EM+K group reaching the zone of local catelectronic responses (Rothman, 1986).

Hyperexcitability due to elevated plasma [K^+] could have occurred during this simulated endurance race. No signs of hyperexcitability were observed during this study. If EKGs had been performed during exercise, some signs could have been observed. In addition, horses were not hypocalcemic, which might have exacerbated clinical signs.

Supplementation during recovery will help to restore plasma [K^+]. Even with access to feed sources rich in K, plasma values will be higher in horses supplied with extra K in the form of electrolyte supplements. Muscle K^+ stores could not be evaluated in this study, but with the presence of higher plasma [K^+] in GP+K supplied horses probably enhanced its uptake into the muscle.

Implications

During this study horses supplied with EM+K had a greater probability to reach a zone of hyperexcitability than horses supplied with EM-K. There was a low probability however for any of the two groups reach the depolarization zone. It remains to be tested how these values would be at speeds similar to the elite endurance races (mean 6.38 m/s). At higher speeds plasma [K^+] of 6.3 mmol/L can be reached and so can depolarization zone.

Apart from supplementing the electrolyte mixtures, only hay was consumed during the rest periods. A feed with a higher glycemic effect would reduce the risk of hypoglycemia.

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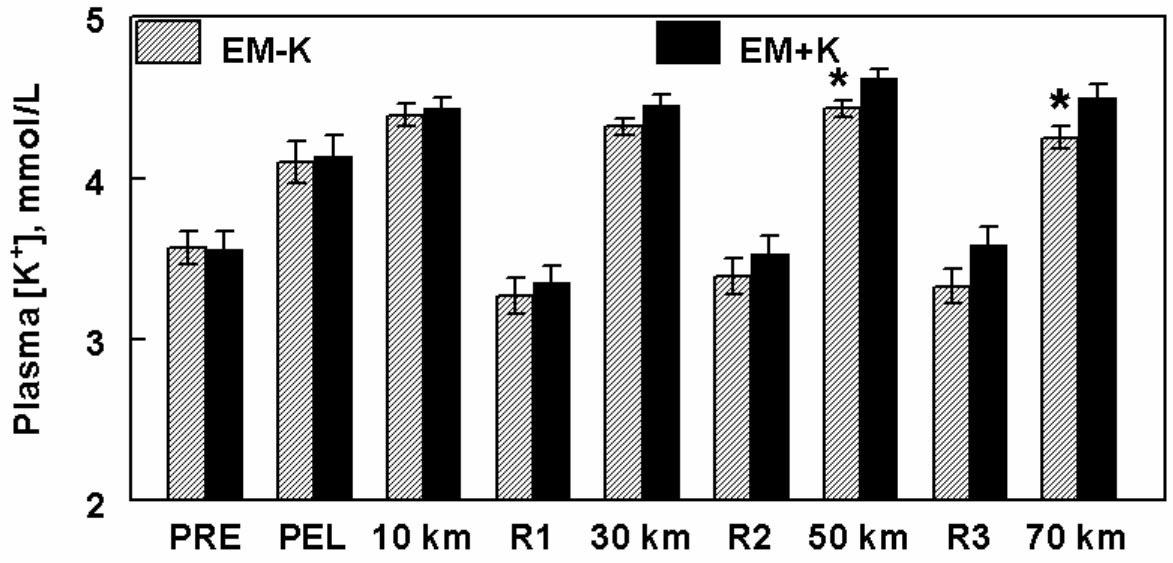

Figure 1. Mean (± SE) plasma [K^+] versus stage of sample collection before, during, and after the 80-km endurance exercise test in EM–K and EM+K treated horses. Notice the increase ($P < 0.05$) with stage from PRE the ride to PEL, 10, 30, 50, and 70 km and decrease ($P < 0.05$) at R1, R2, R3 (EM–K and EM+K treated) horses.

*Significant ($P < 0.05$) differences between EM–K and EM+K treated horses at 50 and 70-km.

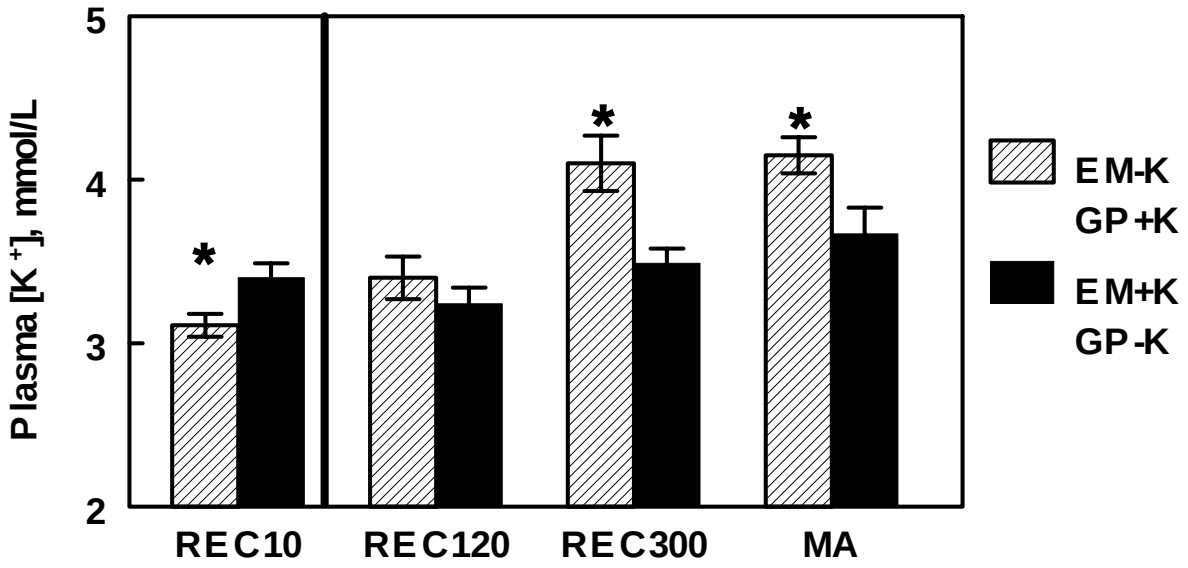

Figure 2. Mean (± SE) plasma [K^+] versus stage of sample collection after the 80-km endurance exercise test in EM–K and EM+K treated horses. Notice the increase ($P < 0.05$) with stage from REC_{10} to REC_{300} (EM–K-GP+K and EM+K-GP treated horses). *Significant ($P < 0.05$) differences between EM–K-GP+K and EM+K-GP treated horses at REC_{10}, REC_{300} and MA.

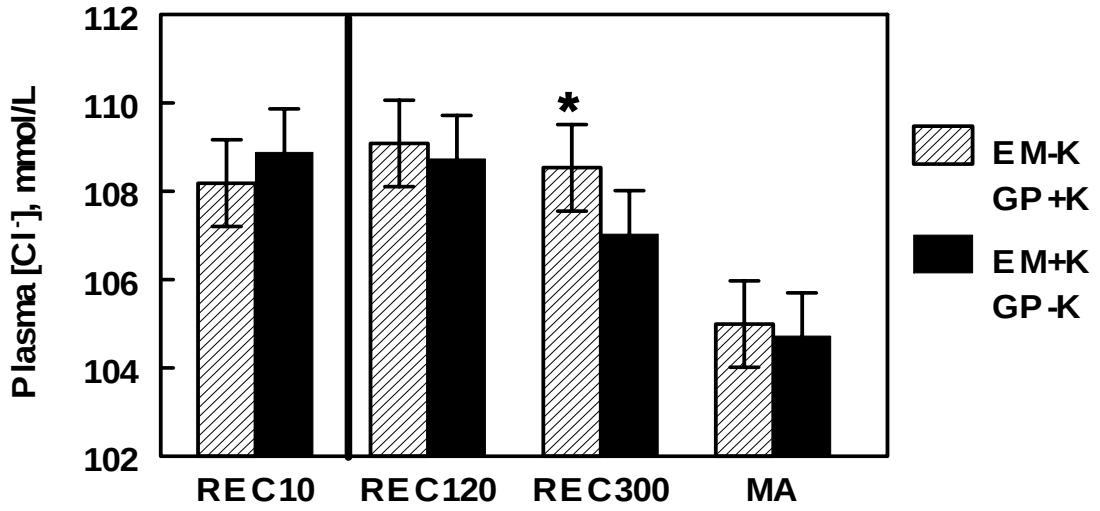

Figure 3. Mean (± SE) plasma [Cl^-] versus stage of sample collection after the 80-km endurance exercise test in EM–K and EM+K treated horses. Notice the decrease ($P < 0.05$) with stage from REC_{300} to MA (EM–K-GP+K and EM+K-GP treated horses).

*Significant ($P < 0.05$) differences between EM–K-GP+K and EM+K-GP treated horses at REC_{300}.

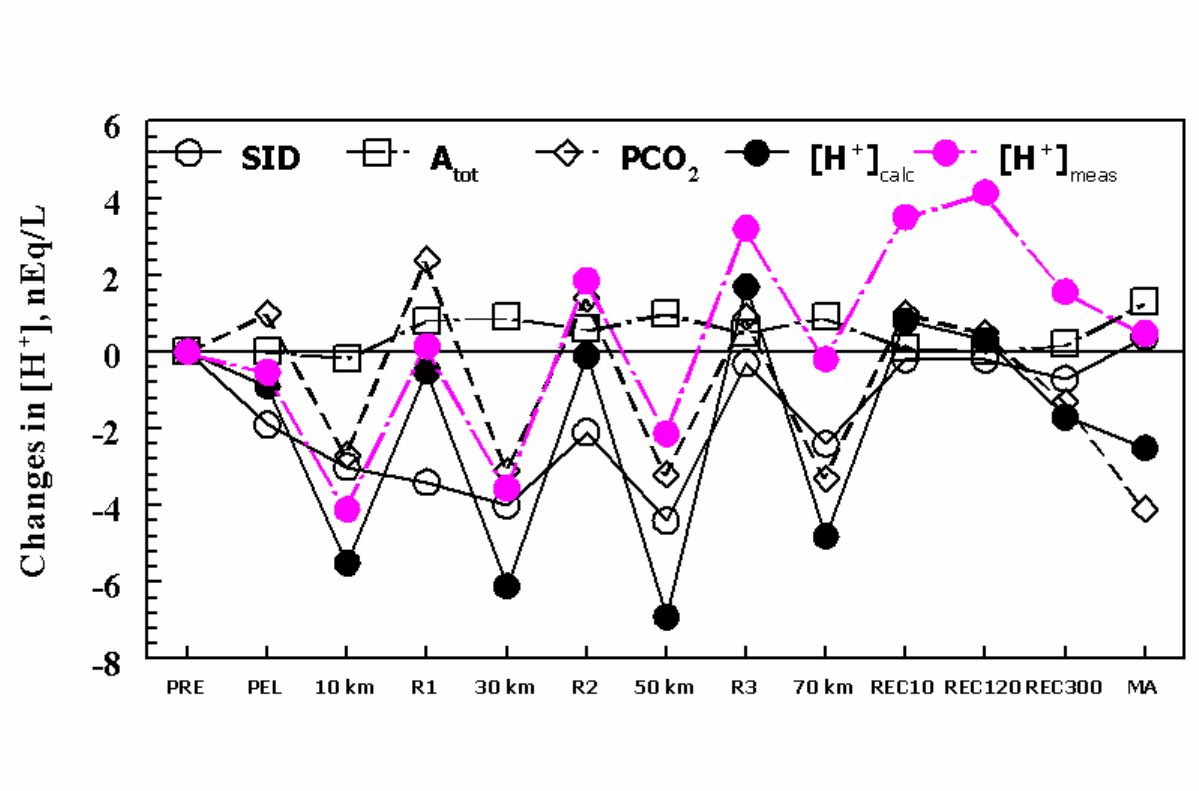

Figure 4. Partitioning of changes in plasma H^+ concentration from resting values (PRE) during the 80-km endurance exercise test and recovery (REC) into contributions from 3 independent variables, the strong ion difference (SID), total weak acids (A_{tot}), and $PvCO_2$ in EM–K and EM+K treated horses.

$[H^+]_{calc}$ is calculated concentration and $[H^+]_{meas}$ is the calculated from the measured pH concentration transformed into $[H^+]$ by the blood gas analyzer.

Table 1. Nutrient composition of feed, grass and hay on a DM basis in the DHI Forage Testing Laboratory (Ithaca, NY).

Nutrient (%DM)	**Feed**	**Pasture**	**Hay**
DM, % as fed	93.3	93.3	94 8
CP, % DM	11.6	23.2	17.3
ADF, % DM	11.5	31	36.5
NDF, % DM	25.4	57.7	64.6
NSC, % DM	36	9.2	7.2
Fat, % DM	12.5	3.7	3.0
Ash, % DM	6.1	11.1	10.28
Ca, % DM	0.9	0.32	0.48
P, % DM	0.53	0.37	0.33
Na, % DM	0.181	0.004	0.003
K, % DM	0.8	3.10	2.95
Mg, % DM	0.22	0.16	0.20
S, % DM	0.22	0.11	0.21
Cl, % DM	0.42	0.66	0.42

	PRE	P.elec	10 km	R1	30 km	R2	50 km	R3	70 km	Rec 10	Rec120	Rec300	MA
Hct (%)	35.3±0.8^{a}	37.6±0.7^{b}	41.7±0.6^{c}	37.5±0.5^{b}	43.9±0.8^{d}	37.2±0.6^{b}	44.1±0.7^{d}	36.4±0.5^{ab}	44.4±0.7^{d}	35.5±0.7^{a}	36.3±0.9^{ab}	39.7±0.7^{e}	41.2±0.7^{c}
Alb (g/dl)	3.25±0.04^{ae}	3.25±0.04^{ae}	3.26±0.05^{ae}	3.42±0.04^{bc}	3.45±0.05^{bf}	3.38±0.05^{cd}	3.46±0.06^{bf}	3.35±0.05^{ce}	3.44±0.06^{bdf}	3.26±0.06^{ae}	3.24±0.06^{a}	3.30±0.05^{e}	3.52±0.05^{f}
Lac(mmol/L)	0.59±0.08^{a}	0.70±0.04^{acf}	0.75±0.13^{acf}	0.78±0.06^{acf}	0.86±0.10^{aef}	0.71±0.07^{acf}	1.16±0.13^{bef}	0.94±0.10^{cef}	1.72±0.24^{d}	1.12±0.13^{ef}	0.93±0.09^{f}	0.93±0.06^{f}	0.75±0.07^{acf}
Na^{+}(mmol/L)	137.8±0.4^{a}	141.5±0.4^{bg}	142.3±0.4^{b}	143.9±0.4^{c}	145.2±0.5^{d}	145.1±0.5^{d}	147.5±0.6^{ef}	145.5±0.5^{d}	148.1±0.6^{e}	146.5±0.4^{f}	146.6±0.5^{f}	145.3±0.6^{d}	141.3±0.5^{g}
K^{+}(mmol/L)	3.57±0.17^{a}	4.12±0.06^{b}	4.39±0.05^{c}	3.31±0.05^{de}	4.30±0.08^{bc}	3.49±0.05^{ad}	4.46±0.08^{c}	3.46±0.07^{ad}	4.38±0.06^{c}	3.26±0.05^{e}	3.32±0.08^{de}	3.80±0.10^{f}	3.92±0.10^{f}
Cl^{-}(mmol/L)	100.7±1.6^{a}	103.4±0.4^{b}	103.5±0.4^{bg}	103.6±0.5^{b}	105.3±0.5±^{cg}	106.2±0.5^{cd}	107.1±0.0^{d}	107.7±0.7^{cd}	108.8±0.4^{f}	108.4±0.4^{ef}	108.8±0.5^{ef}	107.6±0.5^{def}	104.7±0.5^{g}
Ca^{++}(mmol/L)	1.48±0.06^{acf}	1.54±0.01^{bcd}	1.45±0.01^{a}	1.51±0.01^{cdef}	1.46±0.01^{a}	1.54±0.01^{bcd}	1.49±0.01^{acdf}	1.52±0.02^{d}	1.46±0.01^{af}	1.49±0.01^{ae}	1.50±0.02^{ef}	1.46±0.01^{a}	1.50±0.01^{ef}
Mg^{++}(mmol/L)	0.26±0.01^{a}	0.22±0.01^{bde}	0.21±0.01^{cd}	0.22±0.01^{cde}	0.21±0.01^{cd}	0.23±0.01^{bef}	0.22±0.01^{deg}	0.24±0.01^{bf}	0.22±0.01^{eh}	0.24±0.01^{f}	0.24±0.01^{fh}	0.21±0.01^{cg}	0.21±0.01^{ch}
PO_4^{-}(mg/dl)	2.79±0.09^{a}	2.80±0.09^{ade}	3.41±0.10^{b}	3.00±0.11^{c}	3.54±0.11^{d}	2.91±0.10^{ce}	3.71±0.13^{d}	2.98±0.10^{ce}	3.92±0.12^{f}	3.22±0.11^{b}	2.68±0.13^{a}	2.84±0.13^{ac}	1.78±0.10^{g}
pH	7.420±0.007^{af}	7.427±0.003^{a}	7.419±0.003^{b}	7.418±0.003^{af}	7.463±0.003^{b}	7.400±0.003^{c}	7.446±0.004^{d}	7.385±0.004^{e}	7.423±0.003^{af}	7.382±0.004^{e}	7.376±0.003^{e}	7.403±0.004^{c}	7.415±0.006^{f}
PCO_2(mmHg)	47.3±1.3^{ad}	48.5±0.7^{ac}	44.1±0.8^{bd}	50.2±1.0^{c}	43.6±0.7^{b}	48.9±0.6^{ac}	43.5±0.8^{b}	48.3±.9^{a}	43.4±0.7^{b}	48.4±0.4^{ac}	47.9±0.4^{ac}	45.8±0.4^{a}	42.5±0.8^{d}
$[H^{+}]$ (nEq/L)	38.0±0.61^{b}	37.5±0.28^{b}	33.9±0.26^{c}	38.20.26±^{a}	34.5±0.24^{c}	39.9±035^{d}	35.9±0.32^{e}	41.2±0.29^{f}	37.8±0.38^{abg}	41.5±0.28^{fg}	42.2±0.43^{g}	39.6±0.39^{d}	38.5±0.5^{a}
SID (mEq/L)	33.5±3.5^{a}	41.5±0.49^{bcd}	42.4±0.53^{bce}	42.9±0.53^{bcd}	43.4±0.58^{bc}	41.8±0.41^{bde}	43.8±0.58^{c}	40.5±0.47^{de}	42.1±0.47^{bcd}	40.1±0.28^{bde}	40.3±0.56^{e}	40.70.45±^{e}	39.9±0.33^{e}
Osm(mosm/L)	286.5±1.0^{a}	286.7±1.1^{a}	287.8±0.8^{a}	291.1±0.8^{b}	293.8±1.1^{c}	293.5±1.0^{c}	297.8±1.2^{d}	293.9±1.1^{c}	299.3±0.9^{d}	295.8±0.7^{e}	298.9±0.9^{d}	294.6±1.2^{ce}	283.4±1.0^{f}
A_{tot}(mEq/L)	13.2±0.16^{af}	13.2±0.15^{af}	13.3±0.18^{af}	13.9±0.17^{bc}	14.0±0.20^{bg}	13.8±0.20^{ce}	14.1±0.24^{bg}	13.7±0.21^{cf}	14.0±0.23^{beg}	13.3±0.24^{a}	13.2±0.22^{a}	13.4±0.19^{cf}	14.3±0.19±^{g}
Glucose (mmol/L)	5.43±0.21^{acd}	6.03±0.05^{acf}	6.12±0.10^{abf}	5.56±0.11^{abd}	5.97±0.14^{abcf}	6.24±0.90^{bf}	5.32±0.14^{cd}	4.99±0.12^{d}	5.27±0.17^{d}	4.86±0.16^{d}	8.23±0.21^{e}	8.49±0.26^{e}	6.58±0.10^{f}
Insulin (µu/ml)	9.30±0.80^{ac}	9.36±0.81^{ac}	4.10±0.43^{bc}	9.63±1.1^{ac}	3.10±0.38^{bc}	9.96±1.4^{ac}	0.79±0.16b	6.35±0.89^{c}	0.16±0.10^{b}	5.88±1.2^{c}	27.83±2.3^{d}	37.07±5.4^{e}	10.01±0.71^{c}
TG (mg/dl)	30.8±2.8^{ad}	31.1±2.8^{ad}	29.4±2.8^{a}	16.3±2.9^{b}	24.6±2.8^{c}	16.6±2.8^{b}	25.5±2.8^{c}	18.0±2.8^{b}	34.00±2.9^{d}	18.3±2.9^{b}	11.5±3.0^{e}	8.3±3.3^{e}	9.7±3.2^{e}

Table 2. Least square means and Standat error for the sampling times during and after the 80 km endurance exercise test (EET). Different superscripts within rows differ ($P < 0.05$).

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Sample	Horse 1 Period 1 Temp/hum		Horse 1 Period 2 Temp/hum		Horse 2 Period 1 Temp/hum		Horse 2 Period 2 Temp/hum		Horse 3 Period 1 Temp/hum		Horse 3 Period 2 Temp/hum		Horse 4 Period 1 Temp/hum		Horse 4 Period 2 Temp/hum	
PRE	13.7	75	14.3	99	15.3	99	20	89	12	36	9.5	99	8.6	78	8.9	99
10 km	15	78	14.6	99	16.2	99	22.6	73	11.3	36	9.8	99	11.4	67	11	99
R1	18.9	64	14.4	99	17.4	93	26.7	57	12.6	30	10	99	15	58	15.5	81
30 km	20	60	14.4	99	18.7	88	27.4	57	14.4	29	10	99	17.8	55	17.3	75
R2	16.2	62	13.9	99	18.5	89	27.9	54	16.5	28	10.5	98	20	47	18.9	67
50 km	14.6	68	13.7	99	19.9	86	28.2	53	17	27	10.6	99	20	49	20	65
R3	17.1	62	13.7	99	21.7	78	27.6	54	18.6	25	10.6	95	20.1	50	21.4	61
70 km	18.4	60	13.8	99	21.3	77	27.5	55	19.2	24	10.8	98	20.2	51	21.5	62
Mean	16.74	66.16	14.1	99	18.63	88.63	25.99	61.5	15.2	29.4	10.2	98.3	16.6	56.9	16.8	76.1
Sterr	0.79	2.45	0.12	0	0.81	2.96	1.06	4.54	1.1	1.60	0.16	0.5	1.59	3.77	1.67	5.52
Sample	Horse 5 Period 1 Temp/hum		Horse 5 Period 2 Temp/hum		Horse 6 Period 1 Temp/hum		Horse 6 Period 2 Temp/hum		Horse 7 Period 1 Temp/hum		Horse 7 Period 2 Temp/hum		Horse 8 Period 1 Temp/hum		Horse 8 Period 2 Temp/hum	
PRE	13.4	99	15.9	78	11.1	69	16.6	93	11.4	94	12.3	96	12.9	99	12.6	99
10 km	13.6	99	17.3	80	11.3	69	16	95	11.9	92	12.9	96	14.8	99	12.9	99
R1	14.3	97	20	56	10.8	76	16.4	98	13.6	86	13.6	92	19.9	92	13	99
30 km	14.7	94	20.6	52	10.7	80	16.4	97	14.9	84	14.1	91	22.3	86	13.3	99
R2	15.7	91	21.7	52	10.1	78	15.6	97	17.7	79	14.9	88	24.2	78	13.7	99
50 km	15.9	89	22.1	52	10.4	79	15.1	95	19.6	75	15.5	86	24.8	72	13.8	98
R3	16	89	22.6	51	9.8	77	14.5	95	19.5	74	15.9	85	26.4	67	13.7	98
70 km	17.4	90	22.8	50	10	79	15	95	19.7	74	16.1	85	26.5	67	13.8	99
Mean	15.1	93.5	20.4	58.9	10.5	75.9	15.7	95.6	16.04	82.3	14.4	89.9	21.48	82.5	13.4	98.6
Sterr	0.48	1.53	0.90	4.44	0.19	1.56	0.27	0.56	1.24	2.83	0.50	1.62	1.84	4.74	0.17	0.16
Sample	Horse 9 Period 1 Temp/hum		Horse 9 Period 2 Temp/hum		Horse 10 Period 1 Temp/hum		Horse 10 Period 2 Temp/hum		Horse 11 Period 1 Temp/hum		Horse 11 Period 2 Temp/hum		Horse 12 Period 1 Temp/hum		Horse 12 Period 2 Temp/hum	
PRE	20.5	95	14.7	99	16.5	99	14.3	99	14.3	99	12.7	99	14	64	19.9	69
10 km	22.4	88	15.6	99	18.1	99	14.4	99	14.4	99	14.4	96	14.5	62	20.6	69
R1	24.1	75	16.7	96	19.8	96	16	90	16	90	18.2	84	16	59	22	66
30 km	21.2	67	17.4	90	19.2	94	19	81	19	81	19.5	76	16.5	58	23.3	62
R2	22.2	81	18.3	85	17.5	99	19.1	74	19.1	74	21.4	68	17.5	54	25	57
50 km	24.5	69	17.9	87	18	99	19.9	70	19.9	70	21.5	68	16.7	54	26.1	55
R3	24.4	67	17.6	90	18.5	99	20.5	65	20.5	65	20.3	72	18.3	54	26.8	51
70 km	24.4	70	18	90	19.6	98	20.2	65	20.2	65	20.3	71	19.2	53	27.5	48
Mean	22.9	76.5	17.03	92	18.4	97.9	17.9	80.4	18.88	51.8	18.5	79.3	16.59	57.3	23.9	59.6
Sterr	0.56	3.72	0.45	1.89	0.39	0.67	0.92	5.01	0.32	0.84	1.16	4.39	0.63	1.47	1.02	2.87

Table 3. Ambient temperature (^{o}C) and relative humidity (%) during the endurance exercise tests for each horse and period (repetition)

Does usefulness of EM-K supplementation depend on speed?

ABSTRACT: Increases in plasma [K^+] occur with exercise and supplementation of potassium may further increase these concentrations increasing the risk of neuromuscular hyperexcitability. A K-free electrolyte mixture (EM-K) was tested and compared to a K-rich mixture (EM+K) during a incremental exercise test on the treadmill that consisted of three loops (at 6, 7 and 8 m/s) with two 25 minute intervals (R1, R2) between each two loops on thirteen Arabian horses. Electrolytes were supplied 60 min before and at every R. Blood samples were taken during exercise and rest stops and 150 min of recovery. Blood was immediately analyzed for pH, PCO_2, hematocrit (Hct), plasma [Na^+], [K^+], [Cl^-], [Ca^{++}], [Mg^{++}], and glucose. Another sample was centrifuged, and plasma analyzed later for lactate ([La-]), albumin ([alb]), phosphate ([PO_4^-]), and insulin (ins) within two weeks of the test. In addition, plasma [H^+] and osmolality were calculated. Sampling stage effect was found for plasma [H^+], [Cl^-], [Mg^{++}], and [PO_4^-], where increases and decreases were seen for [H^+] and [Cl^-] and decreases for [Mg^{++}] and [PO_4^-]. Potassium supplementation affected plasma [K^+] at certain time points. There was an electrolyte by treatment effect for Hct, TP, [Na^+], osm, [Ca^{++}], glucose and lactate. EM-K horses had lower Hct, TP, glucose, plasma [La-], plasma [Na^+] and osm; they also had higher plasma [Ca^{++}]. Horses were also fed two different diets for three months before the test, one rich in starch (SS) and one rich in fat and fiber (FF). Horses on FF diet had lower albumin, lower PCO_2, lower insulin and glucose compared to SS fed horses. Lower plasma [K^+] and higher plasma [Ca^{++}] may help prevent signs of neuromuscular hyperexcitability. Also lower [La^-] might be a sign of less fatigue in EM-K supplied horses.

Key words: potassium, calcium, DCAB, fatigue, neuromuscular hyperexcitability.

Introduction

The resting membrane potential is mainly dependent on the K^+ distribution across the cell membrane. K^+ is extruded from muscle cells during exercise increasing the extra cellular [K^+]/intracellular [K^+], increasing the resting membrane potential, and consequently, neuromuscular excitability (Sejersted and Sjogaard, 2000). In addition to the intercellular [K^+] increases, the plasma [K^+] also increases. The increase in plasma [K^+] is proportional to the exercise intensity (Harris et al, 1992, Gotlieb-Vedi et al, 1996). However, most studies done during endurance races report decreases in plasma [K^+] and emphasize K sweat losses for the fact (Carlson et al., 1974, Delar et al., 1977, Lindinger and Ecker, 1995; Schott et al, 1997). Just after exercise plasma [K^+] decreases and if blood collection is done some minutes after exercise stops, the concentration will be below resting levels. The same authors that report the lower plasma [K^+] immediately after exercise recommend the supplementation of K during endurance races to replace sweat losses. However, if K^+ supplementation is done just before or during exercise, further increases in the plasma [K^+] may occur and exacerbate neuromuscular excitability. Signs observed by riders that may manifest increased neuromuscular excitability can vary from decreased willingness to continue during a race to cardiac arrhythmias and consequently lower performance. Also, muscle fatigue can be associated with high interstitial [K^+] (Bangsbo et al., 1996). Therefore, further plasma [K^+] increases may be a related to fatigue.

During exercise plasma [Ca^{++}] decreases (Lindinger and Ecker, 1995). A combination between low plasma [Ca^{++}] and high plasma [K^+] can lower the firing threshold and increase resting potential, increasing further more the risk for having increased neuromuscular hyperexcitability (DiBartola and Morais, 2000).

The main purpose of this study is to test if supplementation with K-free electrolyte formulas during exercise at sub-maximal speeds similar competitive races will lead to lower plasma [K^+] and consequently less risk of neuromuscular hyperexcitability.

A second objective was to determine the effects of chronic adaptation to different feed energy sources, fat and fiber versus starch and sugar, and any interactions with K and Na supplementation, on acid base status, strong ion difference, plasma [K^+], [H^+] and [Ca^{++}], and the risk of neuromuscular hyperexcitability.

Materials and methods

This study was undertaken at the Virginia Tech Middleburg Agricultural Research and Extension Center in September of 2004, and was approved by the Institutional Animal Care and Use Committee. Thirteen Arabian and Arabian–cross geldings were alternated every 30 days between two similar mixed grass pastures. They were fed 2 kg/d of a sugar and starch based (SS, n = 6) or fat and fiber based feeds (FF, n = 7). Feed and pasture samples were submitted for nutrient analysis (Table 1). Dietary cation anion balance (DCAB) was calculated for each feed. Three horses of SS received the EM-K formula during the exercise test, and three EM+K. From FF fed horses three received EM+K, and four EM-K.

Horses were trained for 8 wk, three times a week, on alternate days, alternating between treadmill training (Equi Gym Products LLC, Paris, KY) and exercise on a walker (Odyssey Performance horse exerciser, Rockwood, ON, Canada). For another 4 wk, they were submitted to increasing anaerobic intensities on the treadmill and continued to be exercised aerobically on the walker.

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The effects of the two electrolyte formulas one rich in K (EM+K) and one without K (EM-K) were tested in a sub-maximal incremental exercise test on the treadmill (IET). The IET consisted of three loops with two 25 minute intervals (R1, R2) between each two loops. During each R horses had access to water and hay. Ingested water and hay were calculated by subtracting the initial amount from the final amount left over. The first loop consisted of 5 min at 1.8 m/s, 5 min at 4 m/s and 3^{o} incline, followed by 25 minutes at 6 m/s 3^{o} incline, then 5 min at 3.5 m/s and 5 min at 1.8 m/s. The second loop consisted of 5 min at 1.8 m/s, 20 min at 7 m/s and 3^{o} incline, 5 min at 4 m/s, and 5 min at 1.8 m/s. The third loop consisted of 5 min at 1.8 m/s, 15 min at 8 m/s and 3^{o} incline, 10 min at 3.5 /s, and 5 min at 1.8 m/s. Horses were supplied with two doses of either one of the electrolyte formulas 60 minutes before starting the first loop, at 19 minutes of each R and 20 minutes after finishing the last loop. Each dose of EM-K consisted of 21.8 g of sodium chloride, 1.7 g of calcium chloride, 1.1 g of magnesium chloride, and 0.13 g of monosodium monophosphate, amounting 8.6 g of Na^{+}, 14.2 g of Cl^{-}, 0.3 g of Ca^{++}, 0.13 g of Mg^{++}, 0.03 g of P^{-}. Each dose of EM+K consisted of 21.8 g of sodium chloride, 1.7 g of calcium chloride, 1.1 g of magnesium chloride, 13.31 g of K acetate and 0.13 g of monosodium monophosphate, amounting 8.6 g of Na^{+}, 5.3 g of K^{+}, 14.2 g of Cl^{-}, 0.3 g of Ca^{++}, 0.13 g of Mg^{++}, 0.03 g of P^{-}.

Ambient temperature and relative humidity as well as heat stress data were measured throughout the exercise and recovery phases (Microtherm heat stress WBGT Casella, Amherst, NH). Wet bulb globe temperature index (WBGT) was used for comparison between horses (study days). The WBGT index takes into account temperature, humidity, radiation and air movement. The wet bulb component is a function of ambient temperature and relative humidity. The globe temperature comes from the temperature inside the centre of a black globe. When

radiation is strong, the globe temperature is increased, but wind will cool the globe and decrease temperature. The WBGT index is calculated (Marlin and Nankervis, 2003)

WBGT index = 0.7 x wet bulb temperature (^{o}C) + 0.3 x globe temperature (^{o}C)

Horses were weighed before the start of the IET, just after finishing every loop, and two hours after finishing the test. Blood was taken before they got the electrolytes (PRE, at 65 minutes before starting the exercise), then a catheter was placed aseptically into their jugular vein and blood was taken five minutes before starting the exercise (PEL), then at 5 (1.8 m/s), 10 (4 m/s, 3^{o}), 15 (6 m/s, 3^{o}), 25 (6 m/s, 3^{o}), 35 (6 m/s, 3^{o}), 40 (4m/s) and 45 (1.8 m/s) minutes of the first loop. Time continued running on a stopwatch and blood was taken at 50, 60, 65 minutes during R1, then at 75 (1.8 m/s), 80 (7 m/s, 3^{o}), 85 (7 m/s, 3^{o}), 90 (7 m/s, 3^{o}), 95 (7 m/s, 3^{o}), 100 (4 m/s), 105 (1.8 m/s) min during the second loop, then at 110, 120, 130 minutes during R2, at 140 (1.8 m/s), 145 (8 m/s, 3^{o}), 150 (8 m/s, 3^{o}), 155 (8 m/s, 3^{o}), 160 (4 m/s), 170 (1.8 m/s) minutes, during the third loop, and at 175 (REC 5), 180 (REC 10), 185 (REC 15), 190 (REC 20), 200 (REC 30), 210 (REC 40), 230 (REC 60), 260 (REC 90), 290 (REC 120), 320 (REC 150) minutes during recovery and in the morning after (MA). Heart rate and rectal temperatures were measured every time blood was taken.

Jugular blood was sampled into a heparinized 3 cc syringe (Becton Dickinson, Franklin Lakes, NJ), and another sample was collected with a syringe and transferred to heparinized vacutainer tubes (Vacutainer green, Becton Dickinson, Franklin Lakes, NJ). Blood from the 3 cc syringe was analyzed immediately by a blood-gas analyzer (Stat Profile M, Nova Biochemical, Waltham, MA) for pH, PCO_2, hematocrit (Hct), plasma [Na^{+}], [K^{+}], [Cl^{-}], [Ca^{++}], [Mg^{++}], and glucose. The blood in the vacutainer tubes was kept in ice water, and centrifuged within 10 minutes. Plasma was separated, frozen and kept at -80^{o}C for analysis. Spectophotometric assays

were used for determination of plasma concentrations of lactate ([La-]), albumin ([alb]), phosphate ([PO_4^-]), within two weeks of the test (Beckman Instruments Inc., Brea, CA). In addition, plasma [H^+] and osmolality were calculated (Stat Profile M, Nova Biochemical, Waltham, MA). Plasma insulin (ins) was analyzed by radioimmunoassay (Coat-A-Count Insulin, Diagnostic Products, Los Angeles, CA).

Changes in acid-base status during exercise have been analyzed preferably by the comprehensive physicochemical model of Stewart (Kronfeld et al, 1998; Lindinger et al, 1992, Stewart, 1981; Watson, 1996). In this model (Stewart, 1981), concentrations of H^+ and HCO_3^- are dependent upon the strong ion difference (SID), PCO_2, and total weak acids (A_{tot}, mainly proteinates and phosphates). The SID was calculated as the algebraic sum of [Na^+] and [K^+] minus the sum of [Cl^-] and [La^-]; the algebraic sum of other strong ions was assumed to be < 1 mEq/L and to contribute negligibly to changes in SID observed during exercise and recovery. Estimates of weak acids ($A_{tot,}$ mEq/L) were predicted from TP (g/L) multiplied it by 0.211 (Stämpfli et al, 1999).

The partition of changes in plasma [H^+] into contributions from SID, PCO_2 and A_{tot} was performed using software designed for the Stewart system (Watson, 1996). Resting values for SID, PCO_2 and A_{tot} were set in the panel for independent variables, and the predicted value of [H^+] was observed in the panel for dependent variables. Then the series of values of SID at each sampling stage were entered one at a time (without changing PCO_2 and A_{tot}) to yield corresponding series of changes in [H^+] contributed by the changes in SID (Watson, 1996). The procedure was repeated for PCO_2 (without changing SID and A_{tot}) and for A_{tot} (without changing SID and PCO_2).

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Resting membrane potential (E_K, mV) was calculated from extra-cellular [K^+] (K_o) and [K^+] the (K_i) using the Nerst equation (Ganong, 1999).

$$E_K = 61.5 \log [K_o]/[K_i] \text{ at } 37\ ^oC$$

This equation uses the ratio of extracellular/intracellular concentrations of K^+ only, because the permeability constant of K^+ is much higher than those of other ions. Assumptions are needed to test the possible effects of changes in plasma [K^+] on E_K. Measured plasma [K^+] were used for K_o without the approximately equal and opposing adjustments for plasma solids and for lymph. For K_i, a mean equine middle gluteal muscle intracellular [K^+] of 124 mEq/L (K_i mEq/L water) was calculated from data on K (μM/g of wet weight) and water (%) (Johnson et al, 1991).

Data were summarized as least squares means and standard errors. The effects of sampling time (PRE, PEL, 1.8 m/s (3) 4m/s (4), 6m/s (5), 6m/s(6), 6m/s(7), 4m/s(8), 1.8m/s(9), R11, R12, R13, 1.8m/s(13), 7m/s(14), 7m/s(15), 7m/s(16), 7m/s(17), 4m/s(18), 1.8m/s(19), R21, R22, R23, 1.8m/s(23), 8m/s(24), 8m/s(25), 8m/s(26), 4m/s(27), 1.8m/s(28), REC 5 , REC 10, REC 15, REC 20, REC 30, REC 40, REC 60, REC 90, REC 120, REC 150, and MA), treatments (EM-K, EM+K, SS, FF) and their interactions were evaluated by ANOVA with repeated measures in a mixed model and applied to the 13 horses (SAS Institute Inc., Cary, NC). Significance of differences between means was tested by least significant differences covered by a significant F-test for the ANOVA. Simple relationships of plasma [H^+] to SID, A_{tot} and PCO_2, and SID to plasma [Na^+], [K^+], [Cl^-] and [La^-] were tested by linear regression. Statistical significance was inferred from $P < 0.05$. Simple relationships of plasma analyzed variables to WGBT by linear regression. Statistical significance was inferred from $P < 0.05$.

Results

The 13 horses initially weighed 473.5 ± 12.2 kg, decreased to 465.2 ± 12.5 kg after the first, to 457.5 ± 12.2 kg after the second, to 449.52 ± 12.2 kg after the third loop, and 451.2 ± 13.3 kg two hours after finishing the exercise test. Corresponding losses of body weight were 1.76, 3.38, 5.06, and 4.71 %. No differences were found between any treatment groups.

Heart rates increased with exercise and decreased with rest and recovery (Figure 1). No differences were found between treatments. Maximal heart rate mean was at the fastest speed and was 194.7 ± 3.8 beats per minute. No differences in heart rate recovery times were seen between treatments (Table 2). Rectal temperature also varied with time and no treatment effect was seen (Figure 2). Increases occurred with exercise.

Wet bulb globe temperature (WBGT) index variation between horses is in Table 3. The introduction of the WBGT index as a covariate in the model showed effects on PCO_2, Hct, albumin, and TP. Regressions were done with WBGT as the explanatory and these variables as dependent ones. For PCO_2, $r = 0.10$, $P < 0.029$; for Hct $r = 0.14$, $P = 0.014$; for TP $r = 0.52$ and $P < 0.001$, for albumin $r = 0.37$, $P < 0.001$, for Na $r = 0.28$, $P < 0.001$. Electrolyte was included as an explanatory variable with WBGT index for TP and $r = 0.55$, $P < 0.001$. Electrolyte was included as an explanatory variable with WBGT index for Na and $r = 0.30$, $P < 0.001$. Feed was included as an explanatory variable with WBGT for albumin and $r^2 = 0.45$, $P < 0.001$.

Electrolyte intake during the three rest stops was 100.3 g of sodium chloride, 58 g of potassium acetate, 7.8 g of calcium chloride, 5 g of magnesium chloride, 0.6 g of sodium phosphate, totalizing 38 g of Na^+, 1.4 g of Ca^{++}, 0.6 g of Mg^{++}, 23 g of K^+, 62.9 g of Cl^-, and 0.1 g of P in the EM+K group. Electrolyte intake during the three rest stops was 120 g of sodium chloride, 9.3 g of calcium chloride, 6 g of magnesium chloride, 0.7 g of sodium phosphate,

totalizing 45 g of Na^{+}, 1.7 g of Ca^{++}, 0.7 g of Mg^{++}, 75 g of Cl^{-}, and 0.16 g of P in the EM-K group. Calculated DCAB for the electrolytes was – 18 mEq for EM-K and 593 mEq for EM+K. Hay intake during the exercise test until to hours after was 1.40 ± 0.18 and 1.65 ± 0.30 kg for the EM-K and EM+K groups, respectively. Water intake during the exercise test until two hours after was 6.14 ± 1.21 and 10.34 ± 1.29 kg of water for the EM-K and EM+K groups, respectively. There were no significant differences between the groups in hay and water ingestion.

Calculated DCAB for the SS feed was 145 92 mEq/Kg, for the FF feed 185.82 mEq/Kg, for the pastures 393.5 mEq/Kg, and for the hay 353.6 mEq/Kg. Total DCAB for SS plus pasture was 350.4 mEq/Kg and for FF plus pasture 357.4 mEq/kg.

Basal plasma $[H^{+}]$ was 35.9 ± 0.68 nEq/L (Figure 3). It decreased 14.1% during the first loop of exercise ($P < 0.001$) and increased to PRE levels at R1, decreased up to 9.8% during the second loop of exercise ($P < 0.001$) and increased to PRE levels at R2. During the third loop it increased 6.4% ($P < 0.05$) and returned to PRE levels at REC and MA.

Basal plasma $[Cl^{-}]$ was 100.6 ± 0.71 mmol/L (Figure 4). It increased 2% during the 6 m/s and 2.5% during the 8 m/s exercise loops ($P < 0.020$) and returned to PRE at R . The lowest concentrations were seen at REC 20. At MA it was 0.5% higher than PRE ($P = 0.003$).

Basal plasma $[Mg^{++}]$ was 0.57 ± 0.01 mmol/L. It decreased 6.5% during the 6m/s loop ($P < 0.001$), further decreased u to 9% at R1 ($P < 0.001$), increased a little during the 7 m/s loop ($P < 0.003$), decreased up to 13.7% at R2 ($P< 0.009$), increased again to the same levels as the 7m/s loop during the 8 m/s loop (Figure 5). At REC 5 it reached the lowest point decreasing 17.5% and the increased to 3.5% lower than PRE until REC 150. At MA it was still 3% lower than PRE ($P < 0.001$).

Basal plasma $PO_4^{—}$ was 2.81 ± 0.13 mg/dl. It decreased 7.5% after the electrolyte supply ($P = 0.05$) then increased 22% during exercise at the first loop ($P < 0.01$), decreased to PEL during R1, increased again during he second loop to the same level as the first loop, decreased 30% at R2 ($P = 0.009$), then increased up to 35% during the third loop ($P < 0.001$), decreased up to 40% at REC 15 ($P = 0.041$) and increased up to 32% below PRE until REC 150 ($P < 0.001$) (Figure 6) . It was still 16% below PRE at MA ($P < 0.001$).

ANOVA of plasma $[K^+]$ revealed no treatment effect. Baseline $[K^+]$ had a difference so a model should not have given results. Then the difference between the two groups' baseline $[K^+]$ and the increments were analyzed and a sampling by electrolyte ($P = 0.026$) and an electrolyte effect ($P < 0.001$) were revealed (Figure 7). EM+K had higher $[K^+]$ than EM-K at PEL ($P = 0.016$), R1 ($P < 0.048$), at R2 ($P < 0.053$), during the third loop ($P < 0.047$), during REC until REC 60 ($P < 0.049$), at REC 150 ($P = 0.001$) and at MA ($P < 0.001$). Plasma $[K^+]$ increased during exercise and decreased below PRE at R's and REC ($P < 0.001$).

Hematocrit had an electrolyte by sampling ($P = 0.057$) effect and a treatment effect ($P = 0.006$) (Figure 8). No feed effect or interaction was found and it was dropped from the model. EM+K supplied horses had higher hematocrits during exercise in the first ($P < 0.038$), second ($P < 0.05$), third loop ($P < 0.022$) and recovery ($P < 0.038$) until MA ($P = 0.038$). Horses on EM-K had an overall mean hematocrit of 45.1 ± 0.65% and EM+K horses 48.2 ± 0.69 %. Hct increased form PRE during exercise up to 24% in the EM-K group and up to 37% in the EM+K group and decreased at R. It returned to PRE levels at REC and MA.

Total protein had an electrolyte by sampling effect ($P = 0.004$) (Figure 9). At exercise at 8m/s and during cool down in the last loop horses on EM+K had higher TP than EM-K horses ($P < 0.05$). TP increased up to 15.4% in EM-K horses and up to 24.4% in EM+K horses during

exercise and decreased a little during R, but higher than PRE during R and REC. It returned to PRE levels at MA. No feed effect was observed so it was dropped from the model.

Plasma [Na^+] had an electrolyte by treatment effect ($P < 0.001$) (Figure 10). Horses supplied with EM+K had higher plasma [Na^+] after electrolyte delivery ($P = 0.05$) and at the start of exercise ($P = 0.013$), during the first loop ($P < 0.054$), during the second loop ($P < 0.03$), during the third loop ($P < 0.02$), and from REC 40 to REC 150 ($P < 0.05$). Plasma [Na^+] increased progressively up to 4.1% in EM-K and up to 7.3% in EM+K horses during the study, was lower than PRE at MA. No feed effect was found so it was dropped from the model.

Plasma osmolarity had a sampling by electrolyte effect ($P < 0.001$), as well as a treatment effect ($P = 0.039$) (Figure 11). Horses on EM+K had higher osmolarity at the first loop ($P = 0.045$), R1 ($P < 0.04$), third loop ($P < 0.03$), REC 5 ($P = 0.056$), and REC 150 ($P = 0.037$) than horses on EM-K. Osmolarity increased progressively up to 5.0% in EM-K and to 6.8% in EM+K horses during the experiment and returned to PRE levels at MA. Overall plasma osmolarity mean was 292.1 ± 0.47 mosm/L in the EM-K and 293.6 ± 0.50 mosm/L in the EM+K group. No feed effect was observed so it was dropped from the model.

Plasma [Ca^{++}] had an electrolyte by sampling effect ($P = 0.024$) and a treatment effect ($P = 0.020$) (Figure 12). Horses on EM-K had higher plasma [Ca^{++}] at the end of the first loop ($P < 0.039$), during R1 ($P < 0.052$), at R2 ($P < 0.007$), at the start and end of the third loop ($P < 0.039$), throughout REC ($P < 0.025$). Plasma [Ca^{++}] decreased during exercise in the first and second loops and increased during Rs and REC. It returned to PRE at MA. Overall plasma [Ca^{++}] was higher for EM-K horses (1.55 ± 0.014mmol/L) than for EM+K horses (1.49 ± 0.015 mmol/L). No feed effect was found so it was dropped from the model. Simple linear regression

was calculated with [H^+] independent and [Ca^{++}] as dependent variable and a positive relation was found ($r = 0.39$, $P < 0.001$)

Plasma glucose had an electrolyte by sampling ($P = 0.0059$), an electrolyte ($P = 0.031$) and a feed effect ($P = 0.017$) (Figure 13). Plasma glucose was higher in EM+K than EM-K supplied horses from the end of the third loop ($P < 0.041$) and during recovery ($P < 0.05$). Overall plasma glucose was higher in EM+K (7.93 ± 0.23 mmol/L) than in EM-K (7.07 ± 0.23 mmol/L) horses. Also overall plasma glucose was higher in SS (7.91 ± 0.25 mmol/L) than in FF (7.09 ± 0.23 mmol/L) fed horses. No feed by electrolyte interaction was seen, however, horses that got EM-K and FF had lower glucose (6.71 ± 0.32 mmol/L) than those EM+K and SS (8.40 ± 0.37 mmol/L) ($P = 0.001$); and horses on EM-K SS (7.43 ± 0.37 mmol/L) had a trend($P = 0.062$) to have lower glucose than EM+K FF fed horses; and EM+K SS (8.40 ± 0.37 mmol/L) had a trend ($P = 0.074$) to have higher glucose than EM-K FF fed horses (7.42 ± 0.37 mmol/L). Plasma glucose decreased during exercise and increased during R and REC.

Plasma insulin had a sampling by feed ($P = 0.018$) and a feed effect ($P = 0.001$) (Figure 14). Not all times were analyzed, but plasma insulin was higher in the SS fed horses at R1 ($P < 0.001$), R2 ($P = 0.002$), REC 90 ($P = 0.010$), and REC 150 ($P < 0.001$). Plasma insulin decreased at the second ($P = 0.007$) and third ($P = 0.045$) loops during exercise, and increased at REC ($P < 0.001$). No electrolyte effect was found so it was dropped from the model.

Plasma [La^-] had an electrolyte by sampling effect ($P < 0.001$) (Figure 15). Plasma [La^-] was lower in the EM-K supplied than EM+K horses during the third loop ($P < 0.001$) until REC 5 ($P = 0.037$). Plasma [La-] increased during exercise ($P < 0.001$), increased up to 1330% in EM-K and to 2365% in EM+K horses during the last loop ($P < 0.001$) decreasing during REC

and at REC 150 to PRE levels. No feed effects were seen and they were dropped form the model.

Plasma albumin had a feed by sampling effect (P = 0.019) (Figure 16). Plasma albumin was higher in SS fed than FF fed horses at the end of the second loop (P = 0.056), during the third loop (P < 0 .031), at REC 5 (P = 0.049) and REC 60 (P = 0.027). Plasma albumin increased progressively during the study up to 18.3% in FF and to 26.8% in SS fed horses, and returned to PRE at MA. No electrolyte effects were seen and they were dropped from the model.

Plasma PCO_2 had a feed by sampling effect (P = 0.002) (Figure 17). Horses fed SS had higher PCO_2 at the first loop (P = 0.026), second loop (P = 0.035) and third loop (P < 0.033). Plasma PCO_2 decreased during exercise (P < 0.001) and returned to PRE at Rs and REC until REC 150 and MA where it was lower than PRE (P < 0.043). No electrolyte effect was found so it was dropped form the model.

Plasma SID had an electrolyte by sampling effect (P = 0.012). SID had a trend to be greater in EM+K horses at R13, in the beginning of the second loop (P < 0.10) and third loops (P = 0.030). During the end of last loop however, EM-K had higher SID than EM+K horses (P < 0.034)

Mean changes in plasma $[H^+]$ were partitioned among the three independent variables (Figure 18). The initial increase in plasma $[H^+]$ after the first dose electrolytes was administered (PEL) was partitioned in -0.5 for A_{tot} , -1.3 for PCO_2 and 2.1nEq/L for SID. At the second 6m/s sample plasma $[H^+]$ was partitioned in +0.6 for A_{tot}, +2.2 for SID, and -7.1 nEq/L for PCO_2. The main contributor to changes in $[H^+]$ throughout the study was PCO_2. Changes in SID attenuated changes caused by PCO_2. Plasma $[H^+]$ was linearly and positively related to PCO_2 (r = 0.69, P <0.001), negatively to SID (r = 0.10 P <0.033), positively to $[Na^+]$ (r = 0.44, P < 0.001), $[K^+]$ (r =

0.10, P = 0.024), [La-] (r^2 = 0.35, P < 0.001), and A_{tot} (r = 0.10, P = 0.014) and not related to [Cl^-].

Estimated mean E_K before the race were -91.31 and -93.54 mV for the EM-K and EM+K groups, respectively. Assuming no change in K_i during the race, estimated mean changes in E_K from rest to the 8m/s loop were 7.24 mV and 7.5 mV for the EM-K and 9.73 and 9.98 mV for EM+K.

Discussion

At the fastest speed differences in plasma [K^+] concentrations were evident, EM+K supplied horses were in the zone of possible hyperexcitability, whereas EM-K was just at the limit of the hyperexcitability zone. Potassium rich electrolyte supplements induced lower plasma [Ca^{++}] compared to potassium-free ones. A new finding is that K supplementation caused higher plasma glucose and lactate during exercise and recovery. K supplementation may have caused a greater dependence to carbohydrate metabolism. EM-K horses had lower TP, Hct, plasma [Na^+] and osmolarity, meaning that they were less dehydrated. A high fiber diet (FF) yielded a lower plasma albumin during some points of exercise and recovery, indicating more water availability in horses fed this diet. A higher PCO_2 was seen in high carbohydrate fed horses (SS), due to greater reliability on carbohydrate metabolism. Higher insulin and glucose were also seen in horses fed high carbohydrate diets indicating insulin resistance.

Body weight losses are similar to a simulated speed and endurance test in hot and humid or hot and dry conditions (McCutcheon and Geor, 1996), where distance and speeds are similar to the present study and to endurance races (Barton et al, 2002). Water intake was lower than during a treadmill rehydration study (Butudom et al, 2002).

The introduction of WBGT index into the ANOVAs did not change the results, or induce differences between treatments.

Increases of 23 % in plasma albumin found in the present study are similar to increases found after a 100 km endurance race (Rose et al, 1977), where speed was lower but distance greater than the present study, higher than a modified one star three-day even, where speed is similar to the present study (Hinchcliff et al, 1995). Increases of 10% in Hct are similar to endurance races (Barton et al, 2002, Fregin, 1979), but lower than during a modified one star three day event (Hinchcliff et al, 1995). Part of this increase is due to shifts of plasma water into the interstitial compartment and part to dehydration through sweat losses.

Increases of 5% in plasma [Na^+] are comparable to increases seen during an endurance race (Sloet et al, 1991), and during a salt supplementation study (Coenen et al, 1995). Decreases during endurance races, however have been reported (Barton, 2002). Increases in this study are also higher than a repeated sprint study on the treadmill (Taylor et al, 1995) and than in horses competing in a 3 day event (Hinchcliff et al, 1995). Increases of 6% in plasma osmolarity are greater than a previous study (Dusterdieck et al, 1999).

The Na amounts supplied during this study is equivalent to 11.5 and 13.7 L of sweat in the EM-K and EM+K groups, respectively (McCutcheon and Geor, 1996). Since horses lost 5% of their body weights, a deficit of electrolytes of about 12 L of sweat was still present at the end of the study. Despite the supplementation of 38 and 45 g of Na in the EM-K and EM+K groups, respectively the horses drunk very little until two hours after of the study. Even though plasma [Na^+] and osmolarity was higher in EM+K horses and water consumption was also higher than EM-K horses, EM+K horses were more dehydrated during the study (higher total protein).

Plasma [Cl^-] decreased up to 2% during REC and increased up to 2.7% during exercise. Decreases of 3.4% have been reported during repeated sprints (Taylor et al, 1995) and 4.9% after a three–day event (Hinchcliff et al, 1995). In neither of these studies electrolytes were replaced. During endurance races decreases (Barton et al, 2003; Sloet et al, 1991) or no changes (Lindinger and Ecker, 1995) have been reported. During a 62 km endurance race (Nyman et al, 1996) increases were about 10% in salt paste supplied (50 g of Cl^-) horses at 42 km, less than in the present study. However speeds were much higher in this study and speed has been shown to be correlated positively with Cl^- losses (Ecker and Lindinger, 1995). Chloride losses are involved in alkalosis (Carlson and Mansmann, 1974; McCutcheon et al, 1995) but during this study plasma [Cl^-] had no relation with plasma [H^+], probably because decreases in plasma [Cl^-] were small.

Plasma [Mg^{++}] decreased up to 16% at REC 5. During endurance races decreases have been reported due to sweat losses (Rose et al, 1980). No clinical signs were associated with decreased plasma [Mg^{++}] in this study.

Plasma [H^+] decreased during exercise at 6 and 7 m/s, mainly due to decreases in PCO_2, due to hyperventilation (Figure 3 and 18). Increases in [H^+] were seen during the third loop and are mainly due to decreases in SID. During cool down of the last loop a sharp decrease in PCO_2 compensated for the continuous increase in SID. Overall PCO_2 was the predominant contributor to plasma [H^+] during repeated sprints (Kronfeld et al, 1999) and during the current study.

Plasma PO_4^{--} increased during exercise and decreased during Rs. At REC it reached the lowest values. It is released from the working muscles from breakdown of ATP (Fitts, 1992). Greater intensity yielded higher plasma PO_4^{--}. The lowest PO_4^{--} concentrations also coincided

with increases in glucose. Phosphates will enter the cells together with glucose for replenishment of muscle losses after exercise.

Horses in the EM-K group had higher plasma [Ca^{++}] during exercise and recovery. Dietary cation anion balance was similar between both diets but electrolyte DCAB was much lower for EM-K (-18 mEq) than EM+K (593 mEq). Even when adding the hay consumed for both groups during the study DACB was higher for EM+K horses. In cattle high DACB has been associated with alkalosis, PTH ineffectiveness and the cause of periparturient hypocalcemia (Goff and Horst, 1997). In horses a high DCAB diet yielded greater decreases in plasma [Ca^{++}] (-7.05%) after one mile racing, versus low (-6.2%) and basal (-5.5%) DCAB, however differences between treatments were not significant (Cooper et al, 1998). The difference in plasma [Ca^{++}] may have been induced by a higher DCAB in EM+K horses during the study. Higher plasma [Ca^{++}] was positively related to higher plasma [H^{+}] and [H^{+}] negatively related to SID. Lower SID in EM-K at certain points may have contributed to a higher [H^{+}] during the less intense loops (6 and 7 m/s) and to a higher [Ca^{++}].

Plasma glucose was higher in EM+K supplied horses than in EM-K supplied horses, overall but also mainly during recovery. Increases in plasma [K^{+}] stimulate epinephrine release (Fenn, 1940; Silva and Spokes, 1981), which then releases glucose from the liver (Houston, 2001). Epinephrine also induces splenic contraction and this would partially explain higher Hct in EM+K horses. It is known that K also stimulates insulin secretion, but this would lead to more transport of glucose into the cells, yielding lower plasma glucose (Hiatt et al, 1972). No differences in insulin were observed between electrolytes, only between diets.

Plasma [La^{-}] was higher during the highest exercise intensity in EM+K supplied horses. Higher plasma glucose may have led to higher carbohydrate utilization therefore more glycolysis

during the highest intensity of exercise. Also, insulin has been shown to release lactate by skeletal muscle (Novel-Chaté et al, 2001). Although differences were not seen, if more sampling points were analyzed differences in insulin might have been evident. Fatigue is related to higher extra-cellular K (Nielsen, 2001) and lactate (Mainwood and Reanud, 1985). It could be that K supplied horses became more fatigued than EM-K supplied horses. No difference between lactate producing horses was present since in a lactate threshold test done before and horses were distributed between both groups according to lactate threshold.

Higher plasma [Na^+] mainly during exercise was found in EM+K supplied horses as well as higher osmolarity, during some points at exercise and overall. Lower Hct and lower TP were also observed in EM-K supplied horses. Higher Na intake may have led to greater water shifts to the vascular compartment leading to lower plasma [Na^+], osmolarity, lower TP and Hct. However, higher plasma [Na^+] in EM-K horses was not observed at any time that blood was sampled, only at recovery. It is not clear why higher [Na^+] and osmolarity were present despite greater water consumption and lower Na intake in EM+K horses.

Lower albumin was found for FF fed horses. In another study lower TP was found in horses with unlimited access to hay (Danielsen et al, 1995). Higher fiber consumption may lead to higher water consumption and a greater water reservoir in the gut (Meyer, 1987).

Higher PCO_2 was found in SS fed horses during exercise indicating higher CO_2 production in high carbohydrate fed horses (Marlin and Nankervis, 2003).

Horses fed SS diets had higher insulin at R1, R2, REC 90 and REC 150 and glucose throughout the experiment. Inactive gelding fed starch diets have lower insulin sensitivity than the ones fed fat and fiber based diets (Hoffman et al, 2003). Here exercising horses fed starch diets seem to rely more on carbohydrate metabolism during sub-maximal exercise. In an

experiment comparing sweet feed, fiber and fat diets glucose and insulin were higher in sweet feed and fiber diet horses than in fat fed horses during exercise (Crandell et al, 1999). Horses were adapted to the diets and were fed three hours before the exercise.

Higher plasma [K^+] was seen in EM+K supplied horses at the highest speeds and some recovery points. Plasma [K^+] are comparable to levels found during repeated sprints in Arabian horses (Taylor et al, 1995), but lower than Thoroughbred horses during high intensity exercise (Harris and Snow, 1992). Plasma [K^+] increases in proportion to exercise intensity (Harris and Snow, 1992) as K^+ moves out of working muscle cells. Higher plasma [K^+], however, will exert catelectronic effects on E_K and may exacerbate neuromuscular excitability (Ganong, 1999). Eventually high plasma [K^+] reaches a critical level and inhibits action potentials, so muscles and nerves become unable to respond (Ganong, 1999; Mainwood and Renaud, 1985). A value of about +7mV of depolarization leads to a zone of local responses in which cathodal stimuli are facilitated, that is, in which increases in excitability are greater up to the firing threshold (Ganong, 1999). A zone of 7 to 15 mV of depolarization may be considered to represent hyperexcitability, and persistent depolarization greater than about 15 mV to represent prolonged refractory periods and decreased muscle response (Ganong, 1999; Mainwood and Renaud, 1985).

The mean calculated E_K in the present study is –91.31 and -93.54 mV for the EM-K and EM+K groups, respectively. Estimates of –84.31 and –86.54 mV may be predicted for catelectronic local responses and -76.31 and -78.54 mV for firing thresholds, in EM-K and EM+K groups, respectively. The Nerst equation yields corresponding estimates of 5.15 and 4.72 mEq/L for local responses and 7 and 6.4 mEq/L for firing potentials in EM-K and EM+K supplied horses, respectively. The values of -84.07 and -83.81 mV in EM-K horses and -83.81

and -83.56 mV in EM+K horses were calculated for membrane potentials during the highest speeds of exercise from plasma [K^+] averages. Most of the horses were in the local zone of increased excitability, however none showed any signs of neuromuscular hyperexcitability.

The risk of these horses to reach the threshold potential, or firing potential was calculated. The SDs are 0.284 and 0.262 mEq/L for the EM-K group at the two samples during the fastest speed respectively, and 0.366 and 0.438 mEq/L for the EM+K at the same speeds, respectively. Dividing these SD's into the difference between the respective means of 5.19 and 5.24 mEq/L for EM-K and 5.24 and 5.29 mEq/L at these sampling points and 7 mEq/L for EM-K and 6.4 mEq/L for EM+K (+15mV depolarization for a firing potential) will yield respective estimates of 6.39 and 4.81 (EM-K) and 3.17 and 2.53 (EM+K) for z, with corresponding probabilities of less than 1 horse in 10000 for both values the EM-K group and 1 in 1428 and 1 in 166 the EM+K group reaching the firing threshold (Rothman, 1986). Probabilities are small, howevermuch smaller in EM-K horses.

Implications

This study confirmed previous results that potassium-free electrolyte supplementation reduces the risk of neuro-muscular excitability and added emphasis to risk at higher speeds. The supply of potassium-free electrolytes attenuated exercise hypocalcemia reducing the risk of increased neuromuscular hyperexcitability. Higher lactate production in potassium supplied horses also disfavors its supplementation. The potassium free supplements were also associated with less dehydration. Changes in plasma insulin and glucose indicate insulin resistance in carbohydrate fed horses. More experiments on the effect of DCAB on plasma [Ca^{++}] are necessary to confirm results in the prevention of hypocalcemia.

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Journal Article 4

Table 1. Nutrient composition of feed, grass and hay on a DM basis analyzed in the DHI Forage Testing Laboratory (Ithaca, NY).

	Feed		**Pasture**		**Hay**
Nutient	SS	FF	P1	P2	Hay
DM, % as fed	88.2	92.3	94.4	94.5	96.6
CP, % DM	13.3	13.9	22.4	20.9	11.3
ADF, % DM	12	30.6	29.6	30.1	41.7
NDF, % DM	20.8	42.3	52.8	52.7	62.1
NSC, % DM	52.1	11.4	12.2	10.1	8.9
Fat, % DM	4.2	14.6	4.4	3.1	1.9
Ash, % DM	6.6	9.1	9.0	9.2	8.0
Ca, % DM	1.04	1.68	0.47	0.57	0.93
P, % DM	0.68	0.78	0.37	0.32	0.24
Na, % DM	0.16	0.27	0.01	0.01	0.01
K, % DM	1.23	1.37	3.27	3.09	2.08
Mg, % DM	0.32	0.24	0.22	0.22	0.16
S, % DM	0.19	0.18	0.21	0.21	0.11
Cl, % DM	0.44	0.64	1.29	1.24	0.66

Electrolyte	Feed	Time to recovery to 64 bpm, min		
EM-K	SS	5	5	5
EM-K	SS	4	5	3
EM-K	SS	5	5	15
EM+K	SS	3	10	18
EM+K	SS	8	7	17
EM+K	SS	5	5	5
EM-K	FF	5	4	3
EM-K	FF	6	6	8
EM-K	FF	8	2	1
EM-K	FF	1	3	6
EM+K	FF	10	5	30
EM+K	FF	3	4	3
EM+K	FF	1	1	1

Table 2. Feed and electrolyte distribution and time to recover heart rate to 64 beats per minute of 13 horses in a sub-maximal exercise test on the treadmill.

horse	Mean WBGT	SE	min	max
----	20.5	0.1	15.8	25
1	20.7	0.4	17.2	25
2	16.9	0.1	16.3	18.2
3	19.9	0.4	15.8	23.2
4	21.7	0.3	17.4	24.6
5	19.5	0.3	16.4	22.6
6	22.3	0.1	21.7	22.7
7	21.2	0.1	20.1	23
8	19.7	0.1	18.8	20.9
9	21.3	0.4	18	23.7
10	20.8	0.1	19.5	22.4
11	20.5	0.4	16.9	23.8
12	23.2	0.1	22.4	24.7
13	18.5	0.1	18	19.2

Table 3. Wet bulb globe temperature during the study of the 13 horses, SE, minimum and maximum values.

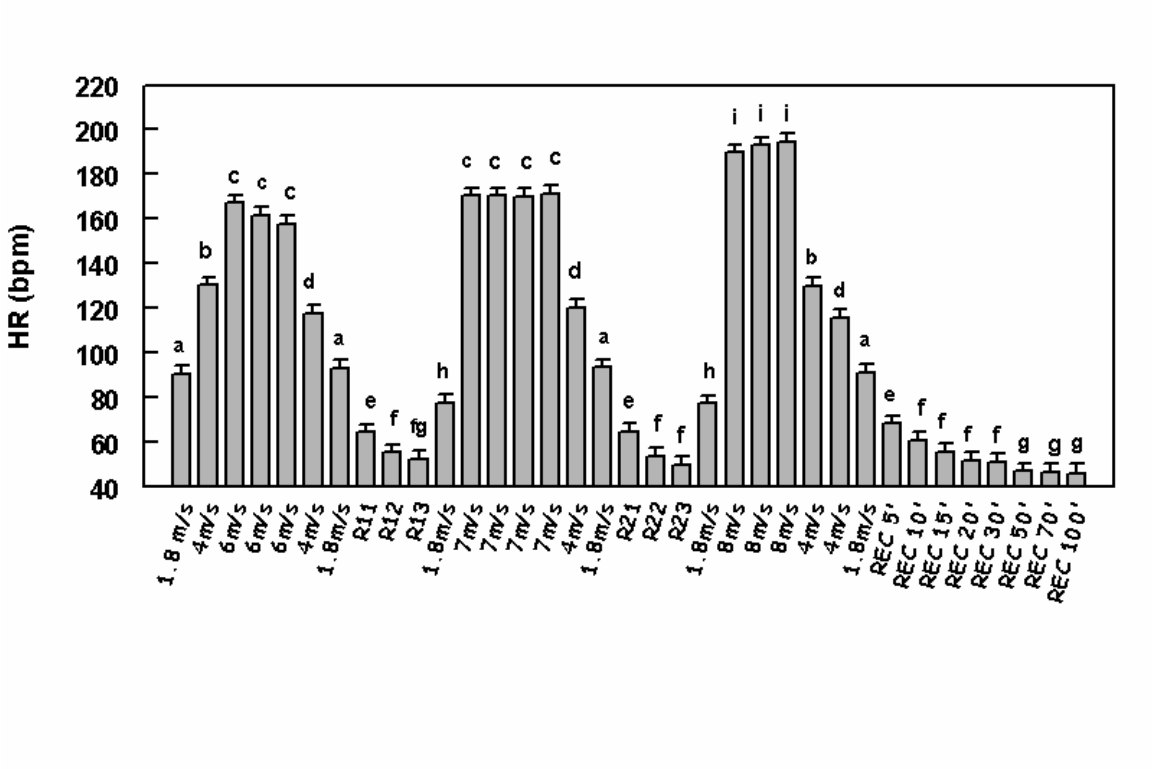

Figure 1. Mean (± SE) heart rate versus stage of sample collection before (PRE), during, and after the sub-maximal exercise test in all combined treatments.

Different letter superscripts differ ($P < 0.05$).

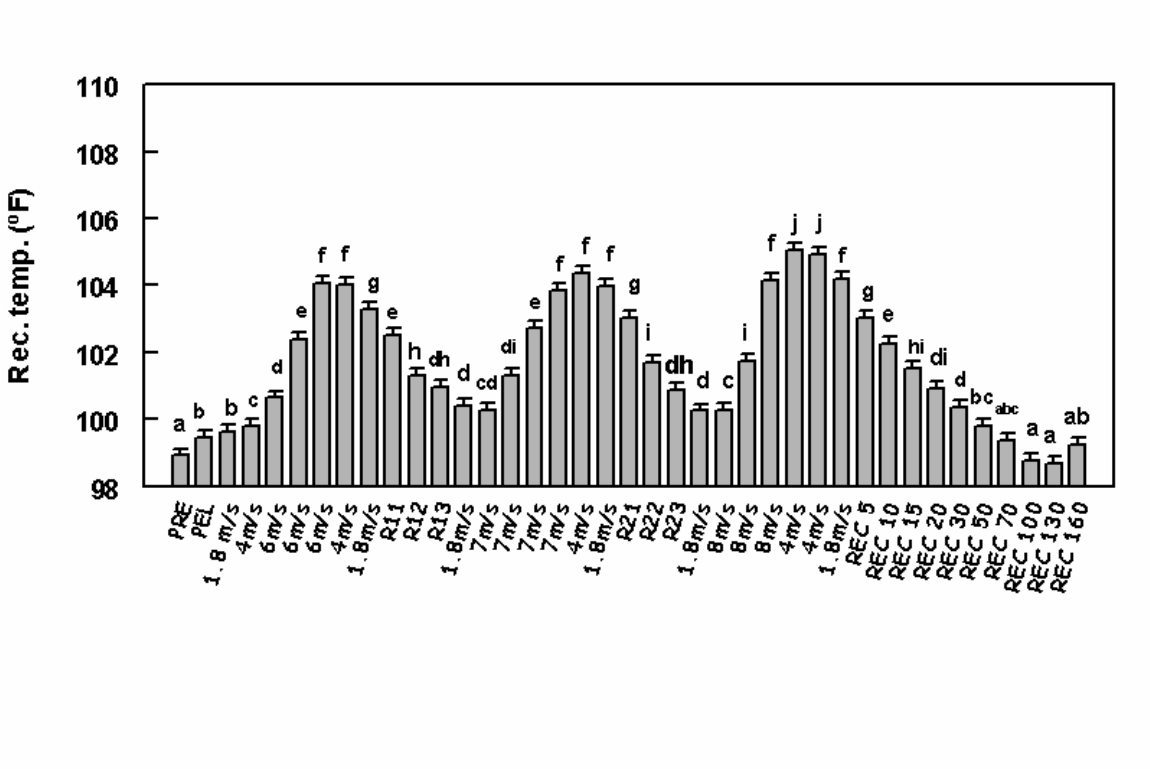

Figure 2. Mean (± SE) rectal temperature versus stage of sample collection before (PRE), during, and after the sub-maximal exercise test in all combined treatments.

Different letter superscripts differ ($P < 0.05$).

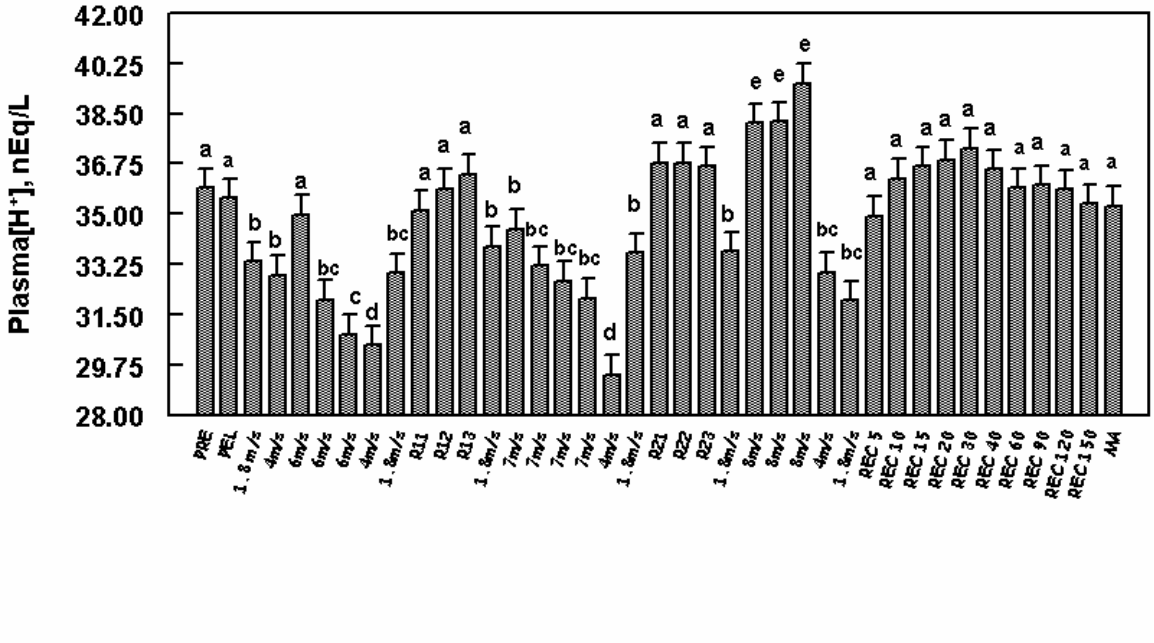

Figure 3. Mean (± SE) plasma [H^+] versus stage of sample collection before (PRE), during, and after the sub-maximal exercise test in all combined treatments.

Different letter superscripts differ ($P < 0.05$).

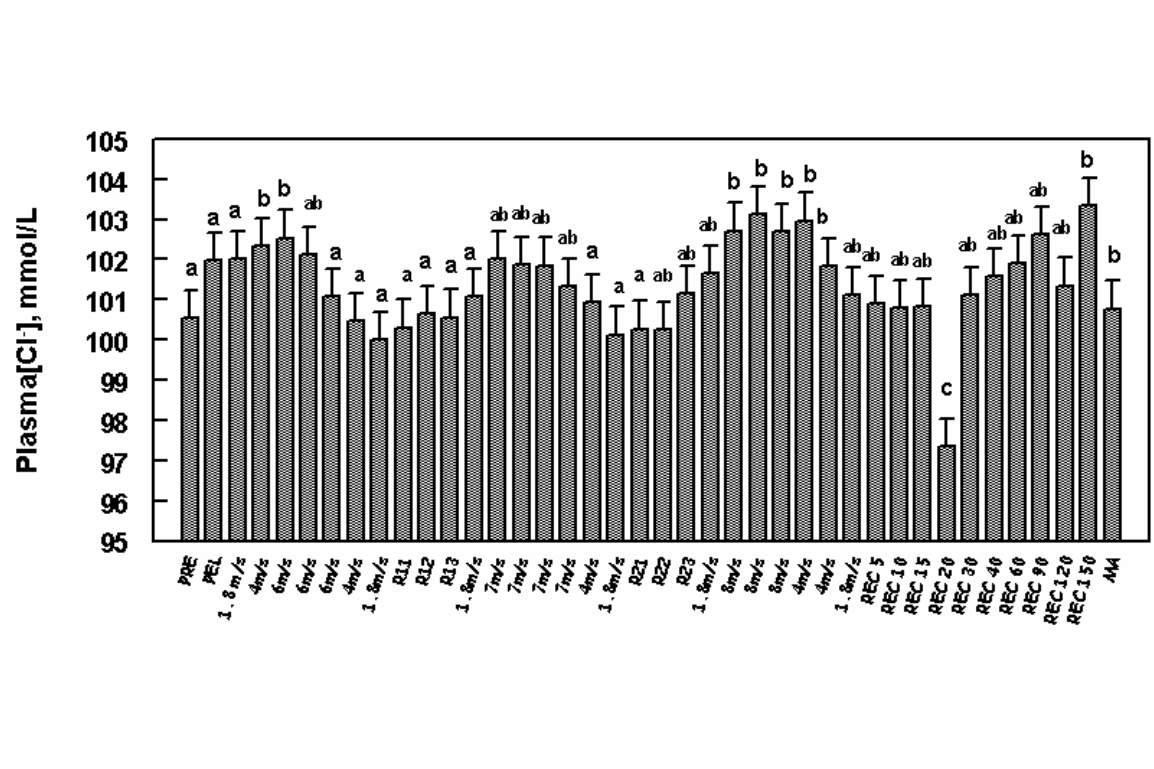

Figure 4. Mean (± SE) plasma [Cl^-] versus stage of sample collection before (PRE), during, and after the sub-maximal exercise test in all combined treatments.

Different letter superscripts differ ($P < 0.05$).

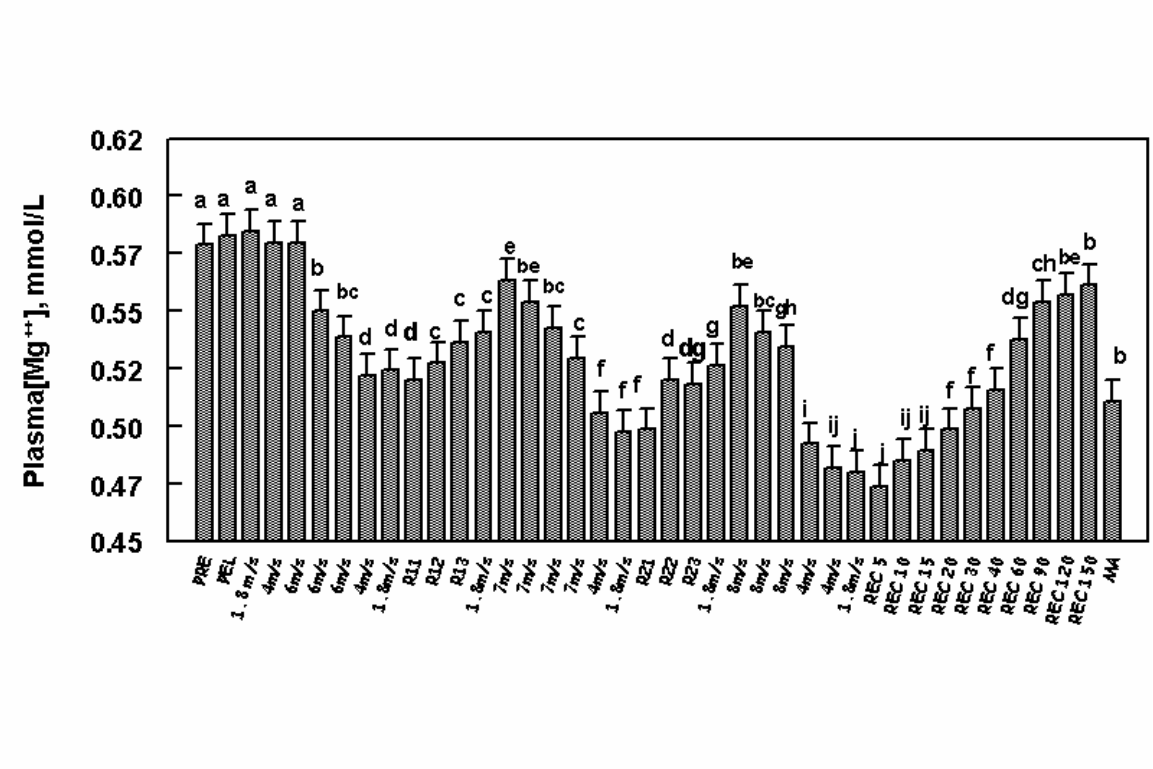

Figure 5. Mean (± SE) plasma [Mg^{++}] versus stage of sample collection before (PRE), during, and after the sub-maximal exercise test in all combined treatments.

Different letter superscripts differ ($P < 0.05$).

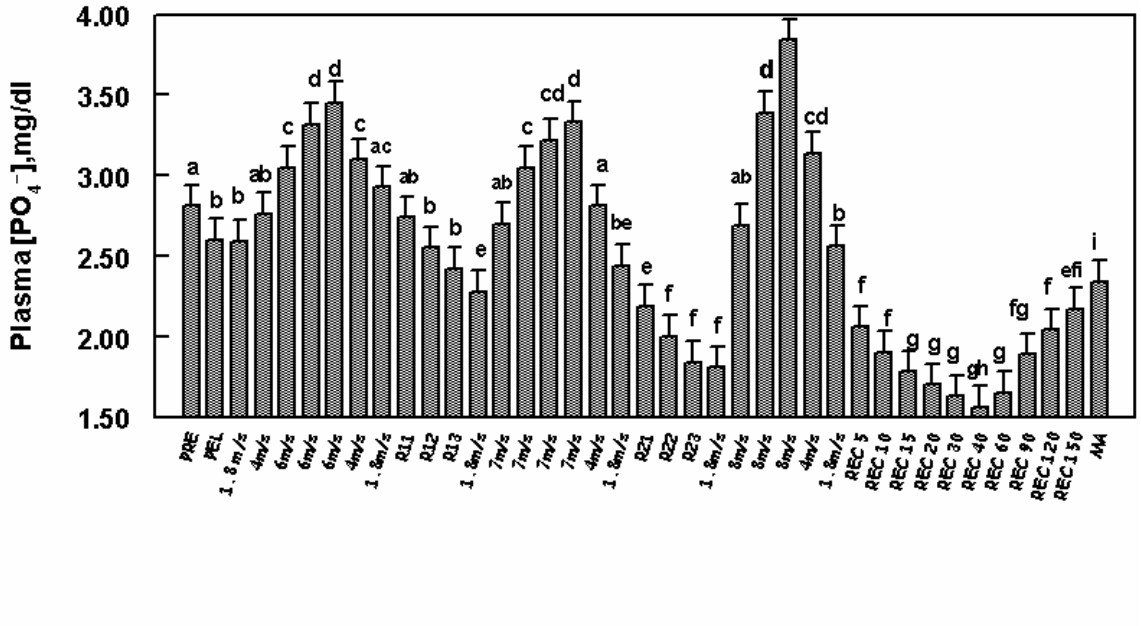

Figure 6. Mean (± SE) plasma [PO_4^-] versus stage of sample collection before (PRE), during, and after the sub-maximal exercise test in all combined treatments.

Different letter superscripts differ ($P < 0.05$).

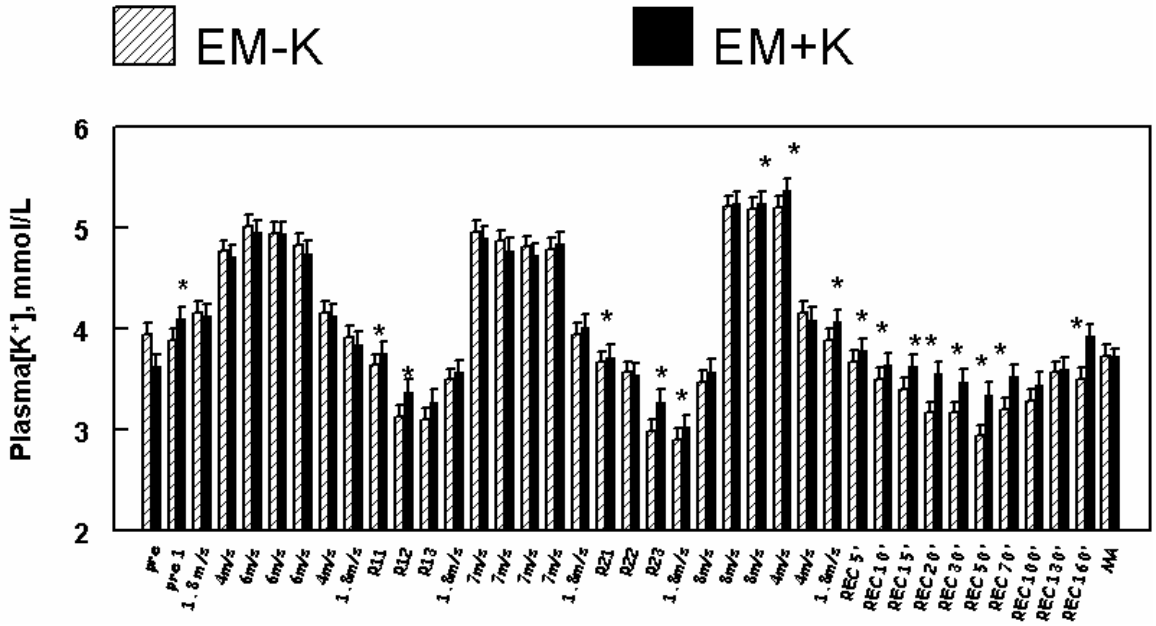

Figure 7. Mean (± SE) plasma [K^+] versus sampling time before, during, and after sub-maximal exercise test in EM–K and EM+K treated horses. Notice the increase($P < 0.05$) with sampling from before the test (PRE) to exercise and the decrease ($P < 0.05$) from exercise in every loop to rest stops and from the last loop to recovery for all (EM–K and EM+K treated) horses. *Significant ($P < 0.05$) differences between EM–K and EM+K treated horses after electrolyte supplementation, at the end of the first, second and third loops, during the last loop, and during recovery.

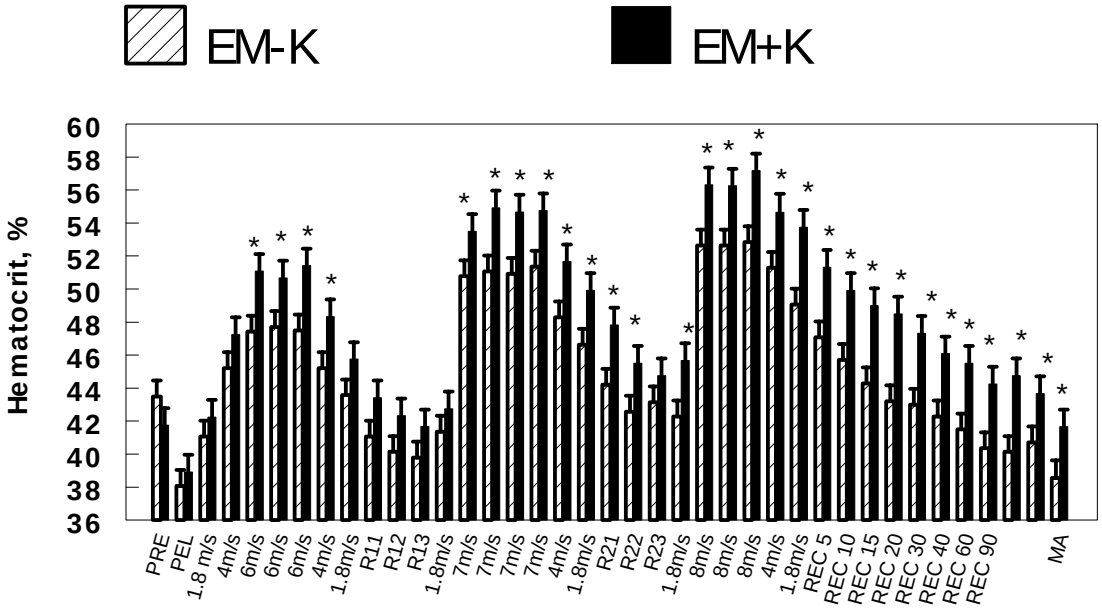

Figure 8. Mean (± SE) plasma hematocrit versus sampling time before, during, and after sub-maximal exercise test in EM−K and EM+K treated horses. Notice the increase ($P < 0.05$) with sampling from before the test to exercise and the decrease ($P < 0.05$) from exercise to every loop rests and from the last loop to recovery for all (EM−K and EM+K treated) horses.

*Significant ($P < 0.05$) differences between EM−K and EM+K treated horses during exercise and recovery.

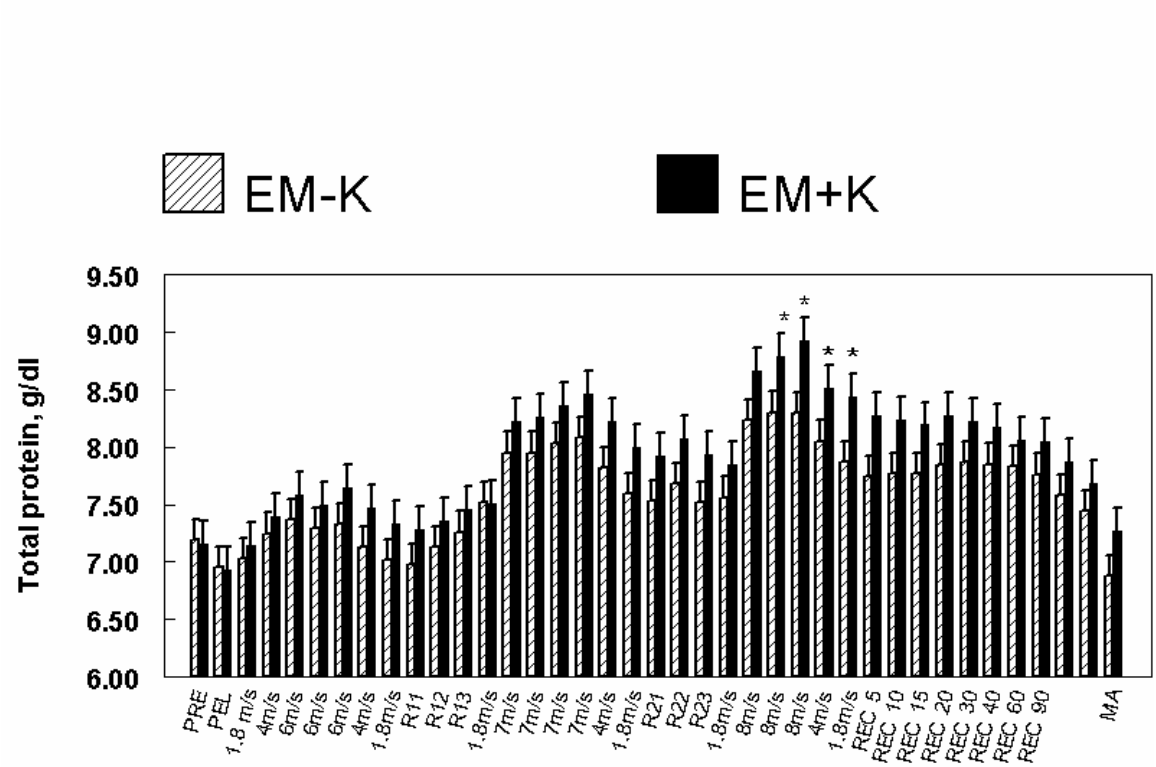

Figure 9. Mean (± SE) total protein versus sampling time before, during, and after sub-maximal exercise test in EM–K and EM+K treated horses. Notice the increase ($P < 0.05$) with sampling from before the test (PRE) to exercise and the decrease ($P < 0.05$) from exercise in every loop to rest stops and from the last loop to recovery for all (EM–K and EM+K treated) horses. *Significant ($P < 0.05$) differences between EM–K and EM+K treated horses during exercise in the last loop.

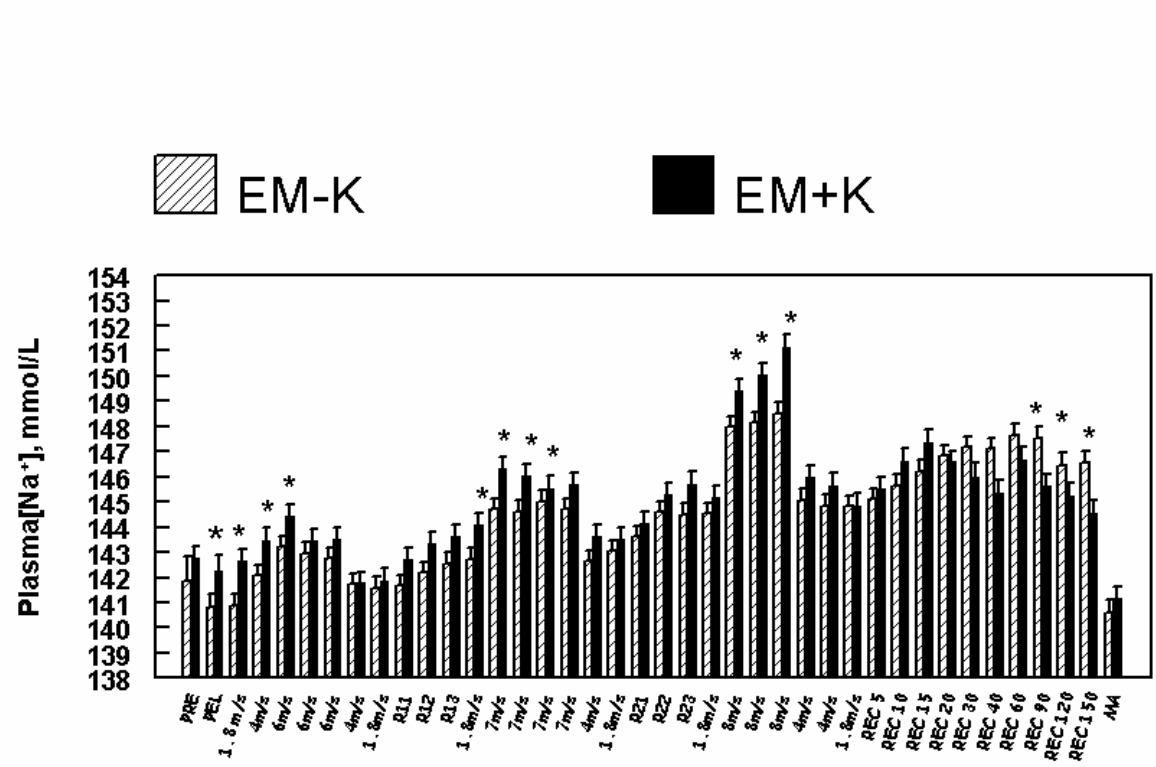

Figure 10. Mean (± SE) plasma [Na^+] versus sampling time before, during, and after sub-maximal exercise test in EM−K and EM+K treated horses. Notice the increase ($P < 0.05$) with sampling from before the test (PRE) to exercise and the decrease ($P < 0.05$) from exercise in every loop to rest stops and from the last loop to recovery for all (EM−K and EM+K treated) horses.

*Significant ($P < 0.05$) differences between EM−K and EM+K treated horses after electrolyte supplementation, during exercise in all loops and recovery.

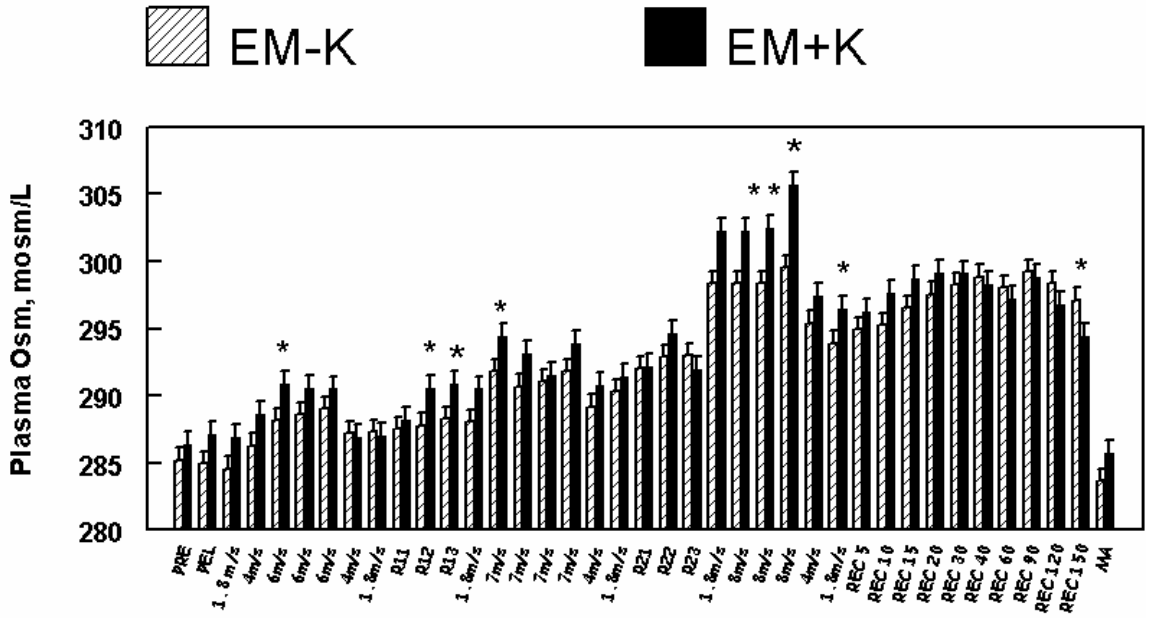

Figure 11. Mean (± SE) plasma osmolarity versus sampling time before, during, and after sub-maximal exercise test in EM–K and EM+K treated horses. Notice the increase ($P < 0.05$) with sampling from before the test (PRE) to exercise and the decrease ($P < 0.05$) from exercise in every loop to rest stops and from the last loop to recovery for all (EM–K and EM+K treated) horses. Also progressive increase from PRE to REC ($P < 0.05$).

*Significant ($P < 0.05$) differences between EM–K and EM+K treated horses during R1, exercise and some recovery points. Also a treatment effect ($P < 0.05$), plasma osmolarity higher in EM+K horses.

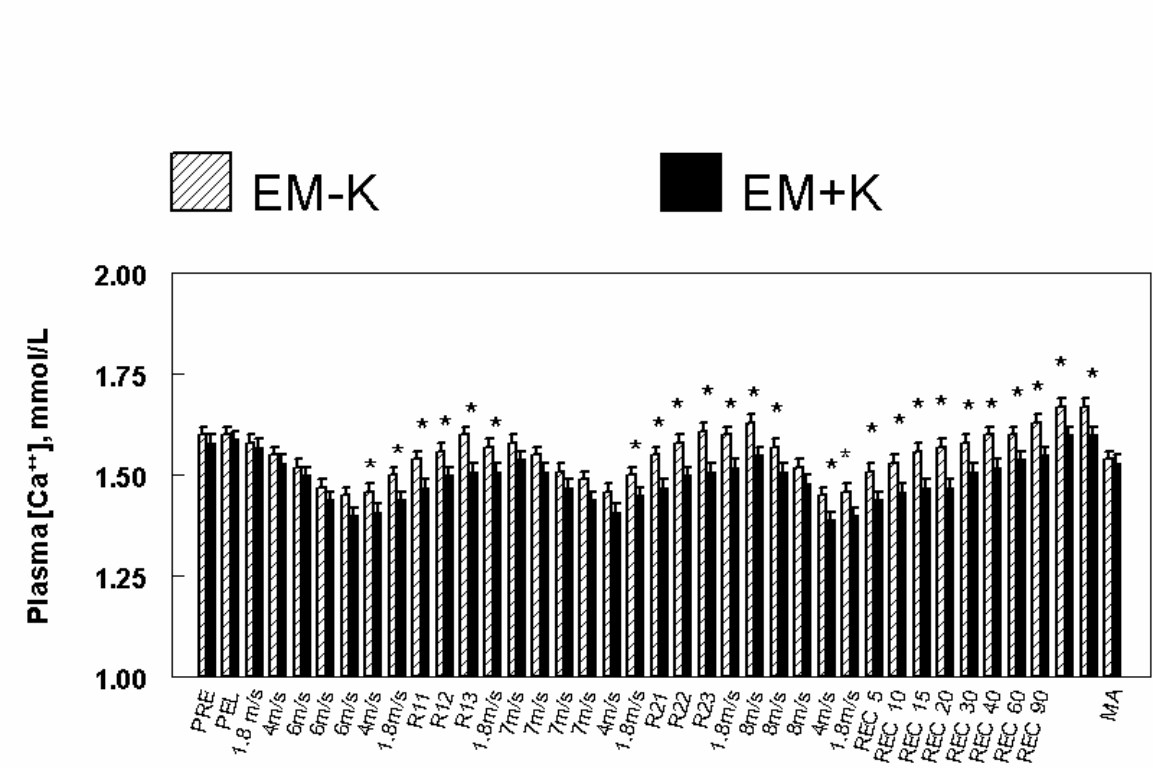

Figure 12. Mean (± SE) plasma [Ca^{++}] versus sampling time before, during, and after sub-maximal exercise test in EM−K and EM+K treated horses. Notice the decrease ($P < 0.05$) with sampling from before the test (PRE) to exercise and the increase ($P < 0.05$) from exercise in every loop to rest stops and from the last loop to recovery for all (EM−K and EM+K treated) horses.

*Significant ($P < 0.05$) differences between EM−K and EM+K treated horses at the end of the first, second and third loop, at every rest stop, and during recovery.

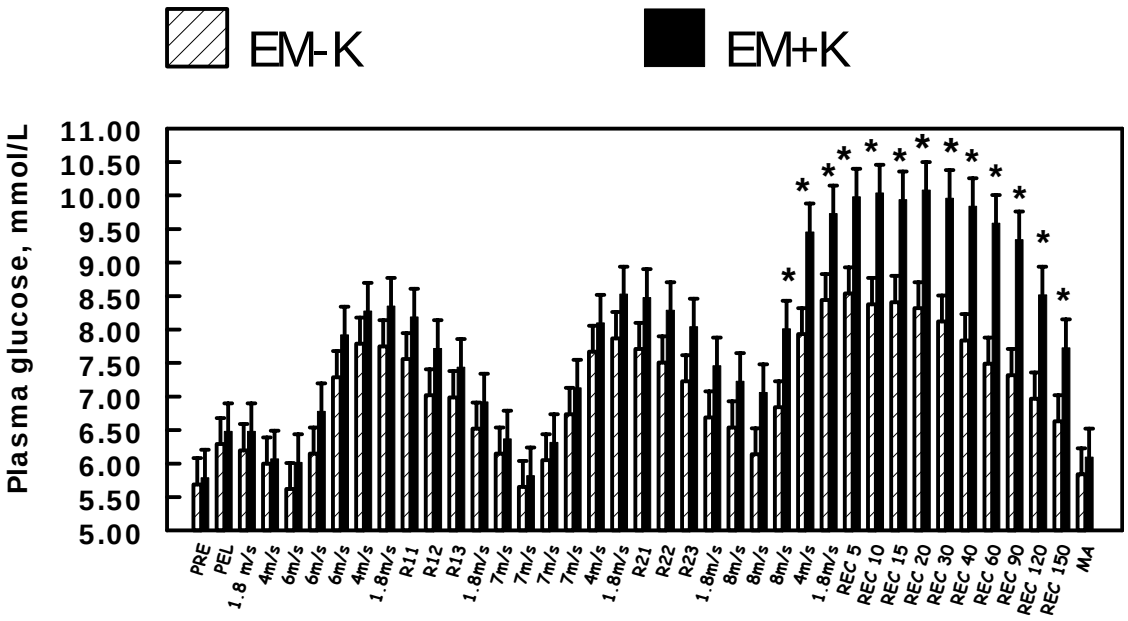

Figure 13. Mean (± SE) plasma glucose versus sampling time before, during, and after sub-maximal exercise test in EM−K and EM+K treated horses. Notice the decrease ($P < 0.05$) with sampling from before the test (PRE) to exercise and the increase ($P < 0.05$) from exercise in every loop to rest stops and from the last loop to recovery for all (EM−K and EM+K treated) horses.

*Significant ($P < 0.05$) differences between EM−K and EM+K treated horses at the end of the third loop, and during recovery. Also overall plasma glucose was higher in EM+K than EM-K horses ($P < 0.05$)

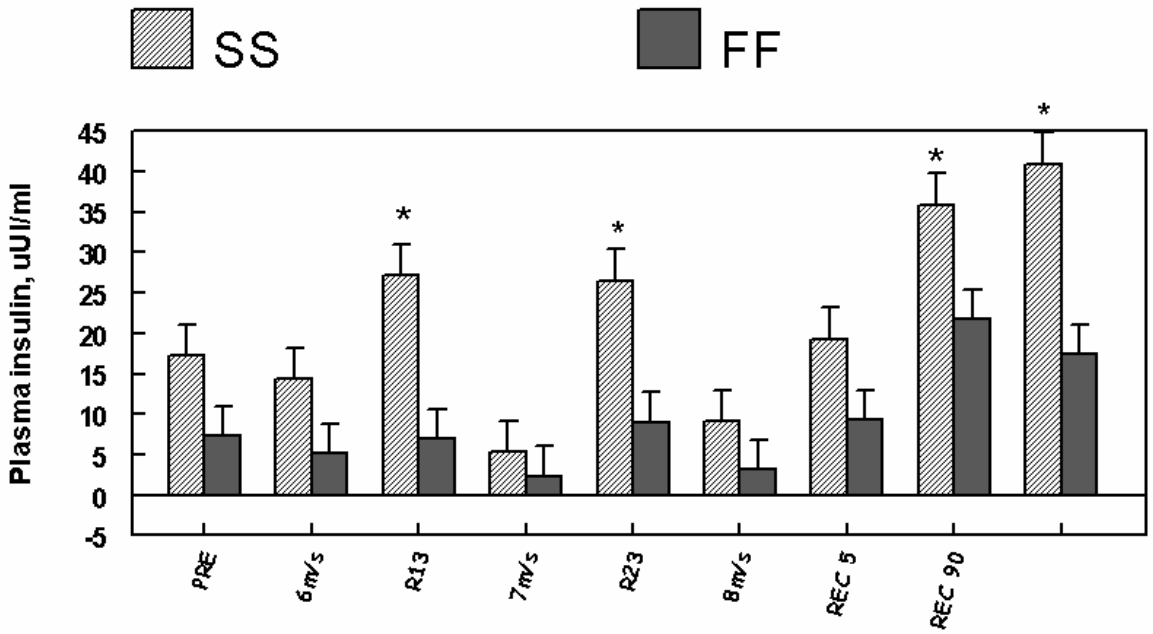

Figure 14. Mean (± SE) plasma insulin versus sampling time before, during, and after sub-maximal exercise test in SS and FF treated horses. Notice the increase ($P < 0.05$) with sampling from before the test (PRE) to the first and second rest stop and the increase ($P < 0.05$) from exercise in every loop to rest stops and from the last loop to recovery for all (EM–K and EM+K treated) horses.

*Significant ($P < 0.05$) differences between SS and FF treated horses at the first, second rest stops, and during recovery.

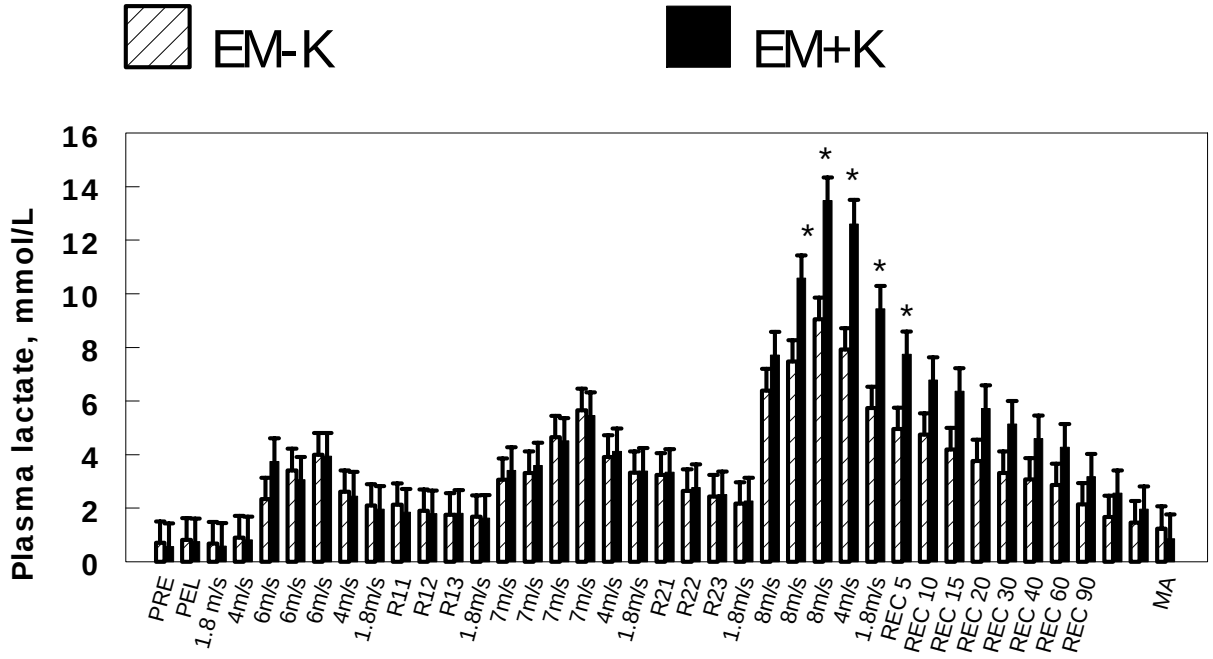

Figure 15. Mean (± SE) plasma [La^-] versus sampling time before, during, and after sub-maximal exercise test in EM−K and EM+K treated horses. Notice the increase ($P < 0.05$) with sampling from before the test (PRE) to exercise and the decrease ($P < 0.05$) from exercise in every loop to rest stops and from the last loop to recovery for all (EM−K and EM+K treated) horses.

*Significant ($P < 0.05$) differences between EM−K and EM+K treated horses at of the third loop.

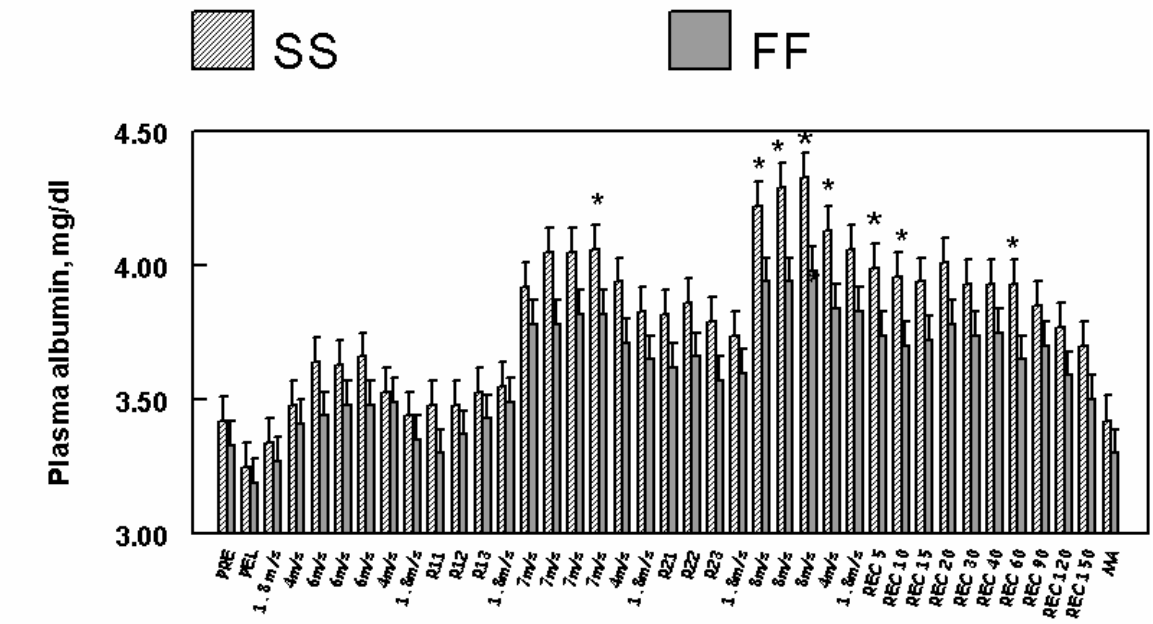

Figure 16. Mean (± SE) plasma albumin versus sampling time before, during, and after sub-maximal exercise test in SS and FF treated horses. Notice the increase ($P < 0.05$) with sampling from before the test (PRE) to exercise and the decrease ($P < 0.05$) from exercise in every loop to rest stops and from the last loop to recovery for all (SS and FF treated) horses.

*Significant ($P < 0.05$) differences between SS and FF treated horses at the second loop, and at the third loop, and during recovery.

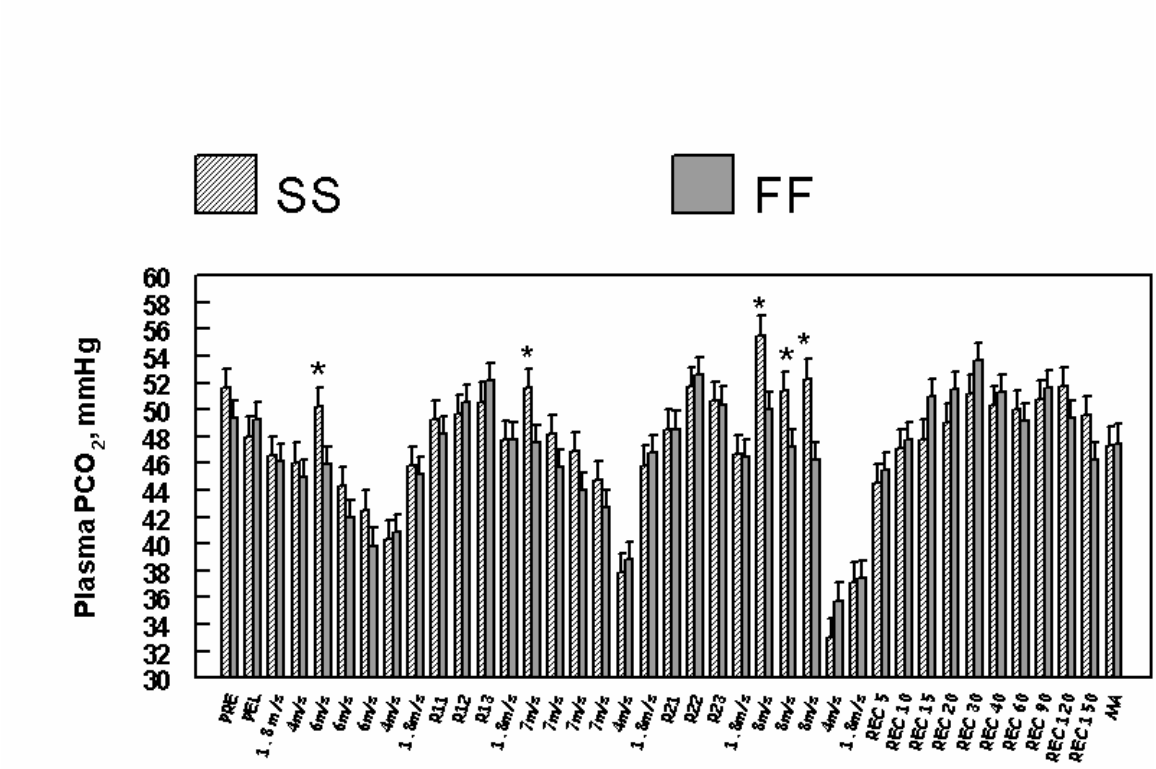

Figure 17. Mean (± SE) plasma PCO_2 versus sampling time before, during, and after sub-maximal exercise test in SS and FF treated horses. Notice the decrease ($P < 0.05$) with sampling from before the test (PRE) to exercise and the increase ($P < 0.05$) from exercise at the end of every loop to rest stops and from the end of the last loop to recovery for all (SS and FF treated) horses.

*Significant ($P < 0.05$) differences between SS and FF treated horses at the beginning of the first, second and third loop.

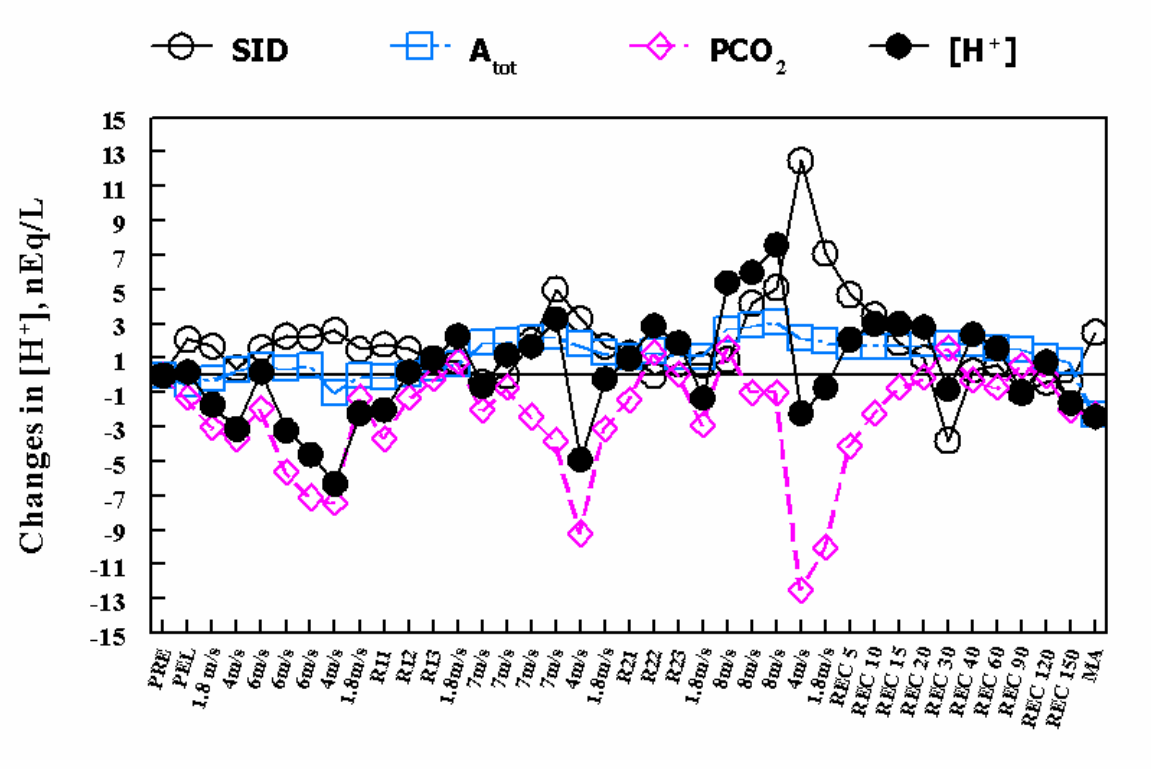

Figure 18. Partitioning of changes in plasma H^+ concentration from resting values (PRE), during the sub-maximal exercise test recovery (REC) into contributions from 3 independent variables, the strong ion difference (SID), total weak acids (A_{tot}), and P_{CO_2}.

Overall Discussion and Summary

The main objectives of these studies were to test the hypothesis that potassium-free electrolytes would lead to lower plasma [K^+] and consequently o fewer chances for the development of increased neuromuscular excitability.

The weakness of the studies lies in the fact that it was not possible to obtain some clinical manifestations of neuromuscular hyperexcitability during exercise. If electrocardiograms of exercining horses were done, differences could have been demonstrated between electrolyte treatments.

During the first experiment a salient finding was that plasma [K^+] increased during prolonged exercise at only 3.4 m/s over hilly terrain. New findings included a biphasic response of plasma [H^+], which decreased initially then increased during prolonged exercise and increased further during recovery. Also new is the finding that changes in plasma [H^+] and [K^+] in the final stage were moderated by supplementation with EM-K. Application of the Stewart comprehensive model revealed the major impacts of SID on plasma [H^+], and plasma [Na^+] on SID. Thus the lower plasma [K^+] and plasma [H^+] in the last stage may be attributable to the absence of K and to the higher amount of Na, respectively, in the EM-K formula.

During the second experiment the results confirmed that supplementation of potassium will affect plasma [K^+]. Further more, lower DCAB in the EM-K horses may have yielded higher calcium concentrations attenuating hypocalcemia during exercise in horses supplemented with experimental feeds. Also, a higher sodium amount in the electrolytes attenuated dehydration in EM-K supplied horses. Plasma [K^+] decreased during the race because exercise intensity was lower than during the first. During the race three eliminated horses had signs of

increased neuromuscular hyperexcitability (two of them were on EM+K) and they had higher plasma [K^+] and lower [Ca^{++}]. These factors might have contributed to the clinical signs of labile heart rate and arrhythmia. The plasma [K^+] level in eliminated horses would lead to the membrane potential being closer to the threshold but would not cause clinical signs by itself. However the combination with hypocalcaemia could lead to a lower firing threshold combined with a higher resting potential leading to the signs that were seen.

The third study once again confirms that supplementation of potassium affects plasma [K^+] and leads to E_K levels much closer to inducing neuromuscular hyperexcitability. Supplementation of potassium after exercise helped restore plasma levels and consequently body stores. This study also confirms that chloride supplementation also will affect plasma [Cl^-] and that it can be maintained despite sweat losses. During exercise increases in SID and decreases PCO_2 caused [H^+] to increase, but during the rest stops increases in PCO_2 helped to restore [H^+] to pre-race levels.

During the fourth study plasma [K^+] changed more in relation to exercise than supplementation. However at the fastest speed differences in plasma [K^+] were evident, and EM+K supplied horses were in the zone of possible hyperexcitability, whereas EM-K was just at the limit of the hyperexcitability zone. Also EM+K horses had a much greater chance to reach the firing threshold than EM-K horses. Potassium-rich electrolyte supplements induced lower plasma [Ca^{++}] compared to potassium-free ones. Possibly, DCAB differences due to differences in the electrolyte composition yielded the difference in plasma [Ca^{++}]. A new finding is that K supplementation caused higher plasma glucose and lactate during exercise and recovery. It may have caused a greater release of epinephrine and consequently led to higher glucose, shifting the horses to carbohydrate use during exercise. EM-K horses had lower TP, Hct, plasma [Na^+] and

osmolarity, meaning that they were less dehydrated, probably because their Na intake was higher. Plasma [H^+] changes were mainly determined by changes in PCO_2, like other studies at higher exercise intensities (Taylor et al, 1995). Changes in PCO_2 moderated changes in SID.

The studies confirmed that at exercise intensities of 4 m/s increases in plasma [K^+] will occur (Kronfeld, 2001). In all studies potassium supplementation affected plasma [K^+], however effects were less evident at higher speeds. The only clinical signs related to increased neuromuscular excitability were seen during the slowest study and a combination of higher plasma [K^+] and lower [Ca^{++}] were related to the clinical cases. Some interesting findings were that at higher exercise intensities potassium supplementation increased plasma glucose and lactate. Higher lactate and potassium are associated with fatigue (Mainwood and Renaud, 1985; Nielsen et al, 2001) therefore supplementation of potassium at sub-maximal speeds is contraindicated.

Implications

Overall these studies have confirmed that plasma [K^+] increases proportional to exercise intensities and shown that potassium supplementation will affect plasma [K^+] values. Also supplementation of potassium during recovery is essential to restore plasma levels and body stores. Supplementation of potassium may affect plasma [Ca^{++}] via a higher DCAB. Higher sodium supplementation also helps to maintain hydration during exercise.

Future Studies

Dietary cation anion balance can affect plasma [Ca^{++}] and hypocalcemia combined with hyperkalemia can lead to clinical signs associated with increased neuromuscular

hyperexcitability. No study has really focused on observing different DCAB diets and endurance exercise. The studies showed effects of different DCAB in cattle on periparturient hypocalcemia (Goff and Horst, 1997), and of horses exercising at high intensities for a short duration of time (Cooper et al, 1998, Baker et al, 1993). Experiments focused in answering if hypocalcemia can be attenuated during endurance exercise through lower DCAB diets could help prevent clinical cases.

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Vita

Tanja Maria Hess, daughter of Karin and Hans Hess, was born on July 30, 1967 in Rio de Janeiro, RJ, Brazil. She attended the Swiss-Brazilian school until 7th grade and then transferred to the Escola Esperimental Corcovado and graduated in 1986. After that she immediately was admitted at the Federal Fluminense University to pursue her veterinary degree. She graduated as a veterinarian in August of 1990. She worked as a resident veterinarian for two years on a farm and then worked as a private practitioner in equine clinics and reproduction, and also trained endurance horses. In 1995 she started her Masters degree at the Federal Rural University of Rio de Janeiro, where she graduated in 1997. Her Masters thesis was titled: Clinical parameters in horses competing in endurance races. In 1997 she won the Brazilian 100-mile Endurance Championship. She worked as a Clinical Instructor and head veterinarian at the Large Animal Hospital of the Federal Rural University of Rio de Janeiro from 1998 to the end of 2000. She was awarded with a Brazilian Scholarship (CNPq) to pursue her doctorate in Equine Nutrition and Exercise Physiology at Virginia Tech. She has been doing her studies since January of 2001.