



Review Article

Current Knowledge of Pathologic Mechanisms and Derived Practical Applications to Prevent Metabolic Disturbances and Exhaustion in the Endurance Horse



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ABSTRACT

Between 30% and 50% of the horses that start international endurance events, over distances of 100–160 km, are eliminated at the vet gates, although elimination rates vary in the different geographical areas and race categories. Elimination rates appear to have increased over recent years, which is a source of concern for the sport's ethics and image. Main reasons for elimination are lameness and metabolic disturbances, associated with dehydration and electrolyte disturbances, and with substrate depletion in active muscle fibers. Moreover, there are severe consequences of these metabolic derangements, including heat stroke, rhabdomyolysis, colic, kidney and liver insufficiency, laminitis, and disseminated intravascular coagulation. The prevention starts with the selection of a fit, healthy horse, free of subclinical diseases. A proper training is one of the best and more secure ways to reduce the risk of these metabolic diseases. Considerations regarding the transport to the place of the event and acclimatization to the new environmental conditions (particularly if weather is hot and humid) should be taken into account. During competition, the control of fluid and electrolyte losses to avoid dehydration and heat accumulation, as well as the control of the substrate utilization to reduce muscle fibers depletion, are of pivotal importance. The management of race intensity is essential, and this can be done by obtaining the lactate aerobic threshold (lactate concentration of 2 mmol/L). Other strategies include ride management, according to the terrain and weather conditions, rider education to detect early signs of critical fatigue, and veterinary examinations.

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1. Introduction

All muscular work if sustained long enough eventually might result in fatigue. In exercises of short duration, recuperation from fatigue is quite rapid, and therefore, it

does not progress to exhaustion. On the contrary, in exercises of long duration, such as endurance rides, the fatigue is not rapidly overcome and it might result in severe physiological changes that may be even life threatening. For that reason, the present paper will be focused in the metabolic diseases of endurance horses, although the same diseases can also appear in 3-day event horses. In addition, horses that experience exhaustion might present serious complications after that characterized by multiorgan dysfunction with renal and hepatic failure, laminitis, colic,

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myonecrosis, pulmonary edema, and disseminated intravascular coagulation [1,2]. Nevertheless, it is important to remark that any horse, that is, exercised more intensely that its fitness level permits, would develop metabolic diseases.

Metabolic diseases are, after lameness, the second reason of elimination of endurance horses in competition. The first epidemiologic study regarding rate of elimination from competition was performed by Burger and Dollinger [3] using the database from the Endurance and Long Distance Ride Conference. These authors showed that of all eliminated horses, 62.7% were eliminated by lameness, 24% for metabolic reasons, 0.3% for other reasons, and in 13% of the eliminations, the reason was not recorded. Some studies performed in the last years from data obtained from the FEI (Fédération Equestre Internationale) in European and Arabian countries reported that approximately 30% of horses are eliminated from lameness and 8%–10% for metabolic reasons [4–6]. More recently, it has been stated that nearly 50% of the horses participating in endurance events are eliminated at veterinary examinations (vet gates) [7]. Unfortunately, elimination rates appear to have increased over recent years, which are a source of concern for the sport's ethics and image [8].

Horses are eliminated by metabolic reasons if the metabolic status is compromised, based on clinical examination, heart rate (HR), cardiac recovery index, color, and moisture of mucous membranes, capillary refill time, presence and intensity of intestinal sounds, and presence of other clinical manifestations of neuromuscular and acid–base disorders [9–12]. These clinical signs are secondary to dehydration, electrolyte and acid–base disturbances, heat accumulation, and substrate depletion [13]. These pathologic mechanisms can be avoided following some strategies before, during, and after competition.

2. Strategies Before the Endurance Competition

2.1. Selection of the Proper Horse

Prevention of metabolic diseases begins with the selection of the horse. Some breeds and some horses are less prepared for endurance work, especially at intense levels of competition or they simply are poor endurance athletes. Breeds particularly successful in endurance exercises are Arabian and cross-Arabian, due to their higher aerobic capacity and better running economy [14–16]. In addition, Arabian and crossbred horses of medium size have more appropriate ratio body size/body surface area for heat dissipation [17].

Other factors to consider are coat color and density of the hair coat. Coat color will affect the quantity of solar heat absorbed, and a long hair coat will limit evaporative heat loss, predisposing these horses to heat stress [18]. Therefore, long hair coat should be clipped to facilitate cutaneous heat loss [19].

2.2. Health Status

Having a healthy and fit horse is one of the best ways of reducing risk of metabolic diseases in competition. Horses

with subclinical anemia, respiratory, cardiovascular diseases, or lameness have increased risk for elimination for metabolic diseases during competition [1,20]. Anemia has been associated with decreased supply of oxygen to the contracting muscles, promoting muscle fiber injuries and rhabdomyolysis [20]. Ventilatory alterations might result in hypoxemia and low alveolar tension oxygen, and therefore, horses with respiratory diseases would have a more anaerobic response to exercise, determining a reduction in performance and earlier onset of fatigue. The integrity of the respiratory system has a great impact on the performance of fast horses (thoroughbreds and standardbreds), but this factor has not been evaluated in endurance horses. However, the report of Fraipont et al [21] showed that endurance horses suffering from respiratory disorders had lower performance parameters in response to a treadmill standardized exercise test. The effects of cardiac abnormalities (including heart murmurs and arrhythmias) on performance have been scarcely evaluated in endurance horses. Previous researchers have demonstrated that Thoroughbred horses with low performance have increased risk of premature ventricular and supraventricular depolarizations [22]. It is supposed that heart arrhythmias would reduce ventricular filling volume, and therefore, cardiac output would be reduced, affecting blood supply to the active tissues during exercise. Fraipont et al [21] did not find significant correlations between valvular regurgitation (both prevalence and degree of regurgitation) and performance during a treadmill standardized exercise test in endurance horses. Moreover, significant differences in valvular regurgitation were not found when comparing groups of horses with different performance. Heart arrhythmias, on the other hand, are common in endurance horses eliminated for metabolic reasons, in association with electrolyte imbalances [23], but also in successful endurance horses, particularly during the recuperation period [24].

As indicated before, lameness is the most common cause of elimination from competition in endurance horses, and it has been proposed that mild or subclinical lameness might contribute to the onset of exhaustion [13,25]. An undetected lameness might cause a horse to move differently, leading to the excessive use of some normally used neuromuscular groups or to the use of different muscle groups than are used normally. These variations in muscle use may result in focal or more diffuse exertional rhabdomyolysis, pain, increased HR, and reduced HR recovery time. In addition, lameness results in increased circulating endogenous catecholamine and cortisol concentrations [26]. Enhanced circulating concentrations of stress hormones might lead to peripheral vasoconstriction and subsequently, to poor blood flow to tissues. This results in an exacerbation of the laminitic effects that often appear in exhaustion and dehydration with hypovolemia [13,25].

2.3. Training and Learning

Any horse, even if it is well suited for endurance exercise, is placed at greater risk of metabolic diseases and exhaustion if ridden competitively when unfit [13,25]. Therefore, arriving at an endurance event with a well-trained horse is the best way to prevent exhaustion. In

order to prescribe a proper training program, it is of pivotal importance to subject the horse to exercise tests, where functionality indices, such as HR_{LA2} , HR at 2 mmol/L of lactate, are calculated. The knowledge of the individual values of HR_{LA2} is also essential for management of exercise intensity during racing [16]. During an exercise with intensity lower than HR_{LA2} , metabolism is mainly dependent on fats, with the consequent sparing effect on glycogen and with a lower production of lactate and metabolic heat. All of these facts interact resulting in a delay of fatigue [16].

The effects of training on the reduction of risk of developing metabolic diseases in endurance horses in competition are described later in this paper. Furthermore, the horses should learn to drink water and eat at every opportunity during training and competition.

2.4. Transport to the Competition

During transport to the competition, a horse can experience a substantial body weight loss (approximately 3 kg/hr of transport) and dehydration, even when access to water and food is maintained [27]. If the transport period was long, the animal would require sufficient time to recover such hydration and electrolyte balances are normal before starting competition [27].

2.5. Acclimatization to Environmental Conditions

It is reasonable to assume that horses that live and train in climate conditions are at greater risk of exhaustion if they compete in new challenged environmental conditions, including high temperature, high humidity, and altitude [25]. In an experimental study, unacclimatized horses tolerate cool and dry and hot and dry conditions but have reduced performance in hot and humid conditions [28]. As environmental temperature increases, the thermal gradient between the skin and the environment is reduced, and sensible heat losses (convective and radiative heat transfer) impaired. Further, when ambient temperature exceeds skin temperature, the gradient for heat transfer is reversed and the horse gains heat from environment.

When humidity rises, the gradient between skin and ambient dew point is reduced and evaporative heat loss is impaired [29,30]. Therefore, under conditions of high ambient temperature and relative humidity, the rate of thermolysis may be inadequate to prevent progressive rise in body temperature, and the horse is in increased risk of thermal stress and hyperthermia [30].

Human studies have demonstrated that the process of heat acclimatization begins within a few (3–5) days of regular exposure to and exercise in the heat, with most adaptations completed within a period of 14–15 days [31–33], and the same appear to happen in the horse [34]. Careful planning is required for horses transport from a temperate weather to a hot humid climate to compete. Horses should have an excellent fitness level before traveling. It has been demonstrated that training in a cool or temperate environment improves physiological responses when athletes are exercised in hot conditions [35]. Fat supplementation has been advocated as a means for reducing thermal stress in horses transported to places with high temperature. A high fat diet (8%–10% on a total diet basis) might reduce the heat, which is greater when the horse consumes carbohydrates [36].

A summary of these strategies are presented in Table 1.

3. Strategies During and After the Endurance Competition

3.1. Ride Management

It has been suggested that weather and terrain can promote exhaustion and alters the interpretation of metabolic and lameness examinations [13]. In human marathon runners, it has been demonstrated that any decrease or increase from the optimal temperature range will result in running speed decrease and reduction of performance [37]. Langlois and Robert [38] reported that more horses needed treatment for metabolic problems at temperatures higher than 22°C than at cooler temperatures. The managers and the veterinarians who hold endurance rides are responsible for judgments and

Table 1

Strategies to follow prior to the competition in order to diminish the risk of exhaustion.

Strategy	Practical Recommendations
Select the best horse for endurance	
Breed	Mainly Arabian and cross-Arabian
Coat	Clip hair coat when is long
Health status	Diagnose and treat (sub)clinical diseases Free of muscle and skeletal diseases
Management of conditions prior to the competition	
Transport	Frequent stops. Provide food and water Avoid stress
Acclimatization to the new environmental conditions	Move to the place of the competition several days before Train properly to tolerate high temperature/humidity Fat supplementation reduces thermal stress
Training	Best way to reduce risk of exhaustion Control training establishing objectives and programming according to the results of periodical exercise tests
Learning	Train to drink water everywhere and any kind of water Train to take electrolytes (paste, oral powder in water or food) Train to eat at each opportunity

modifications of ride conditions. A sum of environmental temperature (°C) and humidity (%) higher than 110 is an indication of inappropriate conditions for competition, exposing the horse to risk of exhaustion and the ride should be curtailed.

3.2. Rider Education

Rider awareness is critical for preventing exhaustion. If the rider is able to interpret the signs of exhaustion early, the horse might have stopped early and the subsequent intensive treatment if underwent might have been altogether unrequired.

HR monitoring during competition is an objective way of assessing metabolic and cardiovascular response to exercise. With the HR, the rider would be able to control the exercise intensity in the different terrains, sparing energy, and maximizing oxygen supply and use. In addition, it could permit an early diagnosis of metabolic alterations or lameness [39].

3.3. Veterinary Examinations

A proper veterinary examination is essential to detect early fatigue and prevent horses to continue exercise at the same speed that eventually leads to exhaustion [13,20]. In fact, Barnes et al [40] demonstrated that veterinarians are able to detect compromised horses using clinical examinations. Consequently, horses must be carefully monitored during the vet gates, where it is decided whether they are fit to continue [10–12]. Precompetition examination is also important to detect horses with infectious diseases, lameness, or dehydration.

3.4. Controlling Fluid and Electrolytes Losses

Sustained prolonged exercise results in generation of considerable intramuscular heat because only about 20%–25% of the energy produced is converted to useful mechanical energy in the form of muscle movement. Core body temperature could increase 0.25°C per minute of exercise if the heat is not dissipated [1]. The heat is shifted from the muscle and the core toward the skin via peripheral vasodilatation and increased skin temperature. The skin is then cooled by convection, radiation (to a limited extent), and especially by evaporation, which is the most efficient means of heat dissipation, about 65% of the total heat load. In addition, sweating could be the only means of thermolysis in a hot environment. The amount of sweat produced by the horse depends on its size, fitness level, exercise intensity, and environmental conditions [17,41]. A sweat rate up to 10–15 L/hr has been described when activity levels are maintained at a high rate (more than 15 km/hr). Thus, during an endurance competition, horses may lose 4%–7% of their body weight [17].

In the first 2 hours of exercise, fluid loss from the extracellular (intravascular) compartment is replaced by the intracellular fluid volume. After this period, there is a decrease in both intracellular and intravascular compartments [1,42]. The hypovolemia reduces perfusion in skeletal muscles and in other vital organs, such as kidneys and

liver. Inadequate tissue perfusion leads to inefficient oxygen and substrate transport, and hampers thermoregulation. Therefore, both frequently applications of cooling methods and maintenance of a proper hydration status are essential to avoid heat stroke [1].

Cooling methods are imperative and should be applied whatever possible. The horses should be kept in the shade during the stop periods to reduce solar heating, particularly if they have dark coat color. The use of fans would improve convective cooling. Devices that release flow of air containing chilled water droplets can reduce ambient temperature [18]. Copious and frequent application of cold water is needed in order to reduce the rate at which heat is stored. The water can be applied with buckets, sponges, or sprays. Application of cold water (with temperatures of 6°C–15°C) appears to be safe when climatic conditions are very hot [43]. Traditionally, application of cold water was not recommended in horses. In human beings, Khogali [44] expressed his concern about cold water inducing dermal vasoconstriction, resulting in a slower rate of cooling. Marlin et al [45], in horses, demonstrated that application of cold water (6°C) did not cause vasoconstriction of blood vessels in the skin, at least during the first phases of cooling. However, skin vasoconstriction was observed later, although it did eliminate the benefits of conductive cooling by further applications of cold water. Water should be scraped from body surface to remove hot water before next application. Water should be applied in the parts of the body surface with higher rate of sweat glands and with superficial large vessels. These vessels become dilated with exercise, extending the area exposed to the environment to facilitate heat elimination [18]. If possible, walking the horse between periods of cooling would probably serve to maintain a higher cardiac output and increased blood flow to areas of effective heat exchange, enhancing cooling [29].

Dehydration has a high cost for the endurance horse. During exercise, large volumes of blood are required by the muscles for oxygen and substrate supply and metabolic waste removal [46]. Hyperthermia increases cardiac output due to increased HR for extra peripheral blood flow for heat dissipation. When hypovolemia appears, there is a competition for blood flow, and thermolysis is impaired by shift of blood to other organs [46,47]. Further, hypovolemia promotes a decrease in blood flow to the splanchnic area, inducing hypoperistalsis—paralytic ileus and clinical signs of colic. With continued dehydration, perfusion of vital organs is reduced, with increased blood viscosity, liver, and kidney hypoperfusion and less blood supply to muscle with rhabdomyolysis and exhaustion [13,46]. Monitoring of rectal temperature is critical to early detection of heat exhaustion and other heat illnesses. Immediately after exercise, an increase in body surface temperature is found, presumably resulting from a greater proportion of cardiac output being directed toward the skin once the muscle demand for blood flow decreased, as demonstrated with thermography [45]. A rectal temperature that exceeds 42°C indicated the need for immediate and vigorous cooling. Persistence of tachypnea after the horse is placed in a cooler environment or is vigorously cooled might suggest that hyperthermia persists, and therefore, the horse is at risk of heat stroke [1,45,46].

Sweating-induced dehydration is always accompanied by electrolyte loss [10–12,48–50]. Equine sweat is isotonic to slightly hypertonic in relation to plasma and contains high concentrations of sodium, chloride, potassium, and some calcium and magnesium [50]. Sweat sodium concentrations are approximately 150 mmol/L, which are similar to those found in plasma. Absolute loss of sodium in sweat may represent a 8.4% of the total plasma sodium content [1]. Plasma sodium concentration in endurance horses in competition can remain within the reference values because both water and sodium are lost in the same proportion [10–13,51,52]. Sodium and fluids loss with sweating may result in hypotonic dehydration, leading to a lack of thirst sensation. Increased serum concentrations are found in those horses that are not allowed or refuse to drink [10–13,53]. Depletion of sodium together with dehydration and hypovolemia has been associated with increased blood viscosity, inadequate tissue perfusion, and inefficient oxygen and substrate supply to active [54,55]. Absolute sodium loss leads to tachycardia, hypotension, neurologic signs, muscle spasms, and fatigue [56].

Because sweat chloride concentrations are twice those of plasma, prolonged sweating leads to a significant reduction in plasma chloride concentrations [9–12]. Hypochloremia is associated with reabsorption of bicarbonate in the kidney in order to maintain a normal anion gap [1]. Consequently, hypochloremic metabolic alkalosis with paradoxical aciduria appears in endurance horses [57]. In addition, a reduction in plasma sodium concentration stimulates the release of aldosterone, with increased absorption of sodium in kidneys and gastrointestinal tract, at the expense of potassium and hydrogen ions, contributing to the alkalosis [10–13,58]. Furthermore, increased alveolar ventilation for heat dissipation results in respiratory alkalosis [1].

Sweat potassium concentration is between 8 and 20 times greater than plasma potassium concentration, and therefore, significant potassium loss occurs during endurance exercise [1,9–13,48,59,60]. During the first phases of exercise, however, there is not a decrease of plasma potassium because potassium moves from muscle fibers to plasma [59]. Plasma potassium concentrations also decrease because of metabolic alkalosis, which drives potassium into the cells and a physiologic reentry into the intracellular compartment on cessation of exercise [9,61]. In addition, as presented before, increased aldosterone concentrations promote hypokalemia [49,58]. These changes, although happen in most of endurance horses in long rides, are more pronounced in those that develop exhaustion and metabolic problems [10–13]. Clinical consequences of hypokalemia are commonly found in exhausted horses and consist in muscle flaccid paralysis, hypoperistalsis, hyperirritability of long nerves (synchronous diaphragmatic flutter), and rhabdomyolysis because of cellular irritability and vasoconstriction [57]. Low intracellular potassium concentration might lead to a low nerve threshold and increased excitability and heart arrhythmias [57].

Calcium losses in sweat and intracellular (muscle cells) calcium shifts might lead to total hypocalcemia [62]. In addition, metabolic alkalosis might decrease plasma

ionized calcium concentrations due to increased protein binding. Hypocalcemia affects nervous sodium channels, resulting in neuro-irritability, involuntary muscle contractions, and synchronous diaphragmatic flutter [1,52,57]. Sweat magnesium concentration is greater than those found in plasma, and therefore, increased sweat production has been associated with hypomagnesemia. Hypomagnesemia increases the release of acetylcholine at the neuromuscular junctions, promoting muscle spasms, and tetanics [63].

As presented before, the prevention of electrolyte and water losses is essential to reduce the risk of exhaustion, and different strategies before, during, and after exercise have been assessed. In human athletes, hyperhydration prior to prolonged submaximal exercise has resulted in better cardiovascular functionality and heat dissipation than exercise performed in an euhydrated state [64], although recently, some studies have failed to demonstrate the same results [65,66]. Similarly, the trials carried out in horses have resulted in equivocal results. Geor and McCutcheon [67] observed that hyperhydration before exercise maintained cardiovascular function and sweating rate and decreased heat storage. By contrast, Sosa León et al [68] found that hyperhydration before exercise helped horses to maintain plasma volume, but cardiovascular function and thermoregulation were not improved, and in addition, fluid administration resulted in arterial hypoxemia. Although the benefits of fluid loading prior to prolonged exercise have not been scientifically established in horses, it could be more critical in the heat when heat dissipation is most dependent on sweat evaporation [67].

Administration of solutions with glycerol is another strategy followed to induce hyperhydration before exercise in human beings. Glycerol administration results in fluid retention because of delayed urinary excretion of excess of fluid [69]. Glycerol administration in horses was not more effective than electrolytes alone in maintaining euhydration during endurance exercise [70]. Administration of a salt supplement together with free access to water is a good strategy before exercise. Electrolyte administration leads to increased water uptake before exercise in humans [71]. The retention of water and electrolytes in the gastrointestinal tract peaks at 4–6 hours after feeding, and therefore, this is the period before exercise for electrolyte supplementation. It is very important to ensure the horses drink water because if electrolytes are supplied without water, osmolality in the gastrointestinal tract may increase sequestration of fluid from the blood. Moreover, an overdose of electrolytes should be avoided. Increased plasma sodium concentrations would promote an increase in blood pressure and, as a result, an increased release of natriuretic peptide leading to excessive excretion of sodium in the urine [72].

Allowing horses to drink salt water (if adapted) during and immediately after exercise appears to be the best way to promote rehydration [73]. This practice enhances total fluid intake during the early phase of recovery and attenuates the magnitude of weight loss [73]. If horses are not used to drink salt water, other options are mixing electrolytes in grain or pelleted feeds or by direct oral administration. Several factors should be considered with oral

electrolyte administrations during competition. The composition, temperature, and tonicity of the solution should be taken into account. In human athletes, fluid absorption is optimal if the oral electrolyte solution is hypotonic, contains glucose, and is below room temperature [74]. Addition of glucose to these solutions appears to increase electrolyte absorption because sodium absorption in the small intestine is coupled with glucose absorption. However, Sosa León et al [75] found that temperature of the solution and the addition of glucose to the electrolyte mix did not affect oral absorption in horses. Other authors, however, recommend using fructose instead of glucose in the electrolyte solutions. Ingestion of fructose produces a smaller increase in blood insulin compared with glucose. Increased blood insulin concentrations may adversely inhibit lipolysis. However, in horses, it has been demonstrated that the insulin response to exercise did not differ between both carbohydrates [76].

Tonicity of the solution did affect gastrointestinal absorption. Hyyppä et al [77] demonstrated that administration of carbohydrate-electrolyte solutions after exercise resulted in a greater plasma volume expansion compared to the same volume of water. Electrolyte administration

directly in the mouth, although is a common practice, has been associated with increased risk of nonglandular ulcers in endurance horses [78] because of hypertonicity.

3.5. Controlling Substrate Depletion

Carbohydrates and fat can be used simultaneously during an endurance activity, but the ratio of utilization depends on the intensity and duration of exercise and on the fitness and training levels of the horse [79]. Horses normally store high concentrations of glycogen in muscle, but these concentrations can be reduced by more than 50% leading to muscle fatigue [46,80,81]. Low muscle glycogen concentration before exercise has been associated with decreased performance, whereas high muscle glycogen content enhances endurance performance [82]. Therefore, feeding strategies before, during, and after exercise that increase or spare muscle glycogen content are important in endurance competitions.

In humans, the term “glycogen loading” refers to maximization of muscle glycogen stores prior to a prolonged competition in which performance might be limited by depletion of muscle glycogen [83]. Studies in horses have

Table 2

Strategies to follow during and after the competition in order to diminish the risk of exhaustion (HR_{LA2} and HR_{LA4}, heart rates at blood lactate concentrations of 2 and 4 mmol/L; HR, heart rate).

Strategy	Practical Recommendations
Ride management	
Cold weather and/or rain	Blanketing during rest periods
High temperature and/or humidity	Change ride conditions if required (duration, velocity control)
Always in the competition	Calculation of individual exercise intensity to keep in competition HR _{LA2} and HR _{LA4} obtained from standardized exercise tests
Rider education	
HR monitoring	Teach the rider to interpret HR changes with exercise Be aware of changes in the attitude of the horse
Veterinary examinations	
Managing temperature	
Cooling methods	Keep the horse in the shade during rest periods Frequent water applications Water scraped from body surface and application of water again Application of water in body parts with high sweat glands content and with big blood vessels Fans (better with chilled water droplets) Walk the horse to maintain blood flow for heat and waste exchanges
Managing fluid and electrolyte losses	Euhydration before exercise Hyperhydration does not work Provide water and electrolytes starting 4–6 hr before racing Avoid overdose of electrolyte Drink salt water during and after exercise (if adapted). Otherwise oral electrolyte powder or pastes. Always with water Better solutions with glucose
Managing substrate repletion	Fat administration during training Carbohydrate-based diet at least 3hr before competition Small amount of grains or pelleted foods during rest periods Hay, grass, or forage of high quality
Proper training	
Improved cardiovascular stability and functionality	Plasma volume expansion Improved heat dissipation Reduced HR in response to exercise and quick recuperation
Improved muscle functionality	Better muscle aerobic capacity Predominant use of fat as energy source Sparing effect of glycogen Increased buffering capacity-better lactate tolerance
Improved thermoregulatory functionality	Lower sweating threshold Decreased water and electrolyte losses Decreased heat production and store

Abbreviation: HR, heart rate.

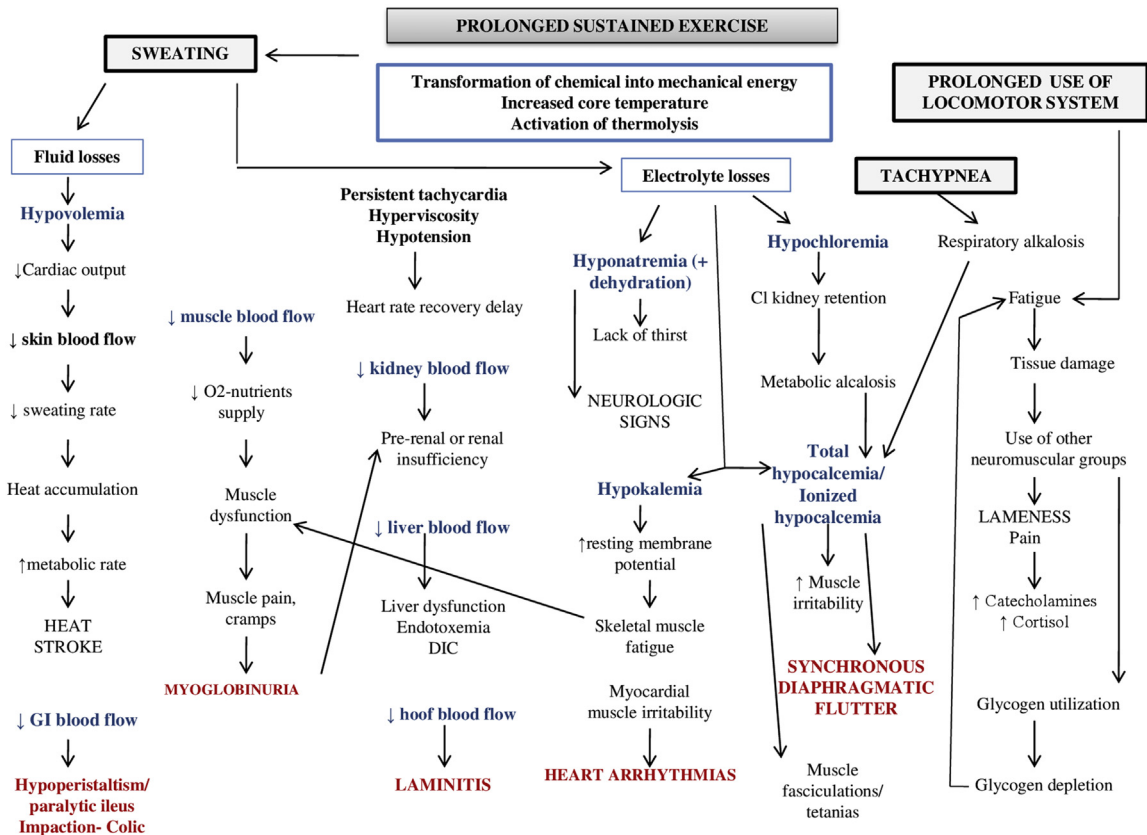


Fig. 1. Complex interrelationships between physiological, pathologic events, and clinical consequences that might eventually happen in an exhausted endurance horse.

shown that only a modest (10%) increase in muscle glycogen content can be achieved through dietary manipulation [79]. Probably, this is due to the high intake in hydrolyzable carbohydrate needed to replenish glycogen muscle, which is not safe for horses because the high risk of gastrointestinal dysfunction and laminitis.

Fat is the greatest energy store for the endurance horse. Muscle fibers can use both triglycerides stored inside of these fibers and free fatty acids from the blood [46,84]. Once the glycogen is depleted in some muscle fibers, exercise can only be maintained at the rate at which free fatty acid are supplied. However, oxidation of fats is limited in absence of carbohydrates [84,85]. Adding fat to the diet of endurance horses during training decreases the amount of grain necessary to meet energy requirements, and in addition, it reduces the risk of gastrointestinal disturbances and laminitis. Fat administration also provides more energy for aerobic metabolism, has a sparing effect on muscle and liver storage of glycogen, delays the onset of fatigue associated with glycogen depletion, decreases lactate concentrations during exercise, and reduces heat production [36,84].

The horse should eat a carbohydrate-based diet (grain or concentrates) at least 3 hours before the event [84]. Carbohydrate ingestion within 3 hours before to competition may be detrimental to performance because suppression of lipid oxidation and accelerated rate of carbohydrate

oxidation. These facts could lead to premature muscle glycogen depletion [86]. It is of the great importance to give the horse as much opportunities as possible to eat on the competition and during the resting periods, in order to assure a replacement of the energy consumed [84]. Small amounts of grain or pelleted feeds can be supplied to replenish energy stores in the muscle fibers. In addition, grass, hay, or forage of excellent quality are essential. The amount of dietary fiber appears to influence the size of the large intestinal fluid in the horse, which acts as a reservoir of water and electrolytes during exercise [84]. Excessive calcium feeding (such as alfalfa forage and other types of feeds with high content in calcium) is generally not recommended during training, even though the effect of performance has not been evaluated in endurance horses. It is thought that excessive calcium in the diet may impair the normal response of the parathyroid gland to hypocalcemia, predisposing to the horse to exhibit clinical signs of calcium deficit. However, it would be of interest to provide this type of feed to the horse the day before and during the competition [57].

3.6. Beneficial Effects of Training

As indicated before, the best way to reduce the risk of exhaustion and other metabolic diseases is to compete with a fit and well-conditioned horse. Training results in benefits

in respiratory, cardiovascular, muscle and skeletal systems, together with changes in heat tolerance and sweat rate. Improvement of cardiovascular function is a direct consequence of training. There is an increase in plasma volume, stroke volume, and cardiac output, contributing to increased cardiac stability [87]. Expansion of plasma volume in the trained horse improves also heat dissipation by reducing the extent to which cutaneous blood flow is compromised. Increased resting plasma volume reduces the reduction of cardiac output during exercise in response to sweat fluid losses. Furthermore, increased stroke volume after training is associated with a reduced HR in order to get the same cardiac output [87].

Muscle fibers undergo intense changes with training in order to increase the ability to transform chemical energy into mechanical energy of muscular movement. The most evident muscle adaptations to endurance exercise are those: increased capillarization to facilitate transport of substrates and waste products and gas exchange, greater storage of fats and glucose; increased muscle enzyme concentrations associated with oxidation of fats and glycogen sparing; increased mitochondrial density in the exercising muscles with increased oxidative capacity; and increased production of cellular proteins to augment the buffering capacity of the muscle cells [88–90]. These changes happen together with an increase in the number of oxidative type IIA fibers.

Training also improves the horse's ability to dissipate heat by evaporation of sweat and respiratory tract [18]. At a given exercise intensity, trained athletes have a higher metabolic rate and, hence, a greater heat production. However, the trained horse is able to maintain a similar core temperature compared to untrained subjects. Training results in an increased sweating sensitivity in horses [91]. Despite these higher sweating rates in trained horses, decreases in sweating rate during recovery result in an overall reduction in sweat fluid losses. This improvement in sweating economy after training may help to minimize demands placed on the circulatory system by reducing the extent of dehydration associated with sweating [91].

These strategies are summarized in Table 2. The complicated network of physiological and pathologic events that might eventually lead to exhaustion in an endurance horse during a competition is presented in Fig. 1.

4. Conclusions

Metabolic diseases (associated to dehydration, electrolyte disturbances, heat accumulation, and substrate depletion) and their clinical consequences are after lameness, an important reason of elimination of endurance horses in competition. This type of alterations, however, is of maximum concern for horse's welfare. Our increased knowledge regarding pathophysiology of the metabolic abnormalities experienced by these horses, together with a better understanding of physiological adaptations to prolonged exercise and training would make us able to develop preventive strategies, in order to be applied before, during, and after competition.

References

- [1] Flaminio MJ, Rush BR. Fluid and electrolyte balance in endurance horses. *Vet Clin North Am Equine Pract* 1998;14:147–58.
- [2] Silverman SC, Birks EK. Evaluation of the i-STAT hand-held chemical analyser during treadmill and endurance exercise. *Equine Vet J* 2002;34:551–4.
- [3] Burger D, Dollinger S. Raisons d'élimination, état de santé et carrière sportive des chevaux dans les raids d'endurance en Europe et dans les pays arabes: approach statistique. *Pract Vet Equine* 1998;30:19–25.
- [4] Nagy A, Murray JK, Dyson S. Elimination from elite endurance rides in nine countries: a preliminary study. *Equine Vet J* 2010;38:637–43.
- [5] Nagy A, Murray JK, Dyson SJ. Descriptive epidemiology and risk factors for elimination from Fédération Equestre Internationale endurance rides due to lameness and metabolic reasons (2008–2011). *Equine Vet J* 2014;46:38–44.
- [6] Nagy A, Murray JK, Dyson SJ. Horse-, rider-, venue- and environmental-related risk factors for elimination from Fédération Equestre Internationale endurance rides due to lameness and metabolic reasons. *Equine Vet J* 2014;46:294–9.
- [7] Younes M, Robert C, Cottin F, Barrey F. Speed and cardiac recovery variables predict probability of elimination in equine endurance events. *PLoS One* 2015;10:e013701.
- [8] Marlin DJ, McEwen J, Sluyter F. Completion and treatment rates in modern endurance racing. In: Proceedings of the 4th International Conference of the International Society for Equitation Science; 2008. Dublin, Ireland.
- [9] Castejón F, Trigo P, Muñoz A, Riber C. Uric acid responses to endurance racing and relationships with performance, plasma biochemistry and metabolic alterations. *Equine Vet J Suppl* 2006;36:70–3.
- [10] Muñoz A, Riber C, Trigo P, Castejón F. Muscle damage, hydration, electrolyte balance and vasopressin concentrations in successful and exhausted endurance horses. *Pol J Vet Sci* 2010;13:373–9.
- [11] Muñoz A, Riber C, Trigo P, Castejón-Riber C, Castejón FM. Dehydration, electrolyte imbalances and renin-angiotensin-aldosterone-vasopressin axis in successful and unsuccessful endurance horses. *Equine Vet J* 2010;38:83–90.
- [12] Muñoz A, Castejón FM, Trigo P, Roldán J, Rubio MD, Gómez-Díez M, Castejón-Riber C, Riber C. Veterinary clinical examination in endurance horses in competition: correlation with laboratory data. In XIX Meeting of the Società Italiana de Veterinari per Equini. February 1st–3rd 2013, Arezzo, Italy.
- [13] Foreman JH. The exhausted horse syndrome. *Vet Clin North Am Equine Pract* 1998;14:205–20.
- [14] Prince A, Geor R, Harris P, Hoekstra K, Gardner S, Hudson C, Pagan J. Comparison of the metabolic responses of trained Arabians and Thoroughbreds during high- and low-intensity exercise. *Equine Vet J* 2002;34:95–9.
- [15] Cottin F, Meayer N, Goachet AG, Jullian V, Slawinski J, Billat V, et al. Oxygen consumption and gait variables of Arabian endurance horses measured during a field exercise test. *Equine Vet J* 2010;38:1–5.
- [16] Castejón-Riber C. Field and treadmill exercise tests in the endurance horse: methodology, measurements and interpretation. PhD Thesis. Spain: University of Córdoba; 2014.
- [17] Hodgson DR, McCutcheon LJ, Byrd SK, Brown WS, Bayly MW, Brengelmann GL, et al. Dissipation of metabolic heat in the horse during exercise. *J Appl Physiol* (1985) 1993;74:1161–70.
- [18] McCutcheon LJ, Geor RJ. Thermoregulation and exercise-associated heat illnesses. In: Hinchcliff KW, Kaneps AJ, Geor RJ, editors. *Equine sports medicine and surgery*. Philadelphia: Elsevier Ltd; 2004. p. 919–36.
- [19] Morgan K, Funkquist P, Nyman G. The effect of coat clipping on thermoregulation during intense exercise in trotters. *Equine Vet J* 2002;34:564–7.
- [20] Muñoz A, Rodríguez RGM, Riber C, Trigo P, Gómez-Díez M, Castejón F. Subclinical Theileria equi infection and rhabdomyolysis in three endurance horses. *Pak Vet J* 2013;33:256–8.
- [21] Fraipont A, Van Erck E, Ramery E, Richard E, Denoix J-M, Lekeux P, et al. Subclinical disease underlying poor performance in endurance horses: diagnostic methods and predictive tests. *Vet Rec* 2011;169:154.
- [22] José-Cunilleras E, Young LE, Newton JR, Marlin DJ. Cardiac arrhythmias during and after treadmill exercise in poorly performing thoroughbred racehorses. *Equine Vet J* 2006;36:163–70.

- [23] Leroux AJ, Schott 2nd HC, Hines MT. Ventricular tachycardia associated with exhaustive exercise in a horse. *J Am Vet Med Assoc* 1995;207:335–7.
- [24] Flethøj M, Kanters JK, Haugaard MM, Pedersen PJ, Carstensen H, Balling JD, et al. Changes in heart rate, arrhythmia frequency, and cardiac biomarker values in horses during recovery after a long-distance endurance ride. *J Am Vet Med Assoc* 2016;248:1034–42.
- [25] Nagy A, Dyson SJ, Murray JK. A veterinary review of endurance riding as an international competitive sport. *Vet J* 2012;194:288–93.
- [26] Rietmann TR, Stauffacher M, Bernasconi P, Auer JA, Weishaupt MA. The association between heart rate, heart rate variability, endocrine and behavioral pain measures in horses suffering from laminitis. *J Vet Med A Physiol Pathol Clin Med* 2004;21:218–55.
- [27] Marlin DJ, Schroeter RC, White SL, Maykuth P, Matthesen G, Mills PC, et al. Recovery from transport and acclimatization of competition horses in a hot humid environment. *Equine Vet J* 2001;33:371–9.
- [28] Marlin DJ, Scott CM, Schroter RC, Mills PC, Harris RC, Harris PA, et al. Physiological responses in nonheat acclimated horses performing treadmill exercise in cool (20 degrees C/40%RH), hot dry (30 degrees C/40% RH) and hot humid (30 degrees C/80% RH) conditions. *Equine Vet J* 1996;22:70–84.
- [29] Geor RJ, McCutcheon LJ, Ecker GL, Lindinger MI. Heat storage in horses during submaximal exercise before and after humid heat acclimation. *J Appl Physiol* (1985) 2000;89:2283–93.
- [30] Marlin DJ, Scott CM, Schroter RC, Harris RC, Harris RA, Roberts CA, et al. Physiological responses of horses to a treadmill simulated speed and endurance tests in high heat and humidity before and after humid heat acclimation. *Equine Vet J* 1999;31:31–42.
- [31] Pandolf KB. Time course of heat acclimation and its decay. *Int J Sports Med* 1998;19:S157–60.
- [32] Rivera-Brown AM, Rowland TW, Ramirez-Marrero FA, Santaana G, Vann A. Exercise tolerance in a hot and humid climate in heat-acclimatized girls and women. *Int J Sports Med* 2006;27:943–50.
- [33] Périard JD, Racinais S, Sawka MN. Adaptations and mechanisms of human heat acclimation: applications for competitive athletes and sports. *Scand J Med Sci Sports* 2015;25:20–38.
- [34] McCutcheon LJ, Geor RJ, Ecker GL, Lindinger MI. Equine sweating responses to submaximal exercise during 21 days of heat acclimation. *J Appl Physiol* (1985) 1999;87:1843–51.
- [35] Nadel ER, Pandolf KB, Roberts MF, Stolwijk JA. Mechanisms of thermal acclimation to exercise and heat. *J Appl Physiol* 1974;37:515–20.
- [36] Kronfeld DS. Dietary fat affects heat production and other variables of equine performance under hot and humid conditions. *Equine Vet J* 1996;22:24–34.
- [37] El Helou N, Tafflet M, Berthelot G, Tolaini J, Marc A, Guillaume M, et al. Impact of environmental parameters on marathon running performance. *PLoS One* 2012;7:e37407.
- [38] Langlois C, Robert C. Epidémiologie des troubles métaboliques chez le chevaux d'endurance. *Pract Vét Equine* 2008;40:51–6.
- [39] Foreman JH, Lawrence LM. Lameness and heart rate elevation in the exercising horse. *J Equine Vet Sci* 1991;11:353–6.
- [40] Barnes A, Kingston J, Beetson S, Kuiper C. Endurance veterinarians detect physiologically compromised horses in a 160 km ride. *Equine Vet J* 2010;38:6–11.
- [41] McCutcheon LJ, Geor RJ, Hare MJ, Ecker GL, Lindinger MI. Sweating rate and sweat composition during exercise and recovery in ambient heat and humidity. *Equine Vet J* 1995;20:153–7.
- [42] Lucke JN, Hall GM. Further studies on the metabolic effects of long distance riding: Golden Horseshoe Ride. *Equine Vet J* 1980;12:189–92.
- [43] Kohn CW, Hinchcliff KW, McKeever KH. Evaluation of washing with cold water to facilitate heat dissipation in horses exercised in hot, humid conditions. *Am J Vet Res* 1999;60:299–305.
- [44] Khogali M. The Makkab body cooling unit. In: Khogali M, Hales JRS, editors. Heat stroke and temperature regulation. Sydney: Academic Press; 1983. p. 139–48.
- [45] Marlin DJ, Schott CM, Roberts CA, Casas I, Holah G, Schoter RC. Post-exercise changes in compartment body temperature accompanying intermittent cold water cooling in the hyperthermic horse. *Equine Vet J* 1998;30:28–34.
- [46] Flaminio MJ, Gaughan EM, Gillespie JR. Exercise intolerance in endurance horses. *Vet Clin North Am Equine Pract* 1996;12:565–80.
- [47] Naylor JRJ, Bayly WN, Gollnick PD, Brengelmann GL, Hodgson DR. Effects of dehydration on thermoregulatory responses of horses during low-intensity exercise. *J Appl Physiol* (1985) 1993;72:994–1001.
- [48] Trigo P, Castejón F, Riber C, Muñoz A. Use of biochemical parameters to predict metabolic elimination in endurance rides. *Equine Vet J* 2010;38:142–6.
- [49] Muñoz A, Riber C, Trigo P, Castejón F. Clinical applications of the renin-angiotensin-aldosterone-vasopressin axis in the horse and future directions for research. *J Equine Vet* 2010;30:607–16.
- [50] McConaghy FF, Hodgson DR, Evans DL, Rose RJ. Equine sweat composition: effects of adrenaline infusion, exercise and training. *Equine Vet J* 1995;20:158–64.
- [51] Muñoz A, Cuesta I, Riber C, Gata J, Trigo P, Castejón FM. Trot asymmetry in relation to physical performance and metabolism in equine endurance rides. *Equine Vet J* 2006;36:50–4.
- [52] Schott 2nd HC, Marlin DJ, Geor RJ, Holbrook TC, Deaton CM, Vincent T, et al. Changes in selected physiological and laboratory measurements in elite horses competing in a 160 km endurance ride. *Equine Vet J* 2006;36:37–42.
- [53] Rose RJ, Hodgson DR, Sampson D, Chan W. Changes in plasma biochemistry in horses competing in a 160 km endurance ride. *Aust Vet J* 1983;60:101–5.
- [54] Adrogué H, Madias NE. Hyponatremia. *N Engl J Med* 2000;342:1581–9.
- [55] Fielding CL, Magdesian KG, Carlson GP. Application of the sodium dilution principle to calculate extracellular fluid volume changes in horses during dehydration and rehydration. *Am J Vet Res* 2008;69:1506–11.
- [56] Arief A, Llach F, Massry SG. Neurological manifestations and morbidity of hyponatremia: correlation with brain water and electrolytes. *Medicine* 1976;55:121–9.
- [57] Trigo P, Riber C, Agüera S, Castejón F. Plasma biochemistry in horses with metabolic alterations during endurance exercise. *J Physiol Biochem* 2005;61:224.
- [58] Tofé E, Muñoz A, Castejón F, Trigo P, Castejón-Riber C, Gómez-Díez M, et al. Behavior of renin angiotensin aldosterone axis during pulling exercise in euhydrated and dehydrated horses. *Res Vet Sci* 2013;95:615–22.
- [59] Rose RJ, Arnold KS, Church S, Paris R. Plasma and sweat electrolyte concentrations in the horse during long distance exercise. *Equine Vet J* 1980;12:19–22.
- [60] Carlson GP. Blood chemistry, body fluids and hematology. In: Gillespie JR, Robinson NE, editors. *Equine exercise physiology 2*. Davis, CA: ICEEP Publications; 1987. p. 393–425.
- [61] Muñoz A, Riber C, Satué K, Lucas RG, Benito M. Relationship between systemic adaptation to physical effort and plasma potassium in untrained and trained Andalusian and Angloarabian horses. *J Equine Sci* 2003;14:13–22.
- [62] Aguilera-Tejero E, Estepa JC, López I, Bas S, Garfia B, Rodríguez M. Plasma ionized calcium and parathyroid hormone concentrations in horses after endurance rides. *J Am Vet Med Assoc* 2001;219:488–90.
- [63] Stewart AJ. Magnesium disorders in horses. *Vet Clin North Am Equine Pract* 2011;27:149–63.
- [64] Goulet ED, Rousseau SF, Lamboley CR, Plante GE, Dionne LJ. Pre-exercise hyperhydration delays dehydration and improves endurance capacity during 2 h of cycling in a temperature climate. *J Physiol Anthropol* 2008;27:263–71.
- [65] Gigou PY, Dion T, Asselin A, Berrigan F, Goulet ED. Pre-exercise hyperhydration induced bodyweight gain does not alter prolonged treadmill running time-trial performance in warm ambient condition. *Nutrients* 2012;8:949–66.
- [66] Hillman AR, Turner MC, Peart DJ, Bray JW, Yalor L, et al. A comparison of hyperhydration versus ad libitum fluid intake strategies on measures of oxidative stress, thermoregulation and performance. *Res Sports Med* 2013;21:305–17.
- [67] Geor RJ, McCutcheon LJ. Hydration effects on physiological strain of horses during exercise-heat stress. *J Appl Physiol* (1985) 1998;84:2042–51.
- [68] Sosa León IA, Davie AJ, Hodgson DR. Effects of oral fluid on cardiorespiratory and metabolic response to prolonged exercise. *Equine Vet J* 1995;18:274–8.
- [69] Van Rosendal SR, Coombes JA. Glycerol use in hyperhydration and rehydration: scientific update. *Med Sports Sci* 2012;59:104–12.
- [70] Düsterdieck KF, Schott 2nd HC, Eberhart SW, Woody KA, Coenen M. Electrolyte and glycerol supplementation improve water intake by horses performing a simulated 60 km endurance ride. *Equine Vet J* 1999;30:418–22.
- [71] Morris DM, Huot JR, Jetton AM, Collier SR, Utter AC. Acute sodium ingestion prior to exercise increases voluntary water consumption resulting in pre-exercise hyperhydration and improvement in exercise performance in the heat. *Int J Sport Nutr Exerc Metab* 2015;25:456–62.

- [72] Kokkonen UM, Pösö AR, Hyyppä S, Huttunen P, Leppaluoto J. Exercise-induced changes in atrial peptides in relation to neuroendocrine responses and fluid balance in the horse. *J Vet Med A Physiol Pathol Clin Med* 2002;49:144–50.
- [73] Butudum P, Schott 2nd HC, Davis MW, Kobe CA, Nielsen BD, Eberhart SW. Drinking salt water enhances dehydration in horses dehydrated by flosemide administration and endurance exercise. *Equine Vet J* 2002;34:513–8.
- [74] Coyle EF, Montain SJ. Benefits of fluid replacement with carbohydrate during exercise. *Med Sci Sports Exerc* 1992;24:S324–30.
- [75] Sosa León LA, Davie AJ, Hodgson DR, Rose RJ. The effects of tonicity, glucose concentration and temperature of an oral rehydration solution on its absorption and elimination. *Equine Vet J* 1995;20:140–6.
- [76] Bullimore SR, Pagan JD, Harris PA, Hoekstra KE, Rose KA, Gardner SC, et al. Carbohydrate supplementation of horses during endurance exercise: comparison of fructose and glucose. *J Nutr* 2000;130:1760–5.
- [77] Hyyppä S, Saastamainen M, Pösö AR. Restoration of water and electrolyte balance in horses after repeated exercise in hot and humid conditions. *Equine Vet J* 1996;22:108–12.
- [78] Holbrook TC, Simmons RD, Payton ME, MacAllister CG. Effect of repeated oral administration of hypertonic electrolyte solution on equine gastric mucosa. *Equine Vet J* 2006;37:501–4.
- [79] Lacombe VA, Hinchcliff KW, Taylor LE. Interactions of substrate availability, exercise performance, and nutrition with muscle glycogen metabolism in horses. *J Am Vet Med Assoc* 2003;223:1576–85.
- [80] Snow DH, Baxter P, Rose RJ. Muscle fibre composition and glycogen depletion in horses competing in an endurance ride. *Vet Rec* 1981;108:374–8.
- [81] Snow DH. Fatigue and exhaustion in the horse. In: *International Conference on Equine Sports Medicine*, Stockholm, Sweden, 21–22 Jul 1990.
- [82] Harris P, Harris NC. Nutritional ergogenic aids in the horse: uses and abuses. In: Pagan J, Geor RJ, editors. *Advances in equine nutrition II*. UK: Nottingham University Press: Nottingham; 2001. p. 491–507.
- [83] Murakai I, Sakuragi T, Uemura H, Menda H, Shindo M, Tanaka H. Significant effect of a pre-exercise high-fat meal after a 2-day high-carbohydrate diet on endurance performance. *Nutrients* 2012;4:625–37.
- [84] Harris P. Feeding management of elite endurance horses. *Vet Clin North Am Equine Pract* 2009;25:137–53.
- [85] Pagan JD, Geor RJ, Harris PA, Hoekstra K, Gardner S, Hudson C, et al. Effects of fat adaptation on glucose kinetics and substrate oxidation during low-intensity exercise. *Equine Vet J* 2002;34:33–8.
- [86] Jose-Cunilleras E, Hinchcliff KW, Sams RA, Devoer ST, Linderman JK. Glycemic index of a meal fed before exercise alters substrate use and glucose flux in exercising horses. *J Appl Physiol* (1985) 2002;92:117–28.
- [87] McKeever KH, Scali R, Geiser S, Kearns CF. Plasma aldosterone concentration and renal sodium excretion are altered during the first days of training. *Equine Vet J* 2002;34:524–31.
- [88] Essén B, Lindholm A, Thornton J. Fibre types, enzyme activities and substrate utilization in skeletal muscles of horses competing in endurance rides. *Equine Vet J* 1984;16:107–12.
- [89] Kim JS, Hinchcliff KW, Yamaguchi M, Beard LA, Markert CD, Devor ST. Exercise training increases oxidative capacity and attenuates exercise-induced ultrastructural damage in skeletal muscle of aged horses. *J Appl Physiol* (1985) 2005;98:334–42.
- [90] McGowan CM, Golland LC, Evans DL, Hodgson DR, Rose RJ. Effects of prolonged training, overtraining and detraining on skeletal muscle metabolites and enzymes. *Equine Vet J* 2002;34:257–63.
- [91] McCutcheon LJ, Geor RJ. Effects of short-term training on thermoregulatory and sweat responses during exercise in hot conditions. *Equine Vet J* 2010;38:135–41.