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Prawit Butudom

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Harold C. Schott II

Major professor

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Prawit Butudom

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ABSTRACT

THIRST AND REHYDRATION IN DEHYDRATED HORSES

By

Prawit Butudom

Body fluid homeostasis is crucial for maintaining normal physiologic function. During exercise, especially in endurance horses, a substantial loss of body fluid by sweating can occur. Replacement of this fluid deficit by drinking is important to restore body fluid homeostasis. The primary stimulus for thirst is an increase in plasma osmolality or, more specifically, an increase in plasma sodium concentration. However, horses typically do not drink adequately to replace sweat fluid losses, even when water or other rehydration fluids are readily available. This phenomenon of incomplete restoration of body fluids, reflected by persisting body weight loss after exercise, has been termed "involuntary dehydration". The objective of my research was to investigate involuntary dehydration and factors affecting thirst and voluntary water intake in horses dehydrated by endurance exercise. Horses are often transported long distances within a few hours after completion of an endurance competition. Therefore, this study focused on manipulating rehydration during the initial hour following exercise in order to enhance recovery prior to transport. The following hypotheses were tested: *The magnitude of "involuntary dehydration" in horses after the first hour of recovery from endurance exercise is affected by: i) volume of water initially offered after exercise-induced dehydration; ii) type of rehydration solution (water vs. saline) initially offered; and iii) temperature of rehydration fluid.*

Limiting the volume of the initial drink of water (to 4 l, 8 l, or an unlimited amount during the first 5 minutes of recovery) had no significant effect on persisting body weight loss after the first hour of recovery. In contrast, initially offering a saline solution (0.45% or 0.9% NaCl solution during the first 5 minutes of recovery) resulted in greater total fluid intake and attenuated of the magnitude of involuntary dehydration than when plain water was initially offered. Next, providing rehydration fluid at near ambient temperature (20°C) resulted in greater voluntary fluid intake by the end of the initial 60-minute recovery period, in comparison to offering fluid at cool (10°C) or warm (30°C) temperatures.

In conclusion, sodium content and temperature of rehydration fluid affected voluntary fluid intake and involuntary dehydration in horses dehydrated by endurance exercise. Thus, an initial drink of salt water (0.9% NaCl) at temperature near 20°C immediately after exercise (or at rest periods during prolonged exercise) appears to be a good strategy for enhancing rehydration of endurance horses, especially during competition under conditions of high heat and humidity.

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LIST OF ABBREVIATIONS

ADH	=	Antidiuretic hormone
ALD	=	Aldosterone
ATP	=	Adenosinetriphosphate
B	=	Blood
BW	=	Body weight
BM	=	Body mass
CSF	=	Cerebrospinal fluid
ECF	=	Extracellular fluid
FI	=	Fluid intake
FI 0'-5' post	=	Fluid intake during 0 to 5 minute of recovery
GER	=	Gastric emptying rate
Hct	=	Hematocrit
hr	=	Hour
HR	=	Heart rate
ICF	=	Intracellular fluid
ISF	=	Interstitial fluid
IV	=	Intravenous
min	=	Minute
MH	=	Metabolic heat
ORS	=	Oral rehydration solution
P_{osm}	=	Plasma osmolality
PP	=	Plasma protein concentration
SDF	=	Synchronous diaphragmatic flutter
S_{osm}	=	Serum osmolality
TBW	=	Total body water
Tcore	=	Pulmonary arterial blood temperature
UW	=	Unlimited water intake
VO_2max	=	Maximal oxygen uptake
W	=	Water
WI	=	Water intake
WI 0'-5' post	=	Water intake during 0 to 5 minutes of recovery
WI 20'-60' post	=	Water intake during 20 to 60 minutes of recovery

Chapter 1

Literature review

Body fluid homeostasis in athletic horses

BODY FLUID HOMEOSTASIS IN ATHLETIC HORSES

Physiology of body fluids

Fluid and electrolyte homeostasis

Body fluids (water and electrolytes) are crucial for maintaining normal physiologic functions. Water and electrolytes are the *Milieu interieur*, the internal medium surrounding and sustaining cells of the body. This concept was formulated by the French physiologist Claud Bernard in the nineteenth century (Peavy 1995). In order for cells of any tissue to function optimally, it is important that the composition of body fluids is maintained and regulated appropriately. Perturbations of body fluids can adversely affect normal physiologic functions, and when severe, can be life-threatening. Therefore, near constancy of composition and distribution of body fluids is essential for maintenance of normal cell functions. Physiologic responses that counteract perturbations of the internal medium (body fluids) and maintain its constancy are collectively called *homeostasis*. Walter B. Cannon, an American physiologist in the 20th century introduced this concept of physiologic self-regulation (Peavy 1995). Homeostatic responses enable the body to maintain near constant composition and disposition of body fluids in the face of normal physiologic events. They include both neural and humoral regulatory mechanisms.

Body water and electrolyte content

Total body water (TBW) accounts for 45 to 50 % of body weight (BW) in women and 55 to 60% of BW in men (Rose 1989). Women have lower body water content, in comparison to men, due to a higher percentage of adipose tissue (Rose 1989). However, a sex difference in TBW has not been described in horses. In addition, body water content also varies with age in that the newborn infant has the highest water content (about 70% of BW); subsequently, water content decreases with age (Stanton and Koeppen 1998). Total body water is distributed between several body fluid compartments that can be expressed as a percentage of BW. The two main compartments are intracellular fluid (ICF) and extracellular fluid (ECF). In humans, ICF accounts for 36% of BW and ECF accounts for 25% of BW (Rose 1989). The ECF compartment is subdivided into interstitial fluid (ISF) and plasma; these compartments are separated by the capillary wall. Interstitial fluid, which represents fluid surrounding cells in various tissues of the body, comprises 75% of ECF volume. Plasma volume constitutes the remaining 25% of ECF volume. Three smaller ECF compartments have also been described: water found in dense connective tissues, such as cartilage and tendons; water in bone; and transcellular water, which is composed of cerebrospinal, pleural, peritoneal, synovial, and intraocular fluid as well as fluid in the lumen of the gastrointestinal tract. (Rose 1989).

In the horse, TBW is about 65 to 70% of total BW (Carlson 1987a; Carlson 1987b). ICF accounts for approximately 43 to 46% of BW and water in interstitial spaces and in plasma represents approximately 10 to 12% and 4 to 6% of BW, respectively (Schott and Hinchcliff 1993). In contrast to man, the contents of the gastrointestinal tract of the horse contain a substantial amount of water (about 6% of BW) (Meyer and Coenen 1989). This

component of transcellular fluid represents an important reservoir for water and electrolytes during prolonged exercise. Figure 1 illustrates the distribution of total body water in a typical 450-kg horse.

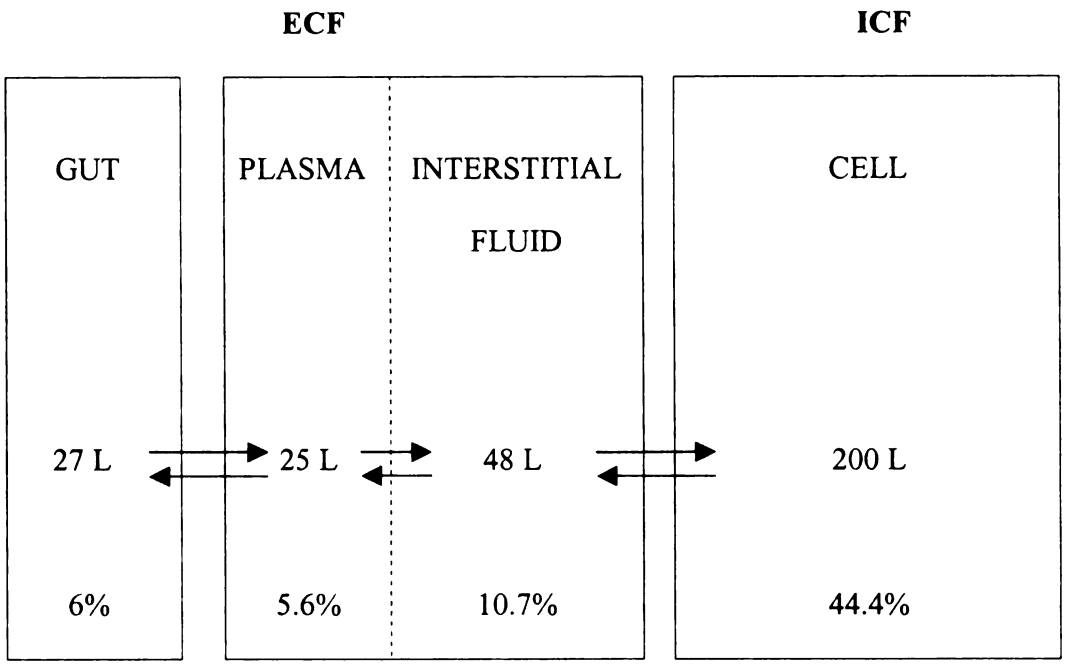

Figure 1. Distribution of total body water in a 450-kg horse presented both as percent of body weight and as absolute volume (L). The four major compartments are plasma, interstitial fluid, intracellular fluid (ICF), and trancellular fluid (mainly in gut). About 200 L of TBW is ICF, and the remaining 100 L is extracellular fluid (ECF). The arrows show body water exchanges between the four compartments.

Table 1 summarizes the distribution of cations and anions between the intracellular and extracellular fluid compartments (Rose 1977). Sodium is the major extracellular cation and potassium is the major intracellular cation. Similarly, the major intracellular anions are proteins and phosphate, whereas the major extracellular anions are chloride and bicarbonate. Because plasma and ISF are separated only by a capillary wall, which is freely permeable to small ions, the ionic composition of these two compartments of the ECF is similar. However, a major difference between the composition of ISF and plasma is that plasma contains a greater amount of protein. The negative charge on plasma protein holds a greater amount of cations (predominantly sodium) in the plasma water and, as a consequence of the Gibbs-Donnan equilibrium, the distribution of cations and anions between ISF and plasma is slighly different (Rose 1977; Kutchai 1998; Stanton and Koeppen 1998; Guyton and Hall 1996). The Donnan effect is the relation that holds any univalent cation and anion pair in equilibrium between the two compartments (Kutchai 1998). Although the Donnan effect can affect the distribution of cations and anions across capillary walls, this effect is normally quite small, and the ionic composition of the ISF and plasma can be considered identical (Stanton and Koeppen 1998; Guyton and Hall 1996). The asymmetric distribution of sodium and potassium ions across the plasma membrane (i.e. between ICF and ECF) is maintained by different membrane permeabilities for sodium and potassium and activity of the Na^+K^+-ATPase. By action of the latter, two sodium ions are pumped out of the cell in exchange for three potassium ions.

Ions	Plasma	Plasma Water[a]	Interstitial Fluid	Skeletal muscle cell
Cations				
Na^+	142.0	152.7	145.1	12.0
K^+	4.3	4.6	4.4	150.0
Ca^{2+}	2.5	2.7	2.4	4.0
Mg^{2+}	1.1	1.2	1.1	34.0
Total	149.9	161.2	153.0	200.0
Anions				
Cl^-	104.0	111.9	117.4	4.0
HCO_3^-	24.0	25.8	27.1	12.0
Phosphate	2.0	2.2	2.3	40.0
$Protein^-$	14.0	15.0	0.0	54.0
Others[b]	5.9	6.3	6.2	90.0
Total	149.9	161.2	153.0	200.0

Table 1. Approximate concentrations (mEq/L) of ions in plasma, interstitial fluid and intracellular fluid (typical muscle cell) (Adapted from Rose 1977). [a] = Plasma water content assumed to be 93%, [b] = This largely represents organic phosphates such as ATP.

Regulation of water balance

A dynamic balance between water loss and gain normally maintains TBW within a narrow range. As reviewed by Anderson (1978), water is lost through urine, feces, sweat, and humidification of inspired air. Water is gained by intake of food, water drinking, and water from metabolism. Fine-tuning of water balance is achieved by action of antidiuretic hormone (ADH) on the collecting ducts in the kidneys. A decrease in TBW initially induces secretion of ADH and subsequently stimulates thirst. Thus, the first response to a decrease in TBW is an increase in ADH secretion to enhance water reabsorption by the kidneys and, with more severe water deficits, drinking is stimulated by thirst. Two regulatory mechanisms work in parallel to correct decreases in TBW: 1) osmotic regulation, which acts in response to reduced volume of cerebral osmoreceptors (cell dehydration); and 2) volume regulation which acts in response to decreases in blood volume (hypovolemia).

1. Osmotic regulation

An increase in the osmolality of body fluids, consequent toa decrease in TBW, is the most important stimulus for water gain. In 1937, Gilman observed that administration of a hyperosmolar NaCl solution resulted in water drinking by euhydrated subjects. In addition, much greater water intake was observed with administration of the hyperosmolar NaCl solution in comparison to administration of an equally hyperosmolar solution of urea (a solute that freely crosses cell membranes). Although both solutes produced similar increase in plasma osmolality (Posm), only the NaCl solution caused a

fall in plasma protein concentration, indicating movement of water from ICF to ECF. Thus, cellular dehydration, not simply an increase in Posm, appears to be important for osmotic regulation of water balance. Specifically, a change in volume of osmoreceptors located in the anterior hypothalamus is necessary for osmotic stimulation of ADH release and thirst.

Initially, rather modest increases in plasma tonicity (e.g., a 3 to 5 mOsm/kg increase in Posm or a 1 to 2 mmol/L increase in plasma sodium concentration lead to secretion of ADH. Again, ADH acts by inserting more water channels in the collecting duct epithelial cells to increase renal water conservation. With greater increase in Posm (e.g., 3%, 3 %, or 3-6 % in humans, ponies and dogs, respectively) thirst is also induced (Wood *et al.* 1977).

2. Volume regulation

Another stimulus for ADH secretion and thirst is a reduction in plasma volume (i.e., hypovolemia). Hypovolemia can induce ADH release and thirst by either cardiovascular reflexes or activation of the renin-angiotensin system (Anderson 1978; Fitzsimmons 1998). Hypovolemia is detected by stretch receptors in the right atrium of the heart that may directly stimulate ADH release and thirst via neural pathways. Hypovolemia, which decreases renal perfusion, also activates the renin-angiotensin system, which provides a further stimulus for ADH secretion and thirst (Fregly 1982; Phillips *et al.* 1982).

The renin-angiotensin system can be activated by a decrease in blood pressure, a decrease in plasma sodium concentration ($[Na^+]$), an increase in plasma potassium concentration ($[K^+]$), or β-adrenergic receptor stimulation via adrenergic nerves or circulating catecholamines. These stimuli increase renin release from the kidneys. After cleavage from angotensinogen by renin, angiotensin I is converted to angiotensin II by converting enzyme in the lungs. Angiotensin II may affect water gain directly by stimulating supraoptic neurons to induce ADH secretion and hypothalamic centers associated with drinking and indirectly by stimulating aldosterone secretion and increasing tubular reabsorption of sodium. A somewhat separate renin-angiotensin system located within the central nervous system (anterior hypothalamus and peoptic region of the brain) also appears to be involved in thirst stimulation. The most sensitive structures to the dipsogenic action of the angiotensin II are the subfornical organ and the organ vasculosum of the lamina terminalis (Fitzsimmons 1998).

Horses drink in response to an isosmotic loss of blood volume and a decrease of plasma volume to 6% is sufficient to stimulate drinking (Hinton 1977). Reduced plasma volume and increased water intake has also been demonstrated in sheep (Zimmerman and Stricker 1978) and rats (Rabe 1975) after furosemide administration. In horses, the dose of furosemide necessary to stimulate significant intake is on the high end (2 mg/kg) of the recommended clinical dose (Sufit *et al.* 1985).

With hypovolemia, activation of the renin-angiotensin system has been suggested to be more important than cardiovascular reflexes in volume regulation of water gain.

Maximal drinking was preceded by an elevation of plasma angiotensin II concentration, suggesting that activation of the renin-angiotensin system, rather than diminished stimulation of cardiovascular distension receptors, was the crucial thirst-eliciting factor. Finally, hypovolemia appears to be a less potent stimulus for water gain than activation of osmoreceptors. For example, experiments in man, monkeys, rats and dogs have demonstrated that at least 10-15% of blood volume has to be lost before an increase in ADH secretion or drinking is observed (reviewed by Greenleaf (1992) and Anderson (1978). In contrast, as mentioned above, an increase of plasma tonicity by as little as 1 to 2% can produce an increase in ADH secretion.

In the equine species, most drinking occurs rapidly (within the first couple of minutes) after water is provided to dehydrated su jects (Sufit *et al.* 1985; Jones *et al.* 1989; Düsterdieck *et al.* 1999). In euhydrated animals most water drinking accompanies eating (peripandial drinking) (Kissileff 1969; Houpt *et al.* 1983; Sufit *et al.* 1985; Jones *et al.* 1989). Management practices should accommodate the horse's drinking patterns by providing water ad libitum in association with feeding. An important difference between humans and horses is the ability of the gastrointestinal tract to function as a reservoir for water and electrolytes, containing up to 6% of BW, that can be utilized by dehydrated horses (e.g., during prolonged exercise or after furosemide administration).

A model of thirst and drinking induction

Although the physiological mechanisms that induce thirst and drinking are not fully understood, a model illustrating the major regulatory mechanisms: osmoreceptors (cellular dehydration), hypovolemia (extracellular dehydration), and activation of the renin-angiotensin system is demonstrated in Figure 2 (Greenleaf 1992). At least six distinct stimuli have been used experimentally for studying the mechanisms of water intake (reviewed by Greenleaf and Fregly 1982): 1) hypertonic saline; 2) β-adrenergic agonist (isoproterenol); 3) angiotensin II; 4) polyethylene glycol; 5) parasympathomimetic agents; and 6) dehydration. The results of these stimuli suggest that multiple factors affect thirst. According to the proposed model, the mechanisms of cellular and extracellular dehydration can act either synchronously or independently to induce fluid intake. In addition to these major pathways, dry mouth and oropharyngeal mechanisms also play a role in fluid intake.

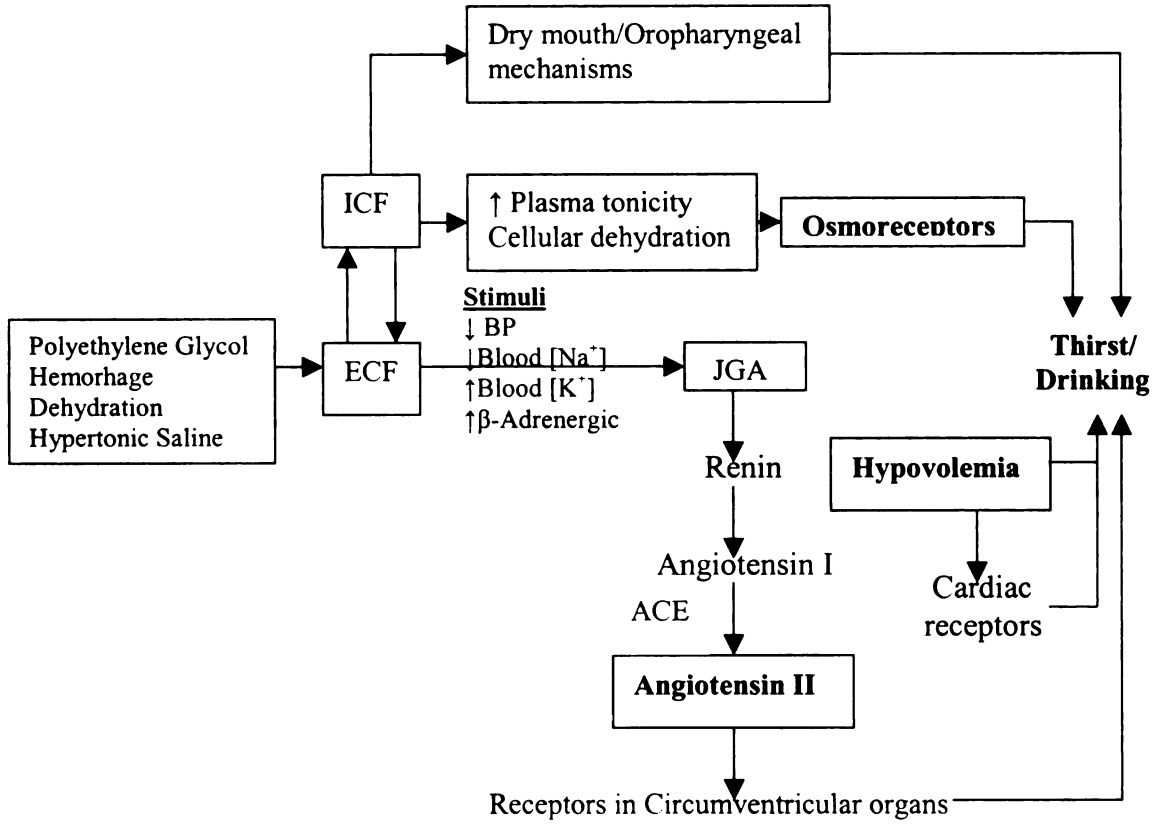

Figure 2. A model illustrating major pathways-osmoreceptors, hypovolemia, angiotensin II-and associated factors for induction of fluid intake. JGA = Juxtaglomerular Apparatus; ACE = angiotensin converting enzyme Adapted from Greenleaf (1992).

Body fluid shifts during exercise

During exercise, metabolism in muscle converts potential chemical energy into kinetic mechanical energy. In addition to work, by-products of increased muscle metabolism include heat, acid, CO_2 and H_2O release from muscle glycogen breakdown (McMiken 1983):

$$\text{Fuel} + \text{oxygen} \rightarrow \text{work} + \text{acid} + \text{heat} + CO_2 + H_2O$$

A wide range of physiological changes in fluid, electrolyte, and acid-base balance occurs in the exercising horse. These changes depend on both the intensity and duration of exercise.

High intensity exercise

Fluid and electrolyte losses by sweat are of minimal importance during short term high-intensity exercise (reviewed by Schott and Hinchcliff 1993). However, fluid and electrolytes shift between fluid compartments during strenuous exercise. The major fluid shift is water movement from vascular and ISF spaces into active muscle cells. This occurs in response to an increase in intracellular osmotic forces due to by products of the increased metabolic rate (i.e., lactate, phosphate and creatine). Some of these metabolic by products, most notably lactate, also leave the cell leading to an increase in ISF osmolality and osmotic pressure. The net effect of this water shift is a reduction in plasma volume and increase in Posm and plasma protein concentration (PP). During high-intensity exercise in horses, Posm may increase from ≈280 mOsm/kg at rest to ≈315 mOsm/kg and PP may increase from 6.0 to 6.5 g/dl to 7.5 to 8.0 g/dl from rest to the end

of exercise (reviewed by Schott and Hinchcliff 1993). This dramatic fluid shift results in substantial hemoconcentration (i.e., packed cell volume >60%) but there is no evidence that it adversely affects performance.

Of perhaps greater interest are the electrolyte changes accompanying high-intensity exercise. Specifically, ($[K^+]$) may approach 10 mmol/l during strenuous exercise in horses (Harris and Snow 1988; Harris and Snow 1992). This marked increase in $[K^+]$ is due to loss of potassium from active muscle cells (Schott *et al.* 2002). Hyperkalemia, in combination with a decrease in intracellular $[K^+]$, may lead to a decrease in contractile force by blocking depolarization (Carlson 1987a; Coffman *et al.* 1978; Schott *et al.* 2002). In summary, during high-intensity exercise, a rapidly increasing body temperature, accumulation of acid, and electrolyte changes, specifically hyperkalemia, may be more important factors contributing to fatigue than body fluid shifts.

Prolonged, low-intensity exercise

During low-intensity exercise, fluid and electrolyte shifts are similar in direction to those occurring during high-intensity exercise, but the magnitude of change is generally smaller (reviewed by Schott and Hinchcliff 1993). In contrast to high-intensity exercise, progressive depletion of fluid and electrolyte stores is a greater challenge to maintenance of homeostasis than is any shift of fluid or electrolyte (Schott and Hinchcliff 1993). Fluid and electrolyte depletion is a consequence of loss of large amounts of fluid and electrolytes by sweating. Equine sweat contains high concentrations of sodium (143.0 ±

9.0 mmol/l), potassium (28.2 ± 2.1 mmol/l), and chloride (158.0 ± 7.1 mmol/l) (McCutcheon *et al.* 1995). Based on loss of body weight, sweating rates in horses during low-intensity exercise have been estimated to approach 10 to 15 liters per hour (Carlson 1985; Carlson 1987a; Carlson 1987b). Depending on environmental conditions, endurance horses may lose 30 to 45 kg (as sweat) over the course of a ride (Carlson 1983; Carlson 1987a; Carlson 1987b). In addition to water loss in sweat, horses also lose large amounts of electrolytes during prolonged exercise. In the approximately 2 hours during which horses completed a treadmill speed and endurance exercise test which simulated running speeds and distances required for this phase of an Olympic level (CCI****) 3-day-event under hot ambient conditions (temperature = 33 to 35°C, relative humidity = 45 to 55%), sweat ion loss (mainly sodium and chloride) was ~6500 mmol (as sweat fluid loss was ~19 liters) (McCutcheon and Geor 1996). Prolonged sweating results in significant chloride loss and a reduction in plasma chloride concentration and endurance horses typically develop a hypochloremic metabolic alkalosis during the later stages of endurance exercise (Schott and Hinchcliff 1993). Endurance horses may also develop respiratory alkalosis during exercise as hyperpnea, hypocapnea, and an increased blood pH were reported in horses exercised on a treadmill at 40% VO_2 max for 60 minutes (Bayly *et al.* 1995). In summary, during prolonged, low-intensity exercise, fluid loss in sweat is likely an important factors limiting performance (i.e., contributing to fatigue).

Effects of body fluid loss on performance

High-intensity exercise

Changes in body fluid balance prior to exercise may affect performance. In human athletes, hyperhydration prior to exercise can improve performance, whereas hypohydration may decrease performance (Sawka and Pandolf 1990; Sawka 1992). However, mild dehydration appears to have little effect on performance of short bouts of high-intensity exercise. In racing horses, hypohydration is commonly induced by furosemide administration (0.5 to 1.0 mg/kg, IV) prior to the race and this practice could be expected to affect performance. Briefly, furosemide-induced dehydration results in an isotonic loss of 5 to 10% of ECF water and electrolyte content (Hinchcliff and Muir 1991). This reduction in ECF also produces a 2 to 3% loss in body weight. A loss of body weight has recently been speculated to actually enhance performance in racing horses (Gross *et al.* 1999).

Prolonged, low-intensity exercise

Metabolic heat production by an endurance horse (speed ~8 m/s) could increase pulmonary arterial blood temperature (Tcore) by ~21°C/hr if no heat were dissipated (Guthrie and Lund 1998). However, more than 90% of metabolic heat generated during prolonged endurance exercise is dissipated (Kingston *et al.* 1997). As previously mentioned, fluid loss via sweat can be substantial and may approach 10 to 15 liters per hour in endurance horses (Carlson 1983). This fluid loss can lead to a decrease in circulating volume and competition between tissues (e.g., active muscle and skin) for blood flow. Furthermore, under hot ambient condition (32° to 34°C), a decreased sweating rate was observed in horses exercising at 50% Vo_2max for 90 minutes

(McCutcheon and Geor 1998). Thus, progressive dehydration may limit continued performance during prolonged exercise by impairing thermoregulation (Schott and Hinchcliff 1993; Schott *et al.* 1997). Initially, mild dehydration (~3% of BW loss) may reduce performance (Dahlborn *et al.* 1995) but as dehydration becomes more severe (7 to10% BW loss), serious medical problems consequent to fluid and electrolyte depletion, hyperthermia, and heat exhaustion can develop (Carlson 1985; Goer and McCutcheon 1996). Characteristic clinical signs exhibited by horses approaching fatigue during prolonged exercise include: reduced effort and responsiveness; reluctance or inability to continue; slow capillary refill time and jugular vein distensability (hypovolemia); lack of gastro-intestinal sounds (poor motility); thermoregulatory failure (elevated rectal temperature); and a persistently elevated heart rate. Further, due to loss of large amounts of electrolytes in sweat, plasma tonicity is decreased. As discussed later, hypo-osmotic dehydration can diminish thirst despite marked dehydration. If these signs are not recognized or appropriate treatment (oral or intravenous fluid) is not instituted, various medical problems may develop. These can include postexertional ileus (Schott and Charlton 1996), synchronous diaphragmatic flutter (SDF) (Schott *et al.* 1997), or a combination of medical problems that have been collectively grouped with the term exhaustive horse syndrome (Carlson 1983; Carlson 1985).

Involuntary dehydration

Involuntary dehydration, also called "voluntary dehydration", is a term used to describe persistent body fluid depletion and a persisting loss in BW resulting from

incomplete voluntary replacement of body fluid losses (Adolph *et al.* 1947). That is, the subject drinks to satiety, but a water deficit remains. Although "involuntary dehydration" is a well-recognized phenomenon, little is known about its physiological mechanism. In humans, involuntary dehydration has been observed following endurance exercise or exposure to hot or cold ambient conditions (Greenleaf 1992). During exercise and heat exposure, fluid loss through sweating appears to be the major factor contributing to involuntary dehydration, while cold exposure appears to lead to peripheral vasoconstriction and increased fluid loss in urine. Greenleaf and co-workers found that the magnitude of involuntary dehydration was directly proportional to the level of exercise or thermal stress on the subject and the greater the body water deficit, the longer it took for complete restoration of that deficit (reviewed by Greenleaf 1992). Although unproven, "involuntary dehydration" may be a protective mechanism to prevent hyponatremia that could develop consequent to full replacement of body fluid losses by water drinking alone (Greenleaf 1992).

Along with the amount of exercise or thermal stress, studies in humans have shown that additional factors can influence the magnitude of involuntary dehydration. An obvious factor is availability of water or other rehydration fluids. Other more subtle factors include type, temperature, and flavoring of fluid available for rehydration (Boulze *et al.* 1983; Sandick *et al.* 1984; Barr *et al.* 1991; Mitchell *et al.* 1994). Further, considerable interindividual variability in voluntary drinking has been reported in human subjects performing exercise or exposed to thermal stress. In fact, human athletes have been characterized as either "better drinkers" (that maintain less than a 2% BW loss) or

"poorer drinkers" (that maintain greater than 2% BW loss) (Szlyk *et al.* 1989). Finally, involuntary dehydration also appears to be affected by social customs that influence what is consumed as well as the capacity and rate of fluid absorption from the gastrointestinal system (reviewed by Greenleaf, 1992).

Mechanisms of fluid intake related to "involuntary dehydration"

According to the proposed model of fluid intake (Figure 2), increases in Posm or $[Na^+]$, hypovolemia, and activation of the renin-angiotensin system are the stimuli for thirst and drinking. Stimulation of thirst is clearly an important factor affecting voluntary rehydration and recovery of water losses. Because sweat contains substantial amounts of Na^+, the increases in Posm and $[Na^+]$ during endurance exercise are less than would be produced by loss of similar volume of pure water. Although small increases are likely corrected by drinking various rehydration beverages, attenuation of these increases appears to be an important factor for development of involuntary dehydration. Next, despite fluid shifts from ISF and ICF into plasma, in attempt to maintain plasma volume, mild hypovolemia may develop during exercise leading to both direct stimulation of thirst and indirect stimulation of thirst by activation of the rennin-angiotensin system. As discussed earlier, "involuntary dehydration" may act as a protective mechanism to guard against excessive plasma dilution leading to hyponatremia.

Involuntary dehydration and equine athletes

Although development of involuntary dehydration in horses performing prolonged exercise has not been specifically described, a number of investigators have reported BW losses of 3 to 7% in horses competing in 3-day events and endurance rides (Lawrence *et al.* 1992; Ecker and Lindinger 1992; Andrews *et al.* 1994; Schott *et al.* 1996; Schott *et al.* 1997). As recently confirmed by Kingston *et al.* (1997), weight loss by horses performing prolonged exercise is an accurate estimate of fluid loss as sweat. Furthermore, a 3 to 4% BW loss can persist after an overnight recovery period (Schott *et al.* 1996; Schott *et al.* 1997), suggesting that substantial time may be required for complete restoration of sweat fluid losses. Persisting BW loss occurred in endurance horses even though horses had free access to water during the ride and recovery period. During a simulated field endurance ride (Nyman *et al.* 1996) voluntarily drinking during the ride replaced only 38% to 45% of BW loss and BW did not return to pre-ride values in any of the horses after 3 hours of recovery despite continuous access to water. Further, after an overnight recovery period (16 hours after the ride) BW deficits were still 0.5 to 1.5% (Nyman *et al.* 1996). All in all, these findings support that involuntary dehydration occurs in endurance horses.

The BW losses discussed above are greater than those reported in most studies of human endurance athletes and suggest that horses may experience a comparatively greater magnitude of involuntary dehydration. In contrast to human sweat, which is typically hypotonic (sodium concentration of 50 to 70 mmol/l) to plasma (Convertino *et al.* 1996), equine sweat is nearly isotonic to plasma due to a higher sodium concentration

(120 to 140 mmol/l) (McCutcheon and Geor 1996). Consequently, horses lose a comparatively greater amount of electrolytes with each liter of sweat produced. As a result, increases in Posm and [Na^+] are even less than in human athletes, leading to even greater blunting of thirst. Thus, it would seem reasonable to speculate that athletic horses may experience a relatively greater magnitude of involuntary dehydration (perhaps a threshold of 3 to 4% BW loss) than their human counterparts.

Problems associated with "involuntary dehydration" in equine athletes

It has been well documented that sweating during exercise is a critical thermoregulatory response for dissipation of metabolic heat (Hodgson *et al.* 1993). As stated earlier, mild dehydration (3% BW loss) may reduce exercise performance (Dahlborn *et al.* 1995), but as dehydration becomes more severe (7 to 10% BW loss), fatigue and exhaustion may ensue (Carlson 1985; Geor and McCutcheon 1996). Therefore, "excessive" involuntary dehydration appears to be an important risk factor for the development of exhaustion and associated medical problems during and after prolonged exercise (Carlson 1985; Schott and Charlton 1996; McCutcheon and Geor 1996). Although discipline specific data are limited, the number of horses that experience medical problems as a consequence of exercise-induced dehydration is substantial. For example, "failure to complete" data for all 1996 endurance rides provided by the American Endurance Ride Conference (AERC) revealed that about 15% and 30% of the nearly 11,000 horses that competed in 50 and 100-mile endurance rides in 1996, respectively, failed to compete the ride. Approximately 20% of the horses that failed to finish were eliminated or "pulled" due to development of "metabolic problems" and 44%

(about 850 horses) required immediate veterinary treatment (Cassotis and Schott 1997). This number did not include horses that may have required treatment several hours later or after a trailer ride home. Similarly, data for 1997 3-day-events provided by the United States Combined Training Association (USCTA) revealed that 25 to 30% of all horses entered in 1 to 4 star competitions failed to complete the event (L Cozzi, USCTA, personal communication 1997). Although the percentage of horses that failed to complete as a consequence of development of dehydration/medical problems was not specifically recorded, an estimate as low as 10% (of the horses that did not finish) coupled with extension to the more than 30,000 horses that compete annually in USCTA events (at all levels) could yield an estimate as high as 750 horses that suffer from heat-related exhaustion and/or illness. Clearly, these data demonstrate that involuntary dehydration is an important problem in equine athletes.

Rehydration following exercise and involuntary dehydration: strategies for fluid rehydration in exercising horses

Although there has been a fairly large recent research effort to study the effects of dehydration on equine endurance performance and treatments to attenuate dehydration (*Equine Veterinary Journal Supplements 20, 1995 & 22, 1996*, reviewed in Schott and Hinchcliff 1998), almost all recent work has involved "forced" hyperhydration or rehydration by administering solutions via a nasogastric tube. Although studies have been limited, another manipulation to enhance rehydration during and following exercise has been administration of electrolytes in feed or as oral pastes in an attempt to increase

voluntary water drinking (Coenen *et al.* 1995; Nyman *et al.* 1996; Düsterdieck *et al.* 1999).

Coenen *et al.* (1995) investigated the effects of pre-exercise electrolyte supplementation by feeding ponies a supplement that contained approximately 300 mmol of sodium (in addition to a dietary intake of 18 g per day) at 1 or 4 hours prior to exercise. Voluntary water intake during the 4 hours before exercise and during recovery was significantly greater for both supplemented groups in comparison to non-supplemented controls. Supplementation of electrolytes was concluded to improve maintenance of body water due to the greater pre-exercise and post-exercise water intake.

In a field study of endurance-trained horses by Nyman *et al.* (1996), $[Na^+]$ increased in horses administered an oral salt paste or offered saline to drink in comparison to horses offered plain water. In addition, fluid intake and BW recovery were greater in the salt paste and saline groups, compared to the plain water group, and at the end of a 3-hour recovery period plasma aldosterone (ALD) was increased in the water group only. These findings suggested that provision of additional Na^+ as a salt paste or salt water during endurance exercise enhanced restoration of body fluid and electrolyte stores.

In another attempt to stimulate greater voluntary water intake during endurance exercise, Düsterdieck *et al.* (1999) demonstrated that supplementation of electrolytes as an oral paste attenuated BW loss and enhanced voluntary water intake by horses performing a simulated endurance ride on a treadmill. Plasma osmolality increased to a

greater extent in horses administered electrolyte pastes in comparison to non-supplemented horses. These investigators also demonstrated that the greater increases in Posm and [Na^+] in the electrolyte supplemented horses were correlated with greater voluntary water intakes, supporting that an increase in plasma tonicity was a stimulus of thirst.

Oral rehydration solutions (ORS) have also been used to restore fluid and electrolyte balance and thereby improve recovery from exercise during acclimatization to hot and humid conditions. Hyyppä *et al.* (1996) demonstrated that rehydration of horses with an ORS (isotonic 0.5% glucose solution containing about 40 mmol/l of sodium) administered via a nasogastric tube 30 minutes after completion of a treadmill exercise bout simulating the endurance phase of a 3-day event resulted in greater voluntary water intake during overnight recovery than in horses administered plain water. Persistent BW loss was also less after overnight recovery in horses given the ORS.

In summary, recent studies in both human and equine subjects have clearly demonstrated that restoration of body fluid stores is more rapid and complete when rehydration solutions containing electrolytes (primarily Na^+ containing solutions) are used in place of water (Maughan and Shirreffs 1994; Shirreffs *et al.* 1996; Marlin *et al.* 1998a, 1998b; Hyyppä *et al.* 1996). In fact, in a 1996 *Position Stand* entitled *Exercise and Fluid Replacement* the American College of Sports Medicine recommended including Na^+ in rehydration solutions at an amount estimated to replace sweat losses,

during exercise lasting longer than 1 hour (Convertino *et al.* 1996). Addition of Na^+ appears to have a further advantage of enhancing palatability of the rehydration solution.

However, a major difference between human and equine athletes is that humans can force themselves to drink rehydration solutions in the absence of thirst while the old saying **"You can lead a horse to water, but can't make it drink"** is as true today as when the saying was originated. To date, there has been limited study of factors affecting voluntary fluid replacement (by drinking) in horses dehydrated by endurance exercise. Thus, a practical limitation of using ORSs in exercising horses is that solutions would ideally be consumed voluntarily, rather than require "forced" rehydration by administering with nasogastric tube or as an oral electrolyte paste.

Factors affecting the magnitude of "involuntary dehydration" in exercising horses

Factors that affect the ability to drink during or after endurance exercise or exposure to stressful environments can contribute to involuntary dehydration. In horses, there has been little study of factors that may affect involuntary dehydration. Over the past few years, studies by Schott and colleagues (Schott *et al.* 1999; Düsterdieck *et al.* 1999; Schott *et al.* 2002a) have demonstrated that the following factors may affect drinking and involuntary dehydration, i) the mechanism by which dehydration is induced (i.e., endurance exercise or furosemide administration); ii) amount and composition of electrolytes administered for rehydration; and iii) variability between horses. To further understand factors affecting involuntary dehydration in exercising horses, other potential factors need to be investigated.

Summary and hypothesis

This chapter reviewed current knowledge about thirst and voluntary drinking in relation to involuntary dehydration in horses. Involuntary dehydration is a perplexing situation that occurs when obviously dehydrated horses drink less or refuse to drink when water is offered. Thus, a body water deficit persists despite free access to water. This phenomenon occurs in humans and horses when they are exposed to exercise or thermal stress, and the magnitude of involuntary dehydration is related proportional to the degree of total stress imposed on the body.

Thirst is stimulated as water is lost from blood and the remaining minerals, especially sodium, become more concentrated in blood, causing an increase in plasma tonicity. Coupled with activation of renin-angiotensin system by hypovolemia, a combination of these physiological changes signals the thirst center in the hypothalamus to induce drinking. Consumption of water will return plasma tonicity to the normal range. Although the physiological mechanisms responsible for involuntary dehydration are not completely understood, a logical explanation is that thirst and drinking are limited due to the isotonic nature of equine sweat. Loss of nearly isotonic fluid as sweat limits the increase in plasma tonicity leaving hypovolemia and activation of renin-angiotensin sytem as perhaps more important, yet less potent, thirst stimuli during and after endurance exercise. Therefore, attenuation of thirst appears to play a role in involuntary dehydration. Human athletes have learned the importance of maintaining body fluid homeostasis during prolonged exercise and will force themselves to drink rehydration

solutions in the absence of thirst. In contrast, modification of drinking behavior is not feasible in equine athletes. The end result is that equine endurance athletes may often be placed at greater risk of developing heat stroke/exhaustion than human athletes.

Many factors can affect voluntary drinking and the degree of involuntary dehydration in human athletes including volume of fluid imbibed and the temperature, flavoring, and electrolyte content of fluid available for rehydration. Further, considerable individual variability in drinking has also been observed. In contrast, factors affecting development of involuntary dehydration in horses have not been well studied. Most prior studies focused on enhancing rehydration during and following exercise, and thereby counteracting involuntary dehydration, have involved "forced" rehydration rather than manipulation to enhance voluntary rehydration. Thus, the objective of my studies was to investigate involuntary dehydration and factors affecting thirst and voluntary water intake in exercising horses. The following hypotheses were tested: *The magnitude of "involuntary dehydration" in horses is affected by: i) volume of water initially made available after dehydration is induced; ii) type of rehydration solution offered (water vs. saline); and iii) temperature of fluid available for rehydration.*

Specific aims:

1. Demonstrate that the magnitude of "involuntary dehydration" (or volume of water imbibed) is affected by the volume of water (4l vs.8l vs. unlimited water intake) initially made available for rehydration following exercise-induced dehydration.

2. Demonstrate that the magnitude of "involuntary dehydration" (or volume of rehydration solution imbibed) is affected by the type of solution (water vs. 0.45% NaCl solution vs. 0.9% NaCl solution) initially made available for rehydration following furosemide-and exercise-induced dehydration.
3. Demonstrate that the magnitude of "involuntary dehydration" (or volume of water imbibed) is affected by temperature of rehydration fluid initially made available for rehydration following furosemide-and exercise-induced dehydration (10°C vs.20°C vs. 30°C).

Of interest, improving the hydration status of equine endurance athletes during the first hour of recovery could be of great benefit by decreasing the magnitude of dehydration prior to further stresses including further exercise or prolonged transport. Initially providing the most appropriate rehydration fluid could enhance total water intake during and after completion of exercise and improved recovery would be expected to decrease the risk of developing medical problems after exercise and transport. If the hypotheses noted above can be substantiated, the results should provide important and practical recommendations for management of equine athletes competing in a number of athletic endeavors.

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Chapter 2

Effect of varying initial drink volume on rehydration of horses

Prawit Butudom, Harold C. Schott II, Shannon M. Axiak,
Brian D. Nielsen, and Susan W. Eberhart

Department of Large Animal Clinical Sciences, College of Veterinary Medicine, and Department of Animal Sciences, College of Agriculture and Natural Sciences, Michigan State University, East Lansing, MI 48824, USA.

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ABSTRACT

Body weight (BW), water intake (WI), and plasma osmolality (P_{osm}) and electrolyte concentrations were measured in six 2-year-old Arabian horses provided either 4 l, 8 l, or an unlimited (UW) amount of water for drinking during the initial 5 min of recovery from 45 km of treadmill exercise. After weighing, horses were placed in a stall and further WI between 20 and 60 min of recovery was measured. During exercise, horses lost 3.3 ± 0.3, 3.2 ± 0.1, and 3.3 ± 0.2% ($p>0.05$) of BW and P_{osm} increased by 7.2 ± 0.5, 7.9 ± 0.8, and 7.7 ± 0.5 mOsm/kg ($p>0.05$) for 4 l, 8 l, and UW, respectively. WI during the first 5 min of recovery was 4.0 ± 0.0, 8.0 ± 0.0, and 9.0 ± 1.3 l and was accompanied by 2.4 ± 0.4, 5.8 ± 0.9, and 6.1 ± 0.7 mOsm/kg decreases ($p<0.05$) in P_{osm} for 4 l, 8 l, and UW, respectively. Between 20 and 60 min of recovery, WI was 6.2 ± 1.5, 1.2 ± 0.6, and 1.0 ± 0.7 l ($p<0.05$) for 4 l, 8 l, and UW, respectively. Thus, total WI was 10.2 ± 1.5, 9.2 ± 0.6, and 10.0 ± 1.1 L ($p>0.05$) for 4 l, 8 l, and UW, respectively. After 60 min of recovery, persisting BW loss was 1.3 ± 0.5, 1.1 ± 0.2, and 1.0 ± 0.2% ($p>0.05$) for 4 l, 8 l, and UW, respectively ($p>0.05$) and P_{osm} had returned to pre-exercise values for all treatments. In conclusion, limiting the volume of water initially provided to horses dehydrated by endurance exercise had no significant effect on total WI during the initial 60 min of recovery; however, persisting BW loss was observed with all treatments. Further, following exercise-induced dehydration, the primary stimulus of thirst was an increase in plasma tonicity rather than hypovolemia.

Introduction

During exercise, maintenance of total body water requires matching water intake (WI) with water loss (via respiration and sweat). However, such a balance rarely occurs such that prolonged exercise usually leads to development of dehydration. Dehydration is often the combined result of lack of intake (when water is not available) and a mismatch between thirst and the magnitude of the body water deficit. However, even when water or other rehydration solutions are readily available, body water losses are only partially replaced by drinking during and shortly after the exercise bout. This incomplete restoration of body fluid, usually measured as persisting body weight (BW) loss, has been termed both voluntary (Hubbard *et al.* 1984) and involuntary dehydration (Greenleaf 1992).

The primary stimulus for voluntary water intake is an increase in plasma tonicity (Fitzsimons 1998). Since sweating results in loss of both water and electrolytes, the increases in plasma osmolality (P_{osm}) and sodium concentration ($[Na^+]$) during exercise are less than would be produced by loss of a similar volume of pure water. Consequently, involuntary dehydration appears to develop because the loss of electrolytes in sweat attenuates development of plasma hypertonicity. The magnitude of involuntary dehydration observed in human endurance athletes is often around a 2% BW loss, although considerable variability has been observed (Hubbard *et al.* 1984; Greenleaf 1992; Szlyk *et al.* 1989). In contrast to human sweat that is hypotonic relative to plasma (Convertino *et al.* 1996), equine sweat is nearly isotonic to plasma (McCutcheon and Geor 1996). As a result, exercising horses lose comparatively greater amounts of

electrolytes with each liter of sweat produced. Horses also have a greater reserve of water and electrolytes in ingesta in the lumen of the gastrointestinal tract (12 to 13% of BW, Meyer 1989a; Meyer 1989b), in comparison to human athletes (1 to 2% of BW, Greenleaf 1992), and this reservoir can be used to partially replace sweat fluid losses during exercise. Due to these species differences, it would be reasonable to expect equine athletes to develop a greater magnitude of involuntary dehydration (BW loss) following endurance exercise. In fact, field studies have demonstrated that endurance horses experience BW losses of 3 to 10% during 80- and 160-km competitions (Ecker and Lindinger 1995; Schott *et al.* 1997; Schott *et al.* 1996), and a 3 to 4% BW loss may still persist after an overnight recovery period (Schott *et al.* 1997; Schott *et al.* 1996). Further, in both human and equine athletes, dehydration consequent to prolonged exercise is a well-recognized risk factor for failure to complete the event and development of medical problems in the recovery period (Sawka 1992; Geor and McCutcheon 1996).

After many competitive events, horses are often limited to a small drink of water immediately after completion. Free access to water is subsequently provided 15 to 60 min later after the horse has been washed off and cooled out. This traditional practice has no basis in science but has been propagated over the centuries because providing an unlimited amount of water to hot horses has been suggested to cause acute abdominal pain or laminitis, a painful condition affecting the hooves (Hinton 1987). We suspected that an initial small drink of water could actually be detrimental for rehydration after exercise as it could produce a rapid decline in plasma tonicity and attenuate thirst when water was again provided later. Thus, we hypothesized that limiting immediate water

intake by horses dehydrated by endurance exercise would decrease total WI during the initial hour of recovery and, thereby, potentiate the magnitude of involuntary dehydration.

Materials and Methods

Horses and conditioning program

Six 2-year-old Arabian horses (three geldings and three fillies) were studied. They were conditioned by performing three 15-km exercise bouts weekly on a treadmill (Mustang 2000, Kagra AG, Fahrwangen, Switzerland) and all horses completed two 30-km exercise bouts before the first experimental run. After each training run, horses were immediately offered water (18 to 22°C) in a hand-held bucket while standing on the treadmill to accustom them to post-exercise water availability. Throughout the training and study period, horses were maintained at pasture with free access to water and no supplemental feed or salt was provided. The training program and experimental protocol were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Michigan State University.

Experimental protocol

All horses performed three 45-km experimental runs and experiments on each horse were separated by a minimum of 10 days. The experimental run (Figure 1) consisted of three 15-km treadmill exercise bouts (0° slope) separated by 15 min rest periods. Each 15-km exercise bout lasted 52 min during which horses worked at speeds varying between 1.6 m/s (walk) and 8 m/s (canter) to simulate a typical 45-km endurance ride.

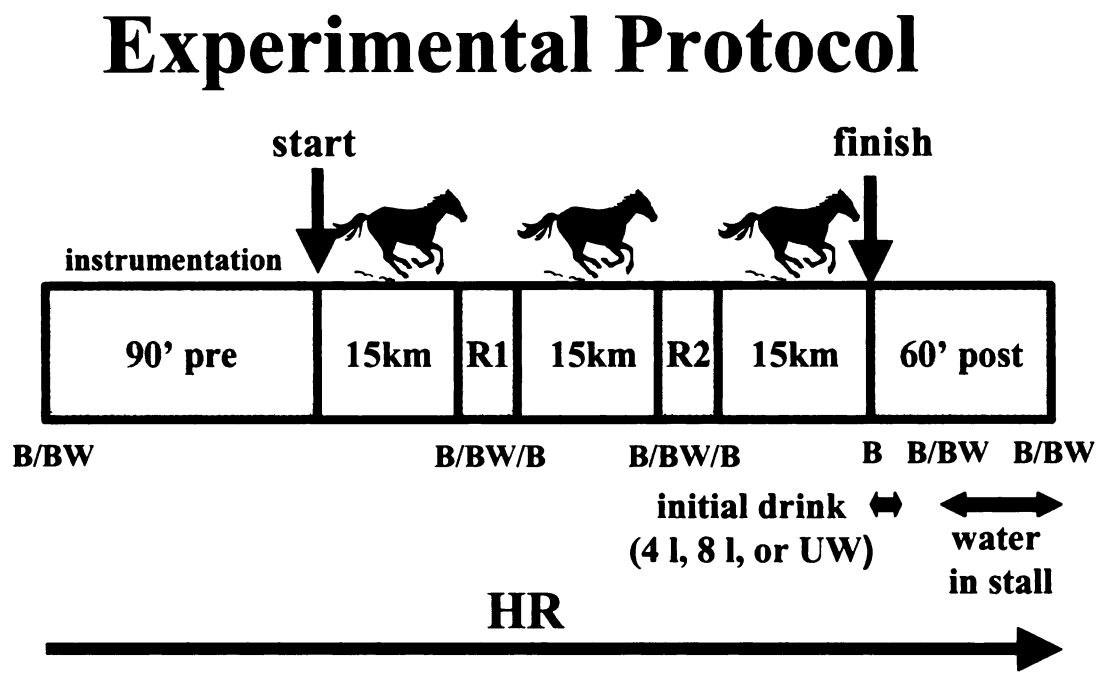

Figure 1. Experimental protocol. Horses were instrumented during the 90 min before exercise. The experimental run consisted of three 15-km exercise bouts separated by two 15 min rest periods. Immediately (within 10 s) after completion of exercise, either 4 l, 8 l, or an unlimited amount (UW) was offered to the horses, in a randomized order, for voluntary drinking (shorter double-headed arrow). After 5 min of recovery, horses were walked off the treadmill and washed down with water. After 20 min of recovery, horses were placed free in a stall (without feed) and further voluntary water intake from 20 to 60 min of recovery was measured (longer double-headed arrow). Blood (B) was collected and body weight (BW) and heart rate (HR) were recorded at various times point during the experiment.

BW was measured on a digital scale (± 0.5 kg) 90 min before the start of exercise (prior to instrumentation), 5 min after completion of each 15-km exercise bout, and 20 and 60 min after completion of the experimental run. All feces produced after initial weighing were collected, and although samples were not collected, urination was also recorded. No water was offered during the experimental run but immediately (within 15 seconds) after completion of the experimental run, water was offered to the horses in a hand-held bucket while they were standing on the treadmill. Horses were offered, in a randomized order, 4 l (~1/2 of a 10 l bucket), 8 l (nearly a full bucket), or an unlimited amount (UW, 2 full buckets were available) of water for voluntary drinking. After 5 min, the bucket was taken away and horses were walked off the treadmill. A nasogastric tube was used to administer any volume of water that was not voluntarily drank when offered 4 or 8 l of water and horses were subsequently washed down with water and weighed. At 20 min post-exercise, horses were placed into a stall without feed but with free access to water in a large bucket (garbage can) and further WI from 20 to 60 min was measured.

Instrumentation, sample collection, and analysis

At 90 min before and at 20 and 60 min after completion of the 45-km run, blood samples were collected by jugular venipuncture and heart rate (HR) was determined by auscultation. During the remaining pre-exercise period, surface electrocardiographic electrodes were applied (for telemetric recording of HR) and a 7 French, 110 cm catheter (Swan Ganz flow directed thermodilution catheter, American Edwards Laboratories, Añasco, Puerto Rico) was aseptically passed into the pulmonary artery via an 8 French introducer inserted into the right jugular vein (for collection of mixed venous blood

samples). HR was recorded at the start, during the 4 m/s (trot) and 8 m/s (canter) phases, and 1 and 5 min after completion of each 15-km exercise bout. Mixed venous blood was collected into heparinized and dry plastic syringes at the start and finish of each 15 km exercise bout and after the initial 5 min of recovery. The heparinized samples were placed on ice until analyzed for plasma electrolyte (Na^+, K^+, and Cl^-) concentrations within 30 min of collection (Stat Profile 9 Analyzer, Nova Biomedical, Waltham, MA). The remainder of each sample was used for measurement of hematocrit (microhematocrit method), plasma protein concentration (PP, by refractometry), and P_{osm} by freezing point depression (Model 3MO plus Advanced Micro-Osmometer, Advanced Instruments, Norwood, MA).

Data analysis

All values provided in the text are presented as means ± SE. Data were initially analyzed by a two way repeated measures ANOVA (SigmaStat, Jandel Scientific, St. Paul, MN) to assess main effects of time and treatment (amount of water offered) and, when F ratios were significant ($p<0.05$), a Student-Newman-Keuls post-hoc test was performed to detect specific differences. One way repeated measures ANOVA was also performed on selected data (total WI and BW loss at the end of the experiment) and, when F ratios were significant ($p<0.05$), a Student-Newman-Keuls post-hoc test was again used. Correlation analyses were also performed to examine the relationships between persisting BW loss and total WI during the 60 min recovery period and between plasma [Na^+] after 20 min of recovery and further WI from 20 to 60 min of recovery.

Results

All six horses completed the three experimental runs. During the experimental runs, ambient temperature ranged between 18.0 and 21.5°C (mean ± SE = 19.5 ± 0.2 °C) and relative humidity ranged between 53 and 92% (mean ± SE = 76.7 ± 2.6%) and there were no differences between treatments. Temperature of the water provided ranged between 18.5 °C and 22.0 °C (mean ± SE = 19.3 ± 0.3 °C). Mean HR ranged from 106.7 ± 1.4 to 110.7 ± 2.0, from 138.6 ± 1.9 to 143.3 ± 1.9, and from 67.8 ± 2.6 to 70.8 ± 2.6 beats/min during trot (4 m/s) and canter (8 m/s) phases of each exercise bout and after 1 min of recovery, respectively. No differences in HR over time (first through third 15-km exercise bout) or between treatments were observed.

By the end of the 45-km run, horses had lost 3.3 ± 0.3, 3.2 ± 0.1, and 3.3 ± 0.2% ($p<0.05$ compared to pre-exercise values and $p>0.05$ for treatment) of their pre-exercise BW for the 4 l, 8 l, and UW treatments, respectively (Table 1 and Figure 2). After 20 min of recovery, persisting BW loss was greater ($p<0.05$) for the 4 l treatment than for the 8 l or UW treatments (Fig. 2) but this was an expected consequence of the experimental design. After 60 min of recovery, persisting BW loss was 1.3 ± 0.5, 1.1 ± 0.2, and 1.0 ± 0.2% ($p<0.05$ compared to pre-exercise values and $p>0.05$ for treatment) for 4 l, 8 l, and UW, respectively. Mean fecal production was 1.0 ± 0.4, 0.9 ± 0.2, and 0.8 ± 0.1 kg ($p>0.05$) for 4 l, 8 l, and UW, respectively. Two horses passed urine during one of the rest breaks or recovery period with the 4 l treatment while three horses passed urine with each of the other treatments.

At the end of exercise, horses drank water immediately after it was offered and the majority of WI was observed within the first minute of recovery. All horses consumed 4 l of water but three horses initially offered 8 l had to have remaining amounts (0.5, 1.5, and 7.5 l) administered by a nasogastric tube. Initial WI for the UW treatment ranged from 4 to 12.5 l (mean 9.0 ± 1.3 l) and no adverse effects of unlimited drinking were apparent. From 20 to 60 min of recovery, horses initially offered 4 l consumed a greater ($p<0.05$) amount of water (6.2 ± 1.5 l) in comparison to horses initially offered 8 l (1.2 ± 0.6 l) or UW (1.0 ± 0.7 l). When combined, total WI during the 60 min recovery period was 10.2 ± 1.5, 9.2 ± 0.6, and 10.0 ± 1.1 l ($p>0.05$) for 4 l, 8 l, and UW, respectively (Figure 3). In addition, there was a significant negative correlation ($r = -0.53$, $p<0.02$) between total WI and persisting BW loss at the end of the 60 min recovery period (Figure 4).

Plasma protein (PP) concentration increased ($p<0.05$) by the end of exercise with all treatments, reflecting a 3 to 5% decrease in plasma volume during exercise, but values were not different from the pre-exercise values at any recovery time (Table 1). By the end of the experimental run, P_{osm} had increased by 7.2 ± 0.5, 7.9 ± 0.8, and 7.7 ± 0.5 mOsm/kg ($p>0.05$) for 4 l, 8 l, and UW, respectively (Table 1). During the first 5 min of recovery, WI was accompanied by decreases in P_{osm} of 2.4 ± 0.4, 5.8 ± 0.9, and 6.1 ± 0.7 mOsm/kg ($p<0.05$) for 4 l, 8 l, and UW, respectively. After 20 min of recovery, P_{osm} had decreased (from end-exercise values) by 3.9 ± 0.9, 7.8 ± 1.4, and 8.2 ± 0.5 mOsm/kg ($p<0.05$) for 4 l, 8 l, and UW, respectively. Although differences between treatments were not observed, P_{osm} remained increased ($p<0.05$) from the pre-exercise value after 20 min of recovery for the 4 l treatment alone. From 20 to 60 min of recovery further WI

was accompanied by decreases in P_{osm} of 3.1 ± 1.1, 0.2 ± 1.1, and 1.5 ± 0.8 mOsm/kg ($p>0.05$) for 4 l, 8 l, and UW, respectively.

Similar to P_{osm}, plasma [Na^+] increased during the experimental run by 3.4 ± 0.6, 3.3 ± 0.4, and 3.1 ± 0.2 mmol/l ($p>0.05$) for 4 l, 8 l, and UW, respectively (Table 1). During the first 5 min of recovery, WI was accompanied by decreases in plasma [Na^+] of 1.4 ± 0.6, 1.8 ± 0.3, and 1.8 ± 0.5 mmol/l ($p>0.05$) for 4 l, 8 l, and UW, respectively. After 20 min of recovery, plasma [Na^+] had decreased (from end-exercise values) by 2.0 ± 0.8, 2.7 ± 0.5, and 3.7 ± 0.6 mmol/l ($p>0.05$) for 4 l, 8 l, and UW, respectively. From 20-60 min of recovery, further WI was accompanied by decreases in plasma [Na^+] of 1.8 ± 0.5, 0.8 ± 0.3, and 0.2 ± 0.5 mmol/l ($p>0.05$) for 4 l, 8 l, and UW, respectively. Further, there was a significant positive correlation ($r = 0.53$, $p<0.02$) between plasma [Na^+] after 20 min of recovery and additional WI from 20 to 60 min of the recovery period (Figure 4). Unlike plasma [Na^+], significant changes in plasma [K^+] and [Cl^-] were not observed during exercise or recovery (Table 1).

		pre-exercise	end exercise	20 min recovery	60 min recovery
BW	4 liters	373.1 ± 13.3	360.7 ± 13.3*	364.6 ± 13.3*	368.3 ± 14.2*
(kg)	8 liters	373.3 ± 13.3	361.4 ± 12.9*	369.4 ± 12.8*	369.0 ± 13.3*
	unlimited	370.8 ± 14.6	358.8 ± 14.2*	367.8 ± 14.7*	367.0 ± 14.6*
Hct	4 liters	34.4 ± 1.4	38.9 ± 1.1*	37.1 ± 0.9	34.4 ± 2.1
(%)	8 liters	34.3 ± 1.0	39.0 ± 1.2*	35.7 ± 1.1	34.0 ± 2.0
	unlimited	34.0 ± 0.8	39.3 ± 1.1*	37.0 ± 1.0	36.7 ± 1.0
PP	4 liters	6.2 ± 0.2	6.5 ± 0.2*	6.2 ± 0.3	6.2 ± 0.3
(g/dl)	8 liters	6.3 ± 0.2	6.6 ± 0.3*	6.4 ± 0.3	6.4 ± 0.3
	unlimited	6.3 ± 0.3	6.5 ± 0.3*	6.3 ± 0.3	6.2 ± 0.2
Posm	4 liters	283.7 ± 1.8	290.8 ± 1.6*	287.0 ± 1.0*	283.9 ± 1.9
(mOsm/kg)	8 liters	284.9 ± 1.2	292.8 ± 1.6*	285.0 ± 1.8	284.8 ± 1.5
	unlimited	287.2 ± 1.6	294.9 ± 1.4*	286.7 ± 1.1	285.2 ± 1.6
$[Na^+]$	4 liters	141.2 ± 0.9	144.6 ± 0.4*	142.6 ± 0.8	141.0 ± 0.5
(mmol/L)	8 liters	141.4 ± 0.5	144.8 ± 0.6*	142.1 ± 0.5	141.3 ± 0.7
	unlimited	141.6 ± 0.7	144.7 ± 0.6*	141.0 ± 0.6	140.8 ± 0.3
$[K^+]$	4 liters	3.3 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	2.8 ± 0.1
(mmol/L)	8 liters	3.3 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	3.0 ± 0.1
	unlimited	3.6 ± 0.1	3.4 ± 0.1	3.1 ± 0.1	3.1 ± 0.1
$[Cl^-]$	4 liters	105.0 ± 0.8	105.9 ± 0.9	105.9 ± 0.9	105.5 ± 0.9
(mmol/L)	8 liters	105.6 ± 1.0	106.6 ± 1.2	105.9 ± 0.9	105.4 ± 0.7
	unlimited	106.0 ± 1.0	107.0 ± 1.0	104.5 ± 0.7	105.0 ± 1.0

* significantly different ($p<0.05$) from the pre-exercise value (within each row)

Table 1. Body weight (BW), hematocrit (Hct), plasma protein (PP), plasma osmolality (Posm), sodium, potassium, and chloride concentrations in six horses before and after completion of 45-km of treadmill exercise and after 20 and 60 min of recovery with rehydration with 4 l, 8 l, or an unlimited amount of water during the initial 5 min of the recovery period.

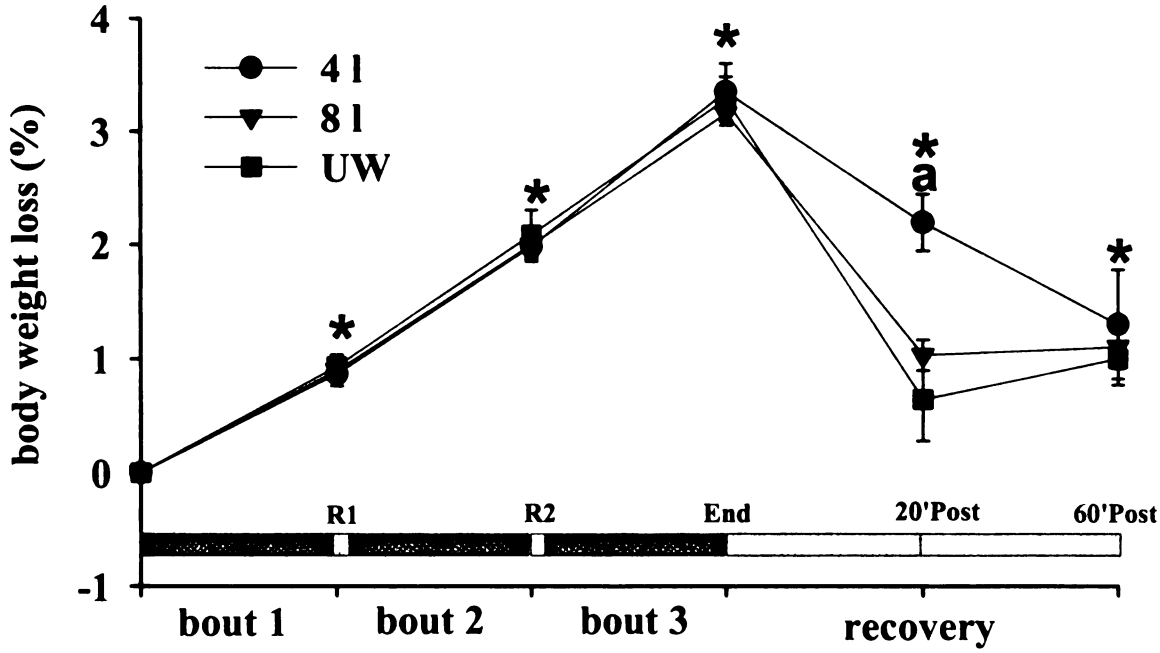

Figure 2. Percent body weight loss during and after 45-km of treadmill exercise for horses offered 4 l (filled circles), 8 l (filled inverted triangles), or an unlimited amount (UW, filled squares) during the initial 5 min of recovery. * = significantly different ($p<0.05$) from the pre-exercise value; a = 4 l significantly different ($p<0.05$) from 8 l and UW; see Figure 1 legend for description of the experimental protocol. R1 = rest 1 (15 min) after the first bout of exercise. R2 = rest 2 (15 min) after the second bout of exercise. 20'Post = 20 min after the end of exercise. 60'Post = 60 min after the end of exercise.

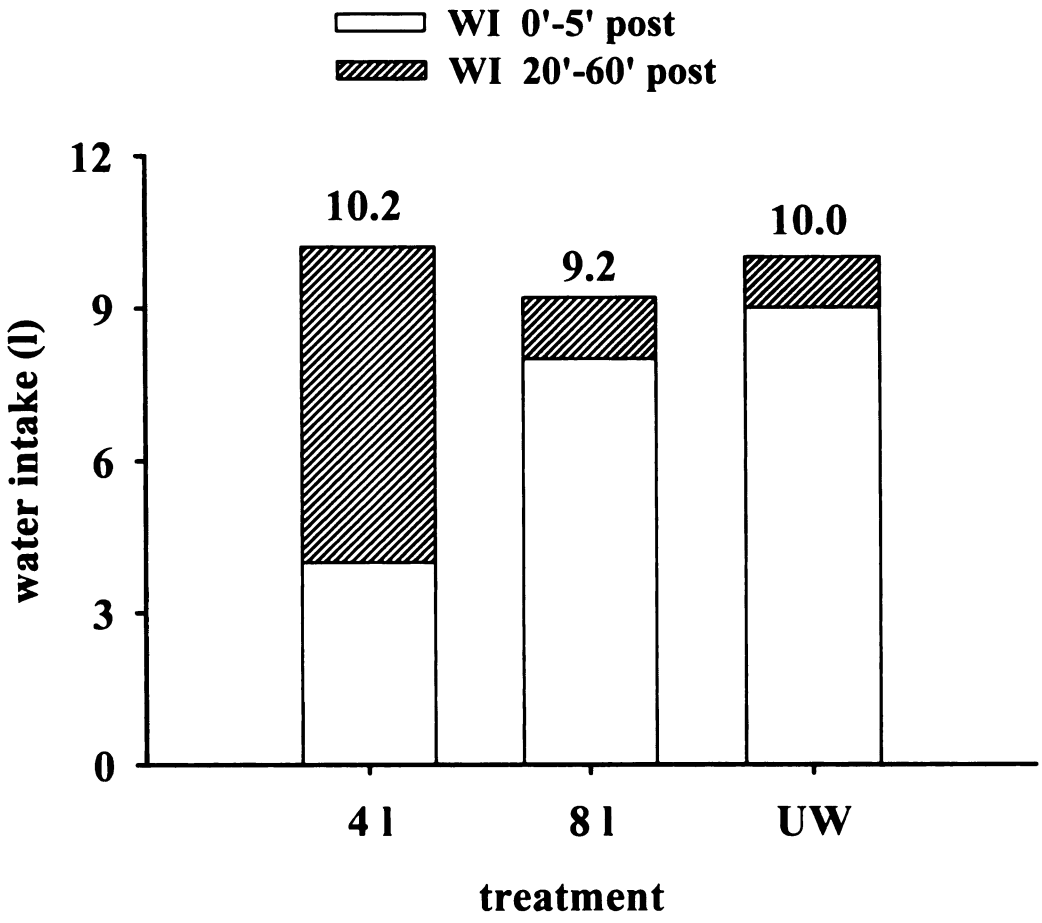

Figure 3. Total water intake during the 60 min recovery period after induction of dehydration by 45-km of treadmill exercise for horses provided 4 l, 8 l, or an unlimited amount (UW) of water during the initial 5 min following exercise and subsequently provided free access to water from 20 to 60 min following exercise. WI 0-5' = water intake during 0 to 5 minute post-exercise; WI 20-60 = water intake during 20 to 60 minute of recovery.

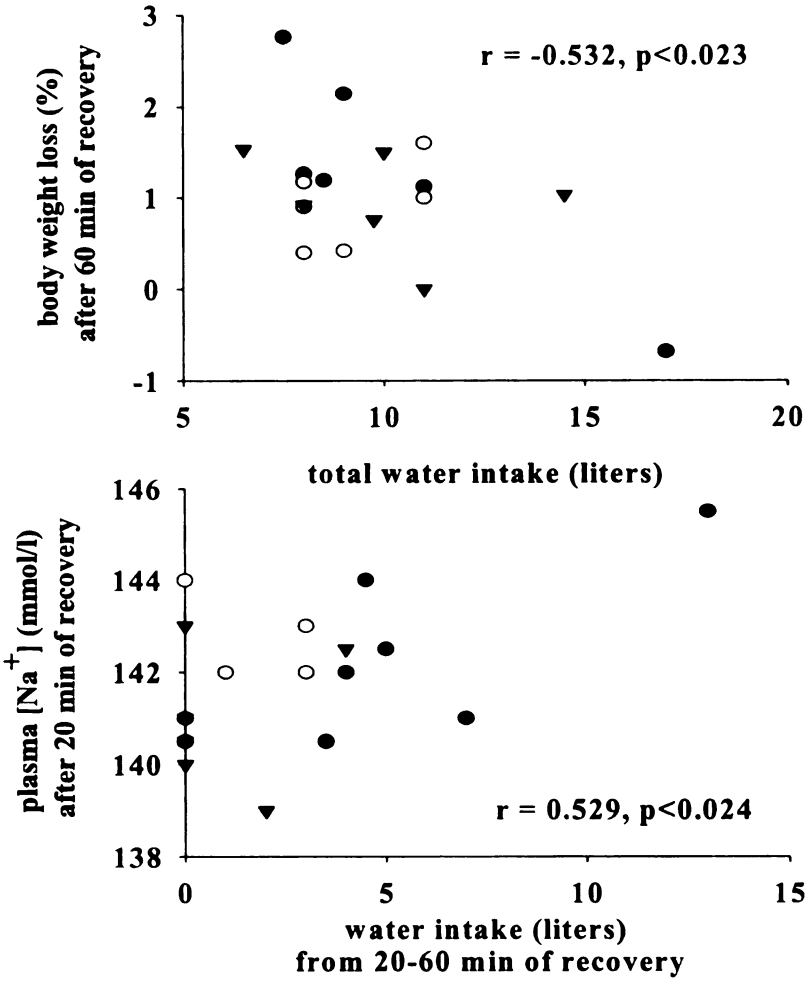

Figure 4. Upper panel. Correlation between body weight loss persisting after 60 min of recovery (magnitude of involuntary dehydration) and total water intake from 0 to 60 min of the recovery period after induction of dehydration by 45-km of treadmill exercise for horses provided 4 l (filled circles), 8 l (open circles), or an unlimited amount (UW, filled inverted triangles) of water during the initial 5 min following exercise and subsequently provided free access to water from 20 to 60 min following exercise. Lower panel. Correlation between plasma $[Na^+]$ after 20 min of recovery and water intake from 20 to 60 min of the recovery period for horses initially provided 4 l (filled circles), 8 l (open circles), or UW (filled inverted triangles) of water.

Discussion

The results of this study demonstrate that offering horses a small drink of water immediately after exercise did not substantially blunt the thirst response when water was again provided after a short cooling down period. Thus, our hypothesis that limiting immediate WI by horses dehydrated by endurance exercise would decrease total WI during the initial hour of recovery, and thereby increase the magnitude of involuntary dehydration, was not supported by these data. However, involuntary dehydration was observed (~1% persisting BW loss [range of 0 to 2.8%] with a mean loss of 4.3 ± 0.7 kg) at the end of the recovery period with all treatments despite free access to water from 20 to 60 min of recovery. The fact that the magnitude of the persisting BW loss was inversely correlated to total WI during the recovery period supports inadequate thirst as the primary mechanism responsible for involuntary dehydration.

Although feces and urine were produced during and after the experimental runs and contributed to BW loss, these losses were only partly responsible for the persisting BW loss after 60 min of recovery. Mean values for fecal production during exercise and recovery were less than 1 kg for all treatments, in comparison to a mean BW loss exceeding 4 kg. Urine was also voided by about half of the horses and explains the tendency for BW loss to increase from 20 to 60 min of recovery with the 8 l and UW treatments (Figure 2) despite further intake of a small amount of water. Although not quantified in this study, mares produced urine at a rate of ~5 ml/min during a similar experimental run in our laboratory (Düsterdieck *et al.* 1999). As a result, it can be estimated that these horses produced 1.5 to 2.0 l of urine from the start to the finish of the

experiment (~5½ h in duration). Assuming that horses passed ~3 l during voiding (including residual urine in the bladder at the start of the experiment), urine losses could be responsible for a mean BW loss of ~1.5 kg (because only half of the horses voided urine). Thus, passage of feces and urine may explain about half, but not all, of the persisting BW loss at the end of the recovery period in these horses.

In contrast to actual competitive events during which horses are sometimes encouraged to drink by frequent offering of small amounts of water, horses in this study were not allowed to drink until the end of the 45-km run. As a consequence, P_{osm} and plasma $[Na^+]$ increased by 2 to 3% during the experimental run indicating that water was lost to a greater extent than Na^+ during exercise. Assuming that equine sweat $[Na^+]$ is similar to that of plasma (McCutcheon and Geor 1996), water would have to be lost via other routes (respiration and urine production) in exercising horses. For these ~370 kg Arabian horses with ~80 l of extracellular fluid (ECF, Lindinger and Ecker, 1995), a 3 mmol/l increase in plasma $[Na^+]$ would represent a loss of ~2 l of free water from ECF. This would equate to 15 to 20% of the total fluid loss during exercise (10 to 12 l), a value that could easily be attributed to respiratory water loss (Hodgson *et al.* 1993). In contrast to $[Na^+]$, plasma $[Cl^-]$ remained unchanged during exercise and this lack of change can be attributed to a greater $[Cl^-]$ in sweat than plasma (McCutcheon and Geor 1996).

Unlike the findings in these horses, increases in plasma $[Na^+]$ from the start to finish of endurance events have not usually been observed in field studies (Ecker and Lindinger 1995; Schott *et al.* 1997; Lindinger and Ecker 1995; Carlson and Mansmann 1974; Lucke

and Hall 1980; Rose *et al.* 1983; Rose *et al.* 1980). However, when blood samples have also been collected near the mid-point of competition (Schott *et al.* 1997; Rose *et al.* 1983) or when the event included faster speed phases (e.g., the steeplechase portion of 3-day-events, Andrews *et al.* 1995), mild increases in plasma [Na^+] have been found. The lack of a persisting increase in plasma [Na^+] at the end of competition can be attributed to WI at rest breaks during the events. Although both an increase in plasma tonicity and a decrease in blood (plasma) volume are well-documented stimuli for thirst (Fitzsimons 1998), an increase in plasma tonicity appears to be the more important stimulus during and after endurance exercise. This statement is supported by the significant positive correlation found between plasma [Na^+] after 20 min of recovery (that remained increased from the pre-exercise value for horses initially offered 4 l) and subsequent WI from 20 to 60 min of the recovery period. Further, PP (and presumably plasma volume) had returned to pre-exercise values after 20 min for all treatments and PP at 20 min of recovery was not correlated ($r=-0.05$, $p>0.83$) with WI from 20 to 60 min of the recovery period. In a previous experiment in which horses completed a similar experimental run, WI was also significantly correlated with the increase in plasma [Na^+] induced by administration of oral electrolyte pastes (Düsterdieck *et al.* 1999). In that experiment electrolyte administration was actually accompanied by a decrease in PP and, presumably plasma volume expansion, during the later stages of the exercise bout.

Consistent with previous studies of dehydrated horses (Houpt *et al.* 1991; Sneddon *et al.* 1993) and ponies (Sufit *et al.* 1985), drinking occurred immediately after water was provided. In our horses essentially all WI occurred within 1 to 2 minutes after stopping

exercise and little further WI was observed during the remainder of the initial 5 min recovery period. When water deprivation was used to induce dehydration, horses and ponies completely recovered the BW loss with initial drinking and the increases in P_{osm} and plasma $[Na^+]$ were corrected within 15 to 30 min of drinking (Sneddon *et al.* 1993; Sufit *et al.* 1985). In contrast, when horses and ponies were dehydrated by furosemide administration (resulting in sodium losses in urine similar to losses in sweat during exercise), initial WI replaced only about two-thirds of the fluid lost in urine (Schott *et al.* 1996; Sufit *et al.* 1985; Schott *et al.* 2002). Thus, similar to endurance exercise, involuntary dehydration was produced when dehydration was induced by loss of urine with a $[Na^+]$ similar to that in plasma. However, regardless of the method used to induce dehydration (water deprivation, furosemide administration, or exercise), rapid declines in P_{osm} and plasma $[Na^+]$ after WI indicate that intestinal function (at least for water absorption) is well maintained in dehydrated horses and ponies (Schott *et al.* 1996; Sneddon *et al.* 1993; Sufit *et al.* 1985). This observation remained true even when the magnitude of induced dehydration (12% BW loss after 72 h of water deprivation in desert dwelling horses) was substantial (Sneddon *et al.* 1993). The fact that more than 50% of the decreases in P_{osm} and plasma $[Na^+]$ observed between the cessation of exercise to 20 min of recovery were observed within the initial 5 min of recovery in our horses further supports that absorption of imbibed water is rapid in dehydrated horses.

Not surprisingly, values for P_{osm} and plasma $[Na^+]$ below the starting values have been measured in horses performing prolonged (160-km) endurance events, especially late in the event or during the recovery period (Schott *et al.* 1997; Carlson and Mansmann 1974;

Rose *et al.* 1980). During and after exercise, WI leads to replacement of ECF losses with a hypotonic fluid that dilutes Na^+ remaining in ECF. In human athletes, development of hyponatremia during prolonged exercise has actually been described as water intoxication (Noakes *et al.* 1985), especially when the competitors drank water frequently during the exercise bout. In addition to ongoing water and Na^+ loss in sweat, a shift of sodium-poor intracellular fluid (ICF) to the ECF during the later stages of prolonged exercise is an additional contributor to dilution of Na^+ remaining in ECF (Lindinger and Ecker 1995). This latter mechanism may be of greater importance in development of hyponatremia in exhausted endurance horses (Schott *et al.* 1997), rather than excessive water drinking. However, prior to development of exhaustion, both mechanisms act to limit increases in P_{osm} and $[Na^+]$, leading to lack of thirst despite a deficit of total body fluid.

In both human and equine athletes, partial replacement of electrolytes lost in sweat is achieved by drinking beverages containing electrolytes or (in horses) oral administration of concentrated electrolyte pastes. In both species, intake of electrolytes may attenuate, but rarely prevents, development of involuntary dehydration following exercise (Greenleaf 1992; Düsterdieck *et al.* 1999). In human athletes, complete recovery of body fluid losses incurred by sweating during endurance exercise actually requires drinking a volume of an electrolyte-containing beverage that exceeds the volume of sweat produced in order to compensate for ongoing urine production (Shirreffs *et al.* 1996). Since athletes would rarely drink such large volumes of a sports drink during or following exercise, full restoration of electrolyte deficits usually accompanies meal ingestion following exercise that is accompanied by further drinking to replace the water deficit (Maughan *et al.*

1996). Although the effects of post-exercise meal consumption on restoration of electrolyte and water deficits in horses have been little studied, it is likely of similar, if not greater, importance to equine athletes that have a large reservoir of water and electrolytes in ingesta in the intestinal tract (Meyer 1989a; Meyer 1989b).

In conclusion, we hypothesized that a small initial drink of water could produce rapid decreases in P_{osm} and plasma $[Na^+]$, thereby satiating thirst. However, total WI during the 60 min recovery period was not different with the three volumes of water initially offered to these horses. Although our hypothesis was refuted, the persisting BW loss at the end of the 60 min recovery period provides further support that horses performing endurance exercise develop involuntary dehydration despite free access to water. It is important to mention that the run distance performed by these horses was relatively short (45-km) in comparison to competitive endurance rides that are typically 80 to160 km. The shorter distance (and duration) of exercise is the most likely explanation for the lesser degree of involuntary dehydration observed in these horses, in comparison to a persisting 3 to 4% BW loss after overnight recovery reported in field studies (Schott *et al.* 1997; Schott *et al.* 1996). To limit or prevent involuntary dehydration, human endurance athletes have learned to force themselves to drink throughout the competition, even when thirst is absent. Unfortunately, "forced" drinking is not an option for horses and when combined with species differences in sweat composition, it is not without merit to suggest that involuntary dehydration may be a greater problem for equine endurance athletes than their human counterparts. In addition, horses are often transported long distances within a few hours after completion of an endurance competition. The muscular work performed

to maintain balance during transport is essentially a continuation of the exercise bout and involuntary dehydration prior to transport could increase the risk of developing exhaustion and associated medical disorders in this species (Hinton 1977; Schott and Charlton 1996). Thus, a practical recommendation would be to offer all horses frequent opportunities to drink during endurance competition and to provide water *ad libitum* immediately after completion of exercise. Finally, our data can also be added to the pool of knowledge used to educate horse owners that providing unlimited water to horses that have just finished exercise is not only a safe practice but is preferable to limiting WI.

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Chapter 3

Drinking salt water enhances rehydration in horses dehydrated by furosemide administration and endurance exercise

Prawit Butudom, Harold C. Schott II, Matthew W. Davis, Christina A. Kobe, Brian D. Nielsen, and Susan W. Eberhart

Department of Large Animal Clinical Sciences, College of Veterinary Medicine, and Department of Animal Sciences, College of Agriculture and Natural Sciences, Michigan State University, East Lansing, MI 48824, USA.

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ABSTRACT

Because the primary stimulus for thirst is an increase in plasma tonicity, we hypothesized that dehydrated horses would voluntarily drink a greater total volume of fluid during the first hour of recovery when they were initially offered salt water. To test this hypothesis, body weight (BW), fluid intake (FI), and [Na^+] were measured in six 2-year-old Arabian horses offered three rehydration solutions. After dehydration was induced by furosemide administration (1 mg/kg, IV) followed by 45 km of treadmill exercise, water (W), 0.45% NaCl, or 0.9% NaCl was offered, in a randomized order, during the initial 5 min after completing exercise. Horses were subsequently placed in a stall and further intake of plain water during the first hour of recovery was measured. By the end of exercise, horses lost 5.2 ± 0.2, 5.6 ± 0.3, and 5.7 ± 0.2 % ($p>0.05$) of BW and FI during the first 5 min of recovery was 10.5 ± 0.7, 11.6 ± 0.8, and 11.6 ± 1.5 l ($p>0.05$) for W, 0.45% NaCl, and 0.9% NaCl, respectively. After 20 min of recovery, [Na^+] had decreased with W but remained unchanged from the end exercise values for both saline solutions. During the initial hour of recovery, further water intake was 0.9 ± 0.4, 5.0 ± 0.5, and 6.9 ± 0.7 l ($p<0.05$) for W, 0.45% NaCl, and 0.9% NaCl, respectively. Thus total FI was 11.4 ± 0.5, 16.6 ± 0.7, and 18.5 ± 1.7 l ($p<0.05$) for W, 0.45% NaCl, and 0.9% NaCl, respectively, and persisting BW loss after 60 min of recovery was greater ($p<0.05$) for W (3.5%) than for the two saline solutions (2.4% for 0.45% NaCl and 1.9% for 0.9% NaCl). In conclusion, providing salt water as the initial rehydration fluid maintained an elevated [Na^+] and resulted in greater total FI and recovery of BW loss during the first hour of recovery, in comparison to offering only plain water.

Introduction

During and after endurance exercise, replacement of water and electrolytes lost in sweat is important for prevention of medical problems that may develop consequent to dehydration (Geor and McCutcheon 1996). However, concurrent loss of water and electrolytes in sweat leads to a condition that has been termed both "voluntary dehydration" and "involuntary dehydration": incomplete voluntary replacement of body fluid losses due to a lack of thirst (Hubbard *et al.* 1984; Greenleaf 1992). The primary stimulus for thirst is an increase in plasma osmolality or, more specifically, an increase in plasma sodium concentration ($[Na^+]$) (Andersson 1978; Fitzsimons 1998). Because sweating results in loss of both body water and electrolytes, the increases in plasma osmolality and $[Na^+]$ concentration during exercise are less than would be produced by loss of a similar volume of pure water. Attenuation of these increases appears to be an important factor for limiting thirst and maintenance of dehydration.

The magnitude of involuntary dehydration in human athletes following endurance exercise is usually about 2% of body weight (BW), although considerable interindividual variability has been observed (Szlyk *et al.* 1989; Greenleaf 1992). As in human athletes, BW loss by horses during prolonged exercise has recently been shown to be an accurate estimate of fluid loss by sweating (Kingston *et al.* 1997). In endurance horses competing in 50 and 100 mile endurance rides (80 and 160 km rides), BW losses of 3 to 4% are common and these losses can persist after an overnight recovery period (Schott *et al.* 1997). Similarly, horses performing a simulated 60 km endurance ride on a treadmill also experienced a ~3% BW loss despite frequent access to water during the exercise bout

(Düsterdieck *et al.* 1999). In both of these studies, ambient conditions were mild but the comparatively greater loss of BW in horses suggests that the magnitude of involuntary dehydration may be greater in this species. This could be a consequence of the higher $[Na^+]$ in equine sweat and a comparatively greater loss electrolyte with each liter of sweat produced (Convertino *et al.* 1996; McCutcheon and Geor 1996).

Recent studies in both human and equine subjects have clearly demonstrated that post-exercise restoration of body fluids and recovery of BW is more rapid and complete when rehydration solutions containing electrolytes (primarily Na^+) are used in place of water (Hypppä *et al.* 1996; Nyman *et al.* 1996; Shirreffs *et al.* 1996; Maughan and Shirreffs 1997; Marlin *et al.* 1998a; Marlin *et al.* 1998b; Monreal *et al.* 1999). Although there have been a number of studies designed to assess treatments used to attenuate or correct dehydration in horses (work up to 1997 reviewed in Schott and Hinchcliff 1998; Marlin *et al.* 1998a; Marlin *et al.* 1998b; Sosa León *et al.* 1998; Düsterdieck *et al.* 1999; Monreal *et al.* 1999; Schott *et al.* 2001; Schott *et al.* 2002), most investigations have studied "forced" hyperhydration or rehydration by administering solutions via a nasogastric tube or use of oral electrolyte pastes to stimulate drinking. In contrast, there has been little study of voluntary rehydration in which horses are allowed to drink solutions of varying composition.

An early investigation by Randall *et al.* (1978) found that euhydrated weanling foals would readily drink saline solutions as long as the concentration did not exceed 0.6% NaCl. More recently, other investigators have shown that euhydrated horses can also be trained to

drink 0.9% NaCl solutions (Houpt *et al.* 1995). Anecdotally, competitors in endurance events have also trained their horses to drink "salt water" but further data is needed to determine if this practice is truly effective before recommendations can be made to competitors. Thus, we tested the hypothesis that horses dehydrated by a combination of furosemide administration and prolonged exercise would voluntarily drink a greater total volume of fluid during the first hour of recovery when they were first offered salt water, in comparison to water, during the initial 5 min of recovery.

Materials and Methods

Horses and conditioning program

Six 2-year-old Arabian geldings ranging in weight from 362 to 449 kg (mean ± SE = 384.4 ± 13.7 kg) were studied. The horses were trained on a treadmill[1] three times a week for a period of 5 weeks and all horses completed two 30 km exercise bouts before the first experimental run. Saline solutions (either 0.45% NaCl or 0.9% NaCl) were offered after each conditioning run to train horse to drink salt water. Throughout the study period (May through August, 2000), horses were maintained at pasture with free access to water and no supplemental feed or salt was provided. The training program and all experimental protocols were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Michigan State University.

Experimental protocol

Horses were brought in from pasture on the afternoon before each experiment and housed overnight in a stall with free access to a grass/alfalfa mixed hay and water. On the

morning of each experimental run, initial dehydration was induced by furosemide[2] administration (1.0 mg/kg IV) 120 min before the start of the 45 km exercise bout. Because the goal was to produce dehydration in excess of a 4% BW loss, furosemide treatment was added to the experimental protocol. Otherwise, 75 to 90 km of treadmill exercise would have been required to produce this degree of dehydration under the mild ambient conditions in our laboratory and a longer exercise bout would have increased the risk of musculoskeletal injury. During the remaining pre-exercise period, surface electrocardiographic electrodes were applied (for telemetric recording of heart rate) and a 7 French, 110 cm Swan Ganz catheter[3] was aseptically passed into the pulmonary artery via an 8 French introducer inserted into the right jugular vein (for collection of mixed venous blood samples). Each horse was studied three times when offered, in a randomized order, either water, 0.45% NaCl, or 0.9% NaCl during the initial recovery period and experiments on each horse were separated by a minimum of 10 days during which further exercise was not performed.

During each experiment BW was measured on a digital scale (nearest 0.5 kg), heart rate was recorded, and a blood sample (~10 ml) was collected before and 120 min after (pre-exercise sample) furosemide administration. Horses then performed 45 km of treadmill exercise at varying speeds to simulate a 25 mile endurance ride (Figure 1). The experimental run consisted of three 15 km exercise bouts (lasting 56 min; speeds between 1.6 [walk] and 8 m/s [canter]; 0° slope) separated by 15 min rest periods. Horses did not have access to water or any other rehydration solution during the run. Immediately (within 10 s) after completion of exercise, one of the three rehydration solutions was

offered for voluntarily drinking from a hand-held bucket. After the initial 5 min of recovery, the rehydration solution was taken away and horses were walked off the treadmill, the pulmonary arterial catheter was removed, and horses were washed with water. The horses had no access to rehydration fluid from 5 to 20 min after the end of exercise. At 20 min of the recovery period, horses were weighed and placed free in a stall (without feed) and further voluntarily water intake and number of drinking episodes from 20 to 60 minutes of recovery was measured. After 60 min of recovery, horses were weighed again and returned to the stall where they remained overnight with free access to hay and water. A final weight was recorded after overnight recovery (~18 h after completion of the 45 km run).

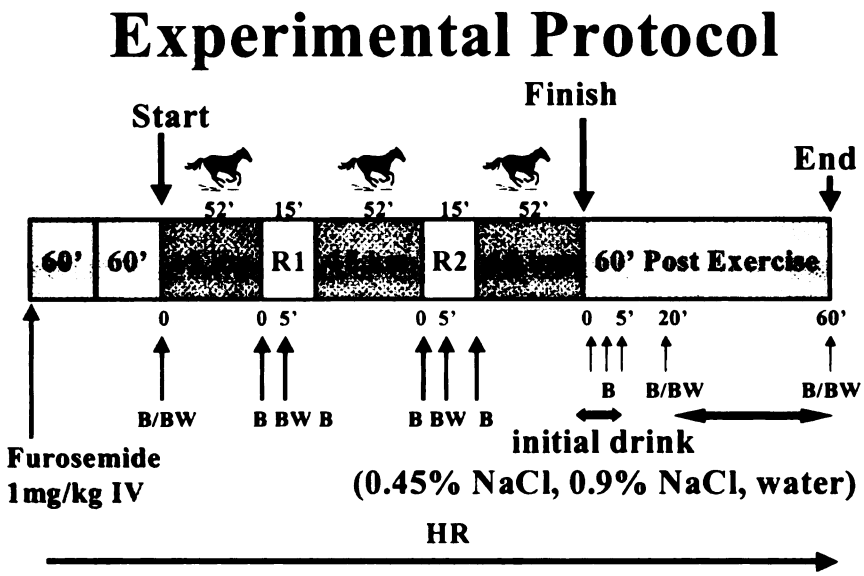

Figure 1. Experimental protocol. Furosemide (1.0 mg/kg IV) was given 2 h prior to and horses were instrumented during the 60 min before exercise. The experimental run consisted of three 15 km exercise bouts separated by two 15 minutes rest periods. Immediately (within 10 seconds) after completion of exercise, one of three rehydration fluids (water, a 0.45% NaCl solution, or a 0.9% NaCl solution) was offered to the horses, in a randomized order, for voluntary drinking. After 5 minutes of recovery, horses were walked off the treadmill and washed down with water. After 20 minutes of recovery, horses were placed free in a stall (without feed) and further voluntary water intake from 20 to 60 minutes of recovery was measured. Blood (B) was collected and body weight (BW) and heart rate (HR) were recorded at various times point during the experiment.

Sample collection and analysis

At 120 minutes before and at 20 min and 60 min and ~18 h after completion of the 45 km run, blood samples were collected by jugular venipuncture and heart rate was determined by auscultation. During exercise heart rate was recorded at the start, during the 4 m/s (trot) and 8 m/s (canter) phases, and 1 and 5 min after completion of each 15 km exercise bout. Mixed venous blood was collected at the start and finish of each 15 km of exercise bout and after 1, 3, and 5 min after completion of the 45 km exercise test. Blood was collected anerobically into heparinised plastic syringes that were placed on ice until analysed for plasma electrolyte (Na^+, K^+, and Cl^-) concentrations within 30 min of collection (Nova Stat Profile 9 Analyzer)[4]. The remainder of each sample was used for measurement of haematocrit (microhaematocrit method) and plasma protein concentration (PP, by refractometry). All feces and urine produced from the time of furosemide administration to the end of the 60 min recovery period were collected and weighed with the exception of urine produced prior to exercise in response to furosemide administration. BW loss (with the exception of ~18 h recovery) was corrected for fecal losses but not for urine losses because the latter was an important factor in the pre-exercise BW loss.

Data analysis

All values provided in the text are presented as means ± SE. Total fluid intake during the recovery period (magnitude of involuntary dehydration) were analyzed using commercial software[5] by a one way repeated measures analysis of variance ANOVA to assess differences between rehydration solutions. When F ratios were significant

($p<0.05$), a Student-Newman-Keuls test was performed to detect specific differences. Changes in parameters measured at multiple times (BW, heart rate, and blood values) were analyzed by a two way repeated measures analysis of variance (main effects of time and treatment) and, when F ratios were significant ($p<0.05$), a Student-Newman-Keuls post-hoc was again performed to detect specific differences. Selected relationships between parameters measured were also examined by Pearson product moment correlation analysis.

Results

All six horses completed the three experimental runs. Ambient temperature in the laboratory ranged between 20 and 27°C (mean 24.6 ± 0.4°C) and relative humidity ranged between 48 and 72% (mean 59.9 ± 1.8%) during exercise and significant differences between the runs for each rehydration solution were not observed. Heart rate during the trot and canter portions of the exercise bouts had mean values of 108 ± 1 and 136 ± 1 beats/min and differences between each 15 km exercise bout or between treatments were not observed.

Furosemide administration produced a BW loss prior to exercise of 1.7 ± 0.3, 1.8 ± 0.2, and 2.1 ± 0.3% ($p>0.05$) for water (control), 0.45% NaCl, and 0.9% NaCl treatments, respectively. The combined effects of furosemide administration and 45 km of treadmill exercise resulted in BW losses of 5.2 ± 0.2, 5.6 ± 0.3, and 5.7 ± 0.2% ($p<0.05$ in comparison to starting values for all treatments; $p>0.05$ for treatment differences) for water, 0.45% NaCl, and 0.9% NaCl treatments, respectively (Table 1 and

Figure 2). After 60 min of recovery, BW remained decreased ($p<0.05$) from starting values for all treatments (3.4 ± 0.1, 2.3 ± 0.2, and 1.9 ± 0.3% for water, 0.45% NaCl, and 0.9% NaCl treatments, respectively). However, BW loss was greater ($p<0.05$) for water in comparison to both saline solutions. None of the horses produced more than 2 kg of feces during the entire experiment and the volume of urine produced from the start of exercise to the end of the 60 min recovery period was not different (3.0 ± 0.7, 2.5 ± 0.7, and 2.4 ± 0.6 l for water, 0.45% NaCl, and 0.9% NaCl treatments, respectively). The loss of ~1% of BW as urine explains the further BW loss observed with the control treatment from 20 to 60 min of recovery (Figure 2). After overnight recovery, BW remained decreased ($p<0.05$) from starting values for all treatments (Table 1) and the magnitude was significantly greater ($p<0.05$) for the water treatment (2.3 ± 0.2%) in comparison to both saline treatments (1.6 ± 0.2 and 1.2 ± 0.2% for 0.45% NaCl and 0.9% NaCl treatments, respectively).

The amount of fluid imbibed during the initial 5 min of recovery was not different among treatments (10.5 ± 0.7, 11.6 ± 0.8, and 11.6 ± 1.5 l for water, 0.45% NaCl, and 0.9% NaCl groups, respectively) and replaced ~50% of lost body fluid (Figure 3). With all rehydration solutions, horses drank immediately when the bucket was offered and the majority of drinking occurred within the first minute of recovery. From 20 to 60 min of recovery, voluntary water intake was greater ($p<0.05$) for both saline treatments (5.0 ± 0.5 and 6.9 ± 0.7 l for 0.45% NaCl and 0.9% NaCl, respectively) than for the control treatment (0.9 ± 0.4 l). When combined, these values resulted in a total voluntary fluid intake during the 60 min recovery period of 11.4 ± 0.5, 16.6 ± 0.7, and 18.5 ± 1.7 l for

water, 0.45% NaCl, and 0.9% NaCl treatments, respectively ($p<0.05$, water less than the two saline solutions, Figure 3). The number of drinking episodes from 20 to 60 min of recovery was also greater ($p<0.05$) for both saline treatments (4.0 ± 0.6 and 4.2 ± 0.7 for 0.45% NaCl and 0.9% NaCl, respectively) in comparison to the control treatment (1.2 ± 0.3). When total fluid intake was compared to persisting BW loss (magnitude of involuntary dehydration) at the end of the 60 min recovery period, a highly significant negative correlation was found (Figure 4).

Haematocrit decreased ($p<0.05$) after furosemide administration and subsequently increased ($p<0.05$) during the exercise bout (Table 1). Plasma protein and Na^+ concentrations increased ($p<0.05$) from starting values during the 45 km exercise bout (Table 1). PP remained elevated for all treatments after 20 min of recovery but values were not different from the starting values by 60 min of recovery. In contrast, plasma $[Na^+]$ after 20 min of recovery decreased to the starting value with water treatment but remained increased for both the 0.45% NaCl and 0.9% NaCl treatments. In fact, after 20 min of recovery, plasma $[Na^+]$ was greater for the 0.9% NaCl treatment, in comparison to both the water and 0.45% NaCl treatments, and after 60 min of recovery, plasma $[Na^+]$ remained increased from the starting value for the 0.9% NaCl treatment. After overnight recovery, plasma $[Na^+]$ was decreased ($p<0.05$) from the starting value for the water treatment but this value was not different from plasma $[Na^+]$ for the two saline treatments at this time point.

Plasma [K^+] decreased ($p<0.05$) after furosemide administration and remained decreased from the starting values for all treatments throughout the 60 min recovery period (Table 1). Plasma [Cl^-] tended to decrease after furosemide administration but this was not a significant finding. In contrast, plasma [Cl^-] was decreased ($p<0.05$) from the starting value after 20 min of recovery with water treatment and remained decreased after 60 min of recovery with this treatment (Table 1).

		pre-furosemide	pre-exercise	end exercise	20 min recovery	60 min recovery	~18 h recovery
BW	water	392.8 ± 11.8	387.1 ± 13.0*	372.5 ± 11.3*	383.0 ± 11.8*	379.3 ± 11.5*	383.8 ± 11.6*
(kg)	0.45%NaCl	391.7 ± 14.4	385.3 ± 13.7*	369.6 ± 13.8*	381.3 ± 14.1*	382.6 ± 14.0*	385.2 ± 14.2*
	0.9%NaCl	386.1 ± 13.4	377.8 ± 13.0*	363.9 ± 12.1*	375.4 ± 13.2*	378.8 ± 13.4*	381.5 ± 13.4*
Hct	water	37.8 ± 2.0	35.0 ± 0.9*	39.8 ± 0.8*	38.2 ± 0.7	37.4 ± 1.2	37.8 ± 1.2
(%)	0.45%NaCl	37.2 ± 1.3	34.1 ± 1.3*	40.6 ± 0.6*	37.8 ± 1.0	36.6 ± 1.2	37.2 ± 1.6
	0.9%NaCl	37.2 ± 1.6	34.3 ± 1.8*	41.0 ± 1.2*	39.2 ± 1.2	36.7 ± 1.0	36.0 ± 0.4
PP	water	58.9 ± 1.8	62.7 ± 1.6*	65.3 ± 1.6*	64.2 ± 1.6*	63.6 ± 1.6	60.5 ± 2.0
(g/l)	0.45%NaCl	58.8 ± 1.6	60.9 ± 1.8*	65.7 ± 1.8*	65.4 ± 1.6*	63.0 ± 1.6	59.4 ± 1.1
	0.9%NaCl	60.4 ± 1.8	66.1 ± 1.6*	68.2 ± 1.6*	69.4 ± 1.6*	65.7 ± 1.6	60.6 ± 2.1
$[Na^+]$	water	139.6 ± 0.7	141.2 ± 0.8*	143.5 ± 0.7*	140.7 ± 1.0 [b]	140.3 ± 0.8	137.6 ± 0.4*
(mmol/L)	0.45%NaCl	139.5 ± 0.8	141.5 ± 0.7*	144.3 ± 0.9*	142.4 ± 0.8* [ab]	140.4 ± 0.9	138.3 ± 0.6
	0.9%NaCl	139.2 ± 0.7	141.7 ± 0.9*	143.4 ± 0.8*	144.3 ± 0.7* [a]	142.4 ± 0.6*	139.1 ± 0.4
$[K^+]$	water	3.3 ± 0.1	3.0 ± 0.1*	3.0 ± 0.1*	2.7 ± 0.1*	2.4 ± 0.1*	3.5 ± 0.2
(mmol/L)	0.45%NaCl	3.4 ± 0.1	2.8 ± 0.1*	3.1 ± 0.1*	2.7 ± 0.1*	2.5 ± 0.1*	3.3 ± 0.1
	0.9%NaCl	3.6 ± 0.1	2.9 ± 0.1*	3.1 ± 0.1*	2.8 ± 0.1*	2.5 ± 0.1*	3.3 ± 0.1
$[Cl^-]$	water	104.8 ± 0.9	103.3 ± 0.9	102.8 ± 0.9	101.9 ± 1.0*	101.3 ± 1.0*	102.8 ± 0.8
(mmol/L)	0.45%NaCl	103.8 ± 0.9	103.0 ± 0.9	102.7 ± 1.0	103.3 ± 0.9	102.4 ± 0.9	103.2 ± 0.4
	0.9%NaCl	103.8 ± 1.0	102.8 ± 0.9	101.9 ± 0.9	102.7 ± 0.9	102.8 ± 1.0	102.8 ± 0.9

* significantly different ($p<0.05$) from the pre-furosemide value (within each row)
[a] different superscripts within a column are significantly different ($p<0.05$)

Table 1. Body weight (BW), haematocrit (Hct), and plasma protein (PP), sodium, potassium, and chloride concentrations in six horses before and 2 hours after (pre-exercise) administration of furosemide (1 mg/kg IV), after completion of 45 km of treadmill exercise, and after 20 and 60 min of recovery with rehydration with water, 0.45% NaCl, or 0.9% NaCl during the initial 5 min of the recovery period.

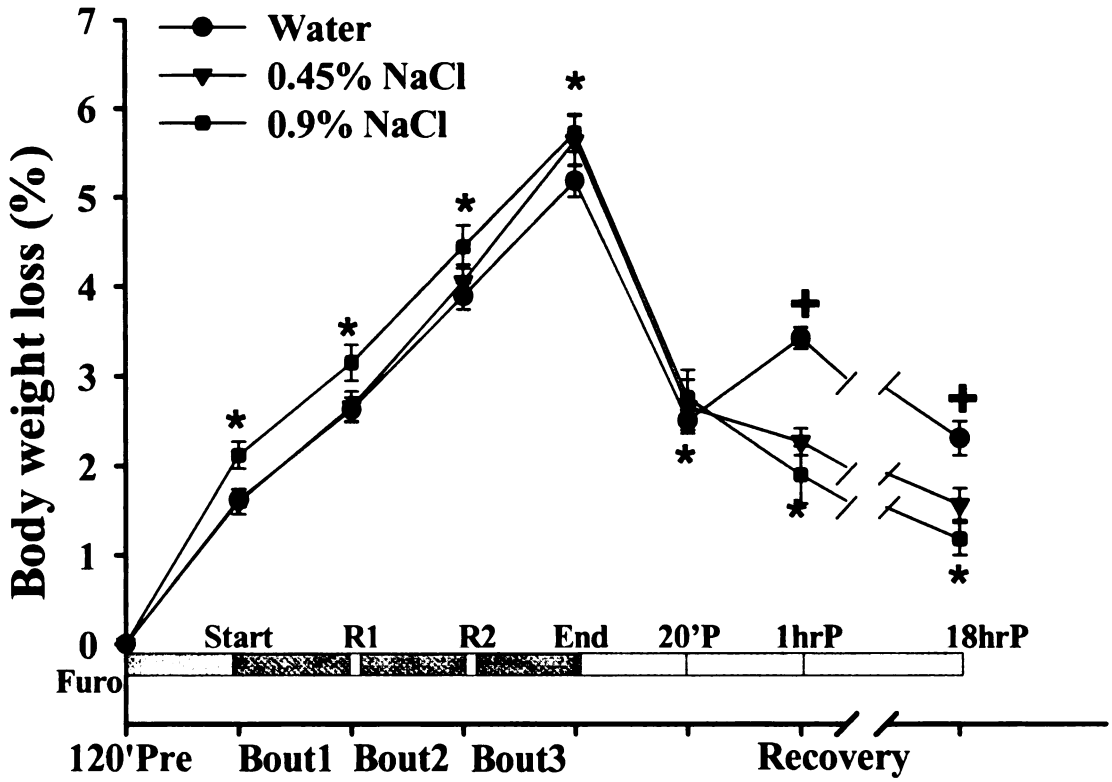

Figure 2 Percent body weight loss after furosemide treatment (1 mg/kg IV) and during and after 45 km of treadmill exercise ride for horses offered water (filled circles), 0.45% NaCl (filled rectangles), or 0.9% NaCl (filled triangles) during the initial 5 min of recovery. See Figure 1 legend for explanation of the experimental protocol. 120'Pre = 120 min pre-exercise. Furo = Furosemide. R1 = rest 1 (15 min. after bout 1). R2 = rest 2 (15 min, after bout 2). 20'P = 20 min after the end of exercise. 1hrP = 1 hour after the end of exercise. 18hrP = 18 hours after the end of exercise. * = significantly different ($P<0.05$) from 120'Pre. $^{+}$ = significantly different ($P<0.05$) from 0.45% and 0.9% NaCl.

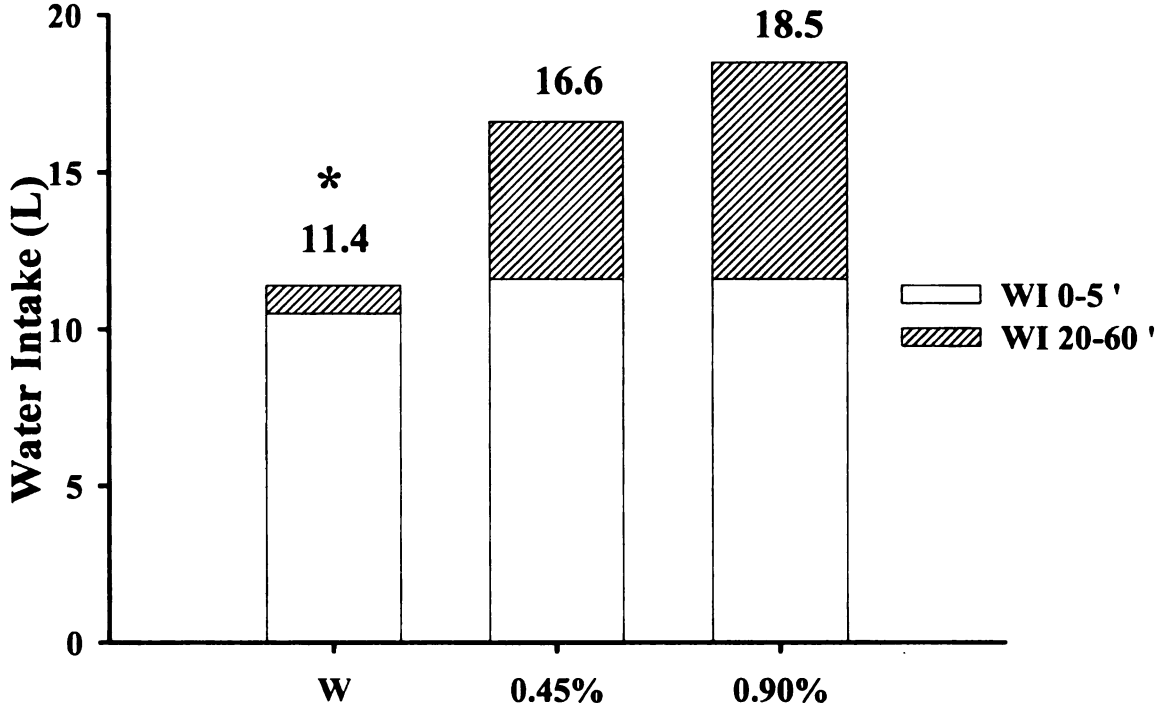

Figure 3. Total fluid intake during the 60 minute recovery period after induction of dehydration by furosemide (1 mg/kg IV) treatment followed by 45 km of treadmill exercise for horses provided water, 0.45% NaCl, or 0.9% NaCl as the rehydration fluid during the initial 5 min following exercise and subsequently provided free access to water from 20 to 60 min following exercise. See Figure 1 legend for explanation of the experimental protocol. WI 0-5' = fluid intake during 0-5 minute post-exercise; WI 20-60 = fluid intake during 20 to 60 minute of recovery; * = Significantly different ($P<0.05$) from 0.45% NaCl and 0.9% NaCl.

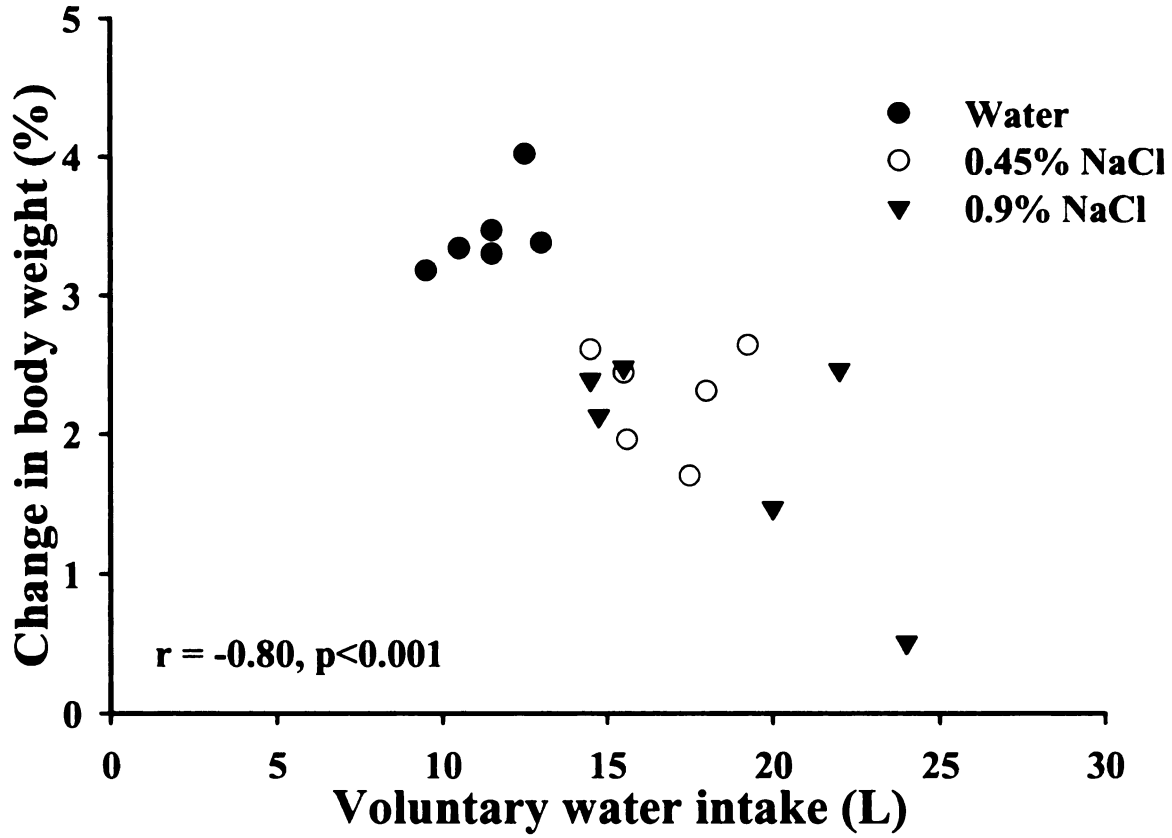

Figure 4. Correlation between total fluid intake from 0 to 60 minutes of the recovery period and persisting body weight loss (magnitude of involuntary dehydration) after 60 minutes of recovery after induction of dehydration by furosemide (1 mg/kg IV) treatment followed by 45 km of treadmill exercise for horses provided water (filled circles), 0.45% NaCl (open circles), or 0.9% NaCl (filled inverted triangles) as the rehydration fluid during the initial 5 min following exercise and subsequently provided free access to water from 20 to 60 min following exercise.

Discussion

The results of this study demonstrate that offering salt water as the initial rehydration solution to horses dehydrated by furosemide administration and endurance exercise can be an effective strategy to increase total fluid intake during the early recovery period. Although initial rehydration with either 0.45% NaCl or 0.9% NaCl did not result in full replacement of body fluid losses, it decreased the magnitude of dehydration to ~2% (for 0.9% NaCl) after the initial hour of recovery, a value comparable to that observed in human endurance athletes after a similar recovery period (Hubbard *et al.* 1984; Szlyk *et al.* 1989; Greenleaf 1992). Although provision of salt water in this study was short in duration and dehydration was induced, in part, by furosemide administration, the data are relevant to horses competing in endurance events that may have limited time to recover before being subjected to a long trailer ride. Even though BW remained decreased from the starting value for all treatments after overnight recovery, initial rehydration with either saline solution attenuated the magnitude of BW loss at this time point as well. The latter finding suggested that most of the electrolytes ingested in the saline rehydration solutions during the initial 5 min of recovery were likely retained in the body fluids of these horses. Retention of the electrolytes imbibed in an initial drink of salt water would clearly be beneficial to horses competing again on the following day.

Of the previous studies investigating rehydration during and after endurance exercise in horses, only one has evaluated voluntary drinking of different rehydration fluids. In that 62 km field study under mild ambient conditions, Nyman *et al.* (1996) offered horses frequent access to either plain water or a 0.9% NaCl solution during the exercise bout and

the initial hour of recovery. At the end of exercise, horses drinking plain water had replaced 38% of their BW loss while those drinking a 0.9% NaCl solution had replaced of 45% of their estimated losses. Although this difference was small, horses that had been offered water alone only drank a further 2 l while those provided the 0.9% NaCl solution drank 14 l of water from 1 to 3 h of recovery and had much greater recovery of BW loss. In our study, horses were not offered any rehydration fluid during the exercise bout but initial drinking of a saline solution also resulted in greater subsequent water intake and recovery of BW from 20 to 60 min of recovery. Taken together, the results of both studies demonstrate that saline solutions can be effectively used, in a variety of manners, to improve rehydration and recovery after endurance exercise.

During the initial 5 min of recovery, the volume of rehydration solution imbibed was not different for any of the treatments (range 8 to 15.5 l). This suggested that composition of the rehydration solution was not an important factor in determining initial fluid intake. Because the volumes drank were similar to the reported capacity of the equine stomach (Pfeiffer and MacPherson 1990), satiation or cessation of drinking was likely a consequence of gastric filling as has been described in rats (Engstrom and Deaux 1974). This suggestion is supported by the observation that the majority of initial drinking also occurred within the first minute after rehydration fluid was offered.

From studies of the diuretic response of horses to furosemide (Freestone *et al.* 1989; Sosa León *et al.* 1998) and sweat electrolyte concentrations in exercising horses (McCutcheon and Geor 1996), the amount of Na^+ lost by these horses likely approached

3000 to 3500 mmol (~1500 mmol after furosemide administration and ~ 1500 to 2000 mmol in sweat [12 to 15 l sweat x 130 mmol/l]). The initial drink of 0.9% NaCl would have provided only 1700 to 1800 mmol of both ions and replaced less than approximately half of the sodium deficit. Nevertheless, when coupled with increased absorption of electrolytes and water from the gastrointestinal tract, the 0.9% NaCl solution may have provided sufficient Na^+ to replace the majority of the deficit. In previous studies of treadmill exercise (Düsterdieck *et al.* 1999) and furosemide-induced dehydration (Schott *et al.* 2002) in our laboratory, supplementation of NaCl as an oral paste at doses estimated to fully replace the electrolytes lost in sweat or urine was accompanied by increased urinary Na^+ losses following electrolyte administration. These results suggested that the doses administered were either greater than the losses (unlikely based on estimated losses) or were greater than needed because a substantial portion of the deficits may have been replaced by intestinal stores.

In horses fed a hay diet, ingesta in the large intestine has been estimated to contain a reservoir of ~15 l of water, containing ~2000 to 2500 mmol each of Na^+, K^+, and Cl^- (Meyer 1989a; Meyer 1989b). This reservoir would provide ~125 to 150 g of NaCl or ~15 l of 0.9% NaCl. Absorption of these intestinal reserves is likely an important reason that horses performing endurance exercise rarely develop significant hyponatremia despite substantial Na^+ losses in sweat and typical replacement with a hypotonic fluid (water). Although not measured in these horses, increases in aldosterone concentration (ALD) have been observed in horses performing endurance exercise under both field and laboratory conditions (Nyman *et al.* 1996; Schott *et al.* 1997; Schott *et al.* 1999). The

ALD response has been attributed to Na^+ losses in sweat and it was either abolished or attenuated in horses administered oral electrolyte pastes or saline water, respectively (Nyman *et al.* 1996; Schott *et al.* 1999). Attenuation of the ALD response when horses in this study drank 0.45% NaCl or 0.9% NaCl would have been expected and would have provided further information as to whether initial drinking of salt water was partly or completely effective in replacing the Na^+ deficit induced by furosemide administration and endurance exercise.

In the horses that initially drank salt water, plasma [Na^+] remained increased from the starting values at 20 min of recovery while rapid intestinal absorption of fluid by the horses that drank water led to a decrease in plasma [Na^+]. Similarly, PP decreased to a greater extent after an initial drink of water while PP remained relatively unchanged after drinking saline. Taken together, these observations could support more rapid intestinal absorption of water than saline. More importantly, the fact that plasma [Na^+] remained elevated was likely an important stimulus for greater water drinking by both saline groups once water was again offered from 20 to 60 min of recovery (Schott *et al.* 1999; Schott *et al.* 2002). Although the composition of the initial rehydration fluid could have also affected gastric emptying rate (GER), it is unlikely that saline solutions slowed GER as Sosa-León *et al.* (1998) found no decrease in GER with saline solutions with osmolalities up to twice that of 0.9% NaCl. The fact that horses that initially drank salt water also readily drank greater amounts of water from 20 to 60 min of recovery further supports that GER was not slowed by the saline solutions offered. Although it remained well within the reference range, plasma [Na^+] actually decreased below the starting value

after overnight recovery in horses that were initially offered water. Thus, it is likely that the greater magnitude of persisting BW loss in the horses offered only water was related to depletion of body electrolyte stores despite the fact that horses had free access to hay during the overnight recovery period. This observation further supports that continued salt supplementation in the form of either salt water, oral pastes, or electrolytes added to concentrate feed, would be an appropriate management recommendation for horses recovering from endurance exercise even when supplementation was provided before and during the competition.

Palatability of the rehydration fluid selected is another important factor that can affect voluntary drinking and post-exercise rehydration in human athletes (Szlyk *et al.* 1989). Although an early study by Randall *et al.* (1978) showed that the upper limit of NaCl concentration that horses would readily drink was 0.6%, that study was performed in weanling foals that were not dehydrated. Subsequent study (Houpt *et al.* 1995) found that horses would willingly drink 0.9% NaCl once they had become accustomed to offering of salt water. Further, adverse effects of drinking salt water at this concentration were not observed in our horses or in these prior studies. Finally, although not statistically significant, there was a tendency for greater water drinking after an initial drink of 0.9% NaCl in comparison to an initial drink of 0.45% NaCl. Thus, in horses that would be provided salt water as a rehydration strategy, a concentration of 0.9% would seem to be a reasonable concentration to employ.

In conclusion, our results and those of Nyman *et al.* (1996) clearly demonstrate that provision of salt water (0.9% NaCl solution) during and following endurance exercise is a better rehydration strategy than providing only water. Drinking salt water can attenuate loss of body fluid stores by stimulating greater subsequent water intake and it appears that the electrolytes imbibed in salt water are largely retained. Rehydration with plain water alone is not recommended because ingestion of this hypotonic fluid can lead to thirst inhibition and could delay overall recovery by an additional day or longer. The recommendation to provide salt water would be of greatest benefit to horses competing in endurance events lasting several days but should only be pursued after horses had been previously trained to drink salt water during and after exercise.

Footnotes

[1] Mustang 2000, Kagra AG, Fahrwangen, Switzerland.

[2] Lasix® (furosemide) injection 5%, Hoechst Roussel Vet, Warren, NJ.

[3] Swan Ganz flow directed thermodilution catheter, American Edwards Laboratories, Añasco, Puerto Rico.

[4] Stat 7 Profile Analyzer, Nova Biomedical, Waltham, MA.

[5]SigmaStat, Jandel Scientific, St. Paul, MN.

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Chapter 4

Rehydration fluid temperature affects voluntary drinking in horses dehydrated by furosemide administration and endurance exercise

Prawit Butudom, Donna J. Barnes, Matthew W. Davis,
Brian D. Nielsen, Susan W. Eberhart, and Harold C. Schott II

Department of Large Animal Clinical Sciences, College of Veterinary Medicine (Butudom, Davis, Barnes, Eberhart, and Schott), and Department of Animal Sciences, College of Agriculture and Natural Sciences (Nielsen), Michigan State University, East Lansing, MI 48824, USA.

ABSTRACT

There has been little study of the effect of rehydration fluid temperature on voluntary intake by dehydrated horses. In human athletes, temperature of the rehydration solution has been demonstrated to affect voluntary intake during the initial recovery period and the magnitude of dehydration. A recent study has been shown that offering a saline solution (0.9% NaCl) to dehydrated horses as the initial rehydration fluid after exercise results in greater total fluid intake (and attenuation of the magnitude of dehydration) than when plain water is offered. We hypothesized that dehydrated horses would voluntarily drink a greater total volume of cool fluid than warm fluid during the first hour of recovery. To test this hypothesis, we measured body weight (BW), fluid intake (FI), serum osmolality (Osm) and $[Na^+]$ in six horses offered three different temperatureS of 0.9% NaCl. After dehydration was induced by furosemide administration (1 mg/kg, IV) followed by 30 km of treadmill exercise; 10°C, 20°C and 30°C 0.9% NaCl was offered, in a randomized order, immediately after completing exercise. The initial rehydration fluid was removed after 5 minutes and horses were washed off and cooled out. After 20 min of recovery, horses were placed in a stall (without feed) and the volume of further voluntary water (at 10, 20, or 30°C) intake from 20 to 60 minutes of recovery were measured and the number of drink episodes was recorded. By the end of exercise, horses lost 4.2 ± 0.3, 4.5 ± 0.3, and 4.5 ± 0.2% of BW ($p<0.05$ in comparison to pre-furosemide values for all treatments; $p>0.05$ for treatment differences) and FI during the first 5 minutes of recovery was 9.8 ± 2.5, 12.3 ± 2.1, and 9.7 ± 2.0 L for 10, 20, and 30°C treatments, respectively. After 20 min of recovery, $[Na^+]$ increased by 3.6 ± 0.4, 2.6 ±

0.5, and 3.7 ± 0.5 ($P>0.05$) mmol/L. Between 20 and 60 min of recovery, the numbers of drinking episodes was not different among treatments ($P>0.05$), but water intake for 20°C (7.7 ± 0.8 L) and 30°C 0.9% NaCl (6.6 ± 1.2 L) was greater than for 10°C 0.9% NaCl (4.9 ± 0.5 L; $P<0.05$). When total fluid intake was compared to persisting BW loss at the end of the 60 min recovery period, a highly significant negative correlation ($r= -0.91$, $P<0.01$) was found. Thus, total FI was 14.7 ± 2.5 L, 19.9 ± 2.5 L ($P<0.05$, in comparison to 10°C 0.9% NaCl), and 16.3 ± 2.4 L for 10°C, 20°C and 30°C 0.9% NaCl, respectively. At 60 min of recovery, persisting BW loss was not different among treatments. In conclusion, providing rehydration fluid at near ambient (20°C) temperature resulted in greater voluntary fluid intake by the end of the initial 60-minute recovery period, in comparison to offering cool (10°C) or warm (30°C) temperatures.

Introduction

Replacement of water and electrolytes lost in sweat during endurance exercise is important for continued work as well as for prevention of medical problems that may develop during the recovery period (Geor and McCutcheon, 1996). However, both human and equine athletes fail to completely replace body fluid losses by voluntary drinking during the first few hours of the recovery period, despite free access to various rehydration solutions (Engell *et al.*, 1987; Schott *et al.*, 1997). This condition, measured as persisting body weight (BW) loss, has been termed both "voluntary dehydration" and "involuntary dehydration" and has been attributed to blunted thirst (Hubbard *et al.*, 1984; Greenleaf, 1992). Because sweating results in loss of both water and electrolytes, exercise-induced dehydration results in lesser increases in plasma osmolality (P_{osm}) and sodium concentration ($[Na^+]$) than dehydration induced by water deprivation. Since the primary stimulus for thirst is an increase in plasma tonicity (Andersson, 1978; Fitzsimons, 1998), attenuation of these increases with sweating appears to be an important factor for limiting thirst and rehydration during and after exercise.

Restoration of body fluids and recovery of BW following exercise, in both human and equine athletes, is more rapid and complete when rehydration solutions containing electrolytes are used in place of plain water (Maughan and Shirreffs, 1994; Hyyppä *et al.*, 1996; Nyman *et al.*, 1996; Shirreffs *et al.*, 1996; Marlin *et al.*, 1998). However, most previous studies have investigated the effects of "forced" hydration by instructing subjects to drink specific amounts or, in horses, by administering solutions via a nasogastric tube. In contrast, there has been less investigation of voluntary drinking

during or after exercise in either species. In human subjects with varying degrees of hypohydration, fluid intake during a one hour recovery period, although insufficient to replace BW losses, was correlated with the increase in P_{osm} during exercise (Engell *et al.*, 1987). Recent studies in our laboratory have shown a similar relationship between the increase in plasma tonicity and voluntary fluid intake by horses dehydrated by endurance exercise (Düsterdieck *et al.*, 1999; Butudom *et al.*, 2002).

In addition to electrolyte content, more subtle factors including temperature and flavoring of rehydration solutions also affect voluntary intake by human athletes (Adolph and Wills, 1947; Boulze *et al.*, 1983; Sandick *et al.*, 1984; Szlyk *et al.*, 1989). The influence of water temperature has been fairly well documented in human athletes: following exercise, they both prefer and drink greater volumes of cool water, in comparison to water at temperatures at or above ambient temperature (Boulze *et al.*, 1983; Sandick *et al.*, 1984; Szlyk *et al.*, 1989). In addition, cool or cold water ingested during and after exercise can act as a heat sink and lower core temperature (Wimer *et al.*, 1997). In horses, there has been little study of the effects of water temperature on voluntary drinking. In the only study we could find, ponies stabled in a cold environment (near freezing) consumed a greater volume when warm (31-48°C) water was offered, in comparison to when cold (0-4°C) water was offered (Kristula and McDonnell, 1994).

There have been no studies in horses examining the effects of water temperature on drinking responses or core temperature changes during or after endurance exercise. Thus, similar to observations in human athletes, we hypothesized that horses dehydrated by a

combination of furosemide administration and prolonged exercise would drink a greater total volume of a cool (10°C) rehydration fluid than fluid at ambient (20°C) or near body (30°C) temperatures during the first hour of recovery. In addition, drinking cool fluid was hypothesized to produce a more rapid decrease in core temperature. Based on prior work in our laboratory (Butudom *et al.*, 2002), we elected to provide 0.9% NaCl at various temperatures as the initial rehydration solution (0-5 minutes post-exercise) followed by water at various temperatures for the remainder of the 60 minute recovery period.

Materials and Methods

Horses and conditioning program

Six 2- to 3-year-old mixed breed horses (three males and three females) ranging in weight from 287-460 kg (mean ± SE = 360 ± 14 kg) were studied. The horses had recently completed a 60 day training study during which they exercised 6 days per week in a free-flow horse walker (Centaur Horse Walker, Inc.). During the final week of that training study they exercised 70 min/day alternating between walking (1.7 m/sec for 40 min) and trotting (3.4 m/sec for 30 min). Between the end of the training study and the start of this study (a 3 week period), horses were accustomed to treadmill exercise (Mustang 2000, Kagra AG) three times per week and all horses completed two 30 km exercise bouts before the first experimental run. Salt water (0.9% NaCl) at different temperatures (10, 20, or 30°C) was offered after each conditioning run to introduce horses to the different treatments. Throughout the study period (May-August, 2001), horses were maintained at pasture with free access to water and no supplemental feed or salt was provided. The training program and all experimental protocols were performed

in accordance with the guidelines of the Institutional Animal Care and Use Committee of Michigan State University.

Experimental protocol

Horses were brought in from pasture on the afternoon before each experiment and housed overnight in a stall with free access to a grass/alfalfa mixed hay and water. On the morning of each experimental run, initial dehydration was induced by furosemide administration (Lasix® [furosemide] injection 5%, Hoechst Roussel Vet, 1.0 mg/kg IV) 120 min before the start of the 30 km exercise bout. Because our goal was to produce dehydration in excess of a 4% BW loss, furosemide treatment was added to the experimental protocol. Otherwise, 60-90 km of treadmill exercise would have been required to produce this degree of dehydration under the mild ambient conditions in our laboratory and a longer exercise bout would have increased the risk of musculoskeletal injury. During the remaining pre-exercise period, surface electrocardiographic electrodes were applied (for telemetric recording of heart rate [HR]) and a 7 French, 110 cm catheter (Swan Ganz flow directed thermodilution catheter, American Edwards Laboratories) was aseptically passed into the pulmonary artery (confirmed by pressure waveform) via an 8 French introducer inserted into the right jugular vein (for collection of mixed venous blood samples and measurement of core temperature). Each horse was studied three times when offered, in a randomized order, 0.9% NaCl at 10, 20, or 30°C during the initial 5 min of recovery and water at 10, 20, or 30°C (same temperature as the initial saline drink) during the remainder of the 60 min recovery period. Experiments on each horse were separated by a minimum of 7 days during which further exercise was not performed.

During each experiment BW was measured on a digital scale (nearest 0.5 kg) before and 120 min after (pre-exercise) furosemide administration. Horses then performed 30 km of treadmill exercise at varying speeds to simulate an endurance ride (Fig. 1). The experimental run consisted of two 15 km exercise bouts (lasting 56 min; speeds between 1.6 [walk] and 8 m/s [cantar]; 0° slope) separated by a 15 min rest period. Horses did not have access to water or any other rehydration solution during the run. Immediately (within 10 s) after completion of exercise, 0.9% NaCl at one of the three temperatures was offered for voluntarily drinking from a hand-held bucket. In addition to documenting total volume imbibed, the number of drinks and total duration of drinking was determined by subsequent review of a continuous video recording collected during the initial 5 min recovery period. The time from which the horse first began sipping water until it raised its lips away from contact with water was recorded as a single drinking episode. After the initial 5 min of recovery, the rehydration solution was taken away and horses were walked off the treadmill, the pulmonary arterial catheter was removed, and horses were washed with water. The horses had no access to rehydration fluid from 5-20 min after the end of exercise. At 20 min of the recovery period, horses were weighed and placed free in a stall (without feed) and further voluntarily water intake (at 10, 20, or $30^{\circ}C$) was measured. In addition, the number of drinking episodes from 20-60 minutes of recovery was recorded by continuous observation during this 40 min period. After 60 min of recovery horses were weighed again and returned to the stall where they remained overnight with free access to hay and water. A final weight was recorded after overnight recovery (~18 h after completion of the 45 km run).

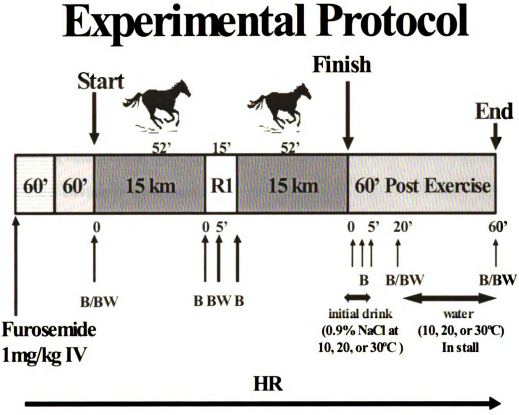

Figure 1. Experimental protocol. Furosemide (1.0 mg/kg IV) was given 2 h prior to and horses were instrumented during the 60 min before exercise. The experimental run consisted of two 15-km exercise bouts separated by one 15-min rest periods. Immediately (within 10 seconds) after completion of exercise, one of three rehydration fluids (10, 20, or 30°C 0.9% NaCl solution) was offered to the horses, in a randomized order, for voluntary drinking (shorter double-headed arrow). After 5 minutes of recovery, horses were walked off the treadmill and washed down with water. After 20 min of recovery, horses were placed free in a stall (without feed) and further voluntary water intake (at 10, 20, or 30°C) from 20-60 minutes of recovery was measured (longer double-headed arrow). Blood (B) was collected and body weight (BW) and heart rate (HR) were recorded at various times point during the experiment.

Sample collection and analysis

At 120 minutes before and at 20 and 60 min and ~18 h after completion of the 30 km run, blood samples (~10 ml) were collected by jugular venipuncture and HR was determined by auscultation. During exercise, HR and pulmonary arterial blood temperature (T_{core}, 9520A Cardiac Output Computer, Baxter Healthcare Corp., Edwards Critical-Care Division) were recorded at the start, during the 4 m/s (trot) and 8 m/s (canter) phases, and 1 and 5 min after completion of each 15 km exercise bout. Blood samples were collected at the start and finish of each 15 km of exercise bout and after 1, 3, and 5 min after completion of the 45 km exercise test. Blood was collected anaerobically into heparinized plastic syringes that were placed on ice until analyzed for plasma electrolyte (Na^+, K^+, and Cl^-) concentrations within 30 min of collection (Stat 7 Profile Analyzer, Nova Biomedical). The remainder of each sample was used for measurement of hematocrit (microhematocrit method) and plasma protein concentration (PP, by refractometry). All feces and urine produced from the time of furosemide administration to the end of the 60 min recovery period were collected and weighed with the exception of urine produced prior to exercise in response to furosemide administration. BW loss (with the exception of ~18 h recovery) was corrected for fecal losses but not for urine losses because the latter was an important factor in the pre-exercise BW loss.

Dissipation of metabolic heat by drinking

In an attempt to determine the heat dissipating effects of the initial period of drinking 0.9% NaCl at 10, 20, or 30°C, the gain in heat content of the ingested fluid was estimated

by assuming that the specific heat of 0.9% NaCl was the same as that of water (1.0 kcal/l/°C) and that all fluid drank was warmed to 38°C after 5 min of recovery. This heat gain by ingested fluid would be equal to the loss of metabolic heat (MH) by the horse. Next, the total MH produced during the two 15 km exercise bouts was estimated to determine the fraction that could be dissipated into the ingested salt water. MH was estimated by the following formula:

MH = oxygen consumption (ml/min/kg) x BW (kg) x k (kcal/l O_2) x duration (min)

where k, a constant for the amount of heat liberated per liter of O_2 consumed, was assumed to be 4 kcal/l O_2 (Hodgson *et al.*, 1993). Horses were assumed to have a maximal oxygen consumption of 120 ml/kg/min and to work at an average load of 30% of maximal oxygen consumption during the two 52 min exercise bouts. Finally, the role of heat transfer in cooling was evaluated by comparing the decline in T_{core} from the end of exercise to 5 min of recovery to the gain in heat content of the ingested fluid.

Data analysis

All values provided in the text are presented as means ± SE. Total fluid intake during the recovery period was analyzed using commercial software (SigmaStat, Jandel Scientific) by a one way repeated measures ANOVA to assess differences between temperatures of the rehydration solutions. When F ratios were significant ($p<0.05$), a Student-Newman-Keuls test was performed to detect specific differences. Changes in parameters measured at multiple times (BW, HR, T_{core}, and blood values) were analyzed by a two way repeated measures ANOVA (main effects of time and treatment) and, when

F ratios were significant ($p<0.05$), a Student-Newman-Keuls post-hoc was again performed to detect specific differences. Selected relationships between parameters measured were also examined by Pearson product moment correlation analysis.

Results

Five of six horses completed all three experimental runs while only five horses completed experiments in which they were offered rehydration solutions at 10°C. Ambient temperature in the treadmill laboratory ranged between 18 and 28°C (mean 25.0 ± 0.7°C) and relative humidity ranged between 46 and 75% (mean 59.9 ± 1.8%) during exercise and significant differences between the runs for each temperature of rehydration solution were not observed. Mean HR ranged from 110.2 ± 3.6 to 117.4 ± 3.1, from 142.8 ± 3.0 to 153.4 ± 4.3, and from 79.0 ± 7.7 to 87.5 ± 5.3 beats/min during the trot (4 m/s) and canter (8 m/s) phases of exercise and after 1 min of recovery, respectively. No differences over time (first and second 15 km exercise bout) or between treatments were observed.

Furosemide administration produced a BW loss prior to exercise of 2.1 ± 0.3, 2.2 ± 0.2, and 2.0 ± 0.1% ($p>0.05$) for 10, 20, and 30°C treatments, respectively. The combined effects of furosemide administration and 30 km of treadmill exercise resulted in BW losses of 4.2 ± 0.3, 4.5 ± 0.3, and 4.5 ± 0.2% ($p<0.05$ in comparison to pre-furosemide values for all treatments; $p>0.05$ for treatment differences) for 10, 20, and 30°C treatments, respectively (Table I and Fig. 2). After 60 min of recovery, BW was not different from the pre-furosemide values for any of the treatments but BW loss for 10 and

30°C treatments (0.8 ± 0.5 and 0.5 ± 0.8%, respectively) tended (p=0.15) to be greater than the value for the 20°C treatment (-0.3 ± 0.8) for which a mild gain in BW was actually observed. Similarly, after 18 h of recovery, BW was not different from the pre-furosemide values for any of the treatments and differences between treatments were not detected (decrease of 0.9 ± 0.7, 0.3 ± 0.5, and 0.3 ± 0.5% for 10, 20, and 30°C treatments, respectively).

The amount of fluid imbibed during the initial 5 min of recovery was not different among treatments (9.8 ± 2.5, 12.3 ± 2.1, and 9.7 ± 2.0 l for 10, 20, and 30°C treatments, respectively) and replaced approximately two-thirds of lost body fluid (Fig. 3). With all rehydration solution temperatures, horses drank immediately when the bucket was offered and the majority of drinking occurred within the first 1 to 2 min of recovery. However, there was a tendency for horses to take fewer (p=0.07), longer drinks when offered the 20°C saline (6.0 ± 1.7 drinks lasting 20.3 ± 5.3 s) in comparison to saline at 10°C (10.3 ± 2.0 drinks lasting 11.6 ± 3.8 s) or 30°C (11.0 ± 2.0 drinks lasting 14.6 ± 2.7 s). From 20 to 60 min of recovery, voluntary water intake was greater (p<0.05) for both 20 and 30°C saline (7.7 ± 0.8 and 6.6 ± 1.2 l, respectively) than for saline at 10°C (4.9 ± 0.5 l). When combined, these values resulted in a total voluntary fluid intake during the 60 min recovery period of 14.7 ± 2.5, 19.9 ± 2.5, and 16.3 ± 2.4 l for 10, 20, and 30°C treatments, respectively (p<0.05, 20°C greater than 10°C, Fig. 3). The number of drinking episodes from 20-60 min of recovery was not different for water at any temperature (4.0 ± 0.8, 4.6 ± 0.8, and 4.3 ± 1.0 for 10, 20, and 30°C treatments, respectively). When total

fluid intake was compared to persisting BW loss at the end of the 60 min recovery period, a highly significant negative correlation ($r= -0.91$, $P<0.01$) was found (Fig. 4).

Hematocrit tended to decrease after furosemide administration but the only significant change was an increase ($p<0.05$) by the end of exercise (Table 1). PP and plasma [Na^+] increased ($p<0.05$) after furosemide administration and remained increased through the end of exercise (Table I). PP tended to decrease within 5 min of recovery and was not different than the pre-furosemide values after 20 min of recovery. In contrast, plasma [Na^+] reached the highest values after 20 min of recovery and remained greater than the pre-furosemide value after 60 min of recovery for the 10°C treatment. After overnight recovery plasma [Na^+] was not different from the pre-furosemide values for any of the treatments. Plasma [K^+] decreased ($p<0.05$) after furosemide administration and remained decreased from the pre-furosemide values for all treatments throughout the 60 min recovery period (Table 1). After overnight recovery plasma [K^+] was not different from the pre-furosemide value for the 10°C treatment but remained decreased ($p<0.05$) for the 20 and 30°C treatments. Plasma [Cl^-] decreased ($p<0.05$) after furosemide administration and remained decreased until 20 min of recovery for all treatments (Table 1).

Mean T_{core} ranged from 38.8 ± 0.2 to 39.0 ± 0.2, from 39.1 ± 0.2 to 39.5 ± 0.2, and from 38.2 ± 0.2 to 38.3 ± 0.2°C during the last canter phases of the first and second exercise bouts and after 1 min of recovery, respectively. Although T_{core} increased ($p<0.05$) from the pre-exercise values during the canter phases of both exercise bouts,

values after 1 min of recovery had returned to the pre-exercise values and no differences between treatments were observed (Fig. 5). Depending on both temperature and volume consumed, estimated MH transferred to the fluid imbibed during the initial 5 min of recovery ranged from 48 to 532 kcal (Table 2). In addition, there was a significant positive linear correlation between the change in T_{core} from 0 to 5 min of recovery and the amount of MH transferred to the ingested fluid (Fig. 6). However, exercise at an intensity of 30% of maximal O_2 consumption for 104 minutes produced an estimated MH load ranging from ~4300 to ~6900 kcal, depending on the BW of the horse (Table 2). Thus, dissipation of heat by warming of fluid imbibed from 0-5 min of recovery accounted for a maximum of ~8% of the total MH generated in one horse while in most horses it was responsible for dissipation of less than 5% of the total MH generated during exercise.

		pre-furosemide	pre-exercise	End 1st bout	end exercise[a]	5 min recovery	20 min recovery	60 min recovery	~18 h recovery
BW	10°C	363 ± 29	356 ± 29*	352 ± 29*	348 ± 29*	NA	358 ± 29*	360 ± 29	360 ± 30
(kg)	20°C	361 ± 26	353 ± 26*	349 ± 25*	345 ± 25*	NA	357 ± 26	362 ± 26	360 ± 26
	30°C	357 ± 24	350 ± 23*	345 ± 23*	341 ± 22*	NA	351 ± 23*	356 ± 24	356 ± 23
Hct	10°C	32.1 ± 1.1	30.2 ± 1.1	34.4 ± 0.7	36.7 ± 2.2*	32.7 ± 1.4	30.6 ± 1.5	29.6 ± 0.9	30.4 ± 0.9
(%)	20°C	33.3 ± 1.1	32.3 ± 1.1	35.7 ± 1.0	39.8 ± 1.5*	35.3 ± 1.3	34.1 ± 0.9	30.5 ± 1.2	32.4 ± 0.8
	30°C	31.4 ± 1.0	29.5 ± 0.8	34.7 ± 0.9	36.0 ± 1.0*	33.9 ± 0.6	33.0 ± 0.5	31.0 ± 0.8	29.2 ± 1.7
PP	10°C	6.5 ± 0.3	6.6 ± 0.2*	6.9 ± 0.3*	7.2 ± 0.3*	7.1 ± 0.3*	6.7 ± 0.3*	6.6 ± 0.2	6.4 ± 0.2
(g/l)	20°C	6.5 ± 0.2	6.8 ± 0.2*	7.0 ± 0.1*	7.0 ± 0.1*	6.9 ± 0.2*	6.5 ± 0.2	6.4 ± 0.2	6.4 ± 0.2
	30°C	6.4 ± 0.3	6.7 ± 0.2*	6.9 ± 0.2*	7.0 ± 0.2*	6.9 ± 0.2*	6.6 ± 0.2	6.5 ± 0.2	6.3 ± 0.2
$[Na^+]$	10°C	137.7 ± 0.9	139.9 ± 0.4*	140.7 ± 0.9*	140.5 ± 0.5*	142.8 ± 1.0*	143.2 ± 0.8*	141.0 ± 1.2*	137.8 ± 0.6
(mmol/L)	20°C	139.5 ± 0.8	140.7 ± 0.8*	141.4 ± 0.7*	141.4 ± 0.9*	141.8 ± 0.9*	142.1 ± 1.1*	139.6 ± 1.2	138.6 ± 1.0
	30°C	138.0 ± 1.0	139.8 ± 0.9*	140.2 ± 0.9*	141.0 ± 0.8*	141.6 ± 0.8*	141.7 ± 0.7*	139.4 ± 0.4	138.5 ± 0.8
$[K^+]$	10°C	4.1 ± 0.1	3.6 ± 0.2*	3.8 ± 0.2*	3.7 ± 0.1*	3.5 ± 0.2*	3.2 ± 0.2*	3.1 ± 0.2*	3.9 ± 0.2
(mmol/L)	20°C	3.8 ± 0.2	3.2 ± 0.1*	3.5 ± 0.1*	3.4 ± 0.1*	3.2 ± 0.1*	3.0 ± 0.1*	2.9 ± 0.2*	3.5 ± 0.1*
	30°C	4.0 ± 0.2	3.3 ± 0.1*	3.5 ± 0.1*	3.4 ± 0.1*	3.3 ± 0.1*	3.0 ± 0.1*	2.8 ± 0.2*	3.5 ± 0.2*
$[Cl^-]$	10°C	104.6 ± 0.7	101.9 ± 0.9*	101.1 ± 0.9*	101.6 ± 0.9*	102.4 ± 0.6	105.2 ± 0.8*	105.3 ± 0.9	104.8 ± 1.0
(mmol/L)	20°C	106.6 ± 1.9	103.3 ± 1.8*	103.0 ± 1.8*	103.6 ± 2.3*	103.2 ± 2.1	104.8 ± 1.9*	105.5 ± 1.7	105.1 ± 1.3
	30°C	105.4 ± 0.7	103.8 ± 0.5*	102.8 ± 0.7*	102.7 ± 0.7*	103.2 ± 0.6	104.4 ± 0.8*	104.4 ± 0.8	105.2 ± 0.5

Table 1. Body weight (BW), haematocrit (Hct), and plasma protein (PP), sodium, potassium, and chloride concentrations in six horses before and 2 hours after (pre-exercise) administration of furosemide (1 mg/kg IV), after completion of 30 km of treadmill exercise, and after 20 and 60 min and ~18 h of recovery with initial rehydration (0 to 5 min of recovery) with 0.9% NaCl at 10, 20, or 30°C followed by rehydration with water at 10, 20, or 30°C from 20 to 60 min of the recovery period. NA = Not available

[a] end exercise body weight estimated as 20 min recovery weight + fluid intake (1 L = 1 kg) + fecal losses from 0 to 20 min of recovery

*significantly different ($p<0.05$) from the pre-furosemide value (within each row)

Horse	BW (kg)	Treat ment	Specific heat for horse (kcal/°C)	Metabolic heat (MH) At 30%VO_2Max (kcal)	Fluid intake 0-5 min recovery (Liters)	Δ Tcore End-5 min Recovery (°C)	Heat content At 10, 20, 30°C (kcal)	Heat content at 38°C (kcal)	Δ Heat content (kcal)	Δ Heat content as a % of MH at 30% VO_2Max	Cooling effects of fluids (°C)
Trouble	322.8	10°C	267.9	5033.6	4.8	-0.01	47.5	180.5	133.0	2.6	0.5
Jabba	391.5	20°C	324.9	6115.2	19	0.79	190.0	722.0	532.0	8.7	1.6
Han Solo	443.7	30°C	368.3	6905.6	7.8	0.42	78.0	296.4	218.4	3.2	0.6
Wilbur	ND	10°C	ND	ND	ND	ND	ND	ND	ND	ND	ND
Houdini	299.5	20°C	248.6	4671.7	10.5	0.68	105.0	399.0	294.0	6.3	1.2
Toby	274.0	30°C	227.4	4272.3	7.0	0.48	70.0	266.0	196.0	4.6	0.9
Trouble	318.5	10°C	264.4	4967.0	17.7	1.28	354.0	672.6	318.6	6.4	1.2
Jabba	408.5	20°C	339.1	6369.0	13.5	0.07	270.0	513.0	243.0	3.8	0.7
Han Solo	429.6	30°C	356.6	6701.8	15.4	0.82	308.0	585.2	277.2	4.1	0.8
Wilbur	304.5	10°C	252.7	4746.6	3.0	-0.17	60.0	114.0	54.0	1.1	0.2
Houdini	307.5	20°C	255.2	4796.5	12.5	0.06	250.0	475.0	225.0	4.7	0.9
Toby	281.5	30°C	233.6	4393.0	11.5	0.77	230.0	437.0	207.0	4.7	0.9
Trouble	327.0	10°C	271.4	5100.2	19.0	0.25	570.0	722.0	152.0	3.0	0.6
Jabba	390.7	20°C	324.3	6094.4	10.3	0.77	309.0	391.4	82.4	1.4	0.3
Han Solo	422.8	30°C	350.9	6597.8	9.2	0.70	276.0	349.6	73.6	1.1	0.2
Wilbur	301.9	10°C	250.6	4709.1	5.6	-0.33	168.0	212.8	44.8	1.0	0.2
Houdini	318.5	20°C	264.4	4967.0	6.0	-0.08	180.0	228.0	48.0	1.0	0.2
Toby	276.0	30°C	229.1	4305.6	8.0	-0.05	240.0	304.0	64.0	1.5	0.3

Table 2. Metabolic heat load and cooling effects of administered fluids at 10°C, 20°C, and 30°C NaCl during 0-5 min recovery after a 30-km exercise run on a treadmill in 6 horses. Exercise composed of a two bouts of 52 min of speeds varying between 4-8 m/s, which was estimated to be 30%VO_2max (125 mlO_2/min/kg). Specific heat for horses is assumed to be 0.83 kcal/kg/°C, and metabolic heat (MH) was estimated from: MH= VO_2 (l/min) x k x exercise duration (min). Heat content was estimated from: Heat content (kcal) = mass (l) x specific heat for water x °C. Specific heat for water is 1 kcal/l/°C. By assumimg that all water heated to 38°C by 5 min after exercise and the pulmonary artery temperature (Tcore) has equilibrated blood, the body heat transfer to warm water (Δ heat content, kcal) and the cooling effects of administered fluid can be estimated on the basis of specific heat capacity and changes in body temperature during the time of fluid administration.

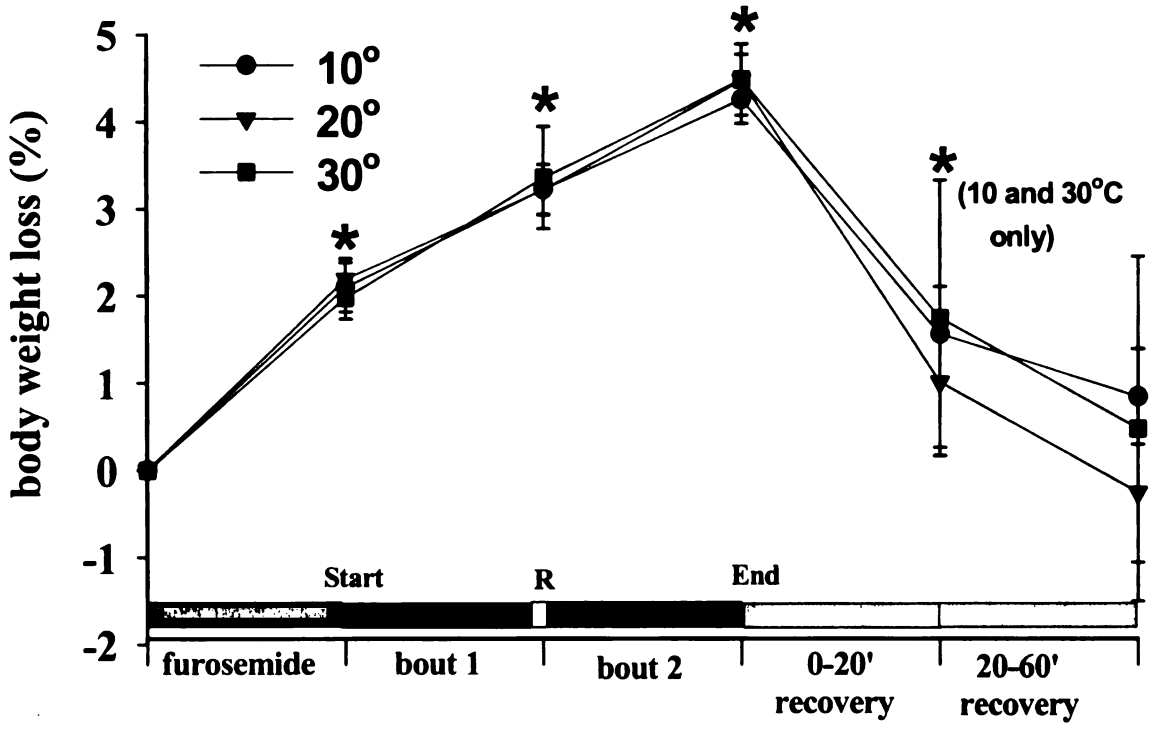

Figure 2. Percent body weight loss after furosemide treatment (1 mg/kg IV) and during and after 45 km of treadmill exercise ride for horses offered 10°C (filled circles), 20°C (filled inverted triangles), or 30°C 0.9% NaCl (filled squares) during the initial 5 min of recovery. * = significantly different ($p<0.05$) from the pre-furosemide value; see Figure 1 legend for description of the experimental protocol.

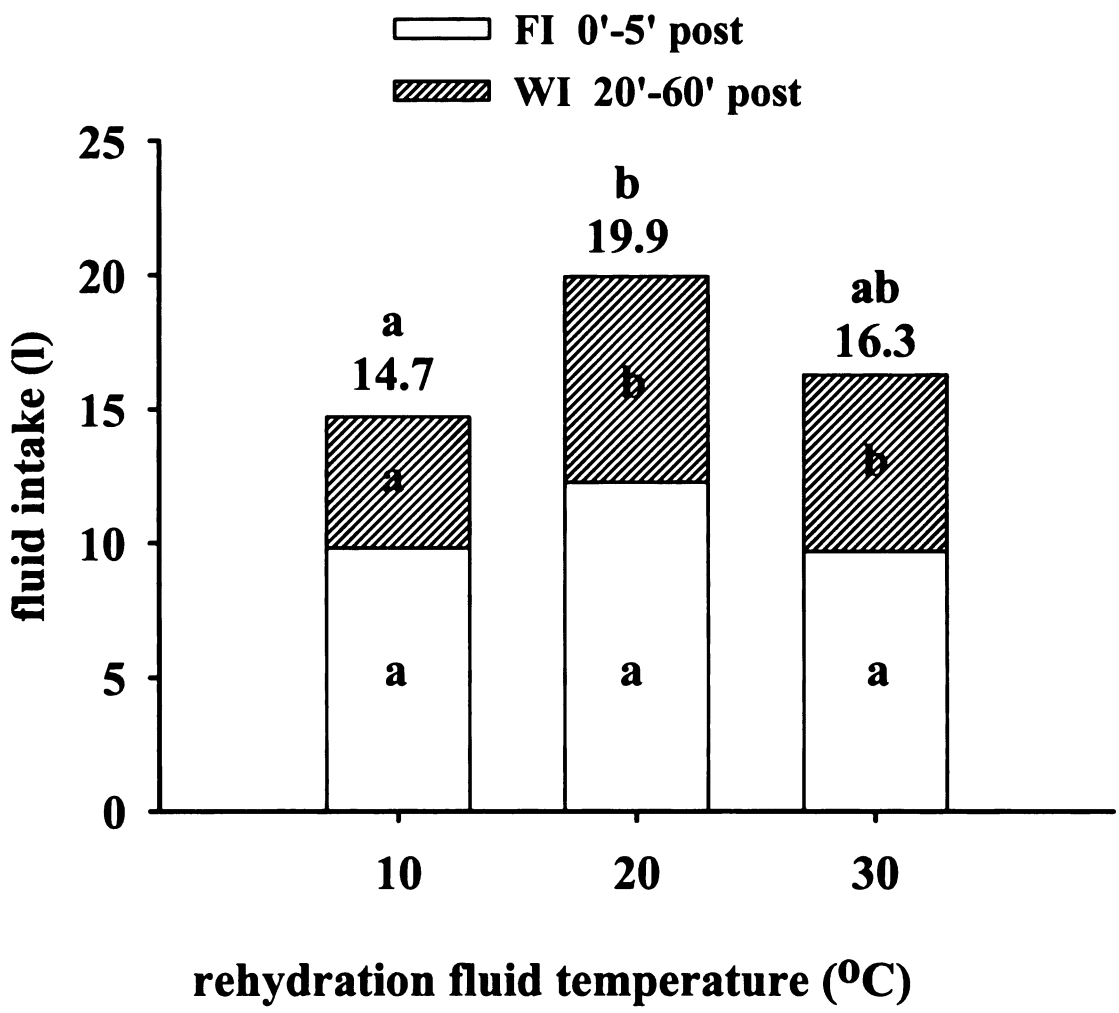

Figure 3. Total fluid intake during the 60 minute recovery period after induction of dehydration by administration of furosemide (1 mg/kg IV) followed by 30 km of treadmill exercise for horses provided 10, 20, or 30°C 0.9% NaCl solution as the rehydration fluid during the initial 5 min following exercise and subsequently provided free access to water (at 10, 20, or 30°C) from 20 to 60 min following exercise. FI 0'-5' = fluid intake during 0 to 5 minute post-exercise; FI 20'-60' = fluid intake during 20 to 60 minute of recovery.

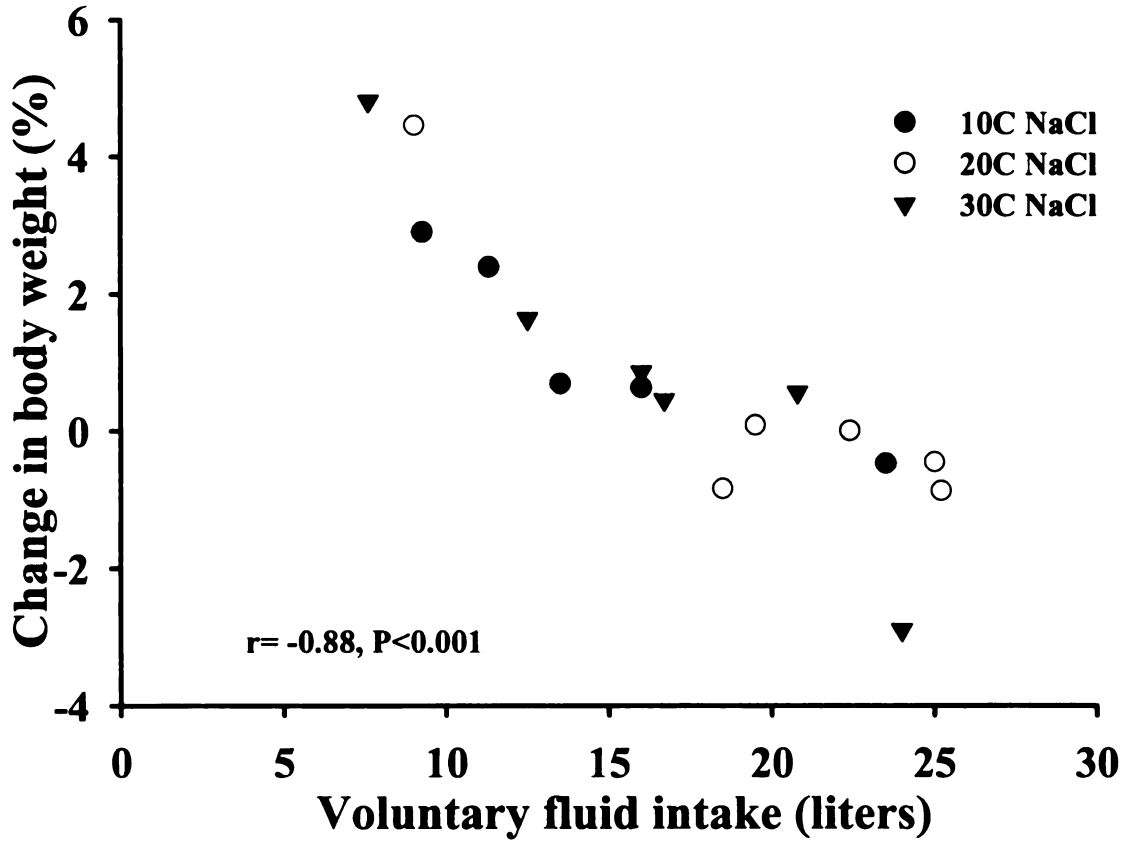

Figure 4. Correlation between total fluid intake from 0 to 60 minutes of the recovery period and persisting body weight loss (magnitude of involuntary dehydration) after 60 minutes of recovery after induction of dehydration by administration of furosemide (1 mg/kg IV) followed by 30 km of treadmill exercise for horses provided 10, 20, or 30°C 0.9% NaCl solution as the rehydration fluid during the initial 5 min following exercise and subsequently provided free access to water (at 10, 20, or 30°C) from 20-60 min following exercise.

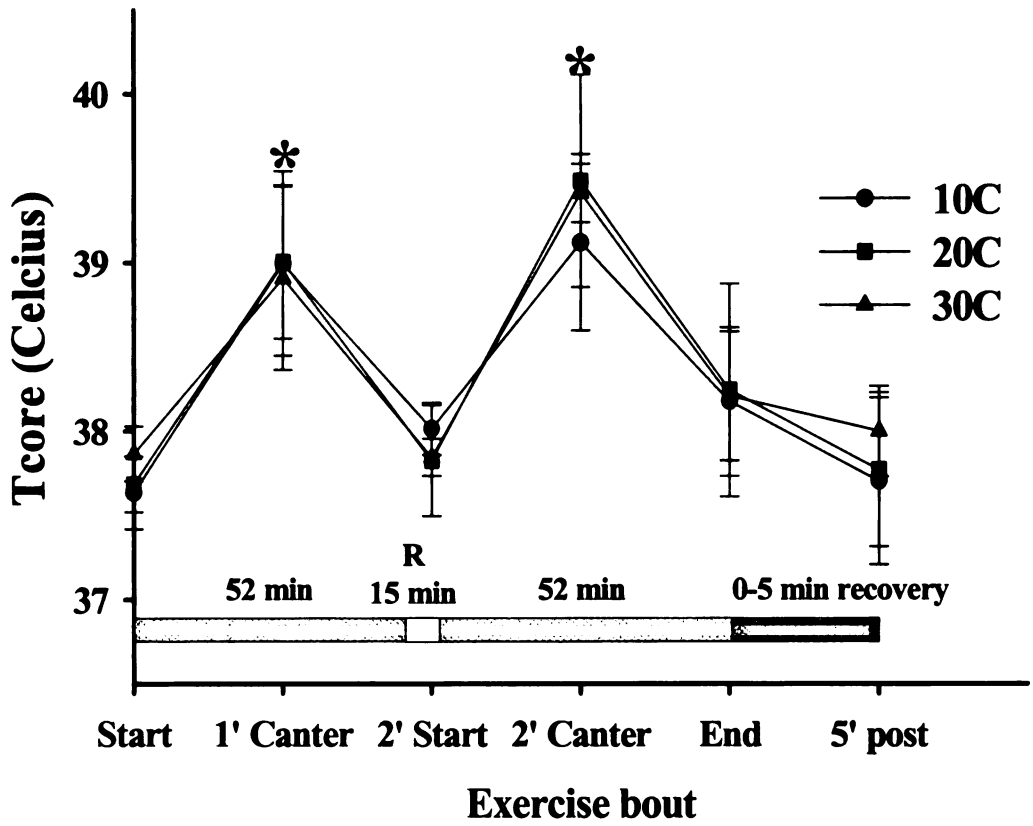

Figure 5. Pulmonary artery blood (Tcore) changes in 6 horses during 2 phases of exercise until 5 minutes of postexercise recovery after induction of dehydration by administration of furosemide (1 mg/kg IV) followed by 30 km of treadmill exercise for horses provided 10, 20, or 30°C 0.9% NaCl solution as the rehydration fluid during the initial 5 min following exercise. * = significant diference from pre-exercise values (all 3)

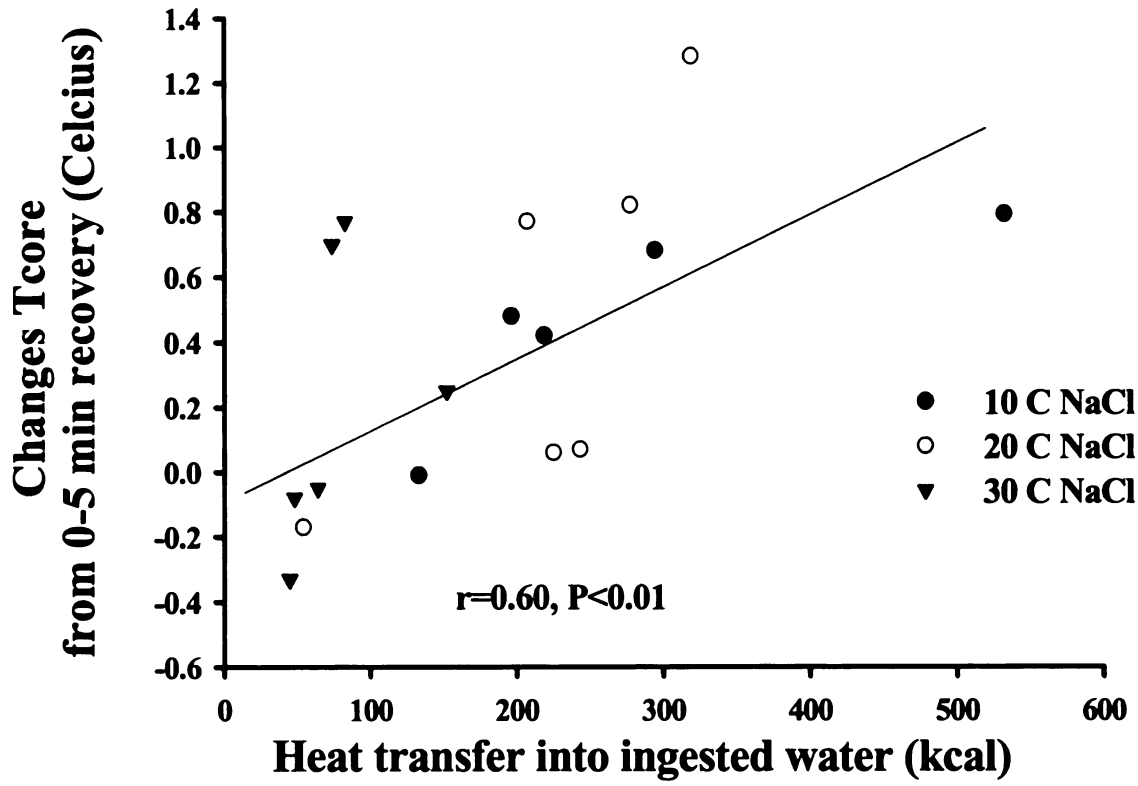

Figure 6. Correlation between changes in Tcore from 0 to 5 minutes of the recovery period and the amount of heat transfer to ingested water (0.9% NaCl) during 0-5 minutes of recovery after induction of dehydration by administration of furosemide (1 mg/kg IV) followed by 30 km of treadmill exercise for horses provided provided 10, 20, or 30°C 0.9% NaCl solution as the rehydration fluid during the initial 5 min following exercise.

Discussion

The results of this study demonstrate that temperature of rehydration fluid influences voluntary drinking in horses recovering from furosemide- and exercise-induced dehydration. Compared to either cooler (10°C) or warmer (30°C) temperatures, offering fluid at near ambient temperature (20°C) resulted in the greatest voluntary fluid intake by the end of the initial 60 min recovery period. Thus, our hypothesis that horses dehydrated by a combination of furosemide administration and prolonged exercise would drink a greater total volume of a cool (10°C) rehydration fluid, than fluid at ambient (20°C) or near body (30°C) temperatures, during the first hour of recovery was refuted. Even though there was a difference in fluid intake between different fluid temperatures during the first hour of recovery, BW loss after 60 minutes of recovery did not differ between treatments. However, BW loss for the 10 and 30°C treatments tended to be greater than the value for the 20°C treatment for which a mild gain in BW was actually observed.

As in a previous study (Butudom *et al.* 2002), initial rehydration with a saline solution attenuated the magnitude of BW loss after the first hour of recovery. Indeed, "involuntary dehydration" did not occur in horses in this study. In addition, this finding suggested that the saline solution drank during the initial 5 minutes of recovery was largely retained in the body fluids of these horses. Drinking salt water (0.9% NaCl) can attenuate loss of body fluid stores by stimulating greater subsequent water intake (Nyman *et al.* 1996; Butudom *et al.* 2002). Adding Na^+ to the rehydration fluid increases P_{osm}, which enhances renal water conservation (Maughan and Shirreffs 1994; Shirreffs *et al.* 1996). In contrast, increased production of dilute urine is observed in human subjects after

consumption of plain water or hypotonic oral rehydration solutions (Mitchell *et al.* 1994; Mitchell *et al.* 2000). In horses, rehydration with water alone is less effective because ingestion of this hypotonic fluid can lead to thirst inhibition and can delay recovery from exercise (Nyman *et al.* 1996; Butudom *et al.* 2002). Thus, initial intake of salt water appears to be beneficial to horses that may have limited time to recover from endurance events before being subjected to further exercise, including transport after finishing the competition.

Although limited to 6 horses, the results of this study demonstrate that horses dehydrated by endurance exercise may prefer rehydration fluids at near ambient temperatures (in a temperate climate). In addition to the volume imbibed, the pattern of drinking may also be used to assess fluid preference. Although the number of drinking episodes during the initial 5 min of recovery was not different for fluid at any temperature, there was a tendency for horses to take fewer, longer drinks when offered the NaCl solution at 20°C, in comparison to 0.9% NaCl at 10°C or 30°C during the first 5 minutes of recovery. In addition to supporting a preference for fluid at 20°C, these results could also suggest a temperature dependence of taste of saline solutions in horses. Studies in humans and laboratory animals have demonstrated that saline solutions taste different at various temperatures (McBurney *et al.* 1973; Lundy and Contreras 1997). For example, human subjects reported that saline tasted salty when the temperature of the solution was below 22°C but that the solution was tasteless when the temperature was above 22°C (McBurney *et al.* 1973).

As in our previous experiment in which the composition of the initial rehydration solution was varied (Butudom *et al.* 2002), horses in this study drank a similar mean volume of 0.9%NaCl at all temperatures during the first 5 minutes of recovery. This particularly striking finding suggests that neither composition nor temperature of the rehydration solution may be an important factor in determining initial fluid intake. Rather, dehydrated horses appeared to drink until satiated by a mechanism independent of fluid composition or temperature. Gastric filling has been suggested to be an important factor in satiation of thirst and cessation of drinking (Towbin 1949; Engstrom and Deaux 1974). Indeed, the volumes drank by these horses were similar to the reported capacity of the equine stomach (Pfeiffer and MacPherson 1990). The suggestion that gastric filling contributes to initial satiation is further supported by the observation that the majority of initial fluid consumption occurred within the first two minutes after completing the exercise bout.

In contrast to laboratory animals that tend to prefer and drink the greatest amount of water at 25-35°C, in a thermoneutral environment (Carlisle 1977), resting humans appear to prefer water at 20°C (Boulze *et al.* 1983). This preference for cooler water by adult humans may be a learned behavior because it is not observed in newborns (Boulze *et al.* 1983). Next, human athletes that become both dehydrated and hyperthermic show a preference for water at 5-15°C (Boulze *et al.* 1983; Sandick *et al.* 1984; Szlyk *et al.*, 1989). Although colder water is preferred following exercise, offering a progressively colder drink (<10°C) can actual decrease volume consumed (Boulze *et al.*, 1983). As a result, hyperthermia, rather than dehydration, has been suggested to be a more important

mechanism for the preference for colder water (Boulze *et al.* 1983) while oropharyngeal cooling has been advanced as a mechanism of greater thirst satiation and decreased intake of colder water (Gold *et al.* 1973; Ramsauer *et al.* 1974; Carlisle 1977). Further, gastric emptying is slower for cold solutions than for warm solutions (Deaux 1973). Thus, intake of cold water could also prolong satiation by slowing gastric emptying rate. Core temperature of horses in the present study at the end of exercise was not increased from pre-exercise values; thus, hyperthermia was not a stimulus for drinking. Lack of hyperthermia, in addition to greater oropharyngeal cooling, could potentially explain why our horses drank the least amount of fluid at 10°C. Although horses cannot rate a fluid preference, our data suggest that, like dehydrated-normothermic human athletes, dehydrated-normothermic equine athletes exercising under moderate environmental conditions may prefer to drink rehydration fluids at near ambient temperature (20°C).

Horses in this study were offered 0.9% NaCl as the initial rehydration fluid during the first 5 min of the recovery period and plasma [Na^+] remained increased from the pre-exercise values after 20 min of recovery. In contrast, drinking during the initial 5 min of recovery caused a prompt return of PP (and presumably plasma volume) to pre-exercise values by 20 min of recovery (except for the 10°C treatment). Although both an increase in plasma tonicity and a decrease in blood (plasma) volume are well-documented stimuli for thirst (Fitzsimons 1998), the increase in plasma tonicity appeared to be the more important stimulus for further drinking from 20 to 60 min of recovery. In another study in which horses maintained plasma volume during a similar experimental run, water intake was significantly correlated with the increase in plasma [Na^+] induced by administration

of oral electrolyte pastes (Düsterdieck *et al.* 1999). Of interest, both plasma [Na^+] and PP were greatest after 20 min of recovery in horses initially offered 0.9% NaCl at 10°C. However, horses drank the least amount of water at 10°C from 20 to 60 min of recovery. This finding could support that oropharyngeal cooling may also have played a role in satiation of thirst with this treatment.

The potential cooling effect of ingesting saline at different temperatures during the initial 5 min of recovery was evaluated by comparing the decline in T_{core} from 0-5 min of recovery to the estimated amount of MH transferred to ingested fluid. Although a significant positive linear correlation between these measures was found, supporting cooling by the ingested fluid, less than 5% of the estimated MH load generated during exercise was likely transferred to ingested fluid. These results indicate that any direct cooling effect of ingested fluid in horses performing endurance exercise is minimal and confirm that the major route of heat dissipation is by evaporation of sweat.

In conclusion, the results of this study clearly show that rehydration fluid temperature influences intake by dehydrated-normothermic horses. Although a significant difference in the volume of saline drank during the initial 5 min of recovery was not found, horses offered saline at 20°C tended to take fewer, longer drinks and consumed 2-2.5 l more during this initial recovery period. Further, due to the low number of experimental subjects, our ability to detect significance was limited by a lack of power. From 20-60 min of the recovery period, horses did drink the greatest amount of water at near ambient temperature (20°C), in comparison to water at either cooler (10°C) or warmer (30°C)

temperatures. These findings support that attention should be paid to rehydration fluid temperature when attempting to maximize short-term water intake during and after endurance exercise. Further, future studies of the effects of oropharyngeal cooling on fluid intake by horses are warranted. When combined with our previous findings (Butudom *et al.*, 2002), these data support that horses exercising for longer than an hour may benefit from initial provision of salt water (0.9% NaCl) at a temperature near 20°C followed by frequent opportunities to drink plain water at temperature near 20°C. Enhancing short-term rehydration should improve recovery and would be expected to decrease the risk of developing medical problems after exercise and transport.

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Chapter 5

General Discussion and conclusions

DISCUSSION AND CONCLUSIONS

Incomplete voluntary replacement of body fluid losses sustained by sweating during endurance exercise, despite free access to water or other rehydration solutions during and after the exercise bout, is well-recognized in both human and equine athletes. This phenomenon, which has been termed both voluntary and involuntary dehydration, is recognized as a persisting body weight loss and has been attributed to blunted thirst. Thus, factors affecting stimulation of thirst and voluntary drinking must contribute to development of involuntary dehydration. The primary stimulus for thirst appears to be an increase in plasma osmolality or, more specifically, an increase in plasma Na^+ concentration coupled with activation of the renin-angiotensin system (Andersson 1978; Fitzsimmons 1998).

In human athletes, a number of factors including volume imbibed, sodium content, temperature, and flavoring of the rehydration solution have been demonstrated to affect the magnitude of involuntary dehydration during the initial recovery period (Boulze *et al.* 1983; Sandick *et al.* 1984; Szlyk *et al.* 1989; Barr *et al.* 1991; Shirreffs *et al.* 1996). In order to prevent involuntary dehydration, the volume of fluid imbibed must actually exceed the volume of fluid lost as sweat to account for ongoing water loss in urine (Shirreffs *et al.* 1996). Rarely are such volumes of rehydration fluid imbibed by athletes recovering from endurance exercise. In addition to adequate volume, a number of studies (Barr *et al.* 1991; Shirreffs *et al.* 1996) have clearly demonstrated that inclusion of electrolytes, particularly sodium, in the rehydration fluid is critical for retention of imbibed fluid. Although consumption of plain water is better than no fluid ingestion, it is

accompanied by a large increase in urine flow leading to poor fluid retention. The finding of numerous studies in the past couple of decades led the American College of Sports Medicine to publish a 1996 *Position stand* entitled *Exercise and fluid replacement* in which sodium is recommended to be included in rehydration fluid at an amount estimated to replace sweat losses for exercise lasting longer than 1 hour (Convertino *et al.* 1996). In addition to electrolyte content, more subtle factors including temperature and flavoring of the rehydration fluid also affect voluntary intake by human athletes (Boulze *et al.* 1983; Sandick *et al.* 1984; Szlyk *et al.* 1989). Finally, considerable interindividual variability in voluntary drinking has also been observed in human athletes and this can be another factor affecting the magnitude of involuntary dehydration (Szlyk *et al.* 1989).

Over the past ten years, there have been a number of studies designed to assess treatments used to attenuate or correct dehydration in equine endurance athletes (work up to 1997 reviewed in Schott and Hinchcliff 1998; Marlin *et al.* 1998a; Marlin *et al.* 1998b; Sosa León *et al.* 1998; Düsterdieck *et al.* 1999; Monreal *et al.* 1999). Although a few of these studies demonstrated that administration of electrolytes as oral pastes can attenuate loss of body fluid stores, primarily by stimulating water intake, most studies involved "forced" hyperhydration prior to exercise or rehydration during and after exercise by administering solutions via a nasogastric tube. In the study by Düsterdieck *et al.* 1999 in which horses completed a 60-km simulated endurance ride on a treadmill, supplementation of electrolytes as an oral paste before and during exercise enhanced voluntary water intake and, thereby, attenuated body weight loss. Electrolyte supplementation also stimulated horses to drink earlier during the course of the exercise

test and greater water intake was demonstrated to be correlated to an increase in plasma osmolality and sodium concentration.

In horses, there has been little study of drinking and strategies to enhance voluntary fluid replacement during and after endurance exercise. In the only study to date to address this question, Nyman *et al* (1996) reported that horses completing a 62-km simulated field endurance ride voluntary drank more water from 1 to 3 hours of recovery when offered a saline solution (0.9% NaCl) during the ride and the first hour of recovery than when offered plain water. Thus, studies of factors affecting voluntary rehydration by horses performing endurance exercise were clearly needed. We investigated the effects of volume (experiment 1, chapter 2), sodium content (experiment 2, chapter 3), and temperature of rehydration fluid (experiment 3, chapter 4) on voluntary rehydration of horses dehydrated by endurance exercise.

In experiment 1 (Chapter 2), limiting the volume of water (to 4 l, 8 l, or an unlimited amount during the first 5 minutes of recovery) initially provided to horses dehydrated by endurance exercise had no significant effects on total fluid intake, but all horses had involuntary dehydration (persisting body weight loss) at 60 minutes of recovery. Experiment 2 (Chapter 3) demonstrated that offering a saline solution (0.45% or 0.9% NaCl) as the initial rehydration fluid after exercise maintained an elevated [Na^+] and resulted in greater total fluid intake (and attenuation of the magnitude of involuntary dehydration) than when plain water was offered. In experiment 3 (Chapter 4), we found that providing rehydration fluid (0.9% NaCl) at near ambient (20°C) temperature resulted

in the greatest voluntary fluid intake by the end of the initial 60-minute recovery period, in comparison to offering cool (10°C) or warm (30°C) temperatures.

Our studies provide further support that an increase in plasma tonicity is a stimulus of thirst in dehydrated horses. More importantly, plasma osmolality declined rapidly (within 5 minutes) after water drinking, supporting rapid absorption of imbibed water. Further, regardless of the rehydration strategiy used, most drinking occurred within the first couple of minutes after the rehydration solution was provided at the end of exercise. This observed of pattern of drinking was similar to that previously described for horses and ponies (Sufit *et al.* 1985; Jones *et al.* 1989; Düsterdieck *et al.* 1999). Next, the observation that the mean volume fluid imbibed during the initial 5 minuites of recovery was typically between 10 and 12 liters suggested that gastric filling may be a limiting factor for rapid rehydration. However, the pattern of drinking was somewhat different when the temperature of rehydration fluid was manipulated (Chapter 4). The number of drinking episodes and duration of drinking was less when the rehydration fluid temperature was below (10°C) or above (30°C) ambient temperature (20°C). As for human athletes, this finding indicated that there is an optimal drink temperature for rehydration fluid for horses. In contrast to human athletes that can provide verbal feedback, it is more difficult to assess fluid preference in horses. In addition to volume imbibed, the difference in drinking patterns observed in experiment 3 should also be assessed in future studies when investigating factors that affect the rehydration process.

In conclusion, even though the exact physiological mechanisms of "involuntary dehdyration" in dehydrated horses remain unclear, we documented several factors that affect voluntary drinking and rehydration of horses dehydrated by endurance exercise. Further, our findings can have immediate practical application to limit or prevent involuntary dehydration following endurance exercise. A strategy to enhance voluntary fluid intake would be to offer all horses an initial drink of salt water at near 20°C during the first few minutes after completion of exercise as well as offering horses frequent opportunities to drink salt water during an endurance ride (i.e., at all rest stops). Before this recommendation is put into practice during competition, training horses to drink salt water should be pursued. Finally, further research needs to be performed to better understand the physiological mechanisms of involuntary dehydration in exercising horses and to design additional strategies to prevent or, at least, attenuate its occurrence.

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Appendices

Appendix 1

Experimental protocol-Exercise speeds

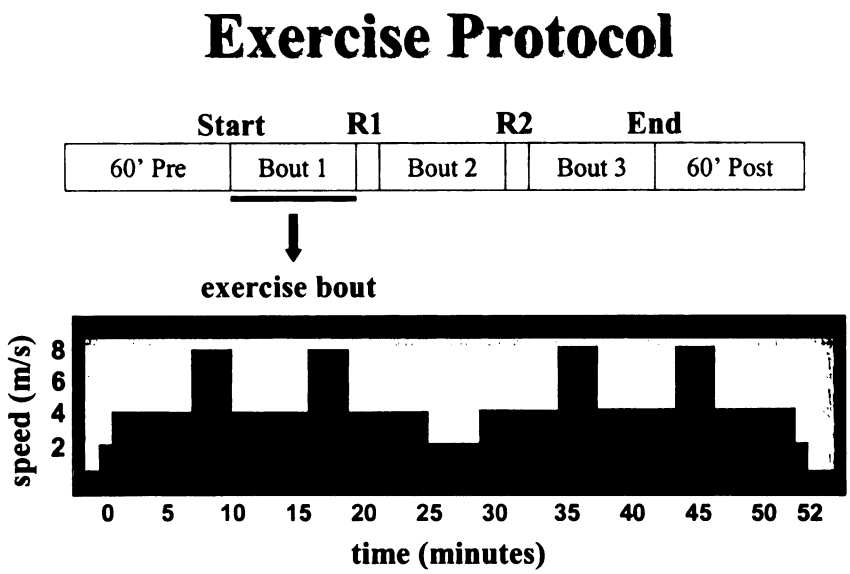

Figure 1.1 *Experimental protocol – Exercise speeds:* All horses performed a 45 km simulated treadmill endurance riding which is similar to the first 25 miles of a typical endurance ride with varying speeds. Each 15 km exercise bout consisted of two 26 min cycles of treadmill exercise at speed varying from 2 (walk), 4 (trot) to 8 (canter) m/s at 0° treadmill slope.

Appendix 2

Mean ± SE changes in plasma (Posm) and serum osmolality (Sosm) and mixed venous serum osmolality (Sosm), and heart rate (HR) in horses at start, and several time points after a completion of 30 to 45-km simulated endurance ride on a treadmill. Values for trot and canter are values for the last trot and canter for the last exercise bout.
* = values significant from 120 Pre-exercise, [a] = Values of body temperature recorded per rectum.

Variables	Protocol	Treatments	Pre-exercise	Canter	Trot	End exercise	5 min recovery	20 min recovery	60 min recovery	18hr recovery
Posm (mOsm/kg)	45-km treadmill exercise	4 liters 8 liters unlimited	283.7 ± 1.8 284.9 ± 1.2 287.2 ± 1.6			290.8 ± 1.6* 292.8 ± 1.6* 294.9 ± 1.4*	288.4 ± 1.4* 287.1 ± 2.0 288.8 ± 1.6	287.0 ± 1.0* 285.0 ± 1.8 286.7 ± 1.1	283.9 ± 1.9 284.8 ± 1.5 285.2 ± 1.6	NA NA NA
Posm (mOsm/kg)	Furosemide (1 mg/kg iv) + 45-km treadmill exercise	Water 0.45% NaCl 0.9% NaCl	286.7 ± 2.9 281.9 ± 2.9 287.2 ± 2.9			293.2 ± 2.9* 290.9 ± 2.9* 299.7 ± 2.9*	289.8 ± 2.9* 289.8 ± 2.9* 298.7 ± 2.9*	286.4 ± 2.9 287.2 ± 2.9* 300.0 ± 2.9*	283.2 ± 2.9* 281.4 ± 2.9 293.7 ± 2.9*	280.2 ± 2.8* 276.3 ± 3.2* 286.7 ± 2.7
Sosm (mOsm/kg)	Furosemide (1 mg/kg iv) + 30-km treadmill exercise	10°C NaCl 20°C NaCl 30°C NaCl	284.4 ± 1.5 282.3 ± 2.0 276.7 ± 3.9			288.3 ± 1.9 287.6 ± 2.7 283.9 ± 4.2	289.2 ± 1.4 287.5 ± 1.9 282.4 ± 3.6	288.9 ± 2.2 286.1 ± 2.8 282.3 ± 4.3	283.2 ± 1.0 278.6 ± 2.4 276.8 ± 3.8	284.5 ± 1.3 279.3 ± 2.0 274.8 ± 3.9
HR (beat/min)	45-km treadmill exercise	4 liters 8 liters unlimited	43.3 ± 2.2 40.7 ± 2.4 47.3 ± 2.3	141.7 ± 2.5* 133.7 ± 2.4* 138.3 ± 2.9*	109.2 ± 1.5* 107.7 ± 2.7* 108.5 ± 1.8*	67.2 ± 4.1 67.0 ± 2.3 70.0 ± 2.3	59.8 ± 2.5 58.5 ± 2.2 61.0 ± 1.7	54.3 ± 3.6 56.3 ± 2.2 53.0 ± 2.3	55.7 ± 4.5 51.7 ± 2.8 51.3 ± 1.6	NA NA NA
HR (beat/min)	Furosemide (1 mg/kg iv) + 45-km treadmill exercise	Water 0.45% NaCl 0.9% NaCl	44.7 ± 1.5 52.0 ± 7.7 50.0 ± 4.3	138.2 ± 3.5* 138.2 ± 5.0* 134.2 ± 9.3*	111.8 ± 2.7* 109.8 ± 6.0* 105.7 ± 0.5*	78.2 ± 2.1 80.4 ± 4.1 76.2 ± 3.8	68.8 ± 3.2 74.5 ± 5.6 69.3 ± 2.2	54.7 ± 1.3 53.2 ± 2.2 54.3 ± 2.2	51.0 ± 2.8 48.7 ± 1.3 48.7 ± 2.6	46.0 ± 2.7 42.6 ± 1.5 41.0 ± 1.4
HR (beat/min)	Furosemide (1 mg/kg iv) + 30-km treadmill exercise	10°C NaCl 20°C NaCl 30°C NaCl	46.7 ± 4.5 44.0 ± 1.5 44.0 ± 2.0	143.4 ± 6.1* 149.0 ± 5.7* 145.0 ± 6.1*	113.0 ± 1.4* 112.7 ± 6.3* 112.8 ± 2.0*	79.0 ± 7.7 87.5 ± 5.3 80.3 ± 2.7	63.4 ± 5.3 71.7 ± 5.4 67.7 ± 3.0	50.8 ± 2.8 50.0 ± 2.0 50.7 ± 2.7	47.2 ± 2.3 52.0 ± 2.7 41.2 ± 2.3	42.0 ± 2.0 44.7 ± 3.5 42.7 ± 0.8

Table 2.1 : Mean ± SE changes in plasma (Posm) and serum osmolality (Sosm) and mixed venous serum osmolality (Sosm), and heart rate (HR) in horses at start, and several time points after a completion of 30 to 45-km simulated endurance ride on a treadmill. Values for trot and canter are values for the last trot and canter for the last exercise bout. * = values significant from 120 Pre-exercise, [a] = Values of body temperature recorded per rectum, NA = not available

Appendix 3

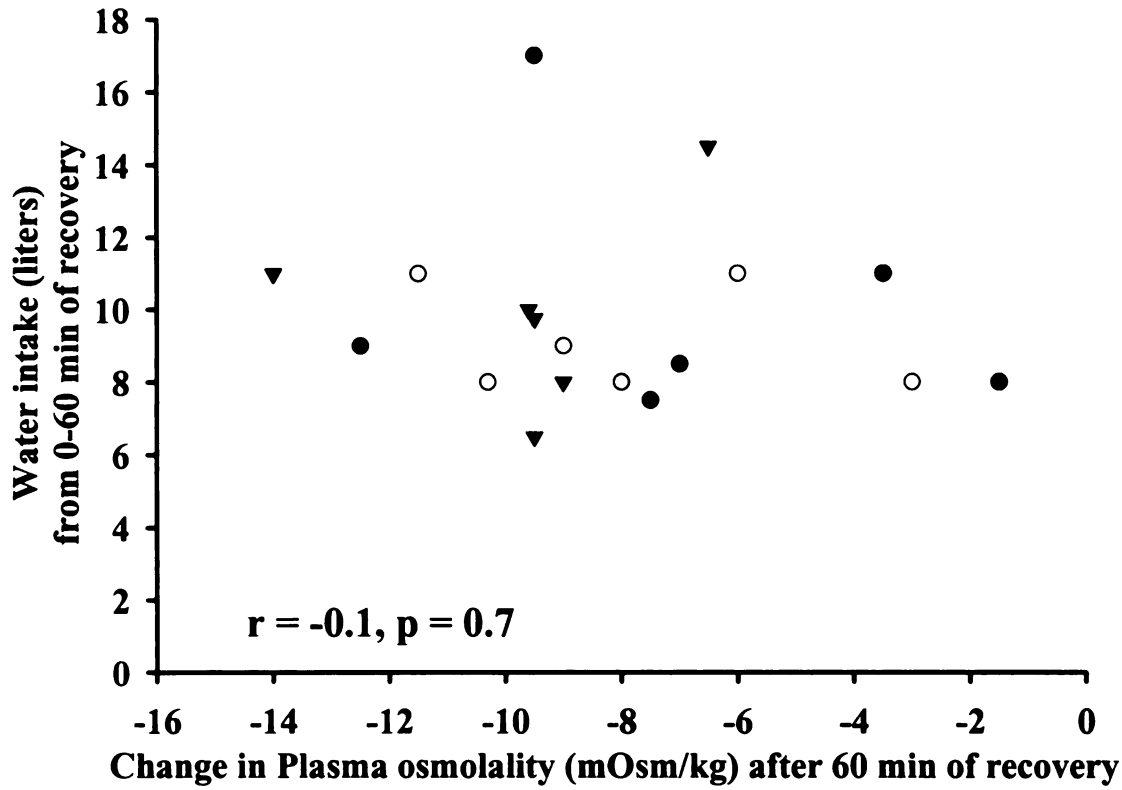

Figure 3.1 Correlation between total water intake (liters) from 0 to 60 minutes of recovery period and change in plasma osmolality (mOsm/kg) after 60 minutes recovery period after induction of dehydration by 45-km of treadmill exercise. Horses were provided 4 l (filled circles), 8 l (circles), or an unlimited amount (UW, filled inverted triangles) of water during the initial 5 min following exercise and subsequently provided free access to water from 20-60 min following exercise (Chapter 2).

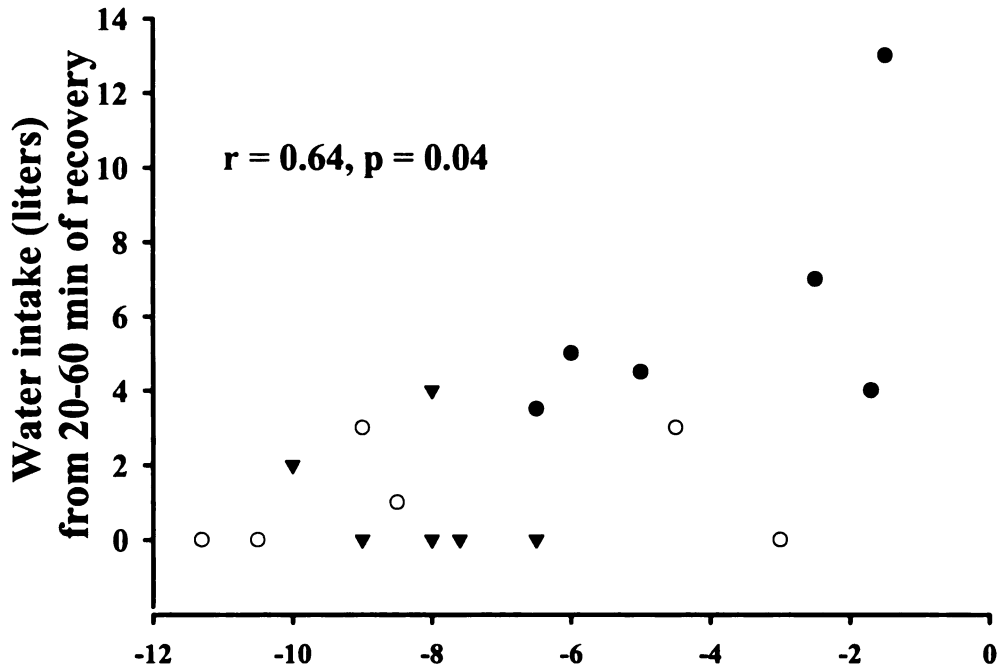

Figure 3.2 Correlation between total water intake (liters) from 20 to 60 minutes of recovery period and change in plasma osmolality (mOsm/kg) after 20 minutes recovery period after induction of dehydration by 45-km of treadmill exercise. Horses were provided 4 l (filled circles), 8 l (circles), or an unlimited amount (UW, filled inverted triangles) of water during the initial 5 min following exercise (Chapter 2).

Appendix 4

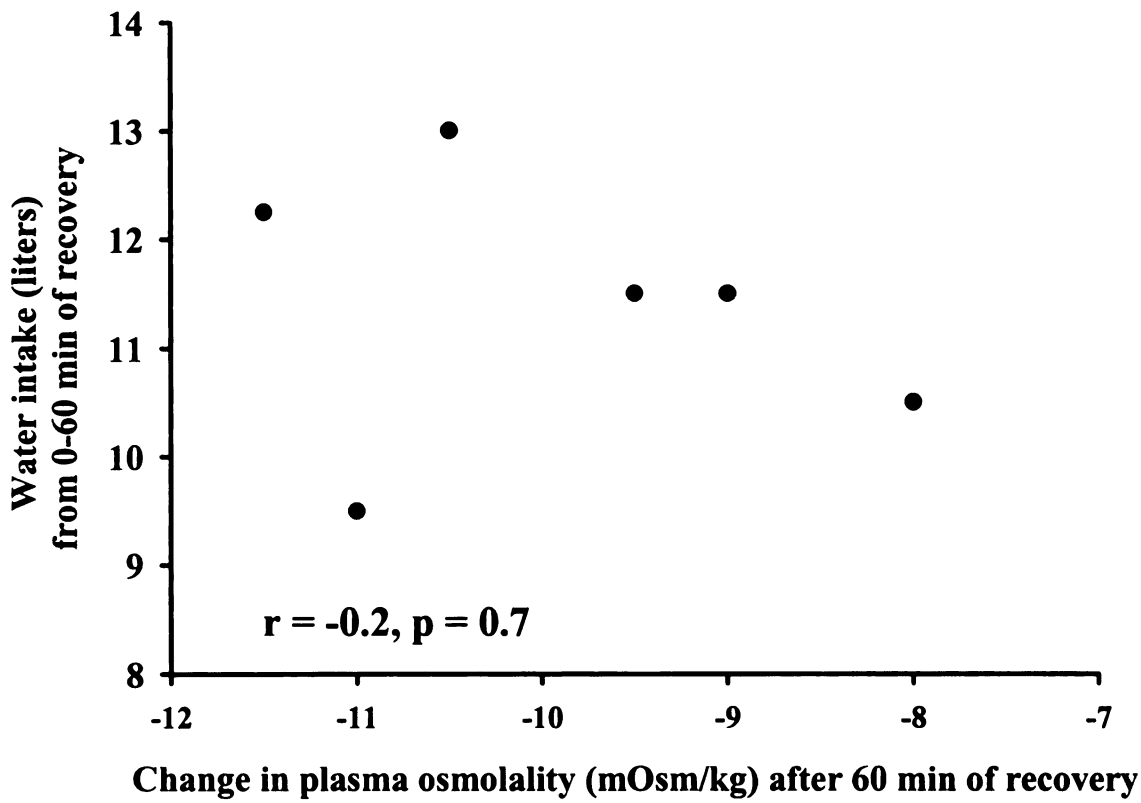

Figure 4.1 Correlation between total water intake (liters) from 0 to 60 minutes of recovery period and change in plasma osmolality (mOsm/kg) after 60 minutes recovery period after induction of dehydration by furosemide (1 mg/kg IV) treatment followed by 45-km of treadmill exercise for horses provided water during the initial 5 min following exercise and subsequently provided free access to water from 20-60 min following exercise (Chapter 3).

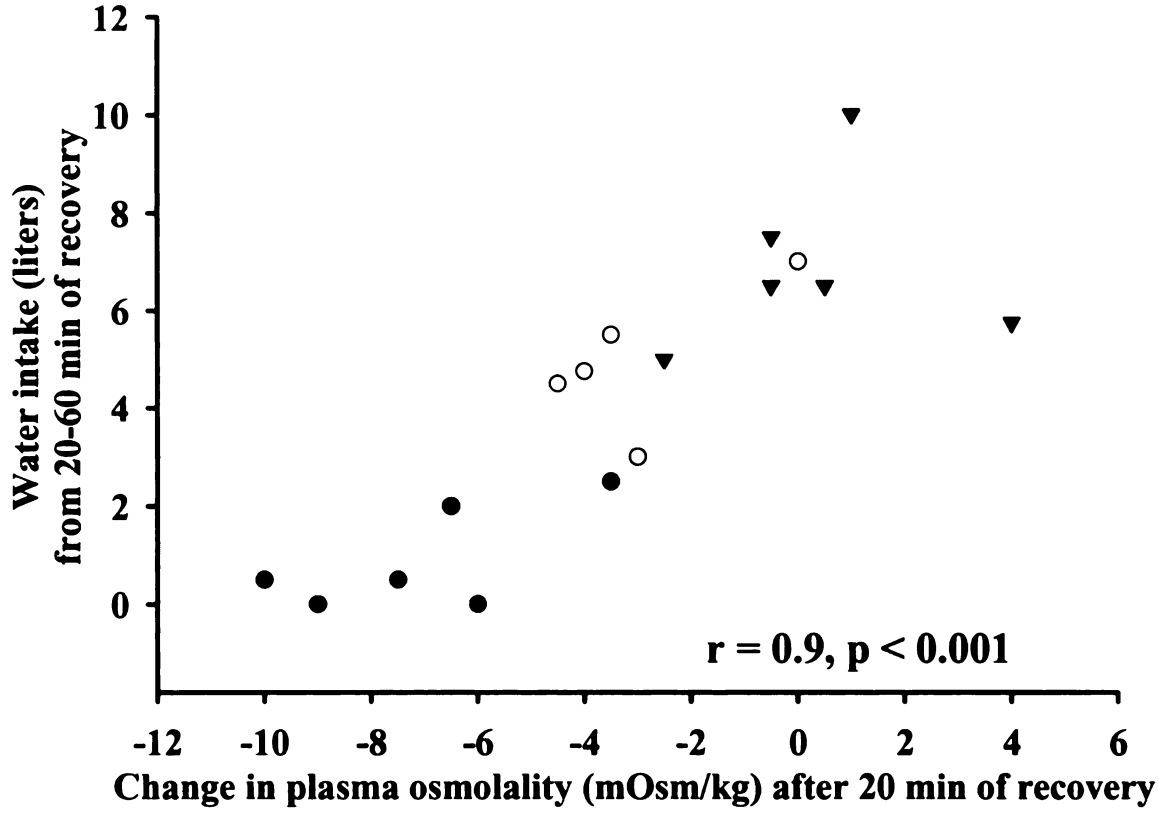

Figure 4.2 Correlation between total water intake (liters) from 20 to 60 minutes of recovery period and change in plasma osmolality (mOsm/kg) after 20 minutes recovery period after induction of dehydration by furosemide (1 mg/kg IV) treatment followed by 45 km of treadmill exercise for horses provided water (filled circles), 0.45% NaCl (open circles), or 0.9% NaCl (filled inverted triangles) as the rehydration fluid during the initial 5 min following exercise and subsequently provided free access to water from 20 to 60 min following exercise (Chapter 3).

Appendix 5

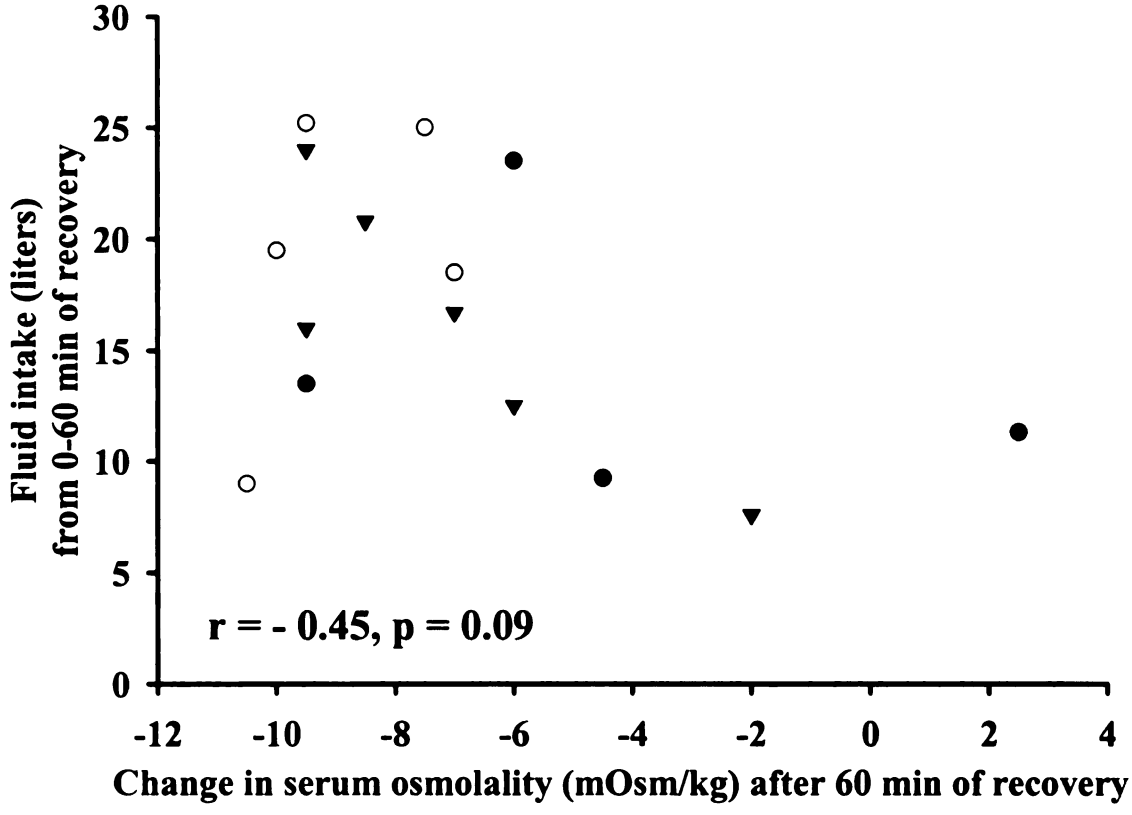

Figure 5.1 Correlation between total fluid intake during the 60 minute recovery period after induction of dehydration by administration of furosemide (1 mg/kg IV) followed by 30 km of treadmill exercise for horses provided 10 (filled circles), 20 (circles), or 30°C (filled inverted triangles) 0.9% NaCl solution as the rehydration fluid during the initial 5 min following exercise and subsequently provided free access to water (at 10, 20, or 30°C) from 20 to 60 min following exercise (Chapter 4).

Appendix 6

Rehydration fluid	Fluid intake 0-5 min of recovery (mean, L)	Drink episodes (mean)	Drink duration (mean, second)
10°C 0.9% NaCl	9.8	10.3	11.6
20°C 0.9% NaCl	12.3	6	20.3
30°C 0.9% NaCl	9.7	11	11.6

Table 6.1 Mean drinking episodes and drinking duration for rehydration fluids (0.9% NaCl solution) at 10, 20 and 30° C during 0 to 5 minutes of postexercise recovery after induction of dehydration by administration of furosemide (1 mg/kg IV) followed by 30 km of treadmill exercise for horses provided 10, 20, or 30°C 0.9% NaCl solution as the rehydration fluid during the initial 5 min following exercise and subsequently provided free access to water (at 10, 20, or 30°C) from 20 to 60 min following exercise (Chapter 4). No significant differences between treatments.

Appendix 7

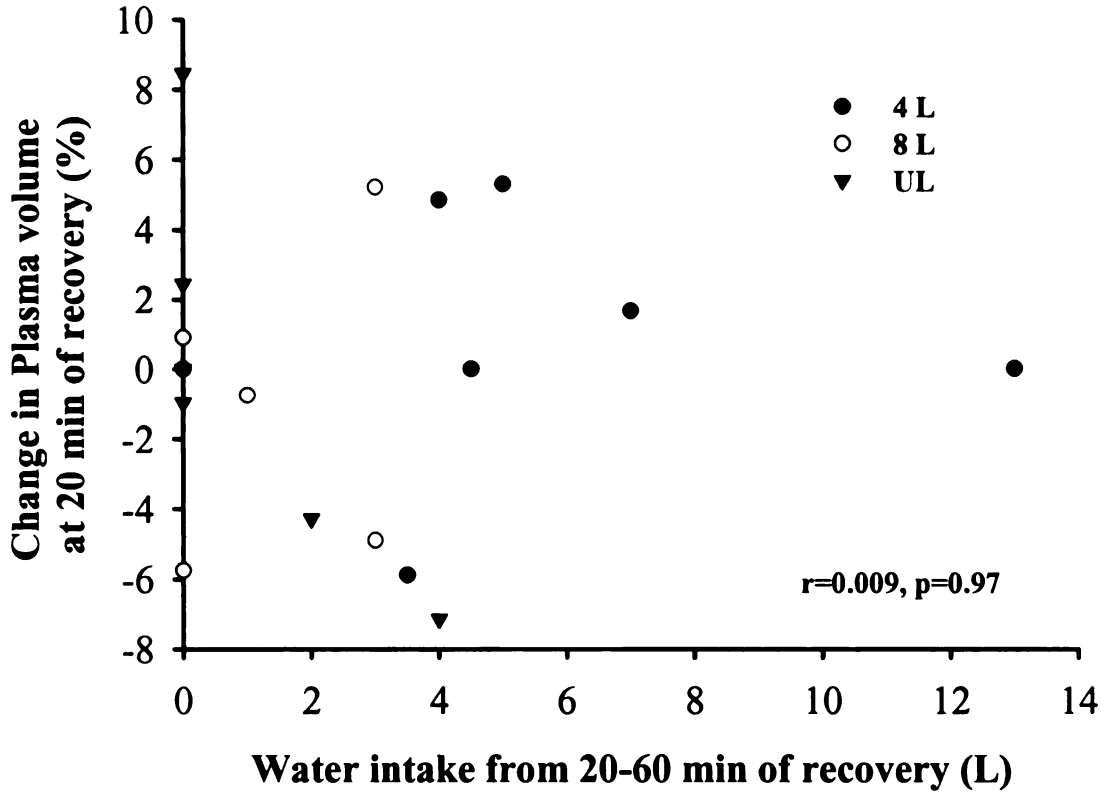

Figure 7.1 Correlation between total water intake (liters) from 20 to 60 minutes of recovery period and change in plasma volume (%) at 20 min recovery period after induction of dehydration by 45-km of treadmill exercise. Horses were provided 4 l (filled circles), 8 l (circles), or an unlimited amount (UL), filled inverted triangles) of water during the initial 5 min following exercise and subsequently provided free access to water from 20-60 min following exercise (Chapter 2).

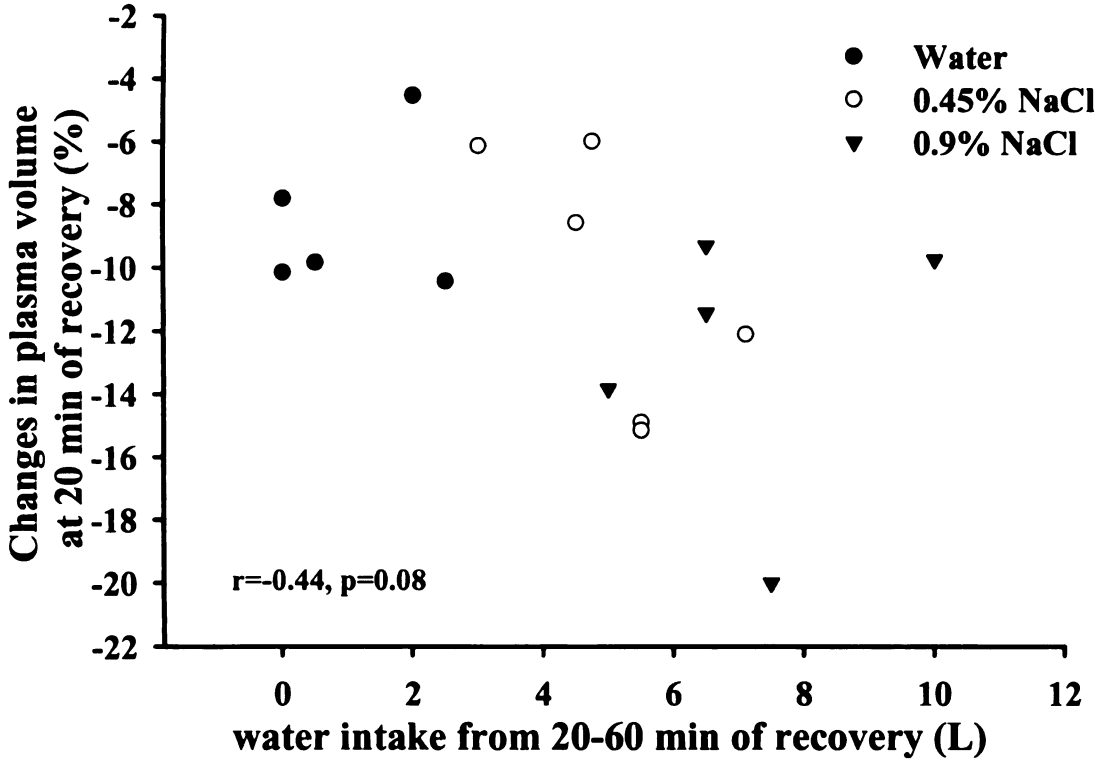

Figure 7.2 Correlation between total water intake (liters) from 20 to 60 minutes of recovery period and change in plasma volume (%) after 20 minutes recovery period after induction of dehydration by furosemide (1 mg/kg IV) treatment followed by 45 km of treadmill exercise for horses provided water (filled circles), 0.45% NaCl (open circles), or 0.9% NaCl (filled inverted triangles) as the rehydration fluid during the initial 5 min following exercise and subsequently provided free access to water from 20 to 60 min following exercise (Chapter 3).

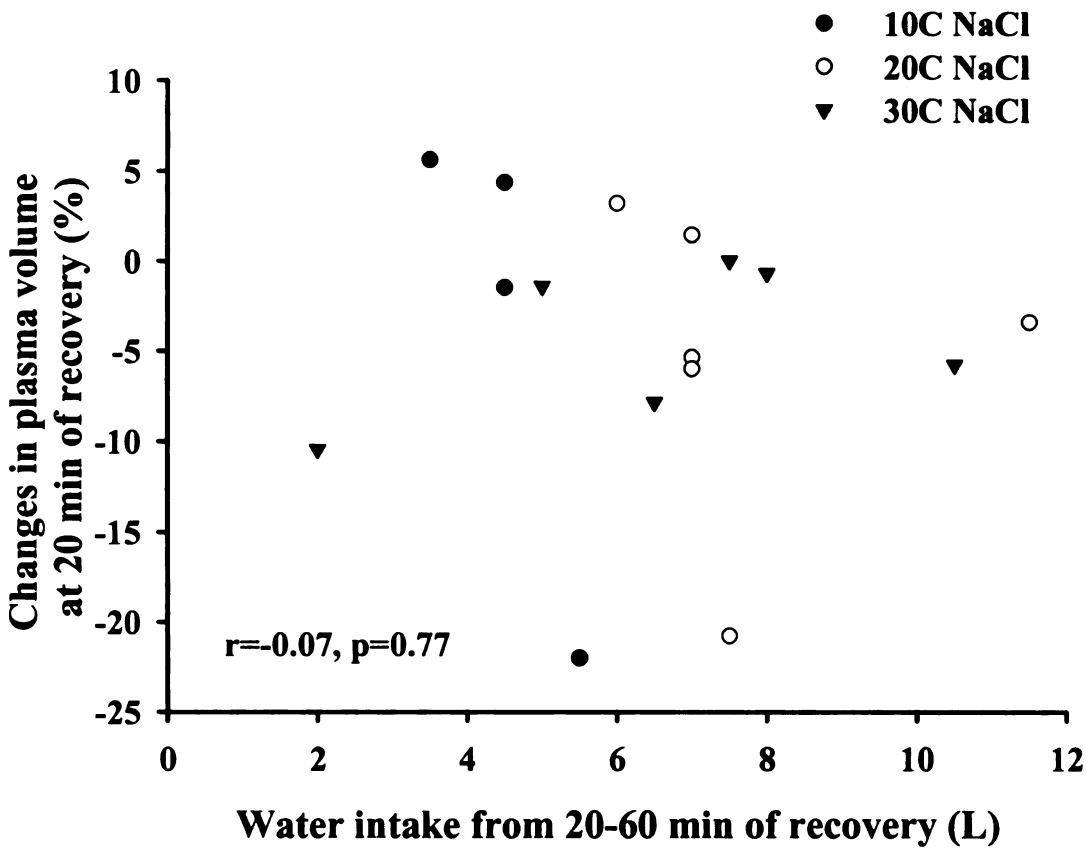

Figure 7.3 Correlation between total fluid intake from 20 to 60 min recovery period after induction of dehydration by administration of furosemide (1 mg/kg IV) followed by 30 km of treadmill exercise for horses provided 10 (filled circles), 20 (circles), or 30°C (filled inverted triangles) 0.9% NaCl solution as the rehydration fluid during the initial 5 min following exercise and subsequently provided free access to water (at 10, 20, or 30°C) from 20 to 60 min following exercise (Chapter 4).