EFFECTS OF HYPERVOLEMIA ON ARTERIAL OXYGENATION IN
THOROUGHBRED HORSES PERFORMING EXERCISE SIMULATING THE SECOND
DAY OF A 3-DAY EQUESTRIAN EVENT

BY

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THESIS

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Exercising horses can lose in excess of 10-12 L/hour of fluid as sweat, and during prolonged exercise, effective circulating volume is diminished. During the second day of an equestrian 3-day event (3DE) there is only a limited opportunity for horses to consume fluids. Dehydration is, therefore, a critical issue for horses performing the second day of a 3DE, particularly if weather conditions are hot and/or humid. Pre-exercise hydration (hyperhydration or ‘fluid-loading’) schemes have been investigated to help maintain blood volume during exercise. However, it has been suggested that pre-exercise hyperhydration might adversely affect arterial oxygenation in horses exercising at ~55-60% of maximum oxygen consumption (Sosa Leon et al. 2002). Exercise induced arterial hypoxemia is not typically seen during moderate exercise in normal horses and hyperhydration (and hypervolemia) does not affect arterial oxygen tension or hemoglobin saturation during short term maximal exertion (Manohar et al. 2003).

Studies were carried out on 7 Thoroughbred horses under control and hyperhydration conditions. Hyperhydration (and hypervolemia) was induced by administering NaCl (0.425 g/kg) via nasogastric tube 5 hours pre-exercise followed by free access to water. Two sets of experiments utilizing different treadmill exercise protocols were performed. The first (Protocol 1) simulated the second day of a mid-level 3DE and was identical to that of Sosa Leon et al. (2002). The second exercise test (Protocol 2) was shorter in duration but incorporated high intensity exercise known to induce arterial hypoxemia and hemoglobin de-saturation. Blood-gas tensions, hemoglobin saturation, and pH were measured pre-exercise and at various times throughout exercise. In addition, plasma protein concentration, hemoglobin concentration, blood lactate concentration, heart rate and core (pulmonary artery) body temperature were monitored during exercise.

Sodium chloride administered as described induced plasma volume expansion estimated on the basis of change in plasma protein concentration of 11.3 ± 3.2% (SE) prior to exercise Protocol 1 and a 15.5 ± 1.1% (SE) increase prior to Protocol 2. Despite establishing significant hypervolemia, we were unable to demonstrate any effect of pre-exercise hyperhydration on arterial oxygen tension or hemoglobin saturation during either exercise test. Arterial CO₂ tension, blood lactate concentrations, and pH were not affected by pre-exercise hyperhydration. In both exercise protocols, there was a reduction in the “arterial to mixed-venous blood O₂ content
difference” in the hyperhydrated treatment during much of the exercise protocol, that was likely offset by an increase in cardiac output.
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CHAPTER 1: INTRODUCTION

The second day of an equestrian 3-day event (3DE) comprises multiple phases, the duration and intensity of which vary with the level of competition (Foreman 2004). During the second day of a 3DE, horses must perform moderate-to-high intensity exercise for a relatively prolonged period interspersed with multiple jumping efforts. Although the format of the second day has undergone changes in an effort to improve competitor safety, this day remains the most physically demanding for both equine and human competitors and is one of the most demanding athletic events that horses participate in.

Prolonged exercise poses a dual challenge for the cardiovascular system in that it must meet both the metabolic requirements of the working musculature and provide for adequate thermoregulation. Maintaining adequate blood volume is, therefore, critical to preserving cardiovascular and thermoregulatory function particularly in species that rely upon sweating to eliminate heat as is the case for both equine and human athletes (Hodgson et al. 1994; Harrison, 1985). Cutaneous vasodilation and an increase in cutaneous blood flow facilitate transfer of heat from the core to the periphery that is then dissipated to the environment by evaporation of sweat. Under severe conditions, exercising horses can lose in excess of 10-12 L/hour of fluid as sweat, and during prolonged exercise, effective circulating volume is considerably diminished (McCutcheon and Geor 1996, 1998). Even relatively mild body water deficits (hypohydration) have been shown to impair athletic performance and effective thermoregulation during exercise in humans (Armstrong et al. 1997; Montain and Coyle 1992; Fortney et al. 1981) and horses (Naylor et al. 1993a).

Dehydration and hyperthermia are therefore critical issues for horses performing extended bouts of exercise, such as the second day of a 3DE, particularly if weather conditions are hot and/or humid. Human athletes are usually encouraged to drink fluids both before and during prolonged exercise to maintain hydration. However, during the second day of a 3DE there is only a limited opportunity for horses to take in fluids and this occurs after the major portion of exercise has been completed. Consequently, a number of pre-exercise hydration (fluid loading or “hyperhydration”) schemes have been investigated (Sosa Leon 1998; Sosa Leon et al. 1996, 2002). However, it has been reported that pre-exercise hyperhydration impairs pulmonary function and adversely affect arterial oxygenation in horses exercising at ~55-60% of maximum
oxygen consumption (Sosa Leon et al. 2002). Because exercise induced arterial hypoxemia is not generally seen at moderate intensity exercise, the primary aim of these studies was to examine the effects of hyperhydration and hypervolemia on pulmonary oxygenation during exercise.

The Equestrian 3-Day Event

The 3DE comprises three tests (dressage, cross-country, and show jumping) completed over consecutive days that, in their entirety, are designed to assess flexibility, fitness, and athleticism (Foreman 2004). The dressage competition, held on the first day, is a low-intensity test during which the horse performs a series of precise movements within a prescribed area. The horse and rider are judged for balance, rhythm, suppleness, and harmony. The second day or cross-country (sometimes known as the endurance day) consists of periods of moderate-to-high intensity exercise interspersed with multiple jumping efforts. The third and final component of the 3DE is an arena show-jumping test over modest jumps and of limited duration, but conducted at a moderate pace. This last trial assesses the technical jumping skills of the horse and rider, but also tests fitness and the ability to perform over multiple days.

It is the second day of the 3DE that is the most physically demanding for both equine and human competitors. In the traditional or long format, the second day includes 4 phases denoted A to D; the duration and intensity of each phase increases as the standard of competition increases (table 1). Phase A is 16-20 minutes in length serving as a warm-up for phase B and is typically conducted at a trot. In phase B, the “steeple chase”, horses travel at a canter or gallop over brush fences for between 3 and 4.5 minutes. Phase C is a relaxed trot of 25-40 minutes duration and is intended to provide a “cool down” period after the steeplechase (Williamson et al. 1996). However, under hot and/or humid conditions, horses might actually accumulate heat during phase C and this portion of the event has been the subject of considerable debate (Foreman et al. 1995). Phases A and C are collectively known as “Roads and Tracks”. Following phase C, there is a brief rest period of 10-15 minutes (Phase X), that is in fact a mandatory veterinary inspection to determine whether the horse is fit to continue on to phase D. Phase D is the actual “cross-country” and is by far the most demanding segment of the day. In this phase, competitors cover distances up to 7.4 km at a strong canter or gallop and are required to jump 24 to 36 fixed, solid obstacles. This final portion of the second day can last up to 13 minutes. Under the traditional format, the entire second day can include over 80 minutes of almost continuous exercise. In
recent years, many 3DE, including the most prestigious four-star competitions, have adopted a shortened format in which phases A, B, and C of day 2 are either curtailed or eliminated completely.

Table 1. Typical speeds and distances of each phase of the second day of an equestrian 3-day event (traditional or long format). In general, the speed remains relatively constant, but the distance covered increases with the level of competition. In both the steeplechase and cross-country portions there are a number of jumping efforts – the height and number of which increases as the level of competition increases (Foreman 2004).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Speed (m/s)</th>
<th>Distance (m)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Roads and Tracks (A)</td>
<td>3.7</td>
<td>3,520-4,400</td>
<td>16-20</td>
</tr>
<tr>
<td>Steeplechase (B)</td>
<td>10.7-11.5</td>
<td>1,920-3,105</td>
<td>3-4.5</td>
</tr>
<tr>
<td>2nd Roads and Tracks (C)</td>
<td>2.7</td>
<td>4,000-6,400</td>
<td>25-40</td>
</tr>
<tr>
<td>Rest (X)</td>
<td>0</td>
<td>0</td>
<td>10-15</td>
</tr>
<tr>
<td>Cross Country (D)</td>
<td>8.7-9.5</td>
<td>3,640-7,410</td>
<td>7-13</td>
</tr>
</tbody>
</table>

Numerous researchers have examined the physiological and biochemical changes occurring in equine athletes during the second day of actual 3DE (Andrews et al. 1994; Andrews et al. 1995; Ecker and Lindinger 1995; Hinchcliff et al. 1995; Kohn et al. 1995; Marlin et al. 1995; White et al. 1995; Williamson et al. 1996). Whilst these studies have garnered useful information, the variables that can be effectively measured are limited and those that are measured often show great variation. This is because there is huge variability between, and even within, studies in terms of environmental conditions, competitor ability (and likely fitness), and length and difficulty of courses. In addition, many variables are measured only before and/or after exercise and might not accurately reflect changes occurring during exercise. There are now a number of studies investigating the effects of prolonged exercise utilizing equine treadmills (Bayly et al. 1995; Geor and McCutcheon 1998a; Kingston et al. 1997; Lindinger et al. 1995, 2004; McCutcheon and Geor 2000; McCutcheon et al. 1999; Naylor et al. 1993a,b; Sosa Leon et al. 1995b). This approach has enabled researchers to measure a wider range of physiological variables, measure those variables during exercise, standardize exercise intensity, and control environment (in terms of heat and humidity). Furthermore, with appropriate training protocols, variations in fitness between individual animals can be somewhat reduced. A growing number of studies have used treadmill exercise protocols to simulate the second day of a 3DE (Harris et al. 1995a,b; McCutcheon and Geor 1996; Marlin et al. 1996, 1999; Sosa Leon et al. 1995a, 1996, 2002; Tennent-Brown et al. 2006a,b). It is difficult in the laboratory to replicate jumping efforts of phases B and D and weight of the rider; however, the exercise intensity of the second day can
be effectively simulated by varying belt speed and treadmill incline (Thornton et al. 1987).

**Body Water Responses to Exercise**

A critical physiological response during prolonged exercise is to maintain an effective circulating volume to provide for perfusion of the contracting muscles and facilitate heat dissipation. In human studies, the response of the body’s fluid compartments to exercise depends on a wide range of factors including: type of exercise performed; posture in which exercise is performed; exercise intensity; ambient temperature and humidity; and heat acclimation, fitness, age, and gender of subjects (Harrison 1985). Both increases and decreases in intravascular volume have been recorded during exercise; however, the predominant response is a decrease in intravascular volume and hemococoncentration shortly after the initiation of both severe- and moderate-intensity exercise (Freund et al. 1987; Harrison et al. 1975; Harrison 1985; Lindinger et al. 1994; van Beaumont et al. 1973).

Under experimental conditions, the initiation of moderate-to-high intensity exercise in humans is typically accompanied by a rapid decrease in plasma volume and hemococoncentration, the extent of which is directly related to the intensity of exercise (Convertino et al. 1981). Although the initial decrease is followed by a slight increase, plasma volume remains below resting levels throughout exercise (Harrison et al. 1975; Harrison 1985). This decrease in plasma volume occurs before significant sweat-fluid losses have occurred and results from water movement out of the vascular space and into the interstitial and intracellular compartments (Lindinger et al. 1994). The driving forces for these fluid shifts are thought to include: increases in interstitial and intracellular osmolality; increased capillary pressure within active muscle beds; and increased arteriolar dilation within contracting muscle. The increase in interstitial and intracellular osmolality is likely due to rapid hydrolysis of phosphocreatine and accumulation of osmotically active creatinine and inorganic phosphate and other substrates within contracting muscles (Hultman and Spriet 1986). During prolonged exercise, plasma volume decreases sharply in the first few minutes of exercise, but then remains fairly static if fluid losses are (at least partially) replaced (Sanders et al. 1999). However, if no effort is made to re-hydrate the exercising subject, plasma volume continues to decrease throughout exercise (Sanders et al. 1999). In humans, it has been estimated that between 23 and 94% of the fluid lost during exercise is derived from the intracellular compartment. The exact value appears to depend upon
the type and duration of exercise performed and form of heat stress utilized.

Similar to the pattern described in humans, a rapid decrease in plasma volume has been reported at the initiation of high intensity exercise in horses (Masri et al. 1990; McKeever et al. 1993). However, during low-to-moderate intensity exercise (40-50% of VO$_{2\text{max}}$), the situation is less clear. Both an increase (Naylor et al. 1993b) and a decrease (Lindinger et al. 1995, 2000, 2004) have been described. Naylor and co-workers reported an increase in plasma volume accompanied by an increase in total plasma protein concentration during exercise of approximately 40% of VO$_{2\text{max}}$ (Naylor et al. 1993b). These workers suggested that this was a result of return of protein-rich fluid from the interstium and lymphatics back into the vascular space. However, in these experiments, plasma volume was only measured 15 minutes into the recovery period following exercise and not during exercise.

Lindinger et al. (1995, 2000, 2004) has more convincingly demonstrated that, during moderate intensity (~50% of VO$_{2\text{max}}$) exercise in horses, there is a rapid initial decrease in plasma volume (of approximately 8 to 9%) in the first 5 minutes of exercise followed by a gradual increase. These findings have been supported by the work of others (Geor and McCutcheon, 1998a; McKeever et al. 1993). As in humans, plasma volume remains below resting levels throughout exercise. After relatively short (< 40 minutes) bouts of exercise, plasma volume returns to pre-exercise values within the first 15 minutes of recovery (Lindinger et al. 1995). However, after prolonged exercise, restoration of total body water might be protracted under both experimental (Lindinger et al. 2004) and field (Andrews et al. 1994) conditions. During prolonged (75-120 minutes), sub-maximal (trotting) exercise, total intracellular fluid volume did not change and the decrease in total body water was borne entirely by the extracellular fluid compartment (Lindinger et al. 2004). In contrast, during shorter bouts of exercise, it appears that the intracellular fluid compartment bears the brunt of net water losses (Lindinger et al. 1995). It is probable that fluid shifts differ between tissues; the intracellular fluid compartment of contracting muscle likely increases during exercise while that of non-contracting tissues decreases (Lindinger et al. 2004).

Some of the differences in fluid shifts between humans and horses could be related to differences in sweat composition between the two species (Kingston et al. 1997; McCutcheon and Geor 1996, 1998). Human sweat is hypotonic to plasma and exercise-induced fluid losses result in plasma hypertonicity (Maughan and Lindinger 1995). Plasma hypertonicity mobilizes
fluid from the intracellular space into the extracellular space and, in effect, helps to defend plasma volume. In contrast, equine sweat is hypertonic to plasma and rich in sodium, chloride and, particularly, potassium (Kingston et al. 1997; McCutcheon and Geor, 1996, 1998). Thus prolonged sweating might be expected to result in plasma (and interstitial) hypotonicity and, potentially, movement of fluid from the extracellular to intracellular compartments (Lindinger et al. 2004).

**Thermoregulation and the Role of Sweating**

Extended bouts of exercise at moderate intensities lead to production and accumulation of heat within the body (Hodgson et al. 1994). Moderate increases in core body temperature improve muscular performance (“warming up”) and generate a rightward shift in the hemoglobin-O₂ dissociation curve that enhances O₂ unloading to the tissues. However, excessive heat accumulation might contribute to exercise fatigue and can have serious adverse consequences. When compared to humans, exercising horses are predisposed to heat stress for two reasons. First, horses have a significantly higher metabolic capacity than humans resulting in a higher rate of muscular heat production during exercise (Maughan and Lindinger 1995). The second reason is that the ratio of body surface area to body mass in the horse is only about 50% of that in man, reducing the capacity for evaporative heat dissipation (Maughan and Lindinger 1995). As a consequence, equine athletes might be unable to dissipate heat rapidly enough to prevent dangerous increases in core temperature during prolonged exercise, particularly under hot and/or humid conditions.

The four mechanisms of heat dissipation for exercising animals are: 1) radiation, 2) convection, 3) conduction, and 4) evaporation (Hodgson et al. 1994). In general, radiation plays only a minor role in heat loss; however, convection, conduction, and evaporation are more important depending on environmental conditions. Convection, the loss of heat due to the movement of air across the skin, is important at lower ambient temperatures, but its contribution is diminished as ambient temperatures increase. Conduction is the process involved in the transfer of heat between surfaces. Indirect conduction (i.e., via the circulatory system) is the major mechanism by which heat is transferred from the working muscles to the heat exchange surfaces (i.e., the skin and respiratory tract). During exercise, a proportion (a maximum of 25% is suggested in one review) of the increase in cardiac output is directed toward augmenting blood
flow to the skin and facilitating heat transfer from the core to the periphery (Rowell 1974).

Evaporative cooling is the primary means of heat dissipation in exercising horses (and humans) (Hodgson et al. 1994). Evaporation of sweat from the skin provides the major route of heat loss although evaporative cooling from the respiratory tract can contribute approximately 25-30% of the total (Hodgson et al. 1993). The driving force for evaporation is the vapor pressure difference between the evaporative surface and surrounding air. When the vapor pressure gradient is diminished (i.e., in very humid conditions) or if the sweating rate is very high, sweat drips from the skin and contributes little to cooling. Therefore, under conditions of high ambient temperature and humidity, the efficacy of evaporative heat loss is reduced and the risk of thermal stress increased (Geor and McCutcheon, 1998a,b).

In mammals that rely on sweating for heat dissipation, prolonged exercise and/or exposure to high heat stressors can result in considerable fluid losses. Sweat fluid losses in exercising horses can exceed 10-12 L/hour (McCutcheon and Geor, 1996). In horses that are normally hydrated pre-exercise, the sweating rate and sweat composition does not change during prolonged exercise even in the face of significant (~6%) hypohydration (Kingston et al. 1997). However, hypohydration induced pre-exercise (by water deprivation or diuretic administration) has been suggested to impair sweat responses and decrease sweating rates (Naylor et al. 1993a).

As mentioned, equine sweat contains high concentrations of the major electrolytes sodium, chloride, and potassium. High sweating rates in equine athletes performing prolonged exercise can therefore be accompanied by substantial electrolyte losses that might, under extreme conditions, impair muscle function presumably by disruption of trans-membrane electrochemical gradients (Maughan and Lindinger, 1995).

**Hypohydration and its Effects on Thermoregulation, Cardiovascular Function, and Performance**

During moderate-intensity exercise, blood flow to the contracting muscles of the brachium and thigh increases 31- to 38-fold and by 70- to 76-fold respectively (Parks and Manohar 1983). As body temperature increases, cutaneous vasodilation occurs and there is an increase in blood flow to the skin effecting heat dissipation. Changes in peripheral perfusion and vascular tone tend to increase total vascular space. During strenuous exercise, vasoconstriction of selected tissue beds (e.g., those supplying the splanchnic tissues) helps to preserve an effective
circulating volume (Manohar 1986a). However, during moderate-intensity exercise, blood supply to these tissue beds is maintained (Parks and Manohar 1983). Thus, the combination of extended, moderate-intensity exercise and heat strain results in competition for a finite blood volume (and cardiac output) that is exacerbated by exercise-induced fluid losses. Therefore, matching fluid intake with losses is a critical problem in athletes performing prolonged exercise, particularly under hot, humid conditions. Even in human athletes ingesting *ad libitum* fluids during exercise, sweat fluid losses often exceed water intake resulting in a body water deficit or hypohydration (Greenleaf, 1992; Sawka et al. 2001).

Both hypohydration and plasma hypertonicity have been shown to impair heat dissipation in exercising humans (Montain and Coyle 1992; Sawka et al. 1985). In humans, blood flow to the working muscles is usually maintained at the expense of flow to the skin (Gonzalez-Alonso et al. 1995, 1997; Montain and Coyle 1992; Nadel et al. 1980). This preferential partitioning of cardiac output toward the musculature is aggravated by hypovolemia and reduces the transfer of heat to the periphery. In addition, hypohydration limits evaporative heat loss by reducing sweating rate and sensitivity (i.e., the increase in sweating rate per unit increase in temperature) (Fortney et al. 1981; Greenleaf and Castle 1971; Sawka et al. 1983, 1985). The onset of sweating is also delayed (i.e., sweating threshold is increased) by hypohydration (Fortney et al. 1981; Montain et al. 1995). Thus, as the magnitude of water deficit increases, there is a concomitant increase in core temperature for the same exercise intensity. Hypohydration not only increases heat accumulation during exercise, but also negates the advantages of aerobic fitness and heat acclimation (Gonzalez-Alonso and Calbet 2003; Sawka et al. 1985, 2001). The increase in plasma osmolality that occurs in humans subsequent to sweat fluid losses also appears to impair thermoregulation (Sawka et al. 1985; Montain and Coyle 1992). In horses, sweating responses and heat storage were not affected by exercise-induced (i.e., sweat fluid losses) hypohydration during prolonged exercise (Kingston et al. 1997). However, pre-exercise hypohydration (3.2-3.9% decrease in body mass achieved by either water deprivation or diuretics) has been shown to increase heat storage in horses exercising at 40% of VO$_{2\text{max}}$ for 40 minutes under mild (21-22°C, 25-40% relative humidity) conditions (Naylor et al. 1993a). Sweating rates and skin temperature were not changed, but core temperature was increased and cardiac output decreased suggesting that transfer of heat to the periphery was impaired (Naylor et al. 1993a) in accord with results from some (but not all) human studies (Montain and Coyle 1992). In a study examining the
effects of hydration during prolonged treadmill exercise (consecutive bouts lasting 45 minutes at 50% VO$_{2\text{max}}$) in a hot (34.5°C) environment, horses receiving oral fluids (equal to approximately 85% of sweat fluid losses) better maintained their plasma volume and had a lower core temperature at the conclusion of exercise (Geor and McCutcheon, 1998a). Hypohydration in the control group was accompanied by decreases in cardiac output, stroke volume, sweating rate, and sweating sensitivity (Geor and McCutcheon, 1998a).

The increase in the volume of the vascular space subsequent to cutaneous vasodilation and changes in peripheral venous tone decreases central venous pressure and cardiac filling. This in turn reduces stroke volume and elicits a compensatory increase in heart rate (Gonzalez-Alonso et al. 1995, 1997; Thomas and Fregin 1990; Montain and Coyle 1992). Exercise-induced fluid losses magnify the decrease in venous return and contribute to the phenomenon of cardiovascular drift (where increases in heart rate are no longer sufficient to compensate for the decreases in stroke volume and cardiac output is reduced) that might adversely affect performance (Thomas and Fregin 1990; Montain and Coyle 1992). In humans performing sub-maximal exercise with little heat strain, hypovolemia decreases stroke volume, but the compensatory increase in heart rate is sufficient to maintain cardiac output relative to the euhydrated state (Gonzalez-Alonso et al. 1995, 1997). However, the combination of heat stress and hypohydration has an additive effect on cardiovascular performance. During sub-maximal exercise with moderate-to-severe heat strain, hypohydration decreases cardiac output below euhydration values because the increase in heart rate is no longer sufficient to overcome the decrease in stroke volume (Gonzalez-Alonso et al. 1997; Montain and Coyle 1992).

Studies in humans have shown that hypohydration can decrease both muscular endurance (Montain et al. 1998) and maximal aerobic power (Sawka et al. 2001). In temperate climates, small water deficits (less than 3% bodyweight loss) do not alter maximal O$_2$ consumption (VO$_{2\text{max}}$) (Sawka et al. 2001). However, VO$_{2\text{max}}$ has been shown to decrease with larger deficits and even small to moderate water deficits (2-4% bodyweight loss) can produce large reductions in VO$_{2\text{max}}$ in hot climates (Craig and Cummings 1966; Sawka et al. 2001). Hypohydration has also been shown to increase O$_2$ consumption (VO$_2$) for a given exercise intensity (Armstrong et al. 1985; Caldwell et al. 1984; Greenleaf and Castle 1971). During severe exercise-heat stress, the reduction in cardiac output might be severe enough to decrease skeletal muscle blood flow with obvious implications for performance (Gonzalez-Alonso and Calbet 2003; Gonzalez-
In addition to immediate adverse effects on performance, hypohydration might have a cumulative effect in athletes competing over several days. Restoration of fluid losses can be delayed in horses that have experienced large sweat-fluid losses (Andrews et al. 1994; Lindinger et al. 2004). In prolonged, sub-maximal exercise in conditioned horses conducted under laboratory conditions, total body water (as assessed by body weight changes) decreased by 4.2 ± 0.5% and plasma volume decreased by approximately 18% (Lindinger et al. 2004). Complete restoration of plasma volume had not occurred by 4 hours post-exercise although it had returned to pre-exercise levels by 13 hours post-exercise (Lindinger et al. 2004). Studies conducted under field conditions have shown that restoration of total body water losses in horses that occur during the second day of a 3DE might not be complete by the time that animals must compete the following day (i.e., 18-24 hours later) (Andrews et al. 1994).

**Fluid Replacement Therapy, Hyperhydration, and the Effects on Performance**

Human athletes performing prolonged exercise are now routinely encouraged to drink fluids or electrolyte solutions both before and during exercise. Studies in humans have shown that performance and thermoregulation are improved if fluid losses are at least partially restored during exercise (Montain and Coyle 1992). The choice of fluid does not appear to be as important as the volume and the best results are achieved with strategies that approximate sweat fluid losses (Sanders et al. 1999). Although appropriate hydration is critical, over the last two decades excessive hydration with hypotonic fluids before or during prolonged exercise has been increasingly associated with life-threatening hyponatremia (Noakes and Speedy, 2006; Rosner and Kirven, 2007). Hormonal (especially abnormalities in vasopressin secretion) and renal abnormalities in water handling that predispose individuals to hyponatremia might be present in some individuals (Rosner and Kirven, 2007). During the second day of a 3DE, there is a very limited opportunity for horses to drink and, even if it were allowed, this occurs late in the event after the majority of the exercise has been completed (i.e., during phase X).

An alternative approach to consuming fluids during exercise is to “fluid load” prior to exercise and create a reserve (i.e., hyperhydration). Hyperhydration has been suggested as a means of maintaining an effective circulating volume in the face of exercise-induced fluid losses thereby protecting cardiovascular and thermoregulatory function. Although results from human
studies have been somewhat inconsistent, several reports have shown that hyperhydration prior to prolonged exercise allows for greater endurance and better cardiovascular and thermoregulatory performance when compared with euhydration (Coles and Luetkemeier 2005; Coutts et al. 2002; Greenleaf and Castle 1971; Greenleaf et al. 1997; Grucza et al. 1987; Lyons et al. 1990; Moroff and Bass 1965). However, a possible detrimental effect of hyperhydration is to decrease hemoglobin concentration and hence, O2-carrying capacity, subsequent to acute plasma volume expansion. The reduction in arterial O2 content might, intuitively, be expected to decrease O2 delivery to the muscles reducing VO2max and exercise performance (Calbet et al. 2006). However, several reports examining the effects of acute plasma volume expansion in humans and horses describe either no change in VO2max (Green et al. 1997; Hopper et al. 1991; Kanstrup and Ekblom 1982; Mier et al. 1996; Warburton et al. 1999; Zavorsky et al. 2002) or an increase (Coyle et al. 1986; Krip et al. 1997). It has been shown in several species that an acute expansion of plasma volume increases cardiac output (primarily through an increase in stroke volume) such that the decrease in O2 carrying capacity is offset and muscle O2 delivery is preserved or even increased during maximal intensity exercise (Gledhill et al. 1999; Horwitz and Lindenfield 1985; Kanstrup and Ekblom 1982; Warburton et al. 2000). In experiments in horses, the effect of hyperhydration (and the subsequent increase in body weight) does not appear to have a significant effect on oxygen consumption (VO2) at workloads below VO2max. Sosa Leon - et al. (2002) found no difference in VO2 between hyperhydrated horses (average increase in bodyweight 21.3 ± 1.2 kg) and control animals. Although horses administered 12 L of water pre-exercise tended to have a slight increase in VO2 compared to euhydrated animals when exercising at 65-70% of maximal heart rate (trotting at 6-7 m/s), this difference was not statistically significant (Nyman et al. 2002).

The extent to which plasma volume expansion can increase cardiac output depends on several factors including training state of subjects (Coyle et al. 1986; Kripp et al. 1997). It is well recognized that endurance trained human (Coyle et al. 1986; Convertino et al. 1980, 1991) and equine (McKeever et al. 1987) subjects have an increased blood volume when compared to untrained individuals. Plasma volume increases within 24 hours of beginning exercise training and accounts for the majority of blood volume expansion in the first 2 weeks of training (Convertino et al. 1980, 1991; Sawka et al. 2000). After approximately 2-3 weeks of exercise training, erythrocyte volume also begins to increase (Sawka et al. 2000). The increased blood
volume, which might be 8-10% greater than pre-training levels, is one of the important mechanisms underlying the increased VO$_{2\text{max}}$ in trained individuals (Kripp et al. 1997). Hyperhydration and plasma volume expansion might, therefore, be of limited or no benefit in trained subjects (Coyle et al. 1986; Kripp et al. 1997; Warburton et al. 2000).

A number of pre-exercise hyperhydration strategies have been investigated for horses undertaking prolonged bouts of exercise (Geor and McCutcheon 1998a; Nyman et al. 2002; Schott et al. 2001; Sosa Leon 1998; Sosa Leon et al. 1995a,b, 1996, 2002;). However, it has been reported that hyperhydration of horses prior to treadmill exercise simulating the second day of a 3DE adversely affected arterial oxygenation (Sosa Leon et al. 2002). In those experiments, mean arterial O$_2$ tension of hyperhydrated horses decreased by ~15 mm Hg during periods of moderate-intensity (approximately 55 to 60% of VO$_{2\text{max}}$) exercise (corresponding to phases B and D of the exercise protocol) when compared to the control group (Sosa Leon et al. 2002). Because exercise is typically accompanied by hyperthermia and acidemia that causes a rightward shift of the hemoglobin-O$_2$ dissociation curve, a drop in arterial O$_2$ partial pressure of 15 mm Hg is likely to be attended by significant hemoglobin de-saturation (Manohar et al. 2004, Nielsen et al. 2002). Sosa Leon et al. (2002) suggested that pre-exercise hyperhydration could adversely affect performance as a result of pulmonary edema subsequent to extravasation of administered fluids.

**Exercise Induced Arterial Hypoxemia**

Exercise induced arterial hypoxemia (EIAH) is a phenomena that occurs during strenuous exercise in equine and a subset of human athletes. Definitions vary between investigators; however, most consider a decrease in arterial O$_2$ tension of 10-15 mm Hg (when adjusted for core body temperature) significant (Dempsey and Wagner, 1997; Prefaut et al. 2000). In addition to arterial hypoxemia, EIAH is accompanied by significant hemoglobin de-saturation (Dempsey and Wagner, 1997; Wagner et al. 1989, 1996). During prolonged or intense exercise, the hemoglobin-O$_2$ dissociation curve is shifted to the right (i.e., decreased affinity of hemoglobin for O$_2$) secondary to hyperthermia and acidemia. The reduction in affinity enhances O$_2$ unloading within the tissues, but might impair O$_2$ uptake by hemoglobin within the lungs. Oxygen loading by hemoglobin would be expected to be further impaired by the decrease in O$_2$ tension that occurs with EIAH. Thus, EIAH likely impairs performance as it limits O$_2$ delivery to
the working musculature (Wagner et al. 1996). Although the definitive cause of EIAH is unknown and likely to be multifactorial, diffusion limitation is thought to be a major contributor, at least in horses (Wagner et al. 1989). It has been suggested that the increase in cardiac output during exercise decreases pulmonary capillary transit time and, therefore, the time available for gaseous exchange (Dempsey and Wagner, 1997; Wagner et al. 1989, 1996; Wilkins et al. 2005). Relative alveolar hypoventilation, as evidenced by an increase in arterial CO$_2$ tension during strenuous exercise, might also account for a portion of the decrease in arterial O$_2$ tension. Increasing ventilation-perfusion (V/Q) mismatching is thought to be an important component to the development of EIAH in strenuously exercising humans, although significant V/Q mismatching does not appear to occur in exercising horses (Wagner et al. 1989; Hopkins et al. 1998; Seaman et al. 1995). Experiments in this laboratory have shown that pulmonary arteriovenous shunting is also unlikely to contribute to EIAH in racehorses (Manohar and Goetz 2005a).

Exercise induced arterial hypoxemia is a well recognized entity in horses working at maximal intensity (Bayly et al. 1989). However, during moderate-intensity exercise, arterial O$_2$ tension is typically well maintained (Bayly et al. 1995) and in experiments conducted on Thoroughbred horses in our laboratory and the laboratories of others, EIAH is not seen until horses are exercising at close to maximal intensity (Bayly et al. 1989, 1995; Goetz et al. 2001; Manohar and Goetz 2003; Manohar et al. 2001a,b, 2002a,b, 2003, 2004). Other investigators have noted arterial hypoxemia to occur at lower work intensities; however, this has typically been in non-Thoroughbred horses (Hinchcliff et al. 1993; Hinchcliff and McKeever 1999; Marlin et al. 1996, 1999). It is interesting to note that EIAH does not occur in all exercising humans, but only in a subset of elite athletes performing at close to maximal intensity (Dempsey and Wagner, 1997; Prefaut et al. 2000).

**Experimental Objective**

In view of the importance of hydration status to optimal performance (Montain and Cole 1992; Naylor et al. 1993a) and the findings of Sosa Leon at al. (2002) suggesting that pre-exercise fluid-loading might impair arterial oxygenation, the primary objective of these studies was to examine the impact of pre-exercise hyperhydration and hypervolemia on arterial oxygenation of exercising Thoroughbred horses. In an initial set of experiments, it was
demonstrated that pre-exercise hypervolemia did not exacerbate arterial hypoxemia occurring in Thoroughbreds performing short-term, high-intensity exercise (Manohar et al. 2003). Thus, it was proposed that, perhaps, a combination of pre-exercise hyperhydration and prolonged exercise was required to induce the arterial hypoxemia observed by Sosa Leon et al. (2002). Therefore, in the first study described here, an exercise protocol identical to that of Sosa Leon et al. (2002) was used to simulate the second day of a mid-level 3DE, although an alternative method was used to induce hyperhydration and hypervolemia rather than the nasogastric administration of a large volume (~26 L) of fluids. In a final series of experiments, the effects of pre-exercise hyperhydration and hypervolemia on arterial oxygenation were examined during a prolonged exercise protocol that incorporated a short burst of high-intensity exercise known to induce arterial hypoxemia and hemoglobin de-saturation.
CHAPTER 2: MATERIALS AND METHODS

Horses Used in Experiments

Experiments were carried out on seven healthy, sound Thoroughbred horses (2 mares and 5 geldings) aged between 3 and 6 years and weighing 454 ± 10 kg. Horses were kept in individual stalls in an air conditioned building and the temperature maintained between 18 and 20°C. They were fed a diet of alfalfa hay and oats and had free access to clean water. Horses were de-wormed with ivermectin (Eqvalan®, MSD-Agvet, Rahway, New Jersey, USA) and inoculated with tetanus toxoid (Fort Dodge Animal Health, Fort Dodge, Iowa, USA) and vaccines against Streptococcus equi equi (Intervet Inc., Millsboro, Delaware, USA) and West Nile Virus (Fort Dodge Animal Health, Fort Dodge, Iowa, USA) infection. Experimental protocols and procedures were approved by the Institutional Laboratory Animal Care and Use Committees.

Exercise-Training of Experimental Horses

These horses had been involved in a previous study that required them to gallop on a high-speed treadmill (Sato®, Upsala, Sweden) at 14 m/s on a 3.5% slope for 120 seconds. For that study, horses were initially familiarized with the stocks and treadmill before being asked to walk, trot, and canter on the treadmill for one week. They were then exercised 3 days per week with the treadmill set at a 0% slope. During this period, horses were walked at 2 m/s for 60 seconds and then trotted at 3, 4, 5 and 6 m/s for 60 seconds. Horses were then cantered at 8 m/s for 60 seconds and galloped at 14 m/s for 120 seconds. The treadmill belt speed was then decreased to 5 m/s for 60 seconds and then 2 m/s for 300 s before stopping. After 4 weeks of training in this manner, the exercise regime was continued in exactly the same fashion but the treadmill was now set at a 3.5% uphill grade. Upon completion of the previous study, horses were immediately assigned to the present study without additional training.

Experimental Procedures

The procedures used in this laboratory for blood-gas and hemodynamic studies have been described previously (Goetz et al. 2001; Manohar and Goetz 2003; Manohar et al. 2001a,b, 2002a,b, 2003, 2004), but are described in detail here. The manes and both sides of the neck
were clipped and shaved to facilitate aseptic preparation of the skin for catheterization of the left and/or right jugular veins. In addition, the left thoracolumbar region was clipped and shaved over the 16th and 17th intercostal spaces to allow catheterization of the abdominal aorta.

Following aseptic skin preparation, 2% lidocaine hydrochloride (Lidocaine HCl, Abbott Laboratories, North Chicago, Illinois, USA) was deposited subcutaneously over the 16th or 17th intercostal space and in 2 locations over the left and/or right jugular veins. Additional subcutaneous lidocaine blebs were placed to facilitate suture placement to anchor the catheters. The abdominal aorta was catheterized percutaneously as follows: the epaxial musculature was desensitized by injection of an additional volume lidocaine via a 20 cm, 18 g spinal needle, a 1.5 cm skin incision was then made, and the aorta was punctured with a 25 cm, 12 g needle (with stylus). This allowed introduction of a 9 F catheter into the aorta that was advanced 50 cm proximally (i.e., toward the heart). Two catheter introducers (Hemaquét®, CR Bard Inc., Boston, Massachusetts, USA) were placed into the left and/or right jugular vein(s) and anchored with suture. The introducer allowed placement of an 8 F cardiac catheter equipped with a tip-manometer (Millar Instruments, Houston, Texas, USA) and thermistor (Edward Laboratories, Santa Clara, California, USA) into the pulmonary artery. A second catheter, also with a tip-manometer was placed in the right ventricle to allow heart rate measurements from the blood pressure waveform. The location of these catheters was confirmed by monitoring for the characteristic phasic blood pressure waveforms on an oscillographic recorder (Gilson, Medical Electronics Inc., Middleton, Wisconsin, USA). This catheter set-up allowed simultaneous sampling of the aortic (arterial) and pulmonary artery (mixed-venous) blood as well as continuous monitoring of pulmonary artery blood (core) temperature and heart rate. After instrumentation, horses stood quietly on the treadmill for 45-50 minutes before exercise studies were undertaken.

In this study, changes in plasma protein concentration were used to assess changes in plasma volume (Boyd 1981). This assumes that the concentration of plasma protein in healthy horses is relatively constant and that acute changes in plasma protein concentration are indicative of changes in plasma volume. Plasma protein concentration was determined by refractometry. The percentage change in plasma volume was calculated as \( [(PP1/PP2) - 1.0] \times 100\% \), where PP1 and PP2 are the initial and test plasma protein concentrations, respectively (Boyd, 1981). Blood for determination of plasma protein concentration and packed cell volume (PCV) was
collected into sodium EDTA (Vacutainer, Becton Dickinson and Company, Franklin Lakes, New Jersey, USA), stored in an ice bath, and analyzed within 3 hours of collection. Packed cell volume was determined by the microhematocrit method. Blood gas samples were collected into 5 mL syringes (Monoject, Tyco Healthcare Group, Mansfield, Massachusetts, USA) that had been coated with approximately 0.3 mL of sodium heparin (Heparin sodium 10,000 USP U/mL, American Pharmaceutical Partners Inc., Schaumburg, Illinois, USA). Following sample collection, syringes were capped with leak-proof caps, stored in an ice bath, and samples analyzed within 90 minutes of collection. Blood-gas tensions, pH, hemoglobin concentration, hemoglobin-O$_2$ saturation, and O$_2$ content were determined using a carefully calibrated blood-gas analyzer/co-oximeter (ABL520 system, Radiometer, Copenhagen, Denmark). All blood-gas tensions and pH data were corrected to the simultaneously measured core temperature. To determine blood lactate concentration, mixed-venous blood samples were obtained at various time points (see experimental design and protocol). These were immediately de-proteinized with chilled perchloric acid (8% w/v) and the supernatant harvested for analysis with a chemical assay (Sigma Diagnostics, Sigma Chemicals, St. Louis, Missouri, USA). All lactate assays were carried out in duplicate.

**Experimental Design and Protocol**

A cross-over experimental design was used in which each horse was studied under both treatment conditions, namely control and hyperhydration. The sequence of treatments was randomized for every horse and at least 7 days were allowed between studies. All experiments were carried out in an air-conditioned laboratory with the ambient temperature maintained between 18 and 20°C. In addition, a large fan was placed in front of the treadmill, providing airflow over the horse during exercise for evaporative cooling. In the control group, horses received no treatment. For the hyperhydration experiments, hypervolemia was induced as described below.

**Induction of Hyperhydration and Hypervolemia**

Before these experiments were performed, a pilot study had been conducted to determine the dose of NaCl that would induce ingestion of water and a significant expansion of plasma volume. In these experiments, a dose of NaCl between 0.30 and 0.45 g/kg administered via a
nasogastric tube was found to induce a decrease in plasma protein concentration of 0.8-1.0 g/dL (Manohar *et al.* 2003). This decrease in plasma protein concentration corresponds to a 15-18% expansion in plasma volume (Boyd 1981). Approximately 30 minutes after receiving NaCl, horses would begin to drink water and would continue to do so intermittently for approximately 3 hours. Plasma protein concentrations reached their lowest point 4.5 – 5.0 hours after NaCl administration and, thereafter, slowly returned to pre-treatment levels.

Based on these results, a NaCl dose of 0.425 g/kg was chosen for the current experiments. Horses were weighed and blood samples collected for determination of baseline plasma protein concentration (pre-NaCl administration). Sodium chloride was then administered in 1,500 mL of luke-warm water via a nasogastric tube. Immediately following NaCl administration, the horses were returned to their stalls and allowed free access to fresh, clean, water. Approximately 3.75 hours after NaCl administration, the horses were taken from their stalls and weighed again immediately before instrumentation (see experimental procedures). Following instrumentation, horses stood quietly on the treadmill for 45-50 minutes before exercise.

At 285-290 minutes after NaCl administration and when heart rate had been stable for 10-15 minutes, pre-exercise samples were collected for plasma protein concentration, PCV, blood-gas, and pH measurements.

*Exercise Protocols*

The exercise protocol (Protocol 1) used in the initial set of experiments described was designed to simulate the second day of a three day equestrian event and is identical to that used by Sosa Leon *et al.* (2002). All exercise was performed with the treadmill set at a slope of 3%. The exercise protocol comprised 4 phases (A, B, C and D; Table 2 and Figure 1) with a 10 minute rest between phases C and D. Protocol 1 consisted of a 30 minute trot at 3 m/s (phase A), 4 minute canter at 8 m/s (phase B), 50 minute trot at 3 m/s (phase C), 10 minute rest, and finally, a 14 minute canter at 7.3 m/s (phase D). Phase D was followed by a short cool-down period (5 minute walk at 2 m/s).
Table 2. Phases of exercise Protocol 1; the exercise protocol used in the initial experiments described here simulating the 2nd day of a 3DE and identical to that used by Sosa Leon et al. (2002).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Speed (m/s)</th>
<th>Gait</th>
<th>Time (min)</th>
<th>Distance (m)</th>
<th>Incline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.0</td>
<td>Trot</td>
<td>30</td>
<td>5,400</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>8.0</td>
<td>Canter</td>
<td>4</td>
<td>1,920</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>3.0</td>
<td>Trot</td>
<td>50</td>
<td>9,000</td>
<td>3</td>
</tr>
<tr>
<td>Rest</td>
<td>0</td>
<td></td>
<td>10</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>7.3</td>
<td>Canter</td>
<td>14</td>
<td>6,132</td>
<td>3</td>
</tr>
<tr>
<td>Cool-down</td>
<td>2.0</td>
<td>Walk</td>
<td>5</td>
<td>600</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>113</strong></td>
<td><strong>23,052</strong></td>
<td></td>
</tr>
</tbody>
</table>

In this protocol, core temperature was measured continuously in addition to the simultaneous arterial and mixed-venous blood samples collected for determination of blood-gas tensions, hemoglobin concentration, hemoglobin-O2 saturation, total O2 content, PCV, and plasma protein concentration. Samples were obtained at: 10, 20 and 30 minutes of phase A; at 1, 2, 3 and 4 minutes of phase B; at 12.5, 25, 37.5 and 50 minutes of phase C; at the end of the 10 minute rest period; and at 5, 10 and 14 minutes of phase D. Heart rate was also determined at these time points. Mixed venous blood lactate concentration was determined pre-exercise, 2 minutes after completing phase B, at the end of phase C and 2 minutes after completing phase D.

A second set of experiments (Protocol 2) was then conducted to investigate whether hyperhydration would induce or exacerbate arterial hypoxemia during prolonged exercise interspersed with periods of maximal intensity work. Exercise was again performed with the treadmill set at an uphill slope of 3% and involved 4 phases (Table 3 and Figure 1). Protocol 2 consisted of a 10 minute trot at 3.7 m/s (phase A), a 2 minute gallop at 14 m/s (phase B), a 20 minute trot at 3.7 m/s (phase C), and an 8 minute canter at 9.2 m/s (phase D). Horses were walked at 1.8 m/s for 10 minutes between phases C and D. The exercise protocol was followed by a brief cool down period during which horses were walked at 2 m/s for 5 minutes.

As with the previous set of experiments, core temperature, arterial and mixed-venous blood-gas tensions, hemoglobin concentration, hemoglobin-O2 saturation, total O2 content, PCV, and plasma protein concentration were measured. Samples were obtained at: 5 and 10 minutes of phase A; 90 and 120 seconds of phase B; 2, 10, 15 and 20 minutes of phase C; the end of the 10 minute walk between phases C and D; and at 2, 4, 6, and 8 minutes of phase D. Heart rate was also determined at these time points. Mixed venous blood lactate concentration was determined pre-exercise, at the end of phase A, 2 minutes after completing phase B, at the end of phase C, and 2 minutes after completing phase D.
Table 3. Phases of exercise Protocol 2; the exercise test included a short burst of maximal intensity exercise known to induce significant arterial hypoxemia.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Speed (m/s)</th>
<th>Gait</th>
<th>Time (min)</th>
<th>Distance (m)</th>
<th>Incline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A First roads and tracks</td>
<td>3.7</td>
<td>Trot</td>
<td>10</td>
<td>2,220</td>
<td>3</td>
</tr>
<tr>
<td>B Steeplechase</td>
<td>14.0</td>
<td>Gallop</td>
<td>2</td>
<td>1,680</td>
<td>3</td>
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<td>C Second roads and tracks</td>
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<td>Trot</td>
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<td>10</td>
<td>1,080</td>
<td>3</td>
</tr>
<tr>
<td>D Cross-country</td>
<td>9.2</td>
<td>Canter</td>
<td>8</td>
<td>4,416</td>
<td>3</td>
</tr>
<tr>
<td>Cool-down</td>
<td>2.0</td>
<td>Walk</td>
<td>5</td>
<td>600</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>55</strong></td>
<td></td>
<td><strong>14,436</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Data Analysis**

All data were subjected to repeated-measures, split-plot design analysis of variance using the SAS statistical software package (SAS version 8.2, SAS Institute, Cary, North Carolina, USA). Treatment comparisons were made by using the least squares significant difference method (Steele and Torrie 1960). Data for the control as well as the hypervolemia experiments were also individually subjected to analysis of variance followed by Newman-Keuls multiple-range test (Steele and Torrie 1960) to determine the significant effects of work intensity and duration within each treatment group. For all statistical analyses, the level of significance was set at $P < 0.05$. Data are presented as mean ± standard error (SE).
Figure 1: A schematic representation of the 2 exercise protocols used in these experiments to simulate the second day of an equestrian 3-day event. All exercise was performed with the treadmill set at a 3% uphill grade. The longer exercise protocol (Protocol 1, upper panel) was used in the first set of experiments and is identical to that of Sosa Leon et al. (2002). The second exercise protocol (Protocol 2, lower panel) incorporated a short burst of exercise known to induce arterial hypoxemia and hemoglobin de-saturation.
CHAPTER 3: RESULTS FROM EXERCISE PROTOCOL 1

Changes in Plasma Protein Concentration (Figure 2; Table 4)

In standing horses, nasogastric administration of NaCl at a dose of 0.425 g/kg caused a marked thirst inducing water consumption of approximately 10-13 liters in individual animals. As a result, plasma protein concentration differed significantly ($P < 0.001$) immediately pre-exercise between the control (6.0 ± 0.1 g/dL) and hyperhydration (5.4 ± 0.1 g/dL) treatments. This change in plasma protein concentration corresponds to an estimated 11.3 ± 3.2% increase in plasma volume in the hyperhydrated horses. Plasma protein concentration remained significantly lower ($P < 0.001$) in the hyperhydration treatment when compared to the control treatment throughout exercise.

During exercise, plasma protein concentration tended to increase during periods of more intense exercise likely subsequent to plasma volume contraction as fluid moved out of the vascular space. In addition, plasma protein concentration increased over the course of the entire exercise protocol as a result of progressive sweat-fluid losses. Absorption of water from the gastrointestinal tract likely continued throughout the exercise protocol as the estimated plasma volume expansion at end-exercise exceeded that at pre-exercise (Table 4; pre-exercise 11.3 ± 3.2%; end-exercise 15.3 ± 2.3%).

Relative changes in plasma protein concentration were similar in nature in both the control and hyperhydration groups. At the initiation of exercise, plasma protein concentration increased in both treatment groups, although this increase was significant only in the control group ($P < 0.001$). During phase B, plasma protein concentration increased sharply and, by the end of this phase, was significantly higher in both treatment groups when compared to the value measured at the end of phase A (control 6.1 ± 0.1 g/dL to 6.6 ± 0.1 g/dL, $P < 0.001$; hyperhydration 5.5 ± 0.2 g/dL to 5.8 ± 0.2 g/dL, $P < 0.001$). Following the completion of phase B, plasma protein concentration then decreased and was significantly lower when measured at 12.5 minutes of phase C (control $P < 0.001$; hyperhydration $P < 0.001$). This variable then increased gradually, but significantly, throughout phase C in both treatment groups (control 6.3 ± 0.1 g/dL to 6.8 ± 0.2 g/dL, $P < 0.001$; hyperhydration 5.5 ± 0.2 g/dL to 5.8 ± 0.2 g/dL, $P = 0.040$). There was a slight, insignificant, decrease in plasma protein concentration during the 10 minute rest period in both treatment groups. Plasma protein concentration then increased sharply
again with the resumption of exercise, but remained stable throughout phase D. The values measured at 5 minutes of phase D (control 7.1 ± 0.2 g/dL; hyperhydration 6.1 ± 0.2 g/dL) were significantly higher than those recorded at the end of the rest period (control $P < 0.001$; hyperhydration $P < 0.001$).

**Changes in Hemoglobin Concentration (Figure 2; Table 4)**

The increased plasma volume in the hyperhydration experiments led to a corresponding decrease in hemoglobin concentration in that treatment group. Hemoglobin concentration was therefore significantly ($P = 0.003$) reduced in the hyperhydration experiments in pre-exercise samples when compared to controls (control 12.8 ± 0.4 g/dL; hyperhydration 11.6 ± 0.6 g/dL). Additionally, all values of hemoglobin concentration measured during exercise were statistically significantly different from pre-exercise values. Hemoglobin concentration was significantly ($P = 0.007$) lower in the hyperhydration treatment when compared to the control group throughout exercise according to ANOVA analysis.

Initiation of exercise in both groups was attended by a large and significant ($P < 0.001$ for both groups) increase in hemoglobin concentration mostly due to the release of erythrocytes from the spleen (although contraction of plasma volume also likely plays a role). Thereafter, hemoglobin concentration followed a pattern similar to that seen with plasma protein concentration. However, hemoglobin concentration tended to remain constant throughout phase C in contrast to the gradual increase in plasma protein concentration observed during that phase. Hemoglobin concentration measured at the end of phase C was not significantly (control $P = 0.185$; hyperhydration $P = 0.685$) different from that recorded at the beginning of the phase in either treatment group. Hemoglobin concentration peaked at the end of phases B (control 18.9 ± 0.5 g/dL; hyperhydration 16.4 ± 0.4 g/dL) and D (control 19.2 ± 0.4 g/dL; hyperhydration 16.4 ± 0.6 g/dL).

**Changes in Core Temperature (Figure 3; Table 4)**

Pulmonary artery blood (core) temperature measured pre-exercise was significantly lower than all values measured during exercise. Pre-exercise temperature was not different between treatment groups ($P = 0.794$). The extent of exercise-induced hyperthermia was not affected by hypervolemia induced by NaCl administration as determined by ANOVA ($P = 0.884$).
From pre-exercise values (control 37.5 ± 0.1°C; hyperhydration 37.5 ± 0.1°C), temperature increased significantly during both phases A and B. Core temperature reached a peak at the end of phase B (control 39.8 ± 0.1°C; hyperhydration 39.9 ± 0.2°C) and subsequently decreased throughout phase C and the rest period. Core temperature measured at 12.5 minutes of phase C (control 38.8 ± 0.1°C; hyperhydration 38.9 ± 0.1°C) was significantly (P < 0.001 for both groups) lower from that measured at the completion of phase B. In these experiments, core temperature measured at the end of the phase C (control 38.4 ± 0.2°C; hyperhydration 38.2 ± 0.1°C) was significantly lower than that recorded at the 12.5 minutes of that phase (control P = 0.019; hyperhydration P < 0.001). Core temperature decreased further during the rest period and then increased steadily throughout phase D reaching the highest recorded values at the end of this phase (control 40.4 ± 0.2°C; hyperhydration 40.1 ± 0.1°C). In both groups, all values recorded for core temperature during phase D were significantly higher than the value recorded at the end of the rest period.

**Changes in Heart Rate (Figure 3; Table 4)**

Heart rate showed predictable changes in response to variation in exercise intensity; heart rate tended to be higher in the hyperhydration group. Although ANOVA analysis for treatment effects on heart rate approached significance, the two groups were not statistically different (P = 0.054). Pre-exercise heart rate (control 40 ± 0 beats/minute; hyperhydration 45 ± 2 beats/minute) was not significantly different between the two groups (P = 0.279), but was significantly lower than all values recorded during exercise.

Initiation of exercise caused significant increases in heart rate in both groups. The highest value for heart rate was recorded after 60 seconds of cantering at 8 m/s during phase B (control 176 ± 6 beats/minute; hyperhydration 172 ± 4 beats/minute). Heart rate had decreased significantly (P < 0.001 for both groups) at 12.5 minutes of phase C (control 110 ± 3 beats/minute; hyperhydration 119 ± 2 beats/minute) and then remained constant throughout this phase of exercise. Heart rate decreased again during the rest period (control 58 ± 4 beats/minute; hyperhydration 60 ± 3 beats/minute), but remained significantly higher than pre-exercise values in both treatment groups (control P < 0.001; hyperhydration P < 0.001). As expected, heart rate at increased at the beginning of phase D (control 150 ± 3 beats/minute; hyperhydration 154 ± 3...
beats/minute at 5 minutes) and then remained constant until completion of this phase.

*Changes in Arterial O₂ Tension and Hemoglobin-O₂ Saturation (Figures 4, 5; Table 4)*

Pre-exercise data for O₂ tension (control 99.1 ± 1.9 mm Hg; hyperhydration 97.4 ± 1.9 mm Hg, \( P = 0.552 \)) and hemoglobin-O₂ saturation (control 98.9 ± 0.1%; hyperhydration 98.7 ± 0.1%, \( P = 0.572 \)) were similar in both groups. Although arterial O₂ tension tended to be lower in the control treatment, particularly during phase C, ANOVA analysis of the data showed no statistically significant \( (P = 0.909) \) difference between treatment groups. In the case of hemoglobin saturation, ANOVA analysis for treatment effects approached, but did reach statistical significance \( (P = 0.054) \). The hyperhydration treatment tended to have slightly higher (although clinically insignificant) hemoglobin saturation than the control treatment.

Arterial O₂ tension remained similar to resting levels throughout the entire exercise test in both treatment groups. Arterial hypoxemia was not observed at any time point during exercise in either treatment group. Furthermore, arterial hemoglobin-O₂ saturation remained within very narrow limits throughout the exercise test and at no time became statistically significantly different from resting levels.

*Changes in Mixed-Venous O₂ Tension and Hemoglobin-O₂ Saturation (Figures 4, 5; Table 4)*

Pre-exercise values for mixed-venous blood O₂ tension were similar in both the control and hyperhydration groups (37.9 ± 0.8 mm Hg and 38.9 ± 1.5 mm Hg, respectively, \( P = 0.197 \)). Similarly, pre-exercise values for mixed-venous hemoglobin-O₂ saturation were not significantly different between treatment groups (control 75.9 ± 1.4%; hyperhydration 75.9 ± 2.5%, \( P = 0.989 \)). Values for both variables measured during exercise were significantly lower than those recorded pre-exercise. Although there was a tendency for values to be lower in the hyperhydration experiments during much of the exercise test, these differences were small and ANOVA did not show statistically significant differences in either mixed-venous O₂ tension \( (P = 0.348) \) or hemoglobin-O₂ saturation \( (P = 0.236) \) between treatment groups.

Changes during exercise in mixed-venous O₂ tension were similar in both treatment groups. Values decreased steadily and significantly during the first 20 minutes of phase A before stabilizing in the final 10 minutes (control 26.7 ± 0.9 mmHg; hyperhydration 27.1 ± 0.8 mmHg). Mixed-venous O₂ tension decreased again during phase B reaching 24.7 ± 1.1 mmHg and 23.6 ±
0.8 mmHg in the control and hyperhydration treatment groups, respectively, at the end of phase B. In both groups, the values recorded at the end of phase B were significantly (control \( P = 0.011 \); hyperhydration \( P < 0.001 \)) lower than those measured at the end of phase A. Mixed-venous \( O_2 \) tension had increased significantly (\( P < 0.001 \) for both groups) when measured at 12.5 minutes of phase C and then remained fairly constant for the remainder of that phase (control \( 28.3 \pm 0.8 \text{ mmHg} \); hyperhydration \( 27.9 \pm 0.7 \text{ mmHg} \)). Mixed-venous \( O_2 \) tension returned toward or slightly exceeded pre-exercise levels by the end of the 10 minute rest period (control \( 39.3 \pm 0.8 \text{ mmHg} \); hyperhydration \( 37.1 \pm 1.3 \text{ mmHg} \)). Mixed-venous \( O_2 \) tension then decreased sharply and significantly (\( P < 0.001 \) for both groups) at the resumption of exercise and then remained steady until the completion of phase D (control \( 24.6 \pm 0.8 \text{ mmHg} \); hyperhydration \( 23.6 \pm 0.2 \text{ mmHg} \)).

The changes observed in mixed-venous hemoglobin-\( O_2 \) saturation were similar in nature to those for mixed-venous \( O_2 \) tension, although more dramatic in magnitude. Values at the end of phase A (control \( 48.8 \pm 2.5\% \); hyperhydration \( 48.5 \pm 2.2\% \)) were significantly lower than those measured pre-exercise (\( P < 0.001 \) for both groups). During phase B, mixed-venous hemoglobin-\( O_2 \) saturation decreased sharply (control \( 34.1 \pm 2.8\% \); hyperhydration \( 31.3 \pm 2.1\% \) at 240 seconds of phase B) and were significantly (\( P < 0.001 \) for both groups) lower than values recorded at the end of phase A. As with mixed-venous blood \( O_2 \) tension, mixed-venous hemoglobin-\( O_2 \) saturation increased and then remained steady during phase C (control \( 52.5 \pm 2.1\% \); hyperhydration \( 50.9 \pm 2.3\% \) at 12.5 minutes of phase C). This value was significantly (\( P < 0.001 \) for both groups) higher than the final value recorded during phase B. Mixed-venous hemoglobin-\( O_2 \) saturation slightly exceeded pre-exercise values by the end of the 10 minute rest period (control \( 77.4 \pm 1.2\% \); hyperhydration \( 73.8 \pm 2.9\% \)), although the difference was not statistically different (control \( P = 0.480 \); hyperhydration \( P = 0.296 \)). During phase D, mixed-venous hemoglobin-\( O_2 \) saturation again decreased sharply (control \( 34.3 \pm 2.8\% \); hyperhydration \( 33.2 \pm 2.4\% \) at 14 minutes of phase D). All values recorded during phase D were significantly (\( P < 0.001 \) for all values in both groups) lower than the value measured at the end of the rest period.
**Changes in Arterial CO₂ Tension (Figure 6; Table 4)**

Pre-exercise values of arterial CO₂ tension were similar in both groups (control 44.5 ± 0.9 mm Hg; hyperhydration 43.8 ± 0.7 mm Hg, \( P = 0.516 \)) and were significantly higher than all subsequent values measured during exercise. ANOVA analysis was unable to detect any significant differences in arterial CO₂ tension between treatment groups indicating similar alveolar ventilation in both groups \( (P = 0.909) \).

In both treatment groups, arterial CO₂ tension decreased significantly \( (P < 0.001 \text{ for both}) \) at the initiation of exercise indicating mild alveolar hyperventilation (control 39.9 ± 1.3 mm Hg; hyperhydration 39.1 ± 0.6 mm Hg at 10 minutes of phase A). Arterial CO₂ tension then re-bounded slightly at 20 and 30 minutes of phase A, although this increase was not statistically significant in either treatment group. Values for arterial CO₂ tension tended to decrease during phase B, but this change only became significant at 240 seconds in the hyperhydration treatment group \((37.1 ± 0.7, P = 0.027)\). Throughout phase C, this variable increased slightly, although insignificantly in both groups. In the hyperhydration group, arterial CO₂ tension returned to nearly pre-exercise values during the rest period \((42.9 ± 0.5 \text{ mm Hg})\), before decreasing significantly \( (P < 0.001) \) at the resumption of exercise. The slight increase in arterial CO₂ tension in the control group during the rest period was not significant. However, as in the hyperhydration group, arterial CO₂ tension in control animals decreased sharply and significantly \( (P = 0.004) \) when exercise was resumed. Arterial CO₂ tension tended to decrease steadily during phase D, although the changes within phase D were not statistically significant. By the end of phase D, the arterial CO₂ tension had reached a nadir and was 35.2 ± 1.9 mm Hg and 35.8 ± 0.7 mm Hg in the control and hyperhydration groups, respectively.

**Changes in Arterial pH (Figure 6; Table 4)**

Pre-exercise values for arterial pH were not significantly different between the control and hyperhydration experiments (control 7.41 ± 0.01; hyperhydration 7.39 ± 0.01. \( P = 0.901 \)). Additionally, ANOVA was unable to detect any statistically significant differences in arterial pH between treatment groups \( (P = 0.710) \).

Arterial pH increased gradually during phase A and, by the end of this phase, arterial pH in the control and hyperhydration groups were 7.44 ± 0.01 and 7.43 ±0.01, respectively. These values were significantly different from the values measured pre-exercise (control \( P = 0.003 \);
hyperhydration \( P < 0.001 \). After cantering at 8 m/s for 240 seconds in phase B, arterial pH had decreased significantly (control \( P < 0.001 \); hyperhydration \( P < 0.001 \)) from the value recorded at the end of phase A and reached their lowest measured value (control 7.39 ± 0.01; hyperhydration 7.39 ± 0.02). By 12.5 minutes of phase C, pH had again increased (control 7.44 ± 0.01; hyperhydration 7.43 ± 0.01) and was significantly \( (P < 0.001 \) in both groups) greater than the value measured at the end of phase B. In the hyperhydration group, pH remained relatively stable during phase C, but then decreased sharply and significantly \( (P = 0.002) \) during the rest period (7.40 ± 0.01). In contrast, arterial pH in the control group decreased steadily throughout phase C and was significantly \( (P = 0.024) \) lower by the end of phase C when compared to the value recorded at 12.5 minutes of the phase (7.41 ± 0.01). At the end of the rest period, arterial pH was essentially identical in both treatment groups (control 7.40 ± 0.01; hyperhydration 7.40 ± 0.01) and not statistically different \( (P = 0.709) \). Following the rest period, pH increased significantly \( (P = 0.011) \) in the hyperhydration group and reached a value of 7.43 ± 0.14 after 14 minutes of cantering (7.3 m/s) in phase D. A similar trend was evident in the control group, although the values measured during phase D for the control group were not significantly different from those obtained at the end of the rest period. At the end of phase D, the arterial pH in the control group was 7.41 ± 0.02; this value, although lower, was not significantly \( (P = 0.419) \) different from the corresponding value recorded in the hyperhydration experiments (7.43 ± 0.14).

**Changes in Arterial and Mixed-Venous O₂ Content (Figure 7; Table 4)**

The effect of hemodilution was reflected in the pre-exercise values for arterial O₂ content that were significantly \( (P = 0.001) \) lower in the hyperhydration treatment group when compared to the control treatment group (control 17.3 ± 0.5 mL O₂/dL blood; hyperhydration 15.7 ± 0.7 mL O₂/dL blood). Values for arterial O₂ content measured while exercising were significantly higher than resting values in both groups as a result of the increased hemoglobin concentration and, to a lesser extent, plasma volume contraction. In addition, ANOVA revealed a statistically significant difference between treatment groups throughout the exercise test \( (P = 0.007) \); the arterial O₂ content was consistently lower in the hyperhydration treatment group reflecting the increased plasma volume and decreased hemoglobin concentration in that group.

Relative changes with time in this variable were similar in both treatment groups. At the start of exercise, arterial O₂ content initially increased significantly \( (P < 0.001 \) in both groups)
Values for arterial O₂ content recorded at the end of phase A were $19.7 \pm 0.5$ mL O₂/dL blood and $17.7 \pm 0.9$ mL O₂/dL blood in the control and hyperhydration treatment groups, respectively. Arterial O₂ content increased significantly during phase B and reached peak values after cantering at 8 m/s for 240 seconds (control $25.3 \pm 0.6$ mL O₂/dL blood; hyperhydration $22.0 \pm 0.6$ mL O₂/dL blood). In both groups, the values recorded throughout phase B were significantly higher than the value measured at the end of phase A. In both treatment groups, arterial O₂ content had decreased significantly ($P < 0.001$ in both groups) when measured at 12.5 minutes of phase C (control $21.6 \pm 0.6$ mL O₂/dL blood; hyperhydration $17.9 \pm 0.6$ mL O₂/dL blood) and thereafter was maintained at a stable level throughout the phase. Arterial O₂ content decreased significantly (control $P = 0.001$; hyperhydration $P < 0.001$) during the rest period in both groups (control $20.5 \pm 0.8$ mL O₂/dL blood; hyperhydration $16.0 \pm 0.7$ mL O₂/dL blood). Recorded values were significantly higher than pre-exercise values in the control group ($P < 0.001$), but not in the hyperhydration group ($P = 0.583$). When compared to values recorded at the end of the rest period, arterial O₂ content increased significantly in both groups during phase D ($P < 0.001$ in both groups). After 5 minutes of cantering at 7.3 m/s, arterial O₂ content in the control and hyperhydration treatment groups were $25.9 \pm 0.7$ mL O₂/dL blood and $21.9 \pm 0.8$ mL O₂/dL blood, respectively. These values remained relatively constant until the completion of phase D.

ANOVA revealed that mixed-venous O₂ content was significantly different between treatment groups ($P = 0.023$), although there was no statistically significant difference in the individual values recorded pre-exercise ($P = 0.109$) and at 30 minutes of phase A ($P = 0.068$). Mixed-venous O₂ content was consistently lower in the hyperhydration group during exercise reflecting the lower hemoglobin concentration in this group as a result of plasma volume expansion. Pre-exercise mixed-venous O₂ content in the control, but not the hyperhydration treatment group was significantly different to all subsequent values recorded during exercise.

Relative changes in this variable throughout exercise were similar in both treatment groups. Mixed-venous O₂ content increased slightly at the initiation of exercise, although after trotting at 3 m/s for 10 minutes in phase A, this increase was only significant in the control treatment group ($P = 0.003$). By the end of phase A, the mixed-venous O₂ content had decreased in both treatment groups and was significantly ($P < 0.001$ in both groups) lower than pre-
exercise levels (control 9.8 ± 0.7 mL O₂/dL blood; hyperhydration 8.8 ± 0.8 mL O₂/dL blood). Mixed-venous O₂ values decreased further during phase B and reached minimum recorded values at 240 seconds in the control treatment group (9.0 ± 0.9 mL O₂/dL blood) and at 120 seconds in the hyperhydration treatment group (7.1 ± 0.5 mL O₂/dL blood). When measured at 12.5 minutes of phase C, mixed-venous O₂ content (control 11.5 ± 0.6 mL O₂/dL blood; hyperhydration 9.3 ± 0.7 mL O₂/dL blood) had increased significantly (control P < 0.001; hyperhydration P < 0.001) when compared to the final value recorded during phase B. Throughout the remainder of phase C, mixed-venous O₂ content did not change appreciably. Values had increased significantly (P < 0.001 in both groups) when measured at the end of the rest period (control 15.6 ± 0.7 mL O₂/dL blood; hyperhydration 11.8 ± 0.8 mL O₂/dL blood) compared to the value recorded at the completion of phase C. Compared to the value recorded at the end of the rest period, mixed-venous O₂ content decreased significantly (P < 0.001 in both groups) after cantering at 7.3 m/s for 5 minutes in phase D (control 9.5 ± 0.9 mL O₂/dL blood; hyperhydration 7.4 ± 0.9 mL O₂/dL blood), but did not change significantly thereafter.

*Changes in Arterial to Mixed-Venous O₂ Content Difference (Figure 7; Table 4)*

Hyperhydration was associated with a significant attenuation of the exercise-induced expansion of the arterial to mixed-venous O₂ content difference (ΔCₐ−vO₂). ANOVA analysis revealed that the ΔCₐ−vO₂ was significantly (P = 0.006) different between the two treatment groups. However, there was no statistically significant (P = 0.141) difference between pre-exercise values for this variable (control 4.4 ± 0.2 mL O₂/dL blood; hyperhydration 3.7 ± 0.3 mL O₂/dL blood). Furthermore, upon analysis of individual data points, there was no statistically significant difference between values recorded at: 20 minutes of phase A; during the first 3 minutes of phase B; and at the end of 10 minutes of rest. Values for this variable were significantly different at all other time points.

Relative changes in this variable with time were similar in both treatment groups and all values recorded during exercise were significantly higher from those recorded pre-exercise in both groups. The arterial to mixed-venous O₂ content difference increased at the initiation of exercise and at 30 minutes (the end) of phase A, ΔCₐ−vO₂ in the control and hyperhydration treatment groups was 9.9 ± 0.3 mL O₂/dL blood and 8.9 ± 0.2 mL O₂/dL blood, respectively.
These values were significantly \((P < 0.001 \text{ in both groups})\) higher than pre-exercise values. During phase B, \(\Delta C_{a-v} O_2\) increased further and reached peak values at 240 seconds of phase B (control 16.2 ± 0.6 mL O\(_2\)/dL blood; hyperhydration 14.8 ± 0.4 mL O\(_2\)/dL blood) that were significantly greater than values recorded at the end of phase A \((P < 0.001 \text{ in both groups})\). Following phase B, \(\Delta C_{a-v} O_2\) had decreased significantly \((P < 0.001 \text{ in both groups})\) when measured at 12.5 minutes of phase C (control 10.1 ± 0.5 mL O\(_2\)/dL blood; hyperhydration 8.6 ± 0.3 mL O\(_2\)/dL blood) and attained a steady state for the remainder of the phase. The arterial to mixed-venous O\(_2\) content difference had decreased significantly \((P < 0.001 \text{ in both groups})\) toward pre-exercise levels by the end of the rest period (control 4.9 ± 0.3 mL O\(_2\)/dL blood; hyperhydration 4.2 ± 0.2 mL O\(_2\)/dL blood) before increasing once again during phase D. At 5 minutes of phase D, the \(\Delta C_{a-v} O_2\) in the control and hyperhydration treatment groups was 16.6 ± 0.8 mL O\(_2\)/dL blood and 14.6 ± 0.5 mL O\(_2\)/dL blood, respectively and these values were significantly higher than those recorded at the end of the rest period \((P < 0.001 \text{ in both groups})\). There was no further statistically significant change in the value of \(\Delta C_{a-v} O_2\) in either treatment group for the remainder of the exercise protocol.

**Changes in Lactate Concentration** *(Table 4)*

There was no difference in lactate concentration (control 0.60 ± 0.04 mmol/L; hyperhydration 0.40 ± 0.04 mmol/L) between the two treatment groups pre-exercise \((P = 0.749)\). Although lactate concentration tended to be lower in the hyperhydration treatment group throughout the exercise test, this difference was not significant \((P = 0.301)\) according to ANOVA analysis.

Lactate concentration in both treatment groups increased significantly \((P < 0.001 \text{ in both groups})\) after the completion of phase B (control 3.57 ± 0.66 mmol/L; hyperhydration 3.07 ± 0.34 mmol/L) when compared to pre-exercise values. By the end of phase C, lactate concentration had decreased significantly \((P < 0.001 \text{ in both groups})\) when compared to the values measured at the completion of phase B. Furthermore, lactate concentration at the end of phase C was not significantly different to pre-exercise concentrations (control \(P = 0.181\); hyperhydration \(P = 0.972\)). At the completion of phase C, lactate concentration was 1.10 ± 0.59 mmol/L and 0.41 ± 0.05 mmol/L in the control and hyperhydration treatment groups, respectively. Lactate concentrations were increased following phase D (control 1.94 ± 0.55
mmol/L; hyperhydration 1.48 ± 0.18 mmol/L) and were significantly (control $P = 0.001$; hyperhydration $P = 0.012$) higher than pre-exercise values.
Table 4. Selected variables (mean ± standard error [SE]) from exercise Protocol 1.

<table>
<thead>
<tr>
<th></th>
<th>Pre-exercise</th>
<th>End Phase A (30min @ 3m/s)</th>
<th>End Phase B (4min @ 8m/s)</th>
<th>Start Phase C (12.5min @ 3m/s)</th>
<th>End Phase C (50min @ 3m/s)</th>
<th>Rest Period (14min @ 7.7m/s)</th>
<th>End Phase D (2min @ 2m/s)</th>
<th>Cool Down (2min @ 2m/s)</th>
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<tbody>
<tr>
<td><strong>Plasma Protein Concentration (g/dL)</strong></td>
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<tr>
<td>Control</td>
<td>6.0 ± 0.1</td>
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<td>6.6 ± 0.1 αΦ</td>
<td>6.3 ± 0.1 αΦ</td>
<td>6.8 ± 0.2 αΦ</td>
<td>6.6 ± 0.2 αΦ</td>
<td>7.1 ± 0.2 αΦ</td>
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<tr>
<td>Hyperhydration</td>
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<td>5.5 ± 0.2 αΦ</td>
<td>5.8 ± 0.2 αΦ</td>
<td>5.7 ± 0.2 αΦ</td>
<td>6.1 ± 0.2 αΦ</td>
<td>6.0 ± 0.2 α</td>
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<td><strong>Arterial Hemoglobin Concentration (g/dL)</strong></td>
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<tr>
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<td>16.4 ± 0.7 α</td>
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<td>16.4 ± 0.4 αΦ</td>
<td>13.1 ± 0.5 αΦ</td>
<td>13.3 ± 0.6 α</td>
<td>11.7 ± 0.5 Φ</td>
<td>16.4 ± 0.6 αΦ</td>
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<tr>
<td>Control</td>
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<td>13.2 ± 3.3</td>
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<td>Control</td>
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<tr>
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<td>94.8 ± 1.5 Φ</td>
<td>103.8 ± 1.5 αΦ</td>
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Table 4. Selected variables (mean ± standard error [SE]) from exercise Protocol 1 (continued).

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<tr>
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<th>End Phase A (30min @ 3m/s)</th>
<th>End Phase B (4min @ 8m/s)</th>
<th>Start Phase C 1 (12.5min @ 3m/s)</th>
<th>End Phase C (50min @ 3m/s)</th>
<th>Rest Period</th>
<th>End Phase D (14min @ 7.7m/s)</th>
<th>Cool Down (2min @ 2m/s)</th>
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<td><strong>Mixed-Venous O2 Tension (mmHg)</strong></td>
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<tr>
<td>Control</td>
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<td>Hyperhydration</td>
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<td>23.6 ± 0.8 αΦ</td>
<td>27.9 ± 0.7 αΦ</td>
<td>27.0 ± 1.2 α</td>
<td>37.1 ± 1.3 αΦ</td>
<td>23.6 ± 0.7 αΦ</td>
<td>31.9 ± 0.4 αΦ</td>
</tr>
<tr>
<td><strong>Arterial Hemoglobin Saturation (%)</strong></td>
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<td></td>
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<tr>
<td>Control</td>
<td>98.9 ± 0.1</td>
<td>98.9 ± 0.1</td>
<td>98.1 ± 0.1 αΦ</td>
<td>98.9 ± 0.0 αΦ</td>
<td>98.3 ± 0.3 αΦ</td>
<td>98.7 ± 0.1</td>
<td>98.2 ± 0.2 αΦ</td>
<td>98.5 ± 0.2</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>98.7 ± 0.1</td>
<td>98.9 ± 0.1</td>
<td>98.3 ± 0.2 αΦ</td>
<td>99.1 ± 0.1 αΦ</td>
<td>99.1 ± 0.1</td>
<td>98.7 ± 0.2</td>
<td>98.7 ± 0.1</td>
<td>98.6 ± 0.2</td>
</tr>
<tr>
<td><strong>Mixed-Venous Hemoglobin Saturation (%)</strong></td>
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<tr>
<td>Control</td>
<td>75.9 ± 1.4</td>
<td>48.8 ± 2.5 α</td>
<td>34.1 ± 2.8 αΦ</td>
<td>52.5 ± 2.1 αΦ</td>
<td>51.2 ± 1.0 α</td>
<td>77.4 ± 1.2 Φ</td>
<td>34.3 ± 2.8 αΦ</td>
<td>57.9 ± 1.4 αΦ</td>
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<tr>
<td>Hyperhydration</td>
<td>75.9 ± 2.5</td>
<td>48.5 ± 2.2 α</td>
<td>31.3 ± 2.1 αΦ</td>
<td>50.9 ± 2.3 αΦ</td>
<td>51.3 ± 3.4 α</td>
<td>73.8 ± 2.9 Φ</td>
<td>33.2 ± 2.4 αΦ</td>
<td>58.4 ± 1.3 αΦ</td>
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<tr>
<td><strong>Arterial CO2 Tension (mmHg)</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>44.5 ± 0.9</td>
<td>41.6 ± 1.1 α</td>
<td>39.7 ± 0.9 α</td>
<td>37.3 ± 2.3 αΦ</td>
<td>39.3 ± 1.7 α</td>
<td>40.0 ± 1.8 α</td>
<td>35.2 ± 1.9 αΦ</td>
<td>35.7 ± 3.0 α</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>43.8 ± 0.7</td>
<td>39.5 ± 0.8 α</td>
<td>37.1 ± 0.7 αΦ</td>
<td>38.4 ± 1.0 α</td>
<td>39.8 ± 1.0 α</td>
<td>42.9 ± 0.5 Φ</td>
<td>35.8 ± 0.7 αΦ</td>
<td>38.6 ± 1.3 αΦ</td>
</tr>
<tr>
<td><strong>Arterial Blood pH</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>7.41 ± 0.01</td>
<td>7.44 ± 0.01 α</td>
<td>7.39 ± 0.01 Φ</td>
<td>7.44 ± 0.01 αΦ</td>
<td>7.41 ± 0.01 Φ</td>
<td>7.40 ± 0.01</td>
<td>7.41 ± 0.02</td>
<td>7.41 ± 0.01</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>7.39 ± 0.01</td>
<td>7.43 ± 0.01 α</td>
<td>7.39 ± 0.02 αΦ</td>
<td>7.43 ± 0.01 αΦ</td>
<td>7.43 ± 0.01 α</td>
<td>7.40 ± 0.01</td>
<td>7.43 ± 0.01 αΦ</td>
<td>7.42 ± 0.01</td>
</tr>
</tbody>
</table>
Table 4. Selected variables (mean ± standard error [SE]) from exercise Protocol 1 (continued).

<table>
<thead>
<tr>
<th></th>
<th>Pre-exercise</th>
<th>End Phase A (30min @ 3m/s)</th>
<th>End Phase B (4min @ 8m/s)</th>
<th>Start Phase C (12.5min @ 3m/s)</th>
<th>End Phase C (50min @ 3m/s)</th>
<th>Rest Period</th>
<th>End Phase D (14min @ 7.7m/s)</th>
<th>Cool Down (2min @ 2m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arterial O₂ Content (mL O₂/dL blood)</strong></td>
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<tr>
<td>Control</td>
<td>17.3 ± 0.5</td>
<td>19.7 ± 0.5 a</td>
<td>25.3 ± 0.6 aΦ</td>
<td>21.6 ± 0.6 aΦ</td>
<td>22.1 ± 0.9 a</td>
<td>20.5 ± 0.8 aΦ</td>
<td>25.8 ± 0.5 aΦ</td>
<td>25.1 ± 0.6 a</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>15.7 ± 0.7</td>
<td>17.7 ± 0.9 a</td>
<td>22.0 ± 0.6 aΦ</td>
<td>17.9 ± 0.6 aΦ</td>
<td>18.1 ± 0.8 a</td>
<td>16.0 ± 0.7 aΦ</td>
<td>22.2 ± 0.7 aΦ</td>
<td>21.8 ± 0.7 a</td>
</tr>
<tr>
<td><strong>Mixed-Venous O₂ Content (mL O₂/dL blood)</strong></td>
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</tr>
<tr>
<td>Control</td>
<td>12.9 ± 0.4</td>
<td>9.8 ± 0.7 a</td>
<td>9.0 ± 0.9 a</td>
<td>11.5 ± 0.6 aΦ</td>
<td>11.3 ± 0.4 a</td>
<td>15.6 ± 0.7 aΦ</td>
<td>9.0 ± 0.7 aΦ</td>
<td>14.7 ± 0.5 aΦ</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>12.0 ± 0.9</td>
<td>8.8 ± 0.8 a</td>
<td>7.2 ± 0.6 aΦ</td>
<td>9.3 ± 0.7 aΦ</td>
<td>9.4 ± 1.0 a</td>
<td>11.8 ± 0.8 aΦ</td>
<td>7.6 ± 0.7 aΦ</td>
<td>12.8 ± 0.7 a</td>
</tr>
<tr>
<td><strong>Arterial to Mixed-Venous O₂ Difference (mL O₂/dL blood)</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>4.4 ± 0.2</td>
<td>9.9 ± 0.3 a</td>
<td>16.2 ± 0.6 aΦ</td>
<td>10.1 ± 0.5 aΦ</td>
<td>10.8 ± 0.6 a</td>
<td>4.9 ± 0.3 aΦ</td>
<td>16.8 ± 0.8 aΦ</td>
<td>10.4 ± 0.4 aΦ</td>
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<tr>
<td>Hyperhydration</td>
<td>3.7 ± 0.3</td>
<td>8.9 ± 0.2 a</td>
<td>14.8 ± 0.4 aΦ</td>
<td>8.6 ± 0.3 aΦ</td>
<td>8.7 ± 0.5 a</td>
<td>4.2 ± 0.2 aΦ</td>
<td>14.6 ± 0.5 aΦ</td>
<td>9.0 ± 0.1 aΦ</td>
</tr>
<tr>
<td><strong>Mixed-Venous Blood Lactate Concentration (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.6 ± 0.04</td>
<td>NA</td>
<td>3.6 ± 0.7 a</td>
<td>NA</td>
<td>1.1 ± 0.6 aΦ</td>
<td>NA</td>
<td>NA</td>
<td>1.9 ± 0.6 aΦ</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>0.4 ± 0.04</td>
<td>NA</td>
<td>3.1 ± 0.3 a</td>
<td>NA</td>
<td>0.4 ± 0.4 aΦ</td>
<td>NA</td>
<td>NA</td>
<td>1.5 ± 0.2 aΦ</td>
</tr>
</tbody>
</table>

★ ANOVA analysis demonstrates statistically significant difference between treatments
α Statistically significant difference from pre-exercise value
Φ Statistically significant difference from immediately preceding value (in table)
1 Samples collected 12.5 min after the beginning of phase C (50 min @ 3.7 m/s)
2 Change in plasma volume calculated as [(PP1/PP2) − 1.0] x 100%, where PP1 and PP2 are the initial and test plasma protein concentrations, respectively (Boyd, 1981).
3 Sample for mixed-venous blood lactate collected at 2 min after the completion of phase B (2m @ 3.7m/s)
Figure 2: Changes in plasma protein concentration (g/dL, left panel) and arterial hemoglobin concentration (g/dL, right panel) in the first (longer) exercise protocol (Protocol 1).

Left panel: Administration of NaCl to horses induced a marked thirst and water consumption resulting in an average estimated increase in plasma volume (based on the decrease in plasma protein concentration) of $11.3 \pm 3.2\%$ prior to the initiation of exercise. In the hyperhydration treatment group, plasma protein concentration remained significantly lower than in the control treatment group throughout the entire exercise test ($P < 0.001$).

Right panel: An increase in plasma volume in the hyperhydration treatment group led to a concomitant and statistically significant ($P = 0.007$) decrease in hemoglobin concentration.

★ ANOVA indicates a statistically significant difference between treatment groups (i.e., hyperhydration and control).
α Significantly different to all subsequent values recorded during exercise.
Φ Significantly different to previous data point.
Δ Indicates that values within phase B are significantly different to the value recorded at $t = 31$ minutes (end of phase A [30 minutes @ 3.0 m/s]).
δ Compares data points within phase B. Labeled points are significantly different to the value recorded at $t = 31$ minutes (end of phase A).
π Compares data points within phase B. Labeled points are significantly different to the value recorded at $t = 32$ minutes (first value recorded during phase B [4 minutes @ 8.0 m/s]).
θ Compares data points within phase C, labeled values are significantly different to the value recorded at $t = 47.5$ minutes (first value recorded during phase C [50 minutes @ 3.0 m/s]).
λ Compares data points within phase D. Labeled points are significantly different to the value recorded at $t = 95$ minutes (end of 10 minute rest period).
Figure 3: Exercise-induced changes in core (pulmonary artery) temperature (°C, left panel) and heart rate (beats/minute, right panel) during an exercise protocol identical to that used by Sosa Leon et al. (2002) simulating the second day of a 3-day event (Protocol 1).

Left panel: The extent of exercise-induced hyperthermia was similar for the 2 treatment groups and no significant effect of hyperhydration on core temperature was observed ($P = 0.884$).

Right panel: Heart rates pre-exercise and throughout the exercise protocol were not significantly different ($P = 0.054$) between treatment groups.

δ Compares data points within phase B, labeled points are significantly different to the value recorded at $t = 32$ minutes (first value recorded during phase B [4 minutes @ 8.0 m/s]).
π Compares data points within phase B, labeled points are significantly different to the value recorded at $t = 33$ minutes (second value recorded during phase B).
For explanation of other symbols, see Fig. 2.
Figure 4: Changes in arterial and mixed-venous blood O₂ tension (mm Hg, left panel) and hemoglobin-O₂ saturation (%) (right panel) identical to that used by Sosa Leon et al. (2002) simulating the second day of a 3-day event (Protocol 1). In contrast to the experiments of Sosa Leon et al. (2002), we were unable to demonstrate any effect of pre-exercise hyperhydration (hypervolemia) on either arterial oxygenation or hemoglobin saturation during phases B and D of the exercise test. Arterial blood O₂ tension ($P = 0.909$) and hemoglobin-O₂ saturation ($P = 0.054$) were not statistically different between treatment groups at any time. Mixed-venous blood O₂ tension ($P = 0.348$) and hemoglobin-O₂ saturation ($P = 0.236$) were also similar between treatment groups and statistically significant differences were not evident. An expanded view of changes in arterial and mixed-venous blood O₂ tensions during phases B and D is shown in figure 6.

μ: Compares data points within phase B with the value recorded at $t = 31$ minutes (end of phase A [30 minutes @ 3.0 m/s]). In the control experiment, values at $t = 34$ and 35 minutes were statistically significantly different from the value recorded at the end of phase A. In the hyperhydration experiment, all values within phase B were statistically significantly different from that point.

δ: Compares data points within phase B. Labeled points are significantly different to the value recorded at $t = 32$ minutes (first value recorded during phase B [4 minutes @ 8.0 m/s]).

For explanation of other symbols, see Fig. 2.
Figure 5: An expanded view of changes in arterial and mixed-venous blood O₂ tension during phases B and D of exercise in Protocol 1. Note the close similarity of data in the control and hyperhydration treatment groups; there were no statistically significant differences in arterial or mixed-venous O₂ tension between the two.

Figure 6: Changes in arterial CO₂ tension (mm Hg, left panel) and arterial pH (right panel) during an exercise test identical to that used by Sosa Leon et al. (2002) simulating the second day of a 3-day event (Protocol 1).

*Left panel:* There were no statistically significant differences in arterial CO₂ tension (P = 0.909) between treatment groups during exercise indicating similar levels of alveolar ventilation in both.

*Right panel:* There were no statistically significant differences (P = 0.710) in arterial pH between treatment groups at any time during exercise.

δ Compares data points within phase B. Labeled points are significantly different to the value recorded at t = 32 minutes (first value recorded during phase B [4 minutes @ 8.0 m/s]).

For explanation of other symbols, see Fig. 2.
Figure 7: Changes in arterial and mixed-venous blood O₂ content (mL O₂/dL blood, left panel) and the arterial to mixed-venous blood O₂ content difference (mL O₂/dL blood, right panel) during the first exercise protocol used in these experiments (Protocol 1).

Left panel: When compared to the control treatment group, arterial blood O₂ content was significantly lower ($P = 0.007$) in the hyperhydration treatment group both before and throughout exercise as a result of hemodilution and decreased hemoglobin concentration. Mixed-venous O₂ blood content was also lower in the hyperhydration treatment group and there was a significant difference between treatment groups ($P = 0.023$), although there was no statistically significant difference in the individual values recorded pre-exercise ($P = 0.109$) and at 30 minutes of phase A ($P = 0.068$).

Right panel: The arterial to mixed-venous blood O₂ content difference ($\Delta C_{a-v}O_2$) increased significantly during phases B and D. Although there was no statistically significant ($P = 0.141$) difference in this variable pre-exercise, hyperhydration was associated with a significant ($P = 0.006$) attenuation of the exercise-induced expansion of $\Delta C_{a-v}O_2$.

★ ANOVA indicates a statistically significant difference between treatment groups (i.e., hyperhydration and control). In some cases, examination of individual data points indicated no statistical difference; these individual points are indicated by the absence of a symbol.

δ Compares data points within phase B. Labeled points are significantly different to the value recorded at $t = 32$ minutes (first value recorded during phase B [4 minutes @ 8.0 m/s]).

π Compares data points within phase B. Labeled points are significantly different to the value recorded at $t = 33$ minutes (second value recorded during phase B).

For explanation of other symbols, see Fig. 2.
CHAPTER 4: RESULTS FROM EXERCISE PROTOCOL 2

Changes in Plasma Protein Concentration (Figure 8; Table 5)

Nasogastric administration of NaCl at a dose of 0.425 g/kg induced water consumption of approximately 10-13 liters as seen in the previous experiments once horses had been returned to their stalls. As a consequence, pre-exercise plasma protein concentration differed significantly \((P < 0.001)\) between the control \((6.0 \pm 0.1 \text{ g/dL})\) and hyperhydration \((5.2 \pm 0.1 \text{ g/dL})\) treatment groups. This decrease in plasma protein concentration equates to an estimated expansion of 15.5 ± 1.1% in plasma volume in the hyperhydration treatment group. Plasma protein concentration remained significantly lower \((P = 0.001)\) in the hyperhydration treatment group compared to the control treatment group over the entire exercise test.

Plasma protein concentration was significantly higher during exercise than immediately pre-exercise and tended to be even higher during periods of more intense effort. The progressive increase in plasma protein concentration over the course of the exercise protocol as a result of fluid loss due to sweating was not as obvious in this set of experiments as it had been during the longer exercise test. However, as in those experiments, absorption of water from the gastrointestinal tract probably continued during exercise as the estimated plasma volume expansion continued to increase until the end of the walking period following phase C, hereafter referred to as the rest period (Table 5, pre-exercise 15.5 ± 1.1%; rest period 18.9 ± 2.1). Furthermore, 2 minutes after the completion of exercise, the estimated plasma volume expansion in the hyperhydration group exceeded that at pre-exercise (Table 5, pre-exercise 15.5 ± 1.1%; cool down 16.6 ± 1.2%).

The relative changes in plasma protein concentration (and therefore plasma volume) during the exercise test were similar in nature in both treatment groups and to those described for the previous exercise protocol. At the initiation of exercise, plasma protein concentration tended to increase in both treatment groups although this was not significant in either. By the end of phase B, plasma protein concentration was significantly \((P < 0.001\) for both groups) higher in both treatment groups when compared to the value measured at the end of phase A (control 6.5 ± 0.1 g/dL to 7.4 ± 0.3 g/dL; hyperhydration 5.5 ± 0.1 g/dL to 6.2 ± 0.1 g/dL). Following completion of phase B, plasma protein concentration decreased significantly (control \(P = 0.003\); hyperhydration \(P = 0.031\)) when measured at 2 minutes of phase C (control 7.0 ± 0.3 g/dL;
hyperhydration 6.0 ± 0.2 g/dL) and had significantly (control $P = 0.002$; hyperhydration $P < 0.001$) decreased further when measured at 10 minutes of phase C (control 6.7 ± 0.3 g/dL; hyperhydration 5.7 ± 0.2 g/dL). Plasma protein concentration tended to decrease through the remainder of phase C and had decreased significantly (control $P = 0.017$; hyperhydration $P = 0.026$) again when measured at the end of the rest period (control 6.2 ± 0.2 g/dL; hyperhydration 5.3 ± 0.1 g/dL). This change is in contrast to the findings in the longer exercise protocol where plasma protein concentration increased gradually, but significantly, throughout phase C in both treatment groups. Plasma protein concentration increased sharply with the resumption of exercise in phase D and then increased slightly, but insignificantly throughout the remainder of that phase. The values measured at 14 minutes of phase D (control 6.8 ± 0.2 g/dL; hyperhydration 5.8 ± 0.2 g/dL) were significantly ($P < 0.001$ for both groups) higher than those recorded at the end of the rest period.

Changes in Hemoglobin Concentration (Figure 8; Table 5)

The increased plasma volume in the hyperhydration experiments was accompanied by a decrease in arterial hemoglobin concentration. Hemoglobin concentration was significantly ($P < 0.001$) lower in pre-exercise samples in the hyperhydration group (11.1 ± 0.3 g/dL) when compared to the control group (12.5 ± 0.4 g/dL). All values for this variable measured during exercise were statistically significantly different from those measured pre-exercise. Hemoglobin concentration was significantly ($P < 0.001$) lower in the hyperhydration treatment group when compared to the control treatment group throughout the exercise test.

In both treatment groups, there was a large and significant ($P < 0.001$ for both groups) increase in hemoglobin concentration at the initiation of exercise primarily as a result of splenic contraction. Thereafter, hemoglobin concentration followed a pattern similar to that seen with plasma protein concentration. Peak values for hemoglobin concentration were recorded at the end of phase B (control 20.7 ± 0.2 g/dL; hyperhydration 18.0 ± 0.3 g/dL) and during phase D (control 19.1 ± 0.3 g/dL; hyperhydration 17.3 ± 0.4 g/dL).

Changes in Core Temperature (Figure 9; Table 5)

Pulmonary artery blood (core) temperature was significantly lower when measured at rest when compared to all subsequent values measured during exercise in both treatment groups.
However, the hyperthermia experienced during exercise was not affected by hyperhydration and no significant \( P = 0.884 \) difference was detected between treatment groups as determined by ANOVA analysis.

From pre-exercise values (control 37.4 ± 0.1°C; hyperhydration 37.4 ± 0.1°C), temperature had increased significantly \( (P < 0.001 \) for both groups) by the end of phase A (control 38.4 ± 0.1°C; hyperhydration 38.3 ± 0.1°C). Core temperature reached a peak at the end of phase B (control 41.0 ± 0.1°C; hyperhydration 40.8 ± 0.2°C) and then decreased throughout phase C and the rest period. Core temperature measured at the end of the phase C was significantly \( (P < 0.001 \) for both groups) lower than that measured at the beginning of the phase (control 40.4 ± 0.1 to 39.7 ± 0.1°C; hyperhydration 40.2 ± 0.1 to 39.6 ± 0.2°C). Core temperature decreased further during the rest period before increasing steadily throughout phase D reaching maximum measured values at the end of this phase (control 41.3 ± 0.1°C; hyperhydration 41.5 ± 0.3°C). In both treatment groups, all the values for this variable recorded during phase D were significantly higher than the value recorded at the end of the rest period.

**Changes in Heart Rate (Figure 9; Table 5)**

Although ANOVA analysis revealed a statistically significant \( P = 0.002 \) difference in heart rate between treatment groups, examination of the individual data points revealed significant differences only at 5 and 10 minutes (end) of phase A and at 2 and 4 minutes of phase D. At each time point that was significantly different, the heart rate was higher (11-14 beats/minute) in the hyperhydration treatment group. However, there was no statistically significant \( P = 0.727 \) difference in pre-exercise heart rate (control 44 ± 2 beats/minute; hyperhydration 42 ± 1 beats/minute) between the two treatment groups. Heart rate measured pre-exercise was significantly lower than all subsequent values recorded during the exercise protocol in both treatment groups.

Heart rate increased sharply and significantly \( (P < 0.001 \) for both groups) in the first 5 minutes of exercise (control 146 ± 5 beats/minute; hyperhydration 158 ± 6 beats/minute). There was then a small and statistically insignificant decrease in rate recorded in both groups at the end of phase A. Exercise Protocol 2 was designed to elicit maximum heart rate during phase B; at the end of this phase, heart rates were 216 ± 2 beats/minute and 214 ± 2 beats/minute in the control and hyperhydration treatment groups, respectively. Heart rate had decreased.
significantly \((P < 0.001\) for both groups) when measured at 2 minutes of phase C (control \(147 \pm 7\) beats/minute; hyperhydration \(146 \pm 6\) beats/minute) and continued to decrease slightly throughout the remainder of this phase, although this decrease was only significant in the control treatment group \((P = 0.043;\) not illustrated in figure 6). Heart rate decreased significantly in both treatment groups \((P < 0.001\) for both groups) during the rest period (control \(109 \pm 7\) beats/minute; hyperhydration \(113 \pm 6\) beats/minute), but remained significantly higher than pre-exercise values \((P < 0.001\) in both groups). As would be expected, heart rate increased sharply (and significantly, \(P < 0.001\) in both groups) with the resumption of exercise (control \(178 \pm 5\) beats/minute; hyperhydration \(189 \pm 4\) beats/minute at 2 minutes of phase D) and then remained fairly constant.

**Changes in Arterial O₂ Tension and Hemoglobin-O₂ Saturation (Figure 10; Tables 5 and 6)**

Pre-exercise values for arterial O₂ tension (control \(102.9 \pm 1.6\) mm Hg; hyperhydration \(102.2 \pm 2.5\) mm Hg, \(P = 0.819\)) and hemoglobin-O₂ saturation (control \(99.1 \pm 0.1\%\); hyperhydration \(99.1 \pm 0.1\%\), \(P = 0.972\)) were similar in both groups. During phase B of this exercise protocol, horses were required to exercise at an intensity known to induce significant arterial hypoxemia and hemoglobin de-saturation. Hyperhydration did not exacerbate either the hypoxemia or hemoglobin-O₂ de-saturation in these experiments and statistically significant differences between treatment groups could not be demonstrated in arterial O₂ tension \((P = 0.385)\) or arterial hemoglobin-O₂ saturation \((P = 0.294)\).

During phase B, arterial O₂ tension and hemoglobin-O₂ saturation were significantly decreased in both groups. Arterial O₂ tension reached the minimum measured value (control \(74.5 \pm 2.2\) mm Hg; hyperhydration \(77.3 \pm 2.0\) mm Hg) at 120 seconds of phase B and the lowest value for hemoglobin-O₂ saturation (control \(85.6 \pm 1.9\%\); hyperhydration \(85.9 \pm 1.4\%\)) was recorded at the same time. In both treatment groups, arterial O₂ tension recorded at 120 seconds of phase B were significantly lower than pre-exercise values \((P < 0.001\) for both groups) and values obtained at the end of phase A \((P < 0.001\) for both groups). Similarly, in both groups, the values for hemoglobin-O₂ saturation measured at 120 seconds of phase B were significantly lower than pre-exercise values \((P < 0.001\) for both groups) and the values measured at the end of phase A \((P < 0.001\) for both groups).

Throughout phases A and C and during the rest period, arterial O₂ tension was
maintained near or exceeded pre-exercise values in both treatment groups. Similarly, hemoglobin-O₂ saturation was also maintained within very narrow limits and close to resting values throughout these phases. During phase D however, both arterial O₂ tension and hemoglobin saturation tended to be lower in the hyperhydration treatment group and, on examination of individual data points, this became statistically significant at 6 and 8 minutes of phase D (not illustrated in figure 10). However, at both time points, these differences were small and the values obtained remained within normal reference ranges; therefore, the clinical significance of this finding is questionable.

**Changes in Mixed-Venous O₂ Tension and Hemoglobin-O₂ Saturation (Figure 10; Tables 5 and 6)**

Venous samples were unable to be obtained due to equipment failure from several horses at several time points toward the end of the exercise protocol during these experiments. Therefore, complete statistical analysis of the data was not always possible.

Pre-exercise values for mixed-venous blood O₂ tension were similar in both treatment groups (control 38.1 ± 0.9 mm Hg; hyperhydration 39.8 ± 1.1 mm Hg, \(P = 0.262\)). Similarly, pre-exercise values for mixed-venous hemoglobin-O₂ saturation were not significantly different between treatment groups (control 76.6 ± 1.7%; hyperhydration 78.5 ± 1.3%, \(P = 0.331\)). ANOVA showed no statistically significant differences in the mixed-venous O₂ tension (\(P = 0.425\)) between groups. A statistically significant difference between treatment groups was revealed by ANOVA for mixed-venous hemoglobin-O₂ saturation (\(P = 0.024\)). However, examination of the available data reveals no significant differences at any of the individual data points. Thus, any statistical differences between groups in mixed-venous hemoglobin-O₂ saturation, if present, are unlikely to be of clinical significance.

Mixed-venous O₂ tension decreased throughout phase A and by the end of the phase, recorded values (control 27.2 ± 0.6 mm Hg; hyperhydration 28.9 ± 1.1 mm Hg) were significantly (\(P < 0.001\) for both groups) lower than pre-exercise values. Mixed-venous O₂ tension decreased further and significantly (\(P < 0.001\) for both groups) during phase B and reached the lowest recorded value at 120 seconds of that phase (control 16.4 ± 0.8 mm Hg; hyperhydration 15.4 ± 0.8 mm Hg). Following the conclusion of phase B, mixed-venous O₂ tension increased and, when measured at 2 minutes of phase C, slightly exceeded pre-exercise
values (control 43.9 ± 2.9 mm Hg; hyperhydration 42.9 ± 2.4 mm Hg). Throughout phase C, values for this variable decreased steadily and at the end of this phase were 31.6 ± 1.4 mm Hg and 30.5 ± 0.8 mm Hg for the control and hyperhydration groups, respectively. Mixed-venous O₂ tension remained constant during the rest period, but decreased again as exercise was resumed in phase D. At 8 minutes of phase D, mixed-venous O₂ tension (control 23.8 ± 0.8 mm Hg; hyperhydration 23.9 ± 0.9 mm Hg) was significantly lower than pre-exercise values (P < 0.001 for both groups) and the values recorded at the end of the rest period (P < 0.001 for both groups).

As in the previous experiments, the changes observed in mixed-venous hemoglobin-O₂ saturation were similar in nature to those seen in mixed-venous O₂ tension although more dramatic in magnitude. At the end of phase A, recorded values for this variable (control 50.2 ± 1.0%; hyperhydration 53.7 ± 3.0%) were significantly lower than those measured pre-exercise (P < 0.001 for both groups). Mixed-venous hemoglobin-O₂ saturation decreased sharply with maximal exercise and reached the lowest recorded values at 120 seconds of phase B (control 7.8 ± 0.8%; hyperhydration 6.9 ± 0.5%). These values were significantly (P < 0.001 for both groups) lower than the values recorded at the end of phase A. At the completion of phase B, values for mixed-venous hemoglobin-O₂ saturation increased and then remained relatively constant throughout phase C. Values recorded through the remainder of phase C were not significantly different from the value recorded at 2 minutes of phase C (control 49.4 ± 1.9%; hyperhydration 48.0 ± 2.4%). During the rest period, mixed-venous hemoglobin-O₂ saturation increased (control 61.8 ± 1.2%; hyperhydration 58.9 ± 1.0%) significantly (P < 0.001 for both groups) before decreasing at the resumption of more intense exercise. During phase D, mixed-venous hemoglobin-O₂ saturation remained constant and at 8 minutes of the phase recorded values were 25.8 ± 1.0% and 23.0 ± 1.9% for the control and hyperhydration groups, respectively. In both treatment groups, these values were significantly lower than those recorded pre-exercise (P < 0.001 for both groups) and at the end of the rest period (P < 0.001 for both groups).

**Changes in Arterial CO₂ Tension (Figure 11; Tables 5 and 6)**

Pre-exercise levels of this variable were similar in both groups (control 44.7 ± 0.6 mm Hg; hyperhydration 43.7 ± 0.7 mm Hg, P = 0.605). Although the values for arterial CO₂ tension tended to be lower in NaCl treated horses, ANOVA was unable to detect a statistical difference
between treatment groups ($P = 0.479$).

At the initiation of exercise, arterial CO$_2$ tension decreased significantly (control $P = 0.011$; hyperhydration $P = 0.009$) before re-bounding slightly, but insignificantly. At the end of phase A, arterial CO$_2$ tension (control 41.2 ± 0.3 mm Hg; hyperhydration 39.6 ± 0.9 mm Hg) was lower than pre-exercise values although this was only significant in the hyperhydration treatment group ($P = 0.029$, not indicated in Figure 11). During maximal exercise (phase B), arterial CO$_2$ tension increased dramatically and exceeded resting values indicating relative alveolar hypoventilation. At 120 seconds of phase B, values for this variable (control 53.3 ± 1.8 mm Hg; hyperhydration 50.5 ± 1.6 mm Hg) were significantly higher than both pre-exercise values ($P < 0.001$ for both groups) and the values recorded at the end of phase A ($P < 0.001$ for both groups). At the completion of phase B, arterial CO$_2$ tension decreased below resting levels corresponding to a period of alveolar hyperventilation (control 27.4 ± 1.1 mm Hg; hyperhydration 27.2 ± 0.9 mm Hg). Throughout phase C, the rest period, and the first 2 minutes of phase D, values for arterial CO$_2$ tension increased steadily. At 2 minutes of phase D, arterial CO$_2$ tension (control 39.5 ± 0.7 mm Hg; hyperhydration 39.0 ± 0.7 mm Hg) was significantly ($P < 0.001$ for both groups) higher than the value at 2 minutes of phase C, but remained significantly (control $P = 0.006$; hyperhydration $P = 0.011$) lower than the pre-exercise value. Arterial CO$_2$ tension decreased slightly during the later portions of phase D before declining sharply during the cool down period (control 29.9 ± 1.6 mm Hg; hyperhydration 28.8 ± 1.9 mm Hg).

**Changes in Arterial pH (Figure 11; Table 5)**

ANOVA analysis revealed a significant ($P = 0.049$) difference in arterial pH between treatment groups. However, examination of individual data points not did reveal any significant differences between the two sets of values. Thus, any differences between treatment groups in arterial pH are unlikely to be of clinical significance.

Pre-exercise values for arterial pH were similar in both groups (control 7.41 ± 0.00; hyperhydration 7.41 ± 0.01) and not statistically different ($P = 0.508$). Arterial pH increased slightly, but insignificantly during phase A before decreasing dramatically with the onset of phase B. Maximal exertion during phase B was accompanied by a significant acidemia (control 7.08 ± 0.04; hyperhydration 7.04 ± 0.04). Values for arterial pH recorded during phase B
differed significantly from pre-exercise \((P < 0.001\) for both groups) and during phase A \((P < 0.001\) for both groups). During phase C, arterial pH increased gradually and, by the end of phase C, pH values (control \(7.37 \pm 0.03\); hyperhydration \(7.36 \pm 0.03\)) were no longer significantly different from pre-exercise values (control \(P = 0.142\); hyperhydration \(P = 0.194\)). Arterial pH decreased again in phase D, but became significantly different from pre-exercise values and the value recorded at the end of the rest period only in the hyperhydration treatment group.

**Changes in Arterial and Mixed-Venous \(O_2\) Content (Figure 12; Table 5)**

The hypervolemia in hyperhydrated horses was accompanied by considerable hemodilution and a significant \((P < 0.001)\) decrease in arterial \(O_2\) content in resting horses (control \(17.0 \pm 0.5\) mL \(O_2/dL\) blood; hyperhydration \(15.1 \pm 0.4\) mL \(O_2/dL\) blood). Values for arterial \(O_2\) content measured while exercising were significantly higher than those measured pre-exercise largely as a result of the increased \(O_2\) carrying capacity subsequent to splenic contraction. Furthermore, ANOVA revealed that there was a statistically significant difference in this variable between treatments \((P = 0.007)\).

Arterial \(O_2\) content increased significantly \((P < 0.001\) in both groups) over the first 5 minutes of exercise as a consequence of a dramatic increase in arterial hemoglobin concentration and slight increase in arterial \(O_2\) tension. The values for arterial \(O_2\) content at the end of phase A were \(22.2 \pm 0.5\) mL \(O_2/dL\) blood and \(20.3 \pm 0.6\) mL \(O_2/dL\) blood in the control and hyperhydration treatment groups, respectively. Despite the decrease in arterial \(O_2\) tension and hemoglobin saturation during maximal exercise, arterial \(O_2\) content increased during phase B reflecting further hemoconcentration during this period. Values for arterial \(O_2\) content at 90 seconds of phase B (control \(24.4 \pm 0.3\) mL \(O_2/dL\) blood; hyperhydration \(21.4 \pm 0.5\) mL \(O_2/dL\) blood) were significantly higher than those recorded at the end of phase A (control \(P < 0.001\); hyperhydration \(P = 0.024\)). At 120 seconds of phase B, arterial \(O_2\) content decreased very slightly (statistically insignificant) before increasing at 2 minutes of phase C as arterial \(O_2\) tension and hemoglobin saturation returned to (or exceeded) resting values. Values measured at 2 minutes of phase C (control \(25.3 \pm 0.4\) mL \(O_2/dL\) blood; hyperhydration \(23.8 \pm 0.3\) mL \(O_2/dL\) blood) were significantly (control \(P = 0.040\); hyperhydration \(P < 0.001\)) higher than the value recorded at 120 seconds of phase B. Arterial \(O_2\) content decreased significantly \((P < 0.001\) for both groups) throughout phase C and then decreased significantly \((P < 0.001\) for both groups)
again during the rest period following the course of hemoglobin concentration. Arterial O₂ content measured at the end of the rest period was 21.9 ± 0.6 mL O₂/dL blood and 19.2 ± 0.5 mL O₂/dL blood in the control and hyperhydration groups, respectively. At the resumption of exercise in phase D, arterial O₂ content again increased in association with an increase in hemoglobin concentration. Arterial O₂ content remained fairly constant during phase D and the values recorded at 2 minutes of the phase (control 25.0 ± 0.5 mL O₂/100mL blood; hyperhydration 22.0 ± 0.5 mL O₂/100mL blood) were not statistically significantly different from subsequent values. All values measured during phase D were significantly (P < 0.001 for both groups) higher than those measured at the end of the rest period.

ANOVA revealed that mixed-venous O₂ content was significantly different between groups (P < 0.001) and, as a consequence of hemodilution, lower in the hyperhydration group. Mixed-venous O₂ content decreased slightly during phase A, although this was only significant in the control treatment group (control 12.9 ± 0.5 to 11.4 ± 0.5 mL O₂/dL blood, P = 0.008; hyperhydration 11.9 ± 0.4 to 11.2 ± 0.9 mL O₂/dL blood, P = 0.236). Values for mixed-venous O₂ content decreased sharply and significantly (P < 0.001 in both groups) with increased exercise intensity in phase B. At 120 seconds of this phase, mixed-venous O₂ content was 2.3 ± 0.2 mL O₂/dL blood and 1.7 ± 0.1 mL O₂/dL blood in control and hyperhydration groups, respectively. At 2 minutes of phase C (control 13.4 ± 0.8 mL O₂/dL blood; hyperhydration 11.7 ± 0.7 mL O₂/dL blood), mixed-venous O₂ content was similar to pre-exercise values and did not change significantly during the remainder of that phase or during the rest period. Compared to the value recorded at the end of the rest period, mixed-venous O₂ content decreased significantly (P < 0.001 in both groups) at the resumption of exercise in phase D, but remained relatively constant thereafter. Mixed-venous O₂ content measured at 2 minutes of phase D (control 7.4 ± 0.6 mL O₂/dL blood; hyperhydration 5.7 ± 0.6 mL O₂/dL blood) was not substantially different from subsequent values during that phase.

**Changes in Arterial to Mixed-Venous O₂ Content Difference (Figure 12; Table 5)**

Hyperhydration was associated with a significant attenuation of the exercise-induced expansion of the arterial to mixed-venous blood O₂ content difference (ΔCₐ₋vO₂). ANOVA revealed that the ΔCₐ₋vO₂ was significantly (P = 0.001) different between the two treatment groups. However, there was no statistically significant (P = 0.064) difference between pre-
exercise values for this variable (control 4.1 ± 0.3 mL O₂/dL blood; hyperhydration 3.3 ± 0.2 mL O₂/dL blood). Relative changes with time in this variable were similar in both treatment groups and all values of ΔCₐ₋ᵥO₂ recorded during exercise were significantly higher than those recorded pre-exercise.

Arterial to mixed-venous blood O₂ content difference increased significantly (P < 0.001 in both groups) during phase A and at the end of the phase values in the control and hyperhydration treatment groups were 10.8 ± 0.1 mL O₂/dL blood and 9.2 ± 0.4 mL O₂/dL blood, respectively. During phase B, ΔCₐ₋ᵥO₂ increased further and reached peak values at 120 seconds of that phase (control 22.0 ± 0.2 mL O₂/dL blood; hyperhydration 19.5 ± 0.3 mL O₂/dL blood) that were significantly greater than values recorded at the end of phase A (P < 0.001 in both groups). This increase corresponds to an O₂ extraction ratio of greater than 90% in both treatment groups. Oxygen extraction ratios were not significantly different between treatment groups at any point during phase B (data not shown). Following completion of phase B, ΔCₐ₋ᵥO₂ had decreased significantly (P < 0.001 in both groups) when measured at 2 minutes of phase C (control 11.9 ± 0.5 mL O₂/dL blood; hyperhydration 12.1 ± 0.5 mL O₂/dL blood). Values for this variable tended to decrease during phase C and had decreased substantially by the end of the rest period (control 8.0 ± 0.2 mL O₂/dL blood; hyperhydration 7.6 ± 0.4 mL O₂/dL blood). The ΔCₐ₋ᵥO₂ increased again as exercise resumed in phase D. During phase D, ΔCₐ₋ᵥO₂ tended to increase and at the end (8 minutes) of that phase, values were 18.7 ± 0.3 mL O₂/dL blood and 17.1 ± 0.4 mL O₂/dL blood for the control and hyperhydration groups, respectively. These values correspond to an oxygen extraction ratio of approximately 75% that was not significantly different between groups.

Changes in Lactate Concentration (Table 5)

There was no statistically significant difference in mixed-venous lactate concentration between the two treatment groups pre-exercise (control 0.54 ± 0.03 mmol/L; hyperhydration 0.47 ± 0.02 mmol/L, P = 0.958). ANOVA was unable to demonstrate a statistically significant difference between treatment groups for this variable during exercise (P = 0.928).

Lactate concentration had not changed significantly from pre-exercise levels when measured at the end of phase A. However, following the completion of phase B, mixed-venous blood lactate concentration (control 18.4 ± 1.9 mmol/L; hyperhydration 18.3 ± 1.5 mmol/L) was
significantly higher than both pre-exercise values ($P < 0.001$ in both groups) and values recorded at the end of phase A ($P < 0.001$ for both groups). Mixed-venous blood lactate concentration had decreased significantly ($P < 0.001$ for both groups) when measured at the end of the rest period (control $3.5 \pm 1.14$ mmol/L; hyperhydration $2.67 \pm 1.21$ mmol/L). This value was higher than pre-exercise measurements, although this finding was only significant in the control group ($P = 0.032$). Lactate concentration increased again during phase D (control $4.94 \pm 0.78$ mmol/L; hyperhydration $6.09 \pm 2.05$ mmol/L), although the increase was significant only in the hyperhydration treatment group ($P = 0.014$). The values for mixed-venous blood lactate concentration measured following phase D were significantly (control $P = 0.002$; hyperhydration $P < 0.001$) higher when compared to pre-exercise levels in both treatment groups.
Table 5. Selected variables (mean ± standard error [SE]) from exercise Protocol 2.

<table>
<thead>
<tr>
<th></th>
<th>Pre-exercise</th>
<th>End Phase A (10m @ 3.7m/s)</th>
<th>End Phase B (2m @ 14m/s)</th>
<th>End Phase C (20m @ 3.7m/s)</th>
<th>Rest Period (10m @ 1.8m/s)</th>
<th>End Phase D (8m @ 9.2m/s)</th>
<th>Cool Down (2m @ 2m/s)</th>
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</thead>
<tbody>
<tr>
<td><strong>Plasma Protein Concentration (g/dL)</strong> ★</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
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<td>6.5 ± 0.1 aΦ</td>
<td>7.4 ± 0.3 aΦ</td>
<td>6.5 ± 0.2 aΦ</td>
<td>6.3 ± 0.2 aΦ</td>
<td>6.8 ± 0.2 aΦ</td>
<td>6.6 ± 0.2 a</td>
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<tr>
<td>Hyperhydration</td>
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<td>5.5 ± 0.1 aΦ</td>
<td>6.2 ± 0.1 aΦ</td>
<td>5.5 ± 0.2 aΦ</td>
<td>5.3 ± 0.1 aΦ</td>
<td>5.8 ± 0.2 aΦ</td>
<td>5.7 ± 0.2 a</td>
</tr>
<tr>
<td><strong>Arterial Hemoglobin Concentration (g/dL)</strong> ★</td>
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</tr>
<tr>
<td>Control</td>
<td>12.5 ± 0.4</td>
<td>16.4 ± 0.4 aΦ</td>
<td>20.7 ± 0.2 aΦ</td>
<td>17.5 ± 0.5 aΦ</td>
<td>16.2 ± 0.5 aΦ</td>
<td>19.1 ± 0.3 aΦ</td>
<td>18.0 ± 0.3 aΦ</td>
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<tr>
<td>Hyperhydration</td>
<td>11.1 ± 0.3</td>
<td>14.9 ± 0.5 aΦ</td>
<td>18.0 ± 0.3 aΦ</td>
<td>15.7 ± 0.3 aΦ</td>
<td>14.3 ± 0.4 aΦ</td>
<td>17.3 ± 0.4 aΦ</td>
<td>16.8 ± 0.4 a</td>
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<tr>
<td><strong>Increase in PV in the hyperhydration study (%)†</strong></td>
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<tr>
<td>Core Temperature (°C)</td>
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<td>16.7 ± 1.8 aΦ</td>
<td>18.3 ± 2.2</td>
<td>18.4 ± 1.4</td>
<td>18.9 ± 2.1</td>
<td>15.7 ± 1.1</td>
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<td>Control</td>
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<td>38.4 ± 0.3 aΦ</td>
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<td>39.7 ± 0.1 aΦ</td>
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<td>41.3 ± 0.1 aΦ</td>
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<td>38.3 ± 0.3 aΦ</td>
<td>40.8 ± 0.2 aΦ</td>
<td>39.6 ± 0.2 aΦ</td>
<td>38.8 ± 0.2 aΦ</td>
<td>41.5 ± 0.3 aΦ</td>
<td>40.6 ± 0.3 aΦ</td>
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<tr>
<td>Heart Rate (beats/min) ★</td>
<td>44.0 ± 2.2</td>
<td>136.6 ± 4.6 aΦ</td>
<td>216.1 ± 2.0 aΦ</td>
<td>135.1 ± 6.1 aΦ</td>
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<td>179.1 ± 4.7 aΦ</td>
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<td>214.4 ± 2.3 aΦ</td>
<td>142.7 ± 4.9 aΦ</td>
<td>113.3 ± 6.1 aΦ</td>
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<td>Hyperhydration</td>
<td>102.9 ± 1.6</td>
<td>105.5 ± 0.7 aΦ</td>
<td>74.5 ± 2.2 aΦ</td>
<td>111.7 ± 2.9 aΦ</td>
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<td>99.9 ± 2.2</td>
<td>119.3 ± 2.9 aΦ</td>
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<td>Arterial O₂ Tension (mmHg)</td>
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<td>107.5 ± 1.5</td>
<td>77.3 ± 2.0 aΦ</td>
<td>105.0 ± 4.0 aΦ</td>
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<td>Mixed-venous O₂ Tension (mmHg)</td>
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<td>27.2 ± 0.6 aΦ</td>
<td>16.4 ± 0.8 aΦ</td>
<td>31.6 ± 1.4 aΦ</td>
<td>32.2 ± 1.0 aΦ</td>
<td>23.8 ± 0.8 ††</td>
<td>33.7 ± 0.9 ††</td>
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<tr>
<td>Control</td>
<td>39.8 ± 1.1</td>
<td>28.9 ± 1.1 aΦ</td>
<td>15.4 ± 0.8 aΦ</td>
<td>30.5 ± 0.8 ††</td>
<td>30.7 ± 0.7 ††</td>
<td>23.9 ± 0.9 a†</td>
<td>35.3 ± 2.3 ††</td>
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<td>Hyperhydration</td>
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<td>99.0 ± 0.1 aΦ</td>
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<tr>
<td>Control</td>
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<td>37.3 ± 0.4 a</td>
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<td>Hyperhydration</td>
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<td>Mixed-Venous Hemoglobin Saturation (%)★</td>
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<tr>
<td>Arterial CO₂ Tension (mmHg)</td>
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<td>33.1 ± 2.2 aΦ</td>
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<td>36.6 ± 1.1 a</td>
<td>28.8 ± 1.9 aΦ</td>
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Table 5. Selected variables (mean ± standard error [SE]) from exercise Protocol 2 (continued).

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<tr>
<th></th>
<th>Pre-exercise</th>
<th>End Phase A (10m @ 3.7m/s)</th>
<th>End Phase B (2m @ 14m/s)</th>
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<th>Rest Period (10m @ 1.8m/s)</th>
<th>End Phase D (8m @ 9.2m/s)</th>
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<td><strong>Arterial Blood pH ★</strong></td>
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<td>Control</td>
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<tr>
<td>Hyperhydration</td>
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<td>7.39 ± 0.04 Φ</td>
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<td><strong>Arterial O₂ Content (vol %) ★</strong></td>
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<tr>
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<td>17.0 ± 0.5</td>
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<td>24.3 ± 0.3 aΦ</td>
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</tr>
<tr>
<td><strong>Mixed-Venous O₂ Content (vol %) ★</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.9 ± 0.5</td>
<td>11.4 ± 0.5 aΦ</td>
<td>2.3 ± 0.2 aΦ</td>
<td>12.6 ± 0.6 Φ</td>
<td>13.9 ± 0.6 Φ</td>
<td>6.8 ± 0.3 ↑†</td>
<td>13.8 ± 0.6 ↑†</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>11.9 ± 0.4</td>
<td>11.2 ± 0.9</td>
<td>1.7 ± 0.1 aΦ</td>
<td>10.7 ± 0.4 ↑†</td>
<td>11.4 ± 0.3 ↑†</td>
<td>5.5 ± 0.4 a↑†</td>
<td>12.9 ± 0.5 ↑†</td>
</tr>
<tr>
<td><strong>Arterial to Mixed-Venous O₂ Difference (vol %) ★</strong></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>4.1 ± 0.3</td>
<td>10.8 ± 0.1 aΦ</td>
<td>22.0 ± 0.2 aΦ</td>
<td>11.1 ± 0.3 aΦ</td>
<td>8.0 ± 0.2 aΦ</td>
<td>18.7 ± 0.3 ↑†</td>
<td>10.8 ± 0.5 ↑†</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>3.3 ± 0.2</td>
<td>9.2 ± 0.4 aΦ</td>
<td>19.5 ± 0.3 aΦ</td>
<td>10.2 ± 0.5 ↑†</td>
<td>7.6 ± 0.4 ↑†</td>
<td>17.1 ± 0.4 a↑†</td>
<td>9.9 ± 0.3 ↑†</td>
</tr>
<tr>
<td><strong>Mixed-Venous Blood Lactate Concentration (mmol/L) ★</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>0.54 ± 0.03</td>
<td>0.67 ± 0.08</td>
<td>18.4 ± 1.9 aΦ</td>
<td>NA</td>
<td>3.50 ± 1.14 aΦ</td>
<td>NA</td>
<td>4.94 ± 0.78 a</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>0.47 ± 0.02</td>
<td>0.69 ± 0.10</td>
<td>18.3 ± 1.5 aΦ</td>
<td>NA</td>
<td>2.67 ± 1.21 aΦ</td>
<td>NA</td>
<td>6.09 ± 2.05 aΦ</td>
</tr>
</tbody>
</table>

★ANOVA analysis demonstrates statistically significant difference between treatments
αStatistically significant difference from pre-exercise value
ΦStatistically significant difference from immediately preceding value (in table)
†Insufficient data to calculate statistical significance when compared to pre-exercise value
‡Insufficient data to calculate statistical significance when compared to immediately preceding value

Change in plasma volume calculated as [(PP1/PP2) - 1.0] x 100%, where PP1 and PP2 are the initial and test plasma protein concentrations, respectively (Boyd, 1981).

3Sample for mixed-venous blood lactate collected at 2 min after the completion of phase B (2m @ 3.7m/s)
Figure 8: Changes in plasma protein concentration (g/dL, left panel) and arterial hemoglobin concentration (g/dL, right panel) in the second exercise protocol described in this report (Protocol 2). This exercise test was shorter in duration than the first protocol used and included short bursts of exercise known to induce arterial hypoxemia and hemoglobin saturation.

**Left panel:** In the second set of experiments, administration of NaCl induced water consumption and a subsequent average estimated increase in plasma volume (based on the decrease in plasma protein concentration) of $15.5 \pm 1.1\%$ prior to the initiation of exercise. Plasma protein concentration remained significantly lower ($P < 0.001$) in the hyperhydration treatment group throughout the exercise test when compared to the control group.

**Right panel:** The increase in plasma volume led to a corresponding decrease in hemoglobin concentration in the hyperhydration treatment group when compared to the control treatment group. This difference between treatment groups remained significant throughout the exercise test ($P < 0.001$).

★ ANOVA indicates a statistically significant difference between treatment groups (i.e., hyperhydration and control).
α Statistically significant difference from all subsequent data during exercise phases A, B, C, and D.
Φ Significantly different to previous data point.
δ Compares data points within phase B. Labeled points are significantly different to the value recorded at $t = 11$ minutes (end of phase A [10 minutes @ 3.7 m/s]).
∆ Compares data points within phase D. Labeled points are significantly different to the value recorded at $t = 43$ minutes (walk [10 minutes @ 1.8 m/s]).
Figure 9: Exercise-induced changes in core (pulmonary artery) temperature (°C, upper panel) and heart rate (beats/minute, right panel) during exercise in Protocol 2.

Left panel: The hyperthermia experienced during exercise was not affected by hyperhydration and no significant ($P = 0.884$) difference was detected between treatment groups.

Right panel: Pre-exercise heart rate was not significantly different between treatment groups ($P = 0.727$). Statistical analysis revealed a significant ($P = 0.002$) difference in heart rate between treatment groups; however, examination of the individual data points revealed significant differences occurred only at a handful of time-points (indicated by ★). At each time point where there was a statistically significant difference, heart rate was slightly (11-14 beats/minute) higher in the hyperhydration treatment group. Note that maximal heart rate was elicited during phase B in both treatment groups and was not different between treatment groups. For explanation of symbols, see Fig. 8.
Figure 10: Changes in arterial and mixed-venous blood $O_2$ tension (mm Hg, upper panel) and hemoglobin-$O_2$ saturation (%) (right panel) occurring during an exercise protocol incorporating a short burst of intense exercise known to induce arterial hypoxemia and hemoglobin de-saturation (Protocol 2).

**Left panel:** Changes in arterial and mixed-venous blood $O_2$ tension were similar in the 2 treatment groups and statistically significant differences between treatment groups in either variable could not be demonstrated. During maximal-intensity exercise in phase B, arterial $O_2$ tension was significantly decreased in both treatment groups (i.e., exercise-induced arterial hypoxemia was induced); however, hyperhydration did not exacerbate arterial hypoxemia.

**Right panel:** Arterial hemoglobin-$O_2$ saturation decreased significantly in both treatment groups during phase B. Mixed-venous blood hemoglobin-$O_2$ saturation decreased significantly during both phases B and D. Statistically significant differences between treatment groups were not observed for either variable.  

$\alpha$ Statistically significant difference from pre-exercise values.

For explanation of other symbols, see Fig. 8.
Table 6. Mean (± standard error of the mean) arterial and mixed-venous blood O$_2$ tension, arterial and mixed-venous blood hemoglobin-O$_2$ saturation, and arterial CO$_2$ tension during phases B and D of exercise in Protocol 2.

<table>
<thead>
<tr>
<th></th>
<th>Pre-exercise</th>
<th>Phase B (120 sec @ 14m/s)</th>
<th>Phase D (8minutes @ 9.2m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>90 sec</td>
<td>120 sec</td>
</tr>
<tr>
<td><strong>Arterial O$_2$ Tension (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>102.9 ± 1.6</td>
<td>75.3 ± 2.3</td>
<td>74.5 ± 2.2</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>102.2 ± 2.5</td>
<td>78.8 ± 1.8</td>
<td>77.3 ± 2.0</td>
</tr>
<tr>
<td><strong>Mixed-venous O$_2$ Tension (mmHg)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>38.1 ± 0.9</td>
<td>17.1 ± 0.8</td>
<td>16.4 ± 0.8</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>39.8 ± 1.1</td>
<td>16.4 ± 0.7</td>
<td>15.4 ± 0.8</td>
</tr>
<tr>
<td><strong>Arterial Hemoglobin Saturation (%)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>99.1 ± 0.1</td>
<td>88.0 ± 1.6</td>
<td>85.6 ± 1.9</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>99.1 ± 0.1</td>
<td>88.5 ± 1.2</td>
<td>85.9 ± 1.4</td>
</tr>
<tr>
<td><strong>Mixed-Venous Hemoglobin Saturation (%)</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>76.6 ± 1.7</td>
<td>9.1 ± 0.9</td>
<td>7.8 ± 0.8</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>78.5 ± 1.3</td>
<td>7.9 ± 0.6</td>
<td>6.9 ± 0.5</td>
</tr>
<tr>
<td><strong>Arterial CO$_2$ Tension (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>44.7 ± 0.6</td>
<td>53.5 ± 1.9</td>
<td>53.3 ± 1.8</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>43.7 ± 0.7</td>
<td>50.7 ± 1.8</td>
<td>50.5 ± 1.6</td>
</tr>
</tbody>
</table>

*ANOVA analysis demonstrates statistically significant difference between treatments.
Figure 11: Changes in arterial CO₂ tension (mm Hg, left panel) and arterial pH (right panel) during exercise during the second exercise test used in these experiments (Protocol 2).

**Left panel:** Arterial CO₂ tension peaked during phase B indicating relative alveolar hypoventilation and then reached a nadir at the beginning of phase C as horses hyperventilated during recovery from maximal exercise. From this point onward, a gradual recovery in CO₂ tension occurred until the beginning of phase D. There was no statistical difference in arterial CO₂ tensions between treatment groups ($P = 0.479$) indicating similarity of ventilation between the two.

**Right panel:** Although hyperhydration did not affect arterial CO₂ tension, arterial pH tended to be somewhat lower than in the control study. ANOVA analysis indicated a significant ($P = 0.049$) difference between treatment groups although examination of individual data points did not reveal any significant differences between individual pairs of values.

α In the left panel, indicates a statistically significant difference to all subsequent values recorded during exercise (except the final value in phase A of the control treatment group [not indicated]). In the right panel, indicates a statistically significant difference from pre-exercise values.

For explanation of other symbols, see Fig. 8.
Figure 12: Changes in arterial and mixed-venous blood $O_2$ content (mL $O_2$/dL blood, left panel) and the arterial to mixed-venous blood $O_2$ content difference (mL $O_2$/dL blood, right panel) in the second exercise protocol examined in this report (Protocol 2).

Left panel: As a result of the diminished hemoglobin concentration, arterial blood $O_2$ content ($C_{aO_2}$) was significantly lower both before and during exercise in the hyperhydration experiments when compared to the control study ($P = 0.007$). The decrease in $C_{aO_2}$ was attended by a significant reduction in mixed-venous blood $O_2$ content ($C_{vO_2}$) during exertion in the hyperhydration experiments ($P < 0.001$).

Right panel: Pre-exercise values of arterial to mixed-venous blood $O_2$ content difference in the control and hyperhydration experiments were similar ($P = 0.064$). Exercise caused the arterial to mixed-venous blood $O_2$ content difference to increase in both treatment groups during phases B, C, and D of the exercise test. However, as in the previous experiments, the arterial to mixed-venous blood $O_2$ content difference was significantly attenuated in the hyperhydration study ($P = 0.001$).

★ ANOVA indicates a statistically significant difference between treatment groups (i.e., hyperhydration and control). However, examination of individual data points indicates that not all are significantly different between treatment groups; data points significantly different between treatment groups are indicated. For explanation of the remainder of symbols, see Fig. 8.
CHAPTER 5: DISCUSSION

The importance of adequate hydration in maintaining cardiovascular and thermoregulatory function and thus optimizing performance during exercise is well recognized (Greenleaf and Castle 1971; Gonzalez-Alonso and Calbet 2003; Gonzalez-Alonso et al. 1995, 1997, 1998, 1999; Montain and Coyle 1992; Montain et al. 1995, 1998; Naylor et al. 1993a; Sawka et al. 1983, 1985, 2000, 2001). In human athletes, pre-exercise “fluid-loading” (hyperhydration) has been investigated as a means to preserve vascular volume in the face of sweat-fluid losses. This approach has shown promise in some, although not all, studies in exercising humans (Coles and Luetkemeier 2005; Coutts et al. 2002; Greenleaf and Castle 1971; Greenleaf et al. 1997; Grucza et al. 1987; Lyons et al. 1990; Moroff and Bass 1965). Creation of a “fluid reservoir” could benefit horses performing prolonged exercise particularly if there is limited opportunity to consume water during exercise. However, using a treadmill exercise test to simulate the 2nd day of an equestrian 3DE, Sosa Leon et al. (2002) reported that pre-exercise hyperhydration produced significant arterial hypoxemia during moderate exercise (55 to 60% VO$_{2\text{max}}$). In those experiments, arterial O$_2$ tension was well maintained throughout exercise in the control group (Sosa Leon et al. 2002). However, a statistically significant decrease of approximately 15 mm Hg in arterial O$_2$ tension occurred in hyperhydrated horses during phases B and D of the test (Sosa Leon et al. 2002). Based on their results, these investigators suggested that pre-exercise fluid loading might be detrimental to arterial oxygenation and athletic performance (Sosa Leon et al. 2002).

In our laboratory, arterial hypoxemia occurs only in horses exercising at close to maximal intensity (Goetz et al. 2001; Manohar and Goetz 2003; Manohar et al. 2001a, 2001b, 2002a, 2002b, 2003, 2004). Therefore, we were interested in the reported effect of hyperhydration on arterial oxygenation observed when horses were exercising at only 55-60% of VO$_{2\text{max}}$ (Sosa Leon et al. 2002). Using an alternate method of inducing hyperhydration (see materials and methods) and hypovolemia, we examined the effects of hyperhydration in Thoroughbred horses in a series of experiments utilizing a variety of treadmill exercise protocols. In an initial study, Manohar et al. (2003) induced an average estimated increase in plasma volume of 18.0 ± 1.8% (based on the decrease in plasma protein concentration) prior to an incremental exercise test eliciting maximal exertion (14 m/s on a 3.5% uphill grade). Despite establishing significant
hypervolemia, there was no effect on the development and/or severity of arterial hypoxemia or hemoglobin-O₂ de-saturation during exercise (Manohar et al. 2003). In a later study, acute plasma volume expansion using hypertonic saline (7.2%) administered intravenously also failed to elicit any differences in arterial O₂ tension between treatment and control horses performing maximal exercise (Manohar et al. 2005b). It was therefore speculated that perhaps the combination of fluid loading and prolonged exercise contributed to the arterial hypoxemia recorded in the experiments of Sosa-Leon et al. (2002). Thus, in the first set of experiments reported here, an exercise protocol identical to that of Sosa-Leon et al. (2002) was utilized. However, despite establishing significant hypervolemia throughout exercise, no decrease in arterial O₂ tension or hemoglobin saturation was observed at any time (Tennent-Brown et al. 2006a). In a final series of experiments, horses were studied during extended exercise that incorporated a burst of high-intensity exercise known to induce arterial hypoxemia and hemoglobin de-saturation. Once again, despite significant plasma volume expansion that persisted throughout exercise, hyperhydration did not alter arterial oxygenation or hemoglobin saturation during either high-intensity exercise (phase B) or prolonged moderate-intensity exercise (phase D) (Tennent-Brown et al. 2006b). From these studies, it was concluded that pre-exercise hypervolemia subsequent to hyperhydration induced in the manner described here is not detrimental to arterial oxygenation of horses performing exercise simulating the 2nd day of a 3DE.

It is likely that differences in experimental procedure explain the divergent results of these experiments and those of Sosa-Leon et al. (2002). The most important of these is the manner in which hyperhydration was induced. In their experiments, Sosa Leon et al. (2002) administered 26 L (~6% of bodyweight) of an isotonic solution as 4 equal doses to experimental horses via nasogastric intubation over 1 hour with last dose administered 1 hour pre-exercise. In the studies described here, NaCl (0.425 g/kg) was administered 5 hours pre-exercise and horses were then allowed to drink water as dictated by homeostatic mechanism(s), over the subsequent 3 hours. All animals in this study had stopped drinking before they were taken from their stalls for instrumentation; the volume of water voluntarily consumed (approximately 10 to 13 L) was considerably smaller than the volume of fluid administered to the horses in the Sosa Leon et al. (2002) study. Additionally in our experiments, a period of 2 hours (after voluntary water intake
had ceased) was available for water absorption from the gastrointestinal tract (GIT) before initiation of exercise.

These two factors (i.e., a smaller volume of water intake [~10-13 L vs. 26 L] and an additional 2 hour period for water absorption from the GIT) likely led to a smaller overall gastrointestinal bulk in our hyperhydrated horses compared to those of Sosa Leon et al. (2002). The smaller bulk of abdominal contents might have posed less restriction to diaphragmatic excursions allowing better maintenance of ventilation and arterial oxygenation during exertion (Figs. 4, 5, and 10). In the initial study described here, horses tended to exhibit a reduction in arterial CO₂ tension throughout the exercise test indicating mild alveolar hyperventilation. In the second set of experiments, arterial CO₂ tension increased during maximal exercise (phase B) indicating alveolar hypoventilation which has been suggested to contribute to exercise induced arterial hypoxemia (EIAH). Importantly, in all our experiments, recorded arterial CO₂ tensions were similar between control and hyperhydration treatment groups (Fig. 6 and 11), indicating similar alveolar ventilation in both groups. It is interesting to note that in the report of Sosa Leon et al. (2002), figure 3 shows an increase in arterial CO₂ tension of approximately 4 mm Hg in the hyperhydrated group at the end of phase B (4 minutes at 8 m/s on a 3% incline). Although this increase was not statistically significantly different from the arterial CO₂ tension measured in the control group at the same time point, it perhaps supports the suggestion that ventilation was impaired in hyperhydrated horses.

A further difference between our experiments and those of Sosa Leon et al. (2002) is that plasma volume was significantly increased (based on plasma protein concentration) at the initiation of exercise in the present studies; however, this was not the case in the study of Sosa-Leon et al. (2002). Examination of the data from that report reveals that there had been no significant change in plasma protein concentration or hematocrit as exercise commenced suggesting that very little of the administered fluid had been absorbed from the gastrointestinal tract (Sosa Leon et al. 2002). In contrast, pre-exercise samples indicate that plasma volume in hyperhydrated horses had increased in the present studies before the initiation of exercise by an average estimated 11.3 ± 3.2% in the first experiment and 15.5 ± 1.1% in the second when compared to the control treatment (Fig. 2 and 8; Tables 4 and 5). In the report of Sosa Leon et al. (2002), fluid did eventually appear to be absorbed from the gastrointestinal tract and led to an estimated 10.8% expansion of plasma volume at end-exercise based on changes in plasma
protein concentration. Continued absorption of water from the gastrointestinal tract during exercise appeared to have also occurred in the present studies as the increase in relative plasma volume at end-exercise exceeded that pre-exercise (Tables 4 and 5). Of particular relevance to this discussion, is that even though the magnitude of hypervolemia in our experiments (both pre-exercise and end-exercise) was greater than that reported by Sosa Leon et al. (2002), we were unable to demonstrate any adverse effects on arterial O$_2$ tension and/or hemoglobin-O$_2$ saturation.

Sosa Leon et al. (2002) suggested that pulmonary edema could be the cause of arterial hypoxemia in their hyperhydration experiments. Exercise-induced arterial hypoxemia (EIAH) is a well recognized phenomenon in horses working at maximal intensity; however, EIAH is not typically observed at lower workloads (Bayly et al. 1989, 1995; Goetz et al. 2001; Manohar and Goetz 2003; Manohar et al. 2001a, 2001b, 2002a, 2002b, 2003, 2004). The definitive cause of EIAH is unknown, but diffusion limitation is thought to be a major contributor in horses (Hopkins et al. 1996, Wagner et al. 1989). Diffusion limitation occurs when the blood-gas barrier is thickened (as might occur with pulmonary edema) or if capillary transit time is sufficiently shortened (Dempsey and Wagner, 1997; Wagner et al. 1989, 1996; Wilkins et al. 2001). In horses (and ponies) performing maximal exercise, pressures within the pulmonary vessels are extremely high and mean pressures in the pulmonary artery of over 100 mmHg are routinely recorded (Goetz and Manohar 1986; Magid et al. 2000; Manohar 1986b; Manohar and Goetz 1996a,b, 1998; Manohar et al. 1998, 2000a, 2000b). However, at lower work rates, pulmonary arterial pressures are correspondingly lower (Goetz and Manohar 1986; Manohar 1986b; Manohar and Goetz 1996a,b; Manohar et al. 1998, 2000a, 2000b). Even at pressures associated with maximal exercise, the structural changes in the blood-gas barrier contributing to stress failure of pulmonary capillaries (West et al. 1993) are not believed to contribute to the pathogenesis of arterial hypoxemia and hemoglobin-O$_2$ desaturation (Manohar et al. 2001a). In addition, using a dual indicator (electrical impedance and temperature) technique, Wilkins et al. (2001) were unable to detect an increase in lung water (i.e., edema) in Standardbred horses performing exercise eliciting maximal heart rate and significant hypoxemia. The experiments described in the present report were more likely than those of Sosa Leon et al. (2002) to produce pulmonary edema for a number of reasons. Hydrostatic pressure was likely higher and oncotic pressure lower (due to greater plasma volume expansion and hemodilution, particularly in the
early phases of exercise) and pulmonary arterial blood pressure was almost certainly greater (as a result of higher exercise intensity in the second exercise protocol) in the current studies. Thus, if pulmonary edema were to occur in hyperhydrated horses and contribute to EIAH, one would have expected to see some evidence of impaired arterial oxygenation in these experiments. Furthermore, it should be noted that Sosa Leon et al. (2002) reported significant arterial hypoxemia during phase B of their exercise test (30 to 34 minutes from the initiation of exercise) when there had been relatively little plasma volume expansion and much of the administered fluid likely remained within the gastrointestinal tract.

In horses, as in humans, there is rapid decrease in plasma volume at the initiation of both moderate- and high-intensity exercise (Geor and McCutcheon, 1998a; Lindinger et al. 1995, 2000, 2004; Masri et al. 1990; McKeever et al. 1993). This occurs before sweat-fluid losses and is thought to be due to movement of fluid out of the vascular space in response to hydrostatic and osmotic forces. The extent of plasma volume contraction during exercise is directly related to exercise intensity (Convertino et al. 1981). During prolonged exercise, plasma volume then rebounds but remains below resting volumes for the duration of exercise and will tend to decrease if no effort is made to replace sweat-fluid losses (Harrison et al. 1975; Harrison 1985). Although it has been suggested that protein is added to the vascular space during exercise (Naylor et al. 1993?), it is generally believed that the changes in plasma protein concentration during exercise reflect movement of fluid into and out of the vascular space (Lindinger et al. 1995, 2000, 2004). In the present study, plasma protein concentration followed the expected changes in plasma volume. At the initiation of exercise, there was an increase in plasma protein concentration and plasma protein concentration increased sharply during periods of more intense (phases B and D) exercise. In addition, plasma protein concentration increased over the course of both exercise protocols as a result of progressive sweat-fluid losses. During intense exercise, the increase in plasma protein concentration (and oncotic pressure) will tend to counteract the increase in hydrostatic pressure and help retain fluid within the vascular space thereby preventing or reducing edema formation.

The effect of hyperhydration (and hypervolemia) on the performance of athletes who experience EIAH is a subject of debate (Zavorsky et al. 2002, 2003). If diffusion limitation as a result of a decrease in pulmonary transit time is a significant component of EIAH, hyperhydration might improve arterial O₂ tension by slowing pulmonary capillary transit if
additional pulmonary capillaries can be recruited (i.e., by increasing pulmonary capillary blood volume). Conversely, plasma volume expansion could worsen pulmonary gas exchange as a result of interstitial fluid accumulation (edema), decreased lung compliance, reduced hemoglobin concentration, or decreased capillary transit time (increased cardiac output). In humans, acute plasma volume expansion has been shown to lengthen red cell pulmonary transit time during severe (stationary cycling) exercise in endurance athletes (Zavorsky et al. 2002). Further, acute hypervolemia improved arterial O₂ tension and decreased the alveolar-arterial O₂ tension difference during cycling exercise in a group of trained athletes that experienced EIAH (Zavorsky et al. 2003). However, the improvement in pulmonary gas exchange was due to improved ventilation-perfusion (V/Q) matching and not the lengthening of transit time (Zavorsky et al. 2003). Plasma volume expansion might improve V/Q matching by increasing nitric oxide (NO) availability in the lungs as occurs in normovolemic anemia (Deem et al. 1999). Increased NO could improve pulmonary function through its broncho- and/or vasodilator functions. Hypervolemia has not improved O₂ tension in horses exercising at maximal intensity when induced over several hours (Manohar 2003) or acutely with hypertonic saline (Manohar 2005). This lack of improvement could be due to the fact that little V/Q mismatching apparently occurs in the lungs of exercising horses (Hopkins et al. 1998; Seaman et al. 1995; Wagner et al. 1989) or might indicate that the pulmonary capillary beds in exercising horses are already fully recruited. Wilkins et al. (2001) showed that transit times from the pulmonary artery to the carotid artery (i.e., across the pulmonary bed and left heart) decreased as cardiac output increased from approximately 70 mL/kg/min (standing) to approximately 250 mL/kg/min (walking) but transit time decreased only slightly as cardiac output increased further. This suggests that complete capillary recruitment occurs in exercising horses at low exercise intensities; additional cardiac output might be accommodated by distention of the pulmonary capillaries so that transit time remains relatively constant (Wilkins et al. 2001).

Expansion of the plasma volume in the present experiments was attended by a significant decrease in arterial hemoglobin concentration in the pre-exercise period and throughout exercise (Fig. 2 and 8). As a consequence, the O₂ content of both arterial and mixed-venous blood was significantly reduced in the hyperhydration treatment. Hemodilution caused a significant attenuation of the exercise-induced expansion of the arterial to mixed-venous blood O₂ content difference for the majority of both exercise tests (Fig. 7 and 12). Whole body O₂ consumption
(VO₂) of both treatment groups should have been similar (Sosa Leon et al. 2002) or possibly slightly increased in the hyperhydrated horses as a consequence of their increased body weight (Nyman et al. 2002). Whole body O₂ consumption is the product of cardiac output and arterial to mixed-venous blood O₂ content difference (Fick principle). At a constant VO₂, the smaller ΔCa-vO₂ observed in our hyperhydrated horses during exercise (Fig. 7 and 12) should have been accompanied by additional augmentation of cardiac output. Since heart rates during exercise were similar between treatment groups in both studies (Fig. 3 and 9), the additional increase in cardiac output during exercise in the hyperhydration treatment group was likely achieved by an increase in stroke volume. An increase in venous return and, consequently, ventricular filling or a decrease in blood viscosity (as a result of hemodilution; Fig. 2 and 8) might have played a role in increasing stroke volume. The possibility of an increased stroke volume is supported by observations in exercising horses (Hopper et al. 1991), dogs (Horwitz and Lindenfeld 1985), and human subjects (Kanstrup and Ekbolm 1982; Warburton et al. 1999) where an acute increase in plasma volume resulted in a significant increase in cardiac output and stroke volume. It should be noted that at several time points during exercise in both exercise protocols, heart rate in hyperhydrated animals was slightly higher than in the control group. At these time points, the increase in heart rate likely contributed to the increase in cardiac output necessitated by the decreased ΔCa-vO₂. The increase in cardiac output might be expected to decrease pulmonary capillary transit time and induce or exacerbate arterial hypoxemia, particularly if recruitment of pulmonary capillaries is complete (Wilkins et al. 2001). That this did not occur might be related to the asymptotic relationship between cardiac output and pulmonary transit times as discussed (Wilkins et al. 2001). Alternatively, V/Q mismatching increases with increasing exercise intensity in human athletes but is attenuated by plasma volume expansion in athletes experiencing EIAH (Zavorsky et al. 2003). However, as mentioned, V/Q mismatching is apparently minimal in exercising horses (Hopkins et al. 1998; Seaman et al. 1995; Wagner et al. 1989) and further research is clearly required!

In agreement with the results of Sosa Leon et al. (2002), hyperhydration had no effect on core temperature measured during exercise in these studies. Hypohydration has been shown to impair thermoregulation in humans (Fortney et al. 1981) and horses (Naylor et al. 1993a). Conversely, hyperhydration improved heat dissipation during exercise in a number of human studies (Greenleaf and Castle, 1971; Gruca et al. 1987; Moroff and Bass, 1965). However, other
studies in humans (Latzka et al. 1998) and horses (Geor and McCutcheon, 1998a) have failed to find any thermoregulatory advantage of hyperhydration. There are a number of reasons why hyperhydration might not have improved thermoregulation in horses in the current study. The most likely is that sweating rate exceeded evaporation rate in both treatment groups; when this phenomenon occurs, sweat drips from the skin and contributes little to cooling. Although a large fan was placed in front of horses during exercise, fan speed might have been insufficient (wind speed was not measured) and therefore limited the ability to increase the rate of evaporative heat loss. Thus, any advantage of hyperhydration (i.e., a higher or a more sustained sweating rate) might have been obscured. It should be noted that Geor and McCutcheon (1992) found no difference in sweating rates in horses that received pre-exercise oral fluids. An additional explanation for the lack of thermoregulatory benefit could be that hyperhydrated horses tended to have a higher body weight and would be expected to have an increased thermal load that could have negated any benefits of fluid loading. Finally, changes in plasma osmolality, and particularly sodium concentration, have been suggested to affect thermoregulatory function in humans (Sawka et al. 1985; Montain and Coyle 1992). It is unclear whether the same phenomenon occurs in horses. Neither plasma electrolyte concentrations nor osmolality were measured in these experiments and it is assumed that homeostatic mechanisms would attempt to normalize both. However, we cannot rule out that changes in plasma electrolyte concentrations occurred and interfered with thermoregulation. Further studies under conditions of high heat and/or humidity are required to determine whether hyperhydration has any thermoregulatory benefit in exercising horses.

Animals in the hyperhydrated group in these studies were weighed immediately before they received NaCl, immediately prior to instrumentation (3 hours after NaCl administration), and again shortly after the conclusion of exercise. Horses in the control group were weighed immediately before instrumentation and shortly after exercise. On average, the horses that received NaCl gained 11.4 ± 0.6 kg in bodyweight (over both experiments) prior to exercise. As a consequence, hyperhydrated horses were heavier than control animals at the beginning of the exercise test in both protocols although this difference was only statistically significant for the second exercise protocol (Table 7). Horses in the hyperhydration group lost more weight during exercise and this difference was statistically significant in both the shorter and longer exercise protocols (Table 7). However, when pre-NaCl administration bodyweights were used to
determine the weight loses sustained during exercise, hyperhydrated horses lost (statistically) significantly less body weight in both exercise protocols (i.e., their post-exercise weight was closer to their pre-NaCl weight; Table 7). This finding is similar to the findings of others examining the effects of pre-exercise fluid administration (Nyman et al. 2002). Although a number of the hyperhydrated horses urinated during the exercise test, it is assumed that the increased gross weight loss in the hyperhydrated group was due to increased sweat fluid losses. Of interest, is the observation that horses in the hyperhydration group subjectively drank less water following exercise. This is also similar to the results reported by Nyman et al. (2002). Those researchers administered 12 L of water to horses 30 minutes prior to performing exercise eliciting 65-70% of maximal heart rate for 40 minutes and also found that those horses drank less post-exercise than euhydrated animals or horses that were dehydrated prior to exercise. Plasma volume was estimated from changes in total plasma protein concentration in that study; in hyperhydrated horses, the decrease in plasma volume was significantly less and plasma volume was restored more quickly than in control horses (Nyman et al. 2002). Under field conditions, complete restoration of exercise-induced fluid losses might not have occurred 18-24 hours after completion of the cross-country phase of a 3DE (Andrews et al. 1994). This delay in restoration could impair performance in animals that must compete over several days. It would be of interest to examine exercise performance over successive days in animals that were fluid loaded pre-exercise compared with horses that had to restore body water losses by voluntary ingestion.

Table 7. Weight changes during exercise in exercise tests simulating an equestrian 3-day event. Protocol 1 is identical to that used by Sosa Leon et al. (2002) and 113 minutes in duration. Protocol 2 is shorter (55 minutes) but includes a burst of maximal intensity exercise. All weights are presented in kilograms and as mean ± SE.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Condition</th>
<th>Pre-NaCl Admin.</th>
<th>Pre-Exercise</th>
<th>Post-Exercise</th>
<th>Weight Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pre-Exerc./ Post-Exerc.</td>
</tr>
<tr>
<td>Protocol 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>NA</td>
<td>455 ± 11</td>
<td>440 ± 10</td>
<td>−14.8 ± 1.2</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>450 ± 8</td>
<td>462 ± 8</td>
<td>443 ± 7</td>
<td>−18.9 ± 1.9†</td>
<td>−7.3 ± 1.6†</td>
</tr>
<tr>
<td>Protocol 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>NA</td>
<td>456 ± 9</td>
<td>444 ± 9</td>
<td>−11.9 ± 1.1</td>
</tr>
<tr>
<td>Hyperhydration</td>
<td>457 ± 9</td>
<td>468 ± 9†</td>
<td>454 ± 9†</td>
<td>−13.4 ± 1.6†</td>
<td>−2.1 ± 1.6†</td>
</tr>
</tbody>
</table>

NA Not applicable.
† Statistically significantly different from control group in the same exercise protocol (P < 0.05)
Performance of muscular work requires energy expenditure; the amount of which is related to body mass and the speed at which an animal is traveling (Taylor and Heglund, 1982). For most species, addition of weight to an animal increases the energy needed to generate a specific speed in direct proportion to the ratio of the added weight and the animal’s body weight (Taylor and Heglund 1982; Pagan and Hintz 1986). Energy needed to perform muscular work is derived from aerobic and anaerobic metabolism. With increasing work intensity, the rate of O$_2$ consumption increases steadily to a maximum value and then plateaus (i.e., VO$_{2\text{max}}$). As work intensity increases beyond the VO$_{2\text{max}}$, an increasing proportion of the required energy is derived from anaerobic metabolism, the contribution of which can be estimated from blood lactate concentration. It is interesting to note that, in agreement with the results of other studies performed in this laboratory (Manohar et al. 2003), there was no significant change in lactate concentrations in hyperhydrated horses in either exercise protocol despite an average increase in their bodyweight of ~11 kg. Although it would seem relatively inconsequential in comparison to a horse’s total bodyweight, an increase in bodyweight of a similar magnitude resulted in a significant increase in plasma lactate concentration in experiments performed by Hinchcliff et al. (1993) and Nyman et al. (2002) in horses performing short, moderate-to-high intensity exercise. Hyperhydrated horses (administered 12 L of water immediately before exercise) in the study of Nyman et al. (2002) actually had slightly lower plasma lactate concentrations during prolonged (40 minutes) exercise eliciting 65-70% of maximal heart rate when compared to euhydrated animals although this difference was not significant. Finally, Green et al. (1997) found decreased muscle lactate production following acute plasma volume expansion (14-21%) in untrained humans performing seated exercise at approximately 46% VO$_{2\text{max}}$. Interpretation of blood lactate concentration in the current experiments is confounded by changes in plasma volume. If the increase in plasma volume is appropriately accounted for, hyperhydrated horses generally produced more lactate than control animals as might be expected on the basis of increased body weight.

There are a number of limitations to this study; the first is the changes in plasma volume were measured indirectly using changes in plasma protein concentration according to the equations of Boyd (1981). This assumes that protein is not removed from or added to the vascular space during exercise which might not be the case (Lindinger et al. 2004; Masri et al. 1990). Although not ideal, this methodology has been used in a number of studies and changes in
plasma protein concentration appear to be a reasonable reflection of changes in plasma volume over the short term (Lindinger et al. 1995; McKeever et al. 1993). In this study, refractometry was used to measure plasma protein; quantitative plasma biochemistry analysis using a plasma chemistry analyzer would have been preferable and more accurate. In addition, both total protein and albumin concentrations could have been measured which might have provided more insight into fluid and protein shifts during exercise. The oxygen tension of blood samples collected into plastic syringes is significantly higher (by approximately 7 mm Hg) when compared to glass syringes although this difference is no longer significant after 10 minutes of storage (Deane et al. 2004). Further, blood samples stored on ice have slightly but significantly higher (by approximately 4 mm Hg) oxygen tensions than those stored at room temperature (Deane et al. 2004). Finally, in blood samples collected into plastic syringes and stored on ice, oxygen tensions increase (by approximately 4 mm Hg) between 10 and 60 minutes after collection (Deane et al. 2004). Thus, collection of blood samples into plastic syringes, storage on ice and a delay in blood gas analysis for greater than 10 minutes (as was the case in this study) could potentially obscure hypoxemia (Deane et al. 2004). However, all samples in the present study were handled in a similar fashion and significant hypoxemia was demonstrated in the second exercise protocol. The accurate measurement of water intake and the measurement of fecal and urinary water losses would have allowed better evaluation of subjects’ water balance and sweat fluid losses but was not performed in this study. The measurement of plasma sodium concentration, osmolality, vasopressin (anti-diuretic hormone), and aldosterone concentrations in these horses would have been of interest but was not performed.

In conclusion, our data demonstrate that pre-exercise expansion of plasma volume induced with NaCl administration to Thoroughbred horses neither adversely affects arterial O₂ tension, nor hemoglobin-O₂ saturation, during an exercise protocol simulating the 2nd day of a 3DE. Nor did significant pre-exercise hypervolemia exacerbate arterial hypoxemia or hemoglobin-O₂ de-saturation experienced by horses performing an exercise protocol incorporating a burst of maximal exercise known to induce EIAH. These findings contrast those of Sosa Leon et al. (2002), who reported that oral administration of a large volume of fluids pre-exercise caused significant arterial hypoxemia. The disparity in results likely reflects the difference in methods used to induce hyperhydration (and hypervolemia). In our opinion, it is unlikely that pulmonary edema was responsible for the impaired oxygenation observed by Sosa
Leon et al. (2002), but an increase in abdominal bulk could have contributed. Hemodilution in the hyperhydrated horses attenuated the exercise-induced arterial to mixed-venous blood O$_2$ content difference during exercise that was likely offset by an augmentation of cardiac output. Under laboratory conditions, we were unable to demonstrate any benefit of hyperhydration on thermoregulation. However, additional experiments in hot and/or humid conditions are required. Based on the observations that restoration of body water deficits can be protracted under field conditions and that hyperhydrated horses drank less water following exercise, further studies are warranted to determine whether hyperhydration might have some advantages for horses that must compete over successive days.
REFERENCES


APPENDIX A

The attached supplemental file contains the SAS printout of the raw data from exercise Protocol 1 simulating the second day of an equestrian 3-day event and is identical to the exercise test used by Sosa Leon et al. (2002).
APPENDIX B

The attached supplemental file contains the SAS printout of the raw data from exercise Protocol 2 simulating the second day of an equestrian 3-day event and incorporating bursts of maximal intensity exercise known to induce arterial hypoxemia.