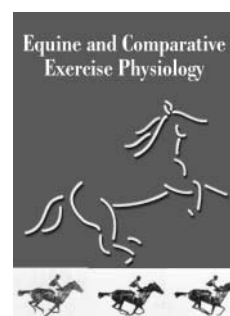


Time course and magnitude of changes in total body water, extracellular fluid volume, intracellular fluid volume and plasma volume during submaximal exercise and recovery in horses



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Abstract

The purpose of the present study was to determine the time course and magnitude of changes in extracellular and intracellular fluid volumes in relation to changes in total body water during prolonged submaximal exercise and recovery in horses. Seven horses were physically conditioned over a 2-month period and trained to trot on a treadmill. Total body water (TBW), extracellular fluid volume (ECFV) and plasma volume (PV) were measured at rest using indicator dilution techniques (D_2O , thiocyanate and Evans Blue, respectively). Changes in TBW were assessed from measures of body mass, and changes in PV and ECFV were calculated from changes in plasma protein concentration. Horses exercised by trotting on a treadmill for 75–120 min incurred a 4.2% decrease in TBW. During exercise, the entire decrease in TBW (mean \pm standard error: 12.8 ± 2.01 at end of exercise) could be attributed to the decrease in ECFV (12.0 ± 2.41 at end of exercise), such that there was no change in intracellular fluid volume (ICFV; 0.9 ± 2.41 at end of exercise). PV decreased from 22.0 ± 0.51 at rest to 19.8 ± 0.31 at end of exercise and remained depressed (18–19 l) during the first 2 h of recovery. Recovery of fluid volumes after exercise was slow, and characterized by a further transient loss of ECFV (first 30 min of recovery) and a sustained increase in ICFV (between 0.5 and 3.5 h of recovery). Recovery of fluid volumes was complete by 13 h post exercise. It is concluded that prolonged submaximal exercise in horses favours net loss of fluid from the extracellular fluid compartment.

Keywords: exercise dehydration; fluid shift; indicator dilution; hydration status; fluid recovery

Introduction

Prolonged exercise or exposure to heat results in a decrease of total body water (TBW) in mammals that sweat for the purposes of thermoregulatory cooling¹. The contributions of the extracellular fluid (ECF) and intracellular fluid (ICF) compartments to the TBW loss of exercise have not been determined in horses, and the literature is limited to a few studies in humans^{2–5} and rats⁶. This human and rat literature reports that the ICF compartment contributes 23–94% of the TBW loss, the remainder being borne by the ECF compartment, with the proportion dependent on the conditions of dehydration and species studied. These studies are lacking in time course data, and even less well studied

are the fluid volume responses that occur during recovery from exercise. There are also no studies that have directly measured ECF and ICF responses to exercise in horses. Therefore, the primary purpose of the present study was to determine the time course and magnitude of changes in TBW, ECF volume (ECFV) and ICF volume (ICFV) during prolonged, submaximal exercise and recovery in horses.

During exercise of the whole organism, the ICF compartment broadly consists of two sub-compartments, one within the contracting skeletal muscle cells and the second within all other cells including non-contracting skeletal muscle^{3,7}. Within contracting skeletal muscle there occurs a rapid increase in intracellular

osmolality, resulting in the net inward movement of ECF and cellular swelling^{3,8,9}; thus contracting skeletal muscle ICFV increases during exercise^{8,10,11}. Together with the net release of osmolytes from contracting muscles into the circulation, the fluid and ion shifts between vascular and contracting muscle compartments result in an increase in plasma osmolality. The increased plasma osmolality promotes a net loss of ICF from non-contracting muscle and other tissues that partially replaces fluid lost from the ECF compartment^{3,8,9}. When the exercise is maximal, this pattern of fluid shift continues until the cessation of exercise, and then is followed by gradual restoration of compartmental fluid volumes, with minor loss of total fluid due to sweating. However, when the exercise is submaximal and prolonged, after the initial ECF-to-ICF shift there is a partial return of fluid from contracting muscle back to the circulation as intracellular osmolality decreases and plasma osmolality increases⁸, resulting in a partial recovery of PV, ECFV and, by inference, contracting muscle ICFV during the period of steady-state exercise in both humans⁸ and horses^{12,13}. Therefore, when whole-body ICFV is determined, the change in ICFV represents the balance between the increases in ICFV within contracting skeletal muscle and decreases in ICFV within non-contracting tissues. After cessation of exercise, and after the initial rapid inter-compartmental fluid shifts have abated, the net changes in ICFV and ECFV from pre-exercise (dehydration) values are equivalent to the decrease in TBW due to sweating⁴.

Based on the results from previous studies, the present study tested the hypothesis that sweating-induced decreases in TBW during prolonged, submaximal exercise in horses will be nearly equally partitioned between the ECF and ICF compartments.

Methods

Animals

Seven horses from the University of Guelph herd were used in the study (Table 1). Horses were fed a diet consisting of grass/alfalfa hay (90%) and grain (10%) in the

morning and afternoon, with access to water at all times. Horses were kept in large grassy paddocks during the day and housed in individual stalls during the evening. The animal care and use procedures were approved by the University of Guelph Animal Care Committee and performed in accordance with the guidelines of the Canadian Council on Animal Care.

Before the study, the horses were conditioned for 12 weeks with a walking and trotting programme on five days per week, of which 2–3 days were spent performing on a treadmill (Equi-Tred International, Alexandria, NSW, Australia) and 2–3 days in an outdoor exerciser (Odyssey Performance Trainer, Campbellville, ON, Canada). The duration and intensity of exercise were increased gradually until the horses were able to trot comfortably on the treadmill for at least 25 min at 3.5 m s⁻¹, 2–3° incline.

Horses were fed in the afternoon 16 h prior to the start of the experiment, which began the morning of the following day; horses had access to water until the start of the experiment. No food or water was provided during the exercise phase of the experiment. Body mass was measured with a large-animal scale (0.5 kg KSL Scales, Kitchener, ON, Canada).

The hair coat over the jugular vein, 10–20 cm below the mandible, was clipped short to the skin on both sides of the neck. Each jugular vein catheterization site was aseptically prepared for insertion of catheters. A topical analgesic, EMLA cream (2.5% lidocaine and 2.5% prilocaine; Astra Pharma, Mississauga, ON, Canada), was applied 30–45 min before insertion of catheters to desensitize the skin. Local anaesthetic (2% Xylocaine; Astra Pharma) was injected subcutaneously to complete the analgesia. Catheters (14-gauge, 5.25 in; Angiocath, Becton-Dickinson, Mississauga, ON, Canada) were inserted antegrade into the left and right veins, and secured with tape and stitches to the skin. Four-way stopcocks with 20 in extensions were attached to the catheters for ease of infusion and blood sampling. Patency of the catheters was maintained with sterile, heparinized 0.9% NaCl (2000 IU l⁻¹ NaCl).

Table 1 Characteristics of horses used in the study

Horse	Breed	Age (years)	Body mass (kg)	TBW (l)	ECFV (l)	ICFV (l)	PV (l)
1	Thoroughbred	12	472.1	316.3	109.8	206.5	24.2
2	Percheron cross	4	513.1	294.5	128.9	165.6	21.7
3	Standardbred	12	511.4	326.6	135.1	191.5	22.4
4	Standardbred	13	443.2	288.5	110.0	178.4	22.2
5	Appaloosa	6	501.0	331.9	104.4	227.4	20.6
6	Standardbred	14	492.1	326.4	130.8	195.6	22.2
7	Standardbred	10	438.9	301.1	112.3	188.7	20.8
Mean		10	481.7	312.2	118.8	193.4	22.0
SE		1.4	11.7	6.6	4.7	7.5	0.5

TBW – total body water; ECFV – extracellular fluid volume; ICFV – intracellular fluid volume; PV – plasma volume; SE – standard error.

Indicator infusion and sampling

Fluid volumes were measured using indicator dilution techniques as described previously¹⁴. Deuterium oxide (Sigma Chemical Co., St. Louis, MO), for measuring TBW, was infused as a 50% v/v solution with 0.9% sterile saline for a total volume of ~50 ml per horse. Sodium thiocyanate, for measuring ECFV, was infused at 3.88 mg kg⁻¹ body mass as a 5% w/v solution in 0.9% sterile saline for a total volume of ~60 ml. Evans Blue (Fisher Scientific, Nepean, ON, Canada), for measuring PV, was administered at 0.10 mg kg⁻¹ body mass as a 5% w/v solution with sterile 0.9% saline.

The prepared indicator solutions were injected into the right jugular vein catheter in sequence (Evans Blue, 10 ml; D₂O, 50 ml; NaSCN, 50 ml) over a period of 4–5 min; blood sampling occurred from the left jugular vein. Sterility of the infusates was ensured by using a non-pyrogenic, sterile, 0.22 μm nylon filter (Millex-Ap/GS Filter; Millipore SA, Mosheim, France) placed between the syringe and extension tubing. Immediately following the final infusion the catheter tubing was flushed with 50 ml of sterile 0.9% saline.

Exercise and recovery

The purpose of the exercise was to induce a 3–5% loss of TBW due to sweating. Horses were exercised on the treadmill at an exercise intensity that maintained heart rate at or below a predetermined value based on each horse's training protocols during the previous week. Horses trotted on the treadmill at an average speed of 3 m s⁻¹ at a 1.5° incline for 60 min, and rested for 20 min while measurements and blood samples were obtained. The horses then performed a second exercise period (30 to 60 min) until each could no longer keep pace with the treadmill.

The first horse in the study was provided with an electrolyte solution (28 g l⁻¹ of Perform'N Win (PNW); Buckeye Nutrition, Dalton, OH) 30 min after completion of the second exercise period. During the initial 3 h after exercise the horse was repeatedly offered water but did not drink. At 4 h, the horse was given 5 kg of wetted hay, which was consumed together with 391 of PNW between 4 and 18 h of recovery. The second horse showed a similar pattern to the first, but was given wetted hay 3 h post exercise and subsequently drank 34.81 of PNW between 4 and 18 h of recovery. Both these horses did not drink until they had eaten. As a result of the slow recovery of the first two horses, the remaining five horses were provided with 5 kg wetted hay and PNW solution 30 min after exercise, and these horses started to eat and drink within 10 min.

Blood analysis

Blood was sampled from the left catheter using 7.5 ml lithium-heparin syringes (Monovette-Sarstedt, Sarstedt,

Germany) before infusion of indicators and 10, 20, 30, 45, 60, 90 and 120 min after infusion to determine TBW, ECFV and PV at rest. Blood samples were taken at 15, 30, 45 and 60 min during exercise, at 15 min during the 20 min rest between exercise sessions, and at 20, 40, 60, 90, 120, 180, 240 and 1060 min of post-exercise recovery.

Each blood sample was immediately transferred into five 1.5 ml Eppendorf centrifuge tubes. Four of these were centrifuged for 5 min at 15 000 g to separate the plasma, which was then transferred into Eppendorf centrifuge tubes and stored at –20°C until analysed for Evans Blue and NaSCN. For D₂O analysis, approximately 1.5 ml of plasma was stored in 1.8 ml screw-cap cryovials at –80°C until analysed for D₂O. Plasma protein concentration was determined (coefficient of variation (CV) 0.83%) by using refractometry (clinical refractometer model SPR-T2; Atago, Tokyo, Japan). Plasma was analysed for Evans Blue concentration (CV 1.3%) via the dual-wavelength method¹⁵ by use of a spectrophotometer (DU-70; Beckman, Mississauga, ON, Canada). Plasma NaSCN concentration was measured (CV 2.1%) spectrophotometrically by a microvolume modification¹⁴ of the method described by Chatterjee *et al.*¹⁶. Analysis of plasma D₂O concentration was performed (CV 1.1%) by Metabolic Solutions (Nashua, NH).

Calculations

TBW was calculated from plasma D₂O concentrations using equations (1) to (3) provided by Metabolic Solutions (Nashua, NH):

$$\text{TBW (mol)} = (WA/18.02a) \times [(\delta_{\text{dose}} - \delta_{\text{tap}})/(\delta_{\text{post}} - \delta_{\text{pre}})], \quad (1)$$

where *W* is grams of water used to dilute the dose, *A* is grams of dose administered to the subject, *a* is grams of dose diluted for analysis, δ_{pre} and δ_{post} are the delta deuterium values determined for the pre-dose and post-dose samples, δ_{dose} is the measured deuterium content of the diluted dose, and δ_{tap} is the measured deuterium content of local (tap) water. To convert TBW to kilograms:

$$\text{TBW (kg)} = \text{TBW (mol)} \times \frac{18.02 \text{ g mol}^{-1}}{1000 \text{ g kg}^{-1}}. \quad (2)$$

The D₂O dilution technique overestimates TBW by 4% because of binding of deuterium to acidic amino acids and other non-exchangeable sites. To correct for the binding of D₂O to non-exchangeable sites, the corrected TBW (TBW_{MS}) was obtained by dividing TBW

from equation (2) by 1.04:

$$TBW_{MS} = TBW/1.04. \quad (3)$$

Percentage changes in PV were calculated from plasma [protein] as:

$$\Delta PV (\%) = (P_0 - P_t)/P_t \times 100, \quad (4)$$

where P_0 and P_t are plasma [protein] at time 0 and time t , respectively.

Statistics

Data are presented as mean \pm standard error. Changes over time were assessed by one-way repeated-measures analysis of variance. When a significant F -ratio was obtained, means were compared using the all pairwise multiple comparison procedure of Holm-Sidak. Statistical significance was accepted when $P \leq 0.05$ at a power of 0.8.

Results

The general characteristics and fluid volumes of the horses at rest before exercise are given in Table 1. Total body water was $64.9 \pm 1.4\%$ of body mass, while ECFV and ICFV were $38.1 \pm 1.6\%$ and $61.9 \pm 1.6\%$ of TBW, respectively.

While all horses easily completed the initial 60 min exercise bout, two horses completed 60 min for the second exercise bout and the other five horses completed 15–25 min. Exercise resulted in a progressive decrease in PV and ECFV (Fig. 1), beginning with the 10 min walking warm-up. The rest break at the end of the first 60 min exercise bout resulted in increases in PV and ECFV; however, when the second exercise bout started, the time course of decrease in PV and ECFV continued until the end of exercise. Similar to the end of the first exercise bout, PV and ECFV were increased 15 min after the end of the second exercise bout. After this, however, both PV and ECFV decreased further ($\sim 18\%$ decrease from pre-exercise) during the first hour of recovery. There was no significant increase in PV and ECFV during the first 4 h of recovery; thereafter there was a gradual restoration of these parameters by 13 h post exercise.

The time course for TBW was calculated from measurements of body mass obtained when the horse was not on the treadmill. Therefore the frequency of measures for TBW and ICFV, calculated from TBW and ECFV (Fig. 2), is less frequent than for PV and ECFV (Fig. 1). TBW decreased by 13.1 ± 1.81 ($4.2 \pm 0.5\%$) during the entire exercise period (Fig. 2a). This entire decrease in TBW was due to loss of volume from the ECFV (Fig. 2b) such that there was no change in ICFV during exercise

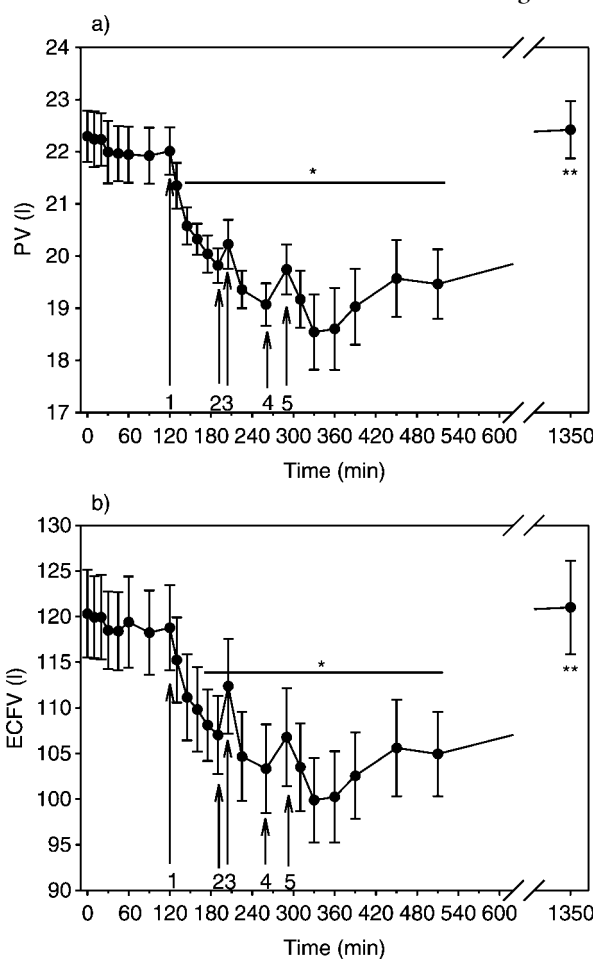


FIG. 1 The time course of (a) plasma volume (PV) and (b) extracellular fluid volume (ECFV) during exercise and recovery. The first 120 min represent the period of indicator dilution within the horses. Values are mean \pm SE for seven horses. Arrows: 1 indicates the start of the 10 min walking warm-up, followed by 60 min of trotting exercise; 2 indicates the end of the first 60 min period of trotting; 3 indicates the 15 min resting sample; 4 represents the end of the second period of trotting exercise; and 5 is the first 20 min post-exercise recovery sample. *Significantly different from the 120 min (pre-exercise) time point; **significantly different from the 270 min (post-exercise) time point

(Fig. 2c). Within 30 min after exercise TBW started to increase (5.5 h time point, 1.5 h of recovery), however ECFV continued to decrease. Taken together, these results translate to an increase in ICFV, which remained elevated for the first 4 h of recovery. After the first 1.5 h of recovery, both TBW and ECFV increased slowly towards pre-exercise values.

The proportion of TBW that was extracellular decreased by $5.1 \pm 0.5\%$ during exercise and the first 30 min of recovery (Fig. 3a). In contrast, ICFV increased from $61.9 \pm 1.6\%$ to $67.0 \pm 1.6\%$ of TBW during the same period (Fig. 3b). The proportionate increase in ECFV and decrease in ICFV persisted during the first 4 h of recovery, and were normalized by 13 h of recovery.

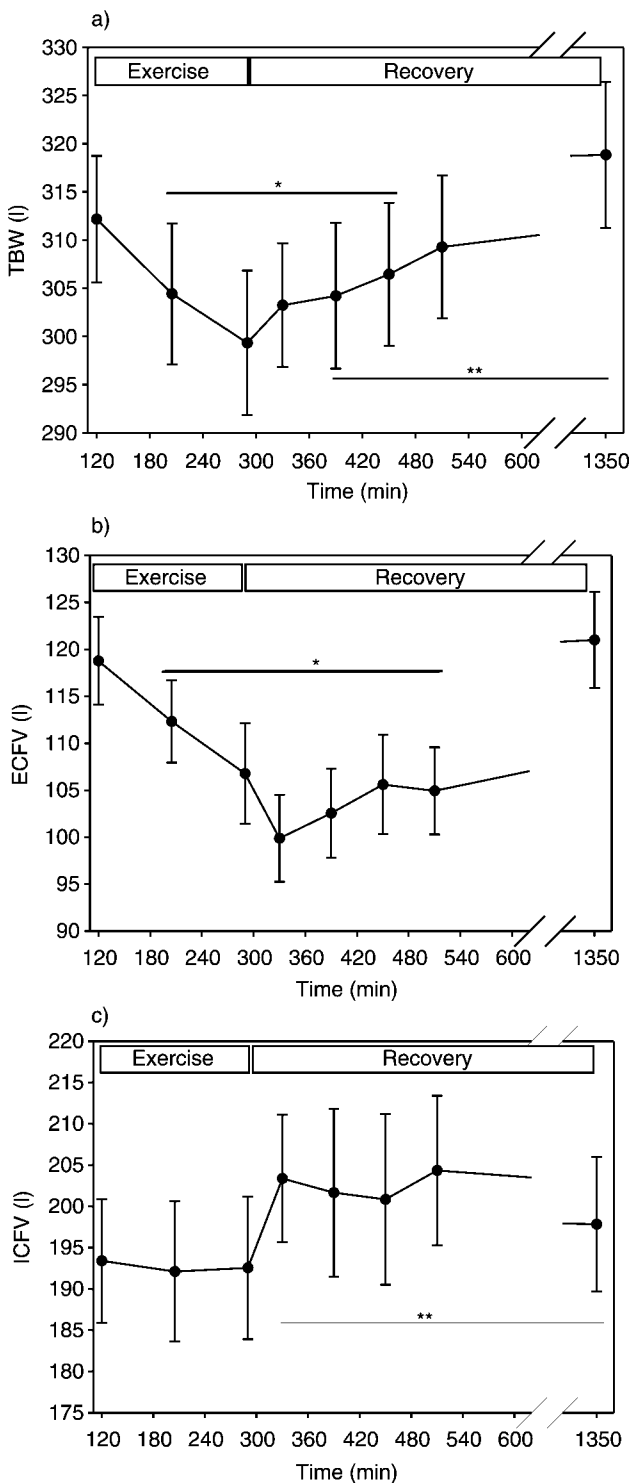


FIG. 2 The time course of (a) total body water (TBW), (b) extracellular fluid volume (ECFV) and (c) intracellular fluid volume (ICFV) during exercise and recovery. Values are mean \pm standard error for seven horses. *Significantly different from the 120 min (pre-exercise) time point; **significantly different from the 290 min (post-exercise) time point

During the period of exercise, the decrease in ECFV (12.0 ± 2.41 at end of exercise) was statistically similar to the decrease in TBW (12.8 ± 2.01 at end of exercise). During the first 30 min of recovery, however,

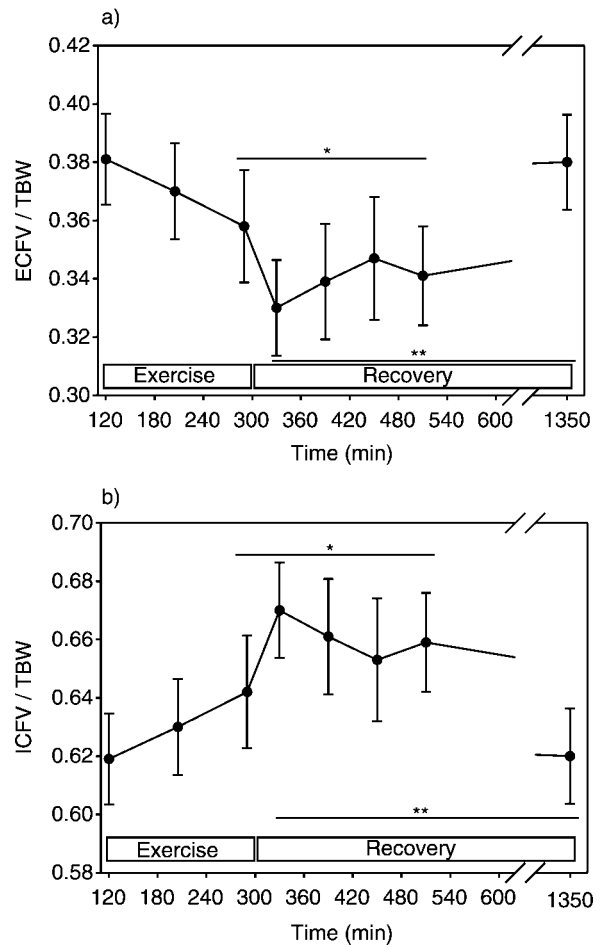


FIG. 3 The time course of (a) the ratio of extracellular fluid volume to total body water (ECFV/TBW) and (b) the ratio of intracellular fluid volume to total body water (ICFV/TBW) during exercise and recovery. Values are mean \pm standard error for seven horses. *Significantly different from the 120 min (pre-exercise) time point; **significantly different from the 290 min (post-exercise) time point

TBW increased while ECFV continued to decrease (Fig. 3). Over the course of the entire experiment, the change in TBW was significantly correlated to the changes in ECFV and to the changes in ICFV (Fig. 4).

Plasma protein concentration, used to calculate the changes in PV and ECFV, increased significantly more than the plasma concentrations of the indicators for PV (Evans Blue) and ECFV (thiocyanate) during exercise (Fig. 5). Extrapolation of the initial 120 min pre-exercise curve into the exercise period shows only a very small increase in measured Evans Blue concentration, and negligible deviation of the measured thiocyanate curve from the extrapolated.

Discussion

The present study appears to be the first to have measured and detailed the time course of changes in

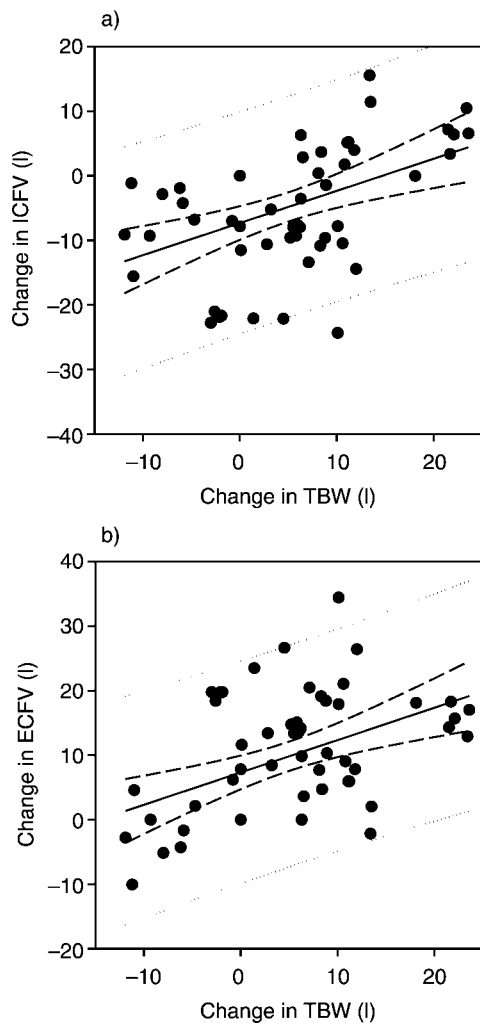


FIG. 4 Linear regression between the change (Δ) in total body water (TBW) and the change in (a) intracellular fluid volume (ICFV) and (b) extracellular fluid volume (ECFV) including all time points within the experiment for seven horses. $\Delta\text{ICFV} = -7.32 \pm 1.30 + (0.498 \pm 0.131 \times \Delta\text{TBW})$; $R^2 = 0.212$, $P < 0.001$. $\Delta\text{ECFV} = 7.32 \pm 1.30 + (0.502 \pm 0.131 \times \Delta\text{TBW})$; $R^2 = 0.214$, $P < 0.001$. Dashed lines represent $+1$ and -1 standard deviation; dotted lines represent 95% confidence interval

multiple body fluid compartments during exercise and recovery in horses. By design, the submaximal exercise bouts decreased TBW by 4–5%. It is noteworthy that, during submaximal exercise, ICFV was completely maintained at pre-exercise values, but this occurred at the expense of volume depletion of the ECF compartment. An important practical outcome from the study relates to the very slow recovery of fluid volumes after completion of exercise. Full recovery of fluid volumes required several hours of rest with food and electrolyte solution.

Limitations

The volumes of the body fluid compartments in the horses at rest, prior to exercise, were similar to

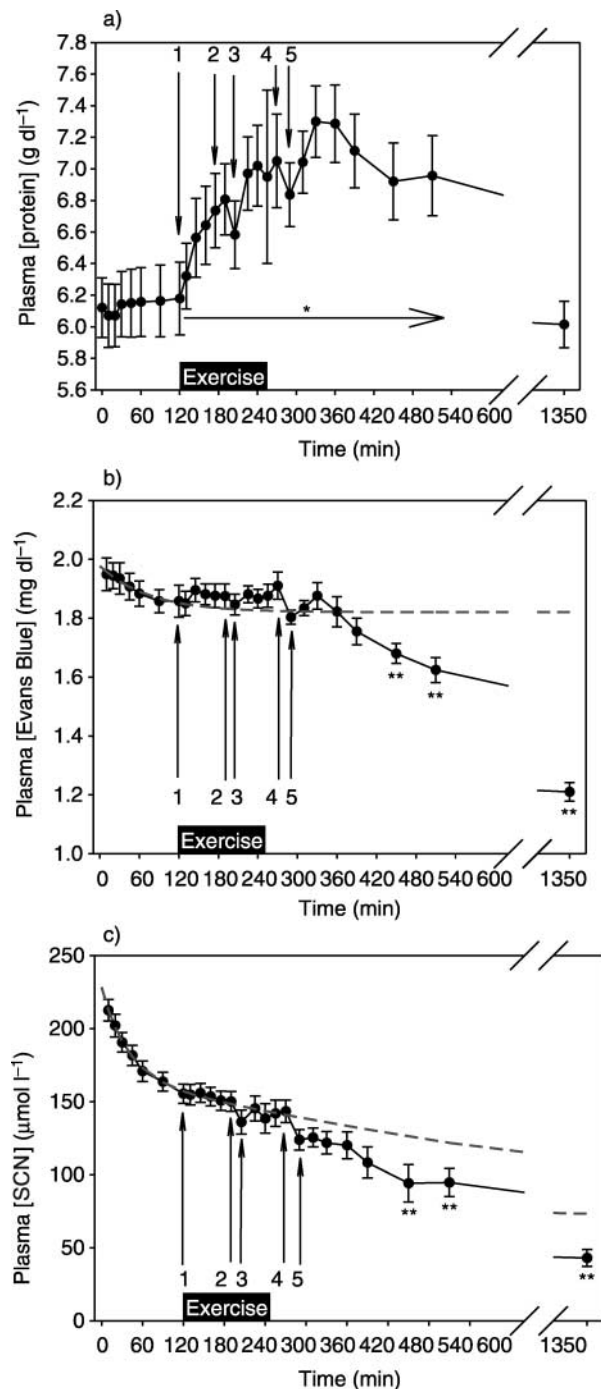


FIG. 5 The time course of plasma (a) protein concentration, (b) Evans Blue concentration and (c) thiocyanate (SCN) concentration in horses before, during and after exercise. The dashed lines in (b) and (c) show the concentrations extrapolated from the pre-exercise (0–120 min) period. Values are mean \pm standard error for seven horses. Arrows: 1 indicates the start of the 10 min walking warm-up, followed by 60 min of trotting exercise; 2 indicates the end of the first 60 min period of trotting; 3 indicates the 15 min resting sample; 4 represents the end of the second period of trotting exercise; and 5 is the first 20 min post-exercise recovery sample. *Significantly different from the 120 min (pre-exercise) time point; **significantly different from the corresponding time point on the extrapolated curve

those reported by others and us (see Forro *et al.*¹⁴ for a literature summary). The decrease in TBW with exercise occurred as a result of sweating, with the non-faecal body mass decrease during the exercise period equating to a mean sweating rate of $8.7 \pm 1.6 \text{ l h}^{-1}$, similar to those reported previously for submaximal exercise^{17,18}. The contribution of water within the gastrointestinal (GI) tract, which may contribute 15–20% of total body water¹⁷, to the changes in ECFV could not be quantified in the present study. It has been reported, but not confirmed, that the water content of the GI tract can decrease by 25% during 1 h of submaximal exercise in ponies¹⁷. Thus in the present study it is possible that all of the decrease in TBW and ECFV occurred from the GI tract. This, however, is unlikely because the decreases in PV, and by inference the entire non-GI tract ECFV in the present and Coenen's study, were appreciable. Coenen¹⁷ noted that a limitation of both the Evans Blue and the thiocyanate techniques for assessing PV and ECFV in response to exercise was an apparent absence of increase in their plasma concentrations; we also saw no increase in the plasma concentrations of these markers during exercise. These two markers cannot be used to assess changes during the exercise period because their non-specific binding to substrate within the body appears to be accelerated during periods of increased cardiovascular activity.

A key assumption in calculating the change in PV and ECFV using plasma protein concentration is that there is no net gain or loss of protein by the vascular compartment during exercise and recovery. However, there is mounting, indirect evidence that a net gain of protein by the vascular compartment may occur during exercise and the initial recovery period^{19,20}. Similar indirect evidence is also found in the present study, where the increases in plasma Evans Blue and thiocyanate concentrations during exercise were markedly reduced or negligible compared with the increase in plasma protein concentration (Fig. 5). If there was no net gain of protein, then there would have had to have been increased rates of loss of Evans Blue and thiocyanate from the vascular compartment. Results from the present study cannot be used to support or refute the assumption; this will require the use of an inert vascular marker that is of sufficient molecular size as to not permeate the vascular endothelium. If the assumption is incorrect and there is net gain of protein from the lymphatic system, as has been suggested²¹, then exercise-induced decreases in PV and ECFV have been overestimated by an amount proportionate to the protein gain.

Compartmental fluid shifts during exercise

Based on the results of human^{2,3,5} and rat⁶ studies, we hypothesized that the exercise-induced decrease in

TBW would be partitioned nearly equally between both ICF and ECF compartments. Depending on the type of heat and exercise dehydration imposed, in humans 23–94% of the decrease in TBW arose from ICF losses^{2,4,5}. ICF fluid losses in rats were approximately 50% of the decrease in TBW⁶. The present study contrasts with these studies in that exercise did not result in any decrease in the absolute ICFV, such that ICFV as a proportion of TBW actually increased. Therefore, the entire decrease in TBW, during the period of exercise, was manifested as a decrease in ECFV. Only during the first 30 min of recovery was there a discrepancy between changes in TBW and ECFV, where ECFV continued to decrease while TBW did not (Fig. 2). Possible reasons for the maintenance of ICFV in exercising horses include increased working muscle mass as a percentage of total body mass, increased intracellular osmotic forces preventing intracellular volume loss, and a sweating rate and composition that minimize exercise-induced increases in extracellular osmolality.

With prolonged, submaximal exercise, humans and horses secrete considerable amounts of sweat onto the skin for thermoregulatory cooling, resulting in decreases in TBW^{1,22,23}. Costill²⁴ proposed the association between sweat losses of water and ions and losses of water and ions from the plasma compartment. Compared with horses, humans produce a dilute sweat that fosters an increased ECF osmolality, whereas equine sweat is isotonic or slightly hypertonic such that sweat production does not contribute to increases in ECF osmolality^{1,23}. In humans, therefore, ECF osmolality is raised by the combined effects of osmolyte release from contracting skeletal muscle and net water loss to the surface of the skin. In contrast, with horses, ECF osmolality is raised primarily by osmolyte release from contracting muscle because the sweat losses of Na^+ , K^+ and Cl^- are so large^{17,18}. Perhaps because horses have a larger proportion of contracting muscle mass per litre of vascular volume than humans performing leg exercise, increases in plasma osmolality during submaximal exercise are similar in both species^{25,26}. This evidence suggests that the net osmotic driving force for net fluid loss from non-contracting tissues into the ECF should be similar in humans and horses.

An increased mass of contracting skeletal muscle, expressed as a proportion of total body mass in horses, compared with humans performing leg-only exercise may explain, at least in part, the apparent ability of horses to maintain ICFV during locomotory exercise. In humans performing two-legged exercise, less than 20% of body mass consists of contracting skeletal muscle²⁷. In contrast, in the exercising horse, ~40% of body mass may comprise contracting skeletal muscle²⁸, a mass that may exceed that of

non-contracting tissues. The contracting muscles swell during the period of exercise, due to the increase in intracellular osmolality. Since there is a greatly reduced proportion of non-contracting tissue from which to lose fluid, compared with humans, the net effect is loss of fluid from non-contracting tissues that is similar to the fluid gain by contracting muscle. Thus, in horses, the decrease in ECFV is similar to the decrease in TBW, with no change in ICFV, during the period of exercise (Fig. 2). It would be interesting to determine if this scenario of fluid responses seen in horses during submaximal exercise would be observed in humans performing prolonged exercise requiring a greater proportion of contracting muscle, such as rowing.

Recovery from exercise

Because of the duration of the exercise and recovery periods, it was important to provide food and water after exercise – this also mimics the situation encountered in the field. There was minimal eating and drinking during the first 90 min of recovery and thus this period represents a true exercise recovery response. Thereafter, the responses increasingly reflect the contributions of ingested food, water and electrolytes.

The rapidity and magnitude of decrease in PV and ECFV during exercise are similar to those seen previously^{12,13}. What is noteworthy is the rapid, transient recovery of PV and ECFV soon after cessation of exercise (arrows 3 and 5 in Fig. 1). Within 15 min of cessation of the first 60 min period of trotting, PV increased by 0.5 l and ECFV by 5 l. The rapidity and magnitude of these changes are consistent with a return of fluid from contracting skeletal muscle to the ECF compartment, concomitant with a decrease in intracellular osmolality and contracting muscle ICFV. The resumption of exercise (between arrows 3 and 4, Fig. 1) appears to have rapidly moved this fluid back into contracting skeletal muscle. Cessation of the second exercise bout also produced increases in PV and ECFV of similar magnitude to those seen at the end of the first exercise bout. However, despite no further exercise, PV and ECFV again started to decrease despite the cessation of noticeable sweating 15–20 min after exercise. As before, the transient increase soon after exercise stopped can be attributed to fluid shift from contracting skeletal muscle back into the ECF compartment. Over the next hour, decreases in PV and ECFV must represent a net shift of fluid into the ICF compartment (Fig. 2c) because there was no further decrease in TBW during this period (Fig. 2a). The only viable explanation for a simultaneous decrease in ECFV and increase in ICFV during this period of exercise recovery is an increase in intracellular osmolality and/or decrease in extracellular osmolality. While the mechanism(s) responsible for these responses remain(s) to be determined, they may be attributed

to an increase in metabolic and ionic osmolytes (glycogen, potassium) within recovering skeletal muscle, to an increase in cellular $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ co-transporter activity in order to restore volume of non-contracting tissues²⁹, and to an increase in cellular $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ co-transporter activity in order to restore intracellular $[\text{K}^+]$ of recovering skeletal muscle^{9,30}.

The recovery of the fluid compartment volumes was remarkably slow. This reflects both the slow rate of ingestion of food and electrolyte solution after exercise and the fact that these horses were not trained to drink to restore volume after periods of exercise. When water and electrolytes are provided by nasogastric intubation immediately before or after exercise, we have seen that these are rapidly (within 10–40 min of administration) taken up by the GI tract into the ECF compartment (Ecker GL, Lindinger MI, unpublished). Perhaps the provision and ingestion of hay (although wetted) prevented a more rapid intestinal absorption of water because the dryness of the hay may have generated a net fluid shift from the ECF compartments into the GI tract³¹. The slow post-exercise recovery of fluid volumes observed in the present study may have direct application to equine endurance racing. Once TBW and fluid volumes have become depleted, it may be very difficult to restore or maintain an adequate state of hydration to be able to continue the exercise at a high or desired level. It is known that those horses that have maintained a good state of hydration perform well over distances of 50–100 miles, often finishing in the top ten³².

Summary and conclusions

Prolonged, submaximal exercise in horses resulted in a 4% decrease in TBW that was borne entirely by the ECF compartment, while whole-body ICFV was maintained. It is likely that ICFV of contracting skeletal muscle was elevated during exercise, while that of non-contracting tissues was reduced. The initial recovery from exercise was accompanied by small, transient increases in PV and ECFV. A further decrease in ECFV during the first 90 min post exercise was accompanied by an increase in ICFV; this probably reflected restoration of ICFV of non-contracting tissues and an increased ECFV of recovering skeletal muscle concomitant with glycogen resynthesis and restoration of intracellular $[\text{K}^+]$. Recovery of fluid volumes required greater than 4 h and was complete within 13 h when food, water and electrolytes were provided for voluntary ingestion.

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