Rate and composition of sweat fluid losses are unaltered by hypohydration during prolonged exercise in horses

JANENE K. KINGSTON,1 RAYMOND J. GEOR,1 AND LAURA JILL McCUTCHEON2

Departments of 1Clinical Studies and 2Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Kingston, Janene K., Raymond J. Geor, and Laura Jill McCutcheon. Rate and composition of sweat fluid losses are unaltered by hypohydration during prolonged exercise in horses. J. Appl. Physiol. 83(4): 1133–1143, 1997.—Rate and ionic composition of sweat fluid losses and partitioning of evaporative heat loss into respiratory and cutaneous components were determined in six horses during three 15-km phases of exercise at ∼40% of maximal oxygen uptake. Pattern of change in sweat rate (SR) and composition was similar during each phase. SR increased rapidly for the first 20 min of exercise but remained at ∼24–28 ml·m⁻²·min⁻¹ during the remainder of each phase. Similarly, the concentrations of Na and Cl in sweat increased until 30 min of exercise but were unchanged thereafter. Sweat osmolality and concentrations of Na and Cl were positively correlated with SR. Sweat K concentration decreased during exercise but was not correlated with SR. Fluid losses were 33.8 ± 1.5 liters, resulting in decreases of ∼21% in plasma volume and ∼11% in total body water. The ∼6% hypohydration was not associated with an alteration in SR, sweat composition, or heat storage. Respiratory and cutaneous evaporative heat loss represented ∼23 and 70%, respectively, of the total heat dissipated, and the partitioning of heat loss was similar in each exercise phase. We conclude that SR and the relative proportions of respiratory and cutaneous evaporative heat loss are unchanged in horses during prolonged low-intensity exercise despite moderate hypohydration.

IN HORSES, as in human subjects, sweating is the principal means of thermoregulation during exercise. However, during exercise in moderate environmental conditions, sweating rates (SR) in the horse (expressed per unit area of skin) have been reported to be more than threefold greater than values in heat-acclimatized human subjects exercising at similar work intensities (11, 28). Whereas cutaneous evaporation represents the primary mechanism for heat dissipation in the horse, respiratory heat loss (RHL) can also contribute substantially. In human subjects, most exercise studies of heat production and dissipation that have partitioned these two major components of evaporative heat loss have used a figure of 10% to account for respiratory losses. Estimates of RHL in horses during low-intensity exercise have ranged from 10 to 30% of the metabolic heat produced (10, 11). This range probably reflects different methods used to estimate each component of heat loss and demonstrates the need for an accurate measurement of total body fluid loss when cutaneous and respiratory evaporative losses are partitioned.

Recently, field investigations that included calculation of body water losses in horses competing in 48- to 163-km endurance events indicated that the majority of the losses occurred during the first half of the event (7, 15). These findings suggest that an alteration in the rate of sweat fluid loss occurs during prolonged exercise. One might speculate that mechanisms for body fluid conservation would result in a decrease in the rate of sweat fluid loss. For instance, in hypohydrated human subjects, there is an increase in the threshold body temperature for onset of sweating and a decrease in sensitivity of the sweating response in direct proportion to the degree of hypohydration (26, 32), suggesting the existence of mechanisms linked to sweating responses that are directed at conservation of body fluid. To date, reports of results from field investigations have not included direct measurements of the rate of sweat fluid and ion losses in the horse. Furthermore, the effect of progressive hypohydration on sweating rate (SR) and on other thermoregulatory responses of horses during prolonged exercise has not been reported.

The objectives of this study were 1) to determine the rate and ionic composition of sweat fluid losses in horses during >3 h of low-intensity exercise in moderate environmental conditions and 2) to partition evaporative heat loss into its respiratory and cutaneous components. We hypothesized that the progressive hypohydration associated with prolonged exercise would evoke a decrease in the rate of sweat fluid loss, thereby conserving fluid and ions. For the purposes of this study, we measured 1) local SR on the lateral thorax; 2) sweat composition; 3) rectal, muscle, and pulmonary artery blood temperatures (T Re, T Mu, and T Pa, respectively) for determination of heat storage; 4) body mass before and after exercise as a basis for measuring total fluid loss; and 5) hematocrit and plasma total protein and osmolality. From measurements of cutaneous evaporative heat loss and changes in body mass (loss of body water), we estimated the partitioning of evaporative heat loss into its respiratory and cutaneous components.

MATERIALS AND METHODS

The care and use of animals followed the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, Ottawa, ON, Canada). All animal experiments were conducted after approval by the Animal Care Committee of the University of Guelph and were performed in compliance with their recommendations.

Experimental animals. Six Thoroughbred horses (3 mares, 3 geldings), ranging in age from 3.5 to 7 yr and weighing 418–501 kg [461 ± 7.5 (SE) kg], were studied. The horses were maintained on a diet that consisted of grain, supplemented with 100 g NaCl and 50 g KCl daily, and mixed timothy grass/alfalfa hay. The diet was designed to meet the National Research Council guidelines for the nutritional requirements of horses.
requirements of horses performing regular exercise and training. The horses were conditioned on a high-speed treadmill (Sato, Sweden) with a program of regular walking and trotting for a minimum of 10 wk before the experiments. Initially, horses were exercised on the treadmill 5 days/wk at 1.8 m/s (walk) for 5 min and 4 m/s (trot) for 15 min. Exercise time at 4 m/s was progressively increased, with the horses covering 8 km at this intensity 5 days/wk by the 4th wk. By the 10th wk, the horses had covered a 30-km distance at least once before the commencement of the experiments. The horses were also trained to drink cool water (−15−20°C) at rest stops during the exercise period. The maximal O2 uptake (VO2max) of each horse was determined by use of an open circuit calorimeter (8) on two occasions after training and before the experiments began. The mean VO2max was 146 ± 6.5 ml·kg⁻¹·min⁻¹.

Experimental protocol and measurements. Before the experiments, food and water were withheld for 2 h. Clinical assessment of hydration status, including measurement of hematocrit and plasma refractive index, indicated that the horses were euhydrated immediately before the commencement of the experimental protocol. A cardiotachometer (Equistat model HR-8A; Equine Biomechanics and Exercise Physiology, Unionville, PA) was applied on the horse's chest to record heart rate (HR). With the treadmill set at a 3° slope, horses exercised at 40% of their predetermined VO2max (3.6–3.8 m/s) for 45 km, with a 15-min rest after each 15-km phase. As a result of the similarity in treadmill speed required for all subjects, each phase of exercise consisted of ~67 min. Exercise was discontinued if the horses demonstrated signs of fatigue (inability to keep pace with the treadmill despite verbal encouragement) or inadequate recovery (HR > 70 beats/minute after 10 min) during the rest phases.

Each experiment consisted of two parts; the same experimental subjects completed identical bouts of exercise with at least 7 days between exercise trials. Duplicate runs were necessary because of the extensive instrumentation required to collect the data from horses during each experiment. During the first part of the experiment (experiment 1), data for measurement of O2 consumption (VO2), change in body mass, change in total body water, and expired air temperature (Tre) and humidity for estimation of RHL were collected. During the second part of the experiment (experiment 2), measurements were made of temperature at selected sites, SR, sweat osmolality, and sweat concentrations of sodium, chloride, and potassium ([Na⁺], [Cl⁻], and [K⁺], respectively). VO2 was measured at 10-min intervals throughout exercise by using an open-flow respiratory-gas collection system (8), and body weight was measured at the end of each phase of exercise. Tre was measured during exercise with a copper/constantan thermocouple made from 0.076-mm-diameter insulated wire (TW40; Physitemp, Clifton, NJ) that was positioned in the false nostril of the horse (40).

Preliminary experiments indicated that the extent of fluid losses incurred during the exercise protocol would result in a degree of hyohydration (deficit in total body water) that could preclude the horse's ability to complete the test. To simulate the opportunity for rehydration available for horses competing in trail and endurance rides and to limit the degree of hyohydration to ~6%, the horses were offered water and feed during each rest phase. The quantity of feed and water consumed was recorded.

The experiments were conducted in an air-conditioned laboratory, with the temperature and relative humidity maintained at 24 ± 2°C and 55 ± 8%, respectively. A fan mounted above and 0.5 m in front of the treadmill was used to maintain an air velocity of 3.5–4 m/s over the anterior and dorsal aspects of the horse. Air velocity was measured with anemometers (Davis Instruments, Hayward, CA) positioned at three sites: lateral midcervical region, lateral and dorsal thorax, and dorsal to the gluteal region of the hindquarters.

Sweat was collected from an area of skin on the lateral thorax by a method previously described for use in the horse (21). This area was chosen after determination of SR at several sites (midcervical, lateral thorax, gluteal region of hindquarters). Although there are regional variations in SR in the horse, previous studies have demonstrated that the SR measured on the lateral thorax is not significantly different from the mean whole body SR estimated from changes in total body water after correction for respiratory water losses (14, 19). A 500-cm² area on the lateral thorax was clipped and shaved, washed, and then rinsed with distilled water. A sealed polyethylene pouch enclosing a 150-cm² area of skin was attached to the skin on all edges with an adhesive. The edges of the pouch were further sealed by dermal tape that covered the pouch/skin margin. A ventral reservoir, formed by a deep fold in the polyethylene, separated accumulating sweat from the skin surface and facilitated the removal of all collected sweat through polyethylene tubing (1.67-mm ID, Intramedic; Becton Dickinson, Parsippany, NJ) incorporated into the lateral margin of the pouch. Sweat samples were collected every 10 min throughout each phase of exercise and at 5 and 15 min in each rest phase. SR, expressed as milliliters per square meter per minute, was calculated on the basis of the volume of sweat collected at the end of each time interval from the measured skin area within the pouch. Therefore, the measured SR represents the average rate of sweat production over a 10-min period. Extrapolation of the local SR, at each time point during exercise and the rest phases, to the horse's total body surface area (SA) was used to calculate a mean whole body SR. SA was calculated by using the formula (11)

\[
SA = 1.090.008 \times \text{body mass (kg)}
\]

Whole body SR, averaged over the duration of each phase of exercise, was also calculated from the total body water losses after correction for estimated respiratory water losses. Horses were weighed before and immediately after exercise (~0.5 kg, KSL Scales, Kitchener, ON). Preexercise total body water was assumed to be 66 ml/kg body weight (4). The change in total body water was calculated from the change in body mass after accounting for food and water intake and including fecal and urinary water losses. Fecal water losses were assumed to be 75% of fecal mass (36).

Body temperatures at the following sites were measured: Tpa, the skin on the left and right dorsal aspects of the thorax (Tsk), Tre, and the middle gluteal muscle (Tmus). Temperatures at sites other than muscle were measured by using copper-constantan thermocouples (Physitemp Instruments, Clifton, NJ) every minute for the first 10 min, then every 10 min throughout exercise, at the end of exercise, and every 5 min during the 15-min rest periods. Tpa was measured by inserting a thermocouple into the pulmonary artery within an 8-Fr polyethylene catheter. The catheter was introduced via a jugular vein, and its position within the pulmonary artery was verified by pressure wave recordings. For measurement of Tsk, flat, 0.5-cm-diameter thermocouples (model SST-1, Physitemp Instruments, Clifton, NJ) were fastened to the skin with adhesive tape and skin sutures. Tre and Tmus were measured preexercise, at the end of exercise, and at the end of each rest period by inserting a needle thermocouple probe ~4 cm into the muscle through the lumen of an
18-gauge 37-mm needle. All thermocouples had response times of ~1°C/s and were calibrated in a heated water bath. All catheterizations and measurements of Tnu were performed after aseptic preparation and local analgesia of the skin.

Pulmonary artery blood samples were collected immediately before exercise, after 2 min of exercise, at the end of each phase of exercise, and after 10 min in each rest phase. Blood samples were analyzed for hematocrit (microhematocrit method) within 5 min of collection. Plasma was then separated from the cells by high-speed centrifugation (14,000 revolutions/min for 5 min) and analyzed for total solids concentration by refractometry (Atago model SPR-T2). The percent change in plasma volume was calculated by using the change in plasma total solids concentration (22). This calculation assumes there is no net movement of protein either to or from the vascular compartment during exercise. The [Na⁺], [Cl⁻], and [K⁺] in sweat samples were determined with an ion-selective analyzer (Statprofile 9 Plus, Nova Biomedical Canada, Mississauga, ON). Plasma and sweat osmolality were determined by freezing-point depression (model 3MO Plus, Awareness Instruments, Needham, MA). All analyses were performed in duplicate.

Estimates of metabolic heat production and total evaporative heat loss. Metabolic heat load (MHL) was estimated from the rate of metabolic V̇O₂ measured at 10-min intervals during exercise. It was assumed that 80% of the calorific value of O₂ consumed during work was released as heat and that each liter of O₂ consumed had an energy equivalent of 21 kJ (11, 16). Therefore, the MHL was calculated by using the formula of Hanson (9) where H₀ = MHL − S

\[ H₀ = MHL - S \]

where H₀ is the total heat dissipated. Stored heat was calculated from the elevation in Tnu at the end and 15 min after each phase of exercise by using the equation S = Tnu × specific heat capacity of body tissue × body mass (kg). As the specific heat capacity of the horse is not known, the value for humans (3.48 kJ · kg⁻¹ · °C⁻¹) was used.

RHL was calculated by using the formula of Hanson (9)

\[ RHL = \dot{V}_E \cdot p_c \cdot (T_e - T_i) + L_a \cdot (W_e - W_i) \text{ (J/min)} \]

where \( \dot{V}_E \) is minute ventilation in liters per minute at standard temperature and pressure, \( p_c \) is the volumetric specific heat capacity of air (J/l), \( T_e - T_i \) is the difference between expired and inspired air temperature (°C), \( L_a \) is the latent heat of vaporization of water (J/g), and \( W_e - W_i \) is the difference between expired and inspired water vapor (g/min). \( \dot{V}_E \) was not measured in this study. However, in a previous study of horses of equivalent mass exercising at a workload equivalent to 40% of V̇O₂max, \( \dot{V}_E \) was linearly related to Tpa (2). Therefore, we estimated \( \dot{V}_E \) by using the following formula (2)

\[ \dot{V}_E = 147 (T_{pa}) - 4,775 \text{ (l/min STPD)} \]

Inspired air temperature and relative humidity were measured with a digital psychrometer (model 37952; Cole Palmer, Vernon Hills, IL). Tpa measured with a thermocouple placed in the false nostril (40), ranged from 33.5 to 34.5°C. Relative humidity of the expired air was assumed to be 100%. Values for \( W_e \) and \( W_i \) content were derived from the respective values for air temperature and relative humidity. Nonrespiratory heat loss was assumed to be the balance of the heat produced after subtraction of RHL and S. It has been reported that heat loss by radiation and convection represent only 1 and 5%, respectively, of the total heat dissipated during exercise in environmental conditions (ambient temperature 22°C, relative humidity 40–50%) similar to those in this study (16, 27). Therefore, 94% of the remaining heat loss was assumed to represent evaporative heat loss from the skin.

Statistical analyses. Results were analyzed by an analysis of variance for repeated measures, with significant differences located by Newman–Keuls post hoc test. The Bonferroni method was used to control for the overall level of significance of the type I error. Linear regression was applied to investigate the relationship between selected variables. Differences were considered significant when \( P < 0.05 \). All results are reported as means ± SE.

RESULTS

Exercise time. One horse was unable to complete the third phase of exercise in one experiment due to fatigue. The mean run times for each exercise phase were as follows: phase I, 67.3 ± 0.5 min; phase II, 67.6 ± 0.5 min; and phase III, 67.5 ± 0.5 min.

Metabolic responses. Mean values for V̇O₂ increased from 4.9 ± 1.0 ml·kg⁻¹·min⁻¹ at rest to 52 ± 4 ml·kg⁻¹·min⁻¹ (range 46–57 ml·kg⁻¹·min⁻¹) during exercise.

Changes in body mass and hydration in response to exercise. After consumption of feed and water were accounted for, and including fecal and urinary water losses, body mass was decreased by 33.8 ± 1.5 kg after exercise (7.3% of preexercise body mass; range 5.1–10.1%). When expressed as a percentage of preexercise total body water (0.66 × body mass), this decrease represented an 11.1% (range 7.8–15.3%) reduction in total body water. The decrease in body mass in phases I (12.1 ± 0.5 kg) and III (11.8 ± 0.4 kg) was significantly (P < 0.01) higher than during phase I (9.8 ± 0.3 kg).

The mean quantity of water consumed during the rest period after each phase of exercise was 6.4 ± 1.1, 3.4 ± 0.7, and 8.1 ± 0.8 liters for phases I, II, and III, respectively (total water consumption was 17.9 ± 1.1 liters). Therefore, total mean volume consumed before the end of exercise was 9.8 liters, with an additional 8.1 liters consumed after the exercise test and before final measurement of change in body mass. Assuming complete intestinal absorption of the water consumed, the net deficits in body mass and total body water at the beginning of phase III were 2.6% (range 1.7–4.1%) and 3.8% (range 2.5–6.5%), respectively. At the end of the exercise protocol, there was a net 5.9% (range 4.1–8.6%) deficit in body mass and an 8.5% (range 6.0–12.6%) deficit in total body water.

Values for plasma total solids, hematocrit, and plasma osmolality during each phase of exercise and in recovery are shown in Table 1. During phase I, there was a rapid and significant (P < 0.001) increase in hematocrit from 39 to 46% within the first 2 min of exercise, consistent with splenic contraction. For the remainder of phase I and the subsequent phases of exercise, the
extent of changes in hematocrit were smaller and paralleled alterations in plasma total solids (hemoconcentration). There were significant (P < 0.001) and progressive increases in plasma total solids during the three phases of exercise. At the end of phases I, II, and III, values for plasma total solids were 7.0, 14.1, and 21.1% higher, respectively, when compared with preexercise values. Plasma volume, as determined by the change in plasma total solids, was decreased by ~21% at the end of the third phase of exercise. Despite the hemoconcentration, there was no significant (P > 0.05) change in plasma osmolality throughout the exercise protocol.

SR. SR during the three phases of exercise, as determined by the volume of sweat collected from the sealed pouch on the thorax, are shown in Fig. 1A. Although there was individual variation in SR, unlike SR in studies in human subjects (23), there was no significant (P > 0.05) difference in SR based on gender of the rest period. The pattern of change in SR was similar during each phase of exercise, with SR increasing rapidly for the first 20–30 min of exercise; SR then remained relatively constant at ~24–28 ml·m⁻²·min⁻¹ during the remainder of each phase. Compared with the corresponding time points in the first exercise phase, SR was significantly (P < 0.001) higher at 10 and 20 min of exercise in phases II and III (Fig. 1A). After each phase of exercise was completed, SR declined rapidly. By 10 min of rest, the hair coat was dry, and no sweat accumulated in the sealed pouch between minutes 5 and 15 of the rest period.

Mean values for whole body SR in experiment 1, calculated from the total body water losses (from change in body mass in each phase) after correction for estimated respiratory water losses, were 23.0 ± 2.2, 26.3 ± 3.1, and 26.1 ± 2.0 ml·m⁻²·min⁻¹ for phases I, II, and III, respectively. These values were not significantly (P > 0.05) different from whole body SR measured by use of direct sweat collection in each exercise phase in experiment 2 (24.8 ± 2.5, 27.8 ± 3.0, and 27.4 ± 2.4 ml·m⁻²·min⁻¹ for phases I, II, and III, respectively).

Sweat composition. Values for sweat osmolality and [Na⁺], [Cl⁻], and [K⁺] are shown in Fig. 1, A–C. During each phase of exercise, the [Na⁺] and [Cl⁻] in sweat were lowest in samples collected after the first 10 min of exercise, were significantly (P < 0.001) increased in the samples collected at 20 and 30 min of exercise, and then did not change significantly (P > 0.05) in samples collected at 40, 50, and 60 min and at the end of exercise (Fig. 1B). The highest value for sweat [Na⁺] (131.2 ± 4.2 mM) was attained in the last 10 min of phase III. There was a similar pattern of change in sweat osmolality. Values were lowest in samples collected at 10 min of exercise and highest in samples collected at 20 or 30 min of exercise in each phase (Fig. 1A). Although mean values for sweat [Na⁺] were higher in phases II and III compared with the corresponding time points in phase I, these differences were not statistically significant (P > 0.05; Fig. 1B). In contrast to initial increases in the [Na⁺] and [Cl⁻] in sweat, [K⁺] (Fig. 1C) was highest in the first 20 min of exercise in phase I, then decreased gradually throughout the remaining phases of exercise, so that the values for [K⁺] were significantly (P < 0.0001) lower by the end of phase III compared with values measured in phase I.

Effect of SR on sweat composition. Linear regression analysis demonstrated a positive correlation between SR and sweat osmolality (r² = 0.70, P < 0.0001), SR and sweat [Na⁺] (r² = 0.62, P < 0.0001), and SR and sweat [Cl⁻] (r² = 0.63, P < 0.0001). The decline in sweat [K⁺] was independent of alterations in SR (r² = 0.11, P = 0.2).

Temperature responses to exercise. Values for these measurements during exercise and recovery are shown in Fig. 2, A and B. There was no significant (P > 0.05) difference between temperature measured at the left and right skin sites and, therefore, these data were pooled. Throughout each phase of exercise, Tpa, Tre, and Ts decreased progressively, and mean values were significantly (P < 0.0001) greater than preexercise values after 10 min of exercise in each phase. In particular, the rate of change in Tpa and Ts was greatest during the first 10 min in each phase of exercise, whereas Tre increased more gradually throughout the entire period of exercise. Compared with the mean value for Tm measured before exercise in phase I (37.3 ± 0.1°C), Tm was significantly (P < 0.0001) increased at the end of exercise in each phase, with no significant (P > 0.05) difference between end-exercise values for all three phases of exercise (phase I, 39.7 ± 0.1°C; phase II, 39.9 ± 0.1°C; phase III, 40.0 ± 0.1°C). In contrast, Tm before the onset of exercise in phases II and III (39.0 ± 0.1 and 39.2 ± 0.1°C, respectively) was significantly (P < 0.0001) increased compared with preexercise Tm in phase I (37.3 ± 0.1°C) and increased <1°C during each of the last two phases of exercise. After each phase of exercise, the Tpa and Ts returned to

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Table 1. Plasma total solids, hematocrit, and plasma osmolality in 6 horses measured preexercise, at the beginning and end of each rest phase and at 10 min in each rest phase after three 15-km phases of exercise at 40% \( V\omega_{2\text{max}} \)

<table>
<thead>
<tr>
<th></th>
<th>Plasma Total Solids, g/dl</th>
<th>Hematocrit, %</th>
<th>Plasma Osmolality, mosmol/kgH₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preexercise</td>
<td>7.1 ± 0.2</td>
<td>39 ± 1</td>
<td>282 ± 2</td>
</tr>
<tr>
<td>Ex2</td>
<td>7.4 ± 0.2</td>
<td>46 ± 1*</td>
<td>283 ± 3</td>
</tr>
<tr>
<td>End</td>
<td>7.6 ± 0.2*</td>
<td>48 ± 1*</td>
<td>283 ± 2</td>
</tr>
<tr>
<td>R10</td>
<td>7.3 ± 0.1</td>
<td>42 ± 1</td>
<td>281 ± 1</td>
</tr>
<tr>
<td>Phase II</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ex2</td>
<td>7.6 ± 0.1†</td>
<td>46 ± 1*</td>
<td>283 ± 2</td>
</tr>
<tr>
<td>End</td>
<td>8.1 ± 0.2†</td>
<td>52 ± 1†</td>
<td>282 ± 1</td>
</tr>
<tr>
<td>R10</td>
<td>7.8 ± 0.2†</td>
<td>46 ± 2†</td>
<td>279 ± 3</td>
</tr>
<tr>
<td>Phase III</td>
<td></td>
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</tr>
<tr>
<td>Ex2</td>
<td>8.0 ± 0.2*</td>
<td>48 ± 1*</td>
<td>282 ± 2</td>
</tr>
<tr>
<td>End</td>
<td>8.6 ± 0.3†</td>
<td>54 ± 2†</td>
<td>283 ± 2</td>
</tr>
<tr>
<td>R10</td>
<td>8.1 ± 0.2*</td>
<td>48 ± 2*</td>
<td>280 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ex2, 2 min of exercise; End, last minute of each exercise phase; R10, 10 min in each rest phase. Significantly different (P < 0.05) compared with *preexercise values; †, corresponding time points in Phase I; ‡, corresponding time points in Phase II.
Fig. 1. Mean sweating rate and sweat osmolality (A), sweat concentrations of Na\(^+\) and Cl\(^-\) (B) and K\(^+\) (C) in 6 horses during 3 phases of exercise at 40% of maximal \(\dot{V}O_2\) uptake (\(\dot{V}O_2\)\text{max}). Values are means \(\pm\) SE.
preexercise values within the first 10 min of each rest phase. In contrast, \( T_r \) remained significantly \((P < 0.001)\) higher \((-0.5^\circ\text{C})\) at the end of each rest phase compared with preexercise \((\text{phase I})\) values, although preexercise \( T_r \) for phases II and III was not significantly different \((P > 0.05)\).

Estimates of metabolic heat production and total evaporative heat loss. The estimated total heat production from the start of exercise in phase I to the completion of phase III was 80,998 ± 1,105 kJ, representing heat production of ~27,000 kJ during each phase of exercise. Estimated values for heat production, dissipation, and storage for each phase of exercise are presented in Fig. 3, A and B. Of the total heat produced, heat storage at the end of exercise in phase III was estimated as 2,386 ± 355 kJ and as 1,316 ± 165 kJ by 15 min of recovery. Total RHL was 18,023 ± 876 kJ \((6,214 + 5,827 + 5,982 \text{ kJ})\) or ~23% of dissipated heat. Of the remaining heat loss, 94% was assumed to be evaporative heat loss from the skin \((55,209 ± 1,850 \text{ kJ})\), and 6% was assumed to include heat loss by radiation and convection \((4,780 ± 150 \text{ kJ})\).

**DISCUSSION**

The major question addressed in this study was whether progressive hypohydration and the associated loss of plasma volume would alter the rate of sweat production or the ionic composition of sweat fluid produced during prolonged, low-intensity exercise in horses. To answer this question, we have measured the rate and composition of sweat fluid losses during >3 h of moderate-intensity exercise in horses. Furthermore, we have measured the changes in body temperature and body mass and, by utilizing the values for cutaneous evaporative heat loss, estimated the partitioning of evaporative heat loss into its respiratory and cutaneous components.
In the present study, we have shown that after an initial rise in the rate of sweat production at the beginning of each phase of exercise, the rate of sweat production did not change significantly throughout the remainder of each phase of exercise. Furthermore, the rate of sweat production at the end of the third phase of exercise was not different compared with values measured at the end of phase I. The similarity in the values for temperatures measured at the end of each phase of exercise, assuming a constant rate of heat production, would indicate that there was no change in the rate of heat loss. Because calculated cutaneous evaporative heat losses were unchanged in each phase of exercise, these findings demonstrate that there was also no significant change in the respiratory component of evaporative heat loss under the conditions of this experimental protocol.

We hypothesized that SR would decrease as a result of progressive hypohydration during prolonged exercise. Furthermore, a decrement in SR would be associated with an increased rate of rise in body temperature (25). Because the rate of sweat production did not change, the contribution of cutaneous evaporative heat loss was assumed to have been unaltered. Moreover, there was no significant change in the osmolality or composition of sweat fluid despite progressive hypohydration (8.5% net decrease in total body water); neither was the hypohydration associated with an increase in plasma osmolality, although plasma volume was estimated to have decreased by ~21% at the end of the third phase of exercise.

The experimental protocol used in the present study was designed to provide low-intensity exercise of sufficient duration to allow comparison of SR and sweat ion composition over time. However, the exercise duration was also long enough and fluid losses were of sufficient magnitude that some of the horses would not have completed the test without some fluid replacement. By providing access to fluid at specified rest stops, the protocol simulated conditions in competitive events. However, allowing voluntary fluid consumption introduced some variability with respect to replacement of fluid losses (15 to 35% of total fluid losses). Furthermore, this fluid intake reduced the degree of hypohydration incurred by the subjects.

Hypohydration of 3% or greater has been associated with significant increases in plasma osmolality in human subjects undergoing moderate exercise (26, 32). In addition, studies of human subjects have demonstrated that hypohydration increases the threshold temperature for sweating and decreases sweating sensitivity in a graded manner, with changes in the sweating response evident at a hypohydration level of 3% (32). This contrasts with the findings of the present study in which the rate of sweat production was unaltered at hypohydration equivalent to a 5.9% decrease in body mass or 8.5% loss of total body water by the end of exercise. This level of hypohydration reflects the net loss of body water after accounting for water consumed (9.8 liters) before the third phase of exercise. Given the uncertainty regarding the rate of gastric emptying and intestinal uptake of the consumed water, our estimate of the actual extent of hypohydration may be conservative.

Sawka and colleagues (32) demonstrated an inverse relationship between the degree of plasma hyperosmo-
lality and SR (r = −0.76; P < 0.05), whereas the relationship between the reduction in plasma volume and SR was less clear. The results of the present study also fail to demonstrate any relationship between plasma volume and SR. Additionally, the rate of sweat fluid loss imposed by the intensity of the exercise and the environmental conditions used in this experiment did not result in an alteration in plasma osmolality. A similar rate of sweat fluid loss has been demonstrated by others during shorter periods of low-intensity exercise. Hodgen et al. (11) measured SR of 24 ± 4 ml·min⁻¹·kg⁻¹ in horses that exercised for 40 min at 40% VO₂max. Naylor and co-workers (28) also measured SR similar to those reported in this study in euhydrated horses and in horses dehydrated before exercise by administration of furosemide or by water deprivation. Despite a significant degree of hypohydration (3.2–3.9% decrease in total body water), these researchers were unable to detect a decrease in SR during 40 min of exercise (4.6–5.5% decrease in total body water by the end of exercise) at the same work intensity undertaken by the horses in the present study. However, Naylor et al. reported that hypohydration decreased the dissipation of heat, as reflected in a reduction in internal transfer of heat from core to periphery. Also, this impairment of thermoregulation, as demonstrated by the increases in temperature of the carotid artery, Tpa, Ttr, gluteal Tmu, and Tsk, was more pronounced from hyperontic vs. isotonic dehydration.

The lack of change in SR in response to the degree of hypohydration demonstrated in the horses in the present study probably represents at least two differences between human and equine subjects. First, the ionic composition of equine sweat during exercise is normally slightly hypertonic, with an osmolality ranging from ~290 to 320 mosmol/kg·H₂O, as reported in the present and previous studies (18, 21). Human sweat is hypotonic relative to extracellular fluid, and the protocol used in this study would have elicited a hypotonic hypovolemia in human subjects. In contrast, despite the extensive sweat fluid losses during the prolonged exercise, the fluid loss incurred by the horses in this study would more closely approximate an isotonic loss and would therefore result in an isosmotic hypohydration. Other studies have demonstrated that the horse is capable of maintaining serum osmolality close to, or even below, resting values during prolonged exercise (31). Because hyperosmolality has been demonstrated to impair thermoregulation (26, 32), an isotonic fluid loss may assist the equine athlete in maintaining SR and adequate heat dissipation in the face of comparatively larger fluid losses.

Second, the interstitial and intestinal fluid volume of the horse represents a significantly larger reserve of fluid and ions potentially available for reabsorption during low-intensity exercise. Webb and Weaver (38) reported that the content of the intestinal tract is ~6% of body mass, or 27 kg for a 450-kg horse. Because >75% of the intestinal content is water (36), the gastrointestinal tract represents a substantial fluid reservoir. Meyer and Coenen (24) demonstrated significant decreases in the water, Na, and Cl content of the gastrointestinal tract of ponies after 1 h of low-intensity exercise. These researchers suggested that, during low-intensity exercise, the fluid reservoir present in the intestinal tract may assist in maintaining plasma volume and the rate of sweat fluid losses.

A decline in SR over time has been reported in horses when sweating is induced by adrenaline infusion (13, 18). Kerr and Snow (13) determined that SR decreased after 1–3 h despite increasing concentrations of adrenaline in an infusion, whereas McConaghy et al. (18) found that some subjects stopped sweating after <30 min of an adrenaline infusion. In the horse, the production of sweat is under sympathetic nervous control (12) and involves β2-adrenoreceptors (33). Although sweating can be induced solely by adrenaline (13, 18), exercise-induced sweating will involve the contribution of circulating adrenaline and sympathetic nervous stimulation (30). The SR and fluid losses incurred by the adrenaline infusion are substantially less than those produced in response to prolonged exercise in this and other studies of endurance exercise (5, 15, 31, 34). The responsiveness of the sweat gland may be altered by prolonged continuous exposure to high blood adrenaline concentrations, resulting in a decline in sweat production. Whereas the sweating response during exercise at >60% VO₂max may reflect a substantial contribution of elevated adrenaline concentration in the blood, the role of adrenaline is probably relatively minor in the formation of sweat produced during prolonged low-intensity exercise.

Differences in [Na⁺] in equine sweat and plasma (~100–125 mM in sweat (Fig. 1A) vs. ~139–145 mM in plasma) suggest there is modification of the ionic composition of extracellular fluid before sweat secretion. Wilson et al. (39) demonstrated reabsorption of Na⁺ by the cells in the equine sweat duct during thermal stimulation, suggesting that some modification of the ionic composition of sweat occurs during its passage through the sweat gland duct. Higher [Na⁺] and [Cl⁻] are present in sweat produced in response to moderate to high-intensity exercise compared with adrenaline infusion. These higher ion concentrations probably reflect the higher SR induced by exercise (13, 18–21, 31). The change in composition of sweat relative to the increase in SR during the initial portion of each exercise phase in the present study adds further support to the probability of some modification of equine sweat.

In contrast to [Na⁺] and [Cl⁻], the [K⁺] in sweat declined by ~12–14% by the end of each phase of exercise when compared with sweat samples collected after the first 10 min of exercise (Fig. 1C). Equine and human sweat glands have been shown to differ from sweat glands in several other domestic species in that there is evidence for K efflux from the gland during secretory activity, and this loss of K ions assists in maintaining secretory drive (12). However, the factors that contribute to sweat [K⁺] that decline with time during exercise are not clear. The lack of a linear relationship between SR and sweat [K⁺] has been demonstrated in studies of human subjects (6, 37).
Adrenergic stimulation of the sweat gland can contribute to secretory drive. However, equine sweat produced in response to epinephrine infusion has a lower $[K^+]$ (18) and, as stated earlier, it is unlikely that there was substantial elevation of blood adrenalin concentrations during exercise in the present study. It has been suggested that changes in $[K^+]$ of interstitial fluid associated with $K^+$ efflux from working skeletal muscle during exercise may be reflected in sweat $[K^+]$ (35). Although reuptake of $K^+$ occurs very rapidly, it is possible that initial increases in plasma $K^+$ could be reflected in the $[K^+]$ in sweat secretions.

To the authors’ knowledge, the present study is the first report of simultaneous measurement in the horse of SR and sweat composition during prolonged exercise. The protocol utilized in this study provided the advantage of greater sampling frequency and controlled exercise conditions compared with previous field studies. Carlson and Ocen (5), Rose et al. (31), and Snow et al. (34) measured ion composition in the sweat of horses during or after prolonged exercise. However, field conditions limited the number of samples that they could obtain and also their choice of collection techniques. Carlson and Ocen (5) and Rose et al. (31) collected samples after completion of the exercise, whereas Snow and colleagues (34) obtained samples after 16, 64, and 80 km of exercise. The researchers were therefore only able to obtain samples at intervals of 1 h or more. In each study, absorbent pads were used to collect sweat, and, although the skin beneath the pad was protected from direct contact with air, some evaporation of sweat fluid occurred, altering the sweat ion concentrations measured. More recently, McConaghy et al. (18) used a revision of this collection technique during low-intensity treadmill exercise and obtained sufficient sweat for ion analysis with samples collected at 15-min intervals. However, sweat ion concentrations measured by McConaghy and colleagues (18) were still slightly higher than values measured by McCutcheon et al. (21) utilizing a sealed pouch, and the former group of researchers attributed these differences to a small degree of evaporative loss. McConaghy et al. (18) also reported variation in sweat $[Na^+]$ and $[Cl^-]$ of as much as 200 mM during 30 min of low-intensity exercise. In the present study, this degree of variation in sweat $[Na^+]$ and $[Cl^-]$ was not evident between individuals or over time. By comparison, there was a greater degree of individual variation in sweat $[K^+]$, and this variation may have precluded the detection of a correlation between sweat $[K^+]$ and SR.

The results of the present study also demonstrated no change in sweat ion concentration with progressive hypohydration during exercise. Although numerous studies of human subjects describe changes in SR and sweat sensory associated with exercise-induced hypohydration and/or hyperthermia, there is relatively little information with regard to human sweat ion composition under these circumstances. Most reports of variation in sweat ion concentration relate to repeated exercise during training or heat acclimation (1, 29). During heat acclimation in human subjects, there is a progressive decrease in sweat $[Na^+]$ during the first 8–10 days of heat exposure. These changes are noted in sweat samples collected from exercise performed on successive days, rather than within a single bout of exercise. Changes in sweat ion composition have also been reported in response to incremental exercise of short duration (35). However, there are few instances of multiple sampling over a prolonged period of low- to moderate-intensity exercise. In the present study, because SR was unaltered, it is perhaps not surprising that the $[Na^+]$ and $[Cl^-]$ in sweat remained unchanged; there appears to be little information that would suggest that the concentrations of these ions would change in a manner other than that associated with an alteration in SR.

There were parallel increases in $T_{pa}$, $T_{re}$, and $T_{sk}$ in response to exercise (Fig. 2, A and B). However, the rate and extent of the increase in temperature were ~50% lower compared with previous reports of horses exercising at a similar work intensity (11, 28). The difference in the rate of rise in body temperature could be accounted for by lower heat production during exercise or by greater efficiency of heat dissipation. Lower heat production is unlikely, because the metabolic rate ($V_{O_2}$) of our horses during exercise was similar to values measured in horses in the studies reported by Hodgson et al. (11) and Naylor et al. (28). Values for HR during exercise were comparable to values reported for the horses in the aforementioned previous studies, providing additional verification of the similarity in the workload (data not shown). A more likely explanation for the difference in rate and extent of the rise in body temperature is greater efficiency of heat dissipation during exercise. In particular, the close matching of treadmill and fan speeds would promote both evaporative and convective heat loss. In the studies by Hodgson et al. and Naylor et al., fan speed was ~50–55% of the horse’s running speed, and most of the exposed skin was covered by unevaporated sweat during exercise. Thus the environmental conditions limited the rate of heat dissipation. In the present study, the moderate room temperature and the close approximation of treadmill speed and wind speed created by the fan resulted in most of the sweat evaporating from the body surface during and after exercise. The greater efficacy of cutaneous heat loss is further supported by the lower $T_{sk}$ and blood temperatures maintained during each phase of exercise in this study compared with the findings of Naylor et al. and Hodgson et al. Also, in the present study, the greater differential between $T_{re}$ and $T_{pa}$ maintained throughout exercise may have reflected a sustained and more substantial effect of the transfer of cooled blood from the skin to the central circulation.

The estimated evaporative heat loss by cutaneous and respiratory routes was unchanged in the three phases of exercise. When estimates of cutaneous evaporative heat loss are based solely on total sweat fluid losses, there is a tendency to overestimate heat loss. This discrepancy largely represents the difficulty in ascertaining the extent of sweat drippage. Total evaporation of the estimated volume of sweat fluid losses...
would have accounted for heat losses of ~19,400, 21,750, and 21,300 kJ during phases I, II, and III, respectively, and would represent 80% of the total heat dissipated during the experimental period. After calculation of RHL, and assuming that radiation and convection accounted for 6% of the total heat dissipated, ~70% of the MHL was dissipated by evaporation of sweat. This difference in estimates reflects sweat dripping during the experimental protocol.

Of the small number of studies that provide estimates of RHL in exercising horses, few reports include accurate measurement of total fluid losses or SR. In the present study, RHL averaged ~90–100 kJ/min throughout the three phases of exercise. Heilemann et al. (10) reported values of ~50 kJ/min in horses during trotting exercise in moderate environmental conditions, whereas Hodgson et al. (11) reported values of ~80 and ~180 kJ/min at the onset and after 35 min of exercise eliciting 40% VO2max, respectively. Lund and colleagues (16) have recently calculated RHL as a much larger component of total heat loss (~200–250 kJ/min) when exercise is brief and maximal and the SR is low. These disparities in estimates of the quantity of heat dissipated via the respiratory tract reflect differences in work intensity, Ve, environmental conditions, and the methods used for calculation of RHL.

Heat loss via the respiratory tract was calculated on the basis of the difference in the temperature and water vapor content of inspired and expired air and an estimate of Ve. Total RHL was calculated from the sum of the convective and evaporative components by using an equation similar to that used by Hanson (9). Given the low specific-heat capacity of air, the majority of the heat was probably lost by evaporation of water from the mucous membranes of the respiratory tract, because the inspired air was saturated with water vapor (16, 27). In this study, we had no direct measure of Ve. However, Bayly et al. (2) demonstrated a linear relationship between Tpa and tidal volume and Ve for horses exercising at 40% VO2max. On the basis of this relationship, we estimated Ve to be in the range of 800–1,100 l/min during exercise. These values are comparable to those obtained from direct measurements of Ve in horses exercising at similar workloads (2, 3). Thus, although we did not directly measure RHL, the results presented here provide some estimate of the relative contribution of respiratory and cutaneous heat loss during low-intensity exercise in moderate conditions. However, it is clear that these proportions could be altered considerably when ambient conditions and/or workloads are changed.

There are few published estimates of heat production, dissipation, and storage in the horse during exercise or of the relative proportions of heat dissipated via the respiratory and cutaneous routes. As previously suggested, such estimates will vary considerably depending on methodology, environmental conditions, and exercise intensity. The advantage of the more prolonged exercise undertaken in this study was that there were periods of ~40 min in each exercise phase during which SR did not change significantly. In other studies in which heat production and dissipation have been estimated (11, 16, 17), a larger proportion of the overall exercise bout represents a period of adjustment to the required workload. As a result, calculations of the quantity of heat dissipated and of the estimated respiratory evaporative component of heat loss vary from 79 to 93% and from 22 to 49%, respectively, of the total MHL. In the present study, ~95% of the MHL was dissipated by the end of exercise, with RHL estimated to represent ~23% of total heat losses.

In summary, during >3 h of low-intensity exercise 1) hypohydration of ~6% was incurred by horses, despite voluntary intake of water during two rest phases during the exercise protocol; 2) SR and the ionic composition of sweat did not change during three phases of exercise; 3) sweat [Na+] and [Cl−] were positively correlated with SR; 4) plasma osmolality was unchanged despite an ~21% decrease in plasma volume and an ~8.5% reduction in total body water; and 5) ~95% of the MHL was dissipated during exercise, of which ~70% was lost by evaporation of sweat and ~23% was dissipated via the respiratory tract. We conclude that SR and the relative proportions of respiratory and cutaneous evaporative heat loss can be maintained unchanged during prolonged low-intensity exercise in horses in thermoneutral conditions despite moderate hypohydration.

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