Heat storage in horses during submaximal exercise before and after humid heat acclimation

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Geor, Raymond J., Laura Jill McCutcheon, Gayle L. Ecker, and Michael I. Lindinger. Heat storage in horses during submaximal exercise before and after humid heat acclimation. J Appl Physiol 89: 2283–2293, 2000.—The effect of humid heat acclimation on thermoregulatory responses to humid and dry exercise-heat stress was studied in six exercise-trained Thoroughbred horses. Horses were heat acclimated by performing moderate-intensity exercise for 21 days in heat and humidity (HH) [34.2–35.7°C; 84–86% relative humidity (RH); wet bulb globe temperature (WBGT) index ~32°C]. Horses completed exercise tests at 50% of peak O2 uptake until a pulmonary arterial temperature (Tpa) of 41.5°C was attained in cool dry (CD) [20–21.5°C; 45–50% RH; WBGT ~16°C], hot dry (HD 0) [32–34°C room temperature (RT); 45–55% RH; WBGT ~25°C], and HH conditions (HH 0), and during the second hour of HH on days 3, 7, 14, and 21, and in HD on the 18th day (HD 18) of heat acclimation. The ratios of required evaporative capacity to maximal evaporative capacity of the environment (Ereq/Emax) for CD, HD, and HH were ~1.2, 1.6, and 2.5, respectively. Preexercise Tpa and rectal temperature were ~0.5°C lower (P < 0.05) on days 7, 14, and 21 compared with day 0. With exercise in HH, there was no effect of heat acclimation on the rate of rise in Tpa (and therefore exercise duration) nor the rate of heat storage. In contrast, exercise duration was longer, rate of rise in Tpa was significantly slower, and rate of heat storage was decreased on HD 18 compared with HD 0. It was concluded that, during uncompensable heat stress in horses, heat acclimation provided modest heat strain advantages when Ereq/Emax was ~1.6, but at higher Ereq/Emax no advantages were observed.

uncompensable heat stress; thermoregulation; humidity; equine

IN SEVERAL SPECIES, IT HAS been unequivocally demonstrated that repeated exposure to exercise-heat stress over many days improves exercise capabilities and reduces core temperature and physiological strain. In human subjects, acclimation to hot conditions results in cardiovascular adjustments that reduce cardiovascular strain and induce changes in sweating responses that elicit earlier onset of sweating, a reduction in the thermal set point for sweating, and higher sweating rates (1, 36, 37). These adaptations are important to the health and performance of human athletes in hot conditions. Now, expanding schedules of international and year-round competition also require elite equine athletes to train and compete in the heat. In fact, several recent top level international 3-day event competitions have been held when the wet bulb globe temperature (WGBT) index levels were >30, a level at which numerous precautions, including reduction in the distance required in the endurance event, are advised to limit overheating during and after competition (14, 34).

The demand for horses to compete in hot dry and hot humid conditions has focused attention on the capacity of equine athletes to adapt to exercise in the heat. Compared with humans, their mass-specific maximal O2 uptake (V02max) is at least twofold higher, and, therefore, at a given work intensity, the metabolic heat load is considerably higher (13, 24). Furthermore, relative to body mass, the surface area for dissipation of heat in the horse is ~50% of that in humans. These thermoregulatory limitations result in a rapid increase in core body temperature to critical levels during exercise, a situation accentuated during exercise in hot conditions (5, 11, 19). The limitations imposed on the exercising horse are further illustrated by calculation of the heat stress index [i.e., the ratio of required evaporative capacity (Emax) to the maximal evaporative capacity of the environment (Emax)]. Because of the horse’s high metabolic rate, ambient conditions with WGBT as low as 15°C can provide a heat stress index of >1 (uncompensable heat stress).

Recognizing the degree to which these thermoregulatory limitations may hinder exercise performance, several recent studies in horses have examined physiological adaptations associated with exercise in the heat (7, 20, 22, 25). In two studies, increases in peak sweating rate during exercise and reductions in sweating threshold and sodium ion concentration in sweat fluid were reported after 10–15 days of daily exposure to and exercise in the heat (22, 25). In addition, one investigation noted improved regulation of plasma volume after 2 wk of heat acclimation (20), whereas an-

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other noted marked increases in respiratory rate in acclimated resting horses that contributed to a lower preexercise core temperature (7). Marlin et al. (22) suggested that heat acclimation may partially restore a reduction in performance noted in unacclimatized horses exercising in hot humid conditions. However, although all studies have used an exercise-heat stress model, differing ambient conditions and exercise tests have provided conflicting information as to the extent of improvements in heat dissipation during exercise.

With the increasing number of demanding equine competitive events held in hot and humid conditions, a need for greater understanding of the physiological effects of these conditions on exercise performance is required. The present study determined the effect of humid heat acclimation on thermoregulatory responses to humid and dry exercise-heat stress in exercise-trained Thoroughbred horses. The specific objectives were to determine 1) whether a period of active humid heat acclimation in horses could decrease heat storage (S) and improve performance, as reflected by the time needed to attain a pulmonary arterial blood temperature (T_{pa}) of 41.5°C during a standardized exercise test (SET) and 2) the effects of heat acclimation on exercise responses under dry (WBGT \sim 25°C; E_{req}/E_{max} \sim 1.6) and humid (WBGT \sim 32°C; E_{req}/E_{max} \sim 2.5) heat stress. These environmental conditions were chosen to reflect typical heat stress levels during equine competitive events. However, given the low evaporative capacity of the humid climate and the horse's heavy reliance on sweating for heat dissipation, we anticipated minimal improvement in exercise performance under severe exercise-heat stress (E_{req}/E_{max} \sim 2.5). Conversely, in environmental conditions with greater evaporative capacity (E_{req}/E_{max} \sim 1.6), we hypothesized that any improvements in sweating responses and other mechanisms for heat loss would reduce S and extend performance.

**MATERIALS AND METHODS**

The care and use of animals in this study followed the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, Ottawa, Ontario). All animal experiments were conducted after approval by the Animal Care Committee of the University of Guelph and performed in compliance with their recommendations. All experiments were conducted during the fall and winter, and the horses received no other controlled exercise during the entire period of study.

**Experimental animals.** Six Thoroughbred horses ranging in age from 3 to 6 yr and weighing 414–505 kg (mean \pm SE, 455 \pm 12 kg) were studied. The horses were maintained on a diet consisting of grass hay and a mixed grain ration (Professional Horse Mix,Ralston Purina). In addition, the horses were provided with 150 g/day of a salt supplement (Na \sim 40 g, K \sim 26 g, Cl \sim 84 g) and had free access to a trace mineral block. Throughout the period of study, the horses were housed individually indoors at an ambient temperature of 16–19°C with free access to a maximum of 36 liters of water provided in two 18-liter buckets that were measured and refilled at 0700 and 1700.

Before the study, the horses were conditioned for 10 wk with a 5 day/wk program of walking, trotting, cantering, and galloping on a high-speed treadmill set on a 3° incline (Sato). The duration and intensity of exercise were gradually increased until the horses were exercising for 40 min at 4 m/s, 4 min at 7 m/s, and at least 2–3 min at 9 m/s by the 10th week of training. All training was conducted under cool dry conditions [20–21.5°C room temperature (RT), 45–50% relative humidity (RH)]. The V_{O2 max} of each horse was determined (8) during the 8th and 10th wk of training and after completion of the subsequent 3-wk period in which exercise was undertaken in the heat. For each horse, regression analysis of the speed-vs.-O_{2} uptake (V_{O2}) data was used to calculate the running speed that elicited 50% of V_{O2 max}.

**Experimental protocol.** After the 10 wk of training in the cool dry conditions, each horse completed a SET under cool dry (CD), hot dry (HD 0) (HD = 32–34°C RT, 45–55% RH), and hot humid conditions (HH 0) (HH = 32–34°C RT, 80–85% RH). WBGT index was calculated as described previously (34), with the assumption that globe temperature was equal to ambient temperature. All SETs were performed at the same time of day, and the order of treatments was randomized with a minimum of 5 days between SETs. The initial SETs (CD, HD 0, and HH 0) were followed by 21 consecutive days in which the horses were exposed to (for 4 h) and exercised in (during the second hour) HH for 4 h between 0700 and 1100. On days 3, 7, 14, and 21 of the period of heat exposure, the horses completed the SET instead of the daily exercise protocol. In addition, all subjects completed a second SET in hot, dry conditions on day 18 of the period of heat acclimation (HD 18).

The daily exercise protocol was undertaken on a treadmill in a climate-controlled exercise laboratory in which HH conditions were maintained throughout the 4-h period. The daily exercise protocol consisted of an initial 1 h before exercise, during which the horse stood on the treadmill while resting measurements were collected. On non-SET days, the second hour consisted of submaximal exercise conducted on a high-speed treadmill set at a 3° incline and included a 5-min warm-up (1.75 m/s), 10 min of trotting (4.2 m/s), 5 min of cantering (6.5 m/s), a further 10 min of trotting (4.2 m/s), followed by 30 min of walking (1.75 m/s) for a total distance of \sim 10,600 m. None of the horses demonstrated signs of impending fatigue, as reflected by an inability to keep pace with the treadmill. During and after daily exercise and the SET, a high-speed fan was used to maintain an air velocity of 3.5–4.0 m/s directed over the anterior and dorsal aspects of the horses. Air velocity was measured with an anemometer (Davis Instruments, Hayward, CA) positioned at three sites: lateral midcervical region, lateral and dorsal thorax, and dorsal to the gluteal region of the hindquarters.

**SET.** Food was withheld overnight (12 h). Water was withheld for a 3-h period before and for the duration of each exercise test. Body mass was measured on a large animal scale (±0.5 kg, KSL Scales, Kitchener, Ontario, Canada) immediately before the exercise protocol and at 60 min of recovery after exercise. Total body sweating rates were calculated from body weight loss adjusted for urine and fecal output.

Resting measurements were obtained during a 1-h period before exercise, during which the horses remained stationary on the treadmill. All exercise was conducted on a treadmill set at a 6° incline. The SET consisted of 5 min of walking (1.5 m/s), followed by exercise at 50% of each subject’s V_{O2 max} (range 3.8–4.3 m/s). Exercise was continued until T_{pa} reached 41.5°C. On cessation of exercise, the horses stood for 5 min and then completed a 25-min walking recovery (1.5 m/s) and a further 30-min standing recovery on the treadmill.
HEAT ACCLIMATION IN HORSES

Measurement of HR and respiratory rate. A cardiotachometer (Equistar model HR-SA, EQB, Unionville, PA) was applied around the horse's chest to record heart rate (HR). HR measurements were obtained at 60 min, 5 min, and immediately before exercise, every minute during exercise, at the end of exercise, and at 5, 15, 30, and 60 min of recovery. Respiratory rate was measured at the same intervals as HR before and after exercise.

Measurement of pulmonary artery, rectal, muscle, and skin temperature. Rectal temperature (T_r) and T_pa were measured by use of copper-constantan thermocouples (Physitemp Instruments, Clifton, NJ) 60 min, 5 min, and immediately before exercise, every minute during exercise, at the end of exercise, and at 5, 10, 15, 30, 45, and 60 min of recovery. T_r was measured by using a thermocouple inserted 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. T_r was measured with a thermocouple inserted 20–30 cm proximal to the anal sphincter. Thermocouples had response times of ~1°C/s and were calibrated in a heated water bath. Middle gluteal muscle temperature (T_mu) was obtained immediately before exercise, at the end of exercise (within 10 s), and at 30 and 60 min of recovery. Muscle temperature was measured by inserting a needle thermocouple (MT-23; Physitemp Instruments) ~4 cm into the muscle through the lumen of an 18-gauge 37-mm needle. All catheterizations and T_mu measurements were performed after aseptic preparation and local analgesia of the skin. Measurements of skin temperature (T_sk) were obtained from a single site (an area of shaved skin on the lateral thorax) by using a flat, 0.5-cm-diameter thermocouple (model SST-1, Physitemp Instruments) fastened to the skin with adhesive tape and sutures.

Heat storage. Heat storage (S) was estimated during exercise and the first 60 min of recovery. For recovery, the change (Δ) in internal temperature (T; T_r or T_pa) was calculated by subtracting the end-exercise temperature from the resting temperature at the end of the 60-min period. For the exercise period, ΔT reflects the change in temperature during the exercise bout. Preexercise body mass was used for calculation of S. The specific heat capacity of the horse is not known; therefore, the value for humans (3.48 kJ·kg⁻¹·°C⁻¹) was used, as in previous studies (7, 22, 23). The ΔS (in kJ/m²) was calculated, on the basis of changes in T_r and T_pa by using the formulas for change in rectal (ΔS_r) = 3.48·body mass (kg)·ΔT_r/body surface area (m²) and change in pulmonary arterial S (ΔS_pa) = 3.48·body mass·ΔT_pa/body surface area. The rates of S during exercise S_r and S_pa (kJ/m²·s) were calculated by dividing ΔS_r and ΔS_pa, respectively, by the run time (in seconds). Body surface area (SA) was calculated by using the formula SA = 1.09 + 0.008 × body mass (13).

Ereq and Emax. Ereq was calculated as previously described (32) by the equation Ereq = M_net + (R + C), where M_net is the rate of metabolic heat production (W/m²), and R and C are radiative and convective heat exchange. M_net was calculated as 20.93 (kJ/O₂ consumed)/0.8 (assuming 20% mechanical efficiency)·V̇O₂ (l/min)·SA⁻¹, where 1 W equals 0.0599 kJ/min. In preliminary trials (32–34°C environment), M_net was calculated from V̇O₂ values obtained at the running speeds used in the exercise-heat stress trials. It was assumed that heat acclimation did not alter the rate of heat production during exercise. R + C was calculated by using the formulas described by Schrotter and Marlin (33). Emax was calculated by the equation Emax = hₜ·(Pₛₖ - Pₜᵢ), where hₜ is the evaporative heat transfer coefficient, Pₛₖ is the saturated pressure of water at skin temperature, and Pₜᵢ is the ambient water vapor pressure (18, 32). The heat stress index (HSI) was calculated as the ratio of Ereq to Emax, expressed as a percentage.

Statistical analysis. Data were analyzed by two-way repeated-measures analysis of variance to compare measures over time and among trials (general linear program of Statistical Analysis System; SAS Institute, Cary, NC). Post hoc multiple comparisons were made by the Tukey method when an F ratio was significant. Significance was determined as P < 0.05. Results are expressed as means ± SE.

RESULTS

V̇O₂ max. V̇O₂ max expressed both in absolute and mass-specific terms, was not significantly altered by the period of heat acclimation. Mean V̇O₂ max of the horses, determined after 8 and 10 wk of exercise training and 3 days after 21 days of active heat acclimation, was 144 ± 6 ml·kg⁻¹·min⁻¹ (66.2 ± 2.7 l/min), 145 ± 9 ml·kg⁻¹·min⁻¹ (66.1 ± 2.8 l/min), and 150 ± 7 ml·kg⁻¹·min⁻¹ (66.9 ± 2.8 l/min), respectively.

Environmental conditions. Mean environmental conditions for the SETs on HH 0, 3, 7, 14, and 21 were not significantly different and ranged from 34.2 ± 0.5°C to 35.7 ± 0.6°C and 84 ± 2.5% to 86.1 ± 2.1% for RT and RH, respectively (mean WBGT 32.5 ± 0.3°C). Mean values for environmental conditions (RT, RH, and WBGT, respectively) during SETs completed in cool dry and hot dry conditions were 20.5 ± 1.7°C, 50.2 ± 2.3%, and WBGT 16.6 ± 0.2°C (CD); 35.2 ± 0.9°C, 53.1 ± 2.1%, and WBGT 24.6 ± 0.3°C (HD 0); and 35.7 ± 0.6°C, 52.6 ± 2.1%, and WBGT 24.7 ± 0.3°C (HD 18).

Biophysical responses. Values for Ereq, Emax, and the HSI are presented in Table 1. Compared with the CD trial, heat stress was ~34% and ~115% higher in the HD and HH conditions, respectively. However, the HSI exceeded 100% in all trials, which indicated that, even in cool ambient conditions, the horses were unable to achieve steady-state thermoregulation during exercise. Ereq, Emax, and the HSI were not different among the HH trials or when HD O and HD 18 were compared.

Changes in body mass and mean sweating rate. The mean decrease in body mass during exercise and 60 min of recovery was significantly reduced in HH after 21 days of heat acclimation (Table 1). In all trials, the reduction in body weight represented <3% of total body weight. Mean sweating rate in HD and HH was also decreased after heat acclimation (Table 1).

Exercise duration. Mean exercise duration for all SETs was based on time to attainment of a T_r of 41.5°C. For the SETs completed on HH 0, 3, 7, 14, and 21, mean exercise time ranged from 19.09 ± 1.41 min on day 0 to 20.92 ± 1.98 min on day 21, and times were not significantly different among SETs (Fig. 1A). On the other hand, exercise duration was ~25% longer in HD 18 (39.00 ± 3.94 min) than in HD 0 (30.14 ± 3.03 min) (Fig. 1B). Mean exercise duration in HD 18 was not significantly different from CD (45.24 ± 3.88 min).

Heart rate and respiratory rate. At rest in HH conditions, there were no differences in HR during the 1 h before exercise. Exercise HR during SETs in all condi-
Heat acclimation in horses during standardized exercise-heat stress tests at 50% of maximal oxygen uptake before and during a 21-day period of humid heat acclimation

Table 1. Biophysical responses of horses during standardized exercise-heat stress tests at 50% of maximal oxygen uptake before and during a 21-day period of humid heat acclimation

<table>
<thead>
<tr>
<th>Variable</th>
<th>CD</th>
<th>HD 0</th>
<th>HD 18</th>
<th>HH 0</th>
<th>HH 7</th>
<th>HH 14</th>
<th>HH 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ereq, W/m²</td>
<td>1.654 ± 0.56</td>
<td>1.756 ± 0.60</td>
<td>1.770 ± 0.45</td>
<td>1.785 ± 0.54</td>
<td>1.743 ± 0.41</td>
<td>1.737 ± 0.52</td>
<td>1.768 ± 0.87</td>
</tr>
<tr>
<td>Emax, W/m²</td>
<td>1.389 ± 0.30</td>
<td>1.106 ± 0.35</td>
<td>1.145 ± 0.30</td>
<td>0.681 ± 0.23</td>
<td>0.683 ± 0.29</td>
<td>0.703 ± 0.42</td>
<td>0.699 ± 0.35</td>
</tr>
<tr>
<td>Heat stress index, %</td>
<td>119 ± 4.8</td>
<td>159 ± 7.2</td>
<td>156 ± 8.0</td>
<td>258 ± 13.1</td>
<td>258 ± 16.8</td>
<td>254 ± 21.1</td>
<td>253 ± 18.0</td>
</tr>
<tr>
<td>Mean sweat rate, g·min⁻¹·m⁻²</td>
<td>24.3 ± 3.2</td>
<td>44.3 ± 8.1</td>
<td>36.4 ± 2.4*</td>
<td>42.1 ± 3.4</td>
<td>40.8 ± 3.9</td>
<td>36.1 ± 2.8†</td>
<td>35.5 ± 2.2‡</td>
</tr>
<tr>
<td>Body weight loss, kg</td>
<td>8.7 ± 0.5</td>
<td>13.7 ± 0.6</td>
<td>12.1 ± 1.4</td>
<td>11.7 ± 1.2</td>
<td>11.5 ± 0.9</td>
<td>10.1 ± 1.3</td>
<td>8.8 ± 0.7†</td>
</tr>
<tr>
<td>% body weight loss</td>
<td>1.9 ± 0.3</td>
<td>3.0 ± 0.8</td>
<td>2.6 ± 0.4</td>
<td>2.5 ± 3.4</td>
<td>2.5 ± 0.5</td>
<td>2.2 ± 0.3</td>
<td>1.9 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 horses. Ereq required evaporative cooling rate; Emax, maximum evaporative cooling of the environment; CD, cool dry exercise trial; HD 0 and HD 18, hot dry trials before and after 18 days of humid heat acclimation; HH 0, HH 7, HH 14, and HH 21, hot humid trials before and after 7, 14, and 21 days of humid heat acclimation. *P < 0.001 vs. HD 0; †P < 0.01 vs. HH 0.

Mean TR, measured on entry to the environmental chamber and before exercise, decreased (P < 0.05) during 21 days of heat exposure. By day 7 of HH, resting TR was significantly lower (37.2 ± 0.1°C) than on HH 0 (37.7 ± 0.2°C), and this difference was maintained throughout the 1-h period of preexercise heat exposure (Fig. 5A). The rate and magnitude of rise in TR was similar for all SETs in HH, resulting in a significantly higher TR at the end of exercise on HH 0 compared with HH 7, 14, and 21. In contrast, the rate of decline in TR after exercise was more rapid on HH 0;
0.10°C) (Fig. 5B). Although the magnitude of rise in $T_{re}$ during exercise was similar in HD 0 and 18 (~3°C), given the difference in exercise duration, the rate of increase in $T_{re}$ was significantly lower in HD 18 (0.077 ± 0.006°C/min) than in HD 0 (0.106 ± 0.008°C/min). There was no difference in the rate of decline in $T_{re}$ in HD 0 vs. 18.

Consistent with the reductions in resting $T_{re}$ and $T_{pa}$, after acclimation, preexercise $T_{mu}$ was lower ($P < 0.05$) in the HH (HH 14 and 21 vs. HH 0) and HD conditions (HD 18 vs. HD 0) (Fig. 6). However, the change in $T_{mu}$ during exercise in SETs on HH 0 to 21 was not different (Fig. 6A). End-exercise $T_{mu}$ in CD and HD (0 and 18) were similar to temperatures measured in middle gluteal muscle at the end of exercise in HH (Fig. 6B). The rate of decline in $T_{mu}$ in HD 0 was significantly increased compared with HD 18; $T_{mu}$ after 60 min of recovery in HD 18 was still significantly higher than in HD 0 (Fig. 6B).

$T_{sk}$ at the onset of exercise was not different for all SETs in HH. In HH 0, $T_{sk}$ increased from 36.0 ± 0.3 to 38.5 ± 0.5°C at the end of exercise. Rate of rise in $T_{sk}$ was similar for all days and absolute values, and change in $T_{sk}$ did not vary by more than 0.2°C for all SETs in HH. The rate of decline in $T_{sk}$ in recovery was slower in HH 14 and 21, such that $T_{sk}$ at 60 min postexercise was significantly higher (0.8 ± 0.1°C) compared with HH 0 (36.2 ± 0.2°C). Values for $T_{sk}$ at the onset of exercise in HD 0 (35.8 ± 0.2°C) and HD 18 (35.6 ± 0.3°C) were not significantly different. Whereas the rate of rise in $T_{sk}$ during exercise in HD 18 (0.083°C/min) was lower ($P < 0.05$) than in HD 0.
DISCUSSION

This paper provides one of the first reports of equine thermal responses to exercise before and during humid heat acclimation. Furthermore, this also appears to be the first study to compare pre- and postacclimation responses in both hot dry and hot humid conditions. Although the climatic conditions were not matched for WBGT (HD: 25°C vs. HH: 32°C), the HD and HH climates were chosen to be representative of environmental conditions encountered by horses competing in endurance rides, the speed and endurance test of a 3-day event, and other prolonged exercise tasks. It is noteworthy that, even in moderate ambient conditions (WBGT ~16°C), $E_{\text{req}}$ exceeded $E_{\text{max}}$ and, by definition, these conditions represented an uncompensable heat stress (Table 1), emphasizing the limitations to thermoregulation in the exercising horse.

After a 3-wk period of combined passive and active (4 h/day) acclimation to hot humid conditions (~35°C, 85% RH, 32°C WBGT), the most significant findings

(0.093°C/min), end-exercise $T_{sk}$ in HD 0 (38.8 ± 0.4°C) and HD 18 (38.9 ± 0.5°C) was not significantly different because of the longer exercise duration in HD 18. There was no difference in the rate of decline in $T_{sk}$ during recovery in HD 0 vs. 18, and $T_{sk}$ after 60 min of recovery was not different from preexercise values.

Heat storage measurements. After exercise in HH, $\Delta S_{re}$ and $\Delta S_{pa}$ were not significantly different for all days in HH (Fig. 7A). In contrast, during the 1 h recovery, $\Delta S_{re}$ on HH 7, 14 and 21, and $\Delta S_{pa}$ on HH 14 and 21, were significantly decreased compared with HH 0 (Fig. 7A). In HD, end-exercise $\Delta S_{pa}$ was also unchanged after heat acclimation, whereas $\Delta S_{re}$ decreased ($P < 0.05$) by ~11.6% in HD 18 (Fig. 7A). Postexercise $\Delta S_{re}$ was significantly lower in HD 18 than in HD 0 (Fig. 7B). The rate of $S$ ($S_{pa}$ and $S_{re}$) during exercise (kW/m²) was not significantly different for all SETs in HH (Fig. 8A), whereas $S$ during exercise was significantly greater in HD 0 compared with HD 18 (Fig. 8B).
were 1) decreases in resting $T_{pa}$, $T_{re}$, and $T_{mu}$; 2) a similar rate of rise in core temperature during moderate-intensity exercise (and therefore no change in exercise duration) in HH; 3) a decrease in $S$ and increase in exercise duration in HD, with postacclimation exercise duration not significantly different from that in cool dry conditions ($\sim 20^\circ C$, 50% RH, 17°C WBGT); 4) a decrease in total fluid loss in both HH and HD; 5) an increase in pre- and postexercise respiratory rate in HH and HD; and 6) a decrease in $\Delta S$ during the first hour of recovery after exercise in both HH and HD.

**Methodology.** Exercise duration during a SET was based on the time needed to attain a $T_{pa}$ of 41.5°C. This temperature criterion was used to prevent increases in core and contracting $T_{mu}$ (to $>43^\circ C$) that may have compromised the health of the horse, particularly in view of the daily exposure to these severe conditions. Another recent study of exercise heat acclimation in horses used a higher $T_{pa}$ criterion (43.5°C), and $T_{pa}$ only approached such high values during a high-intensity (about 75% of $V_{O2max}$) exercise phase; this study also demonstrated a tendency toward increased ability to tolerate high body temperatures (thermal tolerance) after 15 days of heat acclimation (22). The present study was not designed to test for changes in thermal tolerance per se, but rather to examine thermoregulatory responses during a sustained period of steady-state exercise.

One concern with a longitudinal heat acclimation study is that changes in aerobic fitness may confound interpretation of the results. However, there was no change in $V_{O2max}$ after the 3-wk period of daily exercise in the heat. Thus heat acclimation, rather than improved aerobic fitness, likely explains the altered physiological responses.

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Measurements of the rate of S during exercise were calculated on the basis of changes in Tpa and in TRE (Fig. 8). Values calculated by using TRE were 65–75% of those determined by Tpa, reflecting a slower rate of rise and smaller increment in TRE compared with Tpa (Fig. 2.5 vs. 4.0°C). This finding is in agreement with results of previous studies (6, 13, 16). The lag in the TRE response appears to reflect a delay in the transfer of heat to the rectum, perhaps as a result of diversion of blood flow away from the gut during exercise. The implication is that, in the horse, the body cannot be considered a homogeneous mass when determining the effects of exercise-heat stress on S (15).

Our calculations of Ereq were on the basis of measurements of VO2 made before the study and assumed no change in Mnet after acclimation. However, studies in humans have demonstrated an ~3–4% reduction in metabolic rate during submaximal exercise after heat acclimation (31, 38), perhaps as a result of improved muscular efficiency (17). Similarly, Marlin et al. (22) reported an ~5% decrease in heat production in horses during variable-intensity exercise after 15 days of humid heat acclimation. It is therefore possible that we overestimated Ereq and the HSI after acclimation.

Heat acclimation: resting responses. Two weeks of exercise heat acclimation were sufficient to result in beneficial adaptive responses in horses at rest. Resting TRE and Tpa, measured on entry to the laboratory, were decreased by ~0.5°C compared with HH 0 (Figs. 4 and 5). In humans, ~5–10 days of acclimation to HD (9, 30, 35) and HH (2, 4, 26, 27) conditions significantly reduced resting core temperature by ~0.5°C. Similar decreases in resting TRE and Tpa have been reported in horses after 6 (21) and 15 days (22) of humid heat acclimation. In humans, the reduction in resting core temperature after heat acclimation appears to be of benefit because it coincides with decreases in thermoregulatory thresholds for the onset of sweating and cutaneous vasodilation (2, 27). In the present study, a similar decrease in the core temperature sweating threshold was also evident by HH 14 (25), and a similar tendency was observed among the five horses in the study by Marlin et al. (22). In humans, the lower resting body temperature also contributes to improvement in exercise performance by allowing the acclimated individual to exercise for a longer time period before attainment of a critical temperature (10, 28, 29).

In humans (36, 37) and horses (22, 25), the predominant mechanisms for the reduction in resting body temperatures with heat acclimation include an increased ability to dissipate heat by sweating and an improved convective flow of heat from within the body to the skin. In horses, however, the respiratory system also plays an important role in heat dissipation (12). In the present study, the two- to threefold increase in resting respiratory rate in hot conditions during 21 days of heat acclimation strongly suggests that increased respiratory heat loss played a role in lowering TRE and Tpa at rest. Marlin et al. (22) also reported a significant increase in the respiratory rate of horses at rest and during low-intensity exercise (trot) after humid heat acclimation. The mechanism responsible for the increased respiratory rate with the decline in core temperature is not known. However, anticipation of the thermoregulatory demands of exercise by the respiratory controller can be a “learned” response during exercise training (3). In the horses in this study, within 1 wk of exercise heat acclimation, there was a significantly enhanced thermal drive to increase respiratory evaporative heat loss during the 60-min preexercise period.

Exercise responses. In the unacclimated state, the physiological demands imposed on the thermoregulatory system of the horse during moderate-intensity exercise in hot ambient conditions were reflected in the substantial reductions in exercise duration during the SETs in HH and HD compared with CD (Fig. 1 and Ref. 8). In all conditions, exercise at an intensity of 50% of VO2max resulted in uncompensable heat stress (Ereq > Emax). Given the physical limitation to evaporative heat loss in HH (Ereq ~2.5-fold > Emax), we anticipated minimal change in exercise performance after humid heat acclimation. Indeed, 21 days of exercise-humid heat stress had no effect on the rate of rise in Tpa or on time to an
end-exercise $T_{pa}$ of 41.5°C during SETs, and, therefore, there was no increase in duration of exercise performed at 50% of $V_{O2\ max}$. Furthermore, the extent of the increase in $T_{pa}$ and $T_{mu}$ during exercise was unaltered by heat acclimation. Therefore, the lower end-exercise $T_{re}$ evident by day 7 in HH (Fig. 5A) was due to the lower resting (preexercise) $T_{re}$, and $S$ was also unchanged after acclimation (Fig. 7A).

These findings differ from those of other reports of humid heat acclimation in horses. We have previously reported a progressive decrease in $S$ during daily exposure to and exercise in hot humid conditions (WBGT 31–32°C) (7). However, the decrease in $S$ was due to attenuation of the rise in $T_{re}$ during preexercise heat exposure; as in the present study, the net increase in $T_{re}$ during exercise was unchanged during exercise. Marlin et al. (22) reported an increase in $S$ in horses during a simulated speed and endurance test of a 3-day event after 15 days of active heat acclimation. However, because exercise duration was longer and horses attained a higher $T_{pa}$ during the exercise test after acclimation, this increase in $S$ probably reflected the additional work performed.

The similar rate of rise in $T_{pa}$ during exercise in SETs in HH conditions (0.225 vs. 0.093°C/min in CD conditions) reflects the limitations imposed by the combination of high temperature and high RH. This rate of increase in $T_{pa}$ is ~3.4-fold higher than the rate of rise in esophageal temperature in human subjects exercising at 45% $V_{O2\ max}$ in similar hot humid conditions (0.066°C/min) (19, 28). This species difference reflects the higher rate of heat production and the physical constraints on convective, conductive, and evaporative heat loss due to a higher body mass-to-surface area ratio in horses.

In contrast to the circumstance in HH, when horses were exercised in HD ($E_{req} \sim 1.6$-fold $> E_{max}$) conditions after 18 days of humid heat acclimation, they demonstrated a significant ~30% increase in exercise duration. In fact, rate of rise in $T_{re}$ and time to attainment of a $T_{pa}$ of 41.5°C during HD 18 were similar to those achieved during CD 0. The rate of $S$ during exercise in HD was decreased by ~15% (Figs. 7B and 8B). Similar improvements in exercise performance in dry heat conditions have been reported in humans after 7–10 days of heat acclimation (1, 28), but this improvement is mitigated when subjects exercise in warm humid conditions (29).

The reduction in the rate of $S$ during exercise on HD 18, compared with exercise in the heat in the unacclimated state (HD 0), may have been the result of reduced metabolic rate and/or improved heat dissipation. As mentioned, Marlin et al. (22) reported an ~5% reduction in exercise $V_{O2}$ in horses after 15 days of humid heat acclimation. It is also possible that enhanced sweating responses contributed to improved heat dissipation in HD conditions (25), whereas high humidity limited the efficacy of any improvements in cutaneous evaporative heat loss in the HH environment.

In humans, acute exercise-heat stress elicits higher HR than for exercise in temperate conditions. During the first 3–7 days of heat acclimation, there is a gradual reduction in HR during exercise in association with higher stroke volume and lower core ($T_{re}$ or esophageal) and skin temperatures (for review, see Ref. 36). In the present study, acute humid heat stress (HH 0), but not dry heat stress (HD 0), resulted in a significant increase in HR during exercise compared with cooler conditions (CD) (Fig. 2). However, during exercise in the latter SETs in HH, HR was not significantly different from HR during exercise in CD or in HD (0 and 18). The reason for the higher HR during exercise in HH 0 is unclear. In accord with findings in humans, it is possible that the decrease in HR during subsequent SETs in HH reflected a true heat adaptive response. However, the lack of change in exercise HR in HD (0 vs. 18) or alteration in $T_{sk}$ responses (HH and HD) after acclimation argues against this interpretation. Furthermore, Marlin et al. (22) reported that mean HR in horses during exercise was unchanged after a 15-day period of heat acclimation. Perhaps high sympathetic nervous activity associated with naïve exposure to the hot humid conditions is an alternative explanation for the higher HR in HH 0.

**Recovery responses.** Heat acclimation resulted in a decreased rate of heat dissipation during recovery compared with the SETs on HH 0 and HD 0 (Fig. 7). This reduced rate of heat dissipation was reflected in a slowed rate of decline in $T_{pa}$ and $T_{re}$ (Fig. 4 and 5), a more rapid decrease in sweating rate, and lower post-exercise sweating rates (25). An apparent increase in sweating efficiency after heat acclimation, i.e., reduction in excessive sweat fluid loss (25), resulted in an increased duration of elevated core temperature during recovery (Fig. 4). An enhanced sweating efficiency was also evident in HD 18, compared with HD 0, and was associated with a smaller decrease in body water loss, despite the longer exercise duration (Table 1). Presumably this conservation of sweat fluid, and the resultant decrease in cutaneous evaporative cooling, was partially offset by the increased postexercise respiratory rates compared with those measured before acclimation.

In conclusion, this study demonstrated that exposure to and exercise in hot humid conditions for a period of 21 consecutive days resulted in thermoregulatory adaptations at rest and during exercise in dry heat conditions. Heat acclimation resulted in decreased $S$ before (in hot humid and hot dry conditions) and during exercise (in hot dry conditions), enhanced (thermosensitive) ability to dissipate heat by increased respiratory rate, and improved exercise performance as measured by run time to a predetermined $T_{pa}$ (41.5°C) during exercise in hot dry but not in hot humid conditions. It is evident that the greater thermoregulatory limitations to exercise imposed on the equine athlete, compared with their human counterparts, are accentuated in environmental conditions of high temperature and relative humidity. The physical limita-
tions are primarily imposed by the extensive muscle mass, the high mass-specific rate of metabolic heat production, and the low mass-specific surface area for heat dissipation. Therefore, there remains a strong need to carefully consider exercise intensity and duration as key determinants of performance limits in horses exercising in hot or humid conditions.

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REFERENCES


35. Shvartz E, Bhattacharya A, Sperinde SJ, Brock PJ, Sciaraffa D, and Van Beaumont W. Sweating responses dur-

