Heat acclimation improves regulation of plasma volume and plasma Na\(^+\) content during exercise in horses

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Lindinger, Michael I., L. J. McCutcheon, G. L. Ecker, and R. J. Geor. Heat acclimation improves regulation of plasma volume and plasma Na\(^+\) content during exercise in horses. J. Appl. Physiol. 88: 1006–1013, 2000.—This study determined the plasma volume (PV) and ion responses to heat acclimation and exercise in six trained Thoroughbred horses during 21 days of exposure to heat and humidity (33°C, 83% relative humidity) for 4 h/day. During the 2nd h on days 0, 3, 7, 14, and 21, horses performed a standardized treadmill test, running at 50% of peak \(\mathrm{O}_2\) uptake until pulmonary artery temperature reached 41.5°C. Heat acclimation resulted in an increase in PV from 21.3 ± 1.1 liters on day 0 to 24.3 ± 1.0 liters on day 14, returning to 22.6 ± 0.9 liters on day 21. The corresponding total plasma protein contents were 1,273 ± 53, 1,455 ± 81, and 1,377 ± 57 g, respectively, and increases in total plasma Na\(^+\) plus Cl\(^-\) content were 5,145 ± 126, 5,749 ± 146, and 5,394 ± 114 mmol, respectively. Thus changes in PV were accompanied by direct changes in plasma protein and osmolyte contents. With exercise on day 0, PV decreased by 7.1 ± 0.7% at 5 min of exercise and remained decreased (−6.7 ± 1.3%) at 5 min of recovery. By day 21, PV decreased significantly less than on day 0 (by 5.2 ± 0.9% at 5 min of exercise), was decreased by only 2.0 ± 1.6% at 5 min of recovery, and was fully restored at 15 min of recovery. Plasma Na\(^+\) concentration increased 3 meq/l during the first 5 min of exercise and was normalized by 5 min of recovery on day 0 and by end exercise on day 21. It is concluded that improved ability to regulate PV during exercise in response to heat acclimatization is associated with an increased PV and an improved conservation of Na\(^+\).

thermoregulation; heat stress; humidity; Atlanta Summer Olympic Games

COMPARED WITH THE MANY thermoregulatory studies conducted on humans over the past century, reviewed by Werner (39), there has been a dearth of research conducted on the effects of hot environmental conditions on exercising horses until the past six years. Compared with humans, horses are at a physical disadvantage for heat dissipation because of an approximately fivefold lower ratio of contracting muscle mass to skin surface area (for reviews see Refs. 11 and 17). This results in greatly elevated rates of heat storage and a rapid rise to critical core temperatures during exercise at submaximal intensities, even under cool, dry conditions (11, 14, 19). Such rapid increases in core temperature pose a serious problem for horses exercising in hot conditions because of the reduced gradient for heat dissipation to the environment. The underlying purpose of the present study was, therefore, to determine whether horses could acclimatize to hot, humid conditions and, if so, to determine the time required to confer beneficial adaptations associated with improved maintenance of fluid balance, thermoregulation, and exercise performance.

In humans the increase in plasma volume (PV) during daily exposure to the heat is an important mechanism contributing to physiological heat acclimation responses (5, 38, 39). Such an adaptive mechanism is attractive given the impairments to physiological function and exercise performance that occur when body water stores are inadequate to meet simultaneous cardiovascular and thermoregulatory demands (35). The increased thermoregulatory and cardiovascular stability conferred by adaptive increases in PV is of benefit for prolonging exercise, particularly in the heat (5, 38, 43). Similar advantages appear to accrue from the large increases in PV (up to 29%) that occur in horses during exercise training (29).

In humans, specific thermoregulatory advantages include a lower temperature threshold for the onset of sweating (35, 39) and cutaneous vasodilation (25), an increased sweating rate leading to an increase in evaporative cooling (39), increased perfusion of the skin facilitating convective flow of heat from contracting muscles to the environment (25), and a lowered core body temperature at rest (4); the last named has also been reported in horses (14). Together, these adaptations allow for a slowed rate of increase in core temperature during activity, a decreased rate of muscular heat production, and an increased heat storage capacity that may result in increased exercise duration and/or intensity (14). The cardiovascular advantages primarily relate to an increase in stroke volume due to improved maintenance of central venous pressure (5, 16), such that cardiac output is maintained at a lower heart rate for exercise at a given intensity (14, 16).

The present study tested the hypothesis that daily exposure to hot humid conditions (simulating a worst-case scenario for the Atlanta Olympic Games), with a period of exercise performed in these conditions, would result in increased PV at rest and improved maintenance of fluid balance during exercise.

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METHODS

The care and use of animals followed the Guideto 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. All experiments were conducted during the fall and winter, and the horses received no other exercise during the entire period of study.

Animals. Six Thoroughbred horses ranging in age from 3 to 6 yr and weighing 414–505 kg [455 ± 12 (SE) kg] were studied. All the horses were subjected to surgical relocation of the right carotid artery to a subcutaneous position before the study. Horses were housed individually indoors at 16–19°C. During the exercise training and study period, horses were maintained on a diet of grass hay, mixed-grain ration (Professional Horse Mix, Ralston Purina) and 150 g of mineral mix (complete mineral mix, Equine Minerals, Gold River, BC) daily. All horses were maintained on a diet of grass hay, mixed-grain ration (Professional Horse Mix, Ralston Purina) and 150 g of mineral mix (complete mineral mix, Equine Minerals, Gold River, BC) daily.

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 (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 2–3 min at 9 m/s by the 10th wk of training. Access to water was withheld overnight (12 h), and water was provided immediately after exercise. Access to water was provided throughout the training period to train the horses to drink cool (~15°C) water after exercise (within 5 min of completion). All training was conducted under cool, dry (CD) conditions [CD temperature (RT), 20°C room temperature (RT), 45–50% relative humidity]. The maximal O₂ uptake (V\textsubscript{O₂max}) of each horse was determined during exercise in the 8th and 10th wk of training and after completion of the subsequent 3-wk period, in which exercise was undertaken in the heat.

Experimental protocol. After the 10-wk training period, each horse completed a standardized exercise test (SET) that evaluated sweat and ion losses under hot, humid (HH) conditions (HH: RT = 32–34°C, relative humidity = 80–85%). The 10 wk of training in CD and the initial SET (day 0) were followed by 21 consecutive days during which the horses were exposed to, and trained in, HH for 4 h between 0700 and 1100. This daily exercise training protocol was undertaken in a treadmill room in which the stated temperature and relative humidity for HH were maintained throughout the 4-h period. In addition to the initial SET completed on day 0 (before the 21 days of heat training), on days 3, 7, 11, 14, and 21 of the heat acclimation period the horses completed the SET instead of the daily exercise protocol.

SET. Food was withheld overnight (12 h), and water was withheld for 3 h before and for the duration of each experiment. Body mass was measured on a large animal scale (KSL Scales, Kitchener, ON, Canada) immediately before exercise, during which the horses remained stationary for 3 h before and for the duration of each experiment. All blood samples were collected at 10-min intervals to determine PV at rest (9). Samples were centrifuged within 10 min of collection, and the plasma was collected for analysis. Carotid and pulmonary artery blood samples for measurement of hematocrit, total plasma protein content (ctPP), plasma osmolality, and plasma ion concentrations were collected at rest and at 2, 5, 15, and 30 min of exercise, with an additional sample collected during the final minute of exercise, and at 2, 5, 15, 30, and 60 min of recovery.

Analyses. Most (~6 ml) of the blood was transferred to polyethylene centrifuge tubes, and the plasma was separated within 2 min by centrifugation at 15,000 g. The remaining blood was analyzed within 3 min of sampling for packed cell volume, plasma Na⁺ ([Na⁺]), Cl⁻ ([Cl⁻]), and K⁺ ([K⁺]) concentration, pH, and ionized Ca²⁺ ([Ca²⁺]) concentration ([Ca²⁺]) with use of ion-selective electrodes (Statprofile 5 blood gas/ion analyzer, Nova Biomedical, Waltham, MA). A 200-μl aliquot of blood was deproteinized in 600 μl of 6% (wt/vol) perchloric acid, and the supernatant was analyzed for lactate (2). Plasma was analyzed for protein with a clinical refractometer ([protein]r, model SPR-T2, Atago). The remaining plasma was stored at ~20°C and later analyzed for osmolality, determined on duplicate 200-μl samples by freezing-point depression (model 3MO Plus, Advanced Instruments, Needham, MA). The coefficient of variation for duplicate analyses was 1.3%.

Plasma Evans blue dye concentration was determined spectrophotometrically using the dual-wavelength (620 and 740 nm) absorbance technique (9). This method avoids the problems associated with absorbance changes due to the presence of Hb and other interfering substances, of which even very small amounts may render invalid single-wavelength (620 nm) recordings.

Calculations. Total body water loss was taken as the change in body mass after fecal and urine losses and water intake were accounted for.

The PV before exercise was calculated from the dilution of a known amount of Evans blue dye infused into a jugular vein. The increase in plasma absorbance at 620 nm, after correction for absorbance changes at 740 nm due to interfering substances, yields an Evans blue distribution volume for the time of blood sampling. Because the distribution volume increases with time, at least three plasma samples obtained 10 min apart were analyzed. The corrected absorbances were fitted to a linear regression against time, and the initial PV was calculated by extrapolation to time 0 (9).
The change in PV was calculated using plasma protein concentration ([PP]; the percent change in PV (dPV) was calculated as

\[
dPV(\%) = \frac{[PP]_0 - [PP]_t}{[PP]_0} \times 100
\]

where [PP]_0 and [PP]_t are [PP] at time 0 and time t.

Plasma ion content considers the simultaneous changes in plasma ion concentrations and PV that result from the simultaneous net addition or loss of ion and water from the plasma compartment. Plasma ion contents were calculated as the product of measured concentration (mmol/l of plasma) and PV. The PV at time t was calculated from the preexercise PV and the dPV at time t.

Statistics. Values are means ± SE. Data were analyzed by two-way ANOVA with repeated measures to compare measures over time and among trials. A repeated-measures two-way ANOVA was also used to compare measures over time and between blood sampling sites. When a significant F ratio was obtained, a one-way ANOVA with repeated measures was used to compare means among time points, among days of acclimation, or between blood sampling sites. The Bonferroni post hoc test was used to test for differences among means. Significance was accepted at P ≤ 0.05.

RESULTS

There were few differences between the carotid artery and the pulmonary artery for the reported blood and plasma parameters at rest and during exercise and recovery. Values are from the carotid artery sampling site unless otherwise noted.

Resting parameters. The first 14 days of heat acclimation resulted in progressive increases in PV and the total plasma contents of protein, Na\(^{+}\), Cl\(^{-}\), and Ca\(^{2+}\) (Fig. 1). This was followed by a return toward preacclimation values by day 21. The increase in PV was associated with a decrease in hematocrit from 36.3 ± 0.9% on day 0 to 33.3 ± 1.4% on days 3–21. The increase in PV was directly and linearly related to increases in total plasma protein content (ctPP, g), total plasma Na\(^{+}\) content (ctNa, mmol), and total plasma Cl\(^{-}\) content (ctCl, mmol)

\[
\begin{align*}
PV & = 4.72 \pm 2.55 + 0.0134 \pm 0.0019 \\
& \times ctPP \ (n = 30, \ r^2 = 0.644, \ P < 0.001) \\
PV & = -0.38 \pm 0.78 + 0.00745 \pm 0.00025 \\
& \times ctNa \ (n = 30, \ r^2 = 0.969, \ P < 0.001) \\
PV & = 1.27 \pm 0.98 + 0.0092 \pm 0.0004 \\
& \times ctCl \ (n = 30, \ r^2 = 0.949, \ P < 0.001)
\end{align*}
\]

Body mass at rest before exercise decreased from 454 ± 13 kg on day 0 to 452 ± 13, 450 ± 13, 450 ± 12, and 446 ± 12 kg on days 3, 7, 14, and 21, respectively; the mean was significantly less on day 21 than on day 0.

Exercise responses. During exercise on day 0, [PP] increased by 5.0 ± 1.0 g/l within 5 min and remained at these peak values until the end of exercise at 19.1 ± 1.4 min, when a pulmonary artery blood temperature of 41.5°C was reached (Fig. 2). Similar, although smaller, 2–4 g/l increases in [PP] occurred with exercise on days 3–21. A peak [PP] consistently occurred during the first 5 min of exercise, followed by a partial recovery of [PP] during the remainder of the exercise.

A rapid 4–6% decrease occurred in PV during the first 2 min of exercise, with the peak decrease in PV at 5 min with each exercise trial (Fig. 3). The greatest decrease in PV occurred on day 0 (−7.1 ± 0.7%) and the least on day 3 (−4.4 ± 1.3%), when PV was greatest. As exercise continued, there was a partial recovery of PV, except on day 0. The greatest recovery of PV during exercise occurred on day 21, where, at the end of exercise, PV was not significantly different from the preexercise values. Except on day 0, where recovery of PV did not occur until 60 min after cessation of exercise, there was a rapid recovery of PV during the first 15 min of recovery, such that at 30 min the PV values were similar to those preexercise.

The magnitude and time course of the exercise-induced hematocrit responses were similar among all
days. In general, hematocrit increased from 35 to 45% at 5 min of exercise and remained at these high values until exercise stopped. Within 5 min of termination of exercise, hematocrit fell to ~40%, with a further slow decrease to 36% at 60 min.

Plasma \([\text{Na}^+]\) (Fig. 4) and \([\text{Cl}^-]\) (not shown) tended \((P, 0.1)\) to increase during exercise, peaking at 5 min. This was followed by a return to preexercise values by the end of exercise. Plasma \([\text{Na}^+]\) and \([\text{Cl}^-]\) were significantly decreased during the last 30 min of recovery from exercise on all days. The exercise-induced increases in plasma \([\text{Na}^+]\) (Fig. 4B) and \([\text{Cl}^-]\) (not shown) were significantly different between the carotid and pulmonary artery sampling sites. The rate and magnitude of increase in plasma \([\text{Na}^+]\) and \([\text{Cl}^-]\) were about twofold greater in the pulmonary than in the carotid artery; pulmonary artery values remained higher during exercise but were similar during recovery. The greater electrolyte concentrations in pulmonary than in carotid artery blood is thought to reflect a net movement of water into contracting muscle during the first several minutes of exercise (24).

Fig. 2. Time course of plasma protein concentration ([protein]) during exercise and recovery in hot, humid conditions with 0 (■), 3 (△), 7 (●), 14 (●), and 21 (○) days of acclimation to hot, humid conditions. Values are means ± SE; n = 6. *Significantly different from time 0. Time courses on days 3, 7, 14, and 21 are significantly different from that on day 0. Most error bars have been omitted for clarity; error bars shown are representative of those missing.

Fig. 3. Time course of change in plasma volume during exercise and recovery in hot, humid conditions with 0 (■), 3 (△), 7 (●), 14 (●), and 21 (○) days of acclimation to hot, humid conditions. Values are means ± SE; n = 6. *Significantly different from time 0. Time courses on days 3, 7, 14, and 21 are significantly different from that on day 0. Most error bars have been omitted for clarity; error bars shown are representative of those missing.

Fig. 4. A: time course of carotid artery (CA) plasma \([\text{Na}^+]\) concentration ([Na]) during exercise and recovery in hot, humid conditions with 0 (■), 3 (△), and 14 (●) days of acclimation to hot, humid conditions. Values are means ± SE; n = 6. *Significantly different from time 0. There were no differences among days 0–21, and data from days 3 and 21 (CA data in B) have been omitted for clarity. Most error bars have been omitted for clarity; error bars shown are representative of those missing. B: time course of carotid artery (open symbols) and pulmonary artery (solid symbols) plasma \([\text{Na}^+]\) during exercise and recovery in hot, humid conditions with 3 (triangles) and 21 (circles) days of acclimation to hot, humid conditions. Values are means ± SE; n = 6. *Significantly different from time 0. At 2, 5, 15, and 27 (mean end-exercise time) min of exercise, pulmonary artery means are significantly less than corresponding carotid artery means. There were no differences among days 0–21. Error bars have been omitted for clarity; error bars in A are representative.
The present study shows for the first time in horses that acclimation to HH results in an increase in PV and an improved ability to regulate PV and Na\textsuperscript{+} content during exercise in HH. Specifically, heat acclimation resulted in a time-dependent expansion of PV that can be attributed to increases in plasma protein, Na\textsuperscript{+}, and Cl\textsuperscript{−} contents. These adaptations were not induced by exercise training, because these animals were already trained to the required level of activity for \( \geq 10 \) wk before the start of the study, and there was no change in \( V_{O2max} \) before and after 21 days of heat acclimation. It is believed that these PV adaptations confer an improved ability to thermoregulate by cutaneous heat dissipation during submaximal exercise in the heat (14).

**DISCUSSION**

The change in PV during exercise and recovery was determined from [PP]. This calculation assumes that there is no net gain or loss of protein from the vascular compartment during exercise and recovery. Although this assumption appears to hold during exercise in humans (15, 44), there is a rapid net gain of protein, primarily albumin derived from interstitial fluids, by the vascular compartment during recovery from exercise (37, 44). The early report by Senay (37) of net protein influx to the vascular compartment during exercise is based on the assumption of a stable red cell mass during exercise. However, there is good evidence that splenic contraction during exercise in humans accounts for \( \approx 25\% \) of the increase in hematocrit (22, 40). Such an addition of red blood cells to the circulation during exercise would lead to the erroneous interpretation of an “apparent” gain of protein when hematocrit is used to calculate changes in PV. If a loss of vascular albumin occurs during moderate-intensity exercise in horses, as shown during high-intensity exercise (7), then we may have underestimated the changes in PV during exercise and recovery.

In the present study, 3 days of active heat acclimation resulted in a 5% increase in PV, with a total increase of \( \approx 14\% \) on day 7. This is similar to the increase in PV in exercise-trained humans during heat acclimation/acclimatization (37, 41). In the present study, however, after the initial expansion of PV during the 1st wk of heat acclimation, there occurred a 10% reduction in peak PV, such that PV remained expanded by 5% after 21 days of heat acclimation. Mechanisms that may be responsible for the increase in PV with daily active heat exposure include a net influx and retention of protein within the vascular compartment (37), de novo synthesis of protein that is largely retained in the vascular compartment (44), and retention of Na\textsuperscript{+}, Cl\textsuperscript{−}, and water (1). It appears that all three of these mechanisms may be involved, but their relative importance changes during the time course of the exercise recovery/heat acclimation processes.

Retention of plasma proteins. In the present study, plasma protein content increased progressively by 14% during the first 14 days of heat acclimation. In humans, Senay (37) reported a 23% increase in total circulating protein within 6 days of heat acclimation, which then remained stable to day 10. In humans, expansion of PV during heat acclimation (37) and exercise training (5, 6) was correlated to ctPP, such that each 1 g of protein added (about two-thirds of total protein added was albumin) to the vascular compartment is associated with a 15- to 18-ml increase in PV (36). A markedly similar response occurred in the present study, where a mean increase in protein content of 175 g was associated with a 2.8-liter increase in PV (Fig. 1), equivalent to 16 ml water/g protein.

Senay (37) appears to have been the first to demonstrate that, in trained subjects exercising in cool conditions, a net influx of protein into the vascular compartment immediately on recovery from exercise was not accompanied by retention of protein in the vascular compartment; however, when subjects exercised in the...
heat, there was a prolonged “retention” of protein in the vascular compartment. The adaptive mechanisms responsible for prolonged vascular retention of protein with heat acclimation are poorly understood, with most of the recent research focused on protein shifts during and after exercise (6, 15, 44). On the basis of the available evidence, the time course of increase in ctPP resulting from daily exercise in the heat appears to be as follows. With cessation of exercise there is a rapid increase in ctPP that appears to be due to net influx of protein from interstitial compartments (38, 44). In our horses, interstitial fluid volume was ~80 liters (approximately quadruple the PV) with a total protein content of ~2,800 g [protein concentration of 35 g/l (18)], such that ~30 ml of fluid are associated with each 1 g of protein (36). Clearly, the net increases in vascular water and protein contents are small compared with the capacity of the interstitial compartment, but it appears that small disturbances to interstitial fluid homeostasis evoke compensatory mechanisms to restore volume and composition. For example, cessation of exercise is associated with an increase in albumin synthetic rate leading to a net gain of protein in the vascular (44) and interstitial fluid (18) compartments. It is not known how long protein synthetic rates remain elevated. However, it is suggested that repeated exercise in the heat may result in a sustained net increase in the rates of plasma protein synthesis, leading to expansion of vascular and interstitial compartments.

Retention of plasma osmolytes. The expansion of PV during heat acclimation in horses was associated with increases in plasma Na⁺ and Cl⁻ contents. This response is similar to that seen in humans with heat acclimation/acclimatization (1, 38) and postexercise PV expansion (44). Characteristic of all such studies is that the increase in osmistically active particles matches the increase in PV, such that there is no change in plasma [Na⁺] and [Cl⁻] in resting animals. Senay et al. (38) raised the issue of whether the increase in PV is primarily osmotic (1) or oncotic (38) in origin, but this may be a moot point, since both characteristically occur simultaneously. This may imply integration among mechanisms regulating plasma composition or may simply reflect physical constraints that maintain water and ionic equilibria between vascular and interstitial fluid compartments.

At the endocrine level, fluid and electrolyte balance are regulated by a number of hormones that are secreted in response to mechanical and osmotic stimuli, including the renin-aldosterone system, arginine vasopressin (AVP), and atrial natriuretic peptide (ANP). In humans (10, 30) and horses (27), exercise is accompanied by increases in renin activity, aldosterone, AVP, and ANP that appear to occur in response to exercise-induced decreases in PV and, importantly, act to aid in the restoration of PV on cessation of exercise. Although these responses are short term, they appear to lead to long-term control mechanisms when exercise training occurs and probably during heat acclimation as well (5, 20). In the short term, the increase in PV with exercise training in horses is associated with decreased excretion of water, Na⁺, K⁺, and Cl⁻; however, an increase in free water clearance with decreased fractional clearance of electrolytes suggests activation of mechanisms for tubular conservation of electrolytes (28). In humans, similar coupling of Na⁺ and water retention in response to exercise training (6, 10) and heat (30) occurs in association with activation of the renin-angiotensin-aldosterone system with lowering of plasma ANP. In the long term, after 1 and 2 wk of exercise training in horses, once plasma Na⁺ content has increased, plasma AVP and aldosterone concentrations are similar to control values, as is renal Na⁺ clearance (29). Thus, in horses and humans, there appears to be a rapid initial adaptation that involves renal mechanisms of electrolyte reabsorption. After 1 wk of exercise training in horses, a maintained increase in free water clearance with reduced urea and osmolar clearances suggests that reabsorption of urea and other non-Na⁺ osmolytes may contribute to longer-term maintenance of training-induced hypervolemia (29). The contributions of plasma urea and Na⁺ to the regulation of PV remain to be determined during heat acclimation in horses.

Long-term control of vascular volume. In the present study, heat acclimation resulted in an initial increase in PV over the first 14 days that was followed by a partial restoration of PV toward precacclimation values. The mechanisms involved in the control of PV during heat acclimation are poorly understood and have only recently begun to be investigated. It is worth noting that the initial increase in PV may be accompanied by inhibition of the renin-aldosterone system, which allows PV to expand (16). As cellular adaptations occur to thermoregulatory and metabolic effector tissues and organs, which result in increased efficiency of heat dissipation (20), there appears to be a decreased requirement for an expanded PV, and PV consequently decreases from peak values.

Exercise responses. The onset of exercise is associated with a rapid decrease in PV that is attributed to the net movement of fluid from the vascular compartment into the contracting muscle cells as a result of rapidly increasing osmolality in these cells and increases in capillary hydrostatic pressure (22, 44). When ambient conditions are cool, as exercise continues at submaximal intensities, there typically occurs a partial recovery of PV as water returns to the vascular component (23) simultaneously with the partial recovery of muscle phosphocreatine stores and lactate efflux (24). As previously shown (23), the partial recovery of PV did not occur on day 0 during exercise in HH, indicating that the intensity of exercise was sufficiently high to prevent appreciable return of fluid to the vascular compartment.

It is noteworthy that the greatest exercise-induced decrease in PV occurred on day 0, when PV was smallest, and least on day 3, after the initial rapid expansion of PV. When PV was greatest (day 7), the exercise-induced decrease in PV was intermediate in magnitude of response. Heat acclimation also resulted in a gradual improvement in the regulation of PV.
 during the period of exercise. Specifically, by the end of exercise on day 21, PV recovered to values that were not significantly different from preexercise PV. These results are consistent with the reduced sweat fluid loss (and, hence, reduced body weight loss) and a reduction in peak sweating rate seen during the period of exercise on days 14 and 21 (26). Together, these results suggest that improved regulation of vascular volume may be effected through improved sweating efficiency and through shifts of fluid among contracting muscle, non-contracting tissues, and the vascular and lymph compartments (21, 24, 44). Within contracting muscles, a reduced rate of anaerobic energy provision after heat acclimation and improved aerobic energy provision [in humans (8)] during exercise could account for the reduced magnitude of PV decrease at the onset of exercise. The improved recovery of PV during exercise is consistent with a more rapid resynthesis of phosphocreatine (33) and increased rate of lactate efflux from muscle (3). It is suggested that both of these result in an increased rate of restoration of intracellular osmolarity, effectively releasing water that moved into the cell’s with the onset of exercise. Such a shift of fluid from contracting muscle back to the vascular compartment would clearly be beneficial for improving perfusion of skin and contracting muscle. It can be concluded, as occurs with exercise training in humans (5), that adaptations to skeletal muscle metabolism (8) attenuate the net shift of fluid from the vascular compartment to contracting skeletal muscle at the onset of exercise and increase the return of fluid to the vascular compartment during exercise. During the period of heat acclimation, the progressive improvement in recovery of PV after cessation of exercise is also consistent with a decreased contribution of anaerobic muscle metabolism to meet energy demands (8, 33) and decreased sweating rates (26). A markedly slowed rate of heat storage after heat acclimation was associated with a 10% increase in the rate of heat dissipation (14), suggesting that there was also a reduced rate of metabolic heat production.

In humans and as now shown in horses, heat acclimation-induced expansion of PV appears to be a fundamental physiological adaptation conferring thermoregulatory and cardiovascular advantages during exercise. This key adaptation appears to be fundamental to an increased convective flow of blood from heat production sites to the periphery and an earlier onset of evaporative cooling. In humans, this scenario is successful in improving exercise performance/duration, because during exercise in the heat there is no reduction in blood flow to contracting muscles (32, 34); therefore, an increase in vascular volume should result in improved perfusion of the skin. In horses, however, it is possible that metabolic blood flow demands are not being met, because the mass of contracting muscle is so high. If this is so, then heat acclimation-induced increases in vascular volume may be primarily associated with increased perfusion of the contracting muscles, or increased perfusion of the skin and contracting muscle may occur. What is known in horses is that there is little difference in the rate of increase in core tempera-


In conclusion, heat acclimation in horses resulted in an expansion of PV that was linked to increases in plasma protein, Na^+ and Cl^- contents. These adaptations were associated with an improved ability to regulate PV during the period of exercise and a more rapid recovery of PV during recovery from exercise. It is probable that the increase in PV is responsible for improved skin and skeletal muscle perfusion, accounting for the improved transfer from core to body surface shown in our previous study (14).

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