Preexercise hypervolemia does not affect arterial hypoxemia in Thoroughbreds performing short-term high-intensity exercise

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Manohar, Murli, Thomas E. Goetz, and Aslam S. Hassan. Preexercise hypervolemia does not affect arterial hypoxemia in Thoroughbreds performing short-term high-intensity exercise. J Appl Physiol 94: 2135–2144, 2003. First published January 31, 2003; 10.1152/japplphysiol.00973.2002.—It is reported that preexercise hyperhydration caused arterial O₂ tension of horses performing submaximal exercise to decrease further by 15 Torr (Sosa-Leon L, Hodgson DR, Evans DL, Ray SP, Carlson GP, and Rose RJ. Equine Vet J Suppl 34: 425–429, 2002). Because hydration status is important to optimal athletic performance and thermoregulation during exercise, the present study examined whether preexercise induction of hypervolemia would similarly accentuate the arterial hypoxemia in Thoroughbreds performing short-term high-intensity exercise. Two sets of experiments (namely, control and hypervolemia studies) were carried out on seven healthy, exercise-trained Thoroughbreds in random order, 7 days apart. In resting horses, an 18.0 ± 1.8% increase in plasma volume was induced with NaCl (0.30–0.45 g/kg dissolved in 1,500 ml H₂O) administered via a nasogastric tube, 285–290 min preexercise. Blood-gas and pH measurements as well as concentrations of plasma protein, hemoglobin, and blood lactate were determined at rest and during incremental exercise leading to maximal exertion (14 m/s on a 3.5% uphill grade) that induced pulmonary hemorrhage in all horses in both treatments. In both treatments, significant arterial hypoxemia, desaturation of hemoglobin, hypercapnia, acidosis, and hyperthermia developed during maximal exercise, but statistically significant differences between treatments were not found. Thus preexercise 18% expansion of plasma volume failed to significantly affect the development and/or severity of arterial hypoxemia in Thoroughbreds performing maximal exercise. Although blood lactate concentration and arterial pH were unaffected, hemodilution caused in this manner resulted in a significant (P < 0.01) attenuation of the exercise-induced expansion of the arterial-to-mixed venous blood O₂ content gradient.

blood-gas tensions during exercise; hyperhydration; plasma volume; plasma protein concentration

EXERCISE-INDUCED ARTERIAL hypoxemia is routinely observed in strenuously exercising Thoroughbreds (1, 7, 18–21, 29, 30), and it has been reported that the occurrence of arterial hypoxemia limits athletic performance (5, 29). Although “relative” alveolar hyperventilation, as evidenced by the increasing arterial CO₂ tension despite significantly increased alveolar ventilation during strenuous exercise, contributes to the observed reduction in arterial O₂ tension, this mechanism does not account for the entire decrease in arterial O₂ tension observed in exercising horses. Thus it is suggested that both diffusion limitation, probably related to the dramatic shortening of the pulmonary capillary transit time, as well as ventilation-perfusion inhomogeneity also play a role in bringing about exercise-induced arterial hypoxemia in horses (1, 5, 7, 18–21, 29, 30).

Simultaneous with the development of arterial hypoxemia, exercising Thoroughbreds also exhibit significant pulmonary arterial, capillary, and venous hypertension (14–17). The ensuing high transmural pulmonary capillary pressure contributes to the stress failure of pulmonary capillaries (2, 32), resulting in a rather high incidence of exercise-induced pulmonary hemorrhage (EIPH; Refs. 13, 28). Structural changes in the blood-gas barrier associated with stress failure of pulmonary capillaries (10) and exercise-induced arterial hypoxemia are also observed in human subjects performing strenuous exertion (4, 5, 9, 22, 23, 26, 33–35). These observations have aroused considerable interest in determining the role of structural changes in the blood-gas barrier (2, 10, 24, 32) in bringing about exercise-induced arterial hypoxemia. In this context, although there have been several reports in human subjects (4, 5, 9, 26, 33, 34) as well as in Thoroughbred horses (7, 18–21) suggesting that exercise-induced arterial hypoxemia more likely has a functional basis, rather than a structural basis, the issue is far from being completely settled. Arguments against the structural basis for arterial hypoxemia in galloping Thoroughbreds have been that 1) arterial hypoxemia develops rather quickly with onset of strenuous exercise (being well developed at 30 s of high-intensity exercise) and that its severity does not change significantly with increasing exercise duration, even though structural changes in the blood-gas barrier are expected to intensify with increasing exercise duration (7, 18–21); and 2) in healthy Thoroughbreds, during a successive bout of short-term high-intensity exercise performed 6 min after the first high-intensity exercise bout, an accentu-
EXERCISE-INDUCED ARTERIAL HYPOXEMIA IN RACEHORSES

MATERIALS AND METHODS

Horses. Experiments were carried out on seven healthy, sound Thoroughbred horses (3 fillies, 4 geldings), 3–6 yr old, and weighing 459 ± 38 kg. They were exercise trained for a period of 7 wk before undertaking the blood-gas studies (7, 8, 18–21). The horses were housed in an air-conditioned building, and were accustomed to being handled by people. They were fed a diet of alfalfa hay and oats, and free access to water was provided. The horses were dewormed periodically and were inoculated with tetanus toxoid and strangles vaccine. Our protocols and procedures were approved by the Institutional Animal Care and Use Committees.

Exercise training. After the horses were familiarized with walking, trotting, cantering, and galloping on the high-speed treadmill for 1 wk, all horses were exercised 3 days/wk in the following manner with the treadmill set on the flat, i.e., 0% grade. Beginning with a walk at 2 m/s for 120 s, belt speed was increased at the rate of 1 m/s every 60 s until the horse had trotted at 6 m/s for 60 s. Treadmill speed was then raised to 8 m/s, and the horses were cantered for 60 s. Cantering was followed by galloping at 14 m/s for 120 s. Belt speed was then decreased, first to 5 m/s for 60 s and then to 2 m/s for 300 s before the treadmill was stopped. After initial exercise training for 4 wk in this manner, for the next 3 wk, this incremental exercise regimen was performed 3 days/wk with the treadmill set at a 3.5% uphill grade.

Work intensity eliciting EIPH. Because occurrence of EIPH in horses demonstrates that capillary stress failure and related structural changes in the blood-gas barrier have occurred (2, 32), for the present study we intended to use a workload capable of eliciting EIPH consistently. Trials to ascertain work intensity needed to elicit maximal heart rate and EIPH were undertaken on completion of the above-described exercise training. In agreement with previous work (7, 18–21), it was observed that galloping at 14 m/s on a 3.5% uphill grade not only exceeded the normal work load but also induced EIPH in all horses as demonstrated by the presence of fresh blood in the trachea on postexercise airway endoscopic examination (13, 14, 28). It was also observed in these trials that our horses could not sustain galloping at 14 m/s on a 3.5% uphill grade for >120 s despite vigorous humane encouragement. Thus this workload, i.e., 14 m/s on a 3.5% uphill grade, was selected for further experimentation because it represented a strenuous effort eliciting maximal heart rate and EIPH in the experimental horses.

Experimental procedures. Our procedures for blood-gas and hemodynamic studies have been described in detail previously (14–21); therefore, only a brief description is given here. On the day of the study, after local anesthesia in the 17th intercostal space, the abdominal aorta was percutaneously catheterized. Thereafter, with the use of local infiltration of 2% lidocaine HCl, cardiac catheters (8-F) equipped with a tip manometer (Millar Instruments, Houston, TX), fluid-filled lumen, and a thermistor (Edward Laboratories, Santa Clara, CA) were advanced into the right ventricle and the pulmonary artery via introducers inserted into the left jugular vein. The locations of various catheters were confirmed by monitoring the characteristic phasic blood pressure waveforms on an oscillographic recorder. These catheters permitted simultaneous sampling of the aortic and pulmonary arterial (mixed venous) blood as well as continuous monitoring of the pulmonary arterial blood (core) temperature during the experiments. After catheter placement, horses stood quietly on the treadmill for ~45–50 min before blood-gas and pH studies were undertaken.

In the present study, changes in plasma protein concentration were used to assess the changes in plasma volume and hydration status (3). This is because the quantity of circulating plasma protein is constant in healthy animals, and, therefore, acute changes in plasma protein concentration are indicative of the changes in the plasma volume (3). Plasma protein concentration was determined by refractometry, and the percent change in plasma volume was calculated as [(PP1/PP2) – 1.0] × 100, where PP1 and PP2 are the initial and the test plasma protein concentrations, respectively (3). Blood-gas tensions, pH, hemoglobin concentration, hemoglobin O2 saturation, and O2 content were determined by using a carefully calibrated blood-gas analyzer/CO-oximeter (ABL520 system, Radiometer, Copenhagen, Denmark), and all blood-gas tensions and pH data were corrected to the simultaneously measured pulmonary artery blood (core) tem-
perature. Mixed venous blood lactate concentration was determined as described previously (7, 18, 21). In the present study, O₂ extraction (%) was calculated as (arterial-to-mixed venous blood O₂ content gradient/arterial O₂ content) × 100.

**Experimental design and protocol.** In the present study, all horses were studied in the control as well as the hypervolemia (hyperhydration) experiments, which were carried out in random order, 7 days apart. All experimentation was carried out in an air-conditioned laboratory, where the ambient temperature was maintained at 20–21°C.

Before these experiments were undertaken, separate pilot trials were carried out on all horses to determine the dose of NaCl (dissolved in 1,500 ml of lukewarm water and administered via a nasogastric tube) that would induce significant thirst and cause plasma protein concentration to decrease by 0.8–1.0 g/dl (representing an ∼15–20% expansion of the circulating plasma volume). It was observed after NaCl administration in this manner that horses began to drink water within 20–30 min and that water intake continued intermittently up to 2.5–3 h after NaCl administration. Further water intake was not observed in any horse after 3 h or elapsed after NaCl administration. Preexercise trials revealed the NaCl dose to vary between 0.30 and 0.45 g/kg body weight for individual horses and that an interval of 4.5–5 h after NaCl administration was required to achieve the desired reduction in plasma protein concentration. Thereafter, plasma protein concentration of horses began to gradually recover toward the pre-NaCl baseline values. These observations comprised the basis for hypervolemia experiments in the present study.

In the hypervolemia (hyperhydration) experiments, ∼8–10 min after determination of baseline plasma protein concentration in standing horses (rest 1), a nasogastric tube was positioned and the predetermined (from pilot trials above) dose of NaCl (varying between 0.3 and 0.45 g/kg for individual horses) dissolved in 1,500 ml of lukewarm water was administered into the stomach. After NaCl administration, the horses returned to the stall where free access to fresh water was provided. Because pilot trials had revealed that water intake ceased 3 h after NaCl administration, horses were instrumented (cf. **Experimental procedures**) ∼3.75 h after NaCl administration. After instrumentation, horses stood quietly on the treadmill. At 285–290 min after NaCl administration, plasma protein concentration and blood-gas and pH measurements were made in duplicate on quietly standing horses (hereafter, referred to as the preexercise data) when heart rate and aortic and pulmonary vascular pressures had been stable for at least 10–15 min. In the control study, horses did not receive any medications, and the plasma protein concentration and blood-gas and pH measurements were made at corresponding intervals in duplicate on quietly standing horses during steady-state conditions. The sequence of control and hypervolemia experiments was randomized for all horses, and 7 days were allowed between studies on every horse.

In both treatments, after completion of preexercise measurements, exercise began on the high-speed treadmill set at 3.5% uphill grade in the following manner. Beginning with a walk at 2 m/s for 120 s, the belt speed was raised in increments of 1 m/s every 60 s until the speed was 5 m/s. After the horses had trotted at 5 m/s for 120 s, belt speed was rapidly increased to 14 m/s. On completion of 120 s of galloping at 14 m/s on a 3.5% uphill grade, the belt speed was decreased first to 5 m/s (trot) for 60 s and then to 2 m/s. Horses walked at 2 m/s for 300 s before the treadmill was stopped. In this exercise protocol, along with continuous core temperature measurement, simultaneous arterial and mixed venous blood samples were obtained for determining blood-gas tensions, pH, hemoglobin concentration, hemoglobin O₂ saturation, and O₂ content at 60 and 120 s of trotting at 5 m/s; at 30, 60, 90, and 120 s of galloping at 14 m/s on a 3.5% uphill grade; and at 120 s of walk at 2 m/s. Plasma protein concentration was also determined at 120 s of trot at 5 m/s, at 120 s of galloping at 14 m/s, and at 2 min of walk at 2 m/s. Mixed venous blood lactate concentration was determined preexercise, at 120 s of trotting at 5 m/s, and on completion of the high-intensity exercise.

**Postexercise airway endoscopic examination.** In both treatments, using a flexible fiber-optic endoscope (Pentax Fiberscopes, Orangeburg, NY), careful endoscopic examination of the nasopharynx, larynx, and trachea (up to the carina) was undertaken 45–50 min postexercise (13, 14, 28), and the presence of fresh blood in the airway(s) was regarded as indicative of the occurrence of EIPH.

**Data analysis.** All data were subjected to repeated-measures, split-plot design analysis of variance by using the SAS statistical software package (SAS Institute, Cary, NC), and the treatment comparisons were made by using the least squares significant difference method (27). Data for the control as well as the hypervolemia experiments were also individually subjected to analysis of variance followed by Newman-Keuls multiple-range test (Ref. 27; SAS version 8.2, SAS Institute) to determine the significant effects of work intensity and duration within each treatment. For all statistical analyses, the level of significance was set at P < 0.05. The data are presented as means ± SE.

**RESULTS**

Changes in plasma protein concentration at rest and during exercise. Nasogastric administration of hypertonic NaCl solution was well tolerated by the horses, and it induced marked thirst causing a total water consumption of ∼14–19 liters in individual horses within the first 3 h after NaCl administration. Preexercise measurements revealed plasma protein concentration of standing horses to have decreased (P < 0.0001) from its baseline (pre-NaCl administration; rest 1) value of 6.09 ± 0.11 g/dl to 5.16 ± 0.07 g/dl (Fig. 1, top), amounting to an estimated 18.0 ± 1.8% expansion of the circulating plasma volume (Fig. 2, top).

In both treatments, incremental exercise caused a progressive rise (P < 0.0001) in the plasma protein concentration, indicating a marked contraction of the circulating plasma volume in exercising horses (Fig. 1, bottom). Although the magnitude of the exercise-induced contraction in circulating plasma volume between the control and hypervolemia experiments was not different (Fig. 1, bottom), plasma protein concentration during exercise in the hypervolemia experiments remained less (P < 0.0001) than in the control study (Fig. 1, top). At 120 s of exertion at 5 and 14 m/s on a 3.5% uphill grade in the hypervolemia experiments, the plasma protein concentration was 5.67 ± 0.11 and 6.40 ± 0.17 g/dl, respectively [vs. 6.69 ± 0.09 and 7.43 ± 0.19 g/dl, respectively (both P < 0.0001), in the control study]. Thus the mean circulating plasma volume of exercising horses in the hypervolemia experiments exceeded (by 18% during the trot at 5 m/s and by 16% during galloping at 14 m/s on a 3.5% uphill grade) that in the control study (Fig. 2, top).
Changes in hemoglobin concentration at rest and during exertion (Fig. 2, bottom). Increased plasma volume in the hyperhydration experiments also led to a marked hemodilution, causing a drop (P < 0.0001) in hemoglobin concentration of quietly standing horses. Exercise in both treatments was attended by a large rise (P < 0.0001) in the hemoglobin concentration, due probably in large measure to the release of the erythrocyte reservoir on splenic contraction. From its preexercise values of 13.6 ± 0.5 and 12.0 ± 0.6 g/dl, respectively, in the control and hypervolemia experiments, hemoglobin concentration had increased (both P < 0.0001) to reach 22.3 ± 0.3 and 19.8 ± 0.4 g/dl, respec-

tively, at 120 s of galloping at 14 m/s on a 3.5% uphill grade. However, throughout the exercise protocol, hemoglobin concentration in the hypervolemia experiments remained less (P < 0.0001) than corresponding values in the control study.

Changes in core temperature with exercise. The extent of exercise induced hyperthermia was unaffected by hypervolemia induced after NaCl administration. From its preexercise values (37.2 ± 0.1 and 37.3 ± 0.1°C in the control and hypervolemia experiments, respectively), pulmonary artery blood temperature of horses registered progressive increments as exercise duration and intensity increased, reaching 40.7 ± 0.2
and 40.5 ± 0.2°C respectively, at 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and hypervolemia studies. At this workload, the heart rate of horses had approached its maximal value (220 ± 3 beats/min) in both treatments.

Changes in arterial O₂ tension and hemoglobin O₂ saturation with exercise (Fig. 3). Preexercise data for these variables were similar in both treatments. During submaximal exercise performed at 5 m/s, arterial O₂ tension and hemoglobin O₂ saturation were not different from preexercise values in either treatment. Galloping at 14 m/s on a 3.5% uphill grade was attended by a similar large reduction (P < 0.0001) in arterial O₂ tension at 30 s in both treatments, but further changes in arterial O₂ tension were not observed as exercise duration progressed to 120 s. At 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and the hypervolemia experiments, the arterial O₂ tension values were 73.8 ± 2.5 and 74.4 ± 2.9 Torr, respectively.

In both treatments, desaturation of hemoglobin in the arterial blood was also evident at 30 s of galloping at 14 m/s on a 3.5% uphill grade (P < 0.0001), and as exercise duration progressed to 120 s, the desaturation of hemoglobin intensified (cf. Fig. 3, panel at bottom left). Significant differences between the control and the hypervolemia studies were not found, however. The increasing desaturation of arterial hemoglobin observed in going from 30 to 120 s of galloping at 14 m/s on a 3.5% uphill grade probably resulted from the
rightward shift of the hemoglobin O\textsubscript{2} dissociation curve as hypercapnia (Fig. 4), acidosis (Fig. 5), and hyperthermia (from 39.1 ± 0.1 and 39.0 ± 0.2°C, respectively, at 30 s of galloping in the control and the hypervolemia experiments to 40.7 ± 0.2 and 40.5 ± 0.2°C, respectively, at 120 s; both P < 0.0001) intensified with increasing exercise duration. At 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and the hypervolemia experiments, arterial hemoglobin O\textsubscript{2} saturation had decreased to 85.4 ± 1.8 and 86.6 ± 1.4%, respectively.

Changes in mixed venous blood O\textsubscript{2} tension and hemoglobin O\textsubscript{2} saturation with exercise (Fig. 3). Preexercise values of these variables were similar in the control and hypervolemia experiments. The incremental exercise protocol caused work intensity-related reductions in these variables in both treatments. Although there was a tendency for mixed venous blood O\textsubscript{2} tension and hemoglobin O\textsubscript{2} saturation to be lower in the hypervolemia experiments during galloping at 14 m/s on a 3.5% uphill grade, significant differences could not be demonstrated.

Changes in arterial CO\textsubscript{2} tension with exercise (Fig. 4). Hypervolemia induced after nasogastric administration of NaCl did not affect the preexercise values of arterial CO\textsubscript{2} tension. Whereas submaximal exercise at 5 m/s in both treatments was attended by hyperventilation, during galloping at 14 m/s on a 3.5% uphill grade marked hypercapnia (P < 0.01) developed. At 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and the hypervolemia experiments, arterial CO\textsubscript{2} tension had reached 54.9 ± 2.4 and 51.7 ± 1.9 Torr, respectively, and significant differences between the treatments were not found. During the recovery period after high-intensity exercise, a dramatic hyperventilation ensued in both treatments.

Changes in arterial pH and blood lactate concentration with exercise (Fig. 5). Preexercise values of arterial pH and blood lactate concentration were not different between the control and the hypervolemia experiments. In both treatments, arterial pH did not change with exercise performed at 5 m/s. During galloping at 14 m/s on a 3.5% uphill grade, a progressive acidosis of a similar magnitude was observed in both treatments. At 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and the hypervolemia experiments, the arterial pH had decreased to 7.095 ± 0.035 and
Changes in arterial and mixed venous blood O2 contents (Fig. 6). Because of the lower hemoglobin concentration in the hypervolemia experiments (cf. Fig. 2), preexercise values of arterial O2 content (16.3 ± 0.8 ml O2/dl blood) and mixed venous blood O2 content (12.6 ± 0.9 ml O2/dl blood) were also less (P < 0.01) than corresponding values in the control study, but the arterial-to-mixed venous blood O2 content gradient remained similar for both treatments.

In both treatments, a large (P < 0.0001) increment in arterial blood O2 content was observed during exercise due to increased hemoglobin concentration (Fig. 2). However, because hemoglobin concentration of exercising horses was significantly less in the hypervolemia experiments (Fig. 2), the increment in arterial O2 content was also attenuated (P < 0.01) compared with corresponding values in the control study. At 120 s of galloping at 14 m/s on a 3.5% uphill grade, values of arterial blood O2 content in the control and the hypervolemia experiments were 26.0 ± 0.3 and 23.5 ± 0.3 ml O2/dl of blood, respectively.

In both treatments, although work-intensity-related changes in the mixed venous blood O2 content were observed, the mixed venous blood O2 content of exercising horses in the hypervolemia experiments was found to be significantly less (P < 0.05) than corresponding values in the control study. Despite the latter observation, the arterial-to-mixed venous blood O2 content gradient of exercising horses in the hypervolemia experiments did not approach values observed in the control study (P < 0.01). At 120 s of galloping at 14 m/s on a 3.3% uphill grade, the arterial-to-mixed venous blood O2 content gradient in the hypervolemia experiments was 21.4 ± 0.3 ml O2/dl of blood (vs. 23.5 ± 0.4 ml O2/dl of blood in the control study; P < 0.01).

Airway endoscopic observations. All horses were observed to have experienced EIPH in both treatments as demonstrated by the presence of fresh blood in the trachea on postexercise airway endoscopic examination.

DISCUSSION

Our observations in the control study regarding significant arterial hypoxemia, desaturation of hemoglobin, hypercapnia, acidosis, hemoconcentration, increased O2 extraction, and increased blood lactate concentration (Figs. 2–6), as well as significant hyperthermia in horses performing short-term high-intensity exercise are similar to those described previously (7, 18–21). The exercise-induced changes in plasma protein concentration and contraction of plasma volume in the control experiments (Fig. 1) also mirrored data reported previously (8). Our primary objective in the present study was to examine whether significant preexercise expansion of plasma volume would accentuate the arterial hypoxemia observed in Thoroughbreds performing strenuous exercise. In this context, our data revealed that preexercise induction of significant hypervolemia in healthy horses did not significantly affect the development and/or severity of arterial hypoxemia and desaturation of hemoglobin (Fig. 3) during short-term high-intensity exercise that elicited maximal heart rate and caused stress failure of pulmonary capillaries, resulting in EIPH. These findings are in sharp contrast with those of Sosa-Leon et al. (25), wherein an expansion of plasma volume was re-
ported to cause a mean further drop of 15 Torr in arterial O₂ tension of horses performing submaximal exercise, and it was concluded that hyperhydration before exercise is detrimental to respiratory function. However, it should be noted that our observations concur with those in human subjects wherein preexercise expansion of plasma volume did not significantly affect the exercise-induced arterial hypoxemia (35). It is interesting to note that the magnitude of preexercise hypervolemia was much greater (18%) in our experiments [vs. that in the human subjects (~7.7%; Ref. 35) and in Standardbred horses (10.8% at end exercise; Ref. 25)], and yet statistically significant changes in arterial O₂ tension and/or hemoglobin O₂ saturation of galloping Thoroughbreds could not be demonstrated (Fig. 3).

In a comparison of the divergent results of the present study vs. those in the hyperhydration experiments of Sosa-Leon et al. (25), several important differences between the experimental design and protocols of the two studies must be recognized. First, whereas short-term maximal exercise in Thoroughbreds was examined in the present study, in the experiments of Sosa-Leon et al. Standardbred horses performing submaximal exercise at 7.3–8.0 m/s (equivalent to 55–61% VO₂ max) for 4 and 14 min [after prolonged periods (lasting 30–50 min) of mild exercise at 3 m/s] were studied. It has been reported that Thoroughbreds performing exercise at treadmill speeds up to 8 m/s for brief periods (without extended periods of mild exercise before such exercise), typically, do not exhibit significant arterial hypoxemia (7, 18–21). Thus one wonders whether the prolonged periods of mild exercise (at 3 m/s for 30–50 min) before moderate exertion at 7.3–8.0 m/s somehow contributed to the reported further significant reduction (15 Torr) in arterial O₂ tension in their hyperhydration experiments (25). Second, whereas in the present study a significant expansion of plasma volume was achieved preexercise (Figs. 1 and 2), this was not the case in the experiments of Sosa-Leon et al. In that report (25), as exercise was initiated, significant changes in plasma protein concentration and/or hematocrit had not occurred, but apparently, as a result of the continued absorption of the administered fluid from the gastrointestinal tract during the prolonged periods of mild exercise at 3 m/s, a 10.8% expansion of plasma volume developed at end exercise (25). Despite these differences, the fact remains that the magnitude of preexercise hypervolemia in the present study was greater (18.0 ± 1.8% vs. that (10.8% at end exercise) in the experiments of Sosa-Leon et al. Also, the pulmonary capillary blood pressure of maximally exercising horses is known to far exceed that during moderate exercise (14–17). Thus, on the basis of the mechanism proposed by Sosa-Leon et al. that pulmonary edema due to extravasation of the administered fluid may be responsible for the further significant (15 Torr) drop in arterial O₂ tension of exercising horses in their hyperhydration experiments (25), an even larger reduction in arterial O₂ tension of maximally exercised horses would have been expected in our hypervolemia experiments. However, this was not found to be the case (Fig. 3). Therefore, it is believed that the above differences in experimental design and protocols are unlikely to account for the divergent results of the present study regarding the lack of an effect of preexercise hypervolemia on the arterial hypoxemia in Thoroughbreds performing short-term maximal exercise.

A significant expansion of the plasma volume in our experiments was demonstrated by the significantly diminished concentrations of plasma protein and hemoglobin, both preexercise as well as during exercise (Figs. 1 and 2). Whereas the marked hemodilution did not significantly affect the arterial-to-mixed venous blood O₂ content gradient in standing horses, a statistically significant (P < 0.01) attenuation of the exercise-induced expansion of the arterial-to-mixed venous blood O₂ content gradient did occur (Fig. 6). Because we used the same exercise protocol for both treatments, the total (aerobic + anaerobic) energy requirement to perform the same workload in our control and hypervolemia experiments should be identical. However, after nasogastric administration of NaCl to horses, increased water intake augments body weight, which, in turn, necessitates additional ATP generation during exertion. In maximally exercising horses, the latter is probably provided by increased anaerobic metabolism, thereby causing additional lactate production. Because of greater dilution, hypervolemia (in the absence of increased body weight) would be expected to result in a lower blood lactate concentration in maximally exercising horses. However, this was not the case in the present study (Fig. 5). Thus the observed similarity of blood lactate concentration and arterial pH during maximal exercise in our hypervolemia experiments to values in the control study (Fig. 5) may be indicative of the additional lactate production necessitated by the augmented body weight. Whole body O₂ consumption is the product of cardiac output and arterial-to-mixed venous blood O₂ content gradient (Fick principle). Thus, at constant VO₂ max (during galloping at 14 m/s on a 3.5% uphill grade), the smaller arterial-to-mixed venous blood O₂ content gradient of maximally exercising horses observed in our hypervolemia experiments (Fig. 6) would have been attended by an augmented cardiac output. This is in agreement with the observations of Hopper et al. (11) that after preexercise acute (~20%) expansion of plasma volume, a significant (16%) augmentation of cardiac output occurred in maximally exercising horses (11). Because the maximal heart rate achieved during galloping at 14 m/s on a 3.5% uphill grade was unaffected by hypervolemia in our horses, this augmentation of cardiac output would probably have been accomplished via increased stroke volume (11). Similar data regarding an augmentation of cardiac output and stroke volume have also been reported in human subjects performing strenuous exercise after acute hypervolemia (12, 31). It has been reported that the high packed cell volume (hematocrit approaching 65–68% in our control study) in galloping Thoroughbreds contributes to the
observed high values of blood viscosity (6), which pose an impediment to blood flow. Thus hemodilution by way of lowering blood viscosity (and, therefore, resistance to blood flow (i.e., ventricular afterload)) in our hypervolemia experiments may have contributed to an augmentation of stroke volume in galloping horses. Other factors contributing to an augmentation of stroke volume may include enhanced ventricular filling (via Frank-Starling mechanism) and augmented cardiac contractility (11, 12, 31).

The above discussion regarding an augmentation of cardiac output in hypervolemic horses performing maximal exertion (11) is quite relevant to the mechanism(s) for exercise-induced arterial hypoxemia (Fig. 3). Although relative hypoventilation [as demonstrated by increased arterial CO₂ tension (Fig. 4) despite increased alveolar ventilation in galloping horses] and ventilation-perfusion inhomogeneity also contribute (1, 5, 29, 30), the largest contribution to exercise-induced arterial hypoxemia in racehorses is from diffusion limitation to transfer of O₂ (30). The latter is probably related to the significant shortening of the transit time for blood in the pulmonary capillaries as cardiac output increases dramatically (5). The scenario that galloping horses in our hypervolemia experiments may have had augmented cardiac output (11), in turn, suggests that there would have been a further truncation of the already shortened pulmonary capillary transit time during high-intensity exercise, which should have adversely affected the arterial O₂ tension. However, this was not found to be the case in the present study (Fig. 3), presumably because hypervolemia may also have affected pulmonary capillary blood volume and the distribution of ventilation-perfusion mismatching within the lungs, thereby affecting pulmonary gas exchange of exercising horses. Although in a recent report (35) acute hypervolemia also failed to significantly affect the exercise-induced arterial hypoxemia in human subjects, it should be noted that acute hypervolemia in that study was reported to cause a significant increase in the pulmonary transit time despite the lack of a significant change in cardiac output (35). However, it remained unclear as to what fraction of the measured pulmonary transit time (35) represented the “true” pulmonary capillary transit time and whether the latter and/or pulmonary capillary blood volume were affected by acute hypervolemia. The extent of exercise-induced arterial hypercapnia in our experiments was not significantly affected by preexercise induction of hypervolemia (Fig. 4), indicating similarity of alveolar ventilation in the two treatments.

In agreement with previous findings (7, 18–21), in the present study arterial hypoxemia was also found to be well developed at 30 s of galloping at 14 m/s on a 3.5% uphill grade in both treatments, and its severity did not change with increasing exercise duration (Fig. 3). Recently, Préfaut et al. (22, 23) suggested that pulmonary capillary stress failure-induced arterial inflammatory mediator release plays a role in bringing about the exercise-induced arterial hypoxemia. That all horses in the present study had experienced pulmonary capillary stress failure, was demonstrated by the occurrence of EIPH in both treatments. According to the pulmonary injury-evoked airway inflammatory mediator release hypothesis (22, 23), an accentuation of arterial hypoxemia would be expected to occur with increasing exercise duration as structural changes in the blood-gas barrier (2, 24, 32) intensify over time. Although this hypothesis has considerable appeal, the findings of Wetter et al. (33, 34) in exercising young human subjects did not lend credence to it. Similarly, our laboratory’s recent work with pretreatment of Thoroughbreds with dexamethasone (to suppress the airway inflammatory response) and with a potent antihistaminic agent (tripelennamine HCl, an H₁-receptor antagonist) to mitigate the effects of airway inflammatory- and mast cell-released histamine on pulmonary capillary permeability also did not support the pulmonary injury hypothesis for exercise-induced arterial hypoxemia in racehorses (20, 21).

In summary, our data revealed that preexercise 18% expansion of plasma volume failed to significantly affect the development and/or severity of arterial hypoxemia in Thoroughbred horses performing short-term high-intensity exercise that elicited maximal heart rate and caused stress failure of pulmonary capillaries resulting in EIPH. Furthermore, it was observed that blood lactate concentration and arterial pH of maximally exercising horses were unaffected by preexercise hypervolemia. Although hemodilution caused a significant attenuation of the exercise-induced expansion of the arterial to mixed venous blood O₂ content gradient in our horses, it likely was offset by an augmented cardiac output.

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