Determinants of oxygen delivery and hemoglobin saturation during incremental exercise in horses

Clara K. Fenger, DVM, PhD; Kenneth H. McKeever, PhD; Kenneth W. Hinchcliff, BVSc, PhD; Catherine W. Kohn, VMD, MS

Objective—To determine components of the increase in oxygen consumption (VO₂) and evaluate determinants of hemoglobin saturation (SO₂) during incremental treadmill exercise in unfit horses.

Animals—7 unfit adult mares.

Procedures—Horses performed 1 preliminary exercise test (EXT) and 2 experimental EXT. Arterial and mixed venous blood samples and hemodynamic measurements were taken during the last 30 seconds of each step of the GXT to measure PO₂, hemoglobin concentration ([Hb]), SO₂, and determinants of acid-base state (protein, electrolytes, and PCO₂).

Results—Increased VO₂ during exercise was facilitated by significant increases in cardiac output (CO), [Hb], and widening of the arteriovenous difference in O₂. Arterial and venous pH, PaCO₂, and PVO₂ decreased during exercise. Arterial Pcco₂, bicarbonate ([HCO₃⁻]), and [HCO₃⁻] decreased significantly, whereas Pcco₂ and increased. Arterial and venous sodium concentration, potassium concentration, strong ion difference, and venous lactate concentration all increased significantly during exercise.

Conclusions and Clinical Relevance—Increases in CO, [Hb], and O₂ extraction contributed equally to increased VO₂ during exercise. Higher Pcco₂ did not provide an independent contribution to shift in the oxyhemoglobin dissociation curve (ODC) in venous blood. However, lower PaCO₂ shifted the curve leftward, facilitating O₂ loading. The shift of ODC resulted in minimal effect on O₂ extraction because of convergence of the ODC at lower values of PO₂. Decreased pH appeared responsible for the rightward shift of the ODC, which may be necessary to allow maximal O₂ extraction at high blood flows achieved during exercise. (Am J Vet Res 2000;61:1325-1332)

Maximal exercise requires a large increase in energy use and oxygen consumption (VO₂). Oxygen consumption increases with incremental exercise until maximal oxygen consumption (VO₂max) is achieved.¹ ² This increase in the VO₂ is mediated by increases in O₂ delivery and extraction.³ Oxygen extraction is determined by the tissue O₂ diffusion capacity and the O₂ gradient between the capillary and the muscle mitochondria.⁴ Because the tissue diffusion capacity does not change during a single bout of exercise, the increase in O₂ extraction with exercise is attributable to an increase in the diffusion gradient.¹ Increased O₂ driving pressure results from a decrease in PO₂ at the mitochondria and an increase in capillary PO₂ as the oxyhemoglobin dissociation curve (ODC) shifts to the right in the tissue capillary beds.⁵ This rightward shifting of the ODC facilitates the release of hemoglobin-bound O₂.⁶ Affinity of hemoglobin for O₂ is in dynamic flux, depending on meditators in solution and changes from arterial to venous blood. Mediators that change the affinity of hemoglobin for O₂ include CO₂, 2,3-diphosphoglycerate (DPG), chloride, pH, and temperature, all of which change during exercise.⁷ ⁸ ¹⁰

To the authors’ knowledge, studies that have simultaneously examined determinants of oxygen delivery and hemoglobin saturation during incremental exercise in horses have not been published. Thus, there is a need to evaluate the contributions that meditators of oxygen delivery, O₂ extraction, and other factors affecting the ODC provide to the high VO₂max seen in maximally exercised horses. Although many of these plasma constituents have been studied during maximal exercise, their contribution to the state of the ODC and, therefore, O₂ extraction have not been evaluated in maximally exercised horses. Studies have examined the effects of exercise on these measurements separately, and data have been published on changes in arterial versus venous concentrations of plasma constituents; however, published studies have not used those data in an effort to explain shifts in the ODC in horses. Therefore, we hypothesized that incremental exercise would cause a significant change in the arterial and venous concentrations of plasma constituents affecting the ODC in horses. Changes in concentrations of these plasma constituents such as sodium, potassium, chloride, lactate, and protein may also alter strong-ion difference (SID) and, thus, may alter blood pH; however, studies of maximally exercised horses have not focused on the contribution of changes in SID to the observed changes in blood pH. Thus, the purpose of the study reported here was to determine the components of the increase in VO₂ during exercise and evaluate the determinants of hemoglobin saturation during incremental maximal exercise in horses that were not physically fit. An additional purpose of the study was to determine the underlying cause of the decrease in plasma pH that develops with maximal exercise, using the principles delineated by Stewart.¹¹
Materials and Methods

Experimental protocol—The study was performed in accordance with the Guiding Principles in the Animal Care and Use Committee of the Ohio State University. Seven clinically normal adult mares (2 Thoroughbreds, 3 Standardbreds, 1 Appaloosa, and 1 Quarter Horse) weighing between 435 and 560 kg were used in the study. A subcutaneous transposition of the left carotid artery of each mare was surgically performed 6 to 12 months before the study. Mares were housed individually in box stalls to prevent uncontrolled exercise, were accustomed to running on a high-speed treadmill, and were otherwise untreated. During the experiment, each horse performed 3 separate incremental maximal exercise tests on a high-speed treadmill. The first exercise test was a preliminary test, and the 2 subsequent tests were experimental trials. Two experimental exercise trials were used to facilitate timely processing of the samples. Exercise trials were always performed between 8:00 AM and 10:00 AM during the autumn. Mares were not exercised for at least 1 week before each experimental run.

The preliminary exercise test was performed approximately 4 weeks before the initiation of the first exercise trial. This test was performed to determine the velocity (Velmax) at which VO2max was attained. An individualized incremental exercise test was then developed for each horse. The 2 experimental trials were performed at least 4 weeks apart.

Experimental exercise tests—Horses stood on the treadmill for approximately 10 minutes before the onset of exercise during which time they were instrumented. The treadmill was then started at a velocity of 4 m/s and increased by 1 m/s every 90 seconds until the velocity approached Velmax. Treadmill speed was then increased by 0.5 m/s every 90 seconds until fatigue. Fatigue was defined as the point at which the horse was reluctant to continue despite humane coaxing. Each incremental increase in treadmill speed was maintained for 90 seconds to allow 60 seconds after the speed transitions for achievement of near steady-state conditions and 30 seconds for sample collection. Arterial and mixed venous blood samples were obtained at the end of the equilibration period (ie, just before exercise) and during the last 15 seconds of each speed increment. Sampling at VO2max was performed 60 to 90 seconds after Velmax was attained and, therefore, reflected peak oxygen consumption (VO2peak) rather than VO2max.

Instrumentation—Each horse was walked from its box stall, weighed, and brought to the treadmill barn. Two catheter introducers were placed in the right jugular vein, and 1 was placed in the left jugular vein. A saline (0.9% NaCl) solution-filled arterial catheter was placed in the translocated left carotid artery. All catheters were placed by use of sterile techniques under local anesthesia. Two Swan-Ganz catheters were inserted in the pulmonary artery via the right jugular introducers. One catheter was used for sample collection, and the other was used for temperature determinations. The Swan-Ganz thermistor catheters were calibrated from 36 to 42 °C before the experiment by use of an independent mercury thermometer and a water bath. A micro-manometer catheter was inserted into the right ventricle through the left jugular introducer for determination of right ventricular pressure and heart rate. The position of all catheters was verified by the characteristic wave forms displayed on an oscilloscope. With local anesthesia, a muscle temperature probe was placed in the left middle gluteal muscle for measurement of muscle temperature. Oxygen uptake (ml/kg/min) was measured by use of an open-flow calorimeter designed for use with horses.

Data collection—During trial 1, VO2, heart rate, pulmonary artery blood temperature, and middle gluteal muscle temperature were recorded during the last 15 seconds of each speed increment. Samples of carotid arterial (12 ml) and venous (15 ml) blood were collected during the last 30 seconds of each speed increment. All samples were collected anaerobically into tubes containing anticoagulant (lithium heparin; final concentration, 20 U/ml of whole blood) and stored on ice for the remainder of the experimental run. Immediately after the exercise test, samples were divided by use of anaerobic technique into 2 aliquots of arterial blood and 3 aliquots of venous blood. The arterial and venous blood samples were divided into a 2-ml sample for blood gas analysis, a 2-ml sample placed in an evacuated tube for Hct determinations, and a 7-ml sample placed in an evacuated tube for electrolyte, total protein (TP), and albumin concentration determinations. Mixed venous blood was also placed in 3-ml sodium fluoride-potassium oxalate tubes for lactate analysis. During trial 2, 3 ml of venous blood was obtained for determination of blood 2,3-DPG concentration at each step of the exercise test. An additional 20 ml of venous blood was also obtained for tonometry after the standing equilibration period, at the 4-m/s step of the test (38 to 64% VO2peak), and at VO2peak.

Immediately after the exercise test, pH, PO2, and PCO2 were measured in duplicate by use of a blood gas-pH analyzer. The blood gas-pH analyzer was calibrated between measurements, and values were verified by use of the individual mare's blood samples, which were analyzed by tonometry with several gases of different O2 and CO2 concentrations. Samples were handled anaerobically and chilled on ice for hemoglobin O2 saturation analysis. Coefficient of variability for the blood gas analyzer was 0.02% for pH, 0.5% for PCO2, and 5% for PO2. Hemoglobin concentration (Hb) and hemoglobin saturation (SO2) were measured colorimetrically with a co-oximeter. Samples were run at least in triplicate and repeated until 3 consecutive measurements of each variable differed by <10%. Care was taken between samples to prevent contamination with air. Coefficient of variability for the co-oximeter was 0.2% for [Hb] and 0.5% for SO2.

Arterial and venous PCO2 and PO2 (measured at 37 °C) were corrected for pulmonary artery blood temperature by use of constants for equine blood samples. Degree of hemoglobin desaturation attributable to the difference in temperature between pulmonary artery blood and middle gluteal muscle temperature was also calculated by use of the same constants. Immediately after the exercise test, PCV was determined in duplicate by use of the microhematocrit technique. The anaerobic blood samples and oxalate samples were centrifuged at 4 °C at 1,500 X g for 20 minutes, and the plasma was rapidly transferred to plastic storage tubes and stored at −20 °C for later analysis. Venous plasma lactate (La−) concentration was measured by use of a lactate analyzer. The coefficient of variability for La− was 1.2%.

Plasma TP and albumin concentrations were determined colorimetrically by use of the biuret technique and the bromocresol green technique, respectively. All assays were performed at least in triplicate. Coefficients of variability for these assays were 5.4 and 6.7%, respectively.

The plasma sodium (Na+) concentration and plasma potassium (K+) concentration were measured by use of flame photometry. Samples for determination of Na+ and K+ concentration were analyzed in quintuplicate, and values that differed by >10% from the mean were excluded from analysis. Coefficients of variability for Na+ and K+ concentration were 1.0 and 1.5%, respectively. Chloride (Cl−) concentration was determined by use of an ion-specific silver electrode. These samples were analyzed in triplicate, and any samples that varied >10% from the mean were repeated. Coefficient of variability for chloride was 2.5%. All electrolyte values
were corrected for plasma-free water, which was assumed to be constant at 93% plasma.

Blood collected for measurement of 2,3-DPG concentration was maintained in an ice bath until deproteinized immediately after trial 2. A 1-ml sample of venous blood was deproteinized in 3 ml of 8% trichloroacetic acid and centrifuged at 4 C at 1,500 × g for 20 minutes. After centrifugation, the supernatant was pipetted into a plastic storage tube and maintained at 4 C until assayed. All 2,3-DPG assays were performed in duplicate within 3 hours of the exercise test. The 2,3-DPG concentration was determined colorimetrically. The coefficient of variability for the 2,3-DPG assay was 12%.

Determination of the standard oxyhemoglobin dissociation curve (ODCstd) was performed by use of venous blood samples obtained before exercise, at 4 m/s (approx 50% VO2peak), and at VO2peak. The ODCstd was determined by tonometry of blood with calibration gases at 5.6% CO2 (40 mm Hg) and varied concentrations of O2 at 37 C. Blood was initially equilibrated with 0% O2 and equilibrated with 5 g mixtures with sequentially higher O2 concentrations to obtain a range of PO2 values from < 10 mm Hg to approximately 100 mm Hg. All of the gas mixtures had the same percentage CO2 (5.6% or 40 mm Hg at 1 atm barometric pressure). The percentage O2 (certified to be within 0.02%) and corresponding partial pressures at 1 atm were 1.4 (10 mm Hg), 3.5 (25 mm Hg), 5.6 (40 mm Hg), 9.8 (70 mm Hg), and 14% (100 mm Hg). The actual partial pressure was calculated on a daily basis from the measured atmospheric pressure. Five 1-ml aliquots were analyzed concurrently by use of tonometry to minimize the period during which blood was heated and minimize degradation of 2,3-DPG. The remaining 15 ml of blood was kept refrigerated at 4 C for ≤ 4 hours. Samples of blood (0.5 ml) were taken from the tonometer during equilibration for blood gas and SO2 determination. After blood gas determination, the samples were stored anaerobically and chilled on ice. Hemoglobin concentration and SO2 were measured within 6 hours. The ODCstd was determined by use of an iterative least squares analysis based on Hill's equation:

\[ S_O2 = \frac{k\,(P_O2)}{1 + k\,(P_O2)} \]

where $S_O2$ is the saturation of hemoglobin, $P_O2$ is the partial pressure of oxygen, $k$ is Hill’s coefficient, and $n$ is Hill’s constant. The constants and coefficients were determined for each horse at each exercise intensity and used to calculate the oxygen tension at 50% saturation (P50) and the oxygen tension at 5% saturation (P5). The ex vivo SO2 and PO2 were compared with the SO2 from the ODC to determine the PO2 effect on SO2 during exercise.

The contribution of exercise-induced changes in the SID, PCO2, and weak acid (Aweak) concentration to changes in hydrogen ion (H+) concentration and, therefore, SO2 were calculated (Table 1). Lactate concentration of mixed venous blood samples was used for arterial and mixed venous calculation, because there is no measurable difference between arterial and mixed venous blood La concentration during maximal exercise. Changes in concentrations of calcium, magnesium, and other strong ions were ignored because of their small contribution to SID.21 Albumin was considered the primary contributor to $A_{ion}$, because the net charges of the ionizable groups on globulins are zero at physiologic pH.21 The dissociation equation for albumin derived by Figge et al22 (Table 1) was used instead of the dissociation constant derived by Van Slyke et al,23 which is:

\[ [A']/[A_{ion}] = 8.18 - 1.48 \times (pH) \]

and used by Stewart.19 Inorganic phosphate concentration ([P]) was measured before exercise and at VO2peak. The arteriovenous difference (Da-v) in ionized groups on albumin ($D_A\{\text{ionized protein concentration (A)}\}$) was calculated by algebraic manipulation of the \([A']\) equation (Table 1) to yield:

\[ D_A(A') = 1.48 \times (\text{ab}h) \times (pH - p\text{H}L) \]

Statistical analyses—Relationships between the measured variables and relative workload (% VO2peak) were determined by use of least squares multiple linear, polynomial, and exponential regression analysis.1 The best fit of an exponential, first, or second order equation was determined by F-ratio comparison.24 The values of P50, P5, and difference between SO2 in vivo and that predicted by the ODCstd before exercise (at 4 m/s) and at peak were compared by use of repeated measures ANOVA. The concentrations of inorganic phosphate (P) before exercise and at peak were compared by use of a pooled t-test. Results in the text were expressed as mean ± SE. A value of $P \leq 0.05$ was considered significant.

Results

Oxygen delivery and extraction—The significant increase in VO2 (Table 2) measured during exercise was mediated by increases in cardiac output (CO) and the D(a-v) of O2 (Fig 1; Table 2). The increase in CO was primarily the result of increases in heart rate, because stroke volume (1.3 L) did not change ($P = 0.132$) from preexercise values. Oxygen extraction increased during exercise from 20% at rest to 80% at VO2peak ($P < 0.001$).

The P50 of the ODCstd of blood collected at VO2peak reflected an increase (mean increase, 9.44 ± 3.76 mm Hg; $P < 0.001$) from approximately 22 mm Hg before exercise to approximately 31 mm Hg at VO2peak (Fig 2, Table 3). The shift of the ODC was attenuated at low PO2 values; the P5 at VO2peak was only 3.6 ± 1.60 mm Hg higher ($P < 0.001$) than the P5 before exercise. The magnitude of the right shift of the ODC at P50 was equivalent to that mediated by the fixed acid Bohr effect ($\Delta \log P50/\Delta \text{pH} = -0.47 \pm 0.28$) because of the change...
in blood pH. The P5 and P50 of blood collected at 4 m/s (approx 50% VO2peak) were not different ($P = 0.885$ and $P = 0.057$, respectively) than that before exercise. Arterial PCO2 decreased and venous PCO2 increased throughout the exercise bout ($P < 0.001$). Most of the CO2 generated in the tissues during exercise was carried in the blood in the form of bicarbonate ($HCO_3^-$).

The SO2 measured at 37 C in mixed venous blood at any time point in the exercise test was not different ($P = 0.376$) from the SO2 predicted by the ODC determined at 37 C and P CO2 of 40 mm Hg (ODC std). The SO2 measured at 37 C in arterial blood was higher ($P = 0.003$) than the SO2 predicted by the ODC std, reflecting a leftward shift of the ODC at high values of PO2 and indicating that the arterial hypocapnea had a significant effect on the oxygen-hemoglobin affinity in arterial blood. There was no effect of exercise on blood 2,3-DPG concentration ($16.5 \pm 0.5 \mu mol/g$ vs $15.7 \pm 0.05 \mu mol/g$ of hemoglobin before exercise and at VO2peak, respectively; $P = 0.27$).

Gluteal muscle temperature increased at a greater rate than pulmonary arterial temperature during exercise ($P < 0.001$). Pulmonary arterial temperature increased by 2.4 C from a mean of 37.5 C at rest to a mean of 39.9 C during exercise ($P < 0.001$). Gluteal muscle temperature increased by 3.3 C from a mean of 37.7 C at rest to a mean of 41.0 C during exercise ($P < 0.001$). The in vivo PO2 (Fig 4) was obtained by correcting the values measured at 37 C for pulmonary arterial temperature. The in vivo PaO2 at core body temperature decreased in arterial blood to a lesser extent than the measured PO2. Corrected PaO2 decreased from 106 mm Hg before exercise to 86 mm Hg at VO2peak ($P < 0.001$). This actual PaO2 was 16 mm Hg higher than the PaO2 measured at 37 C. The temperature effect was less in venous blood in which temperature corrected PvO2 decreased from 38 mm Hg before exercise to 20 mm Hg at VO2peak ($P < 0.001$).

Changes in the determinants of pH—Arterial pH decreased during exercise (Table 4; $P < 0.001$) and consequentially was equivalent to a substantial increase in H+ concentration. A greater decrease in pH was observed in mixed venous blood during exercise ($P < 0.001$, Table 4). Interestingly, arterial $HCO_3^-$ con-

Table 2—Determinants (mean [range]) of oxygen consumption (VO2) before exercise and at peak oxygen consumption (VO2peak)

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Before exercise</th>
<th>VO2peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2 (ml O2/kg of body weight/min)</td>
<td>4 (3–6)</td>
<td>104* (78–131)</td>
</tr>
<tr>
<td>Cardiac output (ml/kg/min)</td>
<td>154 (40–183)</td>
<td>472* (367–567)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>49 (35–60)</td>
<td>209* (205–216)</td>
</tr>
<tr>
<td>Hemoglobin saturationa (%)</td>
<td>99.1 (98.7–99.9)</td>
<td>93.1* (87.1–94.6)</td>
</tr>
<tr>
<td>Hemoglobin saturationv (%)</td>
<td>75.2 (67.7–87.2)</td>
<td>13.9* (5.7–18.2)</td>
</tr>
<tr>
<td>PSO (mm Hg)</td>
<td>22.9 (21.2–23.9)</td>
<td>31.8* (29.3–35.0)</td>
</tr>
<tr>
<td>[Hb] (g/dl)</td>
<td>13.1 (9.8–16.1)</td>
<td>20.7* (19.5–22.8)</td>
</tr>
</tbody>
</table>

*Significantly ($P < 0.05$) different from value before exercise.

$VO2$ = Oxygen consumption. $PSO = P_{O2}$ in mm Hg at 50% hemoglobin saturation. $[Hb] = $ Hemoglobin concentration.

Table 3—Mean Hill’s coefficients and Hill’s constants obtained during maximal exercise in 7 horses

<table>
<thead>
<tr>
<th>Level of exercise</th>
<th>Hills coefficient (K)</th>
<th>Hill’s constant (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before exercise</td>
<td>2.429 × 10^4</td>
<td>2.849</td>
</tr>
<tr>
<td>At 4 m/s</td>
<td>1.527 × 10^5</td>
<td>2.744</td>
</tr>
<tr>
<td>At V_max</td>
<td>8.690 × 10^1</td>
<td>2.707</td>
</tr>
</tbody>
</table>

$V_{max} = $ Velocity of the last step completed by a horse during an incremental exercise test.

Figure 1—Differences between arterial and venous oxygen content as a function of work intensity (percentage of peak oxygen consumption [%VO2peak]) in 7 horses. Circles represent individual data points.

Figure 2—Oxyhemoglobin saturation curves determined at rest, at approximately 50% of VO2peak, and at VO2peak in 7 horses. Curves were determined at PCO2 of approximately 40 mm Hg in mixed venous blood and represent the mean of values obtained from the 7 horses.

Figure 3—Arterial and venous PCO2 as a function of work intensity in 7 horses. Circles represent individual data points.
...concentration decreased substantially (P < 0.001), whereas HCO₃⁻ concentration in mixed venous blood decreased to a lesser extent (P < 0.001).

Determinants of the metabolic component of the acidosis changed with increasing exercise intensity (Table 4). In arterial blood, Na⁺ concentration increased 8%, and Na⁺ concentration in mixed venous blood increased 8% (P < 0.001). In arterial and mixed venous blood, K⁺ concentration increased 68% with increasing work intensity (P < 0.001). Chloride concentration did not change in arterial (P = 0.291) or mixed venous (P = 0.735) blood. Lactate concentration in mixed venous blood increased exponentially from before exercise to VO₂peak (P < 0.001). Arterial and mixed venous blood constituents in arterial and mixed venous blood. The increase in VO₂ with incremental maximal exercise detected in the study reported here was attributable primarily to PCO₂ (Fig 4). In arterial blood, 45% of net change in H⁺ concentration (9.5 nEq/L of the total 21.1 nEq/L) was attributable to combined changes in SID, A– concentration, and P₇ concentration. The increase in concentration of oxygenated Hb (7.7 g/dl) in arterial blood with maximal exercise could contribute an additional –5.4 mEq/L charge (4.2 nEq H⁺/L). This accounts for another 19% of the total change, although this is an intracellular change and may not be reflected in plasma. The remaining 36% of the increase of arterial H⁺ concentration with maximal exercise could not be accounted for.

**Arteriovenous difference**—The PO₂ D(a–v) increased 580% from 4.6 to 26.7 mm Hg during exercise (P < 0.001). There was no PCV D(a–v) (P = 0.915) and only a slight hemoglobin D(a–v) (< 1 g/dl, P = 0.008).

The H⁺ concentration D(a–v) increased from 3.3 nEq/L at rest to 13.8 nEq/L at VO₂peak (P < 0.001). This D(a–v) was attributable primarily to PCO₂ D(a–v), which increased from –5.7 mm Hg before exercise to –48.2 mm Hg at VO₂peak. There was no D(a–v) detected for concentrations of [Na⁺] (P = 0.385), [K⁺] (P = 0.350), [P₇] (P = 0.96), or albumin (P = 0.708). The [Cl⁻] D(a–v) was constant at 3.3 mEq/L (P = 0.005) for all work intensities. The SID D(a–v) was –4.3 mEq/L and was not different from the [Cl⁻] D(a–v) (P < 0.05).

**Discussion**

Horses are athletic and have the ability to reach maximal aerobic capacities in the range of 145 to 190 ml/kg/min. This tremendous aerobic capacity is the result of many species-specific adaptations including a highly capacious spleen that can deliver as much as 12 L of RBC-rich blood into the circulation. The advent of high-speed equine treadmills has allowed numerous studies of running horses; however, only a limited number of articles have reported the effects of high-intensity exercise on the differences between concentrations of blood constituents in arterial and mixed venous blood. Such differences influence SID, blood pH, and other factors that affect exercise-induced shifts in the ODC.

The increase in VO₂ with incremental maximal exercise detected in the study reported here was attributed to approximately equal contributions from O₂ delivery and O₂ extraction. For example, if only the Hb

---

**Table 4**—Mean values of determinants of hydrogen ion concentration [H⁺] in arterial and mixed venous blood during a maximal exercise test in 7 horses.

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Before exercise</th>
<th>At maximal exercise</th>
<th>Differences at VO₂peak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arterial</td>
<td>Venous</td>
<td>Arterial</td>
</tr>
<tr>
<td>pH (nEq/L)</td>
<td>7.47</td>
<td>7.43*</td>
<td>7.26</td>
</tr>
<tr>
<td>[H⁺] (nEq/L)</td>
<td>8.5</td>
<td>10.6*</td>
<td>7.26</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>145</td>
<td>146</td>
<td>157</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>3.9</td>
<td>3.9</td>
<td>6.8</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>–109</td>
<td>–106*</td>
<td>–109</td>
</tr>
<tr>
<td>Lactate (mEq/L)</td>
<td>N/A</td>
<td>0.6</td>
<td>N/A</td>
</tr>
<tr>
<td>SID (mEq/L)</td>
<td>36.8</td>
<td>42.1*</td>
<td>35.5</td>
</tr>
<tr>
<td>Lactate (mEq/L)</td>
<td>–10.5</td>
<td>–11.9*</td>
<td>–8.9</td>
</tr>
<tr>
<td>Phosphate (mEq/L)</td>
<td>2.9</td>
<td>2.8</td>
<td>4.2</td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>44.0</td>
<td>49.9*</td>
<td>35.5</td>
</tr>
</tbody>
</table>

*pVenous values are significantly (P < 0.05) different from arterial values. tSignificant (P ≤ 0.05) difference from value obtained before exercise.
concentration and CO increased with exercise without change in O2 extraction. O2 delivery would account for only 18.4 ml O2/kg/min or 18% of the total increase in VO2 from before exercise to VO2peak. Interestingly, CO accounted for an increase in VO2 of only 11.6 ml O2/kg/min if O2 extraction and O2 carrying capacity remained constant. If only O2 extraction increased without a concomitant increase in O2 delivery, O2 extraction would be responsible for only 17% of the increase in VO2 with maximal exercise. The compound effect of a higher extraction percentage from a higher O2 delivery at VO2peak accounted for most of the exercise-induced increase in VO2. Increase in O2 delivery was the result of a 4-fold increase in CO and an increase in Hb concentration and, subsequently, O2 carrying capacity of the blood. The latter was attributable to mobilization of blood stored in the splenic reserve. The large contribution of O2 extraction to VO2peak was attributed to the large reserve of arterial O2 in the resting horse. Percentage of O2 extracted in the tissues before exercise was 20%, and this increased to almost 90% at VO2peak. These percentages reflect whole-body O2 extraction, and it is likely that the muscle O2 extraction percentage may be even higher.

The ODCstd, which was determined at a constant PCO2, was shifted to the right during maximal exercise. This observation agrees with published observations in humans and although expected, was, to the authors’ knowledge, the first such observation to be made in horses performing incremental exercise on a treadmill. The PO2 at P50 before exercise (21.2 to 24.9 mm Hg) was similar to that found by Clerbeaux in resting horses (23 ± 0.8 mm Hg), and lower than that found in resting humans (26.6 mm Hg). An attempt was not made to adjust the pH to a standard value, because any addition of strong acid or base would simultaneously change the SID. Therefore, mean pH of the ODCstd before exercise was 7.458, and mean pH of the ODCstd at VO2peak was 7.220. This corresponds to an increase in H+ concentration during maximal exercise of 25 nEq/L. The P50 at maximal exercise (29.3 to 35.0 mm Hg) was increased 9 ± 4 mm Hg, a difference similar to that seen in humans at maximal exercise (12 mm Hg). This increase in P50 could be attributed entirely to the Bohr effect mediated by the metabolic component of exercise-induced acidosis.

Stewart’s approach to acid-base determination was used in an attempt to identify the source of the exercise-induced metabolic acidosis. This approach relies on the accurate analysis of the independent determinants of H+ concentration, which are SID, PCO2, and AHb. The ODCstd was determined at a constant PCO2, and, therefore, only the SID and albumin concentration were included in the evaluation of the difference between H+ concentration at rest and at VO2peak.

The increase in strong cations (Na+ and K+) during exercise was closely matched by the increase in the strong anion, La-. The net result of these changes was an initial increase in SID at the onset of exercise followed by a decrease in SID. In arterial blood, SID at VO2peak was lower than the SID before exercise, whereas in mixed venous blood, SID at VO2peak was slightly higher. Albumin and P1 concentration were the only weak acids that were measured as globulin proteins and do not substantially contribute to the H+ concentration. Keenan found no change in P1 concentration after maximal exercise, but blood samples in that study were taken 10 minutes after completion of exercise and, therefore, may not accurately reflect P1 changes during exercise. Results of a recent study in Greyhounds indicated a substantial increase in P1 concentration during exercise, which could account for 14.9% of the H+ concentration in arterial blood at 2.5 minutes after exercise.

The present study attributes approximately 25% of the increase in H+ concentration during exercise to the increase in P1 concentration. Carbon dioxide, which decreased during exercise in arterial blood and increased during exercise in mixed venous blood, was the final contributor to the H+ concentration.

The total increase in H+ concentration in arterial plasma from rest to VO2peak was 21.1 nEq/L. Decrease in arterial SID was only 1.3 mEq/L, which could account for only 25.6% of the change in H+ concentration or 5.3 nEq/L. Increase in albumin concentration with exercise was minor at only 0.2 g/dl in arterial blood. In addition to the increase in albumin concentration, there was a simultaneous decrease of the albumin dissociation constant caused by the decrease in pH. Therefore, the increase in albumin concentration may have functioned to partially offset the increase in H+ concentration by buffering 1.4 nEq/L. The increase in P1 concentration resulted in the addition of 5.3 nEq H+/L. Together, arterial SID, A– concentration, and P1 concentration contributed 45% (9.5 nEq/L) of the change in H+ concentration. Thus, the source of 11.6 nEq H+/L during maximal exercise was undetermined.

One potential contributor is the weak acid, oxygenated Hb, which increased by 7.7 g/dl with maximal exercise. The Hb constant K+ increases with oxygenation, which could contribute an additional 19% (4.2 nEq/L) of the H+ concentration. Although this change is intracellular in erythrocytes, pH was measured in plasma that was contiguous with erythrocytes, and, therefore, oxygenation of Hb probably contributed to the increased H+ concentration. The remaining 36% of the increase in arterial H+ concentration with maximal exercise could not be accounted for. Therefore, additional factors, probably undetermined anions, must also change during incremental exercise to account for the arterial metabolic acidosis.

A difference was not detected between SO2 at VO2peak in venous blood and the SO2 predicted by the ODCstd at the same PO2. This finding suggests that the independent CO2 Bohr effect does not contribute to improved O2 delivery during maximal exercise. In mixed venous blood, PCD2 at VO2peak was almost double the value before exercise. Therefore, in mixed venous blood, the increase in PCD2 with exercise would be expected to shift the ODC further to the right of the ODCstd in which the PCD2 was constant at 40 mm Hg.

Failure of the high PCD2 at maximal exercise to further decrease Hb O2 affinity was most likely attributable to the low PO2 in mixed venous blood. The PO2 at VO2peak was 12.3 mm Hg, which is at the lowest part of the ODC. The various ODC converge at the left side of the graph near a PO2 value of 0 mm Hg and SO2 value.
of 0%. This phenomenon is also seen in the ODC of human exercising at 65 and 100% VO2max. This convergence of the various ODC at low O2 tensions suggests that deoxygenation of venous effluent from working muscles is independent of the state of the ODC. Despite lack of a detectable CO2 effect, a CO2 effect may be evident at intermediate PO2 values such as those found in capillaries. A shift of the ODC in capillaries may provide a local increase in driving pressure for entry of O2 into muscle fibers. This effect may be important for facilitating O2 uptake during rapid transit of blood through muscle beds. Rate of O2 uptake into tissues may be facilitated by increasing the capillary to mitochondria diffusion gradient.

Arterial Hb was more saturated than predicted by the ODCstd, suggesting a substantial effect of arterial hypoxemia. Arterial ODC at VO2peak was shifted to the left in comparison with the ODC in venous blood measured in blood sample atVO2peak. This difference was attributable to the Bohr effect. The mixed venous blood had a lower pH attributable to high PCO2, whereas arterial blood had a relatively higher pH attributable to hypoxemia. Arterial blood had metabolic acidosis, whereas mixed venous blood had respiratory acidosis.

To determine the cause of the H+ D(a-v), we evaluated the contributors to H+ concentration using Stewart's approach to acid-base determinations. Acidosis in mixed venous blood could almost exclusively be attributed to high PCO2. If no other independent determinants of H+ concentration changed, the PCO2 D(a-v) would have resulted in a H+ D(a-v) of –36.1 nEq/L. The observed H+ D(a-v) was –15.8 nEq/L, reflecting an acid shift from plasma of 20.3 nEq H+/L. This attenuation of the H+ generated by CO2 was partially mediated by the D(a-v) of SID and albumin charge. The change in both of these independent determinants accounted for about 6.1 nEq H+/L. The remaining 14.2 nEq/L of the change in H+ concentration could not be accounted for.

Potential sources of the remaining H+ concentration are uptake of strong cation from plasma proteins, shift of strong anions or weak acids from erythrocytes to plasma, or pH-related decreases in pKa of other weak acids. Ionized calcium and magnesium were not measured but probably changed between mixed venous and arterial blood. Increasing pH results in binding of ionized calcium by albumin, although the precise quantity cannot be estimated. The reduction in this strong cation decreases the SID, contributing H+ to the plasma.

Chloride ions shift across the erythrocyte membrane during the transition through the pulmonary circulation. Horses in the study reported here did not have significant exercise-induced changes in Cl– concentration. However, changes in Cl– concentration in arterial and mixed venous blood have been reported by other investigators along with substantial alterations in D(a-v) attributable to intense exertion. Samples were separated and processed as soon as possible to eliminate in vitro effects on Cl– concentration. Differences between results of our study and those of others may reflect technique variations (chloridometer vs ion-sensitive electrode methods) or other differences in experimental design. Included in the latter may be the lack of physical fitness of the horses used in our study, because other investigations have used horses that were much more physically fit. Other ions such as calcium, magnesium, phosphate, and sulfate could potentially be added or removed from the plasma by similar shifts, although this has not been documented.

Although albumin concentration did not change from venous to arterial blood, the significant change in pH across the pulmonary circulation resulted in a change in the effective pK of albumin concentration. The D(a-v) of albumin concentration increased from 0 mEq/L before exercise to –1.11 mEq/L at VO2peak. The combined albumin and SID D(a-v) resulted in a net charge of –5.4 mEq in the arterial blood at maximal exercise. In the physiologic SID range, this could account for about 6.1 nEq H+/L or 38.5% of the H+ concentration D(a-v). Therefore, the PCO2 D(a-v) likely accounts for the remaining pH D(a-v) during exercise.

The pH-related decrease of pK of albumin concentration contributed to the H+ concentration in the arterial blood. Similar changes occur in the pK of phosphate and sulfate. If the organic phosphate concentration did not change across the pulmonary circulation, the pH D(a-v) would result in a minor decrease in pK. This contribution would be much lower than the contribution from albumin, because the concentration of phosphate in the plasma is relatively low (0.25 to 0.44 mmol/L). Therefore, P concentration could contribute to the change in H+ concentration but could not account for the entire 20 nEq H+/L.

Exercise influenced the plasma concentrations of all these factors except DPG concentration. This was not measured, but it would be in agreement with the findings of Stull et al. In exercised horses, the D(a-v) of SID and albumin charge. The change in both of these independent determinants accounted for about 6.1 nEq H+/L. The remaining 14.2 nEq/L of the change in H+ concentration could not be accounted for.

Potential sources of the remaining H+ concentration are uptake of strong cation from plasma proteins, shift of strong anions or weak acids from erythrocytes to plasma, or pH-related decreases in pKa of other weak acids. Ionized calcium and magnesium were not measured but probably changed between mixed venous and arterial blood. Increasing pH results in binding of ionized calcium by albumin, although the precise quantity cannot be estimated. The reduction in this strong cation decreases the SID, contributing H+ to the plasma.

Chloride ions shift across the erythrocyte membrane during the transition through the pulmonary circulation. Horses in the study reported here did not have significant exercise-induced changes in Cl– concentration. However, changes in Cl– concentration in arterial and mixed venous blood have been reported by other investigators along with substantial alterations in D(a-v) attributable to intense exertion. Samples were separated and processed as soon as possible to eliminate in vitro effects on Cl– concentration. Differences between results of our study and those of others may reflect technique variations (chloridometer vs ion-sensitive electrode methods) or other differences in experimental design. Included in the latter may be the lack of physical fitness of the horses used in our study, because other investigations have used horses that were much more physically fit. Other ions such as calcium, magnesium, phosphate, and sulfate could potentially be added or removed from the plasma by similar shifts, although this has not been documented.

Although albumin concentration did not change from venous to arterial blood, the significant change in pH across the pulmonary circulation resulted in a change in the effective pK of albumin concentration. The D(a-v) of albumin concentration increased from 0 mEq/L before exercise to –1.11 mEq/L at VO2peak. The combined albumin and SID D(a-v) resulted in a net charge of –5.4 mEq in the arterial blood at maximal exercise. In the physiologic SID range, this could account for about 6.1 nEq H+/L or 38.5% of the H+ concentration D(a-v). Therefore, the PCO2 D(a-v) likely accounts for the remaining pH D(a-v) during exercise.

The pH-related decrease of pK of albumin concentration contributed to the H+ concentration in the arterial blood. Similar changes occur in the pK of phosphate and sulfate. If the organic phosphate concentration did not change across the pulmonary circulation, the pH D(a-v) would result in a minor decrease in pK. This contribution would be much lower than the contribution from albumin, because the concentration of phosphate in the plasma is relatively low (0.25 to 0.44 mmol/L). Therefore, P concentration could contribute to the change in H+ concentration but could not account for the entire 20 nEq H+/L.

Exercise influenced the plasma concentrations of all these factors except DPG concentration. This was not measured, but it would be in agreement with the findings of Stull et al. In exercised horses, the D(a-v) of SID and albumin charge. The change in both of these independent determinants accounted for about 6.1 nEq H+/L. The remaining 14.2 nEq/L of the change in H+ concentration could not be accounted for.

Potential sources of the remaining H+ concentration are uptake of strong cation from plasma proteins, shift of strong anions or weak acids from erythrocytes to plasma, or pH-related decreases in pKa of other weak acids. Ionized calcium and magnesium were not measured but probably changed between mixed venous and arterial blood. Increasing pH results in binding of ionized calcium by albumin, although the precise quantity cannot be estimated. The reduction in this strong cation decreases the SID, contributing H+ to the plasma.

Chloride ions shift across the erythrocyte membrane during the transition through the pulmonary circulation. Horses in the study reported here did not have significant exercise-induced changes in Cl– concentration. However, changes in Cl– concentration in arterial and mixed venous blood have been reported by other investigators along with substantial alterations in D(a-v) attributable to intense exertion. Samples were separated and processed as soon as possible to eliminate in vitro effects on Cl– concentration. Differences between results of our study and those of others may reflect technique variations (chloridometer vs ion-sensitive electrode methods) or other differences in experimental design. Included in the latter may be the lack of physical fitness of the horses used in our study, because other investigations have used horses that were much more physically fit. Other ions such as calcium, magnesium, phosphate, and sulfate could potentially be added or removed from the plasma by similar shifts, although this has not been documented.

Although albumin concentration did not change from venous to arterial blood, the significant change in pH across the pulmonary circulation resulted in a change in the effective pK of albumin concentration. The D(a-v) of albumin concentration increased from 0 mEq/L before exercise to –1.11 mEq/L at VO2peak. The combined albumin and SID D(a-v) resulted in a net charge of –5.4 mEq in the arterial blood at maximal exercise. In the physiologic SID range, this could account for about 6.1 nEq H+/L or 38.5% of the H+ concentration D(a-v). Therefore, the PCO2 D(a-v) likely accounts for the remaining pH D(a-v) during exercise.

The pH-related decrease of pK of albumin concentration contributed to the H+ concentration in the arterial blood. Similar changes occur in the pK of phosphate and sulfate. If the organic phosphate concentration did not change across the pulmonary circulation, the pH D(a-v) would result in a minor decrease in pK. This contribution would be much lower than the contribution from albumin, because the concentration of phosphate in the plasma is relatively low (0.25 to 0.44 mmol/L). Therefore, P concentration could contribute to the change in H+ concentration but could not account for the entire 20 nEq H+/L.

Exercise influenced the plasma concentrations of all these factors except DPG concentration. This was not measured, but it would be in agreement with the findings of Stull et al. In exercised horses, the D(a-v) of SID and albumin charge. The change in both of these independent determinants accounted for about 6.1 nEq H+/L. The remaining 14.2 nEq/L of the change in H+ concentration could not be accounted for.

Potential sources of the remaining H+ concentration are uptake of strong cation from plasma proteins, shift of strong anions or weak acids from erythrocytes to plasma, or pH-related decreases in pKa of other weak acids. Ionized calcium and magnesium were not measured but probably changed between mixed venous and arterial blood. Increasing pH results in binding of ionized calcium by albumin, although the precise quantity cannot be estimated. The reduction in this strong cation decreases the SID, contributing H+ to the plasma. Chloride ions shift across the erythrocyte membrane during the transition through the pulmonary circulation. Horses in the study reported here did not have significant exercise-induced changes in Cl– concentration. However, changes in Cl– concentration in arterial and mixed venous blood have been reported by other investigators along with substantial alterations in D(a-v) attributable to intense exertion. Samples were separated and processed as soon as possible to eliminate in vitro effects on Cl– concentration. Differences between results of our study and those of others may reflect technique variations (chloridometer vs ion-sensitive electrode methods) or other differences in experimental design. Included in the latter may be the lack of physical fitness of the horses used in our study, because other investigations have used horses that were much more physically fit. Other ions such as calcium, magnesium, phosphate, and sulfate could potentially be added or removed from the plasma by similar shifts, although this has not been documented.
minants of $O_2$. However, some might criticize the additive effect of experimental error inherent to the multiple variables measured by use of this method. Nevertheless, the Stewart method does allow conduction of a more in-depth examination of acid-base status by use of a multifactorial physiologic approach. Although the use of multiple breeds in the study reported here is the source of some experimental variation, the variation was moderate, and none of the horses were considered to be outliers when the data were tested statistically. In fact, the use of multiple breeds in a descriptive study may make the data more representative of all horses (except draft and pony breeds). Preexercise heart rates and CO measured in our study should not be considered true resting values, because there was moderate increase in heart rate while the horses were wearing the open-flow calorimetry mask. Although this affects comparisons with other studies, it does not detract from the importance of the exercise-induced changes measured in the study reported here.

Sato Treadmill, Uppsala, Sweden.

3. Argon Medical Corp, Athens, Tex.

4. Intracath 18 gauge 12 in, Deseret, Sandy, Utah.

5. No. 240 polyethylene tubing, Becton-Dickinson, Paripsnaj, NJ.


7. Millar Instruments, Houston, Tex.

8. VR-12, PPG Biomedical, Pittsburgh, Pa.

9. $\#13$, Yellow Springs Instruments, Yellow Springs, Ohio.

10. OXymax-XL System, Columbus Instruments, Columbus, Ohio.

11. Becton-Dickinson, Paripsnaj, NJ.


13. Radiometer OSM 3 hemoximeter, Copenhagen, Denmark


15. Chloride titrater No. 4425000, Buchler Instruments, Lenexa, Kan.

16. Sigma kit No. 631, Sigma Chemical Co, St Louis, Mo.

17. Sigma kit No. 655, St Louis, Mo.

18. Praxair Inc, Columbus, Ohio.


References


