Hematological changes and athletic performance in horses in response to high altitude (3,800 m)

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Wickler, Steven J., and Timothy P. Anderson. Hematological changes and athletic performance in horses in response to high altitude (3,800 m). Am J Physiol Regulatory Integrative Comp Physiol 279: R1176–R1181, 2000.—This study had two goals: 1) measure hematologic changes with high-altitude acclimatization in horses; and 2) assess the effect of 9 days at high altitude on subsequent athletic performance at low altitude. Six horses performed standardized exercise tests on a dirt track (before and during time at altitude) and treadmill (pre- and postaltitude exposure). Resting and immediate postexercise blood samples were measured for blood volume, lactate, red cell number, packed cell volume, and 2,3-diphosphoglycerate (DPG) concentrations at 225 m, over a 9-day period at 3,800 m, and shortly after returning to 225 m. Acclimatization produced increases in total red cell volume (38.2 ± 2.4 to 48.1 ± 2.9 ml/kg, \(P = 0.004\)) and DPG/hemoglobin concentrations (19.4 ± 1.7 increased to 29.4 ± 0.4 \(\mu\)mol/g, \(P = 0.004\)). Two performance variables, heart rate recovery postexercise and lactate recovery, were faster after acclimatization.

METHODS

Animals. The sample population consisted of six equids (4 Arabians, 1 Quarter horse, and 1 Shetland pony), four females and two males, with an average age of 9.0 ± 4.5 years (values given as means ± 1 SE). Body masses averaged 467 ± 15 kg for the horses and 257 kg for the pony. The animals were conditioned physically before testing (4–4.5 mo). The conditioning regimen consisted of 3 days per week on a high-speed treadmill (SA¨TO I, Equine Dynamics, Lexington, KY) at the walk, trot, and canter (a total of 30 min of exercise). The training also included a standardized exercise test with rider on a dirt track two days a week. Heart rates (HR) (Polar Vantage XL, Polar CIC, Port Washington, NY) were monitored during all exercise periods. Two and one-half wk before transport to altitude, horses were exercised on the treadmill to determine their individual maximal HR and maximal exercise-induced hematocrits. This preliminary study involved increasing treadmill speed by 1 m/s, at 1-min intervals, after an 8-min warm-up at 3.5 m/s, until the horse would no longer maintain its position on the treadmill without humane encouragement. HR was recorded and blood samples were taken for hematocrit determinations during the last 15 s at each speed. This test was repeated once more, 1 wk later.

Standard exercise test on the track. The standard exercise test on the track \(\text{SET}_{\text{track}}\) was performed on a compacted dirt oval track, ~200 m in length. HR was used to standardize the animal’s work intensity. The warm-up period consisted of a 15-min walk. The \(\text{SET}_{\text{track}}\) consisted of 5 min walking, a 4-min trot (HR 110 beats/min), a 4-min canter (HR 150 beats/min), and a 2-min gallop (HR 180 beats/min). On completion of the gallop phase, the animals were allowed a 15-min recovery period consisting of 5 min walking and 10 min standing. A similar track was prepared at the high-altitude study site (3,800 m), and the \(\text{SET}_{\text{track}}\) at altitude used the same HR to standardize intensity. Care was taken to

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to maintain the same HR during the tests while at altitude to reduce any training effect as a confounding variable.

**Standard exercise test on the treadmill.** The standard exercise test on the treadmill (SET\textsubscript{TM}) consisted of 5 min at 1.6 m/s, 4 min at 3.5 m/s (HR 110 beats/min), and 4 min at 5.0 m/s with a 10% grade (HR 150 beats/min). The animals then exercised for 1 min at maximal speed with a 10% grade. This speed (8.23 ± 0.40 m/s, \(n = 5\)) was one that was sufficient to produce a maximal HR (214 ± 3 beats/min) and to complete splenic contraction (as tested earlier). The SET\textsubscript{TM} concluded with a 15-min recovery. The speeds of the treadmill exercise were reduced for the pony so corresponding HR was similar to those of the horses. Treadmill tests were only performed at low-altitude pre- and postaltitude.

**Experimental protocol and physiological measurements.** With the use of aseptic techniques, catheters (Cook, CheckFlo Blue Introducer Set, 8F) were placed in the jugular vein and secured with sutures. The catheter site was protected with bandaging material, and the catheters were flushed twice daily with heparinized saline. Catheters were replaced approximately every 72 h or as needed. The catheter (nominal volume of 1.5 ml) was used for all blood sampling and for introduction of the Evans blue dye used for plasma volume (PV) determinations. Twenty milliliters of blood were withdrawn before actual blood sampling to ensure residual blood/saline was cleared from the catheter. The 20 ml was a volume that resulted in <2% dilution by fluid left in the catheter; this was validated in a separate in vitro sample with the use of Evans blue dye. For experimental analyses, 30 ml of blood were collected into a heparinized syringe. Resting blood samples were obtained 90 min postprandial (0815). Blood was analyzed for PCV (microhematocrit method), Hb concentration (Sigma Diagnostics, Procedure #525; cyanmethemoglobin method), and RBC# (manual erythrocyte count Unopette T 5855). Two 1-ml aliquots of whole blood were each placed into 2 ml of perchloric acid (PCA) and 3 ml of TCA and stored in liquid nitrogen until analysis of LA (Sigma Diagnostics, #826-UV) and DPG (Sigma Diagnostics, procedure #665) concentrations could be completed, respectively. All samples were analyzed in duplicate and averaged if within 10%. If duplicates differed by >10%, the assay was repeated.

Blood was sampled for 2 days before transport (225 m, mean day ambient temperature \(T_a = 21^\circ\)C and barometric pressure = 743 mmHg). On the third day, the animals were transported to the Barcroft Faculty of the University of California White Mountain Research Station in the White Mountain range (3,800 m), an approximate 13-h journey (500 km). Resting blood samples were obtained on days 2, 4, and 8 of exposure to high altitude (mean day \(T_a = 15^\circ\)C and barometric pressure = 487 mmHg). The animals were transported back to 225 m on day 10. Resting blood samples were withdrawn and analyzed for 2 days after altitude exposure.

The SET\textsubscript{track} was performed on either of the 2 days pre-altitude transport (225 m) and on days 2, 4, and 8 of exposure to high altitude (3,800 m). The SET\textsubscript{TM} was performed at low altitude on either of the 2 days before altitude transport (on the alternate day from the SET\textsubscript{track}) and on each of the 2 days at low altitude after the 10-day altitude exposure (no SET\textsubscript{track} was run at this time because we wished to use the treadmill test to measure performance variables, and we did not want to run both track and treadmill tests on the same day to avoid fatigue as a confounding variable). Blood samples were taken and analyzed for PCV and LA after all phases of the SET\textsubscript{track} and SET\textsubscript{TM}. Blood samples taken after the gallop phase were additionally analyzed for RBC#, Hb, and DPG concentrations. Samples were taken within 30 s of each phase.

Hb values were expressed as mean corpuscular hemoglobin (MCH, in picograms) and expressed per number of circulating red blood cells in grams per deciliters as the mean corpuscular hemoglobin concentration (MCHC). Changes in DPG were expressed as a function of the amount of Hb (\(\mu\)mol/g). Mean corpuscular volume (MCV) was calculated by expressing the PCV as a function of the RBC#.

PV determinations were performed with the use of a 1% Evans blue dye solution (in 0.9% NaCl) injected at 0.05 ml/kg of body weight following procedures outlined by Persson (26). The dye was injected into the venous catheter immediately after the gallop phase and allowed to circulate in the system for exactly 15 min. After the initial addition of the dye solution, the catheters were flushed with heparinized saline to minimize residual dye. The empty syringe was then weighed to determine the amount of dye injected. A blood sample was withdrawn from the catheterized jugular vein after the 15-min equilibration period. The plasma was separated and frozen for later analysis. Plasma samples were analyzed by the method of McKeever et al. (21).

Blood volumes (BV) were calculated (26) with the use of maximal hematocrit values obtained during the SET\textsubscript{TM}. RCV were calculated by taking the difference between BV and PV. Because BV, PV, and RCV will vary with body size, these measurements were expressed in ml/kg of body weight. Fluid volumes were determined on either of the 2 days before altitude transport, on day 4 at altitude, and on either of the 2 days postaltitude exposure.

Performance evaluations before and after high-altitude exposure were on the basis of the SET\textsubscript{TM}. Variables used to assess performance were: 1) HR at the maximum speed (8.23 m/s) determined in the initial SET\textsubscript{TM}; 2) blood lactate concentrations at maximum speed; 3) HR recovery; and 4) lactate clearance at 15-min postexercise. HR recovery was calculated as the time for the HR to decrease by one-half from peak to resting (in seconds).

**Statistical analysis.** Data were analyzed with the use of Statview (Abacus Concepts, Berkeley, CA) with a repeated-measures ANOVA using a Fisher’s protected least-significant differences post hoc test \((P \leq 0.05\) considered significant). Values were expressed as the means ± SE. Comparisons were made between resting values obtained during the entirety of the study to understand the acclimation process. The low-altitude SET\textsubscript{track} values were compared with the SET\textsubscript{track} values obtained at altitude.

The pre- and postaltitude SET\textsubscript{TM} experimental data were compared with the use of a paired Student’s \(t\)-test to assess performance differences based on the physiological changes that occur with acclimatization. \(P \leq 0.05\) was considered significant. All variables measured in the pony were within the range of values for the horses so they were included in the statistical analysis. The one exception was for speed at a given HR (Table 1), and in this instance, the pony was not included in the statistical comparison.

**RESULTS**

**Resting values: low altitude vs. high altitude.** Resting PCV increased with initial exposure to altitude \((P = 0.039)\) and was elevated on return to low altitude (Fig. 1). The initial resting PCV at low altitude was 33.8 ± 1.9% and increased to 44.1 ± 2.7%, but it was not consistently increased at altitude. The resting PCV remained elevated at least 2 days after altitude expo-
sure at a value of 46.1 ± 4.5% (average of postaltitude samples).

There was no change in the average MCV over time with exposure to the high altitude (38.9 ± 1.2 fl pre-altitude and 37.5 ± 1.6 fl postaltitude, P = 0.781). The resting MCH was also not different (14.4 ± 0.5 vs. 13.6 ± 1.1 pg, for prealtitude and postaltitude, respectively, P = 0.564). However, the MCHC decreased over the duration of the study from 37.0 ± 0.1 to 35.5 ± 0.4 g/dl after 8 days of altitude exposure, and it remained decreased for at least 2 days after return (P = 0.005).

The resting DPG/Hb values increased with time on exposure to high altitude (P = 0.001) and remained elevated for at least 2 days after altitude exposure (Fig. 2). The initial low-altitude value of 19.4 ± 1.7 μmol/g increased to a value of 29.4 ± 0.37 μmol/g after 8 days of altitude exposure. The average concentration remained significantly elevated (P = 0.041) for at least 2 days at low altitude, after acclimatization to high altitude.

The resting LA concentrations increased (P = 0.029) from the initial resting value (from 0.74 ± 0.04 mM to 1.26 ± 0.069 mM) on day 2 of exposure to high altitude (Fig. 3). The subsequent LA concentrations were not different from low altitude.

**SET**track: low altitude vs. high altitude. The PCV of blood taken after the gallop phase of the SET increased over rest, but there was no effect with acclimatization (Fig. 1). The DPG/Hb concentrations during exercise were not different from resting samples taken on the same day, but they did increase (P = 0.03) during the 8 days at altitude (Fig. 2). Lactate concentrations did increase with exercise, however, there were no effects of altitude acclimatization on the LA concentrations (Fig. 3).

### Table 1. Velocities for the track SET

<table>
<thead>
<tr>
<th>Gait</th>
<th>HR</th>
<th>225 m</th>
<th>3,800 m 2nd day</th>
<th>3,800 m 4th day</th>
<th>3,800 m 8th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trot</td>
<td>(110)</td>
<td>4.03 ± 0.17</td>
<td>3.37 ± 0.19</td>
<td>3.04 ± 0.38</td>
<td>2.69 ± 0.16</td>
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<tr>
<td></td>
<td></td>
<td>[3.84]</td>
<td>[3.09]</td>
<td>[2.41]</td>
<td>[1.87]</td>
</tr>
<tr>
<td>Canter</td>
<td>(150)</td>
<td>6.84 ± 0.15</td>
<td>5.79 ± 0.27</td>
<td>5.60 ± 0.22</td>
<td>4.13 ± 0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[5.96]</td>
<td>[3.85]</td>
<td>[3.18]</td>
<td>[2.93]</td>
</tr>
<tr>
<td>Gallop</td>
<td>(180)</td>
<td>8.68 ± 0.34</td>
<td>7.47 ± 0.26</td>
<td>7.23 ± 0.39</td>
<td>5.89 ± 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[7.31]</td>
<td>[5.16]</td>
<td>[4.62]</td>
<td>[4.55]</td>
</tr>
</tbody>
</table>

Velocities are calculated as the mean (± SE) of all the horses (n = 5). The values for the pony are included in brackets. Velocity decreased with initial exposure to altitude and with time at altitude. Velocities were set by heart rates (HR). HR measured in beats/min.
LA concentration after the gallop phase was 2.28 ± 0.65 mM initially, and after 8 days of high-altitude exposure, it was 2.30 ± 0.64 mM ($P = 0.270$).

Speeds for SETtrack were decreased at high altitudes. This decrease averaged 15% for the horses (Table 1) on the second day at 3,800 m and almost 30% for the pony. There was a progressive decrease in speed with time at altitude ($P = 0.02$).

**SET$_{TM}$: prealtitude vs. postaltitude.** After the final gallop stage of the SET$_{TM}$, PCV increased ($P = 0.009$) from 50.5 ± 8 to 53.1 ± 0.3% with altitude acclimatization (Fig. 1), but the MCV was not different ($P = 0.95$). The DPG/Hb increased between pre- and post-altitude exposure (Fig. 2) in a manner similar to resting DPG/Hb concentrations ($P = 0.005$). The LA concentrations after the highest speed did not change with altitude acclimatization with a prealtitude value of 7.73 ± 1.40 mM and a value of 6.23 ± 0.63 mM within 2 days postaltitude exposure ($P = 0.35$). The LA concentrations were greater in the SET$_{TM}$ than in the SET$_{track}$ (Fig. 3).

**BV, PV, and RCV.** The total BV increased ($P = 0.008$) over the duration of the study (Fig. 4). The total BV at low altitude was 76.2 ± 4.2 ml/kg, increasing to 91.4 ± 4.8 ml/kg. This increase in BV was due to an increase in the RCV from an initial volume of 38.2 ± 2.4 to 48.1 ± 2.9 ml/kg at the end of the study ($P = 0.004$) and an increase in PV (initial value of 37.9 ± 1.9 and 43.2 ± 1.9 ml/kg).

**Performance variables.** HR during the maximal speed did not change with acclimatization (214 ± 3.2 vs. 220 ± 1.1 beats/min pre- and postaltitude, respectively, $P = 0.18$), but HR recovery, calculated as the time for HR to decrease by one-half from maximal to resting, was faster after acclimatization (91 vs. 57 s, $P = 0.04$). Maximal LA concentrations during the final phase of the SET$_{TM}$ did not change with altitude acclimatization (7.73 ± 1.40 vs. 6.23 ± 0.63 mM, pre- and postaltitude acclimatization, respectively, $P = 0.35$), but they were significantly lower after 15 min of recovery in animals that had been at altitude (4.90 ± 0.32 vs. 3.24 ± 0.31 mM, $P = 0.003$).

**DISCUSSION**

Interpretation of resting hematologic values in the horse can be somewhat ambiguous because the equine spleen is capable of storing up to one-half of the circulating red cells (26, 31), which can be emptied into the circulation in response to varying stressful stimuli, i.e., any stimuli likely to alter sympathetic activity or plasma epinephrine concentrations. For this reason, resting values may not reflect maximal values for blood parameters.

The resting PCV and RBC# in the present study increased with exposure to altitude. There have been conflicting results in similar studies in resting equids with one observing similar increases in mules at altitude (30), and another finding no changes in resting PCV and RBC# in horses over a 7-wk period at 2,100 m (5). It is difficult to assess the changes observed in the resting numbers of circulating erythrocytes in the present study (or the lack of observed changes) because one cannot control the amount of splenic contraction in a “resting” horse. For example, the increase in resting PCV observed on day 1 at 3,800 m was probably a result of transport stress and reduced water consumption during travel and not a result of environmental hypoxia.

The only change in the resting LA concentrations was observed on day 2 of altitude exposure (Fig. 3), and it may be due, in part, to environmental stresses associated with the ascent and not the hypoxia. This conclusion is supported by the observation that resting lactates on days 4 and 8 at altitude were not different from prealtitude exposure values, and lactates after the SET$_{track}$ at altitude on all days were not any higher than values after the SET$_{track}$ at low altitude. However, there was the indication of metabolic adaptation in skeletal muscle (decreased LDH activity) that suggested a decreased reliance on anaerobic metabolism that occurred over the time frame of this study (10). Because the resting DPG concentration was expressed per amount of Hb, it was not related to the degree of splenic contraction and thus can provide insight into the high-altitude acclimatization process in horses. The resting DPG/Hb values at 225 m before transport to high altitude were similar to values for the horse (4), and they increased during 8 days of altitude exposure (Fig. 2), a finding consistent in humans (15, 20). The physiological changes associated with an increase in the concentration of DPG, i.e., a decrease in the Hb affinity for O$_2$, suggest that the increase in DPG with ascent to high altitude is a compensatory mechanism that ensures sufficient O$_2$ delivery to the tissues (14, 29), although this may be offset by pH changes with hyperventilation (33). One reason that concentrations did not plateau, in contrast to the Lenfant study (14),
was perhaps due to the differences in the elevations at which the data were collected.

Exercise increases sympathetic activity in horses and thus increases hematocrit. The number of cells released from the spleen in response to exercise is not “all-or-none,” but rather it is related to the extent of the increase in sympathetic activity that is related to exercise intensity (1, 25). This pattern was evident in our study in that the less stressful SET_{track} increased hematocrit to ~45%, and the maximal tests on the treadmill increased hematocrits to over 50% (Fig. 1). The different exercise intensities between track and treadmill also were evidenced by higher blood lactates in the treadmill tests (Fig. 3).

The SET_{track} was standardized at both elevations to the same HR. The intent here was to try to approximate the same metabolic effort with the use of the linear relationship between HR and oxygen consumption established in the horse (26). Therefore, the similar LA concentrations observed during the SET_{track} at both elevations may be expected because the HR was similar. There also were no differences observed in LA concentrations in horses performing the SET_{TM}. However, the LA concentrations obtained in horses performing on the treadmill were in excess of 300% of the values obtained from the animals when performing on the track (Fig. 3).

Although postexercise DPG/Hb increased with acclimatization, there was no effect of exercise. Data from exercising horses are conflicting in regards to DPG changes with exercise with some work demonstrating an increase and others no change in this organophosphate (18, 19).

The ascent to altitude in the horse is accompanied by an increase in the total BV. Hurtado et al. (11) measured BV changes in humans at 4,500 m and found an increase in the total BV due to an expanded RCV accompanied by a decrease in the PV. The increase in BV in the present study was due, in large part, to the expansion of RCV. The BV values obtained during this experiment are in the reference range for horses (12, 25, 26, 32). The magnitude of the increase with acclimatization (~20%) is consistent with the increases observed with training (25).

Performance. The increased DPG concentrations as a result of high-altitude acclimatization in the horse (Fig. 2) may enhance low-altitude performance (23). After a return to lower elevations after high-altitude acclimatization, the DPG concentrations remained elevated for at least 2 days. The elevated DPG would facilitate O₂ unloading at the tissues. This, coupled with a higher P_{O₂} at low altitude, may act to increase O₂ delivery for aerobic exercise performance (28). Because blood samples were not collected beyond 2 days postaltitude exposure, we are unsure of the time course of DPG change on return to low altitude.

Another hematologic component that could enhance athletic performance in the horse is an increase in the total BV. Persson (25) detailed the positive relationship between total BV and performance in horses. It is clear that athletic performance, at least at submaximal levels, is directly related to the total volume of circulating blood (32).

The variables we used to assess performance included peak HR and blood lactate concentrations. Altitude acclimatization had no effect on either, however, there were significant decreases in recovery times for both HR and blood lactates, suggesting a positive effect of altitude acclimatization. This is also consistent with changes observed in skeletal muscle of these horses (10).

There are a number physiological changes occurring with altitude acclimatization (22) that would seem to support improved athletic performance such as increases in muscle capillarity (2) and increases in metabolic capacity of skeletal muscle (3, 9). Despite that, the benefits of training at altitude are controversial (7). Current theory argues that the best training strategy for athletes is to train at lower altitudes and sleep at higher elevations (17). In the case of our equine athletes, the exposure to high altitude did have a positive effect. However, our horses were not at an elite level. Although BV was within normal values for these horses (32), it was not as high as more athletically elite horses (8). Thus, the question still remains, how might elite equine athletes benefit from high-altitude exposure?

One of the consequences of the hypoxia at altitude is a decreased V_{O₂max} and a concomitant limitation to training intensity that can actually produce poorer performances (16). Initial exposure to 3,800 m produced decreases in the speeds in our horses (Table 1). Additionally, there was a decline in performance over the time course of the study, and all animals were slowest on the last SET_{track} of their stay at altitude. Although it is likely that a prolonged stay at 3,800 m would have led to deconditioning and poorer performance at low altitude, the present study suggests that for a shorter period, there is the potential for increasing performance.

This study was the result of intense efforts by a number of students, faculty, and staff. C. C. Lewis was principle wrangler, H. M. Greene was primary technician, J. Liberatore was ranch manager, and A. W. Eastman oversaw health care issues. The staff at White Mountain Research Station (principally D. Trydahl) were incredibly helpful.

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