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Permalink
https://escholarship.org/uc/item/21f7x9gb

Journal
Journal of equine science, 28(2)

ISSN
1340-3516

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Publication Date
2017

DOI
10.1294/jes.28.41

Peer reviewed
Hypoxic training increases maximal oxygen consumption in Thoroughbred horses well-trained in normoxia

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Hypoxic training is effective for improving athletic performance in humans. It increases maximal oxygen consumption (VO2max) more than normoxic training in untrained horses. However, the effects of hypoxic training on well-trained horses are unclear. We measured the effects of hypoxic training on VO2max of 5 well-trained horses in which VO2max had not increased over 3 consecutive weeks of supramaximal treadmill training in normoxia which was performed twice a week. The horses trained with hypoxia (15% inspired O2) twice a week. Cardiorespiratory valuables were analyzed with analysis of variance between before and after 3 weeks of hypoxic training. Mass-specific VO2max increased after 3 weeks of hypoxic training (178 ± 10 vs. 194 ± 12.3 ml O2/(kg × min), P<0.05) even though all-out training in normoxia had not increased VO2max. Absolute VO2max also increased after hypoxic training (86.6 ± 6.2 vs. 93.6 ± 6.6 l O2/(STPD)/min, P<0.05). Total running distance after hypoxic training increased 12% compared to that before hypoxic training; however, the difference was not significant. There were no significant differences between pre- and post-hypoxic training for end-run plasma lactate concentrations or packed cell volumes. Hypoxic training may increase VO2max even though it is not increased by normoxic training in well-trained horses, at least for the durations of time evaluated in this study. Training while breathing hypoxic gas may have the potential to enhance normoxic performance of Thoroughbred horses.

Key words: hypoxia, treadmill exercise, VO2max
Equine Research Institute, where the study was conducted. Veterinarians regularly inspected all horses used throughout this study and detected no lameness nor unsoundness in any of the horses during the experiment.

**Horses**

Five healthy Thoroughbreds (one male, three geldings, and one female, average age 7.0 ± 2.0 (SD) years, average weight 483 ± 24 kg) were studied. The horses trained on a treadmill by running up a 6% incline while breathing normoxic gas (inspired O\(_2\) fraction 0.2095) twice a week prior to hypoxic training. Following a warm-up (1.7 m/sec for 2 min and 3.5 m/sec for 3 min), the exercise protocol consisted of 2-min exercise intervals at 1.7, 4.0, 7.0, 10.0, 12.0, 13.0, and 14.0 m/sec until the horses could not maintain their position at the front of the treadmill with humane encouragement. On 3 other days, the horses walked at a speed of 7 km/hr for 1 hr in a walking machine; for the other 2 days of the week, the horses rested in their stalls.

**Hypoxic training**

For hypoxic training, we studied 5 horses in which VO\(_2\)\(_{\text{max}}\) had not increased during the 3 prior consecutive weeks of supramaximal treadmill training in normoxia. The horses ran up a 6% incline while breathing hypoxic gas twice a week for 3 weeks. On 3 other days, the horses walked at a speed of 7 km/hr for 1 hr in a walking machine; for the other 2 days of the week, the horses rested in their stalls. For hypoxic training, the horses wore a semi-open flow mask that delivered 15.1 ± 0.2% inspired O\(_2\) during exercise. The running speed on the treadmill was set to elicit exhaustion between 2–3 min after warm-up (1.7 m/sec for 2 min, 4.0 m/sec for 2 min, and 7.0 m/sec for 2 min). The horses had 5 hypoxic exercise training episodes during the span of 3 weeks.

**Cardiorespiratory valuables on a treadmill**

Exercise evaluations were performed to measure O\(_2\) transport variables and the running distance before exhaustion for each horse in normoxia at the end of normoxic training (before hypoxic training) and at the end of hypoxic training (after hypoxic training). The running distance was calculated as the sum of the running speed × 2 min at each running step. Before leading a horse onto the treadmill, a 14-ga Teflon\(^\circledR\) catheter was placed in the left jugular vein following injection of a local anesthetic agent. The exercise protocol when breathing normoxic gas consisted of a warm-up (1.7 m/sec for 2 min and 3.5 m/sec for 3 min) followed by 2-min exercise intervals at 1.7, 4.0, 7.0, 10.0, 12.0, 13.0, and 14.0 m/sec until the horse could not maintain its position at the front of the treadmill with humane encouragement, all with the treadmill inclined to a 6% grade and with the horse breathing normoxic gas (Fig. 1). Horses wore an open-flow mask for measurement of VO\(_2\) and CO\(_2\) production (VCO\(_2\)). Heart rate was measured with a heart rate monitor (S810, Polar, Kempele, Finland), and venous blood was drawn from the jugular catheter to measure the plasma lactate concentration ([LAC]) and packed cell volume (PCV). Blood samples were centrifuged (KH120A, Kubota, Tokyo, Japan) for 5 min (12,000 × g) to separate plasma for measurement of [LAC] in a lactate analyzer (Biosen C-Line Glucose & Lactate Analyser, EKF-diagnostic GmbH, Barleben, Germany).

**Oxygen consumption**

Horses wore a 25-cm diameter open-flow mask on the treadmill, with a rheostat-controlled 3.8-kW blower drawing air through it at bias-flow rates of 6,000–8,000 l (ATP)/min. Air flowed through 20-cm-diameter wire-reinforced flexible tubing affixed to the mask and across a 25-cm-diameter pneumotachograph (LF-150B, G. N. Sensor, Chiba, Japan) connected to a differential pressure transducer (TF-105, G. N. Sensor); this was used to ensure that bias flows during measurements were identical to those during calibrations. Oxygen consumption and VCO\(_2\) were measured with standard mass-balance techniques [1, 8] using an O\(_2\) and CO\(_2\) analyzer (METS-900, VISE Medical, Chiba, Japan), 2-m-long Nafion\(^\circledR\) drying tube with countercurrent dry gas flow (Drierite (CaSO\(_4\))) to remove H\(_2\)O from sample gas, and electronic mass flowmeters (Model DPM3, Kofloc, Tokyo, Japan) for measuring N\(_2\) and CO\(_2\) calibration flows using the N\(_2\)-dilution/CO\(_2\)-addition technique [5]. Gas analyzer and flowmeter outputs were recorded with A/D hardware (DI-720-USB, DATAQ Instruments, Akron, OH,
U.S.A.) and software (WinDaq Pro+, DATAQ Instruments, Akron) on personal computers.

**Statistical analysis**

Comparisons were made using analysis of variance with pre- or post-hypoxic training as the treatment. A *P*-value of ≤0.05 was considered significant.

**Results**

Before hypoxic training commenced, the VO$_2$max values during the 3 consecutive weeks of supramaximal normoxic training, which was performed twice per week were 178 ± 9.1, 180 ± 10.0, and 178 ± 10.1 m l O$_2$/(STPD)/(kg × min), respectively. The total running distance for the final normoxic exercise run, at which time the final measurement of VO$_2$max was obtained before hypoxic training, was 5,174 ± 759 m.

The total running distance during hypoxic exercise was 2,987 ± 567 m. The running speed that elicited exhaustion in hypoxia between 2–3 min was 12.1 ± 0.45 m/sec. After hypoxic training, the average body weight of the horses was 482 ± 30 kg.

Table 1 shows a summary for the results of the cardiorespiratory values before and after hypoxic training. Mb: body mass. Mean values (+ SD) are shown. *Significant difference (P≤0.05) compared with before hypoxic training.

### Table 1. Cardiorespiratory values before and after hypoxic training

| Variable                          | Before       | After       |
|-----------------------------------|--------------|-------------|
| Body mass (kg)                    | 483 ± 26     | 482 ± 30    |
| Total running distance (m)        | 5,174 ± 759  | 5,808 ± 981 |
| Total running time (min)          | 11.33 ± 0.95 | 12.07 ± 1.22|
| Heart rate (beat/min)             | 214 ± 5      | 217 ± 7     |
| VO$_2$/Mb (m O$_2$ (STPD)/(min × kg)) | 178 ± 10    | 194 ± 12*   |
| VCO$_2$/Mb (m CO$_2$ (STPD)/(min × kg)) | 202 ± 12    | 214 ± 20    |
| Respiratory exchange ratio        | 1.14 ± 0.02  | 1.10 ± 0.04*|
| Absolute VO$_2$ (l O$_2$ (STPD)/min) | 86.6 ± 6.2  | 93.6 ± 6.6* |
| Absolute VCO$_2$ (l CO$_2$ (STPD)/min) | 97.8 ± 7.3  | 102.7 ± 8.0 |
| End-run [LAC] (mmol/l)            | 23.1 ± 3.3   | 23.2 ± 6.8  |
| PCV (%)                           | 65.0 ± 3.3   | 66.1 ± 3.2  |

Cardiorespiratory values were recorded during a run at a speed eliciting VO$_2$max.

**Discussion**

This study was conducted to determine the effects of hypoxic training on VO$_2$max using well-trained horses in which VO$_2$max had not increased over 3 consecutive weeks of twice a week supramaximal treadmill training in normoxia. Three weeks of training while inspiring 15% O$_2$ twice a week improved not only the horses’ mass-specific VO$_2$max, but also their absolute VO$_2$max. Total running distance after 3 weeks of hypoxic training had increased 12% compared with that at the onset of hypoxic training, although this difference in total running distance between before and after hypoxic training was not significant. This may have been due to the high variance in the distance-run data resulting in low statistical power to detect a significant difference. These results suggest that hypoxic training is an effective method for increasing aerobic capacities of horses compared with only using normoxic training.

It has been reported that Thoroughbred horses experience severe hypoxemia when exercising at VO$_2$max [3, 14, 15, 22]. Severe hypoxemia may contribute to elevation of aerobic capacity in Thoroughbred horses, as it suggests that horses are maximally utilizing all components of their O$_2$ transport system, even to the point of becoming hypoxemic and experiencing reduced O$_2$ saturation of arterial blood. Therefore, we wondered if hypoxic training might be effective for increasing VO$_2$max and enhancing the performance of well-trained Thoroughbred horses. We found in this study that hypoxic training is effective in increasing VO$_2$max in highly trained Thoroughbred horses compared with their normoxic VO$_2$max. Hypoxic training may generate more severe hypoxemia than does normoxic training, and we speculate that the existence of more severe hypoxemia may contribute to increasing VO$_2$max; it will be useful to measure the arterial blood O$_2$ concentration in hypoxia in
the future.

We have previously found that hypoxic training improves $V\dot{O}_{2\text{max}}$ and that it increases the running distance before exhaustion in untrained horses [12]. However, the running speeds on the treadmill were the same for both normoxic and hypoxic training. In that study, the relative intensity (percentage of $V\dot{O}_{2\text{max}}$) of hypoxic exercise was higher than that of normoxic exercise [12]. Therefore, it is possible that the differences in $V\dot{O}_{2\text{max}}$ and running distance resulted from differences in the relative intensity of the exercise in that study. In this study, it was difficult to directly compare running speed and running distance between normoxic (i.e., before hypoxic) training and hypoxic training because the exercise protocols were not identical. However, the absolute exercise intensity in normoxia was greater than in hypoxia because the total running distance, running speed, and run time in normoxia were greater than in hypoxia. However, both normoxic and hypoxic training were at the same relative intensity in terms of the fact that all horses ran to exhaustion, although the run time in normoxia was longer than that in hypoxia. Nevertheless, hypoxic training appears to be an effective method to improving $V\dot{O}_{2\text{max}}$ in horses, perhaps due to a stimulus resulting from breathing hypoxic gas, given that $V\dot{O}_{2\text{max}}$ had not increased in these horses over 3 consecutive weeks of supramaximal treadmill training in normoxia. It appears that hypoxic training may enhance the aerobic capacities of horses compared with normoxic training.

It has been reported that altitude training in humans increases the hemoglobin concentration, resulting in increased aerobic capacity [6, 19–21]. It has also been reported that altitude training in human endurance athletes may explain more than half of the increase in $V\dot{O}_{2\text{max}}$ that occurs due to increased hemoglobin mass [20]. In contrast, there was no significant difference in PCV between before and after hypoxic training in this study; it is possible that non-hematological changes may play a role in increasing $V\dot{O}_{2\text{max}}$ in Thoroughbreds. It is known that PCV in Thoroughbreds increases when running because of mobilization of erythrocytes stored in the spleen [17]. Therefore, it is not clear whether increased PCV contributes to increased equine performance or not; further study of this question is needed.

Mizuno et al. reported that increases in $O_2$ deficit (29%) and short-term running performance (17%) were observed after 2 weeks of altitude (2,700 m) training in humans, although $V\dot{O}_{2\text{max}}$ remained at the pre-altitude training value [11]. Some reports suggest that high-intensity intermittent training in hypoxia may increase anaerobic performance [7, 11]; some studies have found that performance, e.g., run time to exhaustion, increased without increased $V\dot{O}_{2\text{max}}$ [9, 11]. End-run lactate concentrations were nearly the same values before and after hypoxic training in this study. In Thoroughbreds, the plasma lactate accumulation rate is related to net anaerobic capacity [13, 16]. Therefore, it is possible that hypoxic training may not increase net anaerobic power in Thoroughbreds, as end-run [LAC] was nearly the same at exhaustion in this study when breathing either gas.

It has been reported that altitude training may decrease body weight [2]. In this study, the horses maintained body weight during hypoxic training, likely because the horses used were highly trained in normoxia a priori. In terms of energy expenditure, horses running while breathing hypoxic gas consumed less $O_2$ because their running speeds and running distances were less than those in normoxia. Because the horses were not living in chronic hypoxia but instead only ran acutely while breathing hypoxic gas during experiments, this may have contributed to the horses’ maintaining their body weights during the course of the experiments.

In summary, it appears that training while breathing hypoxic gas may have the potential to enhance the peak normoxic performance of Thoroughbred horses. Although the specific mechanisms responsible for eliciting this difference are not yet known, the effect of the hypoxic stimulus, even during this relatively brief duration of exposure, is striking.

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HYPOXIC TRAINING IN THOROUGHBRED HORSES

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