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Adaptations of racing Thoroughbreds to a hypoxic chamber: A pilot study

Jie Li[1], Brian D. Nielsen[1]*, Cara I. Robison[1], Holly Spooner[2]

1. Department of Animal Science, Michigan State University, East Lansing, MI 48824, USA
2. School of Agriculture, Middle Tennessee State University, Murfreesboro, TN 37129, USA
Li, J.: ORCID: 0000-0003-2522-1777, Nielsen, B. 0000-0002-2570-5731. Robison, C. I.: ORCID: 0000-0002-3421-5064, Spooner, H. : ORCID: 0000-0002-2864-7776.

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ABSTRACT

Hypoxic exposure results in physiological adaptions and enhanced human athletic performance. However, few reports exist regarding responses of horses to similar conditions. The goals of this preliminary work were to evaluate whether horses could be acclimated to a hypoxic chamber (HC) and to monitor their performance. Trial 1: Two 4-yr-old Thoroughbreds were used to establish protocols for adaptation to the HC. Horses were stalled in the HC for 8 to 10 h/d while O2 concentrations were decreased over 2 wk until 13.5% O2 was achieved. On d 37, horses were removed from the HC and shipped to a track for 7 d before reentering the HC for the remainder of the 2-month study. Resting hemoglobin (Hb) was measured on d 0, 37, and 61 and ranged from 14.8 to 15.2 g/dL. Trial 2: Two 2-yr-old Thoroughbreds were maintained in the HC at 13.5% O2 for 8 to 8.5 h/d for 21 d, shipped to a track for 5 d, and then placed back in the HC 8.5 h daily for the remainder of the 31-d trial. Horses were conditioned on the treadmill or track 6 d/wk. Horses underwent a standardized exercise test (SET) prior to being initially placed in the HC. The SET was repeated on d 10 and 31. Peak heart rate (HR) reached during exercise, and HR at 3 and 5 min post-exercise were recorded. Hemoglobin was measured immediately upon cessation of exercise. There was no difference in HR at 3 min (P=0.18), and 5 min post-exercise (P=0.64). Hb was greater on d 31 compared to d 0 (P<0.01). Without controls for comparison, we cannot confirm that differences detected were caused by the effect of HC, due to potential training effects. Results demonstrated horses can be adapted to HC but improvements in race performance were not noted.

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Introduction

Since the 1968 Olympic Games in Mexico City (elevation 2,240 meters), when the benefits of altitude training on athletic performance became evident (Kasperowski, 2009), people started to use hypoxic conditions to improve aerobic performance. It has been recognized that boosting the oxygen transport capacity, either by training or through other means commonly referred to as "blood doping," is the powerful tool for improving athletic performance in aerobic sports (Segura and Lundby, 2014). However, blood doping was banned by the International Olympic Committee (IOC) in 1985, though there was no specific test for it at the time (Milne, 2006). Moreover, Union Cycliste Internationale (UCI) decided to evict the male athlete with hematocrit (HCT) level >50% and female athlete HCT >47% from the competition (Vergouwen et al., 1999). Given the benefits of blood doping, many coaches and athletes still are trying to find ways to improve athletic performance in a similar fashion, but through legal means. Compared with blood doping, the

*Corresponding Author: Brian D. Nielsen
E-mail: bdn@msu.edu

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function of "live high, train low" is to improve aerobic capacity legally and without harmful side effects (Mairbäurl, 2013). In principle, human and animals exposed to high altitude conditions for an extended period of time, such as living on a mountain or sleeping in a hypoxic chamber that can simulate high altitude conditions, can allow humans or animals to obtain physiological acclamations, such as increases in red blood cell (RBC) volume and erythropoietin (EPO), and improvements in heart rate (HR) recovery (Naeije, 2010), thereby improving their aerobic capacity. Thus, athletes can perform the same intensity of exercise more efficiently when they return to lower elevations (Levine and Stray-Gundersen, 1997).

A number of studies have shown the benefits of high-altitude training to human athletes (Wolski et al., 1996; Stray-Gundersen et al., 2001), which raises interest in the horse industry. There is some anecdotal evidence to suggest some success of high-altitude training in horses. For instance, Canonero II, purchased for $1,200 as a yearling, was shipped from Venezuela where he had been racing and training at altitude, shortly before racing in, and winning, the 1971 Kentucky Derby (Hunter, 2010). However, there is limited evidence in the scientific literature proving that horses can benefit from the physiological changes of chronic altitude exposure. Thus, there is interest in exploring the role of altitude training in horse racing, which could bring some scientific insights for horse owners and trainers for the future.

Given they are commonly used by elite human athletes, it is not surprising that "high-altitude" or hypoxic chambers have also been designed for horses (Stewart, 2013). However, testing of such chambers has been limited. This study was therefore designed using a high-altitude chamber with simulated low oxygen conditions to determine whether horses could be acclimated to a hypoxic chamber (HC) and to monitor their performance utilizing a Standardized Exercise Test (SET) and official races. We hypothesized that horses could adapt to a hypoxic chamber.

Materials and methods

The project was approved by the Michigan State University Institutional Animal Care and Use Committee via an exemption approved on June 15, 2018.

Design of hypoxic chamber (HC): Prior to the start of this work, the owner of the Thoroughbred facility retrofitted a room in his barn to make into a HC. The HC had to be air-tight, have an additional power supply, and be equipped to produce hypoxic conditions. Equipment was housed outside the HC and included a rotary screw air compressor with tank and dryer (Aircenter SK15, Kaeser Compressors, Inc., Fredericksburg, VA) to make incoming gas compressed and flow into the Simulated Altitude Generator (Hypoxico Altitude Training Systems, Hypoxico, Inc., New York, NY). This generator provided oxygen-depleted air to a stall having ventilating openings for equivalent pressure inside. The system contained digital internal and external monitoring systems and could control oxygen concentration from 20.9% down to 9%.

The inside chamber contained two separated stalls (3.1×3.1 m) equipped with sealed door and walls, hay bags, water buckets, oscillating fans, air conditioning, and scrubber machines to remove CO_2, methane, and ammonia produced by horses. Moreover, there was an ammonia monitor and oxygen sensor to ensure horses' safety with the HC equipped with an emergency ventilation door open if conditions inside the chambers were to exceed safety margins. The chamber temperature was approximately 18°C in the winter and 21°C in the summer. Stalls were equipped with a drainage system to allow for urine removal. Feces were manually removed every 12 h. Cartridges for CO_2 scrubber were replaced every 36 h.

Experimental design Trial 1: Two 4-yr-old Thoroughbreds, one gelding and one filly, with at least 6 prior racing starts were housed in the HC to test the adaptation of the horses and chamber equipment. All horses in study were owned by, and under the management of, a professional Thoroughbred breeding and racing facility. The horses were in healthy physical condition as needed for racing. At the beginning of the study, the two horses in Trial 1 were administered 200 mg/d iron orally for four wk in case iron deficiency caused by hypoxia might interfere with erythropoiesis.

Taking into consideration the horses' safety and health, the oxygen concentration was gradually decreased daily to target setpoint (13.5%) that was reached after a two-week period to allow horses to adapt to low oxygen conditions. On a daily basis, within the hypoxic system, it took almost 1.5 h to decrease the oxygen concentration from 21% to 18% and then approximately an additional three hours to reach the setpoint 13.5% (simulated altitude at 3,200 m). Thereafter, horses stayed at the HC with 13.5% oxygen content for 8 to 10 h/d. All horses were provided with ad libitum access to water and grass hay while in the HC. When out of the HC, horses stayed in traditional box stalls and were turned out in a paddock if weather permitted. Additionally, horses were conditioned with 30-min training on a high-speed treadmill (Chadwick Engineering Ltd., Canada) outside the HC, which included walking, trotting, and cantering 6 d/wk . On day 37, the horses were sent to a racetrack, at which point their daily exercise was on the dirt track with a rider. After 7 d, they returned to the farm and resumed daily HC occupation and both track and treadmill exercise through the completion of Trial 1 except when transported to the racetrack for racing. Horse 1 raced on day 62 (one day after the trial ended) and Horse 2 raced on day 41 and day 56. All training and racing regimens were under the control of the farm's resident trainer and were similar to what was prescribed for the other Thoroughbreds in race training at the farm. Trial 1 began in September 2018 and ended in November 2018.

Experimental design Trial 2: The second trial began in December 2018 and finished in January 2019, lasting for 31 days. Two 2-yr-old Thoroughbred fillies, with a minimum of 3 prior starts, were stalled in the HC, with the oxygen concentration gradually decreasing to 13.5% during the two-week adaptation. They stayed with a 13.5% oxygen concentration for 8 to 8.5 h/d. When out of the HC, horses were maintained in normal box stalls on the farm. Horses were conditioned on the treadmill or track 6 d/wk. When not provided controlled daily exercise, horses were allowed turnout in a paddock. The conditioning regimen consisted of 30-min of exercise including walk, trot, and canter on the high-speed treadmill or with a rider on a dirt track, consisting of a 15-min walk (warm-up), a 4-min trot, a 4-min canter, a 2-min gallop, and then a 15-min walk (recovery). The choice as to whether the exercise was performed on the treadmill or track was weather-dependent and, again, was made by the resident trainer and was consistent with the work being received by other Thoroughbreds in race training at the facility. The horses were shipped to a track on d 22 for 5 days and then reentered the HC for the remainder of Trial 2 except when transported to the racetrack for racing. Horse 3 raced two days after Trial 2 ended and Horse 4 raced 8 days after Trial 2 ended. Horses received race training on the dirt track with rider during the time between the end of the trial and racing. In this trial, a standardized exercise test (SET) was performed on the treadmill, consisting of walking 1.6 km at 1.8 m/sec with an 8% slope, trotting 1.6 km at 3.4 m/sec with 3% slope, and walking 0.8 km at 1.8 m/sec. Due to equipment limitations, the SET did not include canter exercise. The SET was performed at d 0 and repeated on d 10 and 31. No supplemental iron was provided in Trial 2.

Sample collection Trials 1 and 2: In Trial 1, blood samples were collected for resting hemoglobin (Hb) in the morning on d 0, 37, and at the end of the trial (d 61). In Trial 2, blood samples were taken immediately after exercise ceased in each SET for Hb analysis. Peak HR, and the HR at 3 min and 5 min post-exercise was recorded after each of the SET on day 0, 10, and 31 by heart rate monitor (Polar, Bethpage, NY).

Sample analysis Trials 1 and 2: Blood samples were taken via jugular venipuncture with vacutainers (BD Vacutainer®, Becton Dickenson, Franklin Lakes). Samples were placed in refrigerated bags, transported immediately after collection to Michigan State University, where whole blood was used to analyze Hb levels following the procedure of Stan-bio (HemoPoint® H2). After 4-wk of iron supplementation, blood samples for iron analysis were taken and centrifuged at 3,500 x g for 20 min. The aliquots of serum were stored at -20° C until analysis. Serum iron was determined by a colorimetric method (Wako Pure Chemical Industries, USA) with an automated analyzer. Total iron binding capacity (TIBC) was calculated, following the manufacturer's instructions. The equation follows as:

$$TIBC\ (\mu g/dl) = (Aunk - Ablk) / (Astd - Ablk) \times 400$$

Where Aunk, Ablk and Astd are the absorbance of the plasma unknown, blank, and standard.

Race performance evaluations Trials 1 and 2: All horses' race results were obtained from Equibase (https://www.equibase.com) and average finishing position within a race and the average speed ratings from races before starting the trials were compared to the races after commencing the trials.

Statistics: Data from Trial 1 were not analyzed statistically but were simply used to look for anomalies outside of normal ranges. All data from the SET trial (Trial 2) are presented as mean ± standard error. Hemoglobin concentration was assessed by ANOVA in the proc MIXED program of SAS version 9.0 (SAS Inc., Cary, NC, USA) with the fixed effect of day. Peak HR, and HR at 3 min, and 5 min post-exercise were compared using a paired Student's t-test to assess performance differences based on the physiological changes that occur with acclimatization. Significance was defined as $P \leq 0.05$.

Results

Blood Variables Trials 1 and 2: After four weeks of being on the study in Trial 1 and being supplemented with iron, serum iron concentrations for the two horses were 124 µg/dL and 119 µg/dL, and TIBC were 404 µg/dL and 416 µg/dL. The standard ranges of serum iron and TIBC are 53 to 209 µg/dL and 244 to 480 µg/dL, respectively (Assenza et al., 2016). The concentrations of resting Hb (g/dL) of first two horses in Trial 1 ranged from 14.8 to 15.2 g/dL. The standard range of resting Hb is 12 to 18 g/dL (Stewart et al., 1977).

In Trial 2, Hb measured immediately post-exercise was greater on d 31 (17.0 g/dL) compared to d 0 (16.0 g/dL, SEM=0.1; P<0.01) as seen in Table 1.

Table 1. Hemoglobin concentration in two Thoroughbreds taken immediately after a SET on day 0, 10, and 31 in Trial 2.

	Hb (g/dL)		
	day 0	day 10	day 31
Horse 3	15.8	15.9	17.1
Horse 4	15.9	16.1	16.9
Average	15.9[a]	16.0[ab]	17.0[b]
SEM=0.1, P Value=0.005			

[a b] Means not sharing similar superscripts differ (P < 0.05). Hb = hemoglobin. SEM = Standard error of the mean. SET = Standard exercise test

Heart rate Trial 2: The average peak HR measured on d 0, 10, and 31 during the SET was between 110 and 120 and did not differ between days. The HR at 3 min post-SET ranged from 61 to 68 (SEM=2, p=0.18) and HR at 5 min post-SET ranged from 54 to 63 (SEM=7, p=0.64). No differences in HR were seen between days (Table 2).

Table 2. Average heart rate in two Thoroughbreds during and after a SET on day 0, 10, and 31.

	Average HR (bmp)		
	Peak	3-min post SET	5-min post SET
day 0	120	68	63
day 10	110	68	62
day 31	120	61	54

bmp = beats per minute. HR = Heart rate. SET = Standard exercise test

Race performance in Trials 1 and 2: The race results from all four horses studied in the two trials can be found in Table 3 – contrasting their performances after HC to their races prior to commencing this study. As can be seen, no improvement in the average finish position was noted and the average speed rating decreased in the races performed after HC adaptation compared to prior to HC adaptation.

Table 3. The average finish position and average speed rating score both prior to HC acclimation and after HC acclimation in four Thoroughbred racehorses along with the number of races from which the average was determined. Racetrack elevation ranged from 147 to 262 m.

Trial 1	Average finish position (# of races)	Average Speed Rating
Horse 1 Prior HC	6 (6 races)	52
Horse 1 After HC	5 (1 race)	41
Horse 2 Prior HC	6 (6 races)	23
Horse 2 After HC	9 (2 races)	17
Trial 2	**Average finish position (# of races)**	**Average Speed Rating**
Horse 3 Prior HC	4 (5 races)	36
Horse 3 After HC	5 (1 race)	14
Horse 4 Prior HC	5 (3 races)	31
Horse 4 After HC	5 (1 race)	2

HC = hypoxic chamber

Discussion

This preliminary study has many limitations. There is a small number of animal subjects, a lack of controls, and limited characterization of athletic performance – all of which complicate the interpretation of this study evaluating the use of a HC and prevent any firm conclusions from being made. The changes over time seen in Trial 2 are confounded with potential training effects (Soroko et al., 2019). Despite these major limitations, the tremendous cost associated with establishing and maintaining HC would likely preclude this study to be performed in a normal research setting. Thus, by having the opportunity to study actual racehorses being placed into HC and then compare their performances before and after adaptation enabled some insights into the acclimation of horses in a HC that might not otherwise be afforded.

While not specifically measured, changes in behavior were noted throughout the study. In Trial 1, when the horses were first exposed to 13.5% oxygen, they remained sedentary and just stood in a corner with their heads down – suggesting they were possibly experiencing altitude sickness such as occurs with humans (Moore and Regensteiner, 1983). However, after two weeks of exposure to low oxygen, behavior appeared to return to normal. The return to normal behavior suggested that horses could adapt to a HC, but that adaptation was not immediate and horse welfare needs to be considered.

The interpretation of resting hematological parameters can be somewhat ambiguous. This is because the horse's spleen is capable of storing one-third to one-half of the RBC (Poole and Erickson, 2011). The spleen can make active contractions induced by chemicals called alpha agonists and mobilize stored RBC to the systemic circulation in response to varying stressful stimuli, such as hypoxia, excitement.

Releasing reserved RBC leads to raising systemic hematocrit from 35% at rest to 60 to 70% during maximal exercise (Poole and Erickson, 2011). Moreover, strenuous exercise and training could potentially boost blood values as well (Soroko et al., 2019). In Trial 2, Hb concentrations were increased after 31 days of being in the study and being stabled in the HC. Similar results were also reported among previous human and other animal studies (Heinicke et al., 2003). Wehrlin et al. (2006) showed Hbmass increased by 5.7% in human athletes after 24 days in a "live high, train low" protocol. Hb concentration is a favorable indicator that supports aerobic performance. However, in this pilot study, due to the lack of resting hemoglobin concentrations, the lack of control animals and possible confounding effects of training, post-SET Hb concentrations were not analyzed statistically. Thus, it is not possible to conclude that the changes in blood parameters were due to the HC.

Iron plays a role in enhancing Hb concentration and the activity of RBC. Exercise can induce changes in iron metabolism to mimic iron deficiency, thereby decreasing Hb and ferritin concentrations in humans (Zoller and Vogel, 2004). Iron supplements are often used by elite athletes during extreme exercise (Beutler, 2002; Zoller and Vogel, 2004). In humans, Govus et al. (2015) suggested that sufficient iron stores are required to support increased erythropoiesis during prolonged altitude exposure. Moreover, a three- to five -fold increase in erythropoiesis occurs and was associated with 100% erythroid iron uptake during the first few days of adaptation. Recognizing this, the racehorse facility owner supplemented the two initial horses from Trial 1 with iron to ensure their iron status was sufficient to facilitate erythropoiesis. However, TIBC and serum iron values were similar to previous results with a standard training program (Assenza et al., 2016). Some studies have reported increased serum iron concentrations in horses following exercise, probably resulting from the release of iron from reticuloendothelial cells (Assenza et al., 2016; Assenza et al., 2017). Iron metabolism can be influenced by the duration and intensity of exercise (Assenza et al., 2016).

The 2007 Horse NRC suggested iron 40 ppm in the diet for adult horses. Typically, there is no need to provide additional iron supplements for horses due to an abundance of iron in the diet of horses (Richards and Nielsen, 2018) and it is unusual for horses in training to have an iron deficiency. Chronic excessive iron intake may result in toxicosis or potential disease in equids (Theelen et al., 2019). At this time, there is no indication that iron supplementation is needed or beneficial to racehorses and the owner, after seeing the initial results from the horses in Trial 1, decided to not continue with iron supplementation in Trial 2.

In terms of indoor air control, when opening the door of the HC to lead a horse through, air within the HC is allowed to mix with air from outside the HC resulting in small changes in oxygen concentration (2 to 3%) that were noted for 15 to 20 min before returning to the setpoint. However, with the oxygen sensor near the door, it is unlikely that the oxygen concentration of the whole room was altered much during those brief periods. Regardless, these brief alterations were noted as a reminder that any time the door is opened, there is a period in which the gas concentrations are altered before homeostasis is once regained.

Conditions of air quality and sanitation in the HC can have a direct effect on the health and performance of horses. Humans sleeping in a hypobaric chamber or high-altitude tent experience different environmental conditions than would a horse residing in one. Air pollutants originate mainly from their feces, urine, feed, and bedding. Moreover, horses undergo substantial hindgut fermentation – resulting in carbon dioxide, methane, and ammonia being released from horses – gases which should be removed from the chamber. Horses can potentially produce 20.7 kg of methane gas (Elghandour et al., 2019) and 12.2 kg of ammonia (EPA, 2004) per year. From this standpoint, filter systems are a critical part of the insulated chamber to remove those biochemical wastes and keep the airflow in the chamber clean and fresh. At the time of this study, to purchase cartridges to remove CO_2 for the chamber, the cost would have been around US$350, and the cartridges would need to be replaced every 36 hours - resulting in high operating costs.

Beyond gas production, horses can produce large amounts of sweat and also release substantial moisture through their respiration. After racing poorly, one of the horses in Trial 2 had an endoscopic evaluation done. An accumulation of mucus within the airways was found and it is believed the high humidity in the chambers likely contributed to mucus production and secretion (Gerber, 1973). This likely had a negative effect on racing performance.

As a pilot field study, we confronted various unexpected challenges with using the HC. While this study was not able to demonstrate improved performances with the use of HC, the findings are useful in providing guidance to others that may be interested in exploring work in this area. For example, lowering the oxygen concentration in the chambers lowering the oxygen concentration in the chambers needs to be done over two to three weeks to allow adaptation without compromising animal welfare. When the oxygen concentration was decreased too quickly after initially placing the horses into the HC, a lack of voluntary movement in the chambers suggested adaptation had yet to occur. As horses seemingly adapted to the HC, their behavior returned to normal. While this study was originally designed to evaluate physiological acclimation, the behavioral adaptation should also be taken into consideration.

In terms of oxygen level, a 13.5% concentration, similar to that reported by Wickler and Anderson (2000), was targeted. The initial assumption was that an ideal concentration could be determined and then used for a larger study with more horses. Towards the end of the two-wk adaptation period in Trial 1, the oxygen concentration was temporarily dropped to 13% but the behavior of the horses was concerning (they appeared to be abnormally depressed and lethargic) and the concentration was returned to 13.5% at which point the horses resumed eating and normal behavior within the HC. Again, this study serves as a reminder that, even if improvements in athletic performance were gained, the well-being of the horses needs to always be considered. Adaptation needs to be gradual and an ideal oxygen concentration for an equine HC still needs to be elucidated.

By monitoring horses performance after exposure to HC, we found their performance actually appeared to decrease, it also revealed potential concerns with using such a system. Beyond needing to remove large quantities of gases such as carbon dioxide, methane, and ammonia, high humidity within the chambers may have been the cause of the excess mucus found in the airway of one of the study horses that had an endoscopic evaluation after a poor race performance. These are not issues that would be present to a great degree with human HC but are challenging to deal with in an equine HC. In addition to the gas removal, a dehumidifier should be used to keep moisture within the chambers to an acceptable level.

While a HC allows oxygen concentrations to be controlled allowing horses to "live high, train low" without actually living at a high altitude, there are many limitations. Using a HC is a time-consuming and installing and operating the system is expensive. While there is vast experience using such systems with humans, many modifications need to be made to a HC system to allow it to be used for horses given that horses excrete waste while in the chambers and this waste must be removed promptly. Besides the high cost and large investments of time needed to manage horses in the chambers, horse welfare must be continually emphasized – particularly during the initial adaptation stage as horses are becoming acclimated to decreasing oxygen concentrations.

Conclusions

Although limitations existed in this pilot study, the findings of this study demonstrate that horses could adapt to a HC, but this process takes time. Also, by not having control horses housed outside the HC, any increases in Hb that may be associated with HC use were confounded with a potential effect of training. While having control horses should be the standard for any research project, this rare opportunity to study horses in a HC and undergoing actual racing provided some knowledge that could be used in further controlled studies. Further, this preliminary work showed no improvements in finish position and speed rating after HC. HC is a costly and time-consuming investment and any desire to invest in such should be taken only after great consideration. Whether HC could enhance horse race performance is still in question.

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