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Cardiopulmonary function during supramaximal exercise in hypoxia, normoxia and hyperoxia in Thoroughbred horses

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Supramaximal exercise while inspiring different O_2 gases may induce different responses in cardiopulmonary function at the same relative and/or absolute exercise intensity. The purpose of this study was to compare the effects of supramaximal exercise in hypoxia, normoxia and hyperoxia on cardiopulmonary function in Thoroughbred horses. Using a crossover design, five well-trained horses were made to run up a 6% grade on a treadmill at supramaximal speeds sustainable for approximately 110 sec (approximately 115% $\dot{V}O_2$max) while breathing normoxic gas (NO, 21% O_2) or hypoxic gas (LO, 15.3% O_2) in random order. Horses also ran at the same speed, incline and run time as in NO while breathing hyperoxic gas (HO_{NO}, 28.8% O_2) and as in LO while breathing normoxic gas (NO_{LO}). Runs were on different days, and cardiopulmonary variables were analyzed with repeated-measures ANOVA and the Holm-Šidák method for pairwise comparisons. Supramaximal speeds differed significantly between NO and LO (14.0 ± 0.5 [SD] m/sec vs. 12.6 ± 0.5 m/sec), but run times to exhaustion did not (112 ± 17 sec vs. 103 ± 14 sec). The $\dot{V}O_2$max in NO was higher than that in LO (165 ± 11 vs. 120 ± 15 ml (min× kg)), as was the arterial oxygen tension (66 ± 5 vs. 45 ± 2 Torr). Oxygen consumption was increased in HO_{NO} and NO_{LO} compared with the values in NO and LO, respectively. Supramaximal exercise in hypoxia induces more severe hypoxemia and decreases $\dot{V}O_2$max compared with normoxia at the same relative intensity. Conversely, supramaximal exercise in hyperoxia alleviates hypoxemia and increases $\dot{V}O_2$ compared with normoxia at the same absolute intensity.

Key words: *hyperoxia, hypoxemia, hypoxia, training, $\dot{V}O_2$max*

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Training while inspiring a low oxygen concentration is effective for improving athletic performance not only in humans but also in horses [3, 5, 10–12, 17–19]. Hypoxic training increases maximal oxygen consumption ($\dot{V}O_2$max) more than normoxic training in untrained Thoroughbred horses [18]. We previously reported that hypoxic (15% inspired O_2) training for 3 weeks increased $\dot{V}O_2$max in 5 highly trained horses in which $\dot{V}O_2$max had not increased over 3 consecutive weeks of supramaximal treadmill training in normoxia [17]. It is still unclear what stimulus during exercise in hypoxia is capable of contributing to the increased aerobic capacity in horses.

Severe hypoxia was observed under normoxic conditions in Thoroughbred horses maximally utilizing all components of their O_2 transport systems [2, 14, 15, 22]. The arterial O_2 tensions in these studies were less than 75 torr [2, 14, 15, 22]. It was reported that a run while inspiring 15% O_2 induced more severe hypoxia than that under normoxic conditions [22]. We previously reported that exercise to fatigue for 2 min under hypoxic conditions increased $\dot{V}O_2$max more than that under normoxic conditions [17]. Severe hypoxemia may contribute to elevating aerobic capacity in Thoroughbred horses. However, the severity of the hypoxemia may depend on the exercise intensity, running time and/or inspired O_2

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concentration. Therefore, the purpose of this study was to compare the effects of exercise to fatigue for about 2 min in hypoxia and normoxia on cardiopulmonary function in Thoroughbred horses.

It has also been reported that horses increase their $\dot{V}O_2$ during a run while breathing hyperoxic gas compared with when breathing normoxic gas [6–8, 13, 22, 23]. However, more information is needed regarding the changes in cardiopulmonary function during a run while inspiring hyperoxic gas. Therefore, we also compared the effects of supramaximal exercise in normoxia and hyperoxia on cardiopulmonary function in Thoroughbred horses.

Materials and Methods

The protocols for the study were reviewed and approved by the animal welfare and ethics committee of the Equine Research Institute.

Horses

Five well-trained Thoroughbreds (2 females, 3 geldings; average body weight, 490 ± 38 (SD) kg; age, 5.2 ± 0.5 years old) from the Equine Research Institute herd were studied. The horses underwent a preliminary surgery to move a carotid artery from the carotid sheath to a subcutaneous location to facilitate arterial catheterization.

Treadmill measurements while inspiring different concentrations of oxygen gas

Using a crossover design, horses were made to run up a 6% grade on a treadmill (Mustang 2200, Ansorix Systems AG, Fahrwangen, Switzerland) set at a speed to elicit exhaustion, which was that resulting in a relative intensity of approximately 115% $\dot{V}O_2$max under both conditions, breathing normoxic (NO, 21% O_2) or hypoxic gas (LO, 15.3% O_2), in random order. Horses also ran at the same speed, incline and run time as in the case of NO while breathing hyperoxic gas (HO_{NO}, 28.8% O_2) and as in the case of LO while breathing normoxic gas (NO_{LO}). Runs were performed once a week, and cardiopulmonary variables were measured.

ECG electrodes were placed around the horse's thorax to measure heart rate (HR) with a commercial HR monitor (S810, Polar Electro Oy, Kempele, Finland). An 18-gauge × 6.4 cm Teflon catheter (Surflo, Terumo, Tokyo, Japan) was placed in the horse's carotid artery, and an 8 Fr × 9 cm introducer (MO95H-8.5, Baxter, Tokyo, Japan) was placed in the jugular vein. A Swan-Ganz catheter (Swan-Ganz catheter, Edwards Lifesciences LLC, Irvine, CA, U.S.A.) was passed via the jugular introducer so that its tip was positioned in the pulmonary artery, which was confirmed by measuring the pressure at its tip with a Statham P23d transducer (Statham P23d, Viggo-Spectramed, Tokyo, Japan).

After the catheters and transducers were connected and tested, the horse began its warm-up with 2 min of walking (1.7 m/sec) and 3 min of trotting (4 m/sec) on the treadmill. Horses wore a T-shaped mask for the measurement of $\dot{V}O_2$ and CO_2 production ($\dot{V}CO_2$) during runs on the treadmill. Horses exercised with the treadmill inclined at a 6% grade for 2 min at each of 3 speeds, 1.7, 4.0 and 7.0 m/sec, and then each horse ran at a speed as mentioned above under each of the NO, LO, HO_{NO}, and NO_{LO} conditions. The T-shaped mask was made of lightweight PVC, and it was connected to flexible 20-cm diameter PVC tubing at the two side arms of the T. A rubber diaphragm that fit around and over the horse's muzzle was mounted on the center tube of the mask. Ropes that passed through overhead pulleys and elastic cords attached to the ceiling supported the weight of the mask. All joints in the flow system of the gas analyzers were sealed with duct tape. The bias flow for the different O_2 concentrations was made with a mass flow controller (Model DPM-3, Kofloc, Tokyo, Japan) connected to compressed nitrogen or O_2 gas cylinders through a gas manifold. A mixing chamber was connected to the upstream flexible tubing on the mask. A mixed bias flow of 100–150 *l*/sec was required. We observed the O_2 concentration in the upstream arm of the mask with an O_2 analyzer (METS-900, VISE Medical, Chiba, Japan) and adjusted it with a mass flow controller. Oxygen consumption was measured with the N_2-dilution technique [4] as modified for use in semi-open-flow systems in which the inspired O_2 concentration is not 20.95% [13], and the rate of $\dot{V}CO_2$ was measured by the CO_2-addition technique [1, 9]. Gas samples were measured with O_2 and CO_2 analyzers after they were dried with $CaSO_4$ to remove H_2O (before the O_2 and CO_2 analyses) and NaOH to remove CO_2 (before the O_2 analysis only). An electronic mass flow controller measured the N_2 and CO_2 calibration flows. Gas analyzers and flowmeter outputs were also recorded with A/D hardware (DI-720-USB, DATAQ Instruments, Akron, OH, U.S.A.) and software (WinDaq Pro+, DATAQ Instruments) on personal computers. Respiratory exchange ratio (RER) was calculated as $\dot{V}CO_2/\dot{V}O_2$.

Mixed venous blood samples were drawn from the tip of the Swan-Ganz catheter and arterial samples from the 18- gauge carotid catheter into heparinized syringes and stored on ice until measurements were made. Blood samples were analyzed for arterial O_2 tension (PaO_2), arterial carbon dioxide tension ($PaCO_2$) and mixed-venous O_2 tension (PvO_2) with a blood gas/hemoximeter analyzer (ABL800 FLEX, Radiometer, Copenhagen, Denmark). The Swan-Ganz catheter in the pulmonary artery was connected to a cardiac output computer (COM-2, Baxter) so that the pulmonary artery temperature (Tpa) its thermistor registered could be recorded at each blood sampling and used

to correct the blood gas measurements. Arterial O_2 saturation (SaO_2) and hemoglobin concentration were measured with a hemoximeter (ABL80 FLEX CO-OX, Radiometer Japan, Tokyo, Japan). Arterial O_2 concentration (CaO_2) and mixed-venous O_2 concentration (CvO_2) were calculated as 1.39 × hemoglobin concentration × O_2 saturation + 0.0031 × PaO_2 [20]. Following these measurements, samples of arterial blood were centrifuged (12,000 rpm; KH120A, Kubota, Tokyo, Japan) to measure the packed cell volume (PCV). Plasma lactate concentration was measured with a lactate analyzer (Biosen C-Line Glucose and Lactate analyzer, EKF-diagnostic GmbH, Barleben, Germany). Cardiac output was calculated using the Fick principle, and cardiac stroke volume was calculated as the quotient of the cardiac output and HR. Oxygen delivery was calculated by multiplication of CaO_2 and cardiac output. The difference in O_2 concentration between arterial and venous blood (avdiff) was calculated as avdiff=CaO_2−CvO_2.

Statistical analysis

Results are expressed as the mean ± SD. Analysis of variance was used to test for differences between the runs. The Holm-Šidák procedure was used for *post-hoc* pairwise comparisons ($P \leq 0.05$). Data were analyzed with commercial statistical software (SigmaStat 3.5, Systat Software, Chicago, IL, U.S.A.).

Results

Table 1 shows the cardiopulmonary variables as the horses exercised at the determined speed after reaching a steady state during exercise while inspiring different O_2 concentrations.

Running speed and $\dot{V}o_2max$

Maximal speeds differed significantly between NO and LO (14.0 ± 0.5 [SD] m/sec vs. 12.6 ± 0.5 m/sec), but run times to exhaustion did not (112 ± 17 sec vs. 103 ± 14 sec). Figure 1 shows the changes in $\dot{V}O_2$ and the main variables that affected $\dot{V}O_2$. Maximal O_2 consumption in NO was higher than that in LO (165 ± 11 vs. 120 ± 15 m*l* (min × kg)). Oxygen consumption was increased in HO_{NO} and NO_{LO} compared with the values in NO and LO, respectively. Respiratory exchange ratio in LO was the highest in all groups, and that in NO was higher than those in NO_{LO} and HO_{NO}.

Table 1. Cardiopulmonary variables as the horse exercised at the determined speed while inspiring different O_2 concentrations

Variable	Unit	NO_{LO}	LO (all-out)	NO (all-out)	HO_{NO}
FIO_2	(%)	21.0 ± 0.0[a]	15.4 ± 0.3[b]	21.0 ± 0.0[a]	28.8 ± 0.7[c]
Speed	(m/sec)	12.6 ± 0.5[a]	12.6 ± 0.5[a]	14.0 ± 0.5[b]	14.0 ± 0.5[b]
Run time	(sec)	103.0 ± 14.4	103.0 ± 14.4	111.6 ± 17.2	111.6 ± 17.2
Heart rate	(beat/min)	207 ± 9	203 ± 11	207 ± 9	207 ± 6
$\dot{V}O_2/M_b$	(m*l*/min×kg)	163 ± 13[a]	120 ± 15[b]	165 ± 11[a]	194 ± 22[c]
$\dot{V}CO_2/M_b$	(m*l*/min×kg)	189 ± 20[ab]	170 ± 15[a]	212 ± 16[b]	204 ± 14[ab]
RER		1.15 ± 0.07[a]	1.43 ± 0.12[b]	1.28 ± 0.04[c]	1.06 ± 0.09[a]
Q/M_b	(m*l*/min×kg)	708 ± 72	684 ± 103	710 ± 73	737 ± 124
SV/M_b	(m*l*/kg)	3.36 ± 0.46	3.43 ± 0.38	3.45 ± 0.40	3.53 ± 0.63
CaO_2	(m*l*/d*l*)	26.2 ± 0.9[a]	18.7 ± 1.1[b]	25.1 ± 1.1[a]	29.7 ± 2.2[c]
CvO_2	(m*l*/d*l*)	3.1 ± 1.0[a]	1.3 ± 0.4[b]	1.8 ± 0.5[b]	3.8 ± 0.9[a]
avdiff	(m*l*/d*l*)	23.1 ± 0.7[a]	17.4 ± 0.9[b]	23.3 ± 0.9[a]	25.9 ± 2.1[c]
O_2 deliv	(*l*/min)	18.5 ± 1.8[a]	12.8 ± 1.7[b]	17.8 ± 1.3[a]	21.8 ± 3.5[c]
SaO_2	(%)	83.0 ± 5.2[a]	59.1 ± 2.7[b]	77.9 ± 4.3[c]	93.7 ± 1.1[d]
PaO_2	(Torr)	67.7 ± 6.4[a]	45.3 ± 2.0[b]	66.0 ± 4.5[a]	118.2 ± 13.3[c]
$PaCO_2$	(Torr)	60.4 ± 7.6[a]	51.8 ± 3.9[b]	67.1 ± 7.7[cd]	62.2 ± 7.8[ad]
PvO_2	(Torr)	15.8 ± 3.1[a]	9.2 ± 2.7[b]	13.2 ± 2.2[a]	19.9 ± 2.0[c]
PCV	(%)	60.5 ± 3.4	60.1 ± 3.2	61.3 ± 2.4	60.1 ± 4.5
[Hb]	(g/d*l*)	22.9 ± 1.2	22.9 ± 1.2	23.4 ± 0.8	23.0 ± 1.8
[LA]	(mmol/*l*)	15.1 ± 3.3[a]	21.6 ± 3.8[b]	20.1 ± 1.9[b]	15.8 ± 2.0[a]
Tpa	(°C)	40.5 ± 0.7[a]	40.6 ± 0.6[a]	41.0 ± 0.8[b]	40.8 ± 0.6[ab]

Variables measured or calculated were fraction of inspiratory oxygen (FIO_2), O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$), body mass (M_b), respiratory exchange ratio (RER), cardiac output (Q), stroke volume (SV), arterial O_2 concentration (CaO_2), mixed-venous O_2 concentration (CvO_2), difference in O_2 concentration between atrial and venous blood (avdiff), O_2 delivery (O_2 deliv), arterial O_2 saturation (SaO_2), arterial O_2 tension (PaO_2), arterial carbon dioxide tension ($PaCO_2$), venous O_2 tension (PvO_2), packed cell volume (PCV), hemoglobin concentration ([Hb]), plasma lactate concentration ([LA]) and pulmonary artery temperature (Tpa). Superscript letters indicate that values are significantly different from other values with different superscript letters ($P \leq 0.05$).

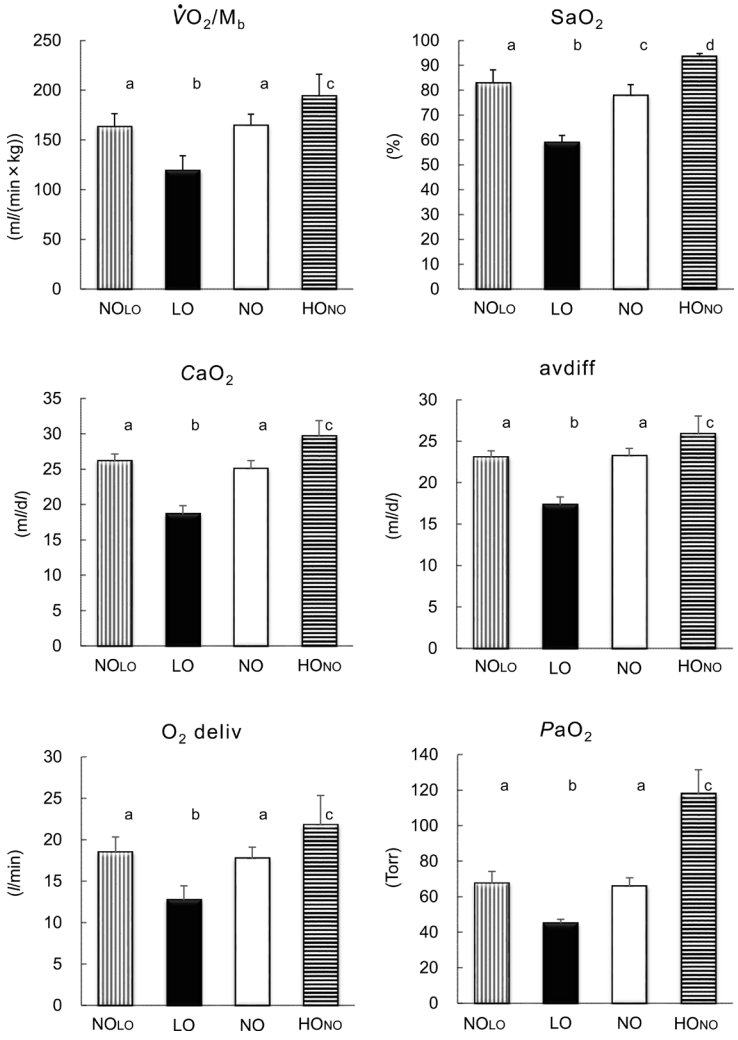

Fig. 1. Cardiopulmonary variables as the horses exercised at the determined speed while inspiring different O_2 concentrations. Variables measured or calculated were O_2 consumption ($\dot{V}O_2$), arterial O_2 saturation (SaO_2), arterial O_2 concentration (CaO_2), difference in O_2 concentration between atrial and venous blood (avdiff), O_2 delivery (O_2 deliv), arterial O_2 tension (PaO_2) and body mass (M_b). Superscript letters indicate that values are significantly different from other values with different superscript letters ($P \leq 0.05$).

Heart rate, cardiac output, stroke volume, hemoglobin concentration and PCV

Maximal HR and stroke volume during runs were not different between the groups. Cardiac output, which was acquired by multiplying the HR and stroke volume, also was not different between the groups (Fig. 2). There were no differences in either the hemoglobin concentration or PCV between the groups.

Oxygen concentrations, saturations, and blood gases

There were significant differences in O_2 delivery, CaO_2, avdiff, SaO_2, PaO_2, $PaCO_2$, and PvO_2 between NO and LO. Atrial O_2 concentrations and SaO_2 in HO_{NO} and NO_{LO} were increased compared with those in NO and LO, respectively (Fig. 1).

Plasma lactate concentrations

The plasma lactate concentration in NO_{LO} was significantly decreased compared with that in LO. Also, the plasma lactate concentration in HO_{NO} was significantly decreased compared with that in NO. There was no significant difference between LO and NO.

Discussion

We have previously reported that hypoxic training for 3 weeks increases aerobic capacity more than normoxic training in well-trained horses [17]. In that study, both hypoxic and normoxic training were at the same relative intensity in terms of the fact that all horses ran to exhaustion, although the running speed in normoxic training was faster

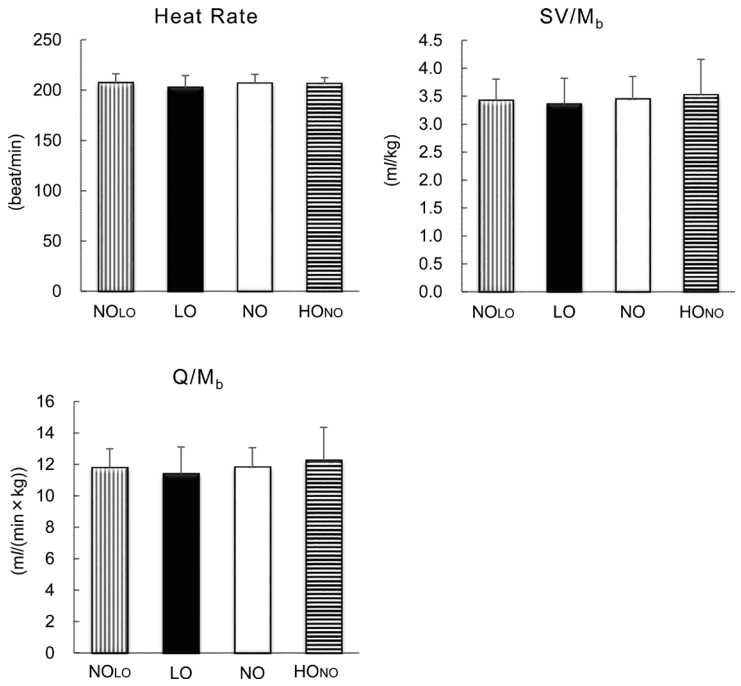

Fig. 2. Cardiovascular variables as the horses exercised at the determined speed while inspiring different O_2 concentrations. Variables measured or calculated were heart rate, stroke volume (SV), cardiac output (Q) and body mass (M_b). There are no significant differences among the groups.

than that in hypoxic training [17]. Few studies have focused on the differences in cardiopulmonary function between hypoxic and normoxic conditions in all-out exercise. We hypothesized that exercise under hypoxic conditions would induce more severe hypoxemia than that under normoxic conditions at the same relative intensity in Thoroughbred horses. We tested this hypothesis by comparing the effects of all-out exercise in both hypoxia and normoxia on cardiopulmonary function. In this study, PaO_2 and SaO_2 in LO were lower than those in NO, although the running speed in LO was slower than that in NO. These show that normobaric hypoxia induced more severe hypoxemia compared with normoxia during runs to exhaustion. These differences between LO and NO may indicate a possible reason for why hypoxic training increased the $\dot{V}O_2$max of highly trained horses in normoxia. These results suggest that the severe hypoxemia while breathing hypoxic gas may have an important role that contributes to the increase in aerobic capacity in Thoroughbred horses.

In this study, the horses ran to exhaustion for approximately 110 sec while breathing normoxic or hypoxic gas to adjust to the relative intensity. We previously reported that the $\dot{V}O_2$max when horses inspired 19.5% O_2 did not change from that under normoxic conditions, although the anaerobic contribution while inspiring 19.5% O_2 was higher than that under normoxic conditions [13]. Therefore, we could not say that breathing hypoxic gas reduces $\dot{V}O_2$max during an exercise bout because Thoroughbred horses may experience severe enough hypoxemia due mostly to diffusion limitation and therefore have much lower SaO_2 during supramaximal exercise [22]. The present study showed that breathing 15% O_2 gas reduced $\dot{V}O_2$max because breathing 15% O_2 gas during an all-out run resulted in decreased SaO_2, CaO_2, avdiff, O_2 delivery and PaO_2 compared with the values during a normoxic all-out run (Fig. 1). Since there were no differences in HR, stroke volume and cardiac output among the groups, breathing 15% O_2 gas had no effect on the circulation of the blood (Fig. 2). These results suggest that breathing 15% O_2 hypoxic gas exacerbates hypoxemia and is the main factor that reduces $\dot{V}O_2$max in Thoroughbred horses.

It was reported that horses increase their $\dot{V}O_2$ while breathing hyperoxic gas compared with while breathing normoxic gas during exercise [6–8, 13, 22]. We previously reported that both breathing 25% and 26% O_2 gas increased $\dot{V}O_2$ in horses [13, 16]. However, we did not measure arterial blood gas during hyperoxic runs in the previous studies. Therefore, it is unclear to what extent breathing hyperoxic gas alleviates hypoxemia and has the effect of increasing $\dot{V}O_2$. In this study, $\dot{V}O_2$ was significantly increased while breathing 29% oxygen gas compared with that when running at the identical speed for the same amount of time under

normoxic conditions. Arterial O_2 saturation while breathing 29% oxygen gas was 93.7% in this study. There may be room for improvement of this value through breathing of higher oxygen concentration gases. These results also showed the possibility that Thoroughbred horses could run at faster speeds while breathing higher O_2 concentrations than when running under normoxic conditions. It has been reported that hyperoxia (50% O_2) increased maximal power output and endurance in humans because of improved arterial, cerebral, and muscle tissue oxygenation [21]. Further studies are necessary to determine the possibility for hyperoxic training in horses.

Breathing 15%, 21% or 29% O_2 gas did not influence the maximal HR, stroke volume, cardiac output and hemoglobin concentration during supramaximal exercise. Breathing gases with different O_2 concentrations may not enhance or attenuate circulatory function in Thoroughbred horses. We can also safely say that functional changes in the circulatory system did not occur among the 4 runs (Fig. 2). It was curious that HR in LO was slightly lower than those under the other conditions, but there were no significant differences.

Running on the treadmill in both LO and NO_{LO} was performed at an identical speed (12.6 ± 0.5 m/sec) for the identical time (103 ± 14 sec). However, there were significant differences in $\dot{V}O_2$, SaO_2, PaO_2 and other variables between LO and NO_{LO}. We have observed similar results between NO and HO_{NO} in horses that also ran at the same absolute intensity as in this study. Running while breathing different O_2 concentrations is thought to be like running with relatively different intensities, when running at the same speed. We thought that the differences in $\dot{V}O_2$, SaO_2, PaO_2 and other variables between LO and NO_{LO} might mainly have resulted from the difference in relative intensity.

Lactate concentrations in LO and NO were similar at exhaustion in this study. It has been reported that the plasma lactate accumulation rate is related to net anaerobic capacity in Thoroughbreds [13, 16]. For exercise to exhaustion, the anaerobic contributions might be equal when horses run for similar durations while breathing either of these gases. On the other hand, when horses in this study ran while breathing higher O_2 concentrations, such as in the case of NO_{LO} and HO_{NO}, compared with LO and NO, respectively, the lactate concentrations in NO_{LO} and HO_{NO} decreased compared with those in LO and NO due to an increase in aerobic capacity. When horses run at the same speed, the energy expenditure is identical even if they breath either gas. Therefore, it is thought that the contributions of the aerobic and anaerobic capacities were different as a result of inspiring gases with different O_2 concentrations during supramaximal exercise.

Supramaximal exercise in normobaric hypoxia induces more severe hypoxemia and decreases $\dot{V}O_2$max mainly due to decreased SaO_2, with no effect on the circulation, whereas supramaximal exercise in hyperoxia alleviates hypoxemia and increases $\dot{V}O_2$ in horses. These findings indicate that it is possible to change the contributions of the aerobic and anaerobic capacities by changing the inhaled oxygen concentration during supramaximal exercise in Thoroughbred horses. They also suggest that the different responses of cardiopulmonary function during supramaximal exercise in hypoxia, normoxia and hyperoxia may induce the different training effects while inspiring different O_2 concentrations.

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