The effects of training in hyperoxia vs. normoxia on skeletal muscle enzyme activities and exercise performance

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Perry CG, Talanian JL, Heigenhauser GJ, Spriet LL. The effects of training in hyperoxia vs. normoxia on skeletal muscle enzyme activities and exercise performance. J Appl Physiol 102: 1022–1027, 2007. First published December 14, 2006; doi:10.1152/japplphysiol.01215.2006.—Inspiring a hyperoxic (H) gas permits subjects to exercise at higher power outputs while training, but there is controversy as to whether this improves skeletal muscle oxidative capacity, maximal O2 consumption (V\textsubscript{O2 max}), and endurance performance to a greater extent than training in normoxia (N). To determine whether the higher power output during H training leads to a greater increase in these parameters, nine recreationally active subjects were randomly assigned in a single-blind fashion to train in H (60% O\textsubscript{2}) or N for 6 wk (3 sessions/wk of 10 × 4 min at 90% V\textsubscript{O2 max}). Training heart rate (HR) was maintained during the study by increasing power output. After at least 6 wk of detraining, a second 6-wk training protocol was completed with the other breathing condition. V\textsubscript{O2 max}, and cycle time to exhaustion at 90% of pretraining V\textsubscript{O2 max} were tested in room air pred and posttraining. Muscle biopsies were sampled pre- and posttraining for citrate synthase (CS), \beta-hydroxyacyl-coenzyme A dehydrogenase (\beta-HAD), and mitochondrial aspartate aminotransferase (m-AsAT) activity measurements. Training power outputs were 8% higher (17 W) in H vs. N. However, both conditions produced similar improvements in V\textsubscript{O2 max} (11–12%); time to exhaustion (~100%); and CS (H, 30%; N, 32%), \beta-HAD (H, 23%; N, 21%), and m-AsAT (H, 21%; N, 26%) activities. We conclude that the additional training stimulus provided by training in H was not sufficient to produce greater increases in the aerobic capacity of skeletal muscle and whole body V\textsubscript{O2 max} and exercise performance compared with training in N.

CITRIC SYNTHASE; \beta-HYDROXYACYL-COEZYME A DEHYDROGENASE; MITOCHONDRIAL OXIDATIVE CAPACITY; HIGH-INTENSITY INTERVAL TRAINING

INSPIRING a hyperoxic gas mixture (H) can improve acute aerobic exercise performance and allows subjects to exercise at higher power outputs for a given heart rate (HR) and with a lower rating of perceived exertion for a given power output compared with breathing normoxic (N) air (2, 9, 13, 16, 25, 26). The ability to exercise at higher power outputs with H also persists during an entire 5- to 6-wk training paradigm compared with N (15, 17). Thus inspiring H allows individuals to train at higher aerobic intensities, which may provide a greater stimulus for enhancing endurance performance. However, it is not clear whether the additional training stimulus from H results in greater adaptive responses in mitochondrial oxidative capacity or exercise performance relative to N.

Ploutz-Snyder et al. (17) reported that even though higher power outputs (~20 W or ~12%) were achieved for a given HR while training in H at ~70% of V\textsubscript{O2 max} each week for 5 wk, no additional increase in V\textsubscript{O2 max} occurred compared with N. Nevertheless, despite the greater training stimulus with H, cytochrome-c oxidase and citrate synthase (CS) activities increased to a greater extent in N relative to H, and \beta-hydroxyacyl-coenzyme A dehydrogenase (\beta-HAD) activity only increased in the N group. However, this study used separate groups of subjects for the H and N training conditions, and the effects of H on training-induced performance increases were not explored. Hence, it is possible that the differences between experimental conditions may be due to genetic factors that influence the individual adaptive responses to training (24).

Perry et al. (15) employed a crossover design to investigate the effects of inspiring H or N at the same exercise HR while training with a high-intensity interval training protocol (HIIT) on V\textsubscript{O2 max} and endurance performance at 90% V\textsubscript{O2 max}. They reported that inspiring 60% O\textsubscript{2} while performing 6 wk of HIIT at 90% V\textsubscript{O2 max} allowed subjects to maintain higher power outputs (~18 W or ~8%) than training in N. This resulted in an ~2-fold greater increase in endurance time to exhaustion at 90% of pretraining V\textsubscript{O2 max} following training (H, 5.1 to 11.1 min; N, 5.6 to 8.5 min) despite similar increases in V\textsubscript{O2 max} for both groups. The authors proposed that the additional stimulus provided by the higher training power outputs in H might have increased skeletal muscle mitochondrial oxidative capacity to a greater extent than training in N.

Thus it is possible that the enhanced endurance performance following training in H or N at the same exercise HR can be explained by greater skeletal muscle metabolic adaptations compared with training in N. Therefore, the primary goal of this study was to determine whether the hyperoxia-induced increases in training power outputs would lead to greater increases than training in room air in the maximal activities of markers of mitochondrial oxidative potential: the tricarboxylic acid cycle enzyme citrate synthase, the \beta-oxidation enzyme \beta-HAD, and the NADH malate-aspartate shuttle enzyme aspartate aminotransferase. Additionally, the relative increases in whole body V\textsubscript{O2 max} and cycling performance time at 90% of pretraining V\textsubscript{O2 max} following training in H and N were also measured to corroborate the skeletal muscle mitochondrial adaptations. The crossover design employed in this study required subjects to complete 6 wk of training in both N and H breathing conditions, separated by at least 6 wk of detraining. We employed a HIIT protocol that required subjects to perform repeated short-term bouts of exercise at ~90% V\textsubscript{O2 max} during each workout.

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METHODS

Subjects. Nine subjects (3 females and 6 males) volunteered to participate in this study. Their mean (±SE) age, height, and weight were 23 ± 1 yr, 178 ± 4 cm, and 72.5 ± 4.0 (Pre-N) and 73.6 ± 4.1 kg (Pre-H), respectively. Their body weight was maintained throughout the entire study. The subjects were not involved in structured training regimens but participated in some form of aerobic activity two to three times per week (e.g., cycling, jogging). Subjects were also advised to refrain from taking any supplements other than multivitamins, and none was on medication. The experimental protocol and associated risks were explained both orally and in writing to all subjects before written consent was obtained. The study was approved by the Research Ethics Boards of the University of Guelph and McMaster University.

Study design. Each subject completed two 6-wk sessions of cycle ergometer interval training separated by at least 6 wk. In a single-blind fashion, subjects were randomly assigned to either N (room air: 21% O2) or H (60% O2) for the first 6-wk session. Following at least 6 wk of detraining, subjects completed a second 6-wk training session under the other condition. All subjects successfully detrained in that they met the criteria of a return in VO2max to their initial detraining level after at least 6 wk of inactivity. There were also no differences in time to exhaustion between both pretrials.

VO2max and cycle to exhaustion protocols. Before each 6-wk training period, subjects completed a continuous incremental cycling test to exhaustion on an electromagnetically braked cycle ergometer (Lode Instrument, Groningen, The Netherlands) to determine pulmonary VO2max using a metabolic measurement system (Vmax Series 229, Sensormedics, Yorba Linda, CA). All subjects practiced cycling to volitional exhaustion at 90% VO2max on the Lode cycle to verify the power output and become familiarized with the procedure. At least 48 h separated the VO2max trial from the experimental performance trial. During this period, subjects refrained from consumption of alcohol and caffeine as well as vigorous physical activity. When the subjects arrived at the laboratory, they rested on a bed while a catheter was inserted into an antecubital vein. A resting blood sample was taken, and saline drip was used to maintain a patent line. For muscle biopsy sampling, an incision was made over the vastus lateralis muscle and associated risks were explained both orally and in writing to all subjects before written consent was obtained. The study was approved by the Research Ethics Boards of the University of Guelph and McMaster University.

Training protocol. Subjects trained on a cycle ergometer (Monark 894 E, Vaxbro, Sweden) at a power output that elicited ~90% VO2max 3 days/wk for each 6-wk period. Subjects completed 10 exercise intervals lasting 4 min and separated by 2 min of rest in each training session. During the first and second exercise sessions of the first 6-wk period, the power output was adjusted to the highest intensity that each subject could tolerate for a complete set of 10 intervals. Each subject’s HR reached a steady state during the final 2 min of intervals 6–10, and this HR averaged 180 beats/min (95% of maximal HR) in both groups and was maintained throughout the remainder of both 6-wk sessions. The power output for each subject was increased to maintain training HR constant throughout the exercise intervals and sessions.

During the exercise training intervals, subjects inspired through a mouthpiece from a 1,000-liter reservoir bag that contained either 21% or 60% O2. Subjects wore nose-clips throughout the work intervals. The subjects were blinded from the filling of the bag by a curtain. The fractional inspired O2 concentration was confirmed with a portable oxygen analyzer (102 series O2 analyzer, Vacumed, Ventura, CA). During the rest intervals, subjects remained on the bike and inspired ambient room air and were also allowed to consume water or sports drink ad libitum.

Subjects were advised to maintain only light levels of aerobic physical exercise and refrain from lower body resistance training throughout the entire study (training and detraining sections). This was verified by activity logs that subjects maintained throughout the study. At the end of the entire study, subjects were asked to respond to a questionnaire indicating which condition they believed they were assigned to in each training period.

Analyses. A small piece of frozen wet muscle (6–10 mg) was removed under liquid N2 for the spectrophotometric determination of CS, β-HAD, and m-AsAT maximal activities (37°C) as described previously (3, 20). An aliquot of this muscle homogenate was then extracted with 0.5 M perchloric acid (HClO4) containing 1 mM EDTA and neutralized with 2.2 M KHCO3 and used for the enzymatic spectrophotometric determination of total creatine (3). CS, β-HAD, and m-AsAT enzyme activities were normalized to the highest total creatine (Cr) content measured in the four biopsies from a given subject (1 per experimental trial). There was no change in the total Cr content in samples obtained before and after training, as previously reported (4, 7).

Venous whole blood was collected in heparinized tubes, and 200 μl was immediately deproteinized with 1 ml of 0.6% (wt/vol) HClO4. The supernatant was stored at −20°C and analyzed for lactate, glucose, and glycerol (3). A second portion of blood was immediately centrifuged, and 500 μl of the plasma supernatant was stored at −20°C and analyzed for free fatty acids with a colorimetric assay (Wako NEFA C test kit, Wako Chemicals, Richmond, VA).

Statistics. A paired t-test was used to compare differences between Pre-N and Pre-H trials for performance time, VO2max, body weight, and enzyme activities to determine if subjects had successfully detrained between the 6-wk training sessions. A two-way ANOVA with repeated measures was used to test for differences in training power output and HR across the 6 wk of training in both N and H conditions, and between detraining and posttraining trials for performance time, VO2max, and muscle enzyme activities. To test for training effects in the blood measurements, a three-way ANOVA was used to compare resting and 5-min time points between detraining and posttraining trials in both N and H. A two-way ANOVA with repeated measures was used for the exhaustion blood measures since this time varied for each subject. When a significant F-ratio was obtained, post hoc analyses were completed using a Student-Newman-Keuls test. The level of significance was established at P < 0.05 for all statistics. Results are expressed as means ± SE.

RESULTS

Training. Training HRs were well maintained during 6 wk of training and were not different between N and H (Fig. 1A). Power outputs in H were greater than N by an average of ~17 W (~8%) throughout the training periods (main condition effect, P < 0.001, Fig. 1B). Power outputs increased (20–25%) to a similar extent during training in N and H (main time effect, P < 0.001). There were no differences in body mass before the two training periods. Despite attempts to blind the subjects to the training condition, they were not blinded as eight of nine subjects successfully identified which condition they were assigned to in each training period.
V\(_\text{O}_2\) max and cycling to exhaustion. There were no statistical differences in the Pre values between N and H for V\(_\text{O}_2\) max or cycling performance. This suggests that the subjects were successfully detrained during the crossover between both training periods. V\(_\text{O}_2\) max increased significantly (\(P < 0.01\)) in both N (12%) and H (11%) (Fig. 2). Time to exhaustion while cycling at 90% of the pretraining V\(_\text{O}_2\) max increased 2-fold following training in both N and H (\(P < 0.001\)) (Fig. 3). There was no statistical difference between the magnitude of increase in both N and H.

Muscle enzyme activities. The maximal activities of CS, \(\beta\)-HAD, and m-AsAT were similar before training and increased to a similar extent following training in N (CS, 32%; \(\beta\)-HAD, 21%; m-AsAT, 26%) and H (CS, 30%; \(\beta\)-HAD, 23%; m-AsAT 21%) (\(P < 0.05\)) (Fig. 4).

Blood metabolites. All venous blood metabolites were similar in the two pretraining cycle to exhaustion trials. Venous whole blood lactate, glucose, glycerol, and plasma free fatty acids were similar before and after training at rest and following 5 min of exercise (Table 1). Venous blood lactate and glycerol were higher at exhaustion following training (\(P < 0.05\)). There were no statistical differences between N and H at any time points for any blood metabolites.

**DISCUSSION**

The present study demonstrated that inspiring 60% \(\text{O}_2\) allowed subjects to exercise at a higher power output than in room air when training at the same HR. It was expected that the higher power outputs in H would provide an even greater stimulus for training adaptations. However, there were no differences between conditions in the magnitude of increases measured in mitochondrial enzyme capacities, V\(_\text{O}_2\) max, and cycle time to exhaustion at 90% of the pretraining V\(_\text{O}_2\) max. This suggests that the higher training power output in H relative to N (8%) was not sufficient to induce greater training adaptations in H above those achieved in N.

Effect of increased training power outputs in H on the adaptive response to HIIT. Many studies have reported that inspiring a hyperoxic gas mixture improves performance during an acute bout of exercise (16, 25, 26). The present study demonstrated that inspiring H allowed all subjects to achieve a higher training power output for a given HR (~17 W or ~8%) compared with N and that this effect was sustained throughout 6 wk of HIIT. This finding supports previous investigations (15, 17) using different training intensities and regimens.
metabolic adaptations to hyperoxic training

It is possible that inspiring H actually dampens the metabolic response to training compared with normoxic training and negates the increased stimulus resulting from the high-intensity training power outputs. For example, it has been shown that H can blunt the decrease in phosphocreatine utilization and reduce the accumulation of free ADP and P_i in the muscle while cycling at 70% VO_2 max or plantar flexing at moderate to high intensities (8, 10, 21, 22). However, it is not known whether this occurs while cycling at 90% VO_2 max as in the present study. Thus H may enhance some adaptive responses via higher training power outputs but limit others by lessening metabolic stimuli.

The present study also reported an increase of ~100% in performance following training in both H and N conditions. These findings did not confirm a previous report of larger increases in high-intensity exercise performance following training in H compared with N (15), despite similar increases in VO_2 max. It is difficult to reconcile the results of the two studies since both incorporated similar methodologies and randomly assigned subjects to each condition using a crossover design. Nevertheless, all of the experimental findings in the present study (VO_2 max, mitochondrial enzyme measurements, and performance) suggest that the greater training power outputs in H were not sufficient to induce greater adaptations relative to training in N. There were also no differences between H and N in venous blood lactate and other blood metabolite responses during the posttraining cycle to exhaustion.

Higher training intensity while inspiring a hyperoxic gas.

The exact mechanisms that allow subjects to cycle at a higher power output at a given HR while exercising in H are not completely understood. However, two potential mechanisms

Table 1. Blood metabolites during a cycling trial to exhaustion at 90% VO_2 max before and after 6 wk of training in hyperoxia and normoxia

<table>
<thead>
<tr>
<th>Metabolite/Trial</th>
<th>Rest</th>
<th>5 min</th>
<th>Exh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate, mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-H</td>
<td>0.6±0.1</td>
<td>4.1±0.8</td>
<td>6.4±0.6</td>
</tr>
<tr>
<td>Pre-N</td>
<td>0.5±0.1</td>
<td>4.0±0.5</td>
<td>5.6±0.7</td>
</tr>
<tr>
<td>Post-H</td>
<td>0.6±0.1</td>
<td>2.8±0.4</td>
<td>6.9±0.9*</td>
</tr>
<tr>
<td>Post-N</td>
<td></td>
<td>4.0±0.2</td>
<td>7.2±0.8*</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-H</td>
<td>4.8±0.3</td>
<td>4.6±0.4</td>
<td>4.9±0.3</td>
</tr>
<tr>
<td>Pre-N</td>
<td>5.0±0.3</td>
<td>4.9±0.3</td>
<td>5.0±0.3</td>
</tr>
<tr>
<td>Post-H</td>
<td>4.6±0.3</td>
<td>4.3±0.4</td>
<td>4.8±0.4</td>
</tr>
<tr>
<td>Post-N</td>
<td>4.6±0.4</td>
<td>5.1±0.3</td>
<td>5.1±0.4</td>
</tr>
<tr>
<td>Glycerol, μM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-H</td>
<td>40.0±6.1</td>
<td>43.5±7.7</td>
<td>46.6±5.1</td>
</tr>
<tr>
<td>Pre-N</td>
<td>35.4±3.8</td>
<td>47.7±4.3</td>
<td>52.4±5.8</td>
</tr>
<tr>
<td>Post-H</td>
<td>38.9±4.3</td>
<td>40.9±6.7</td>
<td>72.1±13.0*</td>
</tr>
<tr>
<td>Post-N</td>
<td>40.0±6.1</td>
<td>43.5±7.7</td>
<td>46.6±5.1*</td>
</tr>
<tr>
<td>Free fatty acids, mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-H</td>
<td>0.23±0.03</td>
<td>0.17±0.02</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>Pre-N</td>
<td>0.17±0.02</td>
<td>0.17±0.01</td>
<td>0.12±0.02</td>
</tr>
<tr>
<td>Post-H</td>
<td>0.16±0.04</td>
<td>0.17±0.03</td>
<td>0.19±0.05</td>
</tr>
<tr>
<td>Post-N</td>
<td>0.17±0.03</td>
<td>0.27±0.07</td>
<td>0.14±0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE for 9 subjects. Pre-H and Post-H, before and after 6 wk of training in hyperoxia, respectively; Pre-N and Post-N, before and after 6 wk of training in normoxia, respectively; VO_2 max, maximal O_2 consumption; Exh, exhaustion. *P < 0.05, greater than Pre for same time point, main effect of training.
have been suggested: an effect on either skeletal muscle metabolism or on central motor output. Studies have demonstrated that inspiring 60% O2 while cycling at 70% \( \dot{V}O_2 \text{max} \) (21), inspiring 100% O2 while cycling at 65 or 90% \( \dot{V}O_2 \text{max} \) (5, 19), or performing repeated bouts of plantar flexion at \( \sim 60\% \) of maximum work rate while inspiring 100% O2 (8) does not alter phosphocreatine utilization or oxidative metabolism at the onset of exercise. Our laboratory has recently shown that inspiring 60% O2 during steady-state exercise at 70% \( \dot{V}O_2 \text{max} \) resulted in a small attenuation in muscle glycogen utilization and phosphocreatine degradation and total lactate production (21, 22). Similarly, Hogan et al. (10) demonstrated that H slowed the depletion of phosphocreatine and accumulation of ATP in calf muscle, particularly at near-maximal power outputs during a test of progressively increasing power outputs. Haseler et al. (8) reported that H augmented the overall fall in muscle phosphocreatine during exercise at 60% of maximum work rate. These findings are supported by the observation that H increases myoglobin saturation during maximal exercise (18), indicating that intracellular PO2 can be increased with H. Thus it may be possible that H attenuates the development of muscle fatigue during repeated bouts at higher power outputs, such as those used in the present study (4-min intervals at 90% \( \dot{V}O_2 \text{max} \)), by reducing the reliance on phosphocreatine and glycolysis and enhancing oxidative phosphorylation. This may be a result of improved arterial and skeletal muscle oxygenation when inspiring H, leading to increased muscle oxygen uptake at either the onset of exercise or at steady state. However, attempts to measure increased oxygen uptake with H reported no effect during the first 2 min of moderate, heavy submaximal, or supramaximal intensities (11, 12, 27), or on leg \( \dot{V}O_2 \) during steady-state exercise at 70% \( \dot{V}O_2 \text{max} \) (22).

On the other hand, previous work has also suggested that central motor output is inversely related to inspired oxygen concentration (14, 23). This suggests that H may lower central neural drive for a given exercise intensity, which may delay the development of neuromuscular fatigue. In support of this, recent evidence suggests that H allows one to achieve a greater power output for a given central motor output. Amann et al. (1) reported that inspiring 100% O2 during a 5-km time trial resulted in a greater central motor output to the contracting quadriceps muscles, which allowed subjects to maintain higher power outputs and achieve a faster time to complete the distance. While it is clear there is a strong relationship between arterial oxygen concentration and central motor output during exercise, it is uncertain whether this is due to a direct effect on the central nervous system or feedback from the periphery (1, 6). Additional research is required to fully elucidate the interactions between peripheral and central systems to explain the ability to exercise at a higher power output with H.

It is important to note that the subjects in the present study began in an untrained state (recreationally active) and trained for 6 wk. The training stimulus in the N condition was intense, and the higher training power output in H (8% greater than in N) did not appear to be sufficient to increase the already impressive metabolic and performance adaptations that occurred with N. However, it would be interesting to determine if H training would be of benefit for already trained, elite athletes who have reached a plateau in their adaptive response to training. For instance, would training twice a week in H permit athletes to exercise at higher power outputs and increase the adaptive responses and improve performance?

In conclusion, the present study employed a crossover design to examine the effects of 6 wk of high-intensity interval training in room air or a hyperoxic (60% O2) environment on \( \dot{V}O_2 \text{max} \), endurance performance at 90% \( \dot{V}O_2 \text{max} \) and skeletal muscle mitochondrial enzymes in recreationally active subjects. The results demonstrated that training in room air produced robust increases in skeletal muscle oxidative enzyme activities, whole body \( \dot{V}O_2 \text{max} \), and endurance performance. Subjects were able to exercise at power outputs that were 8% higher than room air when training while breathing 60% O2. However, this additional training stimulus was not sufficient to produce greater increases in mitochondrial enzyme activities, \( \dot{V}O_2 \text{max} \), or endurance performance above the already large adaptations afforded by training in room air.

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