RESEARCH ARTICLE

Hyperoxia May Reduce Energetic Efficiency in the Trained Rat

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Background: Several studies have been conducted in recent years in the attempt to improve running performance by the use of hyperbaric oxygen, but there is disagreement as to whether this has any beneficial effect. The purpose of this study was to measure the effect of 24 h breathing 100% O_2 in normobaric conditions on energetic efficiency in the trained rat. ***Methods:*** Experiments were carried out on trained rats whose oxygen consumption was evaluated during the training period and on its completion. At the end of the training period, the rats were divided into two groups: 1) rats exposed to air (21% O_2) in normobaric conditions; and 2) rats exposed to 100% O_2 in normobaric conditions. In addition, two groups of sedentary rats were used: 3) sedentary rats exposed to air (21% O_2) in normobaric conditions; and 4) sedentary rats exposed to 100% O_2 in normobaric conditions. Energetic efficiency was estimated by measuring O_2 consumption at submaximal exercise (45 m · min^{-1}, 10° incline). ***Results:*** Training alone reduced O_2 consumption by 18% during submaximal exercise. Exposure to 100% oxygen for 24 h in normobaric conditions reversed the effect of complete training by elevating the O_2 consumption by 17%, which was close to the oxygen consumption of the rats during the incomplete training period. ***Conclusions:*** Our results suggest that prolonged exposure to hyperoxia induces a reduction in the energetic efficiency of the trained rat. The relevance of these findings to sports and diving is discussed.

Keywords: submaximal $\dot{V}O_2$, hyperbaric oxygen, training.

EXPOSURE TO HYPEROXIA occurs in the hyperbaric chamber during hyperbaric oxygen (HBO) therapy, underwater when diving with closed or semi-closed circuit breathing apparatus, and during the administration of ~100% oxygen in normobaric conditions to treat pulmonary insufficiency. A number of recent studies tested the benefit of HBO on running performance. The use of HBO in these studies was in the range of 202–253 kPa for 1–1.5 h before strenuous exercise. The running performance of athletes was evaluated by measuring time to exhaustion, maximal workload, maximal oxygen consumption ($\dot{V}O_{2max}$), maximal heart rate, and blood lactate concentration. Contradictory results were obtained regarding the beneficial effect of HBO on running performance. Some reports (7,11) demonstrated the existence of such an effect, whereas other studies (13,19,23) failed to find evidence of this, stating that there is no scientific basis for the use of HBO to improve running performance.

Improvement in running ability might be related to hemodynamic, pulmonary, and skeletal muscle changes. There are many studies concerning the effect of hyperbaric hyperoxia on hemodynamic changes. In general, hyperbaric hyperoxia affects the cardiovascular system by immediate bradycardia, a decrease in cardiac output, and systemic vasoconstriction (9,22). Most of the studies on the effect of hyperbaric hyperoxia on the cardiovascular system were performed on the intact animal, in which the specific effects on the heart could not be distinguished from other systemic effects. Hence, investigators continue to debate as to whether the above hemodynamic changes were a result of decreased myocardial contractility or other systemic effects (12,18,21). The effect of HBO treatment on the specific function of organs involved in running performance was hardly measured. However, the effect of prolonged breathing of 100% oxygen in normobaric conditions was studied in various organs. In previous reports (4,5), it was shown that isolated working hearts from rats exposed to 100% O_2 in normobaric conditions for 24 h were energetically less efficient in comparison with hearts exposed to air for 24 h. In addition, high doses of epinephrine failed to stimulate hearts from the hyperoxic rats. The authors suggested that cardiac function might deteriorate in the hyperoxic animal during exercise or stress, when the release of catecholamines is elevated. Therefore, it is likely that exercising animals or human subjects may suffer from low energetic efficiency if the exercise follows hyperoxia. Studies investigating the effect of 100% O_2 in normobaric conditions on respiratory (1,2,20) and skeletal muscle function (8,10) presented conflicting results. Whereas Pardy and Bye (20) found that breathing O_2 delays the onset of diaphragm fatigue and decreases the level of perceived dyspnea, Anzueto et al. (2), who studied the effect of hyperoxia (95% O_2 for 24, 48, and 60 h) on diaphragm function, found that oxygen exposure appears to have harmful effects on diaphragm contractility. This effect was associated with a significant increase in glutathione

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disulfide and glutathione disulfide-to-glutathione ratios, i.e., reactive oxygen species may appear in the diaphragm during exposure to hyperoxia. There are also conflicting studies on the effect of hyperoxia on skeletal muscle function. Barclay et al. (8) found reduced muscle fatigue and increased blood flow in dogs, whereas Bredle et al. (10) reported paradoxical tissue hypoxia secondary to local vasoconstriction in the pump-perfused isolated dog hindlimb.

Reduced cardiac function and impaired diaphragm performance subsequent to oxygen exposure may both interfere with exercise efficiency in the normobaric oxygen-exposed animal. In the present study, we tested the hypothesis that 24 h exposure to 100% O_2 in normobaric conditions may cause a decline in the energetic efficiency of trained rats.

METHODS

Animals

Male Sprague-Dawley rats, 220–250 g initial body weight, were used. The animals were divided into two groups: 1) sedentary rats (S) and 2) trained rats (T). Each experimental group consisted of 30 animals. The animals were divided into groups in an arbitrary manner. They were fed on standard laboratory chow and water ad libitum, and were kept at a room temperature of 24 ± 1 °C on an 8:16 h light-dark cycle. The sedentary rats were used as control groups to demonstrate that the training period did in fact elicit an effect, by comparing the heart-to-body mass ratio between the groups. The Animal Care Committee of the Israel Ministry of Defense approved the experimental protocol.

Experimental Training System

Training was carried out on a treadmill (Columbus Instruments, Columbus, OH) equipped with an electric shock grid. The treadmill was also used as a metabolic chamber. The flow of dry compressed air into the front of the chamber was controlled by a needle valve and a flowmeter to yield 10 $L \cdot min^{-1}$. The flowmeter was calibrated before each measurement to an accuracy of ± 1 ml with a flow rate calibrator (VOL-U-METER, Brooks Instruments, Hatfield, PA). A sample of 200 $ml \cdot min^{-1}$ from the gas exiting at the back of the chamber was diverted through a desiccant (Silica Gel) into an oxygen analyzer (S-3A, Electrochemistry Inc., Sunnyvale, CA) calibrated according to the manufacturer's instructions. Oxygen consumption ($\dot{V}O_2$) was measured relative to body mass ($ml\ O_2 \cdot kg^{-1} \cdot min^{-1}$), and was standardized for temperature, pressure, and saturation (STPD). The oxygen consumption rate was calculated from the difference in oxygen concentration between the inflow and the outflow. The breathing gas throughout the training period was air.

Training Protocol

The trained rats ran on a treadmill according to the protocol suggested by Armstrong (6). The rat was placed on the treadmill, and the 7-min exercise session began when the O_2 fraction in the outflowing air reached a plateau. The animals exercised twice a day, in the morning and in the afternoon, 5 d a week for 6 wk, for a total of 30 training days. The running velocity was gradually increased day by day from 10 to 45 $m \cdot min^{-1}$ in steps of 2.5–5 $m \cdot min^{-1}$ on a sequence of 3 different inclines: 0, 5, and 10°. The two exercise sessions each day had the same combination of speed and incline. Oxygen consumption was calculated at the end of the exercise session for each velocity on the 10° incline only. When the rats attained 45 $m \cdot min^{-1}$ on the 10° incline (day 30), this noted the end of what was termed "incomplete training." At that point, the rats ran again progressively at all velocities (one velocity per day) on a 10° incline and the $\dot{V}O_2$ values were measured at the end of each exercise session. These running sessions were termed "complete training." The $\dot{V}O_2$ measurement at the first specific velocity on the 10° incline was taken as the value closest to the $\dot{V}O_2$ of the untrained animals. Since running on a treadmill is a skilled activity for rats, the sedentary animals could not run straight away at 45 $m \cdot min^{-1}$ on a 10° incline, and therefore did not run.

Test Protocol

At the end of the complete training period, immediately after the last exercise session, the sedentary and trained rats were each subdivided into two groups for a total of four groups: 1) sedentary rats exposed to air (21% O_2) in normobaric conditions (S-air); 2) sedentary rats exposed to 100% oxygen in normobaric conditions (S-O_2); 3) trained rats exposed to air (21% O_2) in normobaric conditions (T-air); and 4) trained rats exposed to 100% O_2 in normobaric conditions (T-O_2). The animals were placed for 24 h in two different sealed chambers. The T-O_2 and S-O_2 groups were exposed to 100% oxygen, while the T-air and S-air groups were exposed to air supplied at a rate of 15 $L \cdot min^{-1}$. Soda lime grains were dispersed with sawdust on the chamber floor to absorb CO_2. Under these conditions, the CO_2 level did not exceed 0.5%. Standard laboratory chow and water were provided ad libitum. The ambient temperature was 24 ± 1°C, and the barometric pressure was 101.4 ± 0.6 kPa. All of the rats, trained and sedentary, were weighed at the end of the exposure. The sedentary rats were sacrificed, and the hearts were removed and weighed. The trained rats were immediately placed on the treadmill, to run again for the same length of time as they had earlier at 45 $m \cdot min^{-1}$ on the 10° incline, (test session) at the end of which the $\dot{V}O_2$ was measured. Immediately after this session, the trained rats were sacrificed and the hearts were removed and weighed.

Statistical Analysis

Two-way ANOVA was employed to compare differences in body and heart weights between the sedentary and trained groups at the end of the test session following both gas exposures. Values of $p \leq 0.05$ were considered statistically significant. All of the following analyses were conducted on the trained groups. Differences in $\dot{V}O_2$ at ascending speeds at the first specific velocity on the 10° incline (incomplete training period)

TABLE I. BODY WEIGHT, HEART WEIGHT, AND HEART-TO-BODY WEIGHT RATIO IN THE SEDENTARY (S) AND EXERCISE-TRAINED (T) GROUPS FOLLOWING 24-H AIR (-AIR) OR 24-H OXYGEN (-O_2) EXPOSURES.

	Body Weight (g)	Heart Weight (g)	Heart-to-body Weight Ratio
S-air	449 ± 70	1.12 ± 0.15	0.26 ± 0.04
S-O_2	409 ± 56	1.02 ± 0.13	0.24 ± 0.05
T-air	351 ± 32**	1.06 ± 0.16	0.30 ± 0.05*
T-O_2	346 ± 49**	1.16 ± 0.24	0.34 ± 0.08*

Data are presented as mean ± SD. Significant difference between T-air and both sedentary groups (S-air and S-O_2) and between T-O_2 and both sedentary groups for body weight and heart-to-body weight ratio (*$p < 0.05$, **$p < 0.001$).

and the second time the rats ran (complete training period) were compared using two-way ANOVA with repeated measures. When ANOVA yielded a significant difference, we used the Duncan test to examine for specific differences. Because it is accepted that $\dot{V}O_2$ changes linearly with running speed, we used linear regression of $\dot{V}O_2$ vs. speed to present the coherency of the data. Oxygen consumption measurements at the end of the complete training period and after oxygen or air exposure, all at 45 m · min^{-1} and on a 10° incline, were compared using a paired *t*-test. The comparison between the T-O_2 and T-air groups was made using the Student's *t*-test.

RESULTS

Effect of Training on Body and Heart Weight

Table I presents body weight, heart weight, and heart-to-body weight ratio for all experimental groups. Body weight in the trained rats at the end of the test session subsequent to gas exposure was significantly lower than in the sedentary rats. Heart weight in the trained rats at the end of the test session was not significantly different from the sedentary group. However, the heart-to-body weight ratio was significantly higher in the trained rats compared with the sedentary animals. This difference in the heart-to-body weight ratio between the two groups demonstrates that the training period did in fact elicit an effect. No significant differences in body or heart weight were observed in the trained or sedentary rats following O_2 or air exposure.

Oxygen Consumption

Oxygen consumption in the trained rats as a function of running velocity at the first specific velocity on the 10° incline (incomplete training period) and the second time they ran (complete training period) is presented in **Fig. 1**. At all running velocities except 20 and 35 m · min^{-1}, oxygen consumption at the end of the training period was significantly lower than the values obtained for the incomplete training period. Although 28 training days separated the two measured $\dot{V}O_2$'s at 10 m · min^{-1} and only 10 experimental days intervened between the 45 m · min^{-1} data, a linear regression line can be drawn to describe the $\dot{V}O_2$ for the incomplete training period.

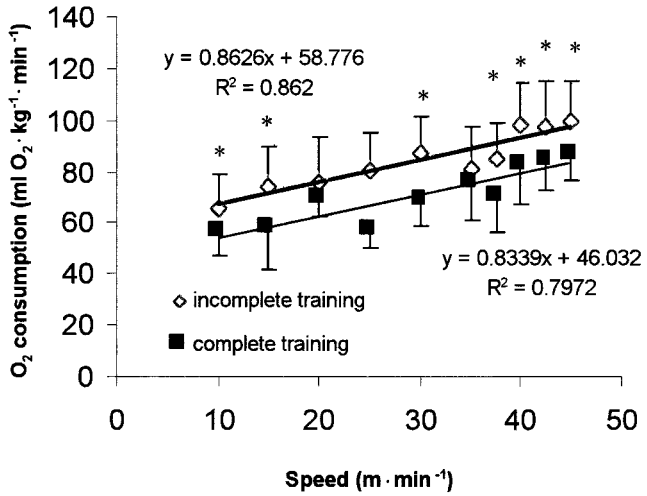

Fig. 1. Oxygen consumption as a function of running velocity on a 10° incline at rest before the exercise session, at the first specific velocity on the 10° incline (incomplete training period), and the second time the rats ran at ascending speeds on the 10° incline (complete training period). Results are presented as +SD for incomplete and –SD for complete training. Lines represent the linear regression of the data (excluding the resting values). n = 30, * indicates a significant difference between the incomplete and complete training periods for each velocity ($p < 0.05$).

The line for the complete training period $\dot{V}O_2$ had a similar slope, but was shifted to a lower $\dot{V}O_2$.

Fig. 2 presents the rats' $\dot{V}O_2$ at the end of the training period, and after oxygen or air exposure. There was no significant difference in $\dot{V}O_2$ between the rats assigned to T-air and T-O_2; therefore, their $\dot{V}O_2$'s before gas exposure were pooled together in Fig. 2. Exposure of 24 h to 100% oxygen in normobaric conditions abolished the effect of training, increasing the O_2 consumption of the complete trained rats by 17% at the end of the test session, to a value similar to that obtained for the incomplete trained rats (99.9 ± 5.4 and 99.7 ± 15.9 ml O_2 · kg^{-1} · min^{-1}, respectively). There was no effect of air

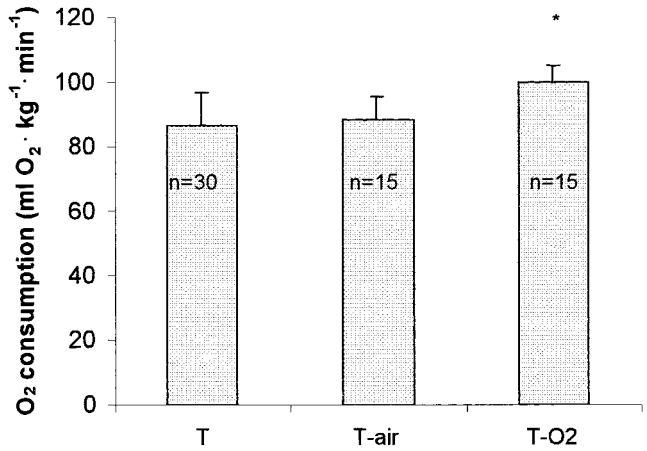

Fig. 2. Oxygen consumption for a speed of 45 m · min^{-1} on a 10° incline at the end of the 7-min exercise session after completion of the training period (T) and for the same speed and incline after 24-h exposure to air (21% O_2, T-air) or 100% oxygen (T-O_2) in normobaric conditions. The animals in the T group were randomly assigned to the other two groups (T-air and T-O_2). Oxygen consumption in the T-O_2 group was significantly higher than in the other two groups (* $p < 0.01$). Values are expressed as mean +SD.

exposure on $\dot{V}O_2$. Thus, the energetic efficiency gained over the last 10 experimental days had disappeared after the O_2 exposure.

DISCUSSION

The present study shows a significant reduction in oxygen demand at the end of the training period, compared with the values obtained at the first specific velocity (incomplete training period) for the same workload (Fig. 1). Running on a treadmill is a skilled activity for rats, so the animals would not run at 45 m · min^{-1} on a 10° incline without training. Since we do not have data for the 10° incline before the start of the training period (for sedentary rats), the effect of training on energetic efficiency should be even greater than that seen in Fig. 1. Lambert and Noakes (15,16) found that for both spontaneous and treadmill training, the $\dot{V}O_{2submax}$ of rats was lower in trained groups, after 4 and 8 wk, but not after 12 wk of training, compared with the control group. As Lambert and Noakes postulated in their study (15), an improvement in running economy ($\dot{V}O_{2submax}$) is the first training-induced adaptation that can be measured in treadmill-trained rats. This reduction in $\dot{V}O_{2submax}$ precedes changes in either $\dot{V}O_{2max}$ or in skeletal muscle oxidative capacity. Therefore, the reduction in oxygen demand following training may be a positive adaptation in rats to exercise stress. The mechanism producing this reduction in oxygen consumption at the end of the training period is unknown.

The only two velocities at which no significant change was found between the incomplete and complete training periods were 20 and 35 m · min^{-1}. It seems reasonable that at these velocities, a change of gait from walking to trotting (20 m · min^{-1}) and galloping (35 m · min^{-1}) was responsible for the increased energetic efficiency (lowering of the $\dot{V}O_2$) during the incomplete training period. The same pattern can be observed during the complete training period, when a gait change occurred at the velocities of 25 and 37.5 m · min^{-1}. The occurrence of gait change in the rats at a higher velocity during the complete training period compared with the incomplete training period may be related to the increase in body size during the training period. This effect of gait change on energetic efficiency is well established for the rat and the squirrel (14).

Prolonged exposure to normobaric oxygen may have harmful effects on respiratory muscles (1), skeletal muscles (10), and the myocardium (4,5) as found in animal models. Thus, it is plausible that exposure to hyperoxia may impair running performance. In the present study, we demonstrated that exposure to 100% oxygen in normobaric conditions for 24 h reversed the training effect on energetic efficiency. These results lead us to conclude that exposure to 100% normobaric oxygen has no ergogenic effect. Furthermore, exposure to normobaric oxygen may even have a deleterious effect on the running capability of rats, as shown by the increased oxygen requirement for the same workload. The reduction in energetic efficiency following 24-h exposure to oxygen in normobaric conditions may be related to the reduction in energetic efficiency of the rat heart (5), which has been partly explained by the change in the heart's response to catecholamines (4). The toxic effect of oxygen has been attributed to the overproduction of reactive oxygen species leading to cell injury. The cellular damage produced by hyperoxia in the myocardium, diaphragm (2), and skeletal muscle (10) might be responsible for the reduction in energetic efficiency.

Recent human studies (19,23) found no significant differences in $\dot{V}O_{2max}$ and $\dot{V}O_{2submax}$ after exposure to hyperbaric hyperoxia in trained subjects, suggesting that HBO treatment does not have ergogenic properties. To assess the possible ergogenic effect of HBO treatment, blood lactate concentration was measured in several human studies as an indication of the degree of metabolic acidosis (7,11,23). Banister et al. (7) found a reduction in blood lactate concentration during severe submaximal exercise performed after 70 min exposure to HBO treatment at 203 kPa followed by 40 min exposure to air in normobaric conditions at rest, compared with the same workload without preoxygenation. This finding was associated with a reduction in $\dot{V}O_{2submax}$. On the other hand, Webster et al. (23) found no change in blood lactate concentration after HBO treatment relative to the baseline tests. The authors suggested that this finding supports the contention that HBO treatment has no ergogenic effect. The measurement of blood lactate concentration was carried out at rest, during last 30 min of exercise for each workload (157, 196, 235, 275, and 314 W), and after a 5 min recovery period. Baseline tests were conducted twice, with a third test after HBO treatment consisting of a 1-h exposure at 203 kPa. In contrast with the studies cited above that used HBO treatment to produce an ergogenic effect in humans, we exposed rats to O_2 at atmospheric pressure for 24 h. It is well known that the phenomenon of oxygen toxicity occurs after a much shorter time in hyperbaric hyperoxia than with 100% O_2 in normobaric conditions (3,17). Thus a short hyperbaric O_2 exposure can be equivalent to a longer period in normobaric hyperoxia.

The results obtained in the present study are relevant to closed-circuit oxygen diving or to diving with oxygen-enriched air, in which divers are repeatedly exposed to hyperbaric hyperoxia. Divers who engage in this kind of professional and military diving are naturally in very good physical condition. Prolonged, strenuous dives in hyperoxic conditions may result in a reduction of energetic efficiency, so that the diver may be unable to do the job to the required standard. Routine exposure to oxygen might be harmful for this population of divers. Whenever the use of hyperoxia is being considered for ergogenic purposes, the deleterious effect of hyperoxia on energetic efficiency and on the response to catecholamines should be taken into account. It is possible that the conflicting results from studies on the ergogenic effect of HBO are related to the dual effect of hyperoxia.

In conclusion, the present study demonstrates that the energetic efficiency achieved by exercise training in rats was abolished following exposure to hyperoxia. The possible impairment of energetic efficiency after hyperoxia should be taken into consideration in numerous branches of sport, as well as in diving.

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