

Intermittent Hypoxia Improves Endurance Performance and Submaximal Exercise Efficiency

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ABSTRACT

Katayama, Keisho, Hiroshi Matsuo, Koji Ishida, Shigeo Mori, and Miharuru Miyamura. Intermittent hypoxia improves endurance performance and submaximal exercise efficiency. *High Alt Med Biol* 4:291–304, 2003.—The purpose of the present study was to elucidate the influence of intermittent hypobaric hypoxia at rest on endurance performance and cardiorespiratory and hematological adaptations in trained endurance athletes. Twelve trained male endurance runners were assigned to either a hypoxic group ($n = 6$) or a control group ($n = 6$). The subjects in the hypoxic group were exposed to a simulated altitude of 4500 m for 90 min, three times a week for 3 weeks. The measurements of 3000 m running time, running time to exhaustion, and cardiorespiratory parameters during maximal exercise test and resting hematological status were performed before (Pre) and after 3 weeks of intermittent hypoxic exposure (Post). These measurements were repeated after the cessation of intermittent hypoxia for 3 weeks (Re). In the control group, the same parameters were determined at Pre, Post, and Re for the subjects not exposed to intermittent hypoxia. The athletes in both groups continued their normal training together at sea level throughout the experiment. In the hypoxic group, the 3000 m running time and running time to exhaustion during maximal exercise test improved. Neither cardiorespiratory parameters to maximal exercise nor resting hematological parameters were changed in either group at Post, whereas oxygen uptake ($\dot{V}O_2$) during submaximal exercise decreased significantly in the hypoxic group. After cessation of intermittent hypoxia for 3 weeks, the improved 3000 m running time and running time to exhaustion tended to decline, and the decreased $\dot{V}O_2$ during submaximal exercise returned to Pre level. These results suggest that intermittent hypoxia at rest could improve endurance performance and submaximal exercise efficiency at sea level in trained endurance athletes, but these improvements are not maintained after the cessation of intermittent hypoxia for 3 weeks.

Key Words: oxygen uptake; altitude; distance running; intermittent hypoxia; cardiorespiratory adaptation; red blood cells

INTRODUCTION

A NUMBER OF STUDIES have demonstrated physiological adaptations as a result of in-

termittent exposure to altitude with or without exercise training, and much of the work on intermittent exposure to altitude was carried out in the former Soviet Union and Common-

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wealth of Independent States in the 1930s (see the reviews by Gippenreiter and West, 1996; Serebrovskaya, 2002). From these studies, it has been proposed that intermittent hypoxia is beneficial as a method of preacclimatization before climbing to high altitude and for the treatment of a variety of clinical disorders (Serebrovskaya, 2002).

Intermittent hypoxia during exercise (i.e., living low training high) has also been utilized frequently by endurance athletes to improve performance, and many studies have indicated the influences of intermittent hypoxia during exercise on endurance performance at sea level and on physiological adaptations in trained athletes (Terrados et al., 1988; Desplanches et al., 1993; Fulco et al., 2000; Meeuwsen et al., 2001). In contrast, some investigators have recently demonstrated the effects of intermittent normobaric or hypobaric hypoxia at rest (1 to 2 h/day) on physiological adaptations in humans (Serebrovskaya et al., 1999; Powell and Garcia, 2000; Katayama et al., 2001b). Garcia et al. (2000) indicated that 2 h daily exposures to normobaric hypoxia (13% oxygen, simulating an altitude of 3800 m) at rest led to an increase in reticulocytes, and Rodriguez et al. (2000) also recently found significant increases in hematological variables after intermittent hypobaric hypoxia (simulating an altitude of 4000 to 5500 m) at rest, 90 min a day, three times per week for 3 weeks. From these data, intermittent exposure to hypoxia at rest has been supported as an ergogenic method to improve endurance performance at sea level (Wilber, 2001). Hellems (1999) and Cedaro (2000) have reported preliminary data from studies of endurance athletes exposed to intermittent normobaric hypoxia at rest while simultaneously training in normoxia; they demonstrated significant improvements in endurance performance and hematological indexes following 2 to 3 hours of daily exposure to hypoxia (9% to 10% oxygen) at rest for 10 to 20 days. However, since these experiments did not include appropriate concurrent control groups, it is unclear whether intermittent hypoxia at rest leads to improvements in endurance performance and hematological parameters in trained athletes. Collectively, data regarding the effects of short-term intermittent hypoxia at rest while simultane-

ously training at sea level on endurance performance are minimal and inconclusive, and cardiorespiratory and hematological adaptations following intermittent hypoxia in trained athletes are unclear.

The purpose of the present study was, therefore, to elucidate the influences of intermittent hypobaric hypoxia at rest on endurance performance and cardiorespiratory and hematological adaptations in trained athletes. Based on the effects of intermittent hypoxia at rest that were reported by Rodriguez et al. (2000), the protocol in the present study consisted of hypobaric hypoxic exposure (simulating an altitude of 4500 m) on alternate days, three times a week, for 3 weeks.

METHODS

Subjects

Twelve trained male endurance runners, who belonged to a collegiate track team, volunteered to participate in this study. All subjects had a maximum oxygen uptake of ($\dot{V}_{O_{2max}}$) $> 65 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and had reported no history of cardiovascular or respiratory disease. Each subject was assigned to either a hypoxic group ($n = 6$) or a control group ($n = 6$). Mean values and standard deviations (SD) for age, height, and body mass were 20.5 ± 2.4 yr, 170.9 ± 4.3 cm, and 55.1 ± 3.9 kg for the hypoxic group and 22.7 ± 3.2 yr, 172.2 ± 2.7 cm, and 58.4 ± 4.8 kg for the control group. There were no significant differences in age or physical characteristics between either group. The subjects were informed of the experimental procedures and possible risks involved in the present study and their informed consent was obtained. This study was approved by the Human Research Committee of the Research Center of Health, Physical Fitness and Sports, Nagoya University.

Experimental procedures

The time course of experimental procedures in this study is presented in Fig. 1. Subjects in the hypoxic group were first familiarized with the hypobaric chamber and the equipment used in this experiment at sea level. Before ex-

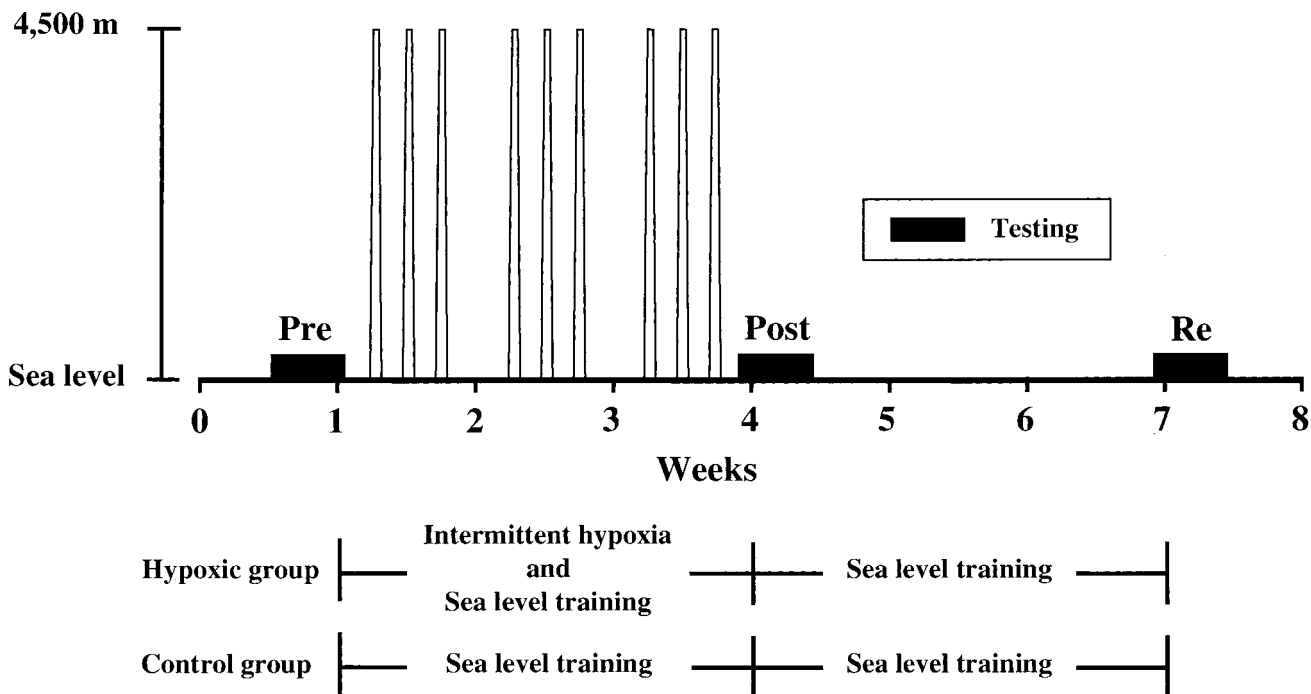


FIG. 1. Time course of the experiment. The measurements were performed before (Pre) and after (Post) intermittent hypoxia and again after the cessation of hypoxic exposure for 3 weeks (Re).

posure to intermittent hypoxia (Pre), a maximal exercise test, a 3000 m time trial, and resting hematology assessment were performed at sea level (Fig. 1). The hypobaric chamber similar to that in our previous studies (Katayama et al., 2001a, b) was utilized for intermittent hypoxia. The barometric pressure in the chamber was lowered to 432 torr, corresponding to a 4500 m altitude over a 30 min period and then held at that level for the next 90 min. The subjects completed the self-assessment portion of the Lake Louise Consensus Questionnaire (Hackett and Oelz, 1992) each day while being exposed to intermittent hypoxia for 3 weeks. The maximal exercise test, a 3000 m time trial and resting hematology assessment at sea level were conducted after intermittent hypoxia, as shown in Fig. 1 (Post). These measurements were repeated again after the cessation of intermittent hypoxia for 3 weeks (Re). The same parameters were measured in the control group, which was not exposed to intermittent hypoxia, at Pre, Post, and Re. The athletes in both groups continued the same training program at sea level throughout the experimental period (Fig. 1).

Maximal exercise test

$\dot{V}_{O_{2max}}$ was determined according to a modified Astrand-Saltin procedure described by Levine and Stray-Gundersen (1997). After a 15 min warm-up, the athletes ran on the treadmill at 9.0 miles/h (mph) at 0% grade for 2 min. The grade was then increased 2% every 2 min until exhaustion. Oxygen uptake (\dot{V}_{O_2}) and carbon dioxide output (\dot{V}_{CO_2}) were measured by using the Douglas bag method; that is, expired gases were collected into a Douglas bag during the last 1 min of each grade until exhaustion. Expired gas volume was measured with a wet-gas meter (model WE, Shinagawa), and gas analyses were performed by means of a mass spectrometer (model ARCO-1000, Arco System). Heart rate (HR) was continuously recorded by a three-lead electrocardiogram (model OEC-6401, Nihon Koden) throughout the test. The peak HR value was expressed as HR_{max} , and the peak pulmonary ventilation (\dot{V}_E , BTPS) value was estimated as $\dot{V}_{Emax} \cdot \dot{V}_{O_2}$ derived during maximal exhaustive exercise and was considered to be $\dot{V}_{O_{2max}}$ when two of the following three criteria were satisfied; iden-

tification of a plateau in \dot{V}_{O_2} with an increase in work rate (<150 mL \dot{V}_{O_2} increase), HR \pm 10% of age-predicted maximum ($220 - \text{age}$), and/or respiratory exchange ratio (RER) > 1.1 .

Endurance performance

Sea-level endurance performance was assessed by 3000 m time trial races on a 400 m track. Time trials were performed at 17:00 to 18:00 in the early evening (temperature 25° to 27°C, relative humidity 64% to 75%, wind 1 to 7 km/h). The subjects were instructed to achieve the best time possible on each time trial. Time was recorded for each subject to the nearest 0.1 sec (Stray-Gundersen et al., 2001). In addition, running time to exhaustion during maximal exercise test was also used as an index for endurance performance at sea level (Mizuno et al., 1990).

Hematological parameters

Venous blood samples taken for hemoglobin concentration, hematocrit, RBC count, reticulocytes, erythropoietin concentration, and ferritin concentration were drawn at 8:00 in the morning. The measurements of the hemoglobin concentration, hematocrit, and RBC count were conducted by an automated cytological cell counter (model SE-9000, Sysmex). Reticulocytes were assayed using flow cytometry (model RAM-1, Sysmex). Erythropoietin and ferritin concentrations were determined by an enzyme-linked immunosorbent assay using a reagent kit (immunoelit EPO, Toyobo) and by a chemiluminescent immunoassay kit (chemilumi ACS ferritin, Bayer Medical).

Statistical analysis

The values were expressed as means \pm SD. The changes in parameters within each group during the experimental periods were analyzed using one-way analysis of variance (ANOVA) with repeated measurements. When significance was found, a Newman-Keuls test was applied to locate differences in the parameters at each session (Pre, Post, and Re) (Green et al., 2000b; Stray-Gundersen et al., 2001). The comparison of parameters between

groups at each session was done by using the Mann-Whitney test. Significance was set at $p < 0.05$.

RESULTS

Baseline descriptive data

There were no changes in physical characteristics in either group throughout the experimental period. During days 1 and 2 of intermittent hypoxia, three of the subjects in the hypoxic group had slight headaches and weakness in the chamber with a simulated altitude of 4500 m, but, thereafter, there was a score of zero for the Lake Louise Consensus Questionnaire for intermittent hypoxia. All athletes in both groups carried out the same training at sea level throughout the study (i.e., the weekly training distance ranged from 100 to 115 km), and the total training volume was not different between groups.

Maximal exercise test

Table 1 indicates \dot{V}_{O_2} , \dot{V}_{CO_2} , \dot{V}_E , RER, and HR obtained at exhaustion in both the hypoxic and control groups during the maximal treadmill test throughout the study. There were no changes in $\dot{V}_{O_{2max}}$, $\dot{V}_{CO_{2max}}$, \dot{V}_{Emax} , RER and HR_{max} in either group throughout the experimental period.

In contrast, \dot{V}_{O_2} in the hypoxic group decreased significantly ($p < 0.05$) after intermittent hypoxia (Post) during the first four stages of the maximal exercise test at grade 0, 2, 4, 6 (%), as shown in Fig. 2A. The changed \dot{V}_{O_2} during submaximal exercise returned to Pre level after the cessation of intermittent hypoxia for 3 weeks (Re, Fig. 2A). Similarly, HR during submaximal exercise tended to decrease after intermittent hypoxia, but the change was not statistically significant (Fig. 2E). RER during submaximal exercise tended to increase after intermittent hypoxia (Post) and returned to the Pre level at Re, although it was not statistically significant (Fig. 2D). \dot{V}_{CO_2} and \dot{V}_E during submaximal exercise in the hypoxic group did not change throughout the study (Fig. 2B and C). In the control group, there were no significant changes in \dot{V}_{O_2} , \dot{V}_{CO_2} , \dot{V}_E , RER, and HR dur-

TABLE 1. MAXIMAL CARDIORESPIRATORY PARAMETERS BEFORE (PRE), IMMEDIATELY AFTER (POST), AND 3 WEEKS AFTER INTERMITTENT HYPOXIA (RE)

	Group	Pre	Post	Re
$\dot{V}O_{2\max}$ (L · min ⁻¹)	H	3.72 ± 0.25	3.69 ± 0.27	3.74 ± 0.29
	C	4.08 ± 0.30	4.13 ± 0.31	4.11 ± 0.34
$\dot{V}O_{2\max}$ (ml · kg ⁻¹ · min ⁻¹)	H	67.4 ± 2.3	67.7 ± 2.8	68.0 ± 2.8
	C	69.4 ± 3.8	69.5 ± 3.1	69.0 ± 3.4
$\dot{V}CO_{2\max}$ (L · min ⁻¹)	H	4.39 ± 0.36	4.40 ± 0.31	4.47 ± 0.30
	C	4.81 ± 0.30	4.78 ± 0.39	4.82 ± 0.45
$\dot{V}E_{\max}$ (L · min ⁻¹)	H	145.8 ± 17.2	144.3 ± 16.6	145.9 ± 17.3
	C	150.9 ± 13.9	149.8 ± 11.6	152.3 ± 14.1
RER	H	1.19 ± 0.05	1.19 ± 0.02	1.20 ± 0.03
	C	1.18 ± 0.03	1.17 ± 0.05	1.18 ± 0.05
HR _{max} (beats · min ⁻¹)	H	189.7 ± 5.6	190.8 ± 6.1	189.8 ± 6.6
	C	192.8 ± 3.9	190.0 ± 4.9	191.7 ± 3.6

Values are means ± SD. $\dot{V}O_{2\max}$, maximum oxygen uptake; $\dot{V}CO_{2\max}$, maximum carbon dioxide output; $\dot{V}E_{\max}$, maximum minute ventilation; RER, respiratory exchange ratio; HR_{max}, maximum heart rate; H, hypoxic group; C, control group.

ing submaximal exercise throughout the experimental period, as shown in Fig. 2.

Endurance performance

Two subjects (one from each group) did not complete the 3000 m time trial all three times, because the subject from the hypoxic group suffered a leg injury before the time trial at Post, and the subject from the control group felt pain in his leg before the trial at Re. For this reason, the data from these two subjects were not included in the time trial analysis. Figure 3 indicates the changes in the 3000 m running time throughout the experiment. In the hypoxic group, the 3000 m performance improved significantly ($p < 0.05$) after intermittent hypoxia [9.38 ± 0.39 (Pre) and 9.25 ± 0.38 (Post) min], and the improved performance tended to decline after the cessation of intermittent hypoxia [9.30 ± 0.37 (Re) min]. On the other hand, there was no significant change in the 3000 m running time in the control group throughout the experimental period, as shown in Fig. 3 [9.46 ± 0.56 (Pre), 9.39 ± 0.53 (Post), and 9.41 ± 0.55 (Re) min].

Figure 4 indicates the changes in the running time to exhaustion during the maximal exercise test throughout the experiment. In the hypoxic group, a significant ($p < 0.05$) increase in running time to exhaustion was observed after intermittent hypoxia [11.5 ± 0.7 (Pre) and 12.6 ± 0.7

(Post) min], and the increased running time decreased significantly ($p < 0.05$) after the cessation of intermittent hypoxia [11.9 ± 0.6 (Re) min]. On the other hand, there was no significant change in the running time during the maximal exercise test in the control group throughout the experimental period, as shown in Fig. 4 [12.0 ± 0.9 (Pre), 11.7 ± 0.9 (Post), and 12.0 ± 0.8 (Re) min].

Hematological parameters

The changes in hemoglobin concentration, hematocrit, RBC count, reticulocytes, erythropoietin concentration, and ferritin concentration are indicated in Table 2. In both groups, all hematological parameters were unchanged throughout the experimental period, and there were no significant differences between groups at Pre, Post, and Re.

DISCUSSION

The major findings of this study are that (1) the 3000 m running time and running time to exhaustion during the maximal exercise test in trained endurance athletes improved significantly in response to 3 weeks of intermittent hypoxia at rest (90 min per day, three times a week, simulating an altitude of 4500 m), and the improved running time declined after the

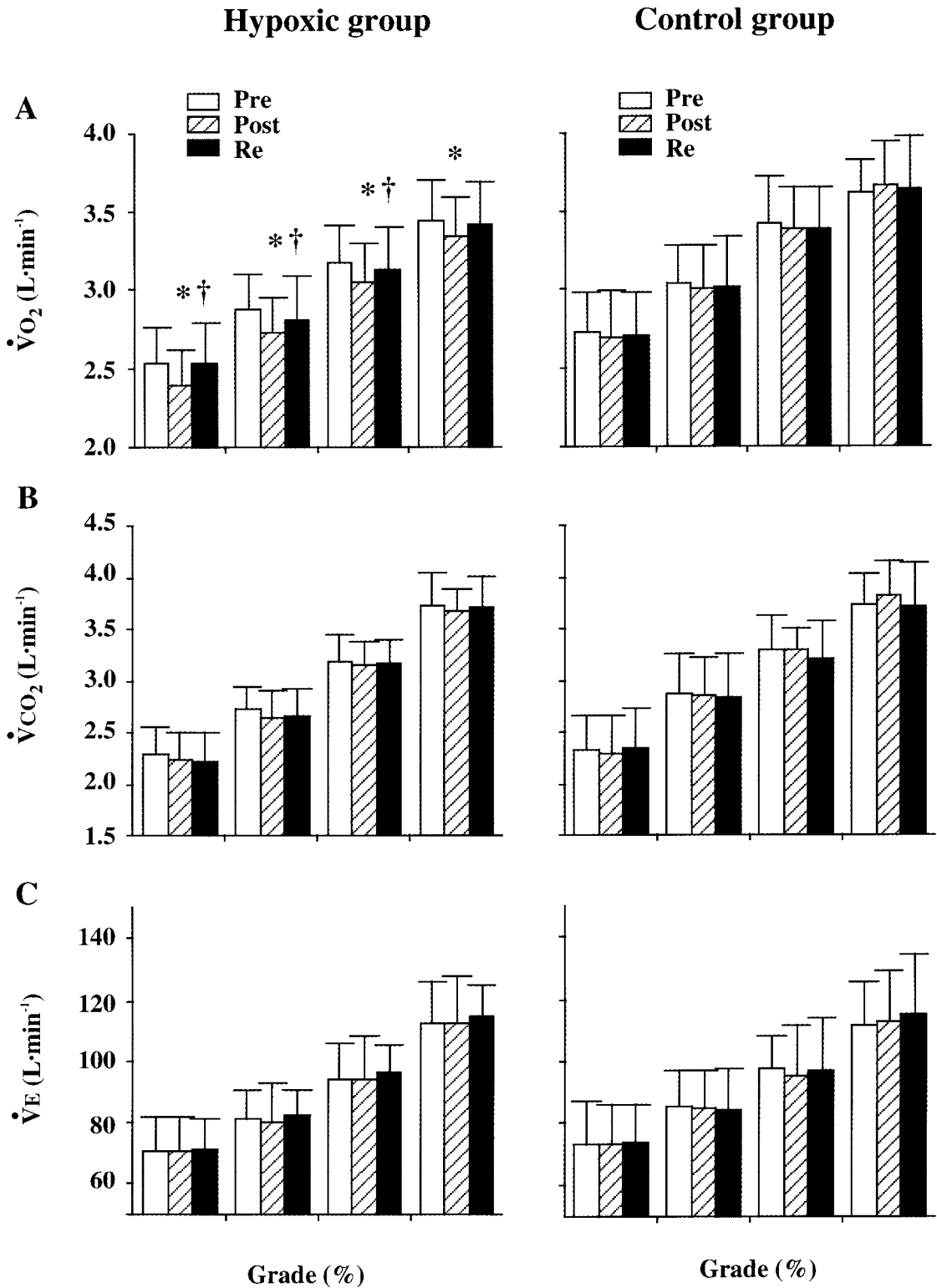


FIG. 2. (A) Oxygen uptake ($\dot{V}O_2$); (B) carbon dioxide output ($\dot{V}CO_2$); (C) expired minute ventilation (VE); (D) respiratory exchange ratio (RER); and (E) heart rate (HR) for the hypoxic and control groups during the first four stages of the maximal exercise test before (Pre) and after intermittent hypoxia (Post) and 3 weeks after hypoxic exposure (Re). Values are means \pm SD. *Pre vs. Post ($p < 0.05$). †Post vs. Re ($p < 0.05$).

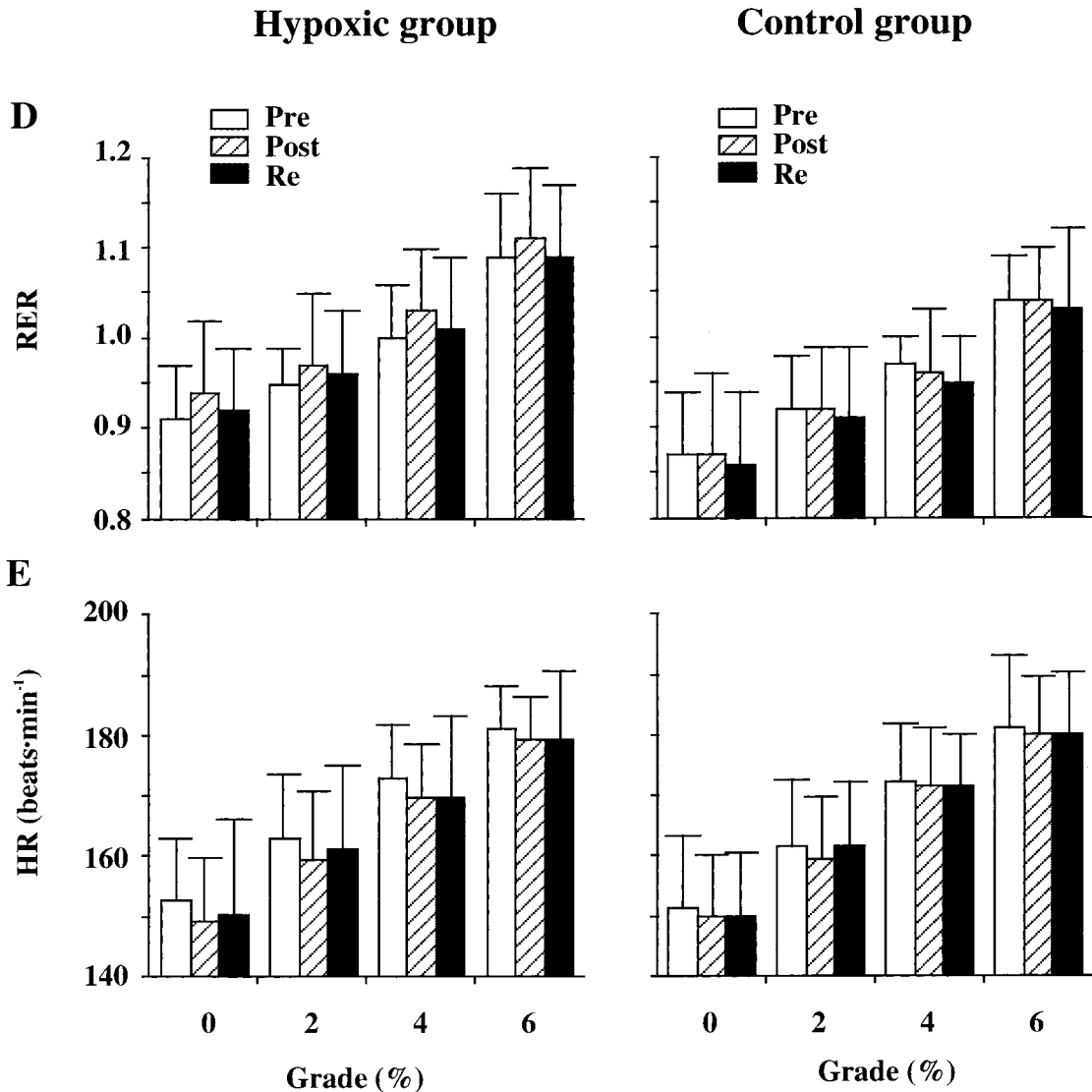


FIG. 2. (continued)

cessation of hypoxic exposure for 3 weeks; (2) cardiorespiratory parameters at exhaustion and resting hematological parameters at rest did not change after intermittent hypoxia; (3) \dot{V}_{O_2} during submaximal exercise in the hypoxic group decreased significantly after intermittent hypoxia at rest, and the changed \dot{V}_{O_2} returned to Pre level after the cessation of intermittent hypoxia for 3 weeks.

Although several altitude or hypoxic training strategies have been used in an effort to incur an advantage in sea-level performance over just sea-level training alone (Wolski et al., 1996; Wilber, 2001), few investigators have examined the effects of intermittent hypoxia at rest while

simultaneously training at sea level on endurance performance or physiological adaptations in trained athletes; these studies have revealed that significant improvements in endurance performance followed intermittent normobaric hypoxia (9% to 10% oxygen, corresponding to 4000 to 6500 m) for 2 to 3 h/day for 10 to 21 days (Hellemans, 1999; Cedaro, 2000). However, since these experiments did not include appropriate control groups, it is unclear whether intermittent hypoxia at rest leads to an improvement in endurance performance in trained athletes. To determine this in the present study, the subjects not exposed to intermittent hypoxia also trained concurrently at

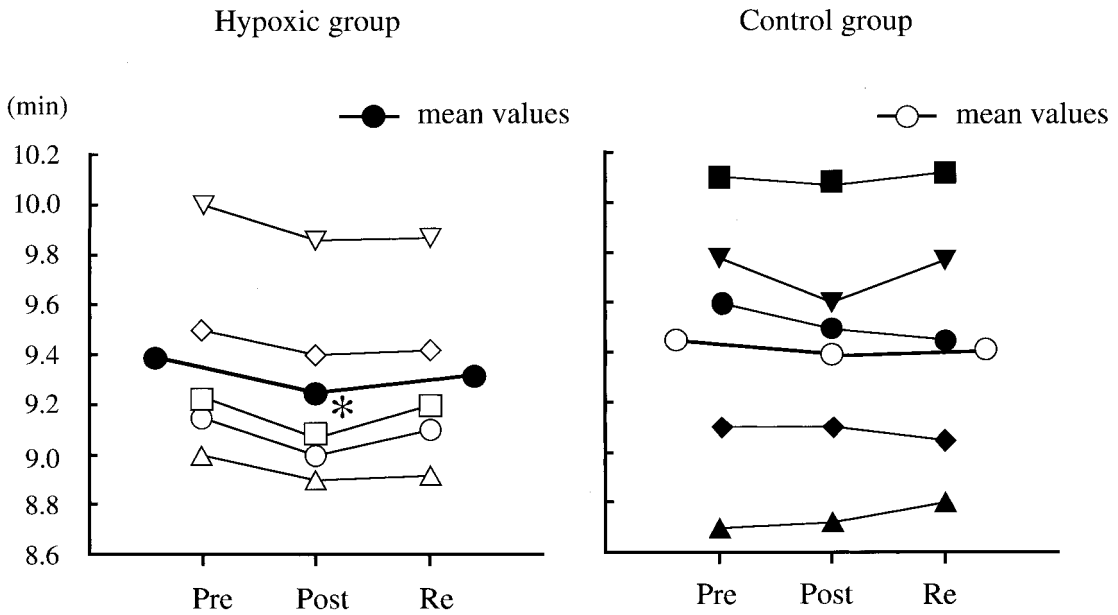


FIG. 3. Individual and mean data for the 3000-m running performance in the hypoxic and control groups before (Pre), after intermittent hypoxia (Post), and 3 weeks after hypoxic exposure (Re). *Pre vs. Post ($p < 0.05$).

sea level with the hypoxic group. Consequently, 3 weeks of intermittent hypoxia at rest while simultaneously training at sea level did significantly improve the endurance running performance in the hypoxic group, but not in the control group, as shown in Figs. 3 and 4.

This result suggests that intermittent hypoxia at rest while simultaneously training at sea level could enhance endurance performance in trained athletes.

Numerous studies have described the influence of altitude training on endurance perfor-

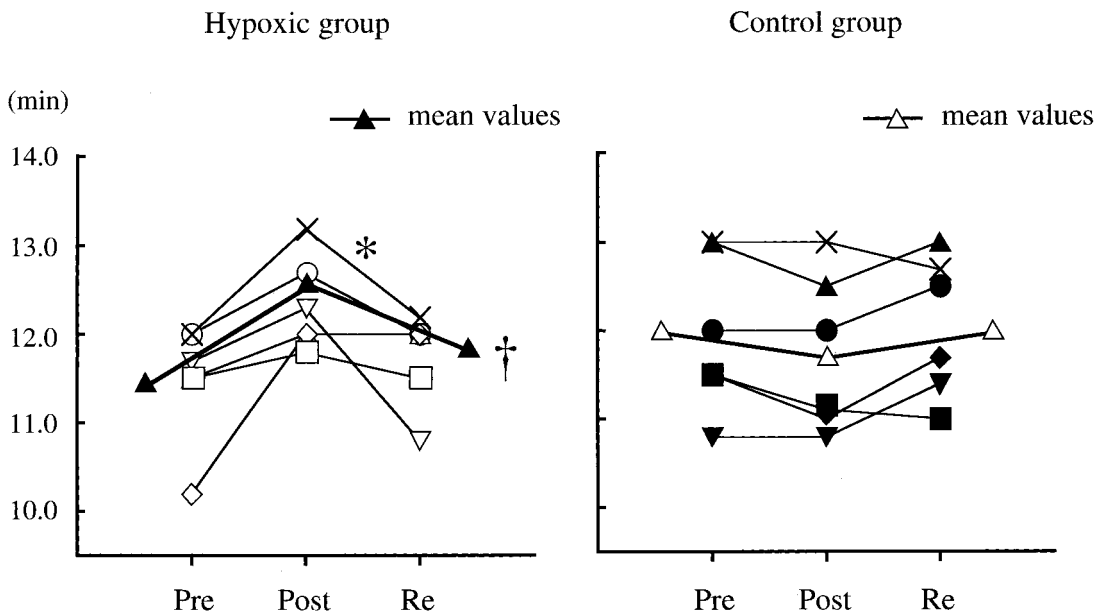


FIG. 4. Individual and mean data for the exercise time to exhaustion during the maximal exercise test in the hypoxic and control groups before (Pre), after intermittent hypoxia (Post), and 3 weeks after hypoxic exposure (Re). *Pre vs. Post ($p < 0.05$). †Post vs. Re ($p < 0.05$).

TABLE 2. HEMATOLOGICAL PARAMETERS BEFORE (PRE), IMMEDIATELY AFTER (POST), AND 3 WEEKS AFTER INTERMITTENT HYPOXIA (RE)

	Groups	Pre	Post	Re
Hemoglobin ($\text{g} \cdot \text{dL}^{-1}$)	H	14.3 ± 0.8	14.6 ± 0.8	14.4 ± 0.7
	C	14.8 ± 0.5	15.0 ± 0.6	14.8 ± 0.7
Hematocrit (%)	H	42.4 ± 2.8	43.0 ± 2.9	42.6 ± 2.1
	C	45.0 ± 2.6	45.5 ± 2.2	45.6 ± 2.4
RBC count ($10^6 \cdot \mu\text{L}^{-1}$)	H	4.79 ± 0.18	4.88 ± 0.18	4.82 ± 0.20
	C	4.89 ± 0.17	4.95 ± 0.17	4.95 ± 0.29
Reticulocytes (%)	H	1.0 ± 0.4	0.9 ± 0.3	1.0 ± 0.4
	C	0.8 ± 0.4	0.8 ± 0.4	0.9 ± 0.5
Erythropoietin ($\text{mU} \cdot \text{mL}^{-1}$)	H	7.23 ± 4.36	8.42 ± 2.94	7.42 ± 3.29
	C	7.68 ± 3.07	7.52 ± 3.40	7.20 ± 3.40
Ferritin ($\text{ng} \cdot \text{mL}$)	H	53.8 ± 26.3	52.2 ± 25.3	50.0 ± 20.9
	C	59.5 ± 35.2	62.8 ± 34.0	61.8 ± 27.1

Values are means \pm SD. RBC count, red blood cell count; H, hypoxic group; C, control group.

mance as mentioned above, but the time course of exercise performance and cardiorespiratory response to exercise after the cessation of altitude training has received little attention. Moreover, there are no reports concerning the effect of the cessation of intermittent hypoxia at rest on endurance performance and physiological adaptations in trained athletes. To elucidate this issue, a 3000 m time trial and maximal exercise test were performed again after the cessation of intermittent hypoxia for 3 weeks in the present study. Accordingly, the improved endurance performance after intermittent hypoxia did not remain at Re, even though the athletes continued their training after the cessation of intermittent hypoxia (Figs. 3 and 4). This result does not concur with those of previous studies, which have shown that 2 to 3 weeks are needed after living high, training high procedures for the athletes to reach a maximum performance (Dick, 1992; Bailey and Davies, 1997) and that the beneficial effects of living high, training low procedures on endurance performance continued for up to 3 weeks post altitude training (Levine and Stray-Gundersen, 1997). The discrepancy between those studies and the present one may be related to several factors, although it is difficult to explain this on the basis of the physiological data obtained here. If the improvement of hematological variables (e.g., RBC) is one of the factors accounting for improved sea-level performance, the improved endurance performance could remain for the lifetime of the RBC.

Therefore, it may be that after living high, training high or living high, training low procedures the improved performance would last for 2 or 3 weeks after returning to sea level. But, as far as we know, the longest time that investigators have documented endurance performance has been up to 3 weeks after altitude training was completed. In contrast, since intermittent hypoxia, as applied in this study, did not lead to the improvement of hematological parameters, it is conceivable that the improved endurance performance did not continue after the cessation of intermittent hypoxia. Thus, one way to explain the inconsistent results between previous studies and the present one may be hematological adaptations after the cessation of hypoxic exposure. Although further investigation is needed to clarify the reasons for these contradictory results, the results in the present study, which used the hypoxic approach, suggest that the change in the endurance performance after the cessation of intermittent hypoxia differs from those of both the living high, training high and living high, training low utilizations.

The hematological adaptations during continuous hypoxia, or sojourns at altitudes, have been well described as an erythropoietic response to hypoxia, which provides a higher transport capacity of the blood. Recently, erythropoiesis has been observed after intermittent hypoxia at rest in several studies (Hellemans, 1999; Cedaro, 2000; Garcia et al., 2000). Rodriguez et al. (2000) also reported that significant increases in RBC count and reticu-

loocytes were found following intermittent exposure to altitude in trained individuals. On the contrary, in the present study, hematological variables did not change after intermittent hypobaric hypoxia, as shown in Table 2. The discrepancy between the present study and others (Hellemans, 1999; Cedaro, 2000; Garcia et al., 2000; Rodriguez et al., 2000) may be related to several factors. As for the magnitude of hypoxia, the hypobaric chamber in the present study was set at a level corresponding to a 4500 m altitude, which was lower than that in previous studies (Hellemans, 1999; Cedaro, 2000), which utilized hypoxic air at 9% to 10% oxygen (equivalent to 5800 to 6400 m altitude). By contrast, Garcia et al. (2000) demonstrated that intermittent exposure at 13% oxygen (3800 m altitude), the magnitude of hypoxia being lower than that in the present study, led to a significant increase in reticulocytes after intermittent hypoxia. Thus, we suspect that the magnitude of hypoxic stimuli (4500 m), as applied in this study, was sufficient to stimulate erythropoiesis. With regard to the exposure period in the present study, the protocol consisted of exposure on alternate days, three times per week for 3 weeks, which was similar to the protocol in Rodriguez et al. (2000), who showed significant increases in RBC count and reticulocytes. However, other studies have reported that a significant increase in hematological variables was found following daily exposure to hypoxia for a period of from 10 to 21 days (Hellemans, 1999; Cedaro, 2000). From the studies above and the results in this study, it is likely that exposure to intermittent hypoxia on alternate days was not enough to increase RBC count and reticulocytes. Concerning the exposure time, the minimum time necessary to be exposed to hypoxia to stimulate erythropoietin production is described as between 84 and 120 min when the inspiratory oxygen content is decreased below 13% (Schmidt, 2002). Frey et al. (2000) also reported no influence on hematological indexes after 75 min of daily exposure to normobaric hypoxia (9% oxygen) for 3 weeks. The athletes in the hypoxic group in the present study were exposed to hypoxia for 90 min/day, which did not differ from the findings of Rodriguez et al. (2000), which showed increases in hematological parameters. From

these data, it is likely that 90 min/day is sufficient to stimulate erythropoiesis. However, Hellemans (1999) and Cedaro (2000) reported preliminary data from studies of endurance athletes that indicated significant increases in hemoglobin concentrations, hematocrit, and reticulocytes following 2 to 3 h of intermittent hypoxia at rest. Moreover, it has been demonstrated that a significant increase in reticulocytes occurs after 2 h/day of intermittent hypoxia (Garcia et al., 2000). Therefore, although we are not certain of the reasons for the discrepancy in the results between the previous studies (Hellemans, 1999; Cedaro, 2000; Garcia et al., 2000; Rodriguez et al., 2000) and the present one, it may be that 90 min exposure is insufficient for erythropoiesis to take place. In other words, it can be speculated that 2 h/day is needed for a vigorous erythropoiesis when intermittent hypoxia at rest is utilized. Further research is required to confirm this speculation.

The most important adaptation to hypoxia or altitude that would improve sea-level performance is an increase in RBC count, which increases the oxygen-carrying capacity of red blood and improves aerobic power (Ekblom et al., 1972; Buick et al., 1980). In fact, several investigators have reported that improvements in endurance running performance after altitude training took place as a result of increases in RBC count (Levine and Stray-Gundersen, 1997; Stray-Gundersen et al., 2001). In those studies, therefore, the mechanism of improved endurance performance could be the result of increases in blood oxygen-carrying capacity. However, the changes in the endurance performance of the hypoxic group in the present study appeared without changes in their hematological variables (Table 2). Thus, the changed endurance performance after intermittent hypoxia and after the cessation of hypoxic exposure in this study could not be explained by a change in blood oxygen-carrying capacity.

A number of previous reports have shown that \dot{V}_{O_2} at given submaximal exercise at sea level remains unchanged after altitude training (Levine and Stray-Gundersen, 1997; Piehl-Aulin et al., 1998) or chronic exposure to hypoxia (Wolfel et al., 1991; Grassi et al., 1996). However, Green et al. (2000b) and Gore et al. (2001) recently found that \dot{V}_{O_2} was significantly

decreased during submaximal exercise subsequent to a sojourn at high altitude or to the living high training low procedure. Interestingly, in the present study, \dot{V}_{O_2} during the first four stages of the maximal exercise test, at grades 0, 2, 4, and 6 (%), in the hypoxic group did decrease significantly ($p < 0.05$) after intermittent hypoxia at rest as shown in Fig. 2A, whereas there was no such change in the control group. These results suggest that intermittent hypoxia at rest, as well as chronic exposure (Green et al., 2000b) and the living high training low model (Gore et al., 2001), also leads to a decrease in \dot{V}_{O_2} during submaximal exercise at sea level. The decreased \dot{V}_{O_2} during submaximal exercise suggests the increased exercise efficiency (Green et al., 2000b; Gore et al., 2001), and the result in the present study is consistent with those of several studies that have indicated higher exercise efficiency in highland residents as compared with lowlanders (Hochachka et al., 1991; Saltin et al., 1995).

Several mechanisms might contribute to the lower \dot{V}_{O_2} during submaximal exercise after intermittent hypoxia. First, because respiratory muscles during exercise consume a significant proportion of the whole-body \dot{V}_{O_2} (Harms and Dempsey, 1999), it may be that a reduction in \dot{V}_{O_2} of the respiratory muscles could contribute to decreased whole-body \dot{V}_{O_2} during submaximal exercise. However, exercise ventilation at sea level did not change after intermittent hypoxia, as shown in Fig. 2C. Thus the lowered \dot{V}_{O_2} during submaximal exercise after intermittent hypoxia could not be explained by the reduction in \dot{V}_{O_2} of the respiratory musculature (Green et al., 2000b; Gore et al., 2001). Second, a shift from oxidative to anaerobic metabolism may contribute to decreased \dot{V}_{O_2} during submaximal exercise. However, previous studies have shown a decreased lactate concentration during submaximal exercise after intermittent hypoxia with and without endurance exercise training (Rodriguez et al., 1999; Casas et al. 2000). Accordingly, it is conceivable that the shift to anaerobic metabolism during exercise after intermittent hypoxia is not the cause of the lowered \dot{V}_{O_2} (Green et al., 2000b; Gore et al., 2001). Third, in the present study, RER during submaximal exercise tended to increase after intermittent hypoxia, but was

not statistically significant, and this result is in agreement with those of others who found lower \dot{V}_{O_2} during exercise after hypoxic exposure (Green et al., 2000b; Gore et al., 2001). An increased RER also coincides with that of highlanders who have higher exercise efficiency (Hochachka et al., 1991). Gore et al. (2001) stated that the higher RER is consistent with the suggestion that at altitude increased carbohydrate flux reflects a shift toward carbohydrate utilization, which optimizes the available energy for a given oxygen consumption (Brooks et al., 1998; Hahn and Gore, 2001). Some reports have shown preferential use of carbohydrate fuels rather than fat during exercise at high altitude (Brooks et al., 1991; Roberts et al., 1996a, b). Judging from these data, the reduction in \dot{V}_{O_2} after intermittent hypoxia may be explained by a shift from fat to carbohydrate oxidation (Green et al., 2000b; Gore et al., 2001; Hahn and Gore, 2001). Fourth, the economization of mitochondrial respiration function during the adaptations to intermittent hypoxia could be included. Intermittent hypoxia caused the reorganization of mitochondrial energy metabolism and favoring NAD-dependent oxidation (see the review by Serebrovskaya, 2002). Serebrovskaya (2002) found that after 14 days of normobaric intermittent hypoxia acute hypoxic stimuli in the presence of succinate results in an increase of the respiratory control index of Chance (state 3/state 4 ratio) and the phosphorylation rate (ATP formed/ O_2 consumed). These results indicate more efficient use of oxygen after intermittent hypoxia, and these adaptations could also be related to the decreased \dot{V}_{O_2} during exercise after intermittent hypoxia in this study. Finally, the mechanisms of the reduction of \dot{V}_{O_2} during exercise after intermittent hypoxia may include a reduction of the ATP-consuming process with skeletal muscle, since Green et al. (1999, 2000a) recently showed that training in hypoxia and sojourns to altitude led to downregulation in muscle $Na^+K^+ATPase$ pump concentration. Therefore, it is possible to assume that intermittent hypoxia results in a downregulation in muscle $Na^+K^+ATPase$, which may also contribute to a reduction in \dot{V}_{O_2} during submaximal exercise (Green et al., 2000b; Gore et al., 2001).

As described previously, the changed endurance performance in this study could not be explained by increases in blood oxygen-carrying capacity, because the affected running time in the hypoxic group of athletes appeared without changes in hematological variables (Table 2). It is interesting to note that the changes in \dot{V}_{O_2} during submaximal exercise both after intermittent hypoxia and after the cessation of hypoxic exposure accompanied the changed endurance performance. The changed \dot{V}_{O_2} during submaximal exercise suggests that the varied exercise efficiency (Green et al., 2000b; Gore et al., 2001) and better exercise efficiency (i.e., lower \dot{V}_{O_2} for a given absolute running speed) can be considered to be advantageous to endurance performance because it will result in the utilization of a lower percentage of the $\dot{V}_{O_{2max}}$ for any particular exercise intensity (Jones and Carter, 2000). Several investigators have demonstrated that running efficiency is correlated with endurance running performance, particularly within groups that are homogeneous in terms of $\dot{V}_{O_{2max}}$ (Conley and Krahenbuhl, 1980; Morgan and Craib, 1992). Thus, it is possible to assume that the potential mechanism underlying the changed endurance performance after intermittent hypoxia in this study is due to the alteration of exercise efficiency. However, it is necessary to confirm this assumption by further study.

In conclusion, the 3000 m running performance and the running time to exhaustion during the maximal exercise test in trained endurance athletes improved significantly after intermittent hypoxia at rest while simultaneously training at sea level, but not in athletes not exposed to intermittent hypoxia. Neither cardiorespiratory parameters to maximal exercise nor hematological parameters at rest changed, whereas \dot{V}_{O_2} during submaximal exercise decreased significantly after intermittent hypoxia. After cessation of intermittent hypoxia for 3 weeks, the increased running time and the decreased \dot{V}_{O_2} during submaximal exercise returned to Pre level. These results suggest that intermittent hypoxia at rest could improve endurance performance and submaximal exercise efficiency at sea level in trained endurance athletes, but these improvements

are not maintained after the cessation of intermittent hypoxia for 3 weeks.

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REFERENCES

- Bailey D.M., and Davies B. (1997). Physiological implications of altitude training for endurance performance at sea level: a review. *Br. J. Sports Med.* 31:183-190.
- Brooks G.A., Butterfield G.E., Wolfe R.R., Groves B.M., Mazzeo R.S., Sutton J.R., Wolfel E.E., and Reeves J.T. (1991). Increased dependence on blood glucose after acclimatization to 4,300 m. *J. Appl. Physiol.* 70:919-927.
- Brooks G.A., Wolfel E.E., Butterfield G.E., Cymerman A., Roberts A.C., Mazzeo R.S., and Reeves J.T. (1998). Poor relationship between arterial [lactate] and leg net release during exercise at 4,300 m altitude. *Am. J. Physiol.* 275:R1192-R1201.
- Buick F.J., Gledhill N., Froese A.B., Spriet L., and Meyers E.C. (1980). Effect of induced erythrocythemia on aerobic work capacity. *J. Appl. Physiol.* 48:636-642.
- Casas M., Casas H., Pages T., Rama R., Ricart A., Ventura J.L., Ibanez J., Rodriguez F.A., and Viscor G. (2000). Intermittent hypobaric hypoxia induces altitude acclimation and improves the lactate threshold. *Aviat. Space Environ. Med.* 71:125-130.
- Cedaro R.L. (2000). Intermittent hypoxic training (IHT): The next frontier in altitude training. *Proceeding of the Third Annual International Altitude Symposium, Flagstaff, AZ*, pp. 54-58.
- Conley D.L., and Krahenbuhl G.S. (1980). Running economy and distance running performance of highly trained athletes. *Med. Sci. Sports Exerc.* 12:357-360.

- Desplanches D., Hoppeler H., Linossier M.T., Denis C., Claassen H., Dormois D., Lacour J.R., and Geysant A. (1993). Effects of training in normoxia and normobaric hypoxia on human muscle ultrastructure. *Pflügers Arch.* 425:263–267.
- Dick F.W. (1992). Training at altitude in practice. *Int. J. Sports Med.* 13 (Suppl):S203–S205.
- Eklblom B., Goldbarg A.N., and Gullbring B. (1972). Response to exercise after blood loss and reinfusion. *J. Appl. Physiol.* 33:175–180.
- Frey W.O., Zenhausem R., Colombani P.C., and Fehr J. (2000). Influence of intermittent exposure to normobaric hypoxia on hematological indexes and exercise performance. *Med. Sci. Sports Exerc.* 32 (Suppl):S65.
- Fulco C.S., Rock P.B., and Cymerman A. (2000). Improving athletic performance: Is altitude residence or altitude training helpful? *Aviat. Space Environ. Med.* 71:162–171.
- Garcia N., Hopkins S.R., and Powell F.L. (2000). Effects of intermittent hypoxia on the isocapnic hypoxic ventilatory response and erythropoiesis in humans. *Respir. Physiol.* 123:39–49.
- Gippenreiter E., and West, J.B. (1996). High altitude medicine and physiology in the former Soviet Union. *Aviat. Space Environ. Med.* 67:576–584.
- Gore C.J., Hahn A.G., Aughey R.J., Martin D.T., Ashenden M.J., Clark S.A., Garnham A.P., Roberts A.D., Slater G.J., and McKenna M.J. (2001). Live high:train low increases muscle buffer capacity and submaximal cycling efficiency. *Acta. Physiol. Scand.* 173:275–286.
- Grassi B., Marzorati M., Kayser B., Bordini M., Colombini A., Conti M., Marconi C., and Cerretelli P. (1996). Peak blood lactate and blood lactate vs. workload during acclimatization to 5,050 m and in deacclimatization. *J. Appl. Physiol.* 80:685–692.
- Green H., Roy B., Grant S., Burnett M., Tupling R., Otto C., Pipe A., and McKenzie D. (2000a). Downregulation in muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ following a 21-day expedition to 6,194 m. *J. Appl. Physiol.* 88:634–640.
- Green H., Roy B., Grant S., Hughson R., Burnett M., Otto C., Pipe A., McKenzie D., and Johnson M. (2000b). Increases in submaximal cycling efficiency mediated by altitude acclimatization. *J. Appl. Physiol.* 89:1189–1197.
- Green H., MacDougall J., Tarnopolsky M., and Melissa N.L. (1999). Downregulation of $\text{Na}^+\text{-K}^+\text{-ATPase}$ pumps in skeletal muscle with training in normobaric hypoxia. *J. Appl. Physiol.* 86:1745–1748.
- Hackett P.H., and Oelz O. (1992). The Lake Louise consensus on the definition and quantification of altitude illness. In: *Hypoxia and Mountain Medicine*. J.R. Sutton, G. Coates, and C.S. Houston (eds.). Queen City Printers, Burlington, VT, pp. 327–330.
- Hahn A.G., and Gore C.J. (2001). The effect of altitude on cycling performance. *Sports Med.* 31:533–557.
- Harms C.A., and Dempsey J.A. (1999). Cardiovascular consequences of exercise hyperpnea. *Exerc. Sports Sci. Rev.* 27:37–62.
- Hellems J. (1999) Intermittent hypoxic training: pilot trial. Proceeding of the Second Annual International Altitude Symposium, Flagstaff, AZ, pp. 145–154.
- Hochachka P. W., Stanley C., Matheson G.O., McKenzie D.C., Allen P.S., and Parkhouse W.S. (1991). Metabolic and work efficiencies during exercise in Andean natives. *J. Appl. Physiol.* 70:1720–1730.
- Jones A.M., and Carter H. (2000). The effect of endurance training on parameters of aerobic fitness. *Sports Med.* 29:373–386.
- Katayama K., Sato Y., Morotome Y., Shima N., Ishida K., Mori S., and Miyamura M. (2001a). Intermittent hypoxia increases ventilation and SaO_2 during hypoxic exercise and hypoxic chemosensitivity. *J. Appl. Physiol.* 90:1431–1440.
- Katayama K., Shima N., Sato Y., Qiu J., Ishida K., Mori S., and Miyamura M. (2001b). Effect of intermittent hypoxia on cardiovascular adaptations and response to progressive hypoxia in humans. *High Alt. Med. Biol.* 2:501–508.
- Levine B.D., and Stray-Gundersen J. (1997). “Living high—training low”: effect of moderate-altitude acclimatization with low-altitude training on performance. *J. Appl. Physiol.* 83:102–112.
- Meeuwse T., Hendriksen I.J.M., and Holewijn M. (2001). Training-induced increases in sea-level performance are enhanced by acute intermittent hypobaric hypoxia. *Eur. J. Appl. Physiol.* 84:283–290.
- Mizuno M., Juel C., Bro-Rasmussen T., Mygind E., Schibye B., Rasmussen B., and Saltin B. (1990). Limb skeletal muscle adaptation in athletes after training at altitude. *J. Appl. Physiol.* 68:496–502.
- Morgan D.W., and Craib M. (1992). Physiological aspects of running economy. *Med. Sci. Sports Exerc.* 24:456–461.
- Piehl-Aulin K., Svedenhag J., Wide L., Berglund B., and Saltin B. (1998). Short-term intermittent normobaric hypoxia—haematological, physiological and mental effects. *Scand. J. Med. Sci. Sports* 8:132–137.
- Powell F.L., and Garcia N. (2000). Physiological effects of intermittent hypoxia. *High Alt. Med. Biol.* 1:125–136.
- Roberts A.C., Butterfield G.E., Cymerman A., Reeves J.T., Wolfel E.E., and Brooks G.A. (1996a). Acclimatization to 4,300-m altitude decreases reliance on fat as a substrate. *J. Appl. Physiol.* 81:1762–1771.
- Roberts A.C., Reeves J.T., Butterfield G.E., Mazzeo R.S., Sutton J.R., Wolfel E.E., and Brooks G.A. (1996b). Altitude and B-blockade augment glucose utilization during submaximal exercise. *J. Appl. Physiol.* 80:605–615.
- Rodriguez F.A., Casas H., Casas M., Pages T., Rama R., Ricart A., Ventura J.L., Ibanez J., and Viscor G. (1999). Intermittent hypobaric hypoxia stimulates erythropoiesis and improves aerobic capacity. *Med. Sci. Sports Exerc.* 31:264–268.
- Rodriguez F.A., Ventura J.L., Casas M., Casas H., Pages T., Rama R., Ricart A., Palacios L., and Viscor G. (2000). Erythropoietin acute reaction and haematological adaptations to short, intermittent hypobaric hypoxia. *Eur. J. Appl. Physiol.* 82:170–177.
- Saltin B., Larsen H., Terrados N., Bangsbo J., Bak T., Kim

- C.K., Svedenhag J., and Rolf C.J. (1995). Aerobic exercise capacity at sea level and at altitude in Kenyan boys, junior and senior runners compared with Scandinavian runners. *Scand. J. Med. Sci. Sports* 5:209–221.
- Schmidt W. (2002). Effects of intermittent exposure to high altitude on blood volume and erythropoietic activity. *High Alt. Med. Biol.* 3:167–176.
- Serebrovskaya T.V. (2002). Intermittent hypoxia research in the former Soviet Union and the Commonwealth of Independent States: history and review of the concept and selected applications. *High Alt. Med. Biol.* 3:205–221.
- Serebrovskaya T.V., Karaban I.N., Kolesnikova E.E., Mishunina T.M., Kuzminskaya L.A., Serebrovsky A.N., and Swanson R.J. (1999). Human hypoxic ventilatory response with blood dopamine content under intermittent hypoxic training. *Can. J. Physiol. Pharmacol.* 77:967–973.
- Stray-Gundersen J., Chapman R.F., and Levine B.D. (2001). "Living high–training low" altitude training improves sea level performance in male and female elite runners. *J. Appl. Physiol.* 91:1113–1120.
- Terrados N., Melichna J., Sylven C., Jansson E., and Kaiser L. (1988). Effects of training at simulated altitude on performance and muscle metabolic capacity in competitive road cyclists. *Eur. J. Appl. Physiol.* 57:203–209.
- Wilber R.L. (2001). Current trends in altitude training. *Sports Med.* 31:249–265.
- Wolfel E.E., Groves B.M., Brooks G.A., Butterfield G.E., Mazzeo R.S., Moore L.G., Sutton J.R., McCullough R.G., Huang S.Y., Sun S.F., Grover R.F., Hultgren H.N., and Reeves J.T. (1991). Oxygen transport during steady-state submaximal exercise in chronic hypoxia. *J. Appl. Physiol.* 70:1129–1136.
- Wolski L.A., McKenzie D.C., and Wenger H.A. (1996). Altitude training for improvements in sea level performance. *Sports Med.* 22:251–263.

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