The effects of nightly normobaric hypoxia and high intensity training under intermittent normobaric hypoxia on running economy and hemoglobin mass

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Neya M, Enoki T, Kumai Y, Sugoh T, Kawahara T. The effects of nightly normobaric hypoxia and high intensity training under intermittent normobaric hypoxia on running economy and hemoglobin mass. J Appl Physiol 103: 828–834, 2007. First published June 7, 2007; doi:10.1152/japplphysiol.00265.2007.—We investigated the effects of nightly intermittent exposure to hypoxia and of training during intermittent hypoxia on both erythropoiesis and running economy (RE), which is indicated by the oxygen cost during running at submaximal speeds. Twenty-five college long- and middle-distance runners (maximal oxygen uptake (VO2max) 60.3 ± 4.7 ml·kg−1·min−1) were randomly assigned to one of three groups: hypoxic residential group (HypR, 11 h/night at 3,000 m simulated altitude), hypoxic training group (HypT), or control group (Con), for an intervention of 29 nights. All subjects trained in Tokyo (altitude of 60 m) but HypT had additional high-intensity treadmill running for 30 min at 3,000 m simulated altitude on 12 days during the night intervention. VO2 was measured at standing rest during four submaximal speeds (12, 14, 16, and 18 km/h) and during a maximal stage to volitional exhaustion on a treadmill. Total hemoglobin mass (THb) was measured by carbon monoxide rebreathing. There were no significant changes in VO2max, THb, and the time to exhaustion in all three groups after the intervention. Nevertheless, HypR showed 5% improvement of RE in normoxia (P < 0.01) after the intervention, reflected by reduced VO2 at 18 km/h and the decreased regression slope fitted to VO2 measured during rest position and the four submaximal speeds (P < 0.05), whereas no significant corresponding changes were found in HypT and Con. We concluded that our dose of intermittent hypoxia (3,000 m for ~11 h/night for 29 nights) was insufficient to enhance erythropoiesis or VO2max, but improved the RE at racetrack speeds of college runners.

running economy; oxygen uptake; intermittent hypoxia

TO ENHANCE AEROBIC PERFORMANCE at sea level, many endurance athletes include altitude or other hypoxic training in their seasonal schedule. The major purpose of altitude training has traditionally been to increase red blood cells, total hemoglobin mass (THb), and subsequent aerobic performance (8, 25). The effect of this training on sea level performance has been documented by many studies, and among these the “living high, training low” (LHTL) approach has also been shown to increase THb and maximal oxygen uptake (VO2max) compared with a control group (24). To overcome the geographical restriction in many countries, which do not have appropriate topography for this approach, normobaric hypoxia has been employed to provide a simulated altitude environment near sea level (22, 32).

LHTL has been reported to provide the athletes with an acclimatization response that includes increases in THb, VO2max, and aerobic performance, while maintaining a similar level of training intensity as at sea level (24, 25, 34, 43). However, some studies using nightly normobaric hypoxia reported no increases in THb and VO2max (1, 11, 41). The extent to which LHTL mediates an increase in red blood cells has recently been the subject of vigorous debate (10, 26), although it has recently been argued that the minimum effective dose of hypoxia to attain a hematological acclimatization effect is >12 h/day for at least 3 wk at an altitude or simulated altitude of 2,100–2,500 m (40). Therefore it is still relevant to further investigate the effects of LHTL on sea level performance in respect of hematological acclimatization.

In addition to a continuous increase in THb, altitude acclimatization affects oxygen delivery and use during submaximal exercise, which may result in the reduction of oxygen uptake of the whole body or working muscles at submaximal intensities (4, 15, 51). The most plausible mechanism that affects lowered oxygen uptake is the transition of fuel supply from fat oxidation toward greater glycolysis because the oxidation of glycerol yields −11% more ATP per mole of O2 compared with the oxidation of fats (15, 16, 41). In favor of these mechanisms, some recent studies documented decreased oxygen consumption at submaximal intensities indicating improvement of athletes’ running economy (RE; 19, 41). The net energy cost at submaximal intensities or RE has been reported as a more predictive parameter of aerobic performance than VO2max (6, 7, 31). Therefore, an improvement of these parameters induced by altitude or hypoxic training would be advantageous for aerobic athletes. However, a number of the studies that demonstrate changes in substrate use after altitude acclimatization have been conducted by using higher altitudes for longer periods than athletes typically adopt (4, 15, 51). Therefore further careful investigation about the efficacy of “living simulated high and training at sea level” in terms of increase in THb and improvement of O2 utilization for the enhancement of athletic performance is warranted.

As an alternative to LHTL, training with intermittent exposure to hypoxia has been investigated for its efficacy on improvement of athletic performance (29, 38, 39, 44). This mode is equivalent to “living low, training high” (LLTH). Brief periods of hypoxia are appealing to athletes whose normal training regimen does not make it practical to spend as long as 12 h/day or more (40) inside a hypoxic chamber to improve their performance. Therefore, training with intermittent exposure to hypoxia may be a viable alternative for athletes seeking to enhance their performance (17, 37), but the evidence for any benefit is inconclusive.

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The purpose of this study was twofold; first to evaluate the effectiveness of LHTL and second to evaluate the performance benefits of training in hypoxia (LLTH). Specifically, we hypothesized that 30 days intermittent nightly exposure to simulated altitude (3,000 m) would increase both THb and improve RE. We also hypothesized that LLTH (for 30 min, 3 times/wk for 4 wk at 3,000 m) would improve athletic performance reflected by improvement of RE without altering THb, since the duration of hypoxia is insufficient to induce a sustained increase in serum erythropoietin even at 5,450 m (21).

**METHODS**

**Subjects.** Twenty-five, male, college long- and middle-distance runners (mean ± SD age 21 ± 2 yr, body mass 58.4 ± 6.0 kg, VO2max 60.3 ± 4.7 ml·min⁻¹·kg⁻¹) participated in this study, which was approved by Japan Institute of Sports Sciences Ethics Committee. The subjects gave their written consent to participate in this study. All the subjects had more than 3 years training history as long- or middle-distance race runners. Their 5,000 m best time before the intervention ranged from 17:52 to 14:57 and the average was 15:57 (min:s).

This cohort was divided into three groups: hypoxic residential group (HypR), hypoxic training group (HypT), and control group (Con). Ten subjects were assigned to HypR and they slept in their own homes in Tokyo. They trained at sea level (altitude) during the intervention. Nine subjects were assigned to HypT (FIO2 0.144) in addition to their ordinary sea level training for the 31-day intervention. Six subjects were assigned to Con, they slept in the accommodation controlled to normobaric normoxia (FIO2 0.144) and they trained in normobaric hypoxia for 12 days of the 31-day intervention. Six subjects were assigned to Con, they slept in their own homes in Tokyo. They trained at sea level (same environment as the HypR group) but had an additional 30 min treadmill running training in normobaric hypoxia for 12 days of the 31-day intervention. Six subjects were assigned to Con, they slept in their own homes in Tokyo and trained at sea level throughout the intervention. The subjects’ sea level training distance, time, and intensity were recorded and controlled to be similar among the three groups, although the hypoxic treadmill training of the HypT group was excluded from this calculation. The physical characteristics and training volume are shown in Table 1.

**Study design.** The study intervention was 31 days (day 0 to day 30). HypR slept at the accommodation controlled to normobaric normoxia on the first night and in normobaric hypoxia for the remaining 29 nights. During the preliminary period, which was set 2 wk before the study intervention, all subjects completed each of the VO2max and THb measurements twice. The THb and VO2max test were conducted once after the intervention. The outline of the study design is shown in Fig. 1.

**Treadmill testing for VO2max and running economy.** VO2 was measured during submaximal and maximal treadmill running. The subjects ran at four submaximal speeds (12, 14, 16, and 18 km/h) each of 4-min duration following 5-min standing resting on the treadmill. Between each submaximal speed, there was a 1-min break to collect a fingertip blood capillary sample for blood lactate concentration (BLa). VO2, minute ventilation (Ve), volume of carbon dioxide produced (VCO2), respiratory exchange ratio (RER), and heart rate (HR) were measured during both submaximal and maximal running. One minute after the fourth submaximal run, the subjects ran to volitional exhaustion commencing at 18 km/h. The speed was increased by 1 km/h each minute up to 20 km/h, and thereafter the treadmill gradient increased by 1% each minute until exhaustion. The time to exhaustion (TTE) during the VO2max stage was used as the marker for performance. HR was measured by telemetry electrocardiograph (WEP-4202, Nihon Kohden) and BLa was measured by Biosen 1,000 (NSI).

**Respiratory gas analysis.** Respiratory gas samples were collected in Douglas bags for 1 min during the last 2 min of each submaximal stage and every 30 or 60 s of the maximal stage. The volume of expired air was measured with a dry gas volume meter (10 liter, custom built, Arco System) after 500 ml of the sample was analyzed for fractions with mass spectroscopy (ARCO-2000, Arco System), which was calibrated by three precision gas mixtures before each test. The custom-designed software was employed to compute VO2, VCO2, Ve, and RER using standard algorithms. The highest 1-min VO2 during the last stage was regarded as VO2max of that test. The higher value of VO2max in two preintervention tests was regarded as preintervention VO2max. The typical error of measurement (TEM) for the two preintervention VO2max tests was 1.4 ml·kg⁻¹·min⁻¹ [95% confidence interval (CI) −2.2 to 1.0 ml·kg⁻¹·min⁻¹] or 2.3% for mean VO2max.

**THb measurement.** Before and after the intervention, THb was measured by carbon monoxide (CO) rebreathing method introduced by Burge and Skiner (5) and modified by Saunders et al. (41). The subjects were dosed with 99.95% CO twice and rebreathed each dose for 10 min each (20 ml for initial dose and 1.50 ml/kg body mass for second dose). Two milliliter blood samples were collected from an antecubital vein to measure the percent of carboxyhemoglobin (%HbCO). The average of %HbCO from eight replicates conducted on each blood sample measured by an ABL OSM3 (Radiometer, Copenhagen, Denmark) for both CO doses was obtained, and the difference of %HbCO between primary and second doses was used to calculate THb (5). The typical error of measurement for duplicate THb measurements conducted during the preliminary period was 0.48 g/kg (95% CI was −1.06 to 0.31 g/kg) or 3.3% of the mean THb. The mean value of THb conducted twice during the preliminary period was regarded as THb value of preintervention.

**Hypoxic environment for HypR and HypT.** In this study, we used the two types of hypoxic environment for the two groups. They were hypoxic accommodation and a training room. The hypoxic environment for both of them was created by filtering compressed air through high-polymer membrane, controlled to yield an FIO2 of 0.144. The volume flow through both the accommodation and training room was sufficient to limit CO2 concentration to below 1,000 ppm. Each HypR subject was accommodated in a single hypoxic room (~50 m³) with bed and bathroom. HypT used the hypoxic training room (~100 m³) equipped with the treadmill for running training.

**Table 1. Subject characteristics, VO2max, THb, and running distance**

<table>
<thead>
<tr>
<th></th>
<th>Con Pre</th>
<th>Post</th>
<th>HypT Pre</th>
<th>Post</th>
<th>HypR Pre</th>
<th>Post</th>
</tr>
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<tr>
<td>Age, yr</td>
<td>20.9 ± 1.5</td>
<td></td>
<td>20.0 ± 2.3</td>
<td></td>
<td>21.1 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>60.0 ± 5.7</td>
<td>60.5 ± 5.3</td>
<td>60.4 ± 4.4</td>
<td>61.1 ± 4.8</td>
<td>55.3 ± 4.0</td>
<td>55.4 ± 4.4</td>
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<tr>
<td>Running distance, km</td>
<td>244.6 ± 82.7</td>
<td></td>
<td>267.8 ± 161.3</td>
<td></td>
<td>248.4 ± 83.6</td>
<td></td>
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</tbody>
</table>

Values are means ± SD. Running distance means total of accumulated running distance during 31-day intervention. THb, total hemoglobin; Con, control; HypT, hypoxic training group; HypR, residential group.
HypT group accumulated a total of 6 h of hypoxia. The hypoxic training sessions were conducted ~3 or 4 days apart at an intensity of 80–90% of maximal heart rate (HRmax) attained during the VO2max test at sea level before the intervention. The subjects started at the speed equivalent to their individual 80%HRmax for the first 10 min and gradually increased the speed during the next 20 min to yield a heart rate of up ~90%HRmax as assessed by telemetry (Heart rate monitor NV, Polar). The standardized warm-up and cool-down before and after each training session was conducted in normoxia.

Statistical analysis. A two-way analysis of variance with repeated measures with groups (HypR, HypT, and Con) and the intervention (pre- and postintervention) was used to test for interaction and main effects. When interactions or main effects reached significance, the Tukey’s post hoc test was used to identify significant differences. All effects were expressed as means ± SD and significance was set at $P < 0.05$. The statistical software package SPSS for Doctors (Nankodo, Japan) was used for all analyses.

RESULTS

Hypoxic exposure. The HypR group accumulated a total of 316 h in hypoxia and their average time of nightly exposure to normobaric hypoxia of HypR was 10:54 ± 0:24 (h:min). The HypT group accumulated a total of 6 h in hypoxia.

VO2max, THb, and time to exhaustion. There were no significant differences among the three groups at baseline in terms of their physical characteristics, running distance, and THb. However, because the Con had a higher absolute body mass, relative values of VO2 and THb were used to evaluate the differences among the groups. There were no significant changes in VO2max and THb within or between groups during the intervention. VO2max tended to decrease in both the Con and HypR groups (by ~5.0 and ~4.2%, respectively), but not in the HypT group (~0.3%). The mean THb remained within 1% of the Pre value for all three groups. The results of THb and VO2max between pre- and postintervention with $F$ and $P$ values were shown in Table 2 and the changes in these variables of each subject are shown in Figs. 2 (relative THb) and 3 (relative VO2max).

TTE was also unchanged between pre- vs. postintervention in all three groups [interaction (groups×time), $F = 1.48$, $P = 0.25$; group main effect, $F = 2.88$, $P = 0.11$] but HypT only showed a tendency to improve TTE after the intervention (pre vs. post in each group, HypT, 220 ± 90 vs. 257 ± 66 s, $P = 0.07$; HypR, 261 ± 49 vs. 279 ± 60 s, $P = 0.17$; Con, 300 ± 37 vs. 294 ± 68 s, $P = 0.78$).

Running economy. At the four submaximal running speeds, VO2 of HypR tended to be reduced at postintervention compared with preintervention values at lower speeds ($P = 0.06$ at 12 km/h and $P = 0.05$ at 14 km/h) and was ~5% reduced at 18 km/h ($P < 0.01$). However, there was no significant change in submaximal VO2 at all four speeds for the HypT and Con groups between pre- and postinterventions (Table 3). The changes of VE, RER, HR, and BLa at submaximal speeds were shown in Table 3. The RER of HypT tended to be higher at postintervention than preintervention ($P < 0.05$ at 12 km/h, $P = 0.06$ at 16 km/h and $P = 0.05$ at 18 km/h). No other significant changes were detected among these variables.

The regression data for the three groups were fitted through the measured VO2 at rest (standing on the treadmill for 5 min) and four running speeds (Fig. 4). There was a significant decrease ($P = 0.04$) in the slopes of VO2 and running speed between pre- and postintervention for HypR, but no significant changes were found in the other two groups ($P = 0.58$ for HypT and $P = 0.64$ for Con).

Table 2. Statistical variables of relative VO2, THb, and TTE

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance</th>
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<tbody>
<tr>
<td>THb, main effect (time)</td>
<td>$F = 0.35$</td>
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<tr>
<td></td>
<td>$P = 0.56$</td>
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<tr>
<td>THb, main effect (group)</td>
<td>$F = 2.49$</td>
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<td></td>
<td>$P = 0.10$</td>
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<tr>
<td>THb, interaction (time×group)</td>
<td>$F = 0.89$</td>
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<tr>
<td></td>
<td>$P = 0.90$</td>
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<tr>
<td>VO2max, main effect (time)</td>
<td>$F = 3.29$</td>
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<tr>
<td></td>
<td>$P = 0.08$</td>
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<tr>
<td>VO2max, main effect (group)</td>
<td>$F = 0.42$</td>
</tr>
<tr>
<td></td>
<td>$P = 0.77$</td>
</tr>
<tr>
<td>VO2max, interaction (time×group)</td>
<td>$F = 0.78$</td>
</tr>
<tr>
<td></td>
<td>$P = 0.47$</td>
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DISCUSSION

The main finding of this study was that 29 nights of sleeping in normobaric hypoxia (~11 h/night at 3,000 m) in long- and middle-distance college runners (HypR) did not increase THb or \( \dot{V}O_2 \)max. Our results contrast with those of Levine and Stray-Gundersen (23) in which the subjects slept and lived at 2,500 m altitude for 28 days (20–22 h/day) and the LHTL group increased red blood cell volume (by 5.3%) and \( \dot{V}O_2 \)max (3.9%). On the other hand, Ashenden et al. (1) had subjects spend 23 nights at 3,000 m (~9 h/night) and observed no change in their THb (1.8% decrease) compared with a control group (0.9% decrease). Other studies using LHTL also reported no change in THb (11, 41). It appears that our study did not have a sufficient hypoxic dose (number of hours per day or a sufficient number of days) to increase THb, because the altitude (3,000 m) is well above that required to stimulate increased erythropoiesis (49) and was 500 m above that used by Levine et al. (23).

The other major finding of this study was that this nightly intermittent exposure to hypoxia improved running economy, which was reflected at reduced whole body submaximal \( \dot{V}O_2 \) and a reduced slope between \( \dot{V}O_2 \) vs. running speed on a treadmill. In particular, HypR showed a significant reduction in submaximal \( \dot{V}O_2 \) at 18 km/h, which is close to race speed of college athletes for a 5,000 m race. Therefore the improvement of RE at 18 km/h was practically worthwhile for these subjects.

![Fig. 2. Changes in relative THb between pre- and postintervention. Individual dashed lines, results of each subject; thick line, means ± SD.](image)

![Fig. 3. Changes in relative maximal oxygen uptake (\( \dot{V}O_2 \)max) between pre- and postintervention. Individual dashed lines, results of each subject; thick line, means ± SD.](image)
**Improved RE in HypR.** The net exercise cost at submaximal workloads has been reported to be more related to endurance athletic performance than \( V^{\text{O}_2}\text{max} \) (6, 7, 31) in a range of locomotions, including swimming (45), cross-country skiing (30), and running (41). Our results showed that \( V^{\text{O}_2} \) during 5-min standing rest was not changed between pre- and postintervention in all three groups. However, the slope between \( V^{\text{O}_2} \) and running speed was reduced only in HypR, which suggests that the LHTL intervention caused the decrease in the net energy requirement during the submaximal exercise. Submaximal \( V^{\text{O}_2} \) is a function of both central factors (such as cardiac output and oxygen carrying capacity) and peripheral factors (such as oxygen use by the working muscle; Refs. 11, 41). The unchanged HR and THb in this study indicate that this LHTL intervention method did not affect “the central” factors. By implication, the reduced slope between \( V^{\text{O}_2} \) vs. running speed postintervention may be due to the change of “peripheral” function of working muscle (15). Our results contribute to the growing body of evidence that natural or simulated altitude reduces \( V^{\text{O}_2} \) in normoxia at submaximal intensities (11, 15, 16, 18–20, 28, 41, 42). In comparison, a large number of studies also reported that submaximal \( V^{\text{O}_2} \) after altitude is unchanged (12, 23, 27, 33, 50). The reasons for the discrepancy between investigators are unclear, but great care was taken with our measurement of \( V^{\text{O}_2} \) as illustrated by our low typical error for submaximal (TEM = 1.0 ml-kg\(^{-1}\)-min\(^{-1}\) with 95%CI; −0.8 to 1.4 ml-kg\(^{-1}\)-min\(^{-1}\) and maximal (TEM = 1.4 ml-kg\(^{-1}\)-min\(^{-1}\) with 95%CI; −2.2 to 1.0 ml-kg\(^{-1}\)-min\(^{-1}\) \( V^{\text{O}_2} \).

**The mechanisms of improved RE.** With regards to mechanisms of reduced submaximal \( V^{\text{O}_2} \) in HypR, a decreased cost of \( V^{\text{E}} \) (15) and greater dependence on carbohydrate use to generate ATP after acclimatization to hypoxia are major candidates to explain improved RE (3, 11, 15). In our results, the former mechanism can be excluded due to no change in submaximal \( V^{\text{E}} \) (nor HR). On the other hand, numerous studies (3, 11, 15, 35, 36) reported that hypoxic acclimatization increases dependence on glucose metabolism instead of fatty acids to generate ATP, where the former are 10% energetically more efficient. However, our results did not show a significant increase in RER after LHTL, which has been reported previously by Gore et al. (11).

In addition to these possibilities to explain improved RE, reduced ATP consumption needs at muscle level after altitude exposure have been reported (13, 14). This change was typically indicated by downregulation of muscle Na\(^{+}\)-K\(^{+}\)-ATPase content. Aughey et al. (2) concluded that 23 nights intermittent exposure to 3,000 m simulated altitude was not sufficient for downregulation of muscle Na\(^{+}\)-K\(^{+}\)-ATPase to influence muscle performance. Our magnitude of hypoxia and duration were very similar to their study and therefore we can speculate that downregulation of muscle Na\(^{+}\)-K\(^{+}\)-ATPase content was unlikely to have influenced the improved RE that we measured. But it is possible that hypoxic exposure resulted in tighter coupling of muscular intracellular bioenergetics, which improved mitochondrial efficiency.

**No effects of high-intensity training in hypoxia on performance of HypT.** Controlled investigations have not been able to elucidate conclusive evidence about intermittent hypoxic training (IHT) with regard to the improvement of athletic performance (9, 39, 46–48), although pooling the results is problematic because there has been a very wide range of duration, frequency, intensity of the training, as well as the level of hypoxia and training models. The study of Dufour et al. (9) is closest to our study with respect of the level of hypoxia, training model, and intensity. Their subjects were distance runners and trained on treadmill at ~80% of \( V^{\text{O}_2}\text{max} \) of normoxia for ~40 min a session and 2 days/wk for 6 wk in normobaric hypoxia simulated to 3,000 m altitude. In contrast to our results, they reported increased \( V^{\text{O}_2}\text{max} \) and increased time to exhaustion during the incremental maximal test. Other
at sea level with or without 60 min of intermittent exposure to hypoxia (3,000 m) had no significant effects on THb, VO2max, or RE. Overall, our chosen dose of LHTL improved RE at race running speed for college level long- and middle-distance runners, but whether international caliber runners also can acquire improved RE at race pace after LHTL requires further research.

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