

## Intermittent normobaric hypoxia does not alter performance or erythropoietic markers in highly trained distance runners

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Submitted 8 September 2003; accepted in final form 10 December 2003

**Julian, Colleen G., Christopher J. Gore, Randall L. Wilber, Jack T. Daniels, Michael Fredericson, James Stray-Gundersen, Allan G. Hahn, Robin Parisotto, and Benjamin D. Levine.** Intermittent normobaric hypoxia does not alter performance or erythropoietic markers in highly trained distance runners. *J Appl Physiol* 96: 1800–1807, 2004. First published December 12, 2003; 10.1152/jappphysiol.00969.2003.—This study was designed to test the hypothesis that intermittent normobaric hypoxia at rest is a sufficient stimulus to elicit changes in physiological measures associated with improved performance in highly trained distance runners. Fourteen national-class distance runners completed a 4-wk regimen (5:5-min hypoxia-to-normoxia ratio for 70 min, 5 times/wk) of intermittent normobaric hypoxia (Hyp) or placebo control (Norm) at rest. The experimental group was exposed to a graded decline in fraction of inspired O<sub>2</sub>: 0.12 (*week 1*), 0.11 (*week 2*), and 0.10 (*weeks 3 and 4*). The placebo control group was exposed to the same temporal regimen but breathed fraction of inspired O<sub>2</sub> of 0.209 for the entire 4 wk. Subjects were matched for training history, gender, and baseline measures of maximal O<sub>2</sub> uptake and 3,000-m time-trial performance in a randomized, balanced, double-blind design. These parameters, along with submaximal treadmill performance (economy, heart rate, lactate, and ventilation), were measured in duplicate before, as well as 1 and 3 wk after, the intervention. Hematologic indexes, including serum concentrations of erythropoietin and soluble transferrin receptor and reticulocyte parameters (flow cytometry), were measured twice before the intervention, on *days 1, 5, 10, and 19* of the intervention, and 10 and 25 days after the intervention. There were no significant differences in maximal O<sub>2</sub> uptake, 3,000-m time-trial performance, erythropoietin, soluble transferrin receptor, or reticulocyte parameters between groups at any time. Four weeks of a 5:5-min normobaric hypoxia exposure at rest for 70 min, 5 days/wk, is not a sufficient stimulus to elicit improved performance or change the normal level of erythropoiesis in highly trained runners.

altitude; athletes; exercise; intermittent hypoxic training; erythropoietin

ENDURANCE ATHLETES OFTEN INCORPORATE altitude training into their training regimens with the expectation that sea-level performance may be improved (8). This practice has led to the proliferation of various terrestrial and simulated methods of altitude exposure, although the efficacy of these protocols for improved performance remains controversial. Historically, studies examining the benefits of altitude training suggest that the combination of living and training at altitude enhances performance at sea level (5, 9). However, when these studies

have been replicated with adequate control groups and comprehensive performance markers throughout the protocol, no benefits were seen beyond those found with sea-level training alone (2, 45). These methods are problematic: although O<sub>2</sub> delivery and utilization may be improved, a lack of proper training adaptations and/or decreased exercise intensity due to hypoxia can lead to a relative detraining effect in some athletes (24). These deficits in training ability/intensity at altitude may mask any performance advantage gained through altitude-induced improvements in O<sub>2</sub> delivery or utilization.

It has been suggested that the ideal approach to altitude training would enable athletes to optimize the stimuli necessary to achieve central and peripheral changes that improve O<sub>2</sub> delivery and utilization while avoiding the detraining effects associated with chronic hypobaric hypoxia (24). This strategy, the “living high-training low” or “Hi-Lo” model, combines living and maintenance training at high altitude (2,500–2,700 m) with intense training sessions at a lower altitude (1,200 m). Significant alterations in red cell volume or soluble transferrin receptor (sTfR; documenting augmented erythropoiesis), Hb concentration ([Hb]), maximum O<sub>2</sub> consumption ( $\dot{V}O_{2\max}$ ), and 3,000- to 5,000-m running performance at sea level have been shown to occur in athletes ranging from competitive collegians and postcollegians (23) to national- and Olympic-caliber athletes (41) using this approach involving 20–22 h of hypobaric hypoxia per day.

Although the Hi-Lo method of altitude training has been demonstrated to be effective (23, 41), logistically and financially it may be difficult to accomplish for many athletes. Furthermore, at 2,200–2,800 m, the degree of erythropoietic response is highly variable (31). Individually tailored altitudes would be one approach to overcome this limitation, but the higher the altitude, the less practical it is to have a training camp and the less likely it is to have easy access to low-altitude areas conducive to high-intensity training. However, one convenient approach to simulated altitude developed in the former Soviet Union more than 60 years ago (38) is breathing a normobaric hypoxic gas (equivalent to ~5,000 m) at rest for ~70 min. This so-called “intermittent hypoxic training” (IHT) comprises 5 min of hypoxia interspersed with 5 min of normoxia and has been proposed as a strategy that would allow a large number of athletes to experience simulated high altitude and individualize exposure with minimal inconvenience.

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Table 1. *Subject characteristics*

	Group	
	Hyp	Norm
Age, yr	25.3 ± 3.9	24.8 ± 2.7
Weight, kg	68.6 ± 3.9	69.6 ± 3.8
$\dot{V}O_{2\max}$ , ml/min	4,875 ± 279	5,050 ± 238
3,000 <sub>TT</sub> , s	522.9 ± 9.8	527.2 ± 14.2
HR <sub>max</sub> , beats/min	188 ± 5	191 ± 8

Values are means ± SD;  $n = 7$ . Hyp, intermittent hypoxia; Norm, placebo normoxia;  $\dot{V}O_{2\max}$ , maximum O<sub>2</sub> consumption; 3,000<sub>TT</sub>, 3,000-m time-trial performance (best of duplicate trials); HR<sub>max</sub>, maximum heart rate (highest of duplicate baseline trials).

This investigation tested the hypothesis that short, repeated bursts of normobaric hypoxia at rest provide a sufficient stimulus to accelerate erythropoiesis, increase  $\dot{V}O_{2\max}$ , and improve sea-level performance in competitive endurance athletes.

## METHODS

### Subjects

Highly trained male ( $n = 14$ ) and female ( $n = 3$ ) distance runners aged 19–34 yr were recruited from collegiate and postcollegiate running teams in the Palo Alto, CA, area (25 m above sea level). All 17 athletes completed the hematologic portion of the protocol (see *Study Design*), and 15 athletes (14 men and 1 woman) completed the performance measures. Subject characteristics are presented in Table 1. The athletes were competitive in distances from 1,500 m to the marathon, with current 5,000-m or equivalent personal-best times of <14:50 (min:s) for men and <16:50 (min:s) for women. Nine of these athletes competed in the US Track and Field National Championships and/or the US Olympic Trials. Six other athletes competed in the National Collegiate Athletic Association Championships, and one athlete was one of the top California state high school athletes and was heavily recruited to the Stanford University track team. Thus these athletes were highly accomplished and competitive on a US national level. The athletes were on similar training schedules and trained together as a squad throughout the study period. In the 3 mo before and during the period of the study, all athletes were training at their typical volume and intensity and continued a similar training schedule throughout the intervention. Training records before the onset of the protocol were based on athlete report. Training was more closely

evaluated from the beginning of the baseline measures to the completion of the study. Training “scores” were based on an estimated fraction of O<sub>2</sub> uptake ( $F\dot{V}O_2$ ) associated with various intensities of running and duration and were developed to estimate the overall stress of workouts as popularized by one of the investigators (J. T. Daniels): minutes ×  $F\dot{V}O_2$ . Subjects were asked to divide their training records into five categories: 1) easy jogging, 2) moderately paced runs approximating marathon pace, 3) threshold/tempo runs, 4) intervals, and 5) “anaerobic” repetitions (e.g., 200-m all-out effort). Each category was assigned an estimated  $F\dot{V}O_2$  according to previously collected  $\dot{V}O_2$  data. For example, a workout consisting of 10 min of warm-up, 30 min of tempo run, and 10 min of cooldown would be calculated as follows: (20 min × 0.70) + (30 min × 0.85) = 39.5. Weekly schedules consisted of two interval sessions, one tempo/threshold session, and one long run. The remainder of the week consisted of easy recovery runs. The athletes were sea-level residents and had not been at altitude >1,500 m for >1 wk during the previous 6 mo. No athlete had exercise-induced asthma or was on medications for reactive airway disease. Voluntary written informed consent approved by the Stanford Institutional Review Board was signed by each athlete before the commencement of the study.

### Study Design

The study was conducted using a matched-pairs, randomized and double-blind design. Subjects were matched by  $\dot{V}O_{2\max}$ , 3,000-m run time-trial (3,000<sub>TT</sub>) performance, gender, and training history and subsequently assigned to the intermittent hypoxic (Hyp) or the placebo normoxic (Norm) group. On the basis of the standard deviation of these athletes for 3,000<sub>TT</sub>, the subject numbers were adequate to detect a 2% (10.6-s) change in performance [approximately twice the typical error (TE) of measurement in this population] with power = 0.85 at an alpha significance of 0.05. The study was divided into three primary segments: baseline, experimental, and recovery (Fig. 1).

The baseline segment served as a familiarization period to minimize learning effects, to introduce subjects to laboratory and field testing, and to establish TE (15) of the measurements. The TE of duplicate baseline measurements were 1.2 and 1.1% for 3,000<sub>TT</sub> performance and  $\dot{V}O_{2\max}$ , respectively. All subjects took an oral iron supplement beginning 2 wk before hypoxic exposure at an individualized dosing schedule (50–450 mg/day of elemental iron based on serum ferritin) to ensure adequate iron stores (40). Doses were based on baseline ferritin measures. Iron supplementation was undertaken by placebo and experimental subjects throughout the study. Duplicate, baseline measurements taken during this phase included [Hb], hematocrit (Hct), percent reticulocytes, and mean cell Hb content of reticu-

Week	Exposure	Measurements
1	<b>Preparation</b>	Hct, Hb, Ret, EPO, sTfr, MCHCr, RetHb, Ferritin, 3000 <sub>TT</sub> , PRF,
2		
3		Hct, Hb, Ret, EPO, sTfr, MCHCr, RetHb, 3000 <sub>TT</sub> , PRF
4 (FIO <sub>2</sub> = 0.12)	<b>Experimental</b> <i>IHT or normoxia</i>	Hct, Hb, Ret, EPO, sTfr, MCHCr, RetHb (days 1, 2, 12 of the experimental protocol)
5 (FIO <sub>2</sub> = 0.11)		
6 (FIO <sub>2</sub> = 0.10)		
7 (FIO <sub>2</sub> = 0.10)		
8		Hct, Hb, Ret, EPO, sTfr, MCHCr, RetHb, 3000 <sub>TT</sub> , PRF
9	<b>Recovery</b>	Hct, Hb, Ret, EPO, sTfr, MCHCr, RetHb (10 days post)
10		
11		Hct, Hb, Ret, EPO, sTfr, MCHCr, RetHb, 3000 <sub>TT</sub> , PRF (26 days post)

Fig. 1. Study design. FIO<sub>2</sub>, fraction of inspired O<sub>2</sub>; Ret, percent reticulocytes; EPO, serum erythropoietin concentration; sTfr, soluble transferrin receptor concentration; MCHCr, mean cell Hb content of reticulocytes; RetHb, reticulocyte Hb; 3,000<sub>TT</sub>, 3,000-m time-trial performance; PRF, performance testing (submaximal and maximal treadmill tests). Blood measurement days are shown in parentheses.

locytes (MCHCr), as well as serum concentration of erythropoietin (EPO) and sTfr. The TE for these variables were 4.0, 4.4, 19.2, 2.4, 21.6, and 5.4%, respectively.

The experimental segment consisted of 4 wk of IHT. Subjects came to the laboratory each day for an IHT session administered by a technician who was not part of the experimental team. The Hyp subjects intermittently breathed hypoxic air delivered through a handheld mask [GO2Altitude Hypoxic Training System (Hypoxicator), Biomedtech] for 70 min five times per week; the Norm subjects breathed normoxic air through the same mask in a randomized, double-blind design. IHT was administered in a ratio of 5:5 (minutes of hypoxic air to minutes of ambient air), and the sessions took place at least 1–2 h before or after exercise training. The temporal and quantitative details of this IHT protocol represent the standard intermittent hypoxic treatment suggested for highly trained athletes according to the manufacturer's instructions and is the protocol most frequently used by competitive athletes to improve performance. The rationale for this protocol is purported to be to provide a hypoxic stimulus severe enough to induce acclimatization, as well as a reperfusion stimulus to induce "oxidative stress." The progressive decrease in fraction of inspired O<sub>2</sub> (F<sub>I</sub>O<sub>2</sub>) over the 4 wk of the study is to provide a maximal tolerable hypoxic stress by the end of the 4-wk session but allow progressive acclimatization to minimize symptoms and improve tolerance. O<sub>2</sub> saturation was measured within the last 5-min "hypoxic" bout using a standard fingertip pulse oximetry clip supplied with the Hypoxicator. The entire protocol is summarized in Fig. 1.

All participants kept diaries of their daily routines, including training volume, duration, and intensity, using a standard form (5). The recovery period required athletes to continue their training routines without an IHT or a normoxia session. After the experimental phase, all subjects performed duplicate performance and hematology tests 1 and 3 wk after the last IHT or placebo session.

#### Laboratory and Performance Evaluation

**Blood parameters.** The measured blood parameters included [Hb], Hct, percent reticulocytes, (reticulocyte number × MCHCr) (RetHb), EPO, and sTfr; [Hb], Hct, reticulocyte parameters, and MCHCr were measured using an Advia 120 flow cytometer (Bayer Diagnostics, Tarrytown, NY). The EPO and sTfr concentrations were determined using an automated solid-phase chemiluminescent immunoassay (Diagnostic Products, Los Angeles, CA) and an automated immunonephelometric assay (Dade Behring), respectively, as reported previously (39). Measurements were taken between 8 and 10 AM twice before the onset of IHT or normoxic exposure, after days 1, 2, and 12 of "exposure," within 48 h of the last exposure, and 10 and 26 days after exposure. Ferritin measurements were determined using a photometric assay (Hitachi 911 Biochemistry Analyzer, Roche Diagnostics, Rotkreuz, Switzerland). Periodization of testing is shown in Fig. 1.

**Time-trial and laboratory treadmill performance evaluation.** The 3,000<sub>TT</sub> was conducted in Stanford, CA, on the same 400-m outdoor track at 9–10 AM under similar weather conditions (16–18°C, wind 0–4 mph, humidity 75–90%). The first 1,200 m of each

time trial was controlled using a pacesetter athlete, who was not part of the study, to better simulate a race situation and to minimize the influence of tactical racing strategies. Laboratory measures were performed at the Sports Medicine Institute International. After a brief warm-up period, subjects ran three separate stages on a treadmill (Fitnax, Custom 36-in. Research Treadmill 2001, Dallas, TX). Two of these stages were 5-min submaximal runs at 0% grade (men at 270 and 320 m/min and women at 230 and 270 m/min). Submaximal economy measures were calculated as  $\dot{V}O_2 \times$  velocity per 1,000 m. The third stage was a progressive  $\dot{V}O_{2\max}$  test. The  $\dot{V}O_{2\max}$  test consisted of 1 min at 0% grade at the second submaximal speed; thereafter, the grade was increased 1%/min until volitional exhaustion. A 2-min rest period was included between each of the three stages. Submaximal and maximal  $\dot{V}O_2$  were measured breath-by-breath and then averaged over 15-s intervals (TrueMax 2400, Parvomedics, Salt Lake City, UT), with the highest four consecutive 15-s values averaged to attain a minute value representative of the exercise bout. Lactate concentrations were determined immediately after the conclusion of each submaximal stage and 2 min after  $\dot{V}O_{2\max}$  via fingertip capillary tube collection and analysis (Sport 2300, Yellow Springs Instruments, Yellow Springs, OH). Heart rate was monitored throughout the protocol (Polar, Advantage, Port Washington, NY).

#### Statistical Analyses

A Kolmogorov-Smirnov test was first performed to ensure that the data satisfied assumptions consistent with a normal distribution. Subsequently, two-factor, repeated-measures ANOVA was used for each of the performance, laboratory, and hematologic measures to compare between groups (IHT vs. Norm) and time (before, during, and after intervention). In the event of significant main effects, Tukey-Kramer multiple-comparison post hoc tests were applied to find the source of difference. Two female placebo subjects were unable to complete the protocol because of injury: one had to abandon the study shortly after commencing, and the other was injured toward the end of the exposure but completed the hematologic measures. As a result, women were excluded from analysis of the laboratory and performance measures to maintain a balanced design. Women were included in hematologic analyses. Significance values were set at  $P < 0.05$ . Values are means  $\pm$  SD.

## RESULTS

### Hypoxic Exposure

O<sub>2</sub> saturation levels for the 4 wk of exposure were 89.9  $\pm$  4.2 and 96.8  $\pm$  2.0% (week 1), 86.3  $\pm$  5.2 and 97.4  $\pm$  1.3% (week 2), 85.9  $\pm$  3.6 and 97.0  $\pm$  1.3% (week 3), and 81.4  $\pm$  4.4 and 97.4  $\pm$  0.7% (week 4) for the Hyp and Norm groups, respectively.

### Training

Training scores were evaluated using a combination of training intensity and duration to describe the overall "stress"

Table 2. Submaximal (270 m/min) performance characteristics

	$\dot{V}O_2$ , ml/min		Lactate, mM		$\dot{V}E$ , l/min		Economy		HR, beats/min	
	Hyp	Norm	Hyp	Norm	Hyp	Norm	HYP	NORM	Hyp	Norm
Baseline	3,363 $\pm$ 200	3,545 $\pm$ 266	1.99 $\pm$ 0.99	1.55 $\pm$ 0.90	77.2 $\pm$ 11.0	79.5 $\pm$ 8.3	180.9 $\pm$ 11.6	184.9 $\pm$ 4.6	152 $\pm$ 6	155 $\pm$ 8
IHT	3,454 $\pm$ 209	3,570 $\pm$ 323	1.48 $\pm$ 0.45	2.33 $\pm$ 0.95	76.6 $\pm$ 7.7	79.9 $\pm$ 10.5	187.6 $\pm$ 6.9	189.6 $\pm$ 8.1	152 $\pm$ 5	157 $\pm$ 10
Post	3,524 $\pm$ 272	3,643 $\pm$ 256	1.35 $\pm$ 0.50	1.85 $\pm$ 0.54	77.3 $\pm$ 11.9	80.5 $\pm$ 9.5	190 $\pm$ 15.0	194.3 $\pm$ 5.8	151 $\pm$ 15	155 $\pm$ 11

Values are means  $\pm$  SD;  $n = 7$ . Economy is measured as  $\dot{V}O_2$  (ml $\cdot$ kg<sup>-1</sup> $\cdot$ min<sup>-1</sup>) per m/min.  $\dot{V}O_2$ , O<sub>2</sub> consumption;  $\dot{V}E$ , minute ventilation; HR, heart rate; baseline, average of baseline measurements; IHT, intermittent hypoxia training measurements within 48-h of protocol completion; Post, 3 wk after IHT; economy,  $\dot{V}O_2$  per meter.

Table 3. Maximal performance characteristics

	$\dot{V}O_{2\max}$				Max Lactate, mM		$\dot{V}_{\max}$ , l/min		HR <sub>max</sub> , beats/min		3,000TT, s	
	ml·kg <sup>-1</sup> ·min <sup>-1</sup>		ml/min		Hyp	Norm	Hyp	Norm	Hyp	Norm	Hyp	Norm
	Hyp	Norm	Hyp	Norm								
Baseline	71.2±4.5	71.9±2.2	4,875±279	5,047±234	10.23±3.73	9.94±2.40	141.4±17.4	147.5±16.0	188±5	191±8	522.8±9.7	525.0±11.3
IHT	71.9±3.9	75.8±3.0	4,917±296	5,293±389	7.58±3.31	10.13±1.98	138.5±13.3	154.8±11.5*	183±7	190±8	526.6±15.5	524.7±13.5
Post	71.6±3.8	76.1±4.4*	4,914±315	5,223±527	8.34±3.09	9.74±1.66	140.8±12.3	153.5±16.8	184±6	188±7	520.6±15.8	520.9±18.7

Values are means ± SD; *n* = 7. Baseline, best or highest of 2 preexposure tests;  $\dot{V}_{E\max}$ , maximum minute ventilation. \**P* < 0.05.

of a work bout. The group mean training scores for the Hyp and Norm groups given as a weekly average were  $118 \pm 20$  and  $122 \pm 32$ , respectively. The technique used for calculating scores is given in detail in METHODS. There was no statistically significant difference between training groups.

### Performance Measures

No significant differences were found between groups in any parameter (Tables 2 and 3). Neither group improved 3,000TT performance when the best baseline performance and the time trial immediately or within 48 h after the experimental phase were compared, although both groups improved their time-trial performance from the first to the second baseline test, presumably as a result of practice and familiarization with this specific time-trial format (Fig. 2). There were also no significant differences between Hyp and Norm groups at any time before, during, or after the intervention for  $\dot{V}O_{2\max}$  or heart rate. Within the Norm group, however,  $\dot{V}O_{2\max}$  was significantly greater (*P* < 0.05) at the 3-wk postexperimental segment of the protocol than at baseline (Fig. 3). Maximal minute ventilation ( $\dot{V}_E$ ) within the Norm group was also significantly greater immediately after the experimental protocol (*P* < 0.05) than at baseline. Submaximal  $\dot{V}O_2$ ,  $\dot{V}_E$ , economy, and heart rate were also unchanged from baseline between the Hyp and Norm groups. Lactate measurements during the faster of the two submaximal speeds within the Hyp group were significantly lower in the final test than at baseline. However, 320 m/min appeared to represent a nonsubmaximal effort (lactate >7.0) in several subjects. Therefore, the decision was made to include only 290 m/min as a measure of submaximal effort. There was no significant difference between groups at any time in this parameter.

**Hematology data.** There were no significant differences between the Hyp and Norm groups for any hematologic

parameters (Fig. 4). EPO in both groups significantly decreased (*P* < 0.01, *F* ratio = 20.42), although there was no difference between groups in this response. Within the Hyp group, each of the postintervention EPO measurements was significantly less than at baseline (*P* < 0.05,  $17.4 \pm 6.8$  vs.  $11.0 \pm 5.3$ ,  $10.8 \pm 4.7$ , and  $9.6 \pm 3.4$  U/l, respectively). The case was similar in the Norm group, where baseline values were significantly greater than values immediately after intervention and 10 days after intervention (*P* < 0.05,  $12.7 \pm 3.3$  vs.  $9.5 \pm 2.9$  and  $9.1 \pm 8.9$  U/l, respectively). Hb measures within the Hyp group were significantly greater 26 days after intervention than after *day 1* of exposure (*P* < 0.05,  $15.3 \pm 0.7$  vs.  $14.7 \pm 0.8$  g/dl); however, neither of these was significantly different from baseline. Within the Norm group, Hb measures were greater at baseline ( $15.7 \pm 0.9$ ) than at *day 1* ( $14.8 \pm 1.3$ ) during the protocol and immediately ( $14.7 \pm 1.1$ ) and 10 days ( $14.7 \pm 1.2$ ) after intervention (*P* < 0.05). In both groups, percent reticulocytes was greater at *day 12* during intervention than 10 days after intervention [*P* < 0.05,  $1.9 \pm 0.5$  vs.  $1.3 \pm 0.2\%$  (Hyp) and  $1.7 \pm 0.3$  vs.  $1.3 \pm 0.2\%$  (Norm)]. Hct within the Norm group at baseline was significantly different from that after *day 1* of intervention and immediately after intervention (*P* < 0.05,  $48.1 \pm 2.9\%$  vs.  $43.8 \pm 4.3$  and  $45.6 \pm 3.7\%$ ). These observations stress the importance of the inclusion of a placebo-controlled design.

### DISCUSSION

This investigation demonstrates that in highly trained distance runners this regimen of IHT (5:5 min hypoxia-to-normoxia ratio of intermittent normobaric hypoxia for 70 min, 5 times/wk for 4 wk) does not alter sea-level 3,000TT performance or  $\dot{V}O_{2\max}$ , nor does it change their hematologic indexes of erythropoiesis. This observation is rein-

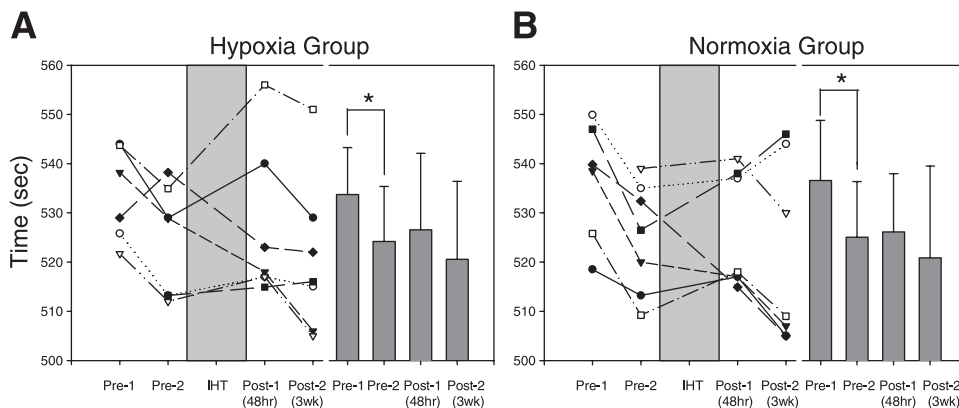


Fig. 2. Individual and group mean ± SD values for 3,000-m time-trial performance including 2 tests (Pre-1 and Pre-2) before intermittent hypoxic training (IHT) and 2 tests after exposure [Post-1(48 h) and Post-2(3 wk)]. \**P* < 0.05. There were no differences between groups.

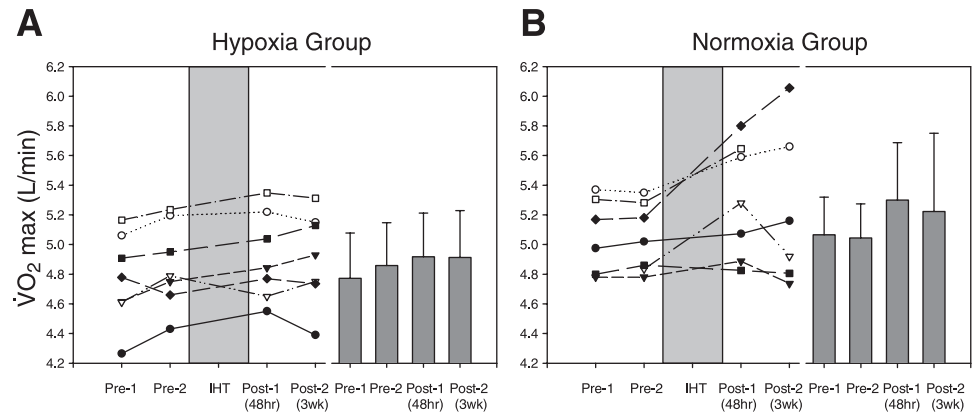


Fig. 3. Individual and group mean  $\pm$  SD values for maximal  $O_2$  uptake ( $\dot{V}O_{2\text{max}}$ ) including 2 tests (Pre-1 and Pre-2) before IHT and 2 tests after exposure [Post-1(48 h) and Post-2(3 wk)]. \* $P < 0.05$ . There were no differences between groups.

forced by the use of well-trained national-caliber athletes in a balanced, matched-pairs, double-blind placebo-controlled design.

#### *Brief Exposures to Normobaric Hypoxia as a Stimulus for Altered Hematology*

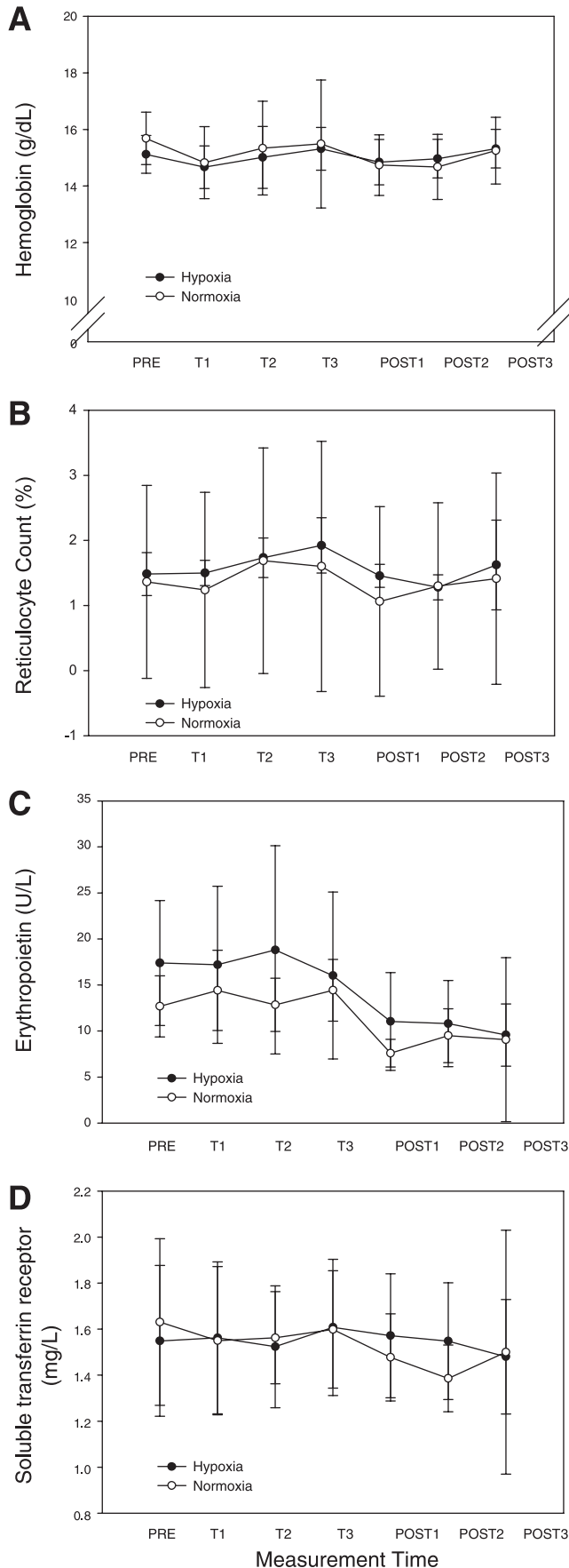
The theory behind intermittent normobaric hypoxia is that, by decreasing the  $O_2$  concentration of inspired air and, thus, decreasing  $O_2$  saturation levels, an increase in the production of EPO and, consequently, red cell mass will be elicited (22, 38). The most salient of the Western studies has demonstrated that at least 120 min of continuous hypoxia or 240 min of intermittent hypoxia at  $F_{I,O_2}$  of 0.105 (21) is required to increase EPO concentration. Previous studies support and refute the assertion that intermittent hypoxic exposure at rest or during exercise is sufficient to produce changes in hematology associated with increased  $O_2$ -carrying capacity, although most of these have used much longer (3–5 h/day) exposures to hypoxia. For example, using intermittent hypoxic exposure techniques, some investigators have reported increases in Hct and [Hb] (33); however, measures of true accelerated erythropoiesis have not been shown. Several studies have shown increases in EPO and reticulocyte counts after 10 days of consecutive 12 h/day exposures to 2,000 or 2,700 m of simulated altitude (25), as well as a combination of 17 days of 3–5 h/day at 4,000–5,500 m and low-intensity exercise (6). Several other investigations (1, 10) indicate that short bursts of 84–114 min (4,000 and 3,000 m, respectively) of hypobaric hypoxia elicit an increase in EPO that reaches a maximum 2–3 h after exposure. It is important to emphasize that all our EPO measurements were performed at the same time of day, in the morning, to minimize any circadian variability in EPO concentrations and to parallel previous studies performed with real altitude exposure. However, this strategy could have prevented the detection of a rise in EPO within the first few hours after the IHT exposure that had returned to baseline by the next morning.

Additional studies suggest that exposure to 5,000 m for 3 h/day for 9 days was sufficient to significantly increase red blood cell count, reticulocyte level, and [Hb] (32). Subsequent studies by the same group of investigators support similar findings with longer-term exposure (33). When the number of acute exposures increased to three for 90 min/wk for 3 wk, red blood cell count, reticulocyte level, and [Hb] increased progressively over time. Peak values were seen at the end of the protocol and/or during the subsequent 2 wk. These results (13,

25, 32, 33) should be interpreted with some caution, however, because several of these studies did not have a control group (13, 32, 33), and it has been demonstrated that training alone modulates reticulocyte levels (34). Moreover, other investigators have emphasized that subacute (1–2 wk) exposure to hypobaric hypoxia may increase reticulocyte counts by increasing the release of immature red blood cells from the bone marrow, without a true acceleration of erythropoiesis (14). Our tightly controlled protocol was unable to produce such effects, despite decreased  $O_2$  saturation levels as low as 82% for the Hyp subjects during hypoxic exposure. Even sTfr was unaltered, and this is considered a prime and sensitive indicator of the erythroid mass. Our results, therefore, dispute the fundamental theory that erythropoiesis results from IHT regimens that combine 5 min of intermittent hypoxia with 5 min of normoxia for 70 min.

#### *Brief Exposures to Normobaric Hypoxia as a Stimulus for Improved Performance*

The primary goal of endurance altitude training for athletes is to enhance performance. Previous studies provide conflicting reports with regard to the assertion that intermittent hypoxic exposure at rest or during exercise improves performance capacity. For example, Casa $\acute{a}$ s and colleagues (6) observed significant improvements in  $\dot{V}O_2$ , pulmonary ventilation, anaerobic threshold, and hematologic measures in athletes combining 17 days of 3–5 h/day at 4,000–5,500 m with low-intensity exercise. Additional investigations by this group showed no difference between passive and active exposure to hypoxia in terms of associated performance benefits (32), implying that hypoxia alone is the primary stimulus for eliciting performance enhancements in athletes exposed to a sufficient stimulus, but neither study included a control group, which is an important limitation for performance assessments. Other carefully controlled investigations have confirmed that supplemental hypoxia administered during training (without sufficient time for “acclimatization”) has no additive effect on performance above and beyond training under normoxic conditions (42). Further investigations employing longer periods of hypoxia at rest found no changes in submaximal and maximal  $\dot{V}O_2$  after 10 days of consecutive 12 h/day exposures to 2,000 or 2,700 m of simulated altitude, even though EPO and reticulocyte counts increased in both groups (25). Finally, a recent controlled study has reported improved performance in



3,000-m run time after 3 h/day for 14 days at  $FI_{O_2}$  of 0.123, without a change in reticulocyte counts (20).

In the present study, there were no changes in laboratory submaximal, maximal, or field 3,000<sub>TT</sub> performance in the Hyp or placebo control (Norm) group after intermittent hypoxic exposure. This observation is important for several reasons. The inclusion of a placebo control group strongly validates the lack of response seen in the experimental group. Furthermore, the lack of change in performance is consistent with our observation of no evidence of hematologic change. Finally, as the single most critical measure of this and any simulated altitude training method, performance did not improve with use of this protocol. In combination, these data lead to the conclusion that a short burst of normobaric hypoxia is a questionable method of altitude simulation, particularly for improving endurance performance at sea level.

### Implications

Previous studies examining the Hi-Lo model, involving 20–22 h of hypobaric hypoxia per day, suggest that individuals with a greater performance enhancement response to altitude training have a more accentuated EPO response initially and throughout the altitude exposure (7). This led to the theory of an EPO “dose” that is required to elicit performance enhancement (22). This dose may be highly individual, with controlled studies in humans demonstrating a more than fourfold difference among subjects in the percent change of EPO in response to sustained (24 h) exposure to simulated altitude of 3,000 m (31). The present study demonstrates clearly that this IHT protocol of short-burst intermittent hypoxic exposure is insufficient to accelerate erythropoiesis. The magnitude (how high), duration (how long per exposure), and frequency (how many exposures per week for how many weeks) of hypoxic exposure necessary to reach a “threshold” for an erythropoietic effect remain uncertain. It remains possible that intermittent hypoxic methods involving more prolonged exposures are efficacious but that the 5-min hypoxic bursts, with 5 min of ambient breathing between each burst, for a total of 70 min, are simply insufficient or ineffective to initiate and sustain the acclimatization process. This may be the case for several reasons based on the understanding of the biological pathways involved in the adaptive response to hypoxia. For example, the principal transcriptional activator of gene expression in hypoxic cells is hypoxia-inducible factor 1 (HIF-1) (35–37, 44). Under normal, well-oxygenated conditions, HIF-1 is hydroxylated via a highly conserved prolyl hydroxylase (the putative cellular “O<sub>2</sub> sensor” in peripheral tissues), which then binds to the von Hippel-Lindau factor, targeting the entire complex for rapid degradation via the ubiquitin-proteasome pathway (11, 17–19). This process is so rapid that, in the presence of O<sub>2</sub> and iron, HIF-1 $\alpha$  has one of the shortest half-lives of any known protein (11, 17, 18, 43). In contrast, under hypoxic conditions, the HIF-1 complex is stable, allowing for transcriptional activation and ultimate stimulation of proteins such as EPO and vascular

Fig. 4. Total Hb (A), reticulocyte count (B), erythropoietin (C), and soluble transferrin receptor (D) at baseline [mean of 2 baseline blood draws (Pre); see text for typical error of each measurement] and after 1, 2, and 12 days of IHT (T1, T2, and T3, respectively) and 1, 10, and 26 days after completion of the 4-wk IHT protocol (Post1, Post2, and Post3, respectively).

endothelial growth factor. Moreover, when altitude natives, or even altitude sojourners, return to sea level, EPO is suppressed (7, 12, 14, 23, 30), iron turnover and bone marrow production of erythroid cell lines are dramatically reduced (16, 26, 27), and red cell survival time is markedly decreased (26, 27, 29). This increase in red cell destruction with suppression of EPO levels has been termed "neocytolysis" and has been observed under other conditions of a relative increase in O<sub>2</sub> content (3, 4, 28). The rapid ubiquitination and destruction of HIF-1 $\alpha$  and neocytolysis (which may be its clinical manifestation) may compromise the ability of short-duration, intermittent hypoxic exposures to induce a sustained increase in the red cell mass.

In conclusion, a 4-wk regimen (5:5-min hypoxia-to-normoxia ratio for 70 min, 5 times/wk for 4 wk) of intermittent normobaric hypoxia is not sufficient to alter 3,000<sub>TT</sub> performance,  $\dot{V}O_{2\max}$ , or indexes of accelerated erythropoiesis in highly trained distance runners.

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