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J Appl Physiol 100:203-211, 2006. First published 22 September 2005;
doi:10.1152/japplphysiol.00808.2005

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Eighteen days of "living high, training low" stimulate erythropoiesis and enhance aerobic performance in elite middle-distance runners

Julien V. Brugniaux,[1] Laurent Schmitt,[1,2] Paul Robach,[1,3] Gérard Nicolet,[2] Jean-Pierre Fouillot,[1] Stéphane Moutereau,[4] Françoise Lasne,[5] Vincent Pialoux,[6] Philippe Saas,[7] Marie-Claude Chorvot,[7] Jérémy Cornolo,[1] Niels V. Olsen,[8] and Jean-Paul Richalet [1]

[1]*Université Paris 13, Laboratoire "Réponses cellulaires et fonctionnelles à l'hypoxie," Bobigny;* [2]*Centre National de Ski Nordique, Prémanon;* [3]*Ecole Nationale de Ski et d'Alpinisme, Chamonix;* [4]*Laboratoire de Biochimie, Hôpital Henri-Mondor, Créteil;* [5]*Laboratoire National de Dépistage du Dopage, Chatenay-Malabry;* [6]*Laboratoire de Physiologie-Biologie du Sport, Faculté de Médecine, Clermont-Ferrand;* [7]*Plateforme de BioMonitoring, Inserm U645/Unité Propre de Recherche et d'Enseignement Supérieur EA2284, Etablissement Français du Sang Bourgogne Franche-Comté, Besançon, France; and* [8]*University of Copenhagen, Department of Pharmacology, The Panum Institute, Copenhagen, Denmark*

Submitted 8 July 2005; accepted in final form 14 September 2005

Brugniaux, Julien V., Laurent Schmitt, Paul Robach, Gérard Nicolet, Jean-Pierre Fouillot, Stéphane Moutereau, Françoise Lasne, Vincent Pialoux, Philippe Saas, Marie-Claude Chorvot, Jérémy Cornolo, Niels V. Olsen, and Jean-Paul Richalet. Eighteen days of "living high, training low" stimulate erythropoiesis and enhance aerobic performance in elite middle-distance runners. *J Appl Physiol* 100: 203–211, 2006. First published September 22, 2005; doi:10.1152/japplphysiol.00808.2005.—The efficiency of "living high, training low" (LHTL) remains controversial, despite its wide utilization. This study aimed to verify whether maximal and/or submaximal aerobic performance were modified by LHTL and whether these effects persist for 15 days after returning to normoxia. Last, we tried to elucidate whether the mechanisms involved were only related to changes in oxygen-carrying capacity. Eleven elite middle-distance runners were tested before (Pre), at the end (Post1), and 15 days after the end (Post2) of an 18-day LHTL session. Hypoxic group (LHTL, $n = 5$) spent 14 h/day in hypoxia (6 nights at 2,500 m and 12 nights at 3,000 m), whereas the control group (CON, $n = 6$) slept in normoxia (1,200 m). Both LHTL and CON trained at 1,200 m. Maximal oxygen uptake and maximal aerobic power were improved at Post1 and Post2 for LHTL only (+7.1 and +3.4% for maximal oxygen uptake, +8.4 and +4.7% for maximal aerobic power, respectively). Similarly oxygen uptake and ventilation at ventilatory threshold increased in LHTL only (+18.1 and +12.2% at Post1, +15.9 and +15.4% at Post2, respectively). Heart rate during a 10-min run at 19.5 km/h decreased for LHTL at Post2 (−4.4%). Despite the stimulation of erythropoiesis in LHTL shown by the 27.4% increase in serum transferrin receptor and the 10.1% increase in total hemoglobin mass, red cell volume was not significantly increased at Post1 (+9.2%, not significant). Therefore, both maximal and submaximal aerobic performance in elite runners were increased by LHTL mainly linked to an improvement in oxygen transport in early return to normoxia and probably to other process at Post2.

intermittent hypoxia; maximal oxygen uptake; carbon monoxide rebreathing technique; soluble transferrin receptor; erythroid burst-forming unit

ONE OF THE MAJOR DETERMINANTS of aerobic performance is the athletes' capacity to deliver oxygen to the tissues. Endurance training has been shown for decades to increase blood volume in both men and women (15). Further investigations found that this enhancement was due to both larger erythrocyte and larger plasma volumes (5). Even if blood volume adaptations represent only one of several mechanisms that permit an increase in maximal aerobic performance, it has been proven to be well correlated with an improvement in maximal oxygen uptake ($\dot{V}O_{2\,max}$) (33).

Exposure to chronic hypoxia is known to invoke blood volume expansion because of an increase in red cell volume (RCV) (16). Hence the underlying hypothesis was that this increase in oxygen delivery capacity could improve maximal aerobic performance (16). Nevertheless, probably due to a complex adaptation of training load in hypoxic conditions, many controlled studies failed to evidence any improvement in aerobic performance at sea level after an altitude training sojourn (12, 22).

An alternative method, namely "living high, training low" (LHTL), was proposed by Levine and Stray-Gundersen (20). It consists of combining nocturnal exposure to hypoxia with sea level training. The underlying hypothesis was to allow stimulation of erythropoiesis during hypoxia while maintaining training intensities at or near sea level. This model has been proved to induce improvement both in red cell mass (18, 21, 31) and in $\dot{V}O_{2\,max}$ (21, 31, 34). On the other hand, other reports did not show any benefit either in red cell mass (1, 2) or in $\dot{V}O_{2\,max}$ (13, 30). As proposed by Levine (22), the magnitude of the modifications in red cell mass and $\dot{V}O_{2\,max}$ is related to the hypoxic stimulus. Various mechanisms could be involved in the changes of aerobic performance. Some authors pled for muscle modifications (14), whereas others thought an improvement in blood oxygen carrying capacity (22) was related to erythropoietin (EPO) release. Nevertheless, the exact cascade leading to an improvement of oxygen transport remains unclear (9).

Another interest among athletes is to have information concerning the prolonged gains on performance. Most of the studies dealt with short-term effects, i.e., within days after the end of the session, whereas the most useful for athletes is a longer effect (i.e., weeks) to prepare for competitions. Thus longer effects are quite poorly documented. Only Rusko et al. (31) and Mattila and Rusko (23) reported an improvement in maximal aerobic performance (31) and cycling performance

Address for reprint requests and other correspondence: J. V. Brugniaux, UFR SMBH, 74 rue Marcel Cachin, 93017 Bobigny cedex, France (e-mail: jbrugniaux@free.fr).

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(23). Similarly, only Levine observed an enhancement of field performance, through a 5,000-m running trial, maintained after 3 wk (21). The rationales of these residual effects (up to 3 wk) are difficult to elucidate. Actually, Levine and Stray-Gundersen (21) explained that global improvements observed in the LHTL group were mediated both by altitude acclimatization and by sea level training effects. Therefore, this aspect of deacclimatization is poorly documented. Another proposal is that this reversible increase in oxygen delivery can maximize training on return to sea level. However, this point of view is not consistent with the reduced training load recommended by Levine and Stray-Gundersen (21) after an altitude sojourn, compatible with persistent enhancement in a 5,000-m time trial.

On another hand, previous work of our team done with swimmers (29) failed to find evidence of any clear increase in maximal performance (+8.1%, $P = 0.09$) after 13 days of LHTL despite an 8.5% increase in RCV. No delayed effects were observed 15 days after the end of the session. Thus the positive effect of the modifications of RCV on performance remains unclear and may be offset by muscle alterations.

From the conclusion of this previous work, in the present study, we aimed to verify whether the possible increases observed in aerobic performance concerns maximal ($\dot{V}O_{2\,max}$) or submaximal performance or both. The second question that arises is to elicit whether these possible improvements persist 2 wk after the end of the session. Finally, we wanted to determine whether the mechanisms involved were due to modifications in blood oxygen carrying capacity at different degrees of maturations of red cells, i.e., early in the differentiation process of stem cells.

METHODS

This work is part of a multicenter study sponsored by the International Olympic Committee and the French Ministry of Sports, during which we have studied three different populations of elite athletes: cross-country skiers, swimmers, and lastly runners.

The Ethics Committee of Paris Necker Hospital approved this study, and all subjects gave their voluntary, written, informed consent to participate in the protocol after a medical check-up and echocardiography.

Subjects

Twelve male runners from the "Fédération Française d'Athlétisme" were recruited for this study, and 11 completed all the testing and training sessions. The one who did not perform the whole study was not included for any analysis. All subjects were classified as elite through their participation in national and international competitions (1,500- to 3,000-m events and cross-country) and were registered on a national list of the French ministry of sports. Our group of athletes was very homogeneous since their personal best time over 1,500 m ranged between 3 min 34.52 s and 3 min 49.92 s (World record: 3 min 26.00 s). Runners were randomly ranked in two groups according to their $\dot{V}O_{2\,max}$ determined at 1,200 m: a LHTL group ($n = 5$) and a "living low, training low" group (CON, $n = 6$). The characteristics (means ± SD) of the CON group were age 23 ± 1 yr, height 178 ± 5 cm, weight 63.5 ± 5.8 kg, and $\dot{V}O_{2\,max}$ 63.3 ± 4.2 ml·min^{-1}·kg^{-1}. Runners in LHTL group were 24 ± 5 yr old, 178 ± 6 cm in height, 66.9 ± 6.7 kg in weight, and with a $\dot{V}O_{2\,max}$ of 63.3 ± 2.5 ml·min^{-1}·kg^{-1}.

Study Design

Testing procedures and training session were led in the "Centre National de Ski Nordique" of Prémanon, Jura, France (1,200 m, barometric pressure = 674 mmHg). The 4-day pretraining period was devoted to pretests (Pre). After these tests, runners preformed an 18-day training period. CON slept in ambient air, i.e., at 1,200 m, whereas LHTL spent 14 h per day in hypoxic rooms. Hypoxic exposure was cut into two parts. LHTL runners were exposed to 6 days at 2,500 m (inspired oxygen fraction = 0.174) before 12 days at 3,000 m (inspired oxygen fraction = 0.164). Normobaric hypoxia was obtained by oxygen extraction from ambient air in each room (OBS, Husøysund, Norway). For safety reasons, oxygen and CO_2 fractions in each room were monitored. Similarly, arterial oxygen saturation by a finger pulse oximeter (NPB-290, Nellcor Puritan Bennett, Pleasanton, CA) was continuously registered for each athlete, and all information was sent to a central control unit with the presence of a medical doctor. Post1 posttests were performed within the first 3 days after return to normoxia. Runners were then allowed to go back home and continue their training with a lower load. Fifteen days after the end of the altitude sojourn, athletes came back to Prémanon for 3 days to test the remanent effects of LHTL (Post2).

Measurements

$\dot{V}O_{2\,max}$ test. $\dot{V}O_{2\,max}$ was measured during an incremental test at Pre and then repeated at Post1 and Post2. The test consisted in an increment of speed or slope on treadmill (Chrono Run Electronic 500 ES, Air Machine, Cesena, Italy). It began with a warm-up at 6 km/h and 6% grade for 3 min. Then, speed was increased by 1 km/h every 2 min until 8 km/h and 6% grade. Thereafter, speed was kept constant at 8 km/h and slope was increased by 2% grade every 2 min up to 14%. Then slope was kept constant at 14% grade and speed was increased by 1 km/h every 2 min until exhaustion. $\dot{V}O_{2\,max}$ corresponded to peak average on 30 s during the last step. Gas exchange (M VMAX series, 29C, SensorMedics, Yorba Linda, CA), heart rate (HR) (S810 Polar Recorder Polar, Kempele, Finland), and oxygen arterial saturation (pulse oximetry, Ohmeda Biox 3740) were continuously monitored. Power output at $\dot{V}O_{2\,max}$ was determined using the speed and slope corresponding to the attainment of $\dot{V}O_{2\,max}$. Power output was expressed in Watts [power output = weight of subject (kg) × speed (km/h) × angle sin (rd)]. Subsequently to the test, we determined the second ventilatory threshold (ST_2), which corresponds to the onset of hyperventilation (4). This threshold is achieved when the ventilation-to-CO_2 uptake ratio increased with the first decrease in end-tidal P_{CO_2}. Oxygen uptake, power output, ventilation, and HR at this threshold were graphically determined.

Endurance performance. A submaximal aerobic field trial was performed at each point of measurement (Pre, Post1, and Post2) in very similar wind and temperature conditions. This test consisted of a 10-min run on a track at the constant speed of 19.5 km/h, during which HR was continuously monitored by a S810 Polar Recorder (Polar, Kempele, Finland). Subjects were informed about the speed during the trial by an alarm that gave them the pace. The chosen running speed corresponded approximately to the sustained velocity over 3,000 m at this period of the season. Moreover, this kind of test presented a good reproducibility because it is a common trial among our runners.

Rationale and quantification for training stimulus. Because runners are elite athletes, we had to precisely define training modalities that matched the standards of the international federation of athletics. Usually, runners develop aerobic performance through relatively high-intensity interval training compared with other activities. Moreover, previous work from our team (29), in which training stimulus was mostly aerobic, failed to evidence an increase in maximal aerobic performance. Hence training was performed with a larger part of interval training than in the preceding study. Nevertheless, in accordance with the observations of Levine and Stray-Gundersen (21) who

found a persistent improvement of aerobic performance over 3 wk after the training session with a reduced training load, runners were allowed to reduce training stimulus during the 15 days between the end of the session and the Post2 measurements.

HR during training was controlled over all the study, including the 15 days of recovery after the end of the LHTL training, with a S810 Polar Recorder. This control aimed to calculate the time spent in different HR zones: below 75% of maximal HR (HRmax; previously determined during $\dot{V}O_{2\,max}$ test), between 75 and 85% HRmax, between 85 and 87% HRmax, and between 87 and 100% HRmax. The last training objective consisted of developing speed and strength capacities.

Intravascular compartments. Total hemoglobin (Hb) mass (nHb), Hb concentration, and intra-vascular compartments (RCV, blood volume, and plasma volume) were determined three times during the study (Pre, Post1, and Post2). Measurement points were standardized in the morning, and the order of subjects remained the same throughout the study. Intravascular compartments were assessed by using a carbon monoxide rebreathing method (circulating blood volume system, CBV-Medical) (6, 26). Hb and carboxyhemoglobin were measured by spectrophotometry (OSM 3, Radiometer, Copenhagen, Denmark). Briefly, it consisted of breathing in a closed circuit including a 2-liter rebreathing bag and a CO_2 absorber. Extra oxygen was administrated into the circuit to compensate for oxygen consumption. The volume of carbon monoxide administered into the rebreathing circuit was 49.34 ml. Before and after the 10-min period of rebreathing, venous blood samples were collected in duplicate. Each sample was analyzed in triplicate in the OSM 3 for determination of Hb concentration and carboxyhemoglobin. Hct was analyzed in triplicate with a micromethod. nHb, RCV, plasma volume, and blood volume were then obtained by calculations (26). The reliability of our methodology was assessed by the calculation of the typical error of measurement for nHb, which is the most directly calculated parameter. We have determined Pre as the reference, and then the error of measurement was calculated for each of the other assays (Post1 and Post2) paired against the reference.

Blood analysis. Resting venous blood samples were collected in the morning, while the subjects were supine, in an overnight fasted state. Measurements were performed at Pre and Post2 periods and also the morning after the last night at 2,500 m (H3000) and 3,000 m, i.e., at Post1 (within 15 min after the subject had left the hypoxic/normoxic room). Hct and percent reticulocytes (Retic) were determined for a Pentra 120 analyzer (ABX, Montpellier, France). The serum samples were assayed for their EPO level by ELISA using human EPO Quantikine IVD from R&D. Soluble transferrin receptor (sTfR) and ferritin levels were determined in sera using the Nichols advantage sTfR and the Nichols advantage Ferritin reagent cartridges, respectively (Nichols Institute Diagnostics). We used the same technique to measure sTfR and ferritin as described by Robach et al. (29).

Colony assays. Erythroid burst-forming units (BFU-E) are immature erythroid precursors that can be identified by culture studies, and their circulating numbers are related to medullary erythroid activity (10). Blood samples were collected on EDTA (BD Vacutainer System, Franklin Lakes, NJ) at Pre and Post1 in the same conditions that other blood samplings to measure the earliest progenitors committed exclusively to erythroid differentiation (BFU-E). Cells were isolated by centrifugation over Ficoll (Histopaque-1077, Sigma, Saint-Quentin Fallavier, France) and washed with Iscove's modified Dulbecco medium before using for colony assays. BFU-E were performed using a commercially available methylcellulose medium containing fetal bovine serum, bovine serum albumin, L-glutamine, 2-mercaptoethanol, and recombinant human erythropoietin in Iscove's modified Dulbecco medium (Methocult H4330, StemCell Technologies, Vancouver, Canada). Cells (8×10^4) were platted per Petri dishes. Colony numbers were counted at *day 14*. Results are expressed as number of cloning unit per 10^5 mononuclear cells.

Iron Intake

Iron mass intake was determined from the dietary recording using GENI software (MICRO6, Villers-lès-Nancy, France) with French (REGAL) and German (SOUCI) tables. The diet was evaluated by the estimation of food and beverage intake. The subject had to record in his notebook all food intake as precisely as possible. All these data were validated using a specific picture book for the estimation of quantities (19). Moreover, runners took daily oral iron supplementation with Tardyféron 80 (256 mg of dried ferrous sulfate).

Statistical Analysis

All values are reported as means ± SD. The Kolmogorov-Smirnov test was first performed to ensure that the data satisfied assumptions consistent with normal distributions. A two-way ANOVA for repeated measures over time was used (two groups: LHTL and CON, and up to 4 measurements: Pre, H3000, Post1, and Post2). In most of the cases, no interactions were found (all parameters except BFU-E and power output at ST_2); therefore, we secondly used a one-way ANOVA for repeated measures over time for each group. The Student-Newman-Keuls post hoc test was used to identify significant differences between means. A Pearson product moment correlation test (r^2, coefficient of determination) was used to analyze the relationship between two quantitative variables. At Post2, some data were lacking for two control subjects. The level of significance was set at $P \leq 0.05$. All statistical analyses were realized by the use of Statistica software version 5 (Statsoft, Tulsa, OK).

RESULTS

Training

Training volume was kept equivalent in both groups along the 18 days of the session (Fig. 1). Training volume was similar in both groups with a mean daily time at 1 h 08 min ($n = 11$). However, mean daily training volume over the posttraining period (15 days) was decreased compared with the training session in both groups ($P < 0.01$).

During the whole study, runners of both groups spent 54% of their training time below 75%, 27% between 75 and 85%, 9% between 85 and 87%, 7% between 87 and 100% of HRmax, and only 3% developed speed/strength.

Performance

$\dot{V}O_{2\,max}$ test. $\dot{V}O_{2\,max}$ ($ml \cdot min^{-1} \cdot kg^{-1}$) measured at 1,200 m was significantly higher for LHTL at Post1 (+9.6%) and Post2

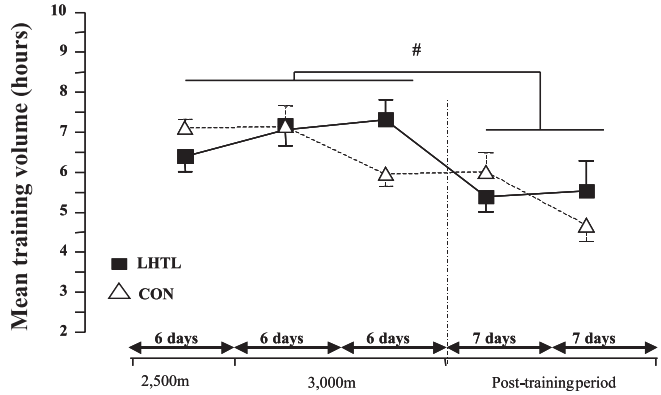

Fig. 1. Daily training volume (expressed in mean h/day over 6 or 7 days). Values are means ± SD. Training parameters are expressed as the mean over 6 days during the session and over 7 days during the 15 days of recovery (following the end of the exposure). CON, control group; LHTL, "live high, train low" group. $^{\#}P \leq 0.01$ training session vs. recovery period in both groups.

Table 1. *Maximal exercise parameters*

	Pre	Post1	Post2
$\dot{V}O_{2max}$, ml·min^{-1}·kg^{-1}			
CON	63.3±4.2	65.9±2.7	65.1±3.8
LHTL	63.3±2.5	69.4±2.9*	66.5±3.3*†
$\dot{V}O_{2max}$, W			
CON	317.7±28.5	322.5±25.6	315.0±41.5
LHTL	344.4±28.8	373.2±30.0*	356.4±45.0†‡
$\dot{V}E_{max}$, l/min			
CON	148.7±11.4	141.8±10.5	145.2±39.4
LHTL	150.2±11.1	153.6±14.1	141.3±22.8
HRmax, beats/min			
CON	191.5±7.3	188.7±5.9	187.8±5.2
LHTL	191.6±9.3	192.4±10.2	187.2±7.8
$PET_{CO_2\,max}$, Torr			
CON	29.6±2.9	30.4±2.5	30.8±3.2
LHTL	32.9±5.4	31.2±5.5	34.9±3.8†

Values are means ± SD. CON, control group; LHTL, "live high, train low" group; $\dot{V}O_{2max}$, maximal oxygen uptake ($\dot{V}O_2$); $\dot{V}E_{max}$, maximal ventilation; HRmax, maximal heart rate (HR); $PET_{CO_2\,max}$, end-tidal P_{CO_2} at $\dot{V}O_{2max}$. Measurements were done before (Pre), at the end (Post1), and 15 days after the end of the training session (Post2). *$P \leq 0.05$ vs. Pre; †$P < 0.05$ vs. Post1; ‡$P = 0.07$ vs. Pre.

(+5.2%) ($P < 0.01$) compared with Pre (Table 1). Similarly, absolute $\dot{V}O_{2\,max}$ (l/min) rises between Pre and both Post1 and Post2 for LHTL (from 4.10 ± 0.2 l/min in Pre to 4.39 ± 0.3 l/min in Post1 and 4.24 ± 0.4 l/min in Post2; $P < 0.05$) and was also higher in Post1 than in Post2 ($P < 0.01$) (Fig. 2). $\dot{V}O_{2\,max}$ in CON remained unchanged along the study (4.01 ± 0.3 l/min in Pre, 4.13 ± 0.4 l/min in Post1, and 4.10 ± 0.5 l/min in Post2). However, no statistical difference was observed between the two groups during the study. Ventilation at $\dot{V}O_{2\,max}$ and HRmax were not modified throughout the study, whatever the group. Power output at $\dot{V}O_{2\,max}$ was increased along the session for LHTL with a maximum at Post1 (+8.4% vs. Pre) ($P < 0.01$), whereas it remained unchanged in CON group along the study. End-tidal P_{CO_2} at $\dot{V}O_{2\,max}$ was not modified for CON during the study, whereas for LHTL it was lower at Post1 with a significant difference at Post2 only ($P = 0.04$ vs. Post1) (Table 1).

Oxygen uptake at ST_2 was higher at Post1 and Post2 compared with Pre for LHTL (from 3.08 ± 0.3 l/min in PRE to 3.57 ± 0.4 l/min in Post1 and 3.51 ± 0.3 l/min in Post2; $P < 0.01$) (Fig. 2). Similarly, power output at ST_2 was increased along the study for LHTL (+14.6% at Post1 and +18.9% at Post2 vs. Pre; $P = 0.01$). There was no alteration in these two parameters for CON throughout the study, with oxygen uptake being 3.12 ± 0.1 l/min in PRE, 3.22 ± 0.2 l/min at Post1, and 3.29 ± 0.4 l/min in Post2. Power output at ST_2 was higher in LHTL than in CON groups at Post1 ($P = 0.03$) and Post2 ($P = 0.04$). HR at ST_2 was not modified in LHTL group. For CON, there was a decrease from Pre to Post1 and Post2 (−3.7 and −4.4%, respectively; $P = 0.01$). The ST_2 appeared at a higher

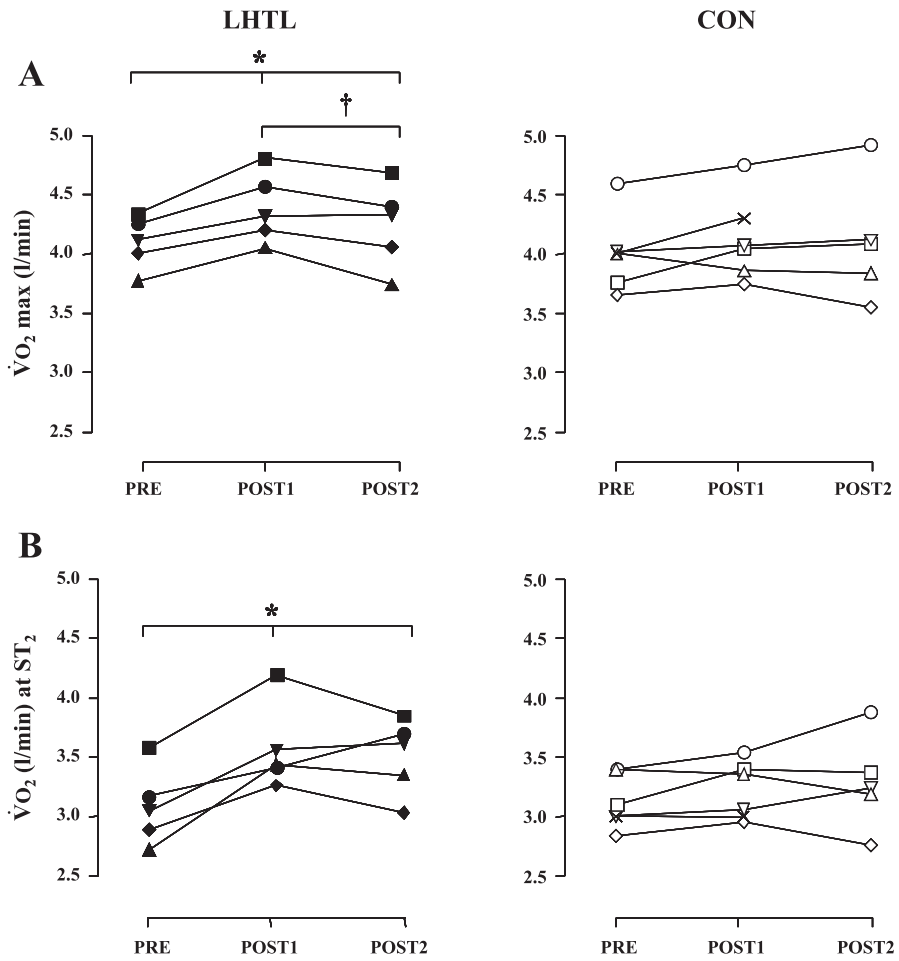

Fig. 2. Individual values of oxygen uptake ($\dot{V}O_2$) at exhaustion (*A*) and at the second ventilatory threshold (ST_2; *B*). Trial was performed before (Pre), at the end (Post1), and 15 days after the end of the training session (Post2). $\dot{V}O_{2\,max}$, maximal $\dot{V}O_2$. One CON subject was missing at Post2. *$P \leq 0.05$ vs. Pre; †$P \leq 0.05$ vs. Post1 (for $\dot{V}O_{2\,max}$ only).

Table 2. *Parameters associated with second ventilatory threshold during the $\dot{V}O_{2max}$ test*

	Pre	Post1	Post2
$\dot{V}O_2$, ml·min^{-1}·kg^{-1}			
CON	49.6±4.0	51.5±2.7	52.1±2.9
LHTL	47.5±2.3	56.4±4.0*	55.3±5.3*
Power output, W			
CON	232.8±23.5	238.2±25.5	229.4±21.2
LHTL	240.8±36.5	276.2±48.7*†	286.6±42.5*†
$\dot{V}E$, l/min			
CON	91.7±10.5	89.6±11.4	91.6±10.2
LHTL	86.4±4.5	97.6±13.1*	98.3±19.5*
HR, beats/min			
CON	173.8±3.8	167.3±2.7*	166.6±3.8*
LHTL	170.6±8.7	166.6±7.0	168.8±8.0

Values are means ± SD. Values during $\dot{V}O_{2max}$ test were measured at the second ventilatory threshold. Measurements were done at Pre, Post1, and Post2. Two-way ANOVA was used for power output. *$P \leq 0.05$ vs. Pre; †$P < 0.05$ LHTL vs. CON.

level of ventilation at Post1 (+12.2%) and Post2 (+15.4%) than at Pre ($P < 0.01$) for LHTL only (Table 2).

Endurance performance. HR during the 10-min trial at constant speed progressively decreased for LHTL to reach significance at Post2 vs. Pre (from 181 ± 11 beats/min in Pre to 176 ± 10 beats/min in Post1, $P = 0.06$ not significant; and 173 ± 8 beats/min in Post2, $P < 0.01$), whereas no change was observed for CON (176 ± 7 beats/min at Pre, 177 ± 5 beats/min at Post1, and 175 ± 4 beats/min at Post2) (Fig. 3).

Hematological Parameters

Intravascular compartments. The typical error for nHb was 3.1 mmol between Pre and Post1 and 2.7 mmol between Pre and Post2. nHb for LHTL was significantly raised at Post1 (+10.1%; $P = 0.05$) but returned to basal level at Post2 (+5.1%), whereas it was not altered in CON group at any point of measurement. RCV for LHTL insignificantly increased from 1.85 ± 0.3 liters at Pre to 2.02 ± 0.2 l at Post1 and was not modified at Post2 compared with Pre (1.93 ± 0.2 liters at Post2). RCV was not modified in CON during the study, with RCV being 1.93 ± 0.2 liters at Pre, 1.94 ± 0.3 liters at Post1, and 1.91 ± 0.3 liters at Post2 (Fig. 4). Plasma volume and blood volume were not modified throughout the study in both groups. No difference was evidenced between groups at any point of measurement for intravascular compartments (Table 3).

Blood analysis. Hct was not modified throughout the study in LHTL. For CON, Hct was higher at H3000 than at Pre (+7.7%) and Post2 (+4%) ($P = 0.01$). Hb concentration measured by spectrophotometry remained unchanged within the study in both groups.

Retic were significantly decreased at Post2 compared with Pre (−26.1%) and H3000 for LHTL (−22.6%) ($P = 0.02$). Similarly for CON, Retic were depleted with the lowest value at Post2 (−31.5% Post2 vs. Pre and −20.3% Post2 vs. H3000 and Post1; $P < 0.01$). Mean serum EPO over the course of the study was significantly higher in LHTL than in CON ($P = 0.04$), but neither CON nor LHTL presented any modification in EPO during the study. Serum ferritin and sTfR were not modified by the session among CON group. In LHTL group, ferritin was not significantly modified either at H3000 or at Post1 ($P = 0.08$) but was depleted at Post2 (−15.7%, $P = 0.04$). Conversely, sTfR in LHTL was increased throughout the study compared with Pre (+17.4% at H3000, +27.4% at Post1, and +24.2% at Post2; $P < 0.01$) (Table 4).

Colony assays. BFU-E was significantly higher at Pre and Post1 in CON than in LHTL ($P = 0.02$). Neither CON nor LHTL presented any significant modification between the two measurements (Table 4).

Iron Intake

Both LHTL and CON groups were supplemented with oral iron during the training session with 40 mg/day. Iron intakes were higher during the 3,000-m stage than during the 2,500-m stage for CON group (14.3 ± 2.2 vs. 13.9 ± 2.8 mg/day, respectively; $P = 0.02$), whereas there was no change for LHTL group during the same period (15.2 ± 3.7 mg/day during the 2,500-m stage and 13.8 ± 2.9 mg/day during the 3,000-m stage). Mean iron intake over the 18 days of hypoxic exposure was lower than during the posttraining period for CON (13.9 ± 2.4 vs. 12.7 ± 1.7 mg/day; $P = 0.04$) and unchanged for LHTL (14.3 ± 2.9 vs. 14.8 ± 4.8 mg/day). Whatever the period, there was no difference in iron intake between groups.

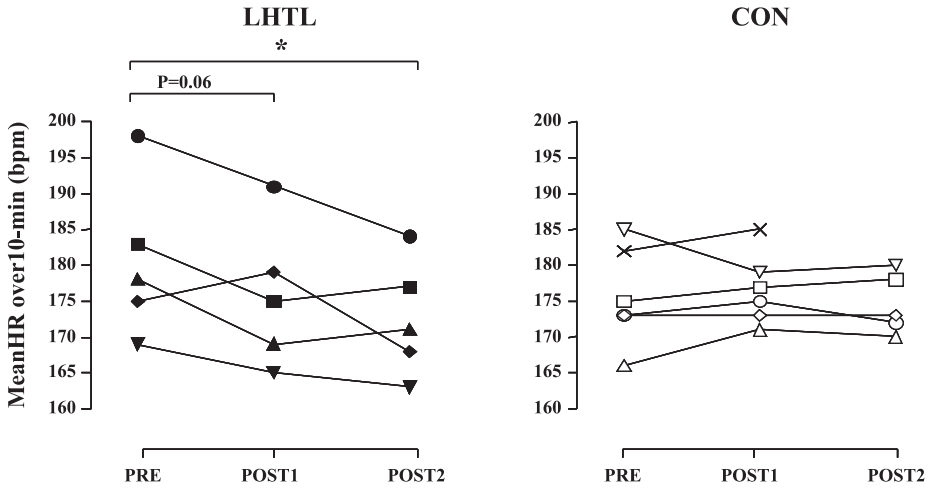

Fig. 3. Individual values of mean heart rate (HR) over a 10-min run at 19.5 km/h. Trial was performed at Pre, Post1, and Post2. *$P \leq 0.05$ vs. Pre. One CON subject was missing at Post2.

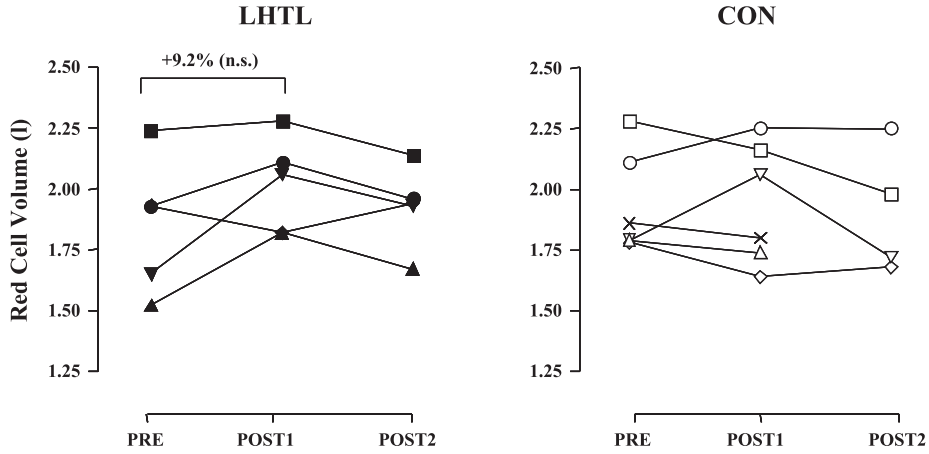

Fig. 4. Individual values of red cell volume. Measurement was realized using the CO-rebreathing method at Pre, Post1, and Post2. Two CON subjects were missing at Post2.

DISCUSSION

This work demonstrated that both maximal and submaximal aerobic performances were increased at the end of a LHTL session. Another interesting finding of our work is that submaximal performance was still enhanced with a 15-day delayed effect, whereas $\dot{V}O_{2\,max}$ alterations began to fade away. We also evidenced a significant stimulation of erythropoiesis at the end of the session as shown by sTfR, ferritin, and nHb but without significant repercussion on RCV.

Immediate Effects of LHTL

One major hypothesis to explain possible improvements in performance following LHTL is related to oxygen delivery (21). In our study, we tried to elucidate which step of erythropoiesis is the most representative of hypoxic influence. Stimulation of erythropoiesis can be assessed at different levels of the synthesis of red blood cells. EPO secretion by kidneys is the primary signal inducing this synthesis. In our study, we were not able to evidence any modifications in EPO levels for two main reasons. First, EPO is known to be secreted within 2–3 h during exposure to hypoxia (7). When we exposed our athletes to 3 h of acute exposure at 3,000 m during the training period (unpublished data), they presented a 37.6% mean increase in EPO (n = 11; range between −4.5 and 105.7%). Moreover, although Ashenden et al. (3) observed a persistent increase in serum EPO after 5 days of LHTL at 2,650 m, sustained hypoxia is a well-known downregulator of EPO secretion (8, 14), especially in well-acclimatized subjects (27). Stray-Gundersen et al. (34) also did not find any increase in plasma EPO after 19 days of LHTL at 2,500 m.

When erythropoiesis is stimulated during LHTL sessions, athletes sometimes experienced a rise in red blood cells. However, from the stimulation of the multipotent stem cells (hemocytoblast) to erythrocytes, many events occur. We did not find any alteration in BFU-E either in CON or in LHTL groups. To our knowledge, changes in erythroid progenitor cells are poorly documented when coupled with altitude training. In fact, differentiation of BFU-E to erythroid colony forming unit, which is the next step leading to red blood cell, is notably mediated by EPO (24). The absence of a significant increase in serum EPO between Pre and Post1 can account for the unchanged BFU-E count at Post1.

Despite this absence of change in mean EPO levels, hematopoiesis was stimulated since serum sTfR for LHTL was

Table 3. *Intravascular compartments*

	Pre	Post1	Post2
nHb, mmol			
CON	45.0±5.3	45.1±6.3	45.4±6.0
LHTL	42.5±5.9	46.9±6.3*	44.7±4.8
Plasma volume, liters			
CON	2.5±0.3	2.3±0.3	2.3±0.4
LHTL	2.1±0.2	2.3±0.3	2.2±0.2
Blood volume, liters			
CON	4.3±0.5	4.2±0.6	4.2±0.6
LHTL	4.0±0.3	4.4±0.5	4.2±0.4

Values are means ± SD. nHb, total hemoglobin (Hb) mass in millimoles of monomeric Hb. Measurements were at Pre, Post1, and Post2. Two CON subjects were missing at Post2. *$P < 0.05$ vs. Pre.

Table 4. *Hematological parameters*

	Pre	H3000	Post1	Post2
EPO, UI/l				
CON	7.4±1.5	7.9±2.6	11.1±4.1	8.4±1.1
LHTL	10.9±3.0	11.2±3.2	10.3±2.2	12.7±2.0
sTfR, nM				
CON	19.7±4.1	21.0±4.1	21.9±3.5	20.1±4.4
LHTL	15.9±2.3	18.7±2.0[a]	20.2±2.5[a]	19.7±0.9[a]
Ferritin, μg/l				
CON	111.4±64.9	101.4±43.9	87.2±52.2	85.5±35.0
LHTL	100.5±118.7	83.6±102.3	84.6±100.0[d]	84.7±99.7[d]
BFU-E,/ml				
CON	593.3±103.7		770.0±352.9	
LHTL	370.4±100.4[c]		435.8±246.6[c]	
Retic, %				
CON	1.6±0.4	1.5±0.5	1.5±0.3	1.2±0.3[a,b,c]
LHTL	1.8±0.4	1.7±0.3	1.5±0.3	1.3±0.3[a]
Hct, %				
CON	44.2±1.3	47.6±2.0[a]	45.7±2.0	45.8±1.8
LHTL	45.4±2.4	47.8±2.5	45.3±1.8	46.3±0.9
Hb, g/dl				
CON	16.9±0.3		17.1±0.6	17.6±0.5
LHTL	17.1±1.1		17.2±0.6	17.2±1.0

Values are means ± SD. EPO, erythropoietin; sTfR, soluble transferrin receptor; BFU-E, burst forming unit erythroid; Retic, percentage of reticulocytes; Hct, hematocrit. Blood samples were obtained at Pre, after the last night at 2,500 m (H3000), at Post1, and at Post2. Two-way ANOVA was used for BFU-E. [a]$P \leq 0.05$ vs. Pre; [b]$P < 0.05$ vs. Post1; [c]$P < 0.05$ LHTL vs. CON; [d]$P = 0.08$ vs. Pre; [e]$P < 0.05$ vs. H3000.

increased, whereas it stayed unchanged for CON. Concomitantly, but to a lesser degree, iron stores assessed by ferritin were depleted for LHTL despite iron supplementation, which represents an increased utilization of iron by erythroid cells. Enhancement in sTfR has previously been shown with chronic exposure to high altitude (28) and with LHTL (34). The magnitude of changes after 6 days at 2,500 m (H3000) in our study was close to the one found after 7 days at 2,500 m (12 h/day) by Koistinen et al. (17) (+17.4% in our study vs. +19% for Koistinen et al.), who also observed an increase in reticulocytes.

In our work, Retic was not modified in both groups. Results are in accordance with other controlled studies using 5–23 nights of LHTL (1, 2, 3) or 26 nights of altitude training (14). Nevertheless, other studies evidenced increased reticulocytes (17, 25). The lack of clear change in the present study can be explained by various factors. First, basal level of reticulocytes in our study was close to the upper physiological limit (close to 2%), suggesting an important turnover among red blood cells probably linked to aerobic training before the study. Moreover, footstrike when running seems to have a major contribution in hemolysis (35). Therefore, we can hypothesize an already upregulated hematopoiesis at the beginning of the study.

The absence of significant changes in RCV (probably due to our small sample size), despite the stimulation of erythropoiesis at Post1, as shown by sTfR and nHb, might be surprising. The main explanation already proposed by various authors (22, 14, 29) is the "dose" of hypoxia. In the studies of Ashenden et al. (1–3), subjects did not spend more than 207 h (9 h/day) in hypoxia throughout the session without any increase in oxygen carrying capacity. Conversely, in the studies of Levine and Stray-Gundersen (22) and Stray-Gundersen et al. (34), athletes probably spent ~450 h in hypoxia (although the authors did not mention the exact ratio between hypoxia and normoxia). In the works of our team, runners have spent 252 h in hypoxia, whereas total exposure was 208 h for swimmers (29). Hence, the present results confirm the importance of the time spent in hypoxia. Nevertheless, contrary to the studies of Ashenden et al. (1, 2), in both of our studies, we evidenced an increase in nHb (+10.1% for runners vs. +7.5% for swimmers). Thus, despite our small sample size, we observed an improvement in oxygen carrying capacity, as shown by nHb. Despite the somewhat high level of possible measurement error, we can be relatively confident in our data owing to the biological variation with time, certainly accentuated by the hypoxic exposure and its interindividual response. Moreover, as expected, nHb and RCV were positively correlated when every point of measurement was pooled ($r^2 = 0.86$, $P < 0.01$).

$\dot{V}O_{2\,max}$ was strongly enhanced at the end of the session for LHTL group. In contrast, there was no change in $\dot{V}O_{2\,max}$ in the CON group despite an equivalent supervised training. However, since there was no difference between groups at Post1, the effect of hypoxia is not so clear. In the report of Levine and Stray-Gundersen (21), improvements in $\dot{V}O_{2\,max}$ were positively correlated with modifications in RCV ($r = 0.37$, $P = 0.02$). In our study, modifications in $\dot{V}O_{2\,max}$ were not related to those in both RCV and nHb between Pre and both Post1 and Post2 with all subjects. This absence of relation could be due to the fact that blood volume adaptations (especially RCV) represent only one of several physiological mechanisms that underlie the increase in $\dot{V}O_{2\,max}$ (21, 33).

Because our training camp was held at the beginning of the season, we cannot exclude an effect of training per se. However, despite the lack of significance, a difference of 4.2% (for absolute $\dot{V}O_{2\,max}$) remains between groups at Post1. Hence, training plays a part in the enhancement for LHTL, and it could explain the lower HR at ventilatory threshold observed for CON.

In our study, submaximal performance was assessed by HR during a 10-min run trial and by parameters associated with ST_2 during $\dot{V}O_{2\,max}$ test. HR during field trial was not statistically modified in both groups at Post1; however, modifications in field HR between Pre and Post1 tended to be correlated with those in $\dot{V}O_{2\,max}$ for LHTL despite the small number of subjects ($P = 0.08$, result not shown). It reinforces the hypothesis that LHTL can elevate both ventilatory threshold (21) and running/cycling economy (32, 13). Similarly, variables associated with ST_2 (oxygen uptake, ventilation, power output) were higher at the end of the session compared with Pre for LHTL group only, with a significant difference between groups for power output. At ventilatory threshold, the increase in nHb could allow a lower cardiac output and, therefore, more peripheral diffusion time and oxygen extraction (21). These results confirm that intermittent hypoxic exposure can enhance not only maximal but also submaximal aerobic performance (especially power output). Nevertheless, the mechanisms involved remain difficult to elicit despite the role of nHb.

Remnant Effects of LHTL

Although sTfR remained increased and ferritin depleted at Post2, it seems difficult to attribute the persistent modifications in performance to central hematological changes since neither RCV nor nHb was modified. Improvements in $\dot{V}O_{2\,max}$ observed for LHTL began to fade away despite persistent significance at Post2. Moreover, parameters at ventilatory threshold (except HR) were still higher than at Pre. Similarly, HR during field trial was lower than in Pre. Hence, the changes in maximal aerobic performance were blunted 15 days after the end of the session, whereas submaximal performance was at its highest level throughout the study. It confirms the results of Levine and Stray-Gundersen (21), who found a persistent effect on 5,000-m performance up to 3 wk, but these authors did not measure $\dot{V}O_{2\,max}$ at this point. Therefore, LHTL session seems to have a positive effect on training when returning to normoxia. Actually, it permits a potentiation of this training despite reduced training load. The mechanisms permitting such an increase remain unclear. Nevertheless, the training design should be an important factor. In another work of our group (29), swimmers experienced an increase in RCV (+8.5%) at Post1 (after 13 days of LHTL), but maximal performance did not change along the study (29). In the present work, RCV was not statistically modified along the study, whereas $\dot{V}O_{2\,max}$ increased. Two main differences emerge from the two protocols that could explain this delayed effect observed consecutively to this session. First, swimmers realized a predominant aerobic training contrary to this one, which involved more high-intensity training (about one-half of the training time was spent above 75% of HRmax). Second, the total duration of the hypoxic exposure was shorter. In this study, the total duration of the session had no delayed effect on RCV within 15 days. Therefore, the training modalities, rather than total volume of

training (during LHTL and normoxia), seem to be a key factor. Indeed, it has already been shown that athletes can reduce the training volume when returning to normoxia without impeding the changes in submaximal aerobic performance (21).

In conclusion, the present study demonstrates that sleeping at 2,500–3,000 m while training at 1,200 m for 18 days improves $\dot{V}O_{2\,max}$ and associated power output at the end of the session, whereas this increase is blunted 15 days after the return to normoxia. This improvement represents a marked increase, especially for elite athletes. The LHTL protocol also seems to have a marked effect on submaximal performance (both at ventilatory threshold and during field trial), which was enhanced in early return to normoxia and even more 15 days after the end of exposure. Although changes in oxygen carrying capacities play an indubitable role, it is not the only responsible factor. It appears that the stimulation of erythropoiesis during LHTL has a major effect within days following return to normoxia, whereas a potentiation of training seems to be the most important factor responsible for the delayed effects.

ACKNOWLEDGMENTS

The authors thank Patrick Bouchet for software development. We also thank Dr. Poul Christensen for allowing us to use his program and carbon monoxide-rebreathing device for the determination of intravascular compartments.

GRANTS

This study was supported by grants from the International Olympic Committee and the French Ministry of Sports.

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