“Live high–train low” using normobaric hypoxia: a double-blinded, placebo-controlled study

Christoph Siebenmann,1 Paul Robach,2 Robert A. Jacobs,1,3 Peter Rasmussen,1 Nikolai Nordsborg,4 Victor Diaz,1,3 Andreas Christ,5 Niels Vidiendal Olsen,6 Marco Maggiorini,5 and Carsten Lundby1

1Center for Integrative Human Physiology, Institute of Physiology, University of Zurich, Zurich, Switzerland; 2Département Médical, École Nationale des Sports de Montagne, Chamonix, France; 3Institute of Veterinary Physiology, University of Zurich, Zurich, Switzerland; 4Department of Exercise and Sport Sciences, University of Copenhagen, Copenhagen, Denmark; 5Intensive Care Unit, Department of Internal Medicine, University Hospital, Zurich, Switzerland; and 6Department of Neuroanaesthesia, The Neuroscience Centre, Rigshospitalet, Copenhagen, Denmark

Submitted 30 March 2011; accepted in final form 21 October 2011

Siebenmann C, Robach P, Jacobs RA, Rasmussen P, Nordsborg N, Diaz V, Christ A, Olsen NV, Maggiorini M, Lundby C. “Live high–train low” using normobaric hypoxia: a double-blinded, placebo-controlled study. J Appl Physiol 112: 106–117, 2012. First published October 27, 2011; doi:10.1152/japplphysiol.00388.2011.—The combination of living at altitude and training near sea level [live high–train low (LHTL)] may improve performance of endurance athletes. However, to date, no study can rule out a potential placebo effect as at least part of the explanation, especially for performance measures. With the use of a placebo-controlled, double-blinded design, we tested the hypothesis that LHTL-related improvements in endurance performance are mediated through physiological mechanisms and not through a placebo effect. Sixteen endurance cyclists trained for 8 wk at low altitude (<1,200 m). After a 2-wk lead-in period, athletes spent 16 h/day for the following 4 wk in rooms flushed with either normal air (placebo group, n = 6) or normobaric hypoxia, corresponding to an altitude of 3,000 m (LHTL group, n = 10). Physiological investigations were performed twice during the lead-in period, after 3 and 4 wk during the LHTL intervention, and again, 1 and 2 wk after the LHTL intervention. Questionnaires revealed that subjects were unaware of group classification. Weekly training effort was similar between groups. Hb mass, maximal oxygen uptake (VO2max), and work output in a simulated 2,500 m and mean power output in a simulated, 26.15-km time trial remained unchanged in both groups throughout the study. Exercise economy (i.e., VO2 measured at 200 W) did not change during the LHTL intervention and was never significantly different between groups. In conclusion, 4 wk of LHTL, using 16 h/day of normobaric hypoxia, did not improve endurance performance or any of the measured, associated physiological variables.

OVER THE PAST FIVE DECADES, endurance athletes have attempted to improve sea-level performance by means of altitude training. In the early 1990s, Levine and Stray-Gundersen (36) introduced the “live high–train low” (LHTL) strategy, where athletes reside and spend the majority of the day at moderate altitude while training closer to sea level. This paradigm aims for athletes to benefit from physiological adaptation to hypoxia, while avoiding the detrimental impact of hypoxia on high-intensity endurance training. After an initial study had provided results indicating that LHTL enhances aerobic performance in competitive runners (34), a bulk of follow-up studies confirmed these benefits across a variety of endurance disciplines (7, 50, 57, 59). None of them, however, used a double-blinded design, and thus it cannot be ruled out that the observed effects, especially on performance measures, were, at least in part, mediated by a placebo effect (11, 56). Our main aim with this study was therefore to investigate the effect of LHTL in a double-blinded, placebo-controlled study.

A further aim was to examine the effect of LHTL on exercise capacity in mild hypoxia. Some endurance disciplines, such as cross-country skiing, frequently perform competitions at moderate altitudes. However, even the mild hypoxia associated with these altitudes may decrease maximal oxygen uptake (VO2max) by reducing arterial O2 content (CaO2) (22, 43), and this mechanism seems to be particularly pronounced in highly trained athletes (30, 31). It appears plausible that this detrimental effect of moderate altitude could be counterbalanced by measures that enhance CaO2. Indeed, LHTL has previously been reported to not only normalize but also even enhance CaO2 at an altitude of 2,340 m so that VO2max was partially recovered (55). However, the study was conducted without a control/placebo group, and it thus remains unclear whether the LHTL intervention was superior to conventional training at low altitude. In the present study, we therefore performed additional VO2max tests at a simulated altitude of 2,500 m to evaluate the impact of LHTL on exercise capacity in mild hypoxia with a placebo-controlled study design.

There has been much controversy as to the mechanisms underlying performance gains following LHTL (20, 35). In the initial LHTL study and the follow-up studies by the same group, performance gains were attributed to altitude-induced polycythemia and a concomitant increase in convective O2 transport capacity (10, 34, 57). However, another subset of LHTL studies failed to reproduce the erythropoietic stimulation by LHTL and instead, observed improvements in exercise economy following LHTL (19, 52, 53). In an attempt to shed more light on the mechanisms underlying performance gains with LHTL, we evaluated both total Hb mass (Hbmass) and exercise economy on five occasions before, during, and after the 4 wk of LHTL.

Thus the major aim of the present study was to determine changes in endurance performance of highly trained endurance athletes after 4 wk of LHTL (minimum 16 h/day at 3,000 m) using a double-blinded, placebo-controlled design. The hypotheses to be tested were: J) LHTL exerts a positive effect on endurance performance (VO2max and time-trial performance),
which is based on altitude-dependent physiological adaptations and not exclusively on a placebo effect; 2) LHTL increases VO2max at moderate altitude; and 3) potential performance gains after LHTL correlate with changes in Hbmass.

METHODS

Subjects

Initially, 24 endurance athletes were recruited as subjects for the present study. However, due to personal reasons, five subjects withdrew participation in the last week before the study. Two further subjects did not show up at the onset of the study for unknown reasons. Finally, 17 highly trained endurance athletes living at or near sea level (16 males, 1 female, age 29 ± 6 years, height 179 ± 8 cm, body wt 69 ± 9 kg) from various countries in North America and Europe attended as subjects in the present study. All of them regularly participated in endurance competitions on at least national levels in disciplines related to cycling, i.e., road cycling, triathlon, cycle cross, and/or mountain bike. To prevent bias from previous altitude acclimatization, we excluded subjects who traveled to altitudes higher than 2,500 m within the last month before the study. All subjects gave written, informed consent to participate, and the study was approved by the local ethical boards (Kanton Zurich and Kanton Waadt, Switzerland). During the course of the experiment, one subject decided to withdraw participation for personal reasons, and hence, his data were not included into the analysis. The remaining 16 subjects all completed the study.

Study Design

At the onset of the study, subjects traveled to Prémamon, France (1,135 m), where they lived for 8 full wk at the Centre National de Ski Nordique, an accommodation of the French state, which is used by national endurance athletes for housing and training. This facility is equipped with fully furnished hypoxic rooms, in which athletes can comfortably live while being exposed to adjustable normobaric hypoxia. All of the experimental procedures of the study were performed at the hospital La Vallée (Le Sentier, Switzerland), located at an altitude of 1,020 m, ~25 km away from Prémamon. Subjects were transported from Prémamon to Le Sentier in mini-buses on experimental days.

The first 2 wk of the study served as a lead-in period, where subjects were familiarized to the natural environment, and baseline testing was performed. For the following 4 wk, the intervention period, subjects were assigned to spend a minimum of 16 h/day in one- to three-person hypoxic rooms containing either normobaric normoxia (placebo group, n = 6) or normobaric hypoxia (LHTL group, n = 10). The group classification was performed in a stratified but not randomized manner to provide optimal equality regarding physiological parameters and regular distribution of athletes of different disciplines. Consequently, the LHTL group was composed of three cross-cyclists, two triathletes, four road cyclists, and one mountainbiker, and the placebo group of two cross-cyclists, one triathlete, two road cyclists, and one mountain biker.

Three days prior to the study start, all rooms were controlled and calibrated with precision gases by the company who built the facilities (Fieldbrook, London, UK). Subjects were blinded toward the environmental condition to which they were exposed in their rooms. Furthermore, all investigators, except for the main investigator (who did not perform any measurements), were blinded toward the group assignment. All rooms had the required hypoxic equipment installed, and the air pumps were constantly turned on. O2 fraction in each room was continuously monitored from two independent O2 probes (TTOX-V, Oxygen GTicelC, City Technology, Portsmouth, UK) connected to a control panel located in a room with restricted access. O2 fraction was controlled by the main investigator 5 days/wk (this person staying in the control room overnight) and by a qualified person from the center, not involved in any measurement, during the 2 other days. In addition, O2 fraction in each room was controlled twice daily via a portable O2 sensor (Vandagraph VN202, Cambridge Sensotec, Cambridge, UK) by either the main investigator or the person from the center.

The LHTL group was exposed to a normobaric hypoxia equivalent to 2,500 m for the first 2 days/nights of the intervention period. Thereafter, the O2 fraction was decreased, equivalent to 3,000 m. As a result, morning arterial O2 saturation (SaO2), estimated by pulse oximetry (NPB-290, Nellcor Puritan Bennett, Pleasanton, CA), was 92 ± 2% in the LHTL group (at 3,000 m) and 97 ± 1% in the placebo group (P < 0.05). Subjects were confined to their rooms from 20:00 to 07:00, from 08:00 to 10:00, and again from 16:00 to 19:00 during these 4 wk. However, they were always allowed to spend more time in their rooms if desired (but this was not recorded). The confinement was rigorously supervised by the main investigator 5 days/wk and by other investigators the remaining 2 days. For psychological reasons and to blind the subjects regarding group classification, all subjects were assigned to different roommates and/or rooms on a weekly basis.

During the last 2 wk of the study, the postintervention period, subjects were relieved of the room confinement and hypoxic exposure. These days had the purpose of monitoring how long potential effects of LHTL would last. However, the blinding was maintained for both subjects and investigators until the end of the study.

Five testing sessions were distributed over the 8 wk of the study. They were scheduled in the first 2 wk (BASELINE), after 3 wk in the LHTL intervention (W 3), on the last 3 days of the LHTL intervention (W 4), and 1 (W 1 POST) and 2 wk (W 2 POST) after termination of the intervention period. To obtain a solid baseline value and allow for test familiarization, all measurements (except for hypoxic VO2max; see below in paragraph) were performed in duplicate during BASELINE, each test separated from its previous by at least 4 days. For the maximal exercise tests and the time trials, the better result of the two tests was adopted as a baseline value, whereas for the measurement of hematological parameters and cycling economy, the average value over the two BASELINE tests was used. In the following four testing sessions, all tests were performed only once, except during W 4, where hematological parameters were again evaluated twice (with calculation of the average value) and where hypoxic VO2max was not tested. All other tests were performed once at this time point. The protocol with the scheduling and content of the five testing sessions is illustrated in Fig. 1.

Measurements

Hbmass. Hbmass was measured by a modified version of a carbon monoxide (CO) rebreathing technique (41). The subject first rested for 20 min in a semirecumbent position. Thereafter, 2 ml blood was sampled from an antecubital vein through a 20-G catheter and analyzed immediately in quadruplicate for l% percent carboxy-Hb (%HbCO) and Hb concentration ([Hb]) on a hemoximeter (ABL800, Radiometer, Copenhagen, Denmark) and hematocrit by the micro-method (4 min at 13,500 rpm). After baseline collection and control, the subject first breathed 100% O2 for 2 min to flush nitrogen from the airways. The breathing circuit (previously O2 flushed) was then closed, and a bolus (1.5 ml/kg) of 99.997% chemically pure CO (CO N47, Air Liquide, Paris, France) was administrated and rebreathed for 8 min. At the end of the rebreathing period, another 2 ml blood sample was obtained and analyzed, following the same procedure. The change in %HbCO between first and second measurement was used for calculation of Hbmass, taking into account the amount of CO remaining in the rebreathing circuit at the end of the procedure (2.2%) (8). Total red blood cell volume (RCV), blood volume, and plasma volume was derived from Hbmass, [Hb], and hematocrit (8). All CO rebreathing tests were performed by the same operator. BASELINE and W 4 values reported here correspond to the average of duplicate measurements conducted on separate days within these testing ses-
A VO2max test was performed, during which subjects were breathing a gas analyzers and the flowmeter of the applied spirometer were monitored as breath-by-breath values (Quark, Cosmed, Rome, Italy). The concentration in the expired gas was continuously measured and total Hb mass; VO2max, maximal oxygen uptake; BASELINE, first 2 wk; W 3, after 3 wk in the LHTL intervention; W 4, on the last 3 days of the LHTL intervention; W 1 POST and W 2 POST, 1 and 2 wk, respectively, after termination of the intervention period.

Fig. 1. Study protocol and testing sessions. The measurements performed in each testing session are listed; (2) indicates that a measurement was performed in duplicate within this session. LHTL, live high–train low; Hbmax, total Hb mass; VO2max, maximal oxygen uptake; BASELINE, first 2 wk; W 3, after 3 wk in the LHTL intervention; W 4, on the last 3 days of the LHTL intervention; W 1 POST and W 2 POST, 1 and 2 wk, respectively, after termination of the intervention period.

To determine cycling economy, we assessed VO2 during submaximal steady-state cycling. For this purpose, the breath-by-breath values of the last minute of the higher warm-up workloads (i.e., 200 W in normoxia and 150 W in hypoxia) were averaged. Since the only female athlete warmed up at lower workloads, she was not included into the evaluation of cycling economy.

Arterial blood sampling. After local anesthesia with 2% lidocaine, a 20-gauge catheter (model 80115.09R, Vygon Laboratories, Ecouen, France) was inserted percutaneously using the Seldinger technique into the radial artery. Arterial blood was sampled anaerobically in heparinized syringes and analyzed immediately for [Hb], SaO2, and lactate concentration by means of the ABL800. Arterial samples were obtained during incremental exercise to exhaustion (VO2max) on two separate occasions, i.e., at BASELINE and at W 3 during the normoxic and hypoxic exercise trials. Samples were collected at 200 W (normoxia) or 150 W (hypoxia) and at exhaustion. In the placebo group, one subject did not undergo catheterization during W 3. In the LHTL group, catheterization was not possible for one subject at BASELINE and for two subjects at W 3. Arterial blood sampling was thus conducted in n = 5 (placebo) and n = 7 (LHTL).

To evaluate exercise performance in a scenario similar to competitions, subjects performed a time trial using their own personal bike mounted on an electrically braked cycle trainer (Fortius Virtual Reality Trainer, Tacx, Rotterdam, Netherlands). The combination with commercially available software allowed for the simulation of a predefined route on a portable computer. We selected the final section of the real Milan-San Remo race with a length of 26.15 km. This route consists of a first flat part (4.8 km, average incline 0.23%), a first climb (5.5 km, 4.19%), a downhill part (3.1 km, –6.79%), a second flat part (8.6 km, 0.25%), and the final climb (4.15 km, 3.13%).

Athletes’ body weight was measured immediately before each trial for software-based calculation of the appropriate resistance. After warming up for a minimum of 10 min, athletes could rest again and start the test whenever they felt ready. To achieve resemblance to actual time-trial cycling competitions, subjects were free to manually change gears and drink and eat ad libitum and allowed visual feedback on the computer screen, which displayed their speed and covered distance. To avoid potential influence from the pressure in the rear tire on the recorded speed/time, the rear wheel of each bike was fitted with a power meter (Powertap Elite+, CycleOps, Madison, WI), and power output over the whole course was measured as outcome variable. Subjects were vigorously encouraged during all tests.
Personal Training and Monitoring

Subjects were instructed to follow their training habits and to keep training intensity and volume as constant as possible throughout the 8 wk. Training was monitored using heart-rate monitors (Polar, Suunto, or Garmin). The investigators advised some athletes on a few occasions to either increase or decrease training volume and/or intensity. Training monitoring did not include experimental exercise tests (which were similar for all athletes) or training time spent at or below 55% of maximal heart rate. Furthermore, subjects frequently rode back from the hospital to the housing facility after testing sessions, and this training time (1 h on average, low to medium intensity) was not recorded either. During weeks 3 and 4, subjects were asked to reduce training the day before the exercise tests to minimize the influence of fatigue.

Sport Nutrition and Iron Supplementation

During the entire study, subjects were provided ad libitum access to commercially available energy drinks and carbohydrate and protein bars (Sponser, Wollerau, Switzerland). The concentrations of ferritin and soluble transferrin receptor (sTfR) were determined by immunoturbidimetric assay on a Hitachi 911 automatic analyzer (Boehringer, Mannheim, Germany) in duplicates during the lead-in phase and demonstrated that no subject was iron deficient at the time of entering the study; the mean value was 80.6 ± 11.9 (range from 62 to 102) ng/ml for ferritin and 2.5 ± 0.3 (range from 2.0 to 3.1) mg/l for sTfR. To prevent any bias from iron deficiency, all subjects were supplemented with a daily oral intake of 256 mg dried ferrous sulfate (Tardyferon 80 mg, Pierre Fabre, Australia), starting upon arrival and continuing until the end of the intervention period.

Efficacy of the Blinding Process

The efficacy of the blinding process was evaluated during the intervention period by questionnaires in which subjects were repeatedly asked to state whether they believed to be living in normoxia or hypoxia.

Statistics

Statistical evaluation of the data was performed by running a two-way ANOVA with repeated measurements, combined with a Tukey post hoc test for multiple comparisons. The statistical software was the commercially available program SigmaPlot 5.0. The data are presented as mean values ± SD. A P value <0.05 was considered statistically significant. A maximum likelihood ANOVA determined whether subject blinding was successful.

RESULTS

Training Effort and Efficacy of the Blinding Procedure

Figure 2 illustrates training volume and intensity in both groups. No difference in total exercise time or heart-rate distribution was observed between the two groups at any point. Although training intensity was well preserved, the recorded training volume was decreased in both groups in the beginning.

Fig. 2. Training. Training intensity (heart rate) and time (insets) over the course of the 8-wk study. In week-2 the volunteers arrived to the research facility, and values only represent 4 days. Exercise effort during the experiments and subsequent bike rides back to the housing facilities are not accounted for in this figure (as it can be assumed to be the same in both groups). Values for exercise intensity are weighted as means with SD and for the total training time, median with 25% quartiles. P and H with short lines indicate mean weighted heart-rate average for placebo and LHTL group, respectively. No differences were observed across time and study group with respect to exercise intensity, whereas the measured total training time decreased significantly with time in both groups but with no difference between the 2 groups.
and toward the end of the study. This was primarily related to the high frequency of testing days in these weeks (exercise tests and the subsequent bike ride from the hospital to the facilities were not included into the monitoring of training effort) and the tapering day before, on which subjects were instructed to avoid long training sessions.

The results of the questionnaires evaluating the blinding process are presented in Table 1. The large variation, with only one-fourth of the subjects guessing right at the end of the LHTL period (equal to the number of subjects guessing wrong), indicates that the blinding process was successful, and subjects were unaware of group classification.

### Hematological Parameters

Hematological parameters are summarized in Table 2 and Fig. 3. Despite a higher reticulocyte count \((P < 0.05)\) in the LHTL group \((17.4 \pm 2.8\%)\) than in the placebo group \((12.7 \pm 3.3\%)\) at the end of the intervention period, \(Hb_{mass}\) was not affected and remained unchanged in both groups (Fig. 3, A and B). Furthermore, neither \([Hb]\) nor hematocrit revealed a statistically significant difference between the two groups. However, during and after the LHTL intervention, both groups experienced a marked hemoconcentration with a parallel decrease in total blood volume and plasma volume and a concomitant increase in \([Hb]\) and hematocrit (Table 2).

At W 4—thus at the end of the LTHL intervention—an increase in \(Hb_{mass}\), which exceeded the typical error of 2.6% for the CO rebreathing procedure, was present in five of 10 subjects within the LHTL group, whereas in three subjects, \(Hb_{mass}\) had decreased by >2.6% (Fig. 3C). The corresponding individual changes in the placebo group are illustrated in Fig. 3D.

No change in urine Epo was detected across time in the placebo group, with values of \(26.5 \pm 13.9\, ng/l\) at BASELINE, \(26.9 \pm 14.3\, ng/l\) after 2–6 days, and \(27.0 \pm 15.2\, ng/l\) after 20–27 days of placebo exposure. In the LHTL group, urine Epo was higher \((P < 0.05)\) after 2–6 days of LHTL exposure \((35.7 \pm 26.8\, ng/l)\) than at BASELINE \((20.8 \pm 15.5\, ng/l)\) but had returned to levels similar to BASELINE after 20–27 days of LHTL exposure \((28.7 \pm 20.2\, ng/l)\). The relative increase in urine Epo between BASELINE and short-term hypoxic exposure \((days 2–6)\) was found to be higher in the LHTL than in the placebo group \((P < 0.05)\). However, absolute values at 2–6 days did not differ significantly between groups, and furthermore, there was no correlation between hypoxia-induced changes in urine Epo and the corresponding variations in either reticulocyte count or \(Hb_{mass}\) at any time point (results not shown).

### Maximal Exercise Capacity

VO\(_2max\) is illustrated in Fig. 4, and Table 3 summarizes further cardiorespiratory parameters obtained during maximal bicycle-ergometer exercise. The 8 wk of training increased VO\(_2max\), expressed in ml/min\(^{-1}\), by an average of 1.0 ± 3.8% in all subjects \((P = 0.06)\). However, VO\(_2max\) remained unaffected by the intervention in the LHTL group and did not differ between groups at any time point. At W 4—thus at the end of the LHTL intervention—VO\(_2max\) was increased by 2.0 ± 1.6% in the placebo group and 0.0 ± 2.8% in the LHTL group \((P = 0.50\) for factor “time”, and \(P = 0.65\) for interaction between factors time and “group”). Correlation analysis indicated that in both groups, individual changes in VO\(_2max\) were not correlated to changes in either \(Hb_{mass}\) or \([Hb]\).

Similar to the normoxic trials, VO\(_2max\) in hypoxia remained unaffected by the intervention (Fig. 4C) in both groups.

### Arterial Blood Parameters During Exercise

Arterial blood parameters are summarized in Table 4, A (normoxic exercise) and B (hypoxic exercise at 2,500 m). The main result is that in both environmental conditions, 3 wk of LHTL exposure induced an increase in arterial \([Hb]\), which supports the hemoconcentration observed in venous blood during CO rebreathing.

### Cycling Economy

Cycling economy, expressed as average VO\(_2\) during the higher warm-up workloads in normoxia (200 W) and hypoxia (150 W), is presented in Fig. 5. During the normoxic trials at W 1 Post (Fig. 5A), cycling economy at 200 W was decreased

---

Table 1. Efficacy of subject blinding

<table>
<thead>
<tr>
<th>Days of LHTL</th>
<th>Guessed right</th>
<th>Guessed wrong</th>
<th>Could not decide</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>8 (50%)</td>
<td>3 (19%)</td>
<td>5 (31%)</td>
</tr>
<tr>
<td>8</td>
<td>7 (44%)</td>
<td>6 (38%)</td>
<td>7 (44%)</td>
</tr>
<tr>
<td>11</td>
<td>5 (31%)</td>
<td>4 (25%)</td>
<td>3 (19%)</td>
</tr>
<tr>
<td>18</td>
<td>7 (44%)</td>
<td>6 (38%)</td>
<td>3 (19%)</td>
</tr>
<tr>
<td>25</td>
<td>8 (50%)</td>
<td>4 (25%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>28</td>
<td>4 (25%)</td>
<td>8 (50%)</td>
<td>8 (50%)</td>
</tr>
</tbody>
</table>

Number and fraction of subjects, able or unable to determine whether exposed to hypoxia or not. Time effect was not significant \((P = 0.639)\). “Guessed wrong” and “Could not decide” answers were pooled. The maximal likelihood for the subjects to guess right was \(P = 0.30\). The maximal likelihood for them to guess wrong or could not decide was \(P = 0.06\). LHTL, live high–train low.

Table 2. \([Hb]\), hematocrit, and intravascular volumes evaluated by carbon monoxide rebreathing

<table>
<thead>
<tr>
<th></th>
<th>Placebo group ((n = 6))</th>
<th>LHTL group ((n = 10))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BASELINE</td>
<td>W 3</td>
</tr>
<tr>
<td>([Hb]) (g/d)</td>
<td>14.1 ± 0.3</td>
<td>15.2 ± 0.6*</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>43.0 ± 0.7</td>
<td>46.8 ± 1.6*</td>
</tr>
<tr>
<td>RCV (l)</td>
<td>3.00 ± 0.33</td>
<td>3.01 ± 0.27</td>
</tr>
<tr>
<td>PV (l)</td>
<td>3.98 ± 0.37</td>
<td>3.43 ± 0.38*</td>
</tr>
<tr>
<td>BV (l)</td>
<td>6.97 ± 0.70</td>
<td>6.44 ± 0.61*</td>
</tr>
</tbody>
</table>

\([Hb]\), venous Hb concentration; Hct, hematocrit; RCV, total red blood cell volume; PV, blood plasma volume; BV, total blood volume; BASELINE, first 2 wk; W 3, after 3 wk in the LHTL intervention; W 4, on the last 3 days of the LHTL intervention; W 1 POST and W 2 POST, 1 and 2 wk, respectively, after termination of the intervention period. Values are means ± SD; \(*P < 0.05\) versus BASELINE; no significant differences between groups were observed.
by 6.9% in all subjects ($P < 0.05$). However, the economy was never significantly different between the two groups. Economy evaluated during the hypoxic trials was unchanged throughout the entire study in both groups and never differed between groups (Fig. 5B).

**Time-Trial Performance**

Average power output during the 26.15-km time trial, expressed in Watt, is illustrated in Fig. 6. We observed a constant trend toward an increase in time-trial performance in all of our subjects over the course of the 8 wk, averaging 5% at the end of the study ($P = 0.12$). However, there was no effect of the LHTL intervention, and thus time-trial performance was always similar between the two groups. Separate analysis of five specific segments of the time trial (including climbs, descents, and flat sections) revealed no difference between groups either. At the end of the intervention period, i.e., at W 4, time-trial performance was increased by 5% in the placebo group and by 2% in the LHTL group ($P = 0.12$ for factor time, and $P = 0.33$ for interaction between factors time and group).

**DISCUSSION**

In the present study, we applied a double-blinded and placebo-controlled design to investigate the effects of LHTL on endurance performance of highly trained endurance cyclists. Our main finding is that LHTL did not affect Hb\textsubscript{mass}, 26-km time-trial performance, VO\textsubscript{2max}, or exercise economy in normoxia and moderate normobaric hypoxia, indicating that 4 wk of LHTL, using normobaric hypoxia, may not be superior to conventional endurance training.

**Impact of LHTL on Aerobic Performance**

Our findings are in contrast to the results of the initial LHTL study (34) and several follow-up studies, where improvements in VO\textsubscript{2max} (7, 16, 50, 57) or endurance performance (57, 59) have been reported. This is intriguing, since we designed the LHTL intervention according to generally accepted recommendations, which for simulated altitude, range from 4 wk at 2,500 – 3,000 m (12–16 h/day) (61) to 18 days at 2,500 m (12 h/day) (44). Our total hypoxic dose exceeded 440 h at 2,500–3,000 m and was among the highest compared with studies.
conducted in the past (7, 19, 33, 44, 48–50, 52, 53, 58, 59, 61). Together with the strict control of the administrated atmosphere in the two groups, lower-pulse SaO2 confirms hypoxemia in our subjects, whereas the elevated urine Epo levels and higher reticulocyte counts in the LHTL group suggest an accordingly physiological response. It is therefore unlikely that our results were associated with an insufficient hypoxic stimulus in the LHTL group. Furthermore, daily oral iron supplementation was provided, and the intake was controlled to avoid iron insufficiency. Finally, training intensity was well preserved in all groups over the course of the study, and the slight (nonsignificant) increase in VO2max and time-trial performance in our already highly trained subjects indicates that the training stimulus was adequate. This raises the question as to why the LHTL group did not benefit from the 4 wk of the intervention. In this context, it is important to note that in the present study, the intervention failed to reproduce the physiological adaptations that have previously been proposed to underlie performance gains following LHTL, and accordingly, the lack of an ergogenic impact appears as a logical consequence. The absence of physiological adaptations to hypoxia in the LHTL group is discussed further below.

With the assessment of measures for aerobic performance using a double-blinded design, we had speculated to demonstrate that the ergogenic impacts of LHTL are independent of a placebo effect. Since earlier LHTL studies were always conducted with an unblinded design, it cannot be ruled out that a placebo effect may at least in part be responsible for the performance gains. This limitation may be particularly crucial in LHTL studies where the expected effects are minor (20). In fact, a recent meta analysis of 51 LHTL studies reported that the potential performance gains in elite athletes is ~4% (5). It is important to note in this context that with this large number of studies, the design and the quality are subject to great variation, which may have affected the outcome of the meta analysis. It nevertheless remains intriguing that an earlier study investigating the influence of a placebo effect on time-trial performance of competitive cyclists observed improvements that were in the same range as reported in the meta analysis (11). Furthermore, an open-label study design, as has been used in all previous LHTL studies, may not only have led to performance gains in the intervention group but may also have hampered the control group by a “nocebo effect” (3), i.e., the detrimental impact of low expectations and demotivation. This may have further widened the gap between subjects of the control and the intervention groups and ultimately created a statistically significant difference in some studies. To overcome this limitation, the present study was performed with a placebo-controlled design, and as illustrated in Table 1, our strategy to blind the subjects was successful. Nevertheless, since in our study, LHTL failed to improve aerobic performance, the present results cannot exclude that the performance benefits of LHTL reported in the past were, at least in part, related to a placebo effect. It could be retorted that if LHTL would benefit athletes by a placebo effect, our placebo group should have improved performance. Nevertheless, our subjects were always uncertain about their respective group classification, which may have prevented a potential placebo effect, as it may occur in open-label studies.

In summary, our study provides no indication for LHTL, using normobaric hypoxia, to improve time-trial performance or VO2max of highly trained endurance cyclists more than conventional training. Given the considerable financial and...
logistic effort of performing a LHTL camp, this should be taken into consideration before recommending LHTL to elite endurance athletes.

**Potential Mechanisms Underlying LHTL**

In their initial LHTL study, Levine and Stray-Gundersen (34) related the aerobic improvements following LHTL to a 8% gain in RCV, which correlated (r = 0.37; P = 0.02) to the increase in VO2max. They hence concluded that the mechanism underlying LHTL is an enhanced, convective O2 transport capacity, secondary to erythropoiesis stimulated by hypoxia. This approach seems convincing, since convective O2 transport is the primary limiting factor of VO2max (32, 51) and because acute manipulations of blood O2 carrying capacity entail changes in VO2max accordingly (14, 18, 40). Nevertheless, although the hypoxic exposure in the present study conformed with the generally accepted recommendations (44, 61) and despite quantifying Hbmass on five different occasions with duplicate measurements during BASELINE and 4 W, we failed to detect an increase in Hbmass in the LHTL group. In both groups, however, we observed some individuals to increase or decrease Hbmass by the >2.6% typical error associated to our measurement, but these were all in a range that can be explained by natural variations over time (42). Accordingly, the increase in [Hb] in both groups was not caused by erythropoiesis but by a reduction in plasma volume. The latter may have counteracted a potential ergogenic effect of a higher O2 carrying capacity secondary to the enhanced [Hb] (27). This explanation is supported by the fact that individual changes in [Hb] were not correlated to changes in VO2max.

The intriguing observation that Hbmass remained unaffected raises the question as to why the hypoxic stimulus failed to reproduce a erythropoietic response similar to that observed by Levine and Stray-Gundersen (34). Since Hbmass appears insensitive to a placebo effect, this finding cannot be explained by the applied study design, and accordingly, other explanations should be considered.

In the initial LHTL study (34), subjects lived at natural altitude, as opposed to the artificially created normobaric hypoxia used in the present study, and it is tempting to conclude that this might explain the different outcomes of the two studies. Since performing a LHTL camp at natural altitude is related to a considerable logistic effort and due to the geographical conditions not feasible in many countries, the use of normobaric hypoxia is an appealing alternative for athletes. However, this is based on the assumption that the response to altitude is solely dependent on O2 partial pressure and unrelated to barometric pressure. This has been questioned recently (12), and several studies have reported slight differences between the effects of normobaric and hypobaric hypoxia (37, 46, 54). Most importantly, in the present context, the reduction in CaO2 seems to be more marked in hypobaric than in equivalent normobaric hypoxia (54). For this reason, the recommended minimal “altitude”

---

**Table 3. Workload and respiratory parameters during maximal cycling exercise**

<table>
<thead>
<tr>
<th></th>
<th>Placebo group (n = 6)</th>
<th>LHTL group (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BASELINE</td>
<td>W 3</td>
</tr>
<tr>
<td>Workload (W)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>411 ± 23</td>
<td>412 ± 31</td>
<td>422 ± 28</td>
</tr>
<tr>
<td>VCO2 (l/min)</td>
<td>5.35 ± 0.46</td>
<td>5.25 ± 0.45</td>
</tr>
<tr>
<td>VE /VCO2</td>
<td>37.3 ± 2.7</td>
<td>37.0 ± 2.2</td>
</tr>
<tr>
<td>Ve /VE</td>
<td>34.4 ± 2.3</td>
<td>34.2 ± 1.1</td>
</tr>
<tr>
<td>RER</td>
<td>1.08 ± 0.04</td>
<td>1.08 ± 0.04</td>
</tr>
<tr>
<td>Ve (l/min)</td>
<td>187 ± 5</td>
<td>183 ± 13</td>
</tr>
<tr>
<td>F (min−1)</td>
<td>60 ± 8</td>
<td>59 ± 8</td>
</tr>
</tbody>
</table>

---

**Table 4A. Arterial blood parameters during exercise in normoxia**

<table>
<thead>
<tr>
<th></th>
<th>Placebo group (n = 5)</th>
<th>LHTL group (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BASELINE</td>
<td>W 3</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 W</td>
<td>95.3 ± 1.2</td>
<td>94.5 ± 1.7*</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>92.5 ± 4.0</td>
<td>91.7 ± 3.4</td>
</tr>
<tr>
<td>[Hb]art (g/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 W</td>
<td>14.9 ± 0.4</td>
<td>15.2 ± 0.5</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>15.6 ± 0.7</td>
<td>16.3 ± 0.7</td>
</tr>
<tr>
<td>[La]art (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 W</td>
<td>1.4 ± 0.5</td>
<td>1.6 ± 0.6</td>
</tr>
<tr>
<td>pHart</td>
<td>7.43 ± 0.02</td>
<td>7.41 ± 0.02</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>7.22 ± 0.03</td>
<td>7.22 ± 0.06</td>
</tr>
</tbody>
</table>

**Table 4B. Arterial blood parameters during exercise in acute normobaric hypoxia (2,500 m)**

<table>
<thead>
<tr>
<th></th>
<th>Placebo group (n = 5)</th>
<th>LHTL group (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BASELINE</td>
<td>W 3</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 W</td>
<td>88.3 ± 2.9</td>
<td>88.4 ± 1.9</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>86.7 ± 5.0</td>
<td>83.2 ± 5.3</td>
</tr>
<tr>
<td>[Hb]art (g/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 W</td>
<td>14.9 ± 0.6</td>
<td>14.9 ± 0.9</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>15.8 ± 0.5</td>
<td>16.2 ± 0.7</td>
</tr>
<tr>
<td>[La]art (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 W</td>
<td>1.2 ± 0.5</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>pHart</td>
<td>16.2 ± 3.6</td>
<td>15.2 ± 3.4</td>
</tr>
</tbody>
</table>

Values are means ± SD; *P < 0.05 versus BASELINE; †P < 0.05 versus placebo.
for LHTL is also higher for normobaric hypoxia than for hypobaric hypoxia (44, 61), and this was taken into account in the present study. It should also be recognized that a previous study, conducted in the very same facilities, applied an equal degree of normobaric hypoxia and observed a pronounced increase in Hbmass in the intervention group (7). We therefore conclude that neither the form nor the degree of hypoxia applied in the present study can explain the absence of an erythropoietic impact in the LHTL group.

A further potential explanation may be the loss of plasma volume, which was observed in both groups and accordingly, not a response to hypoxic exposure per se. Although the increase in urinal Epo concentration in the LHTL group speaks against it, the resulting hemoconcentration might have suppressed Epo synthesis and therefore, counteracted a potential erythropoietic stimulus in the LHTL group (4). We can indeed not explain the observed hemoconcentration, but it might have been a consequence of using normobaric hypoxia, which required our subjects to reside inside the hypoxic rooms for a considerable part of the day and thus limited their involvement in regular daily activities. It may be argued that these unusually long periods of inactivity might have triggered a reaction similar to that observed during bed-rest confinement, where a loss of plasma volume has been reported (23). Since the impact of room confinement in elite athletes remains uncertain, a potential effect on the plasma volume contraction in our subjects cannot be excluded, although the observation that plasma volume did not normalize after the end of the intervention period argues against it.

Although the absence of an increase in Hbmass in our study was unexpected, it is partially supported by a retrospective analysis of the original data of Levine and Stray-Gundersen and coworker (10), where an increase in RCV was observed only in those subjects who also experienced an increase in endurance performance, which corresponded to 55%. This indicates a considerable interindividual variation in the response to LHTL, which is supported further by other studies reporting that the increase in serum Epo during prolonged

![Fig. 5. Exercise economy during submaximal cycling in normoxia (A) and normobaric hypoxia (B). Economy in both conditions was evaluated as VO2, averaged over the last minute of the higher warm-up workload (i.e., 150 W in hypoxia and 200 W in normoxia). The columns represent mean values in both groups, and SDs are indicated by error bars. The normoxic BASELINE values represent the average of the duplicate measurement performed. At W 1 POST, economy was slightly decreased in both groups compared with BASELINE (as indicated by *), but no significant differences between groups were observed.](http://jap.physiology.org/content/japplphysiol/109/5/114/F5)

![Fig. 6. Time-trial performance is expressed as mean power output over a 26.15-km-simulated time trial. Individual results of the subjects in the LHTL group (A) and the placebo group (B) are presented. The BASELINE values represent the better results of the duplicate measurement performed. Group average values are represented by the triangles in both figures. No significant changes or differences between groups were observed.](http://jap.physiology.org/content/japplphysiol/109/5/114/F6)
exposure to high altitude is variable by a factor >40 between individuals chronically exposed to very high altitude (45) or from −41 to 400% in subjects acutely exposed to moderate hypoxia (17). It thus appears that in LHTL studies, the presence of an effect on Hbmass crucially depends on the random composition of the subject groups. This may, in combination with differences in study design, be the reason for the various outcomes of previous LHTL studies regarding gains in Hbmass/RCV and perhaps also explain the lack of an increase in Hbmass in the present study. In this context, it could be considered whether the already high BASELINE Hbmass in our subjects could have contributed to our negative findings. Indeed, the BASELINE values for Hbmass and RCV in our subjects were considerably higher than those reported in previous LHTL studies, demonstrating the most marked erythropoietic effects (7, 34, 59). The high Hbmass may have blunted a potential erythropoietic response compared with less-trained subjects with accordingly lower Hbmass. This, however, needs further research to be confirmed.

In summary, while the results of some earlier studies (7, 34, 53, 57, 59) indicate that LHTL may stimulate erythropoiesis in some athletes, our results demonstrate that this response is not certain in all subjects, which is probably explained by different individual responses to hypoxia (25). Coaches and athletes should be aware of this issue and remember that an individual’s erythropoietic response to LHTL is, to date, difficult to predict.

The other common explanation for LHTL to increase performance is related to skeletal muscle adaptations to hypoxia (20). Gore and coworkers (19, 52, 53) base this on the observation of a higher exercise economy or muscle-buffer capacity (19) following LHTL. Exercise economy is, next to VO2max and the percentage of VO2max at which exercise is performed, one of the main components that determine the velocity in endurance events (13, 24, 26). Hence, an increased economy following LHTL would likely have improved our subjects’ performance in the time-trial tests. However, we found no indications for LHTL to affect whole-body VO2 during submaximal cycling performed on numerous occasions. Although an unchanged economy following LHTL has been reported previously (39), it is difficult to explain the difference with the studies in which a reduced submaximal exercise VO2 has been observed (19, 28, 29, 52, 53). Nevertheless, none of these studies provides an objective description of training intensity, which was either controlled by subjective feedback from the athletes (19, 52) or not at all (28, 29, 53). It is therefore appealing to speculate that the open-label treatment and a concomitant placebo effect motivated LHTL subjects to train harder than their control counterparts, which in turn, may have improved running economy (15).

In summary, the present results provide no indication for LHTL to enhance whole-body exercise economy to an extent that improves aerobic performance. This is in line with our primary finding that LHTL did not enhance any of the parameters that usually determine endurance performance.

**Effect of LHTL on VO2max in Hypoxia**

Winter sports, as well as disciplines from other sports, regularly hold competitions at altitudes ~2,500 m. Even the mild hypoxia associated with such altitudes impairs VO2max by reducing CaO2 (22, 43), and this appears to be pronounced in highly trained individuals (30, 31). Athletes may attenuate this undesirable effect by means that stimulate erythropoiesis and thus normalize arterial blood O2 content. Accordingly, preliminary acclimatization may partly restore VO2max in moderate hypoxia (2, 9). Furthermore, the ergogenic effect of erythropoietic stimulation by recombinant human Epo treatment is pronounced when exercise is performed at moderate altitude (47). Consequently, preliminary altitude exposure may be crucial for the success in endurance races at altitude, particularly on an elite level, where differences in the range of 0.5% may determine the outcome (21). However, during the time span required for altitude acclimatization, athletes have to acquiesce the detrimental impact of hypoxia on high-intensity training quality (6), which may counteract a potential advantage obtained by erythropoiesis. LHTL appears to be an elegant approach to avoid this dilemma, since it may allow for altitude acclimatization while training intensities can be maintained. This was supported by a recent study, where 3 wk of LHTL partially restored VO2max at an altitude of 2,340 m (55). However, this study was designed without a placebo or even a control group, and thus the contribution of a placebo effect and/or training effect cannot be ruled out.

In contrast to these previous findings, the present data do not support the hypothesis that LHTL partly normalizes VO2max at the simulated altitude of 2,500 m. The observation that VO2max in moderate hypoxia was unchanged after LHTL, in spite of a significant increase in [Hb], suggests that either maximal cardiac output or O2 extraction or both were reduced at that time. We conclude that for competitions at moderate altitude, precedent LHTL does not provide an athletic advantage, at least in elite cyclists.

**Limitations**

As discussed earlier, we observed a significant hemococoncentration in both groups, which may have suppressed Epo in the LHTL group (4). Although an Epo response was suggested in the urinal samples obtained from the LHTL group, it has to be emphasized that urinal measurements provide neither the temporal nor quantitative validity of blood measurements (38). Accordingly, we can only draw limited conclusions regarding the contribution of the hemococoncentration to our negative findings.

Another potential limitation is related to the training prescription for the LHTL group, as subjects were instructed to maintain training as constant as possible. Although this has not been scientifically confirmed, a slight reduction of training effort at the onset of a LHTL camp may be preferable in practice to avoid overtraining. However, since we considered the blinding of our subjects toward the group classification most relevant, we could not adapt the training prescriptions to the (simulated) living altitude. Nevertheless, we are convinced that this issue did not mask any effects of the intervention, since in previous studies in which ergogenic effects of LHTL were observed, training efforts were also equal between groups (7, 34, 50). Furthermore, since our subjects were competing in various disciplines and different countries, the present study included both—athletes preparing for an upcoming season (n = 10) and others that had finished their season prior to the study (n = 6). To avoid potential bias from this issue, we equally distributed the athletes of different disciplines, as well
as pre- and postseason athletes, over both groups and clearly instructed them to maintain training effort throughout the study. Nevertheless, we cannot exclude a slight influence of this issue on performance/motivation of our subjects, and the resulting noise of our measurements may have covered small effects of the intervention.

It should also be considered in this context whether the training effort of our subjects was sufficient to prevent detraining, which may also have masked an ergogenic impact in the LHTL group. However, the positive development of time-trial performance and VO\textsubscript{2max} in our already highly trained athletes speaks against this.

It is important to note that our protocol did not allow subjects to schedule a pronounced recovery period prior to exercise tests, which is in contrast to preparation for an actual competition. To avoid disturbance of the subjects’ training regime, we instructed them to perform only one easy training day prior to exercise tests, which is just a minimal form of tapering and might have slightly hampered performance in the tests. This was, however, the same for both groups, and it is thus doubtful that differences between groups were masked by fatigue.

Furthermore, it has to be considered whether the moderate elevation of our living and testing facilities contributed to the results of our study. However, a beneficial impact of a erythropoietic response would, if at all, be more pronounced in mild hypoxia (47). It may also be argued that the initial 2 wk at 1,135 m (the lead-in period) might have triggered a slight physiological response that has hampered a further adaption in the LHTL group during the intervention period. This is, however, unlikely, since even at the higher altitude of 1,600 m, no effects on [Hb] or hematocrit are usually observed (60). Most importantly, it must be acknowledged that in a previous LHTL study conducted in the same facilities and with a very similar protocol, the intervention improved the aerobic performance of runners (7). Hence, the natural elevation of the housing/testing facilities seems not to be a relevant factor for the outcome of our study.

Finally, we chose to perform the LHTL study using normobaric hypoxia, and we can thus draw limited conclusion regarding LHTL at natural altitude. Future studies will have to establish whether athletes respond differently to these two forms of hypoxia.

Conclusion

Contrary to our hypothesis, the present results indicate that LHTL, using normobaric hypoxia, may not improve endurance performance at sea level or moderate altitude more than conventional training. Our results further suggest that the erythropoietic response to LHTL is subject to considerable interindividual variation and may be blunted in elite athletes, where baseline Hb\textsubscript{mass}/RCV is already high. Finally, it is indicated that daily room confinement in elite athletes may lead to a reduction in plasma volume and thereby, counteract a potential erythropoietic response to normobaric LHTL. Future studies are encouraged to investigate the parameters underlying the interindividual variation in the response to LHTL and to compare the effects of LHTL at terrestrial altitude with those obtained with normobaric hypoxia.

GRANTS

Funding for this study was through grants obtained from Bundes Amt für Sport (BASPO; Switzerland), Team Denmark (Denmark), Ministère des Sports (France), and Institut National du Sport, de l’Expertise et de la Performance (France).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.S., P. Robach, R.A.J., P. Rasmussen, N.N., V.D., M.M., and C.L. conception and design of research; C.S., P. Robach, R.A.J., P. Rasmussen, N.N., V.D., A.C., and N.V.O. performed experiments; C.S., P. Robach, R.A.J., P. Rasmussen, N.N., V.D., and N.V.O. analyzed data; C.S., P. Robach, and C.L. interpreted results of experiments; C.S. prepared figures; C.S. drafted manuscript; C.S. edited and revised manuscript; C.L. approved final version of manuscript.

REFERENCES


