Live high-train low for 24 days increases hemoglobin mass and red cell volume in elite endurance athletes

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Wehrlin, Jon Peter, Peter Zuest, Jostein Hallén, and Bernard Marti. Live high-train low for 24 days increases hemoglobin mass and red cell volume in elite endurance athletes. J Appl Physiol 100: 1938–1945, 2006. First published February 23, 2006; doi:10.1152/japplphysiol.01284.2005.—The effect of live high-train low on hemoglobin mass (Hbmass) and red cell volume (RCV) in elite endurance athletes is still controversial. We expected that Hbmass and RCV would increase, when using a presumably adequate hypoxic dose. An altitude group (AG) of 10 Swiss national team orienteers (5 men and 5 women) lived 1,800 and 1,000 m above sea level for 24 days. Before and after altitude, Hbmass, RCV (carbon monoxide rebreathing method), blood, iron, and performance parameters were determined. Seven Swiss national team cross-country skiers (3 men and 4 women) served as “sea level” (500–1,600 m) control group (CG) for the changes in Hbmass and RCV. The AG increased Hbmass (805 ± 209 vs. 849 ± 225 g; P < 0.01) and RCV (2,353 ± 611 vs. 2,470 ± 653 ml; P < 0.01), whereas there was no change for the CG (Hbmass: 849 ± 197 vs. 858 ± 205 g; RCV: 2,373 ± 536 vs. 2,387 ± 551 ml). Serum erythropoietin (P < 0.001), reticulocytes (P < 0.001), transferrin receptor (P < 0.001), soluble transferrin receptor (P < 0.05), and hematocrit (P < 0.01) increased, whereas ferritin (P < 0.05) decreased in the AG. These changes were associated with an increased maximal oxygen uptake (3,515 ± 837 vs. 3,660 ± 770 ml/min; P < 0.05) and improved 5,000-m running times (1,098 ± 104 vs. 1,080 ± 98 s; P < 0.01) from pre- to postaltitude. Living at 2,500 m and training at lower altitudes for 24 days increases Hbmass and RCV. These changes may contribute to enhance performance of elite endurance athletes.

The concept of living at “high” altitude and training at “low” altitude (“live high-train low,” LHTL) has been increasingly used in recent years by endurance athletes with the expectation that sea-level performance may as a consequence be improved (36). The LHTL strategy combines living at moderate altitude, to increase hemoglobin mass (Hbmass) and red cell volume (RCV), with training at low altitude to maintain a high absolute training intensity (26). This concept has been shown to be superior to normal sea-level training or classical live high-train high (LHTH) altitude training for improving sea-level performance in elite endurance athletes (24). However, studies of whether exposure to moderate altitude increases Hbmass and RCV in elite endurance athletes have given controversial results (2, 16, 25). Results from the only published LHTL study that reported increase in RCV (24) have been discussed (2, 17), because RCV was measured indirectly with the Evans blue dye method, for which the adequacy for estimating RCV after hypoxic exposure has been questioned (13, 16, 27). LHTL studies that directly measured Hbmass with the carbon monoxide (CO)-rebreathing method did not report increased Hbmass and RCV (2, 3, 8).

However, two LHTH studies, in which subjects generally spend more time at altitude, have recently reported increased Hbmass after exposure to moderate altitude (9, 18). Thus, it has been hypothesized that the hypoxic dose (living altitude combined with the duration of the altitude exposure) is the key factor (23, 28). To our knowledge, no controlled LHTL study has been published that has used a presumably adequate hypoxic dose at real altitude similar to the study by Levine and Stray-Gundersen (24) and measured Hbmass directly with the CO-rebreathing technique. Therefore, the purpose of our study was to investigate the effects of living at an altitude of ~2,500 m and training at lower altitudes for 24 days on erythropoiesis in elite endurance athletes by using direct measurement of Hbmass.

MATERIAL AND METHODS

Subjects

Ten athletes (5 women and 5 men) from the Swiss national orienteering team, aged 23 ± 4 yr, and seven athletes from the Swiss national cross-country team (4 women and 3 men), aged 21 ± 1 yr, gave written, informed consent to participate in the study, which was approved by the institutional review board of the Swiss Federal Institute of Sport and was carried out according to the recommendations of the Helsinki Declaration.

Study Design

The orienteering athletes were assigned to the altitude group (AG) and completed a 24-day LHTL phase, living 18 h per day at 2,456 m and training at 1,800 and 1,000 m above sea level, in the Swiss Alps. The cross-country skiers were assigned to the control group (CG), completing a normal training phase, which consisted of living and training between 500 and 1,600 m for 24 days. The study was carried out during the preseason for both groups (different time of the year for orienteers and cross-country skiers). An outline of the study design is presented in Fig. 1. Approximately 4 wk before the LHTL phase (AG) and before the experimental phase (CG) (A), blood samples were taken for measurement of serum ferritin (Ftn) to assess bone marrow iron stores. At the pretest (B), 1 day before the LHTL phase began, a blood sample was taken and the athletes from both groups performed a maximal oxygen uptake (V̇O₂ max) test in the laboratory. About 7–10 h later on the same day, the AG ran a 5,000-m time trial on a 400-m track. The blood volume parameters were measured the next day (AG and CG). Additional blood samples where taken from the AG athletes at day 1 (C), day 12 (D), and day 24 (E) of the LHTL phase. Eight days after the 24-day phase (F), the athletes performed the posttest with identical measurements as at the pretest B with the exception that the CG did not perform the V̇O₂ max test.

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Evaluation of Blood Volume Parameters

Hb\text{mass}, RCV, plasma volume (PV), and blood volume (BV) were determined by the CO-rebreathing method according to Burge and Skinner (6) with minor modifications (20). The method is briefly described here. After 15 min in a sitting position, four capillary blood samples (30\,\mu l) were taken from an earlobe and analyzed for carboxyhemoglobin (HbCO) by a hemoximeter (ABL 520, Radiometer A/S, Copenhagen, Denmark). The mean of the four HbCO values was taken as the baseline HbCO value. Subjects were then connected to a Krogh spirometer filled with a mixture of oxygen (5 liters) and CO. The volume of inspired CO varied between 50 and 100 ml depending on gender, barometric pressure, measured V\text{O}_2\text{max}, and body mass with the goal of reaching a \Delta HbCO (difference between baseline values and plateau values) between 5 and 7%. The athletes breathed the gas mixture in the closed system for 12 min. If necessary, oxygen was refilled. Every 2 min, earlobe blood samples were taken for assessment of HbCO. All blood samples for measurement of HbCO were immediately analyzed (10\,s). The HbCO plateau was normally seen after 6–10 min and the mean of three adjacent HbCO values between the 6th and the 12th minute was taken as the plateau value of HbCO. Hb\text{mass}, RCV, BV, and PV were then calculated as described elsewhere (20). For the RCV, PV, and BV calculations, the hemoglobin (Hb) and hematocrit (Hct) values from a venous blood sample taken on the same day were used. The same equipment was used by the same investigators for all tests. The reproducibility of the blood volume parameters (coefficient of variance; CV) was determined in a separate experiment, in which Hb\text{mass}, RCV, PV, and BV were measured in other subjects two times separated by 24 h. We did this twice, once during the LHTL phase of the AG (n = 11) and once during the experimental phase of the CG (n = 7). The mean measured CV during the AG and CG were Hb\text{mass} = 1.7 and 1.4%, RCV = 2.2 and 2.1%, PV = 4.8 and 4.5%, BV = 3.4 and 3.5%, respectively.

Blood Sample

All blood samples were drawn from a cubital vein under standardized conditions (between 7:00 and 8:00 AM before breakfast, in supine position after 15 min of rest). Blood samples from both groups were analyzed for Hb concentration (modified cyanomethemoglobin method, Coulter Gen S, Beckmann), Hct (Coulter Gen S, Beckmann), and serum Ftn (photochemiluminescence; Advia Centaur, Bayer, Leverkusen, Germany). Blood samples from the AG were also analyzed for serum erythropoietin (sEpo; chemiluminescence immunoassay; Advantage, Nichols Institute Diagnostics, San Juan Capistrano, CA), reticulocytes (Rct; flow cytometry; Epics XL, Beckmann), transferrin (TF; immunoassay; Cobas Integra 800; Roche Diagnostics, Basel, Switzerland), and soluble transferrin receptor (sTfR; immunoturbidimetric assay; Cobas Integra 800; Roche Diagnostics). At the pretest, all blood samples were analyzed twice and the CV for the different parameters was calculated: Hb = 0.3%, Hct = 0.6%, Ftn = 4.5%, sEpo = 9.8%, Rct = 6.7%, TF = 0.64% and sTfR = 2.0%.

Evaluation of Performance

5,000-m time trial (AG). The pre- and posttest 5,000-m time trials were conducted on a 400-m track at 400 m above sea level at 7 PM under similar conditions (temperature 19.8 and 20.3°C, humidity 79 and 67%; air pressure 969 and 972 hPa, for pre- and posttest, respectively). Heart rate was monitored throughout the run, and rating of perceived exertion (RPE) was recorded immediately after the run by using the category scale of Borg (5). A capillary blood sample was taken from the earlobe to measure blood lactate concentration.
Vo₂max tests. The AG performed Vo₂max tests at pre- and posttest
to determine Vo₂max, maximal ventilation, maximal heart rate
(HRmax), maximal blood lactate ([La-1]b-max.), and time to exhaustion
(TTE). These tests were conducted on a treadmill in the laboratory
at the Swiss Olympic Medical Center (SOMC) in Magglingen, located
900 m above sea level. After a 10-min warm-up jog, the athletes
began running at their individual anaerobic threshold intensity
(previously determined). The speed was increased by 1 km/h every
minute until the subjects reported having ~90 s left until exhaustion.
The treadmill incline was set at 0% throughout the test. Identical “individ-
ual” tests were used for the pre- and the posttest. Gas exchange was
measured breath by breath with an open-circuit system (Oxycon Pro,
Jaeger-Toennies, Hochberg, Germany), heart rate was monitored with
Polar Accurex plus (Polar Electro, Kempele, Finland), and blood
lactate was analyzed with Ebbio-Plus (Eppendorf, Germany). The CG
performed a Vo₂max test at pretest only, at the SOMC Bad Ragaz
located 400 m above sea level, using identical equipment, but another
protocol: After a warm-up jog, the male athletes began running at 13
km/h. The speed was increased by 1 km/h every minute for the first 3
min of the test and thereafter by 0.5 km/h every 30 s until exhaustion.
The female athletes followed the same protocol but started at 11 km/h.
The treadmill incline was set at 7% throughout the test. During the
Vo₂max tests, both athletes and experimenters were blinded for any
result.

Training regimen. The AG completed low- and moderate-intensity
training at an altitude of 1,800 m (1–2 training sessions per day),
whereas the high-intensity training was performed at 1,000 m
above sea level (twice per week). The AG completed all training (1–2
training sessions per day) at altitudes between 400 and 1,600 m. For
both groups, ~85% of the training completed was at low and mod-
erate intensity and 15% was at high intensity.

Supplementation (AG). The AG athletes started a combined iron
(Ferrum Hausmann, 100 mg Fe2+ /day orally; Astellas Pharma, Le-
derdorp, Netherlands), multivitamin (Burgerstein multivitamin-min-
eral ABC 25; Burgerstein Nährstoffe; Rapperswil, Switzerland), and
vitamin C (Burgerstein vitamin C; Burgerstein Nährstoffe) supple-
mentation when the LHTL phase began. Despite preliminary testing
and oral supplementation, two female and one male athlete had Fe
levels below 20 µg/l at the start of the LHTL phase. The low Fe
values must be seen in the light of high PV, and we assume that these
athletes actually had no relevant iron deficiency under normal sea-
level training conditions. Because we wanted to be on the safe side for
the novel circumstances at altitude, these athletes received venous iron
supplementation (Venofer; Novartis, Basel, Switzerland) in the first
week. The iron status results of these three subjects were therefore
excluded from the data. The changes in Hbmass, and performance
values during LHTL in these subjects did not differ from those
observed in the other athletes. We therefore did not exclude other data
from these three athletes.

Statistics

Data are presented as means ± SD in tables and as means ± SE
in figures. The effect of time on several blood parameters measured
before, during, and after the LHTL phase was evaluated with
one-factor analysis of variance for repeated measures. When the F
value was considered statistically significant (P < 0.05), the Bonferroni
correction was used to evaluate differences at the different time points
in relation to the pretest value at sea level. Differences between pre-
and posttest within the groups were evaluated by paired Student’s t-tests,
and differences between the two groups were analyzed by comparing the absolute group
differences between pre- and posttest with unpaired t-tests. The relationship
between increase in Hbmass and the increase in Vo₂max
was compared by linear regression and Pearson’s coefficient. All
statistical tests were done with the SPSS statistical package 13.0
(SPSS, Chicago, IL). Significance was set at P < 0.05; P < 0.1
was called a trend.

RESULTS

There was no difference in height, weight, body-mass index,
Hbmass, RCV, PV, and BV between the groups, but the cross-
country skiers (CG) had higher Vo₂max values than the orient-
teers (AG) (Table 1 and Fig. 2).

Blood Volume Parameters

Hbmass increased by 5.3% and RCV increased by 5% from
pre- to posttest (P < 0.01) in the AG, whereas there was no
change in Hbmass or RCV in the CG (Table 2 in absolute
values and Fig. 2 in individual body weight-adjusted val-
ues). The changes in Hbmass and RCV were different be-
 tween the groups (P < 0.01). Neither BV nor PV changed
for either group.

Blood Samples

The time course of Hct, sEpo, Rct, Ftn, TF, and sTfR are
presented in Fig. 3. Hct increased during the LHTL phase
(P < 0.01), and post hoc analysis indicated elevated values
at day 24 (P < 0.05), but values returned to pretest level at
posttest. sEpo was affected by the LHTL phase (P < 0.001),
and post hoc analysis showed higher sEpo values at day 1
(P < 0.001; +120%) and day 12 (P < 0.05; +34%) than at
pretest, whereas the values at day 24 and the posttest were
not different from the pretest values. Rct values were
affected by the LHTL phase (P < 0.001), and post hoc
analysis reported higher values at posttest (17.5 ± 4.2%e; P < 0.05)
than at pretest (12.2 ± 2.9%e). Ftn decreased (P < 0.05),
TF increased (P < 0.001), and sTfR increased (P < 0.05)
during the LHTL phase. For the CG, Hct (43.9 ± 3.7
vs. 42.7 ± 3.5%), Hb (15.7 ± 1.2 vs. 15.5 ± 1.1 g/dl),
and Ftn (65 ± 17 vs. 62 ± 19 µg/l) did not change during
the experimental period (pre- and posttest values, respec-
tively).

Table 1. Anthropometric data and maximal oxygen uptake of the altitude group and the control group

<table>
<thead>
<tr>
<th>Altitude Group</th>
<th>Control Group</th>
</tr>
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<tbody>
<tr>
<td>Height, cm</td>
<td>Height, cm</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>Weight, kg</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>BMI, kg/m²</td>
</tr>
<tr>
<td>Vo₂max, m/kg⁻¹/min⁻¹</td>
<td>Vo₂max, m/kg⁻¹/min⁻¹</td>
</tr>
<tr>
<td>Men 179±5</td>
<td>181±56</td>
</tr>
<tr>
<td>69±4</td>
<td>74±3</td>
</tr>
<tr>
<td>21.5±0.8</td>
<td>22.7±0.4</td>
</tr>
<tr>
<td>62±3</td>
<td>73±2*</td>
</tr>
<tr>
<td>Women 168±5</td>
<td>169±2</td>
</tr>
<tr>
<td>55±2</td>
<td>59±4</td>
</tr>
<tr>
<td>19.6±0.6</td>
<td>20.5±0.7</td>
</tr>
<tr>
<td>51±2</td>
<td>66±6*</td>
</tr>
<tr>
<td>All 174±8</td>
<td>175±7</td>
</tr>
<tr>
<td>62±8</td>
<td>66±8</td>
</tr>
<tr>
<td>20.5±0.6</td>
<td>21.6±1.4</td>
</tr>
<tr>
<td>57±7</td>
<td>70±6*</td>
</tr>
</tbody>
</table>

Values are means ± SD. The altitude group consisted of 5 female and 5 male national team orienteers, and the control group consisted of 4 female and 3 male
national team cross-country skiers. BMI, body mass index; Vo₂max, maximal oxygen uptake. *P < 0.05 and †P < 0.01, differences between the groups.
Performance Parameters (AG Only)

\( \dot{V}O_2_{\text{max}} \) test. \( \dot{V}O_2_{\text{max}} \) increased by 4.1% from pre- to posttest (women: 50.8 ± 2.1 vs. 54.5 ± 2.8 ml·kg\(^{-1}\)·min\(^{-1}\); men: 62.3 ± 5.2 vs. 63.8 ± 5.5 ml·kg\(^{-1}\)·min\(^{-1}\); \( P < 0.05 \), women and men together). TTE increased by 41 s (\( P < 0.05 \)), \( HR_{\text{max}} \) decreased by 3 beats/min (\( P < 0.05 \)) whereas \([La^-]_{\text{b-max}}\) did not change (Table 3). Maximal ventilation increased from 129 ± 33 to 133 ± 32 l/min; \( P < 0.05 \) during the LHTL phase. Spearman’s correlation coefficient for the change in

![Fig. 2](image-url)
Fig. 3. Time course of hematocrit, erythropoietin, reticulocytes, ferritin, transferrin, and the soluble transferrin receptor in the altitude group (5 female and 5 male national team orienteers) measured before and after (day 1 and day 18) and during a “live high” (2,456 m)–“train low” (1,800 and 1,000 m) altitude training camp (day 1, day 12, and day 24). Data are means ± SE. P value indicates the effect of time on the parameter. *P < 0.05 and **P < 0.001, post hoc differences from sea-level conditions (day –1).

Table 2. Blood volume parameters measured before and after the 24-day training period in the altitude group and the control group

<table>
<thead>
<tr>
<th></th>
<th>Altitude Group</th>
<th>Control Group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Hbmass, g</td>
<td>RCV, ml</td>
</tr>
<tr>
<td>Pre</td>
<td>805 ± 210</td>
<td>2,353 ± 611</td>
</tr>
<tr>
<td>Post</td>
<td>849 ± 226*</td>
<td>2,470 ± 65*</td>
</tr>
</tbody>
</table>

Values are means ± SD. The altitude group consisted of 5 female and 5 male national team orienteers; the control group was 4 female and 3 male national team cross-country skiers. Pre, before training; Post, after training; Hbmass, hemoglobin mass; RCV, red cell volume; PV, plasma volume; BV, blood volume BV. *P < 0.01, differences between pre- and posttest.
Hb\text{mass} (\Delta \text{Hb}\text{mass}, \% \text{ and the change in } V_{O2}\text{max} (\Delta V_{O2}\text{max}, \% \text{and}}) \text{ were } r = 0.68 (P = 0.21) \text{ for the men, } r = 0.75 (P = 0.15) \text{ for the women, and } r = 0.35 (P = 0.29) \text{ for men and women together.}

5,000-m time trials. The athletes improved 5,000-m running time by \sim 18 \text{ s (1.6\%}; P \leq 0.05\text{, with no difference in } \text{HR}_{\text{max}}, \text{[L}\text{a}^-\text{]}_{\text{b,-max}} \text{ (Table 3), or } \text{RPE} (18.7 \pm 0.7 \text{ vs. } 19.0 \pm 1.0)\text{.}

**DISCUSSION**

The main results of the present study show that Hb\text{mass} and RCV increased by \sim 5\% after living at 2,456 m while training at lower altitudes (1,800 and 1,000 m) for 24 days. There was no change in Hb\text{mass} and RCV in a control group living and training at altitudes between 500 and 1,600 m for 24 days. The improvements in Hb\text{mass} and RCV in the AG were associated with increased s\text{Epo}, \text{Ret}, \text{TF}, \text{ and sTFR, and decreased Ftn values as well as improved } V_{O2}\text{max and 5,000-m running times.}

**Limitations of the Study**

Any research conducted with elite athletes will encounter the challenge to have an appropriate control group, ideally with a randomized design. To have a sufficient number of elite endurance athletes for one altitude group and one control group, we recruited national team member athletes of two different endurance disciplines. It was not possible to randomly assign athletes to either altitude or normal training, because the athletes and coaches in each discipline preferred to train together. Thus the allocation to the AG or CG was based on the specific endurance discipline. This nonrandomized classification raises the question of whether the athletes in both groups have similar endurance characteristics. Indeed, the cross-country skiers had higher V_{O2}\text{max values than the orienteers, but their } V_{O2}\text{max test was conducted at a lower altitude, which may partly explain the higher results (35). However, both groups consisted of elite athletes who have trained seriously for many years, suggesting that the differences in aerobic capacity were more a genetic predisposition than a difference in training status. Importantly, both Hb\text{mass} and RCV were not different between the groups at pretest. We did not perform specific doping tests during the study, but it must be noted that Switzerland has one of the toughest anti-doping programs, which includes numerous unannounced doping controls. In addition, the reasonable increases in Hb\text{mass} and RCV as well as the small variation between the subjects do not support the suspicion of blood doping during this study. It was not possible to measure s\text{Epo}, \text{Ret, TF, and sTFR in the CG. However, these parameters have been measured in previous controlled studies with a sea-level control group (1, 8), so our results may be compared with these. With the above considerations, we feel that the design adaptations made in this study to evaluate the effect of LHTL training on Hb\text{mass} and RCV did not compromise the validity of the results.}

**Increased Hb\text{mass} and RCV After the LHTL**

The influence of 3- to 4-wk altitude exposure at 2,100–2,500 m on Hb\text{mass} and RCV in endurance athletes is controversial (2, 16, 23, 25, 27) and has recently been part of a point (25)-counterpoint (16) debate in this journal. Within this debate, it is important to differentiate between a number of methodological distinctions, such as different altitudes chosen for living and training, different methods of measuring changes in Hb\text{mass} or RCV (16), and the different performance levels of the athletes. In the classical well-controlled LHTL study conducted by Levine and Stray-Gundersen (24), RCV increased by \sim 5\% in the LHTL group after a 4-wk period of living at 2,500 m and training at 1,250 m. These results have been debated (2, 17), because they measured RCV indirectly with the Evans blue dye method, for which the adequacy for estimating RCV after hypoxic exposure has been questioned (16, 27). Studies using the CO-rebreathing method to directly measure Hb\text{mass} have shown contradictory results. Most of these studies failed to show increased Hb\text{mass} and RCV after altitude training (either LHTL or LHTH) (2, 3, 8, 15, 34). However, as we previously pointed out, it seems obvious that the hypoxic dose is a key factor in this debate. There is no doubt about elevated Hb\text{mass} in lifelong residents (19) of moderate altitude (2,600–3,550 m), including athletes (30). It has therefore been suggested that the hypoxic dose in these studies (2, 3, 8, 15, 34) was too low to significantly increase Hb\text{mass} and RCV (23, 28). It is likely that either the living altitudes were too low (8, 15, 34) and/or the durations of altitude exposure were too short (2, 3), compared with regimens that increased Hb\text{mass} or RCV after LHTL or LHTH (28). The hypoxic dose used in our study was very similar to the one used in the LHTL study conducted by Levine and Stray-Gundersen (24), as the athletes lived at the same altitude (2,456 m in our study, 2,500 m in the Levine and Stray-Gundersen study) for a similar duration (24 vs. 28 days) and trained at a similar altitude (1,800 and 1,000 m vs. 1,250 m). RCV results were also very similar, with RCV increasing by 5\% and Hb\text{mass} by 5\% in our study and RCV increasing by 5\% in the Levine and Stray-Gundersen study. In addition, the increases in Hb\text{mass} and RCV in our study related well (21) to the measurement reproducibility (the increase was 3.1 times higher than the CV for the Hb\text{mass} and 2.3 times higher than the CV for RCV). To our knowledge, no other study has been published that used the LHTL protocol at real altitude and found an increased Hb\text{mass} and RCV. Two recently published studies that used the LHTH protocol also found increased

Table 3. \text{V}_{O2}\text{max test and 5,000-m time trial results measured before and after the 24-day “live high-train low” altitude training camp in the altitude group}  

<table>
<thead>
<tr>
<th>\text{V}_{O2}\text{max Test}</th>
<th>5,000-m Time Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>\text{V}_{O2}\text{max, ml/min}</td>
<td>\text{TTE, s}</td>
</tr>
<tr>
<td>Pre</td>
<td>3,515\pm387 *</td>
</tr>
<tr>
<td>Post</td>
<td>3,660\pm770†</td>
</tr>
</tbody>
</table>

\*Values are means \pm SD. \text{TTE, time to exhaustion; } \text{HR}_{\text{max}, \text{maximal heart rate; } \text{[L}\text{a}^-\text{]}_{\text{b,-max}, \text{maximal blood lactate; Time, 5,000-m time trial time. } \* P < 0.05 and † P < 0.01, differences between pre- and posttest.}
Hb\textsubscript{mass} in endurance athletes after a 3-wk altitude training camp at real altitude (9, 18). Hb\textsubscript{mass} increased 6% after 3 wk of LHTH at 2,100–2,300 m in junior swimmers (9) and 9% in elite biathlon athletes living and training for 3 wk at 2,050 m (18). The higher increase in Hb\textsubscript{mass} in the biathlete study was mainly due to the result of one athlete, whose Hb\textsubscript{mass} increased by 18%, whereas Hb\textsubscript{mass} increased by 7% for the rest of the group (31). Unfortunately, neither LHTH study included a control group, reported the reproducibility of Hb\textsubscript{mass} measurement, or controlled for blood doping (33). In addition, they used a relatively small amount of CO in the CO-rebreathing method in relation to the barometric pressure and the estimated magnitude of the athletes’ Hb\textsubscript{mass}, which may lead to a high measurement error (6). There is only one study (LHTH protocol, no study with LHTL protocol) that used an estimated adequate hypoxic dose and found no increase in Hb\textsubscript{mass}: Gore et al. (14) reported no increase in absolute Hb\textsubscript{mass} after 31 days LHTH at 2,690 m. However, the authors pointed out that all athletes in the study succumbed to illness during the period, a condition that can have depressive effects on the erythropoiesis (11).

Changes in Blood and Iron Parameters After LHTL

The increases in Hb\textsubscript{mass} and RCV in this study were in line with changes in several iron and blood parameters during the LHTH phase. sEpo was elevated 120% at day 1 at altitude compared with the pretest. Such an increase has also been seen in other studies, in which sEpo increased, with considerable individual variation, from sea level to ~2,500 m by 50–150%, measured after ~24 h of altitude exposure (1, 8, 12, 24). The increase in sEpo in the AG in our study was even higher than the 60% reported in the study by Chapman et al. (7) after 30-h altitude exposure for the “responder” group of their athletes. Our results are also supported by Ge et al. (12), who measured the sEpo responses at different altitudes and concluded that the threshold altitude for a robust increase in sEpo is ~2,100–2,500 m. In our study, sEpo was still elevated at day 12 at altitude, which has been determined to be an important factor for a relevant increase in RCV (7). However, an increased sEpo is not necessarily associated with an increase in Rct and Hb\textsubscript{mass}: Ashenden et al. (1) showed no different changes in Rct between an altitude group with three 5-day LHTL exposures at 2,650 m in an altitude house and a sea-level control group. It must be noted that Rct is affected not only by altitude exposure but also by normal endurance training (29). The absolute changes in Rct in controlled altitude training studies that did not show a change in Hb\textsubscript{mass} were within 2–4‰ in the sea-level control groups (2, 3, 8) during controlled periods as long as 30 (2) and 70 (3) days. Thus an absolute change in the reticulocytes of ~2–4‰ reflect normal changes due to training. In our study, the mean absolute change in Rct was within 3‰ during the first 12 days of the LHTL phase but was increased by 7‰ (from 10.2 to 17.5‰) from day 12 to the posttest. It is interesting that this increase occurred at the end of and even after the LHTL phase. We do not know how much of this increase in Rct is due to the altitude-induced increase in erythropoiesis and how much can be attributed to changes in training intensity. However, these results may suggest the importance of spending a sufficient amount of time at altitude. TF and sTfR also increased in the AG, whereas Ftn values decreased, even if the absolute changes were smaller than in the study by Levine and Stray-Gundersen (24). Such changes in iron metabolism have been interpreted to occur with increased erythropoiesis (4) and therefore support our findings of increased Hb\textsubscript{mass} and RCV.

Performance Parameters

Because the aim of our study was primarily to investigate the effect of the LHTL phase on hematological parameters, performance parameters were only measured in the AG and we do not know what performance changes may have taken place in the CG. Therefore, the improvement in performance in the AG should be interpreted with caution, because it could have been influenced by training and their own expectation of improved performance after the LHTL camp. Changes in the AG performance are reported, however, because it cannot be taken for granted that our athletes improved performance after 4 wk of LHTL training only. Both V\textsubscript{O2 max} (+4.1%) and TTE (+11.6%) were increased after the LHTL phase. The almost identical [Lac]\textsubscript{max} values at the pre- and posttest support that the athletes ran with a similar volitional exhaustion in the tests. The 5,000-m time trial performance improved (~1.6%), and measurement of HR\textsubscript{max}, [Lac]\textsubscript{max}, and RPE indicated that volitional exhaustion was similar for both trials. Considering the small number of subjects, the correlation between the increase in Hb\textsubscript{mass} and the increase in V\textsubscript{O2 max} must be put into perspective. However, the decrease in 5,000-m time and the increase in V\textsubscript{O2 max} were very similar to those of Levine and Stray-Gundersen (24, 32), and it is known that increased Hb\textsubscript{mass} and RCV is associated with increased endurance performance (22). Therefore, the improved performance parameters may be supported by the increases in the hematological parameters.

We conclude that Hb\textsubscript{mass} and RCV in elite endurance athletes are increased by ~5% after living at 2,500 m and training at 1,800 and 1,000 m for 24 days.

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