Similar Hemoglobin Mass Response in Hypobaric and Normobaric Hypoxia in Athletes

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[1]Section for Elite Sport, Swiss Federal Institute of Sport, Magglingen, SWITZERLAND; [2]Department of Physiology, Faculty of Biology and Medicine, ISSUL, Institute of Sport Sciences, University of Lausanne, SWITZERLAND; [3]National School of Mountain Sports/National Ski-Nordic Centre, Prémanon, FRANCE; [4]Departmental Section of Physical Education and Sports, University of Alicante, SPAIN; and [5]Swiss Laboratory for Doping Analyses, University Center of Legal Medicine, Geneva & Lausanne, Center Hospitalier Universitaire Vaudois & University of Lausanne, SWITZERLAND

ABSTRACT

HAUSER, A., L. SCHMITT, S. TROESCH, J. J. SAUGY, R. CEJUELA-ANTA, R. FAISS, N. ROBINSON, J. P. WEHRLIN, and G. P. MILLET. Similar Hemoglobin Mass Response in Hypobaric and Normobaric Hypoxia in Athletes. *Med. Sci. Sports Exerc.*, Vol. 48, No. 4, pp. 734–741, 2016. **Purpose**: To compare hemoglobin mass (Hb_{mass}) changes during an 18-d live high–train low (LHTL) altitude training camp in normobaric hypoxia (NH) and hypobaric hypoxia (HH). **Methods**: Twenty-eight well-trained male triathletes were split into three groups (NH: $n = 10$, HH: $n = 11$, control [CON]: $n = 7$) and participated in an 18-d LHTL camp. NH and HH slept at 2250 m, whereas CON slept, and all groups trained at altitudes <1200 m. Hb_{mass} was measured in duplicate with the optimized carbon monoxide rebreathing method before (pre-), immediately after (post-) (hypoxic dose: 316 vs 238 h for HH and NH), and at day 13 in HH (230 h, hypoxic dose matched to 18-d NH). Running (3-km run) and cycling (incremental cycling test) performances were measured pre and post. **Results**: Hb_{mass} increased similar in HH (+4.4%, $P < 0.001$ at day 13; +4.5%, $P < 0.001$ at day 18) and NH (+4.1%, $P < 0.001$) compared with CON (+1.9%, $P = 0.08$). There was a wide variability in individual Hb_{mass} responses in HH (−0.1% to +10.6%) and NH (−1.4% to +7.7%). Postrunning time decreased in HH (−3.9%, $P < 0.001$), NH (−3.3%, $P < 0.001$), and CON (−2.1%, $P = 0.03$), whereas cycling performance changed nonsignificantly in HH and NH (+2.4%, $P > 0.08$) and remained unchanged in CON (+0.2%, $P = 0.89$). **Conclusion**: HH and NH evoked similar Hb_{mass} increases for the same hypoxic dose and after 18-d LHTL. The wide variability in individual Hb_{mass} responses in HH and NH emphasizes the importance of individual Hb_{mass} evaluation of altitude training. **Key Words:** ALTITUDE TRAINING, LIVE HIGH-TRAIN LOW, SIMULATED ALTITUDE, PERFORMANCE, ENDURANCE ATHLETES, INDIVIDUAL RESPONSE

The altitude training method live high–train low (LHTL) is well accepted and frequently used by elite endurance athletes to improve sea-level performance (25,27,42). In contrast to classic altitude training (living and training at altitude), LHTL allows athletes to maintain exercise intensity and O_2 flux comparable to sea level as well as to obtain the physiological benefits of altitude acclimatization (20). For elite endurance athletes, the aim of LHTL is to improve their sea-level endurance performance, which is primarily obtained by an increase in hemoglobin mass (Hb_{mass}) (14,33). Altitude training studies have shown a significant increase in Hb_{mass} that is estimated to be 1.1%/100 h of hypoxic exposure at ≥2100 m (14). There is also a large consensus for recommending daily exposure >12 h and a total hypoxic exposure of approximately 300 h to substantially increase Hb_{mass} (7,25,27). Since LHTL is associated with time-consuming travel effort from high to low altitudes and to provide a more logistically convenient environment for athletes, the original LHTL method (20) was further developed by using technical devices (e.g., hypoxic chambers or tents) to simulate an altitude environment (e.g., normobaric hypoxia [NH] using nitrogen dilution or oxygen extraction) (25,42).

To date, it is still debated whether NH and hypobaric hypoxia (HH) evoke different or similar physiological responses (9,11,24). Short-term exposure (<24 h) to HH seems to lead to greater hypoxemia and lower oxygen arterial saturation (34), reduced ventilatory response (10,21), and impaired nitric oxide bioavailability (10) compared with NH. However, the practical significance of these differences for an athlete's preparation is still unclear. Particularly, the effects of NH versus HH on Hb_{mass} changes are unknown, because no data on a direct comparison of long-term exposure to NH and HH with the same hypoxic dose exist. The latter is of particular importance, because it may influence an athlete's altitude training adaptation. Only one study compared the differences between prolonged exposure to HH

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and NH in endurance athletes during an 18-d LHTL training camp (30). In this study, however, the HH group demonstrated a larger total hypoxic dose after the LHTL camp compared with the NH group (300 vs 220 h).

Because thus far no study has compared Hb_{mass} changes with normobaric and hypobaric LHTL with the same hypoxic dose, it remains unclear for endurance athletes whether an LHTL training camp under normobaric or hypobaric hypoxic conditions evokes similar Hb_{mass} responses. This study therefore aimed to compare (i) Hb_{mass} changes between normobaric and hypobaric LHTL after the same hypoxic dose (230 h at the same altitude) and (ii) differences in Hb_{mass} and performance changes after an 18-d LHTL training camp (higher hypoxic dose in HH, but same training load between groups) in either HH or NH in comparison to a control (CON) group.

METHODS

Subjects. Twenty-eight well-trained male triathletes, living at or near sea level (age, 26 ± 5 yr; height, 179 ± 6 cm; body mass, 70 ± 6 kg) participated in the study. The inclusion criteria for participation and data analysis were as follows: 1) a minimum of 5 yr of endurance training and frequent participation in endurance competitions and 2) initial ferritin levels >30 $\mu g \cdot L^{-1}$ (no iron supplementation during the study). All athletes provided written informed consent to participate in the study. The study was approved by the local ethical committee (NCPP EST I: 2014/33; Dijon, France), and all procedures were conducted in accordance with the Declaration of Helsinki.

Study design. Within a 3-wk period, all athletes completed an 18-d training camp and two testing sessions immediately before (pre) and after (post) (Fig. 1). After the pretests, the athletes were assigned to one of the three training groups matched to their 3-km running time: 1) LHTL with normobaric hypoxic exposure (n = 10; 3-km time: 623 ± 47 s, NH), 2) LHTL with hypobaric hypoxic exposure (n = 11; 3-km time: 643 ± 57 s, HH), and 3) the CON group (n = 7; 3-km time: 632 ± 59 s, CON). Both altitude groups slept at an altitude of 2250 m under either simulated (NH) or natural (HH) hypoxic conditions, whereas the CON group lived and all groups trained at altitudes <1200 m. Before the training camp, first Hb_{mass} in duplicate and hematological parameters were measured, and then the performance tests (incremental cycling test and 3-km run) were conducted. At day 13 of the LHTL camp, an additional duplicate Hb_{mass} measurement was performed in the HH group, as it corresponded to the expected hypoxic dose in NH after 18 d (the same hypoxic dose in the HH and NH groups). After the training camp, first, the performance tests were performed and then the Hb_{mass} and hematological measurements. All 3-km running tests were performed near sea level (390 m), whereas the other measurements were performed at 1150 m. During the training camp, the training load and the hypoxic dose were continuously recorded.

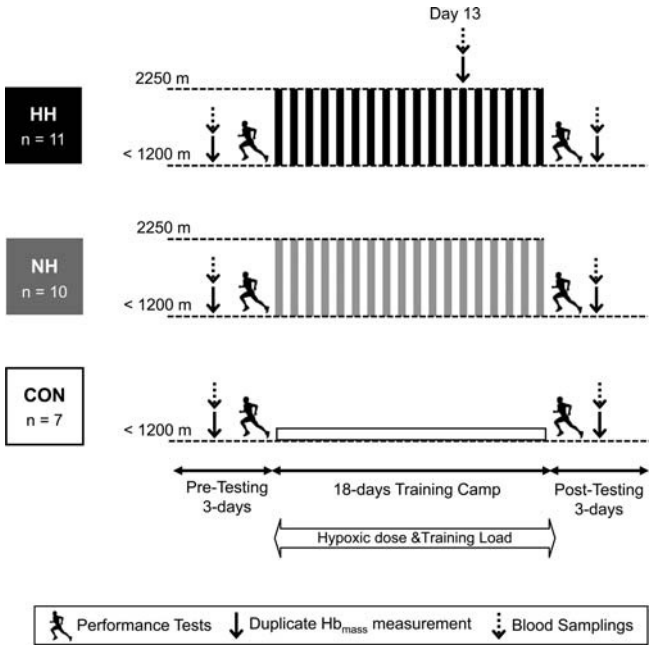

FIGURE 1—Illustration of the study design in HH, NH, or normoxia (CON).

Hypoxic exposure. The HH group lived at Fiescheralp, Switzerland (2250 m, inspired oxygen pressure (P_iO_2) 111.7 ± 0.7 mm Hg; inspired oxygen fraction (F_iO_2) 20.9% ± 0.0%, barometric pressure (P_B) 580.8 ± 3.3 mm Hg) and traveled by cable car twice daily to the valley (altitude <1200 m) for training. The daily hypoxic dose in the HH group amounted 17.4 ± 1.2 h. At day 13 during the training camp, the total hypoxic dose in the HH group was 229.5 ± 1.3 h, and after 18 d, the dose was 316.4 ± 2.3 h. The NH group lived in Prémanon, France (1150 m) and was exposed to NH equivalent to 2250 m in hypoxic rooms (medium size: 15 ± 1 m^2). NH was obtained by extracting oxygen from ambient air in hypoxic rooms (P_iO_2, 112.7 ± 0.1 mm Hg; F_iO_2, 18.1% ± 0.1%; P_B, 668.2 ± 2.5 mm Hg). In each hypoxic room, the gas composition was continuously monitored with oxygen and carbon dioxide analyzers (FIELDBROOK Ltd, London, UK), which were connected to a central monitoring station under the CON of an experienced physiologist. The NH group in Prémanon left the hypoxic rooms on average five to six times per day to eat and train. The daily hypoxic dose in the NH group was 13.1 ± 1.6 h, and the total hypoxic dose after 18 d in the NH group amounted 238.2 ± 10.6 h. For both groups, the time spent in hypoxia was monitored daily and recorded manually.

Training load. All training sessions during the training camp were supervised with the volume and intensity matched for all groups by two experienced certified coaches. The HH and NH group trained separately, because they were located at two different places. The CON group lived in Prémanon (n = 4) and nearby (n = 3) the NH group and trained most of the time together with the NH group. The training consisted of cycling, running, and swimming. Training load quantification

was performed using the Objective Load Scale (ECO) (4), which was specially developed for training load quantification in triathlon. Briefly, the ECO were calculated by multiplying the total duration of a training session (time in minutes) with a scoring value between 1 and 50, depending on the HR-based training zone (1 to 8), and by a factor of 1.0, 0.75, or 0.5 for running, swimming, or biking, respectively. The daily training loads (ECO) of each subject were measured based on each subject's physical characteristics and training program intensity.

Running and cycling performance. Running performance was evaluated during a 3-km run performed on a 400-m outdoor synthetic track at sea level. Starts were individual in a time-trial form (i.e., 30 s between each start), to avoid group or pacing effects. Pre- and post-3-km runs were performed under equivalent conditions: 22°C, P_B 738.4 mm Hg, 62% humidity, and 2.5 $m \cdot s^{-1}$ wind speed and 20°C, P_B 739.5 mm Hg, 60% humidity, and 1.9 $m \cdot s^{-1}$ wind speed for the pre- and postruns, respectively. Cycling performance was assessed with the determination of the maximal aerobic power during an incremental cycling test on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). After a 5-min warm-up period at a workload of 90 W, the workload was subsequently increased by 30 $W \cdot min^{-1}$ until voluntary exhaustion.

Hemoglobin mass. During each testing session, Hb_{mass} was measured in duplicate by using a slightly modified version of the optimized carbon monoxide (CO) rebreathing method described by Schmidt and Prommer (35). Briefly, subjects spent 5 min in a sitting position before three capillary blood samples (35 μL) were taken from the earlobe and analyzed immediately for baseline carboxyhemoglobin (%HbCO) values (ABL 800flex; Radiometer A/S, Copenhagen, Denmark). Subjects then rebreathed for 2 min a gas mixture of 100 mL pure CO (Multigas SA, Domdidier, Switzerland) and 3.5 L oxygen in a closed circuit system (glass spirometer; Blood Tec GbR, Bayreuth, Germany). During the rebreathing period, a CO gas analyzer (Dräger PAC 7000; Dräger Safety, Lübeck, Germany) was used to check for possible CO leakage at the nose, mouthpiece, and spirometer system. At 6 and 8 min after CO rebreathing started, two final capillary blood samples were taken from the earlobe and averaged as a 7-min post %HbCO value. Directly before and 2 min after the rebreathing, the same CO gas detector was used to measure the end-tidal CO concentration in parts per million. Hb_{mass} was calculated from the mean change in %HbCO before and after CO rebreathing, as described previously by Steiner and Wehrlin (37). Both measurements were performed on two consecutive days (12- to 24-h time lag between the measures), and the results were averaged. In this study, the typical error (TE) of the CO-rebreathing method was 1.9% in our mobile laboratory. Because averaged duplicate measurements reduce the TE by a factor of $1/\sqrt{2}$, the TE for the averaged duplicate measurements was 1.3% (17).

Blood samples. On the first morning in pretesting and posttesting, venous blood samples were drawn from an antecubital vein (4.9 ML EDTA tube; Sarstedt, Nümbrecht, Germany) immediately after the athletes woke up (7:00 a.m.). To determine red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), and reticulocyte (Ret) percentage, blood was analyzed via fluorescent flow cytometry and hydrodynamic focusing (XT-2000i, Sysmex Europe, Norderstedt, Germany). The coefficient of variation, which was determined using internal quality controls, was below 1.5% for Hb and 15% for Ret. Plasma EPO was measured using a standard procedure with an enzyme-linked immunosorbent assay kit (Stemcell Technologies, Grenoble, France). CV determined with three internal quality controls (levels: low, medium, and high) were below 15%. Additionally, serum ferritin (Ftn) was quantified using standard laboratory procedures (Dimension EXL; Siemens Healthcare Diagnostics SA, Zürich, Switzerland). To exclude the potential risk of misuse of recombinant human erythropoietin, all athletes were tested for doping by an accredited laboratory (Swiss Laboratory for Doping Analyses, Lausanne, Switzerland) according to the standards of the Athlete Biological Passport (31). All plasma samples were analyzed in duplicate, and the mean values were used for this study.

Statistical analyses. Data are presented as mean ± SD. The collected data were tested for normality (the Shapiro–Wilk test) and equal variance. A two-way repeated-measures ANOVA was applied to evaluate the group differences between the pre- and postmeasurements and group–time interactions. When a significant global effect was indicated, Tukey's *post hoc* test was performed to identify significant differences between the time points and the groups. A linear regression was used to determine the relationship between the percent changes in relative Hb_{mass} and the 3-km running time. Correlation classification of Hopkins (19) was used to interpret the size of the correlation. An α of $P < 0.05$ was considered significant. All analyses were processed using Sigmaplot 11.0 (Systat Software, San Jose, CA). To estimate the magnitude of the changes within the groups, the effect size Cohen's d was calculated (8), which was classified as follows: small effect $d = 0.20$, moderate effect $d = 0.50$, and large effect $d = 0.80$ (8).

To quantify the likelihood that the true mean of percent changes in Hb_{mass} and performance parameters was relevant (i.e., more extreme than the smallest worthwhile change (SWC) of Hb_{mass} and performance, set to ±1%), a contemporary statistical approach was used (18). The magnitude of the change in the mean and the spreads of the 90% confidence limits (CL) were used to classify the effects (positive, trivial, or negative) (19). The magnitude of the change was determined with the following descriptors (1): <1%, almost certainly not; 1%–5%, very unlikely; 5%–25%, unlikely or probably not; 25%–75%, possibly or may be; 75%–95%, likely or probably; 95%–99%, very likely; >99%, almost certainly. The magnitude of change was termed "unclear" if the CL overlapped the positive and negative SWC thresholds. To detect significant individual effects, the 95% CL for percent changes of Hb_{mass} were derived from the present

TABLE 1. Hb_{mass} and hematological parameters before (pre) and after (post) the 18-d LHTL training camp for HH, NH, and CON. As well for the similar hypoxic dose (230 h and 238 h) in HH and NH.

Group	Time	Hypoxia (h)	Hb_{mass} (g)	Hb_{mass} ($g \cdot kg^{-1}$)	RBC ($\mu \cdot \mu L^{-1}$)	Hb ($g \cdot dL^{-1}$)	Hct (%)	Ret (%)	Ftn ($\mu g \cdot L^{-1}$)	EPO ($mU \cdot mL^{-1}$)
HH	Pre	0	886 ± 80	12.9 ± 0.9	5.2 ± 0.6	15.2 ± 1.3	45.4 ± 3.6	1.1 ± 0.3	119.3 ± 128.1	5.0 ± 1.3
	Day 13	230	927 ± 105*	13.5 ± 1.0*	5.0 ± 0.6	14.8 ± 1.6	44.4 ± 4.2	1.0 ± 0.4	75.8 ± 48.3	5.9 ± 1.7
	Post	316	927 ± 95*	13.5 ± 1.0*	5.2 ± 0.5	15.3 ± 1.1	45.8 ± 3.1	1.0 ± 0.4	77.5 ± 68.4*	3.0 ± 0.7*
NH	Pre	0	955 ± 83	13.6 ± 1.4	5.1 ± 0.5	15.1 ± 1.3	45.2 ± 3.7	1.3 ± 0.5	91.3 ± 49.9	6.3 ± 2.4
	Post	238	994 ± 81*	14.1 ± 1.1*	5.3 ± 0.4*	15.7 ± 0.9*	47.1 ± 2.5*	1.2 ± 0.2	87.2 ± 44.7	3.1 ± 1.4*
CON	Pre	0	945 ± 128	13.1 ± 0.7	5.2 ± 0.5	15.1 ± 1.0	44.6 ± 3.4	1.3 ± 0.6	141.1 ± 91.9	4.8 ± 1.4
	Post	0	963 ± 137	13.2 ± 0.7	5.2 ± 0.3	15.2 ± 0.7	45.1 ± 2.4	1.1 ± 0.4	147.1 ± 98.2	4.4 ± 1.6
ANOVA (interaction group–time)		$P < 0.05$	0.18	0.15	0.25	0.18	0.24	0.93	0.15	0.003

*Significant difference between different levels of time ($P < 0.05$).
Data are mean ± SD.

TE of the Hb_{mass} measurement (95% CL = ±1.96 TE √2 1/√2) (17).

RESULTS

Hemoglobin mass. After the same hypoxic dose, the absolute Hb_{mass} of the HH ($d = 0.5$, $P < 0.001$, +4.4%) and NH ($d = 0.5$, $P < 0.001$, +4.1%) groups increased to the same extent (Table 1). Similar increases were also observed for the relative Hb_{mass} values in the HH ($d = 0.6$, $P < 0.001$, +4.3%) and NH ($d = 0.4$, $P < 0.001$, +3.8%) groups. After 18 d, Hb_{mass} was not further increased in the HH group either for absolute ($d = 0.5$, $P < 0.001$, +4.5%) or relative ($d = 0.6$, $P < 0.001$, +4.5%) values. No significant change in the CON group was observed either for absolute ($d = 0.1$, $P = 0.08$, +1.9%) or relative ($d = 0.2$, $P = 0.46$, +1.0%) values. Absolute and relative Hb_{mass} changes did not differ between the groups with the same hypoxic dose ($P > 0.75$), as well as after 18 d ($P > 0.12$). The likelihood of $\%Hb_{mass}$ changes in the altitude groups was likely beneficial compared with CON (>79% positive), with an unclear effect (>50% trivial) between the HH and NH groups after the same hypoxic dose and after 18 d (Table 2). Individual absolute Hb_{mass} responses ranged from −0.1% to +10.6% in the HH group, from −1.4% to +7.7% in the NH group, and from −3.3% to +6.0% in the CON group. The 95% CL for $\%Hb_{mass}$ changes was ±3.7%, and the upper CL was exceeded by most of the subjects in the altitude groups (Fig. 2).

Performance. In the posttest compared with pretest, the 3-km running time decreased with a moderate effect in the HH (from 643 ± 57 s to 618 ± 51 s, $d = 0.5$, $P < 0.001$, −3.9%) and NH (from 623 ± 47 s to 602 ± 36 s, $d = 0.5$, $P < 0.001$, −3.3%) groups and had a small effect in the CON group (from 632 ± 59 s to 619 ± 56 s, $d = 0.2$, $P = 0.031$, −2.1%). Cycling maximal aerobic power did not change significantly in the HH (405 ± 51 W vs 414 ± 45 W, $d = 0.2$, $P = 0.08$, +2.4%), NH (393 ± 36 W vs 402 ± 35 W, $d = 0.3$, $P = 0.08$, +2.4%), or CON (423 ± 57 W vs 424 ± 58 W, $d = 0.0$, $P = 0.89$, +0.2%) group. Running ($P = 0.27$) and cycling ($P = 0.5$) performance changes did not differ between the groups. The performance gains in the altitude groups were likely higher compared with the CON group (>64% positive), with an unclear effect (>39% trivial) between the HH and NH groups (Table 2). There was a large correlation between the relative Hb_{mass} and 3-km running time percent changes from the pretest to the posttest in the altitude groups ($r = -0.64$, $P < 0.001$) (Fig. 3).

Blood parameters. Table 1 lists all hematological parameters. After the training camp, there was a moderate increase in Hct ($d = 0.6$, $P = 0.04$, +4.6%), Hb ($d = 0.6$, $P = 0.02$, +4.8%), and RBC ($d = 0.4$, $P = 0.03$, +4.2%) for NH with no such changes in the HH and CON groups ($d < 0.2$, $P > 0.58$). Ftn decreased to a small extent in the HH group ($d = 0.4$, $P = 0.02$), but not in the NH ($d = 0.1$, $P = 0.92$) or CON ($d = 0.1$, $P = 0.79$) group. A decrease in EPO in the HH ($d = 1.9$, $P < 0.001$, −39.4%) and NH ($d = 1.6$, $P < 0.001$, −51.3%) group compared with the CON ($d = 0.3$, $P = 0.48$, −8.4%) group was observed. A group–time interaction was detected only for EPO ($P < 0.001$), whereas other hematological parameters did not differ between the groups.

Training load and body weight. No differences were found in daily training loads between the groups (213.6 ± 29 vs 205.2 ± 16 vs 155.4 ± 71 ECO for the NH, HH, and CON groups, respectively) during the training camp ($P = 0.21$).

TABLE 2. Differences in Hb_{mass} and performance improvements after 18-d LHTL camp between HH, NH, and CON.

Parameter	Compared Groups	ΔMean (%)	90% CL	Qualitative Outcome[a]	Positive	Trivial	Negative
Hb_{mass}	HH vs CON	2.6	±2.4	Likely beneficial	88%	11%	1%
	NH vs CON	2.2	±2.6	Likely beneficial	79%	19%	2%
	HH vs NH	0.4	±2.0	Unclear	30%	57%	13%
	HH vs NH (same dose)	0.3	±2.5	Unclear	30%	50%	20%
3-km run	HH vs CON	1.9	±1.9	Likely beneficial	80%	19%	1%
	NH vs CON	1.3	±1.5	Possibly beneficial	64%	36%	1%
	HH vs NH	0.6	±2.0	Unclear	37%	55%	8%
P_{max}	HH vs CON	2.1	±3.0	Likely beneficial	74%	22%	4%
	NH vs CON	2.1	±2.5	Likely beneficial	78%	20%	2%
	HH vs NH	0.0	±3.3	Unclear	31%	39%	30%

[a]With references to an SWC of 1% for performance and Hb_{mass}. Group comparison was calculated first group minus second group.
P_{max}, maximal power output; Δmean, differences in mean.

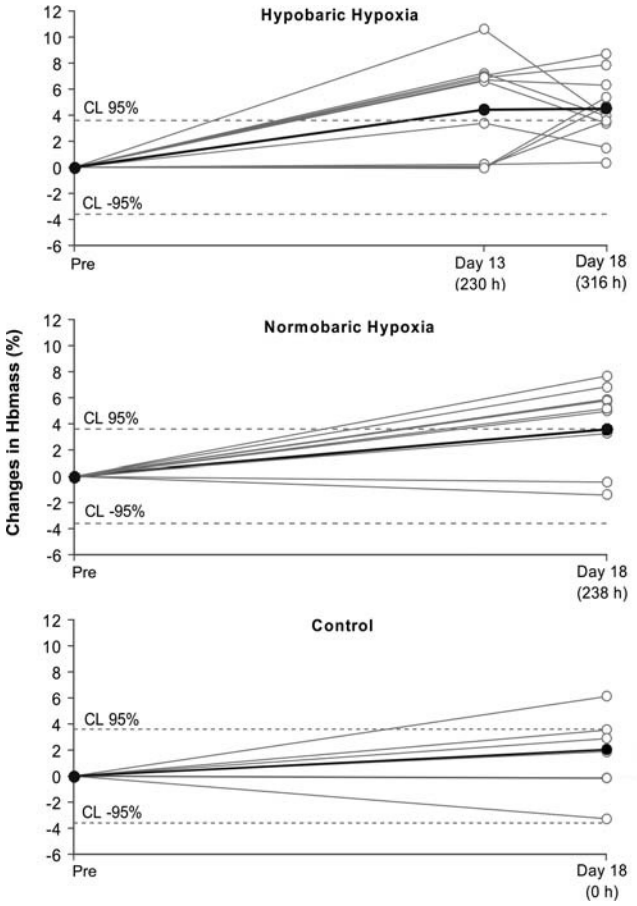

FIGURE 2—Percent changes in hemoglobin mass of each athlete (*open circle*) and mean changes of each group (*filled circle*) after 18-d LHTL and after the same hypoxic exposure (230 h). The 95% CL are indicated by *dotted lines*.

Body weight did not differ ($P = 0.76$) between the groups. Prebody weight was 68.6 ± 6.5, 70.4 ± 4.8, and 72.1 ± 8.2 kg, and postbody weight was 68.6 ± 5.6, 70.6 ± 4.9, and 72.7 ± 8.5 kg for the HH, NH, and CON groups, respectively.

DISCUSSION

To our knowledge, the present study is the first to compare Hb_{mass} response after the same hypoxic dose (approximately 230 h) in normobaric and hypobaric LHTL training camps. The main findings indicate that HH and NH yield a similar group mean increase in Hb_{mass} after the same hypoxic dose and that the difference between HH and NH was unclear with a tendency to be trivial. After the 18 d of LHTL, NH and HH likely had beneficial effects on Hb_{mass} and on performance indicators compared with the CON group, and despite a larger hypoxic dose in the HH group (316 h), the differences between HH and NH remained unclear. There was a wide variability in individual Hb_{mass} response to NH and HH after the same hypoxic dose and after 18 d.

Mean Hb_{mass} responses. The altitude groups demonstrated a similar group mean increase in Hb_{mass} after the same hypoxic dose (+4.4% vs +4.1%) to LHTL at 2250 m. The Hb_{mass} increase was of similar magnitude to that observed by other LHTL studies (12,15). It is well accepted that an adequate hypoxic dose of >12 $h \cdot d^{-1}$ at sufficient altitude for >21 d (25,27), that is, approximately 300 h (7) is recommended to substantially increase Hb_{mass}. However, in the current study, both altitude groups enhanced their Hb_{mass} by approximately 4% after approximately 230 h of hypoxic exposure at 2250 m, which is in accordance with other studies (12,26). These studies also showed a measurable increase in Hb_{mass} (3.0%–3.5%) after 210 h of normobaric hypoxic exposure at 3000 m (26) and after 236 h of HH at 2760 m (12). Furthermore, due to the nature of natural altitude, the HH group accumulated hypoxic hours much faster than the NH group (17 $h \cdot d^{-1}$ vs 13 $h \cdot d^{-1}$) and achieved a similar hypoxic dose (approximately 230 h) after 13 d of altitude training compared with the NH group (18 d), with no additional group mean Hb_{mass} increase in HH (+4.4% vs +4.5%) by day 18 (316 h). This suggests that approximately 230 h of hypoxic exposure at 2250 m in either HH or NH is sufficient to increase Hb_{mass} in endurance athletes and that these erythropoietic adaptations were feasible within a shorter duration of hypoxic exposure than commonly recommended (26). Otherwise, altitude studies have shown that Hb_{mass} increases at a mean rate of 1.1% per 100 h of exposure (14), expecting a further Hb_{mass} increase of approximately 1% from day 13 to day 18 in the HH group. However, there is a wide individual variability in the time course of Hb_{mass} response to altitude training (7,12), which was also present in the HH group from day 13 to day 18 (Fig. 2). Some of the athletes could further increase their Hb_{mass} from day 13 to day 18 (+0.9% to +5.4%), whereas in others Hb_{mass} decreased from day 13 to day 18 (−1.8% to −6.0%). Furthermore, even using duplicate Hb_{mass} measurements, it is still difficult to certainly detect Hb_{mass} changes smaller than the TE (1.3%). Therefore, it might be possible that the lack of increase in Hb_{mass} from day 13 to day 18 in HH is due to individual

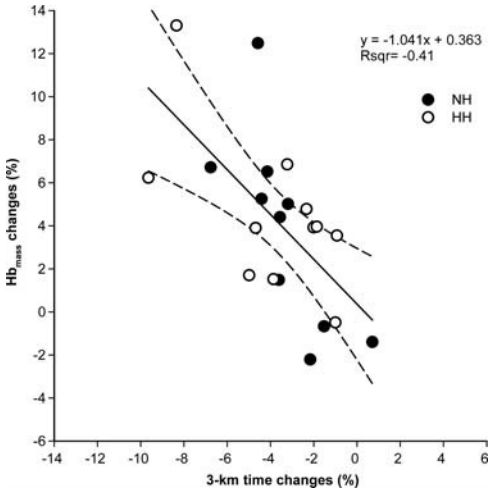

FIGURE 3—Linear regression (and 95% CL) for percent changes from preintervention to postintervention in HH and NH between relative Hb_{mass} and 3-km running time. Regression slope (*solid line*) and 95% CL (*dashed lines*) are shown.

variation in the time course of Hb_{mass} responses and due to measurement error. Lastly, the $\%Hb_{mass}$ changes in both altitude groups were likely beneficial (>79% positive) in comparison with the CON group, indicating that LHTL either in HH or NH is advantageous for Hb_{mass} increase compared with sea-level training. However, the difference between Hb_{mass} response in the NH and HH groups was unclear with a tendency to be trivial after the same hypoxic dose (50%) and after 18 d of LHTL (57%) (Table 2).

Individual Hb_{mass} responses. There was large variability in the individual responsiveness in Hb_{mass} for HH (ranging from −0.1% to +10.6%) and NH (from −1.4% to +7.7%) after the same hypoxic dose and 18 d of LHTL. The 95% CL for $\%Hb_{mass}$ changes were ±3.7%, and the upper CL was exceeded mainly by the athletes in the altitude groups (HH: 7 of 11 and NH: 6 of 10), whereas only one athlete in the CON group exceeded the 95% CL (Fig. 2). Because in all athletes, no depleted ferritin stores (Ftn >30 $\mu g \cdot L^{-1}$) (16), doping abuse (doping CON scores within normal ranges (31)), or different daily training loads during the altitude stay were detected, and all measures were performed in duplicate with no measurement outliers, it can be expected that the athletes who exceeded the 95% CL were "true" Hb_{mass} responders to altitude training at 2250 m in either NH or HH. Individual variability in Hb_{mass} response to LHTL training camps (2700–3000 m) in either HH or NH has been shown and discussed before (7,15,29). However, studies (7,12,15,23,26,29,40) that focused on individual Hb_{mass} response were mainly based on single measures of Hb_{mass} with the optimized CO rebreathing method, which makes the differentiation between physiological and technical variation more difficult. The optimized CO rebreathing method is a very precise tool for determining Hb_{mass} in athletes with a TE of approximately 2% (14). However, a greater certainty about individual Hb_{mass} measures can be attained with duplicate Hb_{mass} measurements, which improve the measure precision, as they reduce the TE by a factor of √2 (30%) (12) and help detect heavy measurement outliers (14). The more precise the Hb_{mass} measurements, the greater the certainty about the individual responsiveness to an altitude training. Thus, it seems to be certain that within a mean Hb_{mass} response of +4.1% to +4.5% after the LHTL camp, individual responsiveness in Hb_{mass} from −1.4% to +10.6% exists.

The cause of such individual variability is still uncertain and may be related to several factors, such as a greater acute and sustained increase in erythropoietic and training velocity response to altitude exposure (6). It has been suggested that the individual variability in Hb_{mass} response may be explained by the initial Hb_{mass} level, assuming that athletes with an already high initial Hb_{mass} level have a limited ability to further increase their Hb_{mass} after altitude training (28). However, in the current study, even athletes with an initial high Hb_{mass} level could increase their Hb_{mass} above the 95% CL (e.g., 1024–1075 g, +5%). Overall, there was a trivial relationship between the baseline Hb_{mass} (g) and the relative increase in absolute Hb_{mass} (%) ($r = 0.02$, $P = 0.92$), indicating that even endurance athletes with already high Hb_{mass} can benefit from LHTL training for further Hb_{mass} improvement. To ensure the wide individual variability in Hb_{mass} response to HH and NH, a cross-over study with the same athletes and a similar hypoxic dose of NH and HH would be needed.

Performance. Changes in running and cycling performance were likely beneficial (64%–80% positive) in the HH and NH groups compared with the CON group (Table 2). The greater performance improvement in the altitude groups (+1.2% to +2.2%) compared with the CON group is of similar magnitude as reported in other LHTL training interventions under normobaric conditions (13,29) and under hypobaric conditions (39,41), whereas the differences between HH and NH in the magnitude of performance changes were unclear. Bonetti and Hopkins (3) reported in a recent meta-analysis on altitude training that natural LHTL might be more beneficial for elite (4.0%; 90% CL ±3.7% vs 0.6%; ±2.0%) and subelite (4.2%; 90% CL ±2.9% vs 1.4%; ±2.0%) athletes than artificial protocols. However, due to the unequal hypoxic doses in the present study and the conflicting results reported in the literature (i.e., uncontrolled studies, poor study design, differences in duration and intensity of hypoxic exposure and subject training status (22)), the present results and literature cannot reflect a direct comparison of LHTL in HH versus NH in performance responses. Therefore, a cross-over study with the same athletes exposed to HH and NH is needed to confirm the present results.

Currently, one of the most recognized physiological mechanisms leading to enhanced sea-level performance after LHTL is a hypoxia-induced increase in Hb_{mass} (14,39). Changes in Hb_{mass} directly affect $\dot{V}O_{2max}$, a key parameter in endurance performance (22,36); accordingly, cross-sectional studies showed that an increase of 1 g in Hb_{mass} results in an approximate 4 $mL \cdot min^{-1}$ rise in $\dot{V}O_{2max}$ at sea level (32,36). There is also evidence that the gain in $\dot{V}O_{2max}$ after altitude training is related to the increase in Hb_{mass} (22,29,32), whereas an increase in Hb_{mass} was reported with different performance outcomes (13,15,29). The present study demonstrated a large correlation between the percent changes in relative Hb_{mass} ($g \cdot kg^{-1}$) and 3-km running time for both altitude groups ($r = -0.64$, $P = 0.002$) (Fig. 3). Because 3-km running time is close to velocity at $\dot{V}O_{2max}$ (2), it can be suggested that in the present study, the improvement in running performance may be directly linked to the changes in Hb_{mass} after 18-d LHTL in either HH or NH.

Blood parameters. The majority of the hematological parameters were similar between the HH and NH groups before and after the 18-d LHTL training camp. EPO was lower in both groups after the LHTL training camp compared with the CON group, which is in line with previous findings (5,7,40), showing that EPO increases at the beginning of altitude exposure and peaks within 2–3 d before beginning to decrease toward sea-level values. It has been suggested that low iron stores (Ftn <30 $\mu g \cdot L^{-1}$) interfere

with Hb_{mass} responses to hypoxic exposure and may reduce the effectiveness of altitude training (38). In the present study, the ferritin levels were above >30 $\mu g \cdot L^{-1}$ in all athletes and only a small correlation between the initial ferritin level and the Hb_{mass} responses ($r = 0.3$, $P = 0.095$) was detected. However, one cannot rule out that low ferritin levels may limit Hb_{mass} responses to altitude training.

Study limitations. This study primarily aimed to compare Hb_{mass} changes after the same hypoxic dose and after 18-d LHTL training camps in either NH or HH. Important notes for consideration in evaluating the findings are that the study settings replicated common real altitude training practices of endurance athletes (e.g., daily exposure, total hypoxic doses under NH and HH conditions, respectively). Thus, the reported total (238 h vs 316 h) and daily (13 h vs 17 h) hypoxic exposure in the present study was lower in the NH group than in the HH group. To directly compare the same hypoxic dose between the two conditions, we performed an additional Hb_{mass} measurement in the HH group at day 13 of the training camp (230 h vs 238 h for HH and NH, respectively). However, one cannot rule out that the unequal nature of the daily hypoxic dose in HH and NH could have influenced the results. Because the primary aim of the study was to compare Hb_{mass} changes between normobaric and hypobaric LHTL after the same hypoxic dose, and the secondary aim was to compare differences in Hb_{mass} and performance changes after 18-d LHTL in either HH or NH, it was planned not to measure performance parameters on day 13 because it would have influenced the training load quantification. Therefore, we cannot exclude putative differences in running or cycling performance with the same hypoxic dose between HH and NH. Another key consideration is the small sample size in the three training groups, which could explain the missing statistical significance between the altitude groups and the CON group, but the magnitude of changes in Hb_{mass} and performance was still likely positive for the NH and HH groups compared with the CON group. Furthermore, we cannot exclude that the hematological concentration values were slightly affected by the suboptimal standardization of the venous blood sampling (travel, fluid intake, etc.). Lastly, to control our findings regarding individual variability in Hb_{mass} response to HH and NH, a cross-over design with a similar hypoxic dose of NH and HH would be needed. However, because of the different periods of the athlete's training (e.g., competition period, off-season, tapering or peaking), a cross-over design with athletes is only feasible if the interventions take place at the same time point of the season.

CONCLUSION

Hypobaric and normobaric LHTL evoked a similar group mean increase in Hb_{mass} (4.4% vs 4.1%) after same hypoxic dose (230 vs 238 h): The difference between HH and NH was unclear with a tendency to be trivial. After the 18-d LHTL training camp, both NH and HH likely have beneficial effects on Hb_{mass} and on performance indicators compared with the CON group, whereas the differences between HH and NH were also unclear, despite a larger hypoxic dose in the HH group (316 h). Individual Hb_{mass} responses demonstrated a large variability in the altitude groups, underlining the importance of individual evaluation of Hb_{mass} responses to altitude training.

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