Individual hemoglobin mass response to normobaric and hypobaric “live high–train low”: A one-year crossover study


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Running title: Individual Hbmass responses in normobaric and hypobaric LHTL

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ABSTRACT

Purpose: To compare individual hemoglobin mass (Hb_mass) changes following a live high–train low (LHTL) altitude training camp under either normobaric hypoxia (NH) or hypobaric hypoxia (HH) conditions in endurance athletes. Methods: In a crossover design with a one-year washout, 15 male triathletes randomly performed two 18-d LHTL training camps in either HH or NH. All athletes slept at 2250 m and trained at altitudes < 1200 m. Hb_mass was measured in duplicate with the optimized carbon monoxide rebreathing method before (pre-) and immediately after (post-) each 18-d training camp. Results: Hb_mass increased similarly in HH (916 to 957 g, 4.5 ± 2.2%, P < 0.001) and in NH (918 to 953 g, 3.8 ± 2.6%, P < 0.001). Hb_mass changes did not differ between HH and NH (P = 0.42). There was substantial inter-individual variability among subjects to both interventions (i.e., individual responsiveness, or the individual variation in the response to an intervention free of technical noise): 0.9% in HH and 1.7% in NH. However, a correlation between intra-individual delta Hb_mass changes (%) in HH and in NH (r = 0.52, P = 0.048) was observed. Conclusion: HH and NH evoked similar mean Hb_mass increases following LHTL. Among the mean Hb_mass changes, there was a notable variation in individual Hb_mass response, which tended to be reproducible.

Key words: altitude; training; hypoxia; LHTL; athletes
NEW & NOTEWORTHY

This is the first study to compare individual Hb$_{\text{mass}}$ response to normobaric and hypobaric LHTL using a same-subject crossover design. The main findings indicate that hypobaric and normobaric hypoxia evoked a similar mean increase in Hb$_{\text{mass}}$ following 18-d LHTL. Notable variability and reproducibility in individual Hb$_{\text{mass}}$ responses between athletes was observed, indicating the importance of evaluating individual Hb$_{\text{mass}}$ response to altitude training.
INTRODUCTION

Simulated and natural altitude training methods are commonly used by elite endurance athletes to enhance sea-level performance (25, 45). The question as to, whether simulated (normobaric hypoxia) altitude and natural (hypobaric hypoxia) altitude differ considerably regarding physiological and performance responses is still debated (5, 26, 32). A frequently used altitude training method, which can be performed under either hypobaric or normobaric conditions, is the “live high–train low” (LHTL) model (22, 41), where athletes live and sleep at a certain altitude but train at a lower altitude or near sea-level (1, 45). However, researchers have rarely directly compared the possible differences between the effects of hypobaric and normobaric LHTL on relevant physiological responses, such as hemoglobin mass \( (H_{\text{b mass}}) \) (16) and performance responses (32). Thus far, only one study (16) has compared individual \( H_{\text{b mass}} \) responses between normobaric and hypobaric LHTL training camps after the same duration (18 d) and the same hypoxic hours (approximately 230 h) in endurance athletes. Interestingly, these results showed that hypobaric and normobaric LHTL evoked similar group mean increases in \( H_{\text{b mass}} \) (4.1% vs. 4.5%) and that there was no difference between the two hypoxic conditions. In line with previous studies (6, 8, 24, 30, 38, 43), individual \( H_{\text{b mass}} \) responses demonstrated a wide variability (–1.4% to 10.6%) in hypobaric and normobaric LHTL. As the number of athletes was small within the hypobaric hypoxia (HH) and normobaric hypoxia (NH) groups (\( n = 10, 11 \)), an uneven distribution of athletes who responded positively or less positive to altitude in \( H_{\text{b mass}} \) may have affected the outcome. Thus, the question whether normobaric and hypobaric LHTL results in similar \( H_{\text{b mass}} \) responses has not been conclusively answered. The straightforward option to diminish the observed effect is to conduct a same-subject crossover design.
Paragraph Number 2 The primary aim of the present study was to investigate whether Hb\text{mass} responses differ between 18-d hypobaric and normobaric LHTL with a same-subject crossover design. The secondary aim was to quantify individual Hb\text{mass} responsiveness in HH and NH.
METHODS

Subjects

*Paragraph Number 3* Fifteen well-trained male triathletes, living at or near sea level (age: 23.9 ± 4.0 yr, height: 178.5 ± 4.9 cm and weight: 64.9 ± 7.6 kg) completed both altitude training camps and fulfilled the following inclusion criteria for participation and data analysis: 1) a minimum of 5 yr of endurance training and frequent participation in endurance competitions, 2) initial ferritin levels > 30 µg·L⁻¹, and 3) no doping abuse (OFF score within reference range (11)). All athletes provided written informed consent to participate in the study. The study was approved by the local ethical committees (Commission Cantonale Valaisanne d’Ethique Médicale, CCVEM; Agreement 051/09 and French National Conference of Research Ethics Committees; N°CPP EST I: 2014/33; Dijon, France), corresponding to the two training locations. All procedures were conducted in accordance with the Declaration of Helsinki.

Study design

*Paragraph Number 4* Originally, it was planned to perform a single parallel group study design (camp 1). To get a crossover study design, we decided after the first training camp to extend the study with another training camp (camp 2), but not all athletes from the first training camp were able to participate a second time. Thus, the present study was based on two training camp phases performed over one year. In the first year (camp 1), a total of 24 athletes were randomly assigned to either a hypobaric or a normobaric hypoxic 18-d LHTL training camp. In the second year (camp 2), at the same time point during the year and during the competitive season, 15 of the 24 athletes performed a second 18-d LHTL training camp with the opposite hypoxic condition (HH or NH). Individual Hb\textsubscript{mass} responses of one single training camp have been published;
for details see Hauser et al. (16). To have a same-subject crossover design (Fig. 1), only the results of these 15 athletes were used in this study. The athletes’ data were pooled for each hypoxic condition from both camps of the study as follows: HH condition included the pooled values from the HH athletes in camp 1 \((n = 5)\) and the HH athletes in camp 2 \((n = 10)\); the same athletes were considered for the NH condition but reversed \((n = 10 \text{ in camp 1 and } n = 5 \text{ in camp 2})\). During the one-year washout period, the athletes did not perform any additional altitude training. Under both hypoxic conditions (NH and HH), athletes slept at an altitude of 2250 m and trained at altitudes < 1200 m. Immediately before (pre-) and after (post-) each training camp, \(Hb_{mass}\) was measured in duplicate, and venous blood samples were collected. At day 13 of the second training camp in HH (camp 2), in 10 of 15 subjects, an additional duplicate \(Hb_{mass}\) measurement was performed, as it corresponded to the expected hypoxic hours in NH after 18 d (matched hypoxic hours in HH and NH). All measurements were performed at 1150 m. During the training camp, training load and hypoxic hours were continuously recorded.

***Figure 1 near here***

Hypoxic exposure

*Paragraph Number 5* For the LHTL training camps under HH, the athletes lived in Fiescheralp, Switzerland (2250 m, inspired oxygen pressure \((P_iO_2)\) 111.6 ± 0.6 mm Hg, inspired oxygen fraction \((F_iO_2)\) 20.9 ± 0.0%, barometric pressure \((P_B)\) 580.2 ± 2.9 mm Hg) and traveled by cable car twice daily to the valley (altitude < 1200 m) for training. Daily hypoxic exposures in HH totaled 17.3 ± 2.3 h. The total hypoxic hours after 18 d were 311.6 ± 7.8 h and after 13 d (only measured in the second camp, \(n = 10\)) 229.5 ±
1.2 h, respectively. For the LHTL training camps under NH, the athletes lived in Prémanon, France (1150 m) and were exposed to normobaric hypoxia equivalent to 2250 m in hypoxic rooms (medium size: 15 m²). Normobaric hypoxia was obtained by extracting oxygen from ambient air in hypoxic rooms \( (P_{O_2} 111.9 \pm 0.6 \text{ mm Hg}, F_{O_2} 18.05 \pm 0.1\%\), \( P_B 666.6 \pm 3.6 \text{ mm Hg}\). 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 control of an experienced physiologist. In Prémanon, the athletes left the hypoxic rooms on average 5–6 times per day to eat and train. Daily hypoxic exposures in NH totaled 12.5 ± 0.4 h, and the total hypoxic hours after 18 d were 225.3 ± 9.0 h. During all training camps, the time spent in hypoxia was monitored daily and recorded manually.

**Training load**

*Paragraph Number 6* All training sessions during the training camps were advised and supervised by two experienced certified coaches. The intervention groups trained separately (located at two different places: Fiesch, Switzerland and Prémanon, France) under the supervision of one coach. The training consisted of cycling, running, and swimming. Training load quantification was performed using the Objective Load Scale (ECOs; (2)), which was specially developed for training load quantification in triathlons. Briefly, the ECOs 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 heart rate 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 (ECOs) of each subject were measured based on each subject’s physical characteristics and training program intensity.
**Hemoglobin mass**

**Paragraph Number 7** $H_{bmass}$ was measured in duplicate using a slightly modified version of the optimized carbon monoxide (CO)-rebreathing method described by Schmidt and Prommer (36). Briefly, a CO dose of 100 mL (Multigas SA, Domdidier, Switzerland) was administered and rebreathed with 3.5 L oxygen for 2 min in a closed circuit system (glass spirometer, Blood Tec GbR, Bayreuth, Germany). Capillary earlobe blood samples (35 µl) were collected three times before the CO-rebreathing procedure and once at minute 6 and 8 after CO rebreathing was started. Blood samples were analyzed for carboxyhemoglobin (%HbCO) using a CO-oximeter (ABL 800flex, Radiometer A/S, Copenhagen, Denmark). $H_{bmass}$ was calculated from the mean change in %HbCO before and after CO rebreathing, as described previously by Steiner and Wehrlin (39). Both measurements were performed on two consecutive days (12–24 h time lag between the measures), and the results were averaged. The typical error (TE) of $H_{bmass}$ measurement was calculated from duplicate measurements as the standard deviation (SD) of the difference score divided by $\sqrt{2}$ (17). To provide a dimensionless measure of reliability, which is comparable between subjects and studies (17), the TE was translated into a coefficient of variation (CV). The CV is calculated by dividing the TE by the mean value of $H_{bmass}$ and is expressed in percent. Averaged multiple measurements reduce the TE by a factor of $1/\sqrt{n}$, where $n$ is the number of measurements (17). In this study, the TEs for duplicate measurements of $H_{bmass}$ at the different time points were as follows: pre-camp 1: 1.8% (90% confidence limits (CLs): 1.3–2.5%); post-camp 1: 1.0% (0.7.1–1.3%); pre-camp 2: 0.9% (0.7.1–1.3%); day 13: 1.9% (1.3–2.6%); post-camp 2: 1.1% (0.8–1.6%). In our mobile laboratory, the overall TE of the CO-rebreathing method was 2.0% (1.5–2.6%), and the TE for the average duplicate measurements was 1.4% (1.1–1.8%).
Ferritin and OFF score

Paragraph Number 8 On the first morning in the pre- and post-testing of both training camps, venous blood samples were drawn from an antecubital vein (4.9 ML EDTA tube, Sarstedt, Nümbrecht, Germany) immediately after the athletes woke up (7 am). To identify iron-deficient athletes (initial ferritin levels > 30 µg·L⁻¹), serum ferritin concentration analysis was determined with a biochemistry analyzer (Dimension EXL, Siemens Healthcare Diagnostics SA, Zürich, Switzerland). The CV, which was determined using internal quality controls, was 4.5%. To exclude the potential risk of illegal blood manipulation, athletes were tested for doping by an accredited laboratory (Swiss Laboratory for Doping Analyses, Lausanne, Switzerland). Therefore, the OFF score (OFF score = Hb (g·L⁻¹) - 60√(reticulocytes in %)) according to Gore et al. (11) was calculated and compared to cut-off limits for athletes tested at altitude > 610 m with a false positive rate of 1:100.

Statistical analyses

Paragraph Number 9 Values are presented as means ± SD. All data were checked for normality (Shapiro-Wilk test) and equality of variance. A two-way repeated measure analysis of variance was applied to evaluate the differences between the conditions (HH and NH) over time. When a significant global effect was indicated, Tukey’s post-hoc test was performed to identify significant differences between different levels of time and conditions. For a comparison of the training load between HH and NH, a paired t-test was performed. Linear regressions were used to determine the Pearson’s correlation coefficient (r) between individual delta Hb mass changes (%) in HH and in NH. The level
of significance was set at $P < 0.05$. All analyses were processed using SigmaPlot 11.0 (Systat Software, San Jose, CA, USA).

**Paragraph Number 10** To assess the likelihood that the differences in percent change in Hb_{mass} between HH and NH were relevant (i.e. more extreme than the smallest worthwhile change in Hb_{mass}, set to $\pm 1\%$) a contemporary statistical approach according to Hopkins (18) was used. This approach calculates the chances (in %) that the true value of an effect is positive, trivial or negative. To classify the magnitude of the effects (positive, trivial, or negative), the change in mean and the 90% CL of the individual change scores were used (19). The effect was termed “unclear” if its CL overlapped the positive and negative smallest worthwhile changes. Individual Hb_{mass} responsiveness (i.e. the individual variation in the response to an intervention free of TE (17)) for NH and HH is expressed as the SD from the mean Hb_{mass} change and was calculated as the square root of the difference between the variance of the Hb_{mass} change scores in the intervention and the variance in change scores arising from TE only $((\text{TE} \cdot \sqrt{2})^2)$. To detect significant individual effects, the 95% CL for percent changes of Hb_{mass} was derived from the present overall TE of the Hb_{mass} measurement $(95\% \text{ CL} = \pm 1.96 \cdot \text{TE} \cdot \sqrt{2} \cdot 1/\sqrt{2}; (17))$. 


RESULTS

Mean Hb\textsubscript{mass} responses

Paragraph Number 11 After 18 d (n = 15), Hb\textsubscript{mass} increased similarly in HH (916.0 ± 84.6 g to 957.1 ± 93.5 g, 4.5 ± 2.2%, \( P < 0.001 \)) and NH (918.0 ± 86.5 g to 952.6 ± 92.7 g, 3.8 ± 2.6%, \( P < 0.001 \); see Fig. 2). For matched hypoxic hours (n = 10), Hb\textsubscript{mass} increased by 4.9 ± 3.7% (891.7 ± 81.7 g to 936.2 ± 106.1 g, \( P < 0.001 \)) in HH and by 3.4 ± 2.2% (883.4 ± 72.4 g to 914.0 ± 82.5 g, \( P = 0.005 \)) in NH. Hb\textsubscript{mass} changes did not differ between the conditions after 18-d LHTL (\( P = 0.42 \)) or for same hypoxic hours (\( P = 0.29 \)). The chance in percent Hb\textsubscript{mass} changes being greater in HH compared to NH was 36% following 18-d LHTL and 61% for matched hypoxic hours (Table 1).

Individual Hb\textsubscript{mass} responses

Paragraph Number 12 Percent changes in individual Hb\textsubscript{mass} ranged from +0.4% to +8.7% in HH and from −1.4% to +7.7% in NH (Fig. 3) after 18-d LHTL. The 95% CL for individual percent Hb\textsubscript{mass} changes was ± 3.9%, and the upper CL was exceeded by eight out of 15 athletes in HH and by seven out of 15 athletes in NH. Individual responsiveness was ±0.9% in HH and ±1.7% in NH. For matched hypoxic hours, individual responsiveness was ±3.4% in HH and ±0.9% in NH. There was a significant correlation between individual delta Hb\textsubscript{mass} changes (%) in HH and in NH after 18-d LHTL (\( r = 0.52, P = 0.048 \))
Ferritin and OFF score

Paragraph Number 13 Initial ferritin levels were > 30 µg·L⁻¹ in all athletes. Pre-ferritin values were 108.1 ± 36.0 µg·L⁻¹ and 107.3 ± 36.3 µg·L⁻¹ in HH and NH, respectively. All athletes were within the cut-off limits for the OFF scores (< 125.3) for pre- (91.7 ± 5.4 vs. 94.6 ± 14.1) and post- (97.2 ± 6.3 vs. 97.9 ± 5.1) testing in HH and NH, respectively.

Training load and body weight

Paragraph Number 14 No differences were found in daily average training loads between the two groups, HH (217.6 ± 87.9 ECOs) and NH (229. ± 80.0 ECOs), during the 18-d LHTL training camps of the crossover study (P = 0.54). In camp 1, the daily training load was similar to that in camp 2 in HH (231.7 ± 42.1 vs. 210.6 ± 105.6 ECOs, P = 0.68) and NH (229.4 ± 25.2 vs. 228.6 ± 7.9 ECOs, P = 0.98). Body weight did not differ over time between HH and NH after 18 d (P = 0.72). The average pre-body weight was 70.3 ± 6.3 kg and 71.6 ± 7.6 kg, and the average post-body weight was 69.8 ± 5.3 kg and 70.6 ± 6.4 kg — for HH and NH, respectively.
DISCUSSION

**Paragraph Number 15** This is the first study to compare individual Hb$_{\text{mass}}$ responses to normobaric and hypobaric LHTL using a same-subject crossover design. The main findings indicate that HH and NH evoked a similar mean increase in Hb$_{\text{mass}}$ following 18-d LHTL. The mean changes in Hb$_{\text{mass}}$ did not differ between HH and NH. Notable variability in individual Hb$_{\text{mass}}$ responses following 18-d LHTL in HH and NH was observed as well as a significant correlation between individual delta Hb$_{\text{mass}}$ changes (%) in HH and in NH.

**Mean Hb$_{\text{mass}}$, responses**

**Paragraph Number 16** Both hypoxic conditions (HH vs. NH) demonstrated a similar mean Hb$_{\text{mass}}$ increase (+4.5% vs. +3.8%) following 18-d LHTL. Furthermore, the chance in percent Hb$_{\text{mass}}$ changes being greater in HH compared to NH was only 36%. Recently, the part study (16) of the crossover study also reported similar Hb$_{\text{mass}}$ responses after an 18-d LHTL training camp in either HH or NH, despite larger total hypoxic hours in HH compared to NH. A recent meta-analysis estimated that Hb$_{\text{mass}}$ increases at a mean rate of 1.1%/100 h of exposure at simulated or natural altitude (14), which would have expected lower mean Hb$_{\text{mass}}$ responses (1% to 2%) in the present study. However, in this meta-analysis, the “upper 95% individual response limits” for 225 h and 310 h were around 5% and 6%, respectively, indicating that group composition can noticeably influence the mean Hb$_{\text{mass}}$ response. The present mean Hb$_{\text{mass}}$ increases were of similar magnitude to previous LHTL studies with longer hypoxic exposures (> 300 h; (15, 44)) and were of greater magnitude than in LHTL studies with similar hypoxic hours (4, 20, 28). The current recommendation suggests an adequate hypoxic exposure of > 12 h/day at natural or simulated altitude > 2000 m for >
299 21 d; that is, approximately 300 h is required to substantially increase Hbmass (4, 31). However, the data for the NH group after 18 d (225 h) and for the HH group after 13 d (230 h) suggest that a relevant Hbmass increase can be achieved with less hypoxic hours (< 300 h) in some subjects. Recently, studies have examined earlier time courses (8, 43) and shorter hypoxic exposure (9, 27) on changes in Hbmass to moderate altitude (2500–3000 m). The data from these studies showed measurable Hbmass increases (2.1% to 3.7%) within a shorter time period (11–13 d) or lower hypoxic exposure (< 210 h) than recommended (14, 31). However, the present study and the reported studies (8, 9, 27, 43) used different athlete populations and applied different altitude protocols, which may limit generalization. Therefore, further research is needed to better understand the time course and dose–response relationship of Hbmass to different altitude protocols in different athlete populations.

Paragraph Number 17 An hypoxia-induced increase in Hbmass seems to be one of the main physiological mechanisms leading to improved sea-level endurance performance after altitude training (14, 22, 23, 42). Hbmass is closely related to maximal oxygen uptake (V\text{̇}O_{2\text{\text{\text{max}}}}) – that is, a gain of 1 g in Hbmass results in a 4 mL-min⁻¹ increase in V\text{̇}O_{2\text{\text{\text{max}}}} under normoxic conditions (37). Further, Hbmass correlates with time trial performance and maximal incremental power output in highly trained endurance athletes (21). In both 18-d LHTL camps, the athletes performed a 3-km running time trial near sea level before and after each camp. The mean performance data of both LHTL camps have been already published (34). If we correlate the percent changes in individual Hbmass data (in g·kg⁻¹) of the present article with the individual performance data from the already published article (34), we obtain a correlation of r = -0.47 (P = 0.07) in HH and a correlation of r = -0.57 (P = 0.03) in NH. This is comparable to our previously published paper (16), where we reported also a correlation (r = -0.64, P = 0.002) between running performance improvements and increase in Hbmass (g·kg⁻¹) after
18-d LHTL (n = 21), suggesting that the enhancement in endurance performance was
directly linked to changes in Hb$_{\text{mass}}$ after LHTL. Whereas, there was no significant
correlation between percent changes in individual performance and Hb$_{\text{mass}}$ (in g) in HH
(r = -0.14, P = 0.61) and in NH (r = -0.35, P = 0.20). This in turn supports the literature
showing an increase in Hb$_{\text{mass}}$ following altitude training with different performance
outcomes (7, 12, 30). Further, it seems that also nonhematological mechanisms such as
improved mitochondrial efficiency and/or muscle pH regulation (13) can contribute to
enhanced sea-level performance following altitude training. Thus, the impact of Hb$_{\text{mass}}$
increase on performance benefits following altitude training remains unclear.

Paragraph Number 18 To date, whether the type of hypoxia (e.g., NH or HH) differs
considerably regarding physiological and performance responses is still debated (5).
Short-term exposure (< 26 h) to HH seems to evoke greater hypoxemia, lower oxygen
arterial saturation (35), and more altered cycling time trial performance (33) compared
to NH. Whereas long-term exposure of the same duration (e.g., following LHTL) to HH
and NH induced similar Hb$_{\text{mass}}$ (16) and performance improvements (32, 34). The
present crossover study confirmed that 18-d LHTL training at 2250 m either in HH or in
NH induced similar mean Hb$_{\text{mass}}$ responses, despite a larger number of hypoxic hours in
HH compared to NH. Thus, from a practical point of view it seems that both hypoxic
conditions (HH or NH) can be used equally for LHTL camps to enhance Hb$_{\text{mass}}$.
However, it must be considered that HH conditions can accumulate hypoxic hours much
faster than NH, while NH conditions are logistically easier and more customizable than
HH.

Individual Hb$_{\text{mass}}$ responses and reproducibility

Paragraph Number 19 Individual variability in Hb$_{\text{mass}}$ response to altitude training
camps in either HH or NH has previously been shown and discussed (6, 8, 16, 38, 43);
however, not many altitude training studies quantified individual responsiveness (24, 27, 29, 30). In the present study, individual Hb\textsubscript{mass} responsiveness (measure of individual responses that is free from the TE) was ±0.9% in HH and ±1.7% in NH, which was slightly lower compared to other studies demonstrating individual Hb\textsubscript{mass} responsiveness of ±1.3% to ±2.6% in HH (24, 29) and of ±1.4% to ±2.9% in NH (27, 30). Interestingly, after the same hypoxic hours in HH, the magnitude of individual Hb\textsubscript{mass} responsiveness was ±3.4%. This result was much greater than expected, suggesting that it was due to measurement imprecision and that even with duplicate Hb\textsubscript{mass} measurements there is still a chance of random noise (14). The reason for individual variability in Hb\textsubscript{mass} response to altitude training remains to be clarified and can be attributed to many factors, such as individual variation in erythropoietic response to hypoxia (3, 6), genetic predisposition (46), occurrence of a mild neocytolysis after descending after return to sea level (6) or different baseline conditions such as low pre-altitude ferritin levels (40). Regarding the latter, in the present study, all individual ferritin levels were above > 30 µg·L\textsuperscript{-1} and an inverse correlation between the pre-altitude ferritin level and Hb\textsubscript{mass} (in g) changes ($r = -0.30$, $P = 0.10$) was shown suggesting that in the present study initial ferritin levels did not influence individual variability in Hb\textsubscript{mass} response. However, there is also evidence that low iron stores (< 30 µg·L\textsuperscript{-1}) may impair Hb\textsubscript{mass} production and thus an individualized iron supplementation strategy during altitude training is recommended (10).

Paragraph Number 20 To detect significant individual Hb\textsubscript{mass} responses, the 95% CLs for the percent changes of Hb\textsubscript{mass} were derived from the present overall TE, which was ±3.9%. The upper CL was exceeded by half the athletes in both hypoxic conditions (HH: eight of 15 and NH: seven of 15, Fig. 3). Because Hb\textsubscript{mass} was measured in duplicate, which reduces the TE by a factor of $1/\sqrt{2}$ (17) and thus enhances the measurement precision, the athletes who exceeded the 95% CL were likely responders.
in Hbmass to the altitude training in the current study. Further, most of the athletes who
increased their Hbmass during the first LHTL altitude camp demonstrated a reproducible
Hbmass response after the second LHTL altitude camp, suggesting that those athletes
who responded once to altitude training will very likely respond another time regardless
of the type of hypoxia. Previous studies focusing on reproducibility of Hbmass responses
in athletes to altitude training camps (24, 43) have demonstrated reproducible mean
percent Hbmass changes but only a small trend toward reproducible individual Hbmass
changes, which is not in line with the present results. Thus, whether reproducibility in
individual Hbmass responses to altitude training camps and/or to different hypoxic
conditions (HH vs. NH) exists remains unclear. Overall, the variability in individual
Hbmass response to hypoxia detected in the present study emphasizes the importance of
evaluating the individual Hbmass response of an athlete to altitude training camps.
Therefore, we recommend measuring Hbmass in duplicate directly before and after an
altitude training camp within a time lag of less than 24 h between the two
measurements.

CONCLUSION

Paragraph Number 21 The findings of the present crossover study indicate that
hypobaric and normobaric LHTL evoked a similar mean increase in Hbmass following
18-d LHTL. There was no difference in Hbmass changes between HH and NH. Notable
variability in individual Hbmass responses between athletes was observed, indicating the
importance of individual evaluation of Hbmass responses to altitude training.
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GRANTS

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DISCLOSURES

Paragraph Number 24 No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.H., L.S., G.P.M., and J.P.W. conceived and designed the work. A.H., S.T., L.S., J.J.S., N.R., R.C.A., R.F., T.S., G.P.M., and J.P.W. performed the research. A.H., S.T., L.S., J.J.S., N.R., R.C.A., R.F., T.S., G.P.M., and J.P.W. analyzed or interpreted the data for the work. A.H. and J.P.W. drafted the manuscript. All authors edited and revised the manuscript critically and approved the final version of the manuscript.
REFERENCES


**FIGURE LEGENDS**

**FIGURE 1.** Illustration of the study design (*n* =15).

**FIGURE 2.** Individual Hb<sub>mass</sub> (g) for before (Pre) and after (Post) 18 d of LHTL in either hypobaric or normobaric hypoxia, *n* = 15.

**FIGURE 3.** Individual hemoglobin mass (Hb<sub>mass</sub>) changes (%) after 18 d of LHTL in hypobaric hypoxia (HH, 312 h) or in normobaric hypoxia (NH, 225 h). The 95% limits (95% CLs) are indicated by dotted lines.
Hypobaric Hypoxia

\(<1200 \text{ m}

2250 \text{ m}

\)

Camp 1

Normobaric Hypoxia

\(<1200 \text{ m}

2250 \text{ m}

\)

Camp 2

Normobaric Hypoxia

\(<1200 \text{ m}

2250 \text{ m}

\)

1 Year Wash out

Day 13

Pre-

18-days LHTL Camp

Post-

Pre-

18-days LHTL Camp

Post-

\(\downarrow \text{\ Hb_mass Measure} \)

\(\uparrow \text{ Blood Samplings} \)
Table 1 Likelihoods of magnitudes of hemoglobin mass (Hbmass) changes between hypobaric hypoxia (HH) and normobaric hypoxia (NH) after 18-days LHTL camp and after matched hypoxic hours (230 h and 225 h).

<table>
<thead>
<tr>
<th>Compared Groups</th>
<th>Parameter</th>
<th>ΔMean (%)</th>
<th>90% CL</th>
<th>positive</th>
<th>trivial</th>
<th>negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH vs. NH 18-days LHTL</td>
<td>Hbmass (g)</td>
<td>0.7</td>
<td>± 1.4</td>
<td>36%</td>
<td>61%</td>
<td>3%</td>
</tr>
<tr>
<td>HH vs. NH Same hypoxic hours</td>
<td>Hbmass (g)</td>
<td>1.4</td>
<td>± 2.3</td>
<td>61%</td>
<td>34%</td>
<td>5%</td>
</tr>
</tbody>
</table>

ΔMean = differences in mean, CL = confidence limits. With references to a smallest worthwhile change of 1% for Hbmass. Comparison of groups always first group minus second group.