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

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Running title: Individual Hb_{mass} 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_{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_{mass} following 18-d LHTL. Notable variability and reproducibility in individual Hb_{mass} responses between athletes was observed, indicating the importance of evaluating individual Hb_{mass} response to altitude training.

INTRODUCTION

Paragraph Number 1 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 (Hb_{mass}) (16) and performance responses (32). Thus far, only one study (16) has compared individual Hb_{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 Hb_{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 Hb_{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 Hb_{mass} may have affected the outcome. Thus, the question whether normobaric and hypobaric LHTL results in similar Hb_{mass} responses has not been conclusively answered. The straightforward option to diminish the observed effect is to conduct a same-subject crossover design.

79 ***Paragraph Number 2*** The primary aim of the present study was to investigate whether
80 Hb_{mass} responses differ between 18-d hypobaric and normobaric LHTL with a same-
81 subject crossover design. The secondary aim was to quantify individual Hb_{mass}
82 responsiveness in HH and NH.
83

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 $\mu g \cdot L^{-1}$, 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_{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 in camp 1 and n = 5 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 m^2). Normobaric hypoxia was obtained by extracting oxygen from ambient air in hypoxic rooms (P_iO_2 111.9 ± 0.6 mm Hg, F_iO_2 18.05 ± 0.1%, P_B 666.6 ± 3.6 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.

161

162 **Hemoglobin mass**

163 ***Paragraph Number 7*** Hb_{mass} was measured in duplicate using a slightly modified
164 version of the optimized carbon monoxide (CO)-rebreathing method described by
165 Schmidt and Prommer (36). Briefly, a CO dose of 100 mL (Multigas SA, Domdidier,
166 Switzerland) was administered and rebreathed with 3.5 L oxygen for 2 min in a closed
167 circuit system (glass spirometer, Blood Tec GbR, Bayreuth, Germany). Capillary
168 earlobe blood samples (35 µl) were collected three times before the CO-rebreathing
169 procedure and once at minute 6 and 8 after CO rebreathing was started. Blood samples
170 were analyzed for carboxyhemoglobin (%HbCO) using a CO-oximeter (ABL 800flex,
171 Radiometer A/S, Copenhagen, Denmark). Hb_{mass} was calculated from the mean change
172 in %HbCO before and after CO rebreathing, as described previously by Steiner and
173 Wehrlin (39). Both measurements were performed on two consecutive days (12–24 h
174 time lag between the measures), and the results were averaged. The typical error (TE) of
175 Hb_{mass} measurement was calculated from duplicate measurements as the standard
176 deviation (SD) of the difference score divided by √2 (17). To provide a dimensionless
177 measure of reliability, which is comparable between subjects and studies (17), the TE
178 was translated into a coefficient of variation (CV). The CV is calculated by dividing the
179 TE by the mean value of Hb_{mass} and is expressed in percent. Averaged multiple
180 measurements reduce the TE by a factor of $1/\sqrt{n}$, where n is the number of
181 measurements (17). In this study, the TEs for duplicate measurements of Hb_{mass} at the
182 different time points were as follows: pre-camp 1: 1.8% (90% confidence limits (CLs):
183 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:
184 1.9% (1.3–2.6%); post-camp 2: 1.1% (0.8–1.6%). In our mobile laboratory, the overall
185 TE of the CO-rebreathing method was 2.0% (1.5–2.6%), and the TE for the average
186 duplicate measurements was 1.4% (1.1–1.8%).

187

188 **Ferritin and OFF score**

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

201

202 **Statistical analyses**

203 ***Paragraph Number 9*** Values are presented as means ± SD. All data were checked for
204 normality (Shapiro-Wilk test) and equality of variance. A two-way repeated measure
205 analysis of variance was applied to evaluate the differences between the conditions (HH
206 and NH) over time. When a significant global effect was indicated, Tukey's *post-hoc*
207 test was performed to identify significant differences between different levels of time
208 and conditions. For a comparison of the training load between HH and NH, a paired *t*-
209 test was performed. Linear regressions were used to determine the Pearson's correlation
210 coefficient (r) between individual delta Hb_{mass} changes (%) in HH and in NH. The level

211 of significance was set at $P < 0.05$. All analyses were processed using Sigmaplot 11.0
212 (Systat Software, San Jose, CA, USA).

213 ***Paragraph Number 10*** To assess the likelihood that the differences in percent change in
214 Hb_{mass} between HH and NH were relevant (i.e. more extreme than the smallest
215 worthwhile change in Hb_{mass}, set to ± 1%) a contemporary statistical approach
216 according to Hopkins (18) was used. This approach calculates the chances (in %) that
217 the true value of an effect is positive, trivial or negative. To classify the magnitude of
218 the effects (positive, trivial, or negative), the change in mean and the 90% CL of the
219 individual change scores were used (19). The effect was termed "unclear" if its CL
220 overlapped the positive and negative smallest worthwhile changes. Individual Hb_{mass}
221 responsiveness (i.e. the individual variation in the response to an intervention free of TE
222 (17)) for NH and HH is expressed as the SD from the mean Hb_{mass} change and was
223 calculated as the square root of the difference between the variance of the Hb_{mass} change
224 scores in the intervention and the variance in change scores arising from TE only
225 $((TE \cdot \sqrt{2})^2)$. To detect significant individual effects, the 95% CL for percent changes of
226 Hb_{mass} was derived from the present overall TE of the Hb_{mass} measurement
227 (95% CL = $\pm 1.96 \cdot TE \cdot \sqrt{2} \cdot 1/\sqrt{2}$; (17)).
228

RESULTS

Mean Hb_{mass} responses

Paragraph Number 11 After 18 d (n = 15), Hb_{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_{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_{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_{mass} changes being greater in HH compared to NH was 36% following 18-d LHTL and 61% for matched hypoxic hours (Table 1).

Table 1 near here

Figure 2 near here

Individual Hb_{mass} responses

Paragraph Number 12 Percent changes in individual Hb_{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_{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_{mass} changes (%) in HH and in NH after 18-d LHTL ($r = 0.52$, $P = 0.048$

Figure 3 near here

Ferritin and OFF score

Paragraph Number 13 Initial ferritin levels were > 30 $\mu g \cdot L^{-1}$ in all athletes. Pre-ferritin values were 108.1 ± 36.0 $\mu g \cdot L^{-1}$ and 107.3 ± 36.3 $\mu g \cdot L^{-1}$ 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_{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_{mass} following 18-d LHTL. The mean changes in Hb_{mass} did not differ between HH and NH. Notable variability in individual Hb_{mass} responses following 18-d LHTL in HH and NH was observed as well as a significant correlation between individual delta Hb_{mass} changes (%) in HH and in NH.

Mean Hb_{mass} responses

Paragraph Number 16 Both hypoxic conditions (HH vs. NH) demonstrated a similar mean Hb_{mass} increase (+4.5% vs. +3.8%) following 18-d LHTL. Furthermore, the chance in percent Hb_{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_{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_{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_{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_{mass} response. The present mean Hb_{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 Hb_{mass} (4, 31).
300 However, the data for the NH group after 18 d (225 h) and for the HH group after 13 d
301 (230 h) suggest that a relevant Hb_{mass} increase can be achieved with less hypoxic hours
302 (< 300 h) in some subjects. Recently, studies have examined earlier time courses (8, 43)
303 and shorter hypoxic exposure (9, 27) on changes in Hb_{mass} to moderate altitude (2500–
304 3000 m). The data from these studies showed measurable Hb_{mass} increases (2.1% to
305 3.7%) within a shorter time period (11–13 d) or lower hypoxic exposure (< 210 h) than
306 recommended (14, 31). However, the present study and the reported studies (8, 9, 27,
307 43) used different athlete populations and applied different altitude protocols, which
308 may limit generalization. Therefore, further research is needed to better understand the
309 time course and dose–response relationship of Hb_{mass} to different altitude protocols in
310 different athlete populations.

311 ***Paragraph Number 17*** An hypoxia-induced increase in Hb_{mass} seems to be one of the
312 main physiological mechanisms leading to improved sea-level endurance performance
313 after altitude training (14, 22, 23, 42). Hb_{mass} is closely related to maximal oxygen
314 uptake ($\dot{V}O_{2max}$) – that is, a gain of 1 g in Hb_{mass} results in a 4 $mL \cdot min^{-1}$ increase in
315 $\dot{V}O_{2max}$ under normoxic conditions (37). Further, Hb_{mass} correlates with time trial
316 performance and maximal incremental power output in highly trained endurance
317 athletes (21). In both 18-d LHTL camps, the athletes performed a 3-km running time
318 trial near sea level before and after each camp. The mean performance data of both
319 LHTL camps have been already published (34). If we correlate the percent changes in
320 individual Hb_{mass} data (in $g \cdot kg^{-1}$) of the present article with the individual performance
321 data from the already published article (34), we obtain a correlation of r = -0.47 (P =
322 0.07) in HH and a correlation of r = -0.57 (P = 0.03) in NH. This is comparable to our
323 previously published paper (16), where we reported also a correlation (r = -0.64, P =
324 0.002) between running performance improvements and increase in Hb_{mass} ($g \cdot kg^{-1}$) after

18-d LHTL (n = 21), suggesting that the enhancement in endurance performance was directly linked to changes in Hb_{mass} after LHTL. Whereas, there was no significant correlation between percent changes in individual performance and Hb_{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_{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_{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_{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_{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_{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_{mass} responses and reproducibility

Paragraph Number 19 Individual variability in Hb_{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_{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_{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_{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_{mass} measurements there is still a chance of random noise (14). The reason for individual variability in Hb_{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 $\mu g \cdot L^{-1}$ and an inverse correlation between the pre-altitude ferritin level and Hb_{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_{mass} response. However, there is also evidence that low iron stores (< 30 $\mu g \cdot L^{-1}$) may impair Hb_{mass} production and thus an individualized iron supplementation strategy during altitude training is recommended (10).

Paragraph Number 20 To detect significant individual Hb_{mass} responses, the 95% CLs for the percent changes of Hb_{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_{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 Hb_{mass} to the altitude training in the current study. Further, most of the athletes who increased their Hb_{mass} during the first LHTL altitude camp demonstrated a reproducible Hb_{mass} 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 Hb_{mass} responses in athletes to altitude training camps (24, 43) have demonstrated reproducible mean percent Hb_{mass} changes but only a small trend toward reproducible individual Hb_{mass} changes, which is not in line with the present results. Thus, whether reproducibility in individual Hb_{mass} responses to altitude training camps and/or to different hypoxic conditions (HH vs. NH) exists remains unclear. Overall, the variability in individual Hb_{mass} response to hypoxia detected in the present study emphasizes the importance of evaluating the individual Hb_{mass} response of an athlete to altitude training camps. Therefore, we recommend measuring Hb_{mass} 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 Hb_{mass} following 18-d LHTL. There was no difference in Hb_{mass} changes between HH and NH. Notable variability in individual Hb_{mass} responses between athletes was observed, indicating the importance of individual evaluation of Hb_{mass} responses to altitude training.

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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

1. **Bonetti DL, Hopkins WG**. Sea-level exercise performance following adaptation to hypoxia: a meta-analysis. *Sports Med* 39: 107-127, 2009.

2. **Cejuela Anta R, Esteve-Lanao J**. Training load quantification in triathlon. *JHSE* 6: 218-232, 2011.

3. **Chapman RF, Stray-Gundersen J, Levine BD**. Individual variation in response to altitude training. *J Appl Physiol* 85: 1448-1456, 1998.

4. **Clark SA, Quod MJ, Clark MA, Martin DT, Saunders PU, Gore CJ**. Time course of haemoglobin mass during 21 days live high:train low simulated altitude. *Eur J Appl Physiol* 106: 399-406, 2009.

5. **Coppel J, Hennis P, Gilbert-Kawai E, Grocott MP**. The physiological effects of hypobaric hypoxia versus normobaric hypoxia: a systematic review of crossover trials. *Extrem Physiol Med* 4: 2, 2015.

6. **Friedmann B, Frese F, Menold E, Kauper F, Jost J, Bartsch P**. Individual variation in the erythropoietic response to altitude training in elite junior swimmers. *Br J Sports Med* 39: 148-153, 2005.

7. **Garvican LA, Pottgiesser T, Martin DT, Schumacher YO, Barras M, Gore CJ**. The contribution of haemoglobin mass to increases in cycling performance induced by simulated LHTL. *Eur J Appl Physiol* 111: 1089-1101, 2011.

8. **Garvican LA, Martin DT, Quod MJ, Stephens B, Sassi A, Gore CJ**. Time course of the hemoglobin mass response to natural altitude training in elite endurance cyclists. *Scand J Med Sci Sports* 22: 95-103, 2012.

9. **Garvican Lewis LA, Clark SA, Polglaze T, McFadden G, Gore CJ**. Ten days of simulated live high:train low altitude training increases Hbmass in elite water polo players. *Br J Sports Med* 47 (Suppl 1): i70-73, 2013.

10. **Garvican Lewis LA, Govus AD, Peeling P, Abbiss CR, Gore CJ**. Iron Supplementation and Altitude: Decision Making Using a Regression Tree. *J Sports Sci Med* 15: 204-205, 2016.

11. **Gore CJ, Parisotto R, Ashenden MJ, Stray-Gundersen J, Sharpe K, Hopkins W, Emslie KR, Howe C, Trout GJ, Kazlauskas R, Hahn AG**. Second-generation blood tests to detect erythropoietin abuse by athletes. *Haematologica* 88: 333-344, 2003.

12. **Gore CJ, Hopkins WG**. Counterpoint: positive effects of intermittent hypoxia (live high:train low) on exercise performance are not mediated primarily by augmented red cell volume. *J Appl Physiol (1985)* 99: 2055-2057; discussion 2057-2058, 2005.

13. **Gore CJ, Clark SA, Saunders PU**. Nonhematological mechanisms of improved sea-level performance after hypoxic exposure. *Med Sci Sports Exerc* 39: 1600-1609, 2007.

14. **Gore CJ, Sharpe K, Garvican-Lewis LA, Saunders PU, Humberstone CE, Robertson EY, Wachsmuth NB, Clark SA, McLean BD, Friedmann-Bette B, Neya**

462 **M, Pottgiesser T, Schumacher YO, Schmidt WF**. Altitude training and haemoglobin
463 mass from the optimised carbon monoxide rebreathing method determined by a meta-
464 analysis. *Br J Sports Med* 47 (Suppl 1): i31-39, 2013.

465 15. **Gough CE, Saunders PU, Fowlie J, Savage B, Pyne DB, Anson JM, Wachsmuth N,**
466 **Prommer N, Gore CJ**. Influence of altitude training modality on performance and total
467 haemoglobin mass in elite swimmers. *Eur J Appl Physiol* 112: 3275-3285, 2012.

468 16. **Hauser A, Schmitt L, Troesch S, Saugy JJ, Cejuela-Anta R, Faiss R, Robinson N,**
469 **Wehrlin JP, Millet GP**. Similar Hemoglobin Mass Response in Hypobaric and
470 Normobaric Hypoxia in Athletes. *Med Sci Sports Exerc* 48: 734-741, 2016.

471 17. **Hopkins WG**. Measures of Reliability in Sports Medicine and Science. *Sports Med* 30: 1-
472 15, 2000.

473 18. **Hopkins WG**. A spreadsheet for analysis of straightforward controlled trials [Online]
474 Sportscience 7. sportsci.org/jour/03/wghtrials.htm. [Aug 2016].

475 19. **Hopkins WG, Marshall SW, Batterham AM, Hanin J**. Progressive statistics for studies
476 in sports medicine and exercise science. *Med Sci Sports Exerc* 41: 3-13, 2009.

477 20. **Humberstone-Gough CE, Saunders PU, Bonetti DL, Stephens S, Bullock N, Anson**
478 **JM, Gore CJ**. Comparison of Live High: Train Low Altitude and Intermittent Hypoxic
479 Exposure. *J Sports Sci Med* 12: 394-401, 2013.

480 21. **Jacobs RA, Rasmussen P, Siebenmann C, Diaz V, Gassmann M, Pesta D, Gnaiger E,**
481 **Nordsborg NB, Robach P, Lundby C**. Determinants of time trial performance and
482 maximal incremental exercise in highly trained endurance athletes. *J Appl Physiol (1985)*
483 111: 1422-1430, 2011.

484 22. **Levine BD, Stray-Gundersen J**. "Living high-training low": effect of moderate-altitude
485 acclimatization with low-altitude training on performance. *J Appl Physiol* 83: 102-112,
486 1997.

487 23. **Levine BD, Stray-Gundersen J**. Point: positive effects of intermittent hypoxia (live
488 high:train low) on exercise performance are mediated primarily by augmented red cell
489 volume. *J Appl Physiol (1985)* 99: 2053-2055, 2005.

490 24. **McLean BD, Buttifant D, Gore CJ, White K, Kemp J**. Year-to-year variability in
491 haemoglobin mass response to two altitude training camps. *Br J Sports Med* 47 (Suppl 1):
492 i51-58, 2013.

493 25. **Millet GP, Roels B, Schmitt L, Woorons X, Richalet JP**. Combining hypoxic methods
494 for peak performance. *Sports Med* 40: 1-25, 2010.

495 26. **Millet GP, Faiss R, Pialoux V**. Point: Hypobaric hypoxia induces different physiological
496 responses from normobaric hypoxia. *J Appl Physiol (1985)* 112: 1783-1784, 2012.

497 27. **Neya M, Enoki T, Ohiwa N, Kawahara T, Gore CJ**. Increased hemoglobin mass and
498 VO2max with 10 h nightly simulated altitude at 3000 m. *Int J Sports Physiol Perform* 8:
499 366-372, 2013.

28. **Robach P, Schmitt L, Brugniaux JV, Nicolet G, Duvallet A, Fouillot JP, Moutereau S, Lasne F, Pialoux V, Olsen NV, Richalet JP**. Living high-training low: effect on erythropoiesis and maximal aerobic performance in elite Nordic skiers. *Eur J Appl Physiol* 97: 695-705, 2006.

29. **Robertson EY, Aughey RJ, Anson JM, Hopkins WG, Pyne DB**. Effects of simulated and real altitude exposure in elite swimmers. *J Strength Cond Res* 24: 487-493, 2010.

30. **Robertson EY, Saunders PU, Pyne DB, Aughey RJ, Anson JM, Gore CJ**. Reproducibility of performance changes to simulated live high/train low altitude. *Med Sci Sports Exerc* 42: 394-401, 2010.

31. **Rusko HK, Tikkanen HO, Peltonen JE**. Altitude and endurance training. *J Sports Sci* 22: 928-945, 2004.

32. **Saugy JJ, Schmitt L, Cejuela R, Faiss R, Hauser A, Wehrlin JP, Rudaz B, Delessert A, Robinson N, Millet GP**. Comparison of "Live High-Train Low" in normobaric versus hypobaric hypoxia. *PLoS One* 9: e114418, 2014.

33. **Saugy JJ, Rupp T, Faiss R, Lamon A, Bourdillon N, Millet GP**. Cycling Time Trial Is More Altered in Hypobaric than Normobaric Hypoxia. *Med Sci Sports Exerc* 48: 680-688, 2016.

34. **Saugy JJ, Schmitt L, Hauser A, Constantin G, Cejuela R, Faiss R, Wehrlin JP, Rosset J, Robinson N, Millet GP**. Same Performance Changes after Live High-Train Low in Normobaric vs. Hypobaric Hypoxia. *Front Physiol* 7: 138, 2016.

35. **Savourey G, Launay JC, Besnard Y, Guinet A, Travers S**. Normo- and hypobaric hypoxia: are there any physiological differences? *Eur J Appl Physiol* 89: 122-126, 2003.

36. **Schmidt W, Prommer N**. The optimised CO-rebreathing method: a new tool to determine total haemoglobin mass routinely. *Eur J Appl Physiol* 95: 486-495, 2005.

37. **Schmidt W, Prommer N**. Impact of alterations in total hemoglobin mass on VO 2max. *Exerc Sport Sci Rev* 38: 68-75, 2010.

38. **Siebenmann C, Robach P, Jacobs RA, Rasmussen P, Nordsborg N, Diaz V, Christ A, Olsen NV, Maggiorini M, Lundby C**. "Live high-train low" using normobaric hypoxia: a double-blinded, placebo-controlled study. *J Appl Physiol (1985)* 112: 106-117, 2012.

39. **Steiner T, Wehrlin JP**. Does hemoglobin mass increase from age 16 to 21 and 28 in elite endurance athletes? *Med Sci Sports Exerc* 43: 1735-1743, 2011.

40. **Stray-Gundersen J, Alexander C, Hochstein A, deLemos D, Levine BD**. Failure of red cell volume to increase to altitude exposure in iron deficient runners. *Med Sci Sports Exerc* 24: S90, 1992.

41. **Stray-Gundersen J, Chapman RF, Levine BD**. "Living high-training low" altitude training improves sea level performance in male and female elite runners. *J Appl Physiol (1985)* 91: 1113-1120, 2001.

42. **Stray-Gundersen J, Levine BD**. Live high, train low at natural altitude. *Scand J Med Sci Sports* 18 Suppl 1: 21-28, 2008.

43. **Wachsmuth NB, Volzke C, Prommer N, Schmidt-Trucksass A, Frese F, Spahl O, Eastwood A, Stray-Gundersen J, Schmidt W**. The effects of classic altitude training on hemoglobin mass in swimmers. *Eur J Appl Physiol* 113: 1199-1211, 2013.

44. **Wehrlin JP, Zuest P, Hallen J, Marti B**. 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.

45. **Wilber RL**. Application of altitude/hypoxic training by elite athletes. *Med Sci Sports Exerc* 39: 1610-1624, 2007.

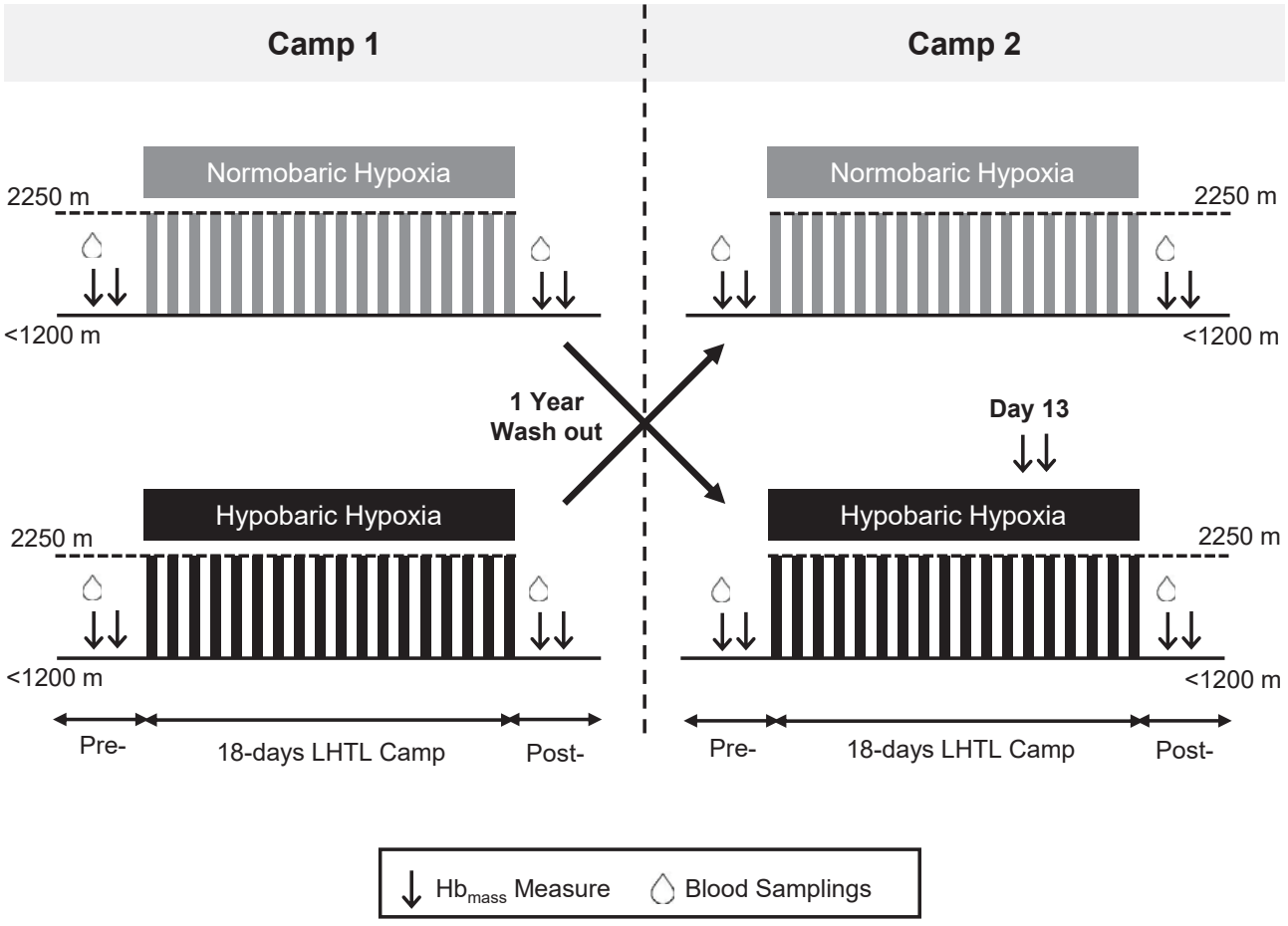
46. **Wilber RL, Stray-Gundersen J, Levine BD**. Effect of hypoxic "dose" on physiological responses and sea-level performance. *Med Sci Sports Exerc* 39: 1590-1599, 2007.

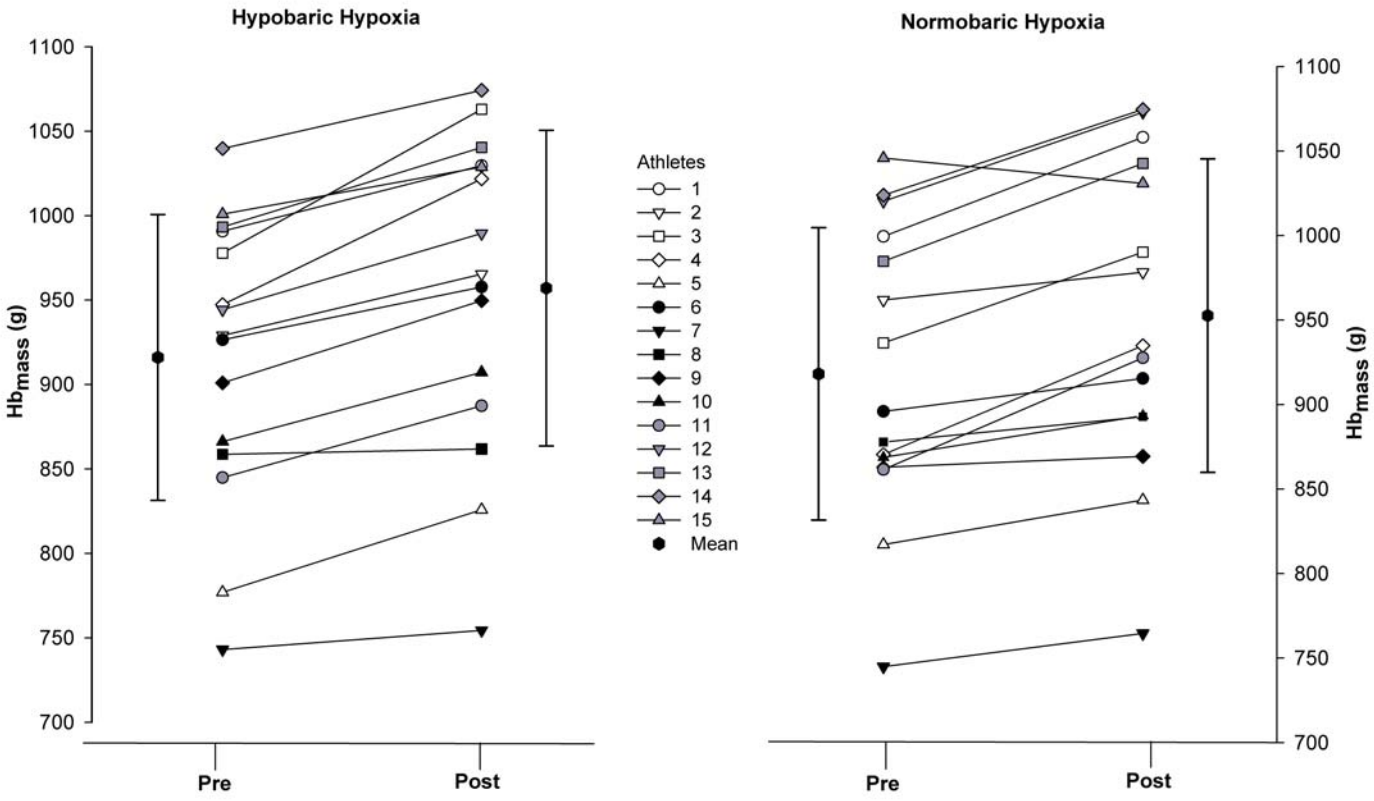
FIGURE LEGENDS

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

FIGURE 2. Individual Hb_{mass} (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_{mass}) 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.





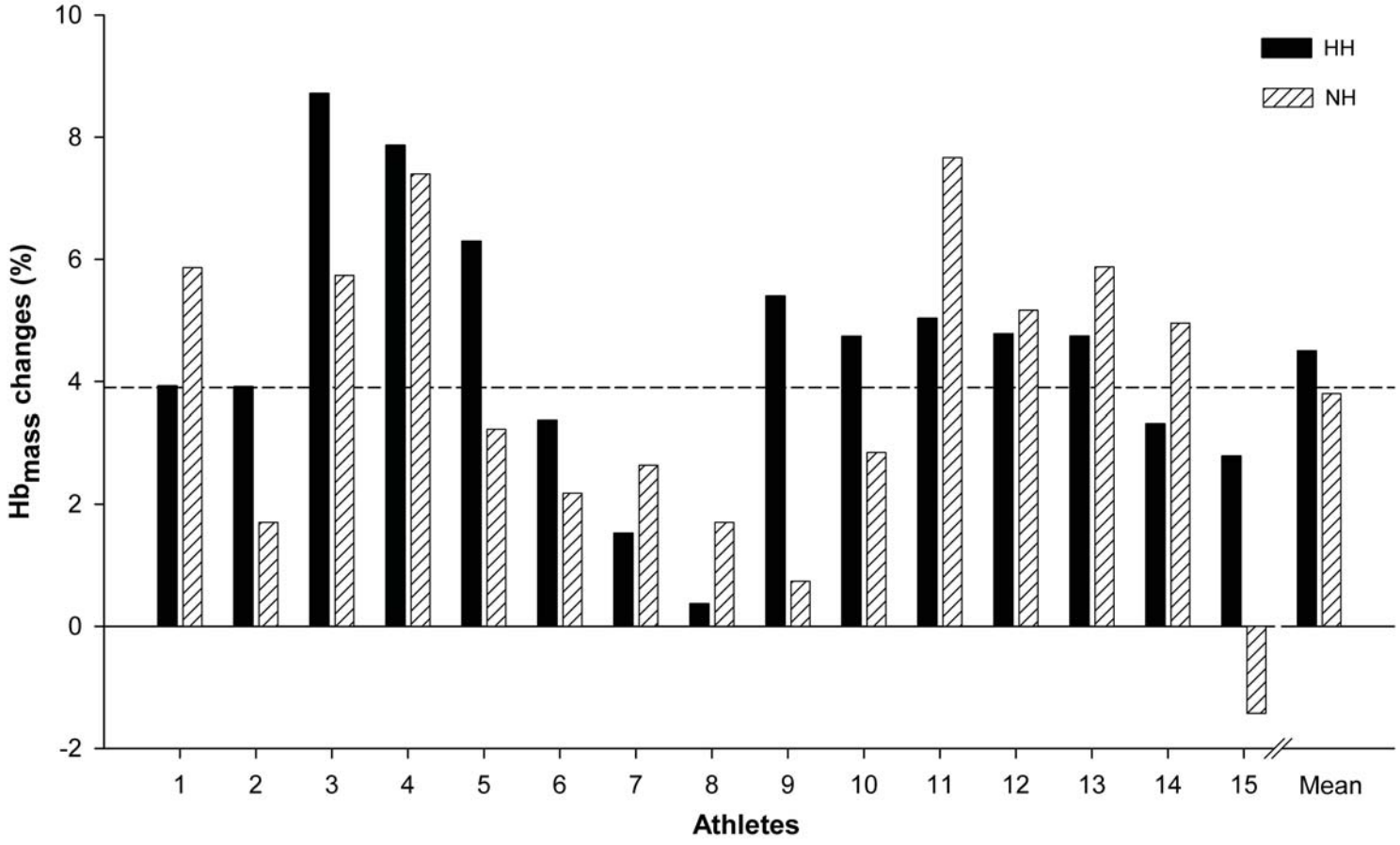


Table 1 Likelihoods of magnitudes of hemoglobin mass (Hb_{mass}) changes between hypobaric hypoxia (HH) and normobaric hypoxia (NH) after 18-days LHTL camp and after matched hypoxic hours (230 h and 225 h).

Compared Groups		Parameter	ΔMean (%)	90% CL	positive	trivial	negative
HH vs. NH	18-days LHTL	Hb_{mass} (g)	0.7	± 1.4	36%	61%	3%
HH vs. NH	Same hypoxic hours	Hb_{mass} (g)	1.4	± 2.3	61%	34%	5%

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