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Do male athletes with already high initial hemoglobin mass benefit from ‘live high–train low’ altitude training?

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What is the central question of this study?
It has been assumed that athletes embarking on an LHTL camp with already high initial Hb\text{mass} have a limited ability to further increase their Hb\text{mass} post-intervention. Therefore, the relationship between initial Hb\text{mass} and post-intervention increase were tested with duplicate Hb\text{mass} measures and comparable hypoxic doses in male athletes.

What is the main finding and its importance?
There were trivial to moderate inverse relationships between initial Hb\text{mass} and percentage Hb\text{mass} increase in endurance and team-sport athletes after LHTL camp, indicating that even athletes with higher initial Hb\text{mass} can reasonably expect Hb\text{mass} gains post-LHTL.

Abstract
It has been proposed that athletes with high initial values of hemoglobin mass (Hb\text{mass}) will have a lower Hb\text{mass} increase in response to ‘live high-train low’ (LHTL) altitude training. To verify this assumption, the relationship between initial absolute and relative Hb\text{mass} values and their respective Hb\text{mass} increase following LHTL in male endurance and team-sport athletes was investigated. Overall, 58 male athletes (35 well-trained endurance athletes and 23 elite male field hockey players) undertook an LHTL training camp with similar hypoxic doses (200-230 h). Hb\text{mass} was measured in duplicate pre- and post-LHTL with the carbon monoxide rebreathing method. While there was no relationship \((r = 0.02, P = 0.91)\) between initial absolute Hb\text{mass} (g) and percentage increase in absolute Hb\text{mass}, a moderate relationship \((r = -0.31, P = 0.02)\) between initial relative Hb\text{mass} (g·kg\(^{-1}\)) and percentage increase in relative Hb\text{mass} was detected. Mean absolute and relative Hb\text{mass} increased to a similar extent \((P \geq 0.81)\) in endurance (from 916 ± 88 to 951 ± 96 g, +3.8%, \(P < 0.001\) and from 13.1 ± 1.2 to 13.6 ± 1.1 g·kg\(^{-1}\), +4.1%, \(P < 0.001\)) and team-sport (from 920 ± 120 to 957± 127 g, +4.0%, \(P < 0.001\) and from 11.9 ± 0.9 to 12.3 ± 0.9 g·kg\(^{-1}\), +4.0%, \(P < 0.001\)) athletes following LHTL. The direct comparison study using individual data of male endurance and team-sport athletes and strict methodological control (duplicate Hb\text{mass}-measures, matched-hypoxic dose) indicated that even athletes with higher initial Hb\text{mass} can reasonably expect Hb\text{mass} gain post-LHTL.

Key words: LHTL, hypoxia, CO rebreathing method

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Introduction

Paragraph Number 1 Many elite endurance athletes perform altitude training with the aim to enhance their oxygen-carrying capacity and eventually their sea-level performance (Wilber, 2007; Bonetti & Hopkins, 2009; Millet et al., 2010). During the last decade, hypoxic/altitude training interventions have become increasingly popular in team sports and innovative methods fitting their physical requirements (combination of aerobic and anaerobic adaptations) have been introduced (McLean et al., 2014; Brocherie et al., 2017). Compared to endurance athletes, team-sport athletes are generally characterised by a lower maximal aerobic capacity (Girard et al., 2013) and possess lower relative hemoglobin mass (Hb\text{mass}) (Heinicke et al., 2001; Wachsmuth et al., 2013a). The potential main benefit derived from the popular ‘live high–train low’ (LHTL) altitude training intervention seems to rely on an increase in Hb\text{mass} (Levine & Stray-Gundersen, 2005; Gore et al., 2013; Wehrlin et al., 2016). In a particular sport (e.g. endurance or team sports), considerable individual variation in Hb\text{mass} response to altitude training has been reported (Friedmann et al., 2005; Garvican et al., 2012; Siebenmann et al., 2012; Garvican Lewis et al., 2013; Wachsmuth et al., 2013b) and quantified as a standard deviation (SD) from the mean change of ±1.7% to ±2.2% (McLean et al., 2013; Hauser et al., 2017). Although sources of this variability still remain unclear, aspects such as erythropoietic response to hypoxia (Chapman et al., 1998; Friedmann et al., 2005), genetic predisposition (Wilber et al., 2007), residual fatigue and training history (Garvican et al., 2007) and/or intra-individual conditions (Wachsmuth et al., 2013b) likely play a role.

Paragraph Number 2 Another suggested reason for this variability relies on the individual’s initial Hb\text{mass} level before embarking upon the altitude training camp. Hence, it has been proposed that athletes with already high initial Hb\text{mass} have a limited ability to further increase (i.e. ceiling effect) their Hb\text{mass} following altitude training (Gore et al., 1998; Robach & Lundby, 2012; McLean et al., 2013). A recent analysis of nine LHTL studies (Robach & Lundby, 2012) demonstrated a high correlation ($r = 0.86$, $P < 0.01$) between initial relative (expressed in g·kg$^{-1}$) Hb\text{mass} and post-intervention percentage increase in relative Hb\text{mass}. However, this analysis had several important limitations, which limit the strength of comparison between the different LHTL training studies. First, different methods (Evans blue dye vs. CO-rebreathing methods) for the determination of Hb\text{mass}, with different accuracy/reliability levels, have been used (Wehrlin et al., 2016). Furthermore, these studies examined different genders - female athletes demonstrate a 10% lower level of Hb\text{mass} compared to male athletes (Schmidt & Prommer, 2008), and ‘hypoxic doses’ varied greatly – between a total of 200–500 hours of hypoxic exposure. Lastly, the data analysis was based on averaged values and not on individual values. Nevertheless, one should be cautious when interpreting group mean data since considerable inter-individual variation in Hb\text{mass} response to altitude training exists (Friedmann et al., 2005; Garvican et al., 2012; McLean et al., 2013; Hauser et al., 2016).

Paragraph Number 3 Since LHTL is primarily used by elite endurance athletes typically presenting elevated Hb\text{mass} values compared to team-sport athletes, the hypothesis that athletes embarking on an LHTL camp with already high initial Hb\text{mass} have a limited ability to further increase their Hb\text{mass} post-intervention (or at least to a lower extent than their team-sport counterparts) needs to be
tested with a more robust study design. Thus, the aim of the present study, which reanalyzed already existing data, was to examine the relationship between individual initial Hbmass prior to LHTL (absolute and relative values) and percentage Hbmass increase following a LHTL camp with comparable hypoxic doses in male endurance and team-sport athletes.

Methods

Ethical approval

Paragraph Number 4 All altitude training studies were approved by the local ethical committees: Commission Cantonale Valaisanne d’Ethique Médicale, CCVEM (Agreement 051/09), French National Conference of Research Ethics Committees (N*CPP EST I: 2014/33; Dijon, France) and by the Anti-Doping Lab Qatar institutional review board (SCH-ADL-070; Doha, Qatar). All experimental procedures were conducted in accordance with the Declaration of Helsinki guidelines, and all athletes provided written informed consent to participate in the respective studies. The study was not registered in a database.

Study design

Paragraph Number 5 Data from three altitude training interventions (studies I, II and III), with similar hypoxic doses (200–230 h) and identical Hbmass measurement procedures, were re-analysed to determine the nature of the association of individual Hbmass increase the individual initial absolute and relative Hbmass. The details of the experimental design of the three altitude training interventions have been published elsewhere (see Study I (Saugy et al., 2014), Study II (Hauser et al., 2016) and Study III (Brocherie et al., 2015)).

Participants

Paragraph Number 6 For studies I and II, 35 well-trained male endurance athletes (age 24.0 ± 4.5 years, height 177.9 ± 4.8 cm, weight 70.2 ± 6.2 kg, training 10-12 h per week) were recruited. For study III, 23 elite male field hockey players (age 24.4 ± 4.0 years, height 179.7 ± 9.1 cm, weight 77.5 ± 8.7 kg, training 7-9 h per week) were included. A total of 58 athletes were included in the final sample. Inclusion criteria for analysis were as follows: initial ferritin levels >30 μg·L⁻¹ (sufficient ferritin stores), male (exclusion confounding factor ‘gender’), endurance or team sport athlete (guarantee high and low initial Hbmass values within the data analysis) and completion of an LHTL altitude training camp with all Hbmass measures done in duplicate by the same investigator prior to and after the intervention.

Altitude interventions

Paragraph Number 7 For studies I and II (normobaric groups), 24 endurance athletes performed an 18-d LHTL altitude training camp under normobaric hypoxic conditions (~12.5 h·day⁻¹ and 225 ± 9 h total hypoxic dose), during which the athletes trained at <1200 m and were exposed to normobaric
hypoxia equivalent to 2250 m (Wehrlin et al., 2016) in hypoxic rooms (P,O₂ 111.9 ± 0.6 mm Hg; F,O₂ 18.1 ± 0.1%; P₈ 666.6 ± 3.6 mm Hg; 1150 m). For study II (hypobaric group), since normobaric and hypobaric hypoxia induces similar Hbₘₐₜₜ and endurance performance responses after LHTL altitude training (Saugy et al., 2014; Hauser et al., 2016), an additional 11 endurance athletes were included, who completed a 13-d LHTL camp under hypobaric hypoxic conditions with similar total hypoxic hours (230 ± 1 h, ~17.5 h·day⁻¹). Those athletes lived at 2250 m (P,O₂ 111.7 ± 0.7 mm Hg; F,O₂ 20.9%, P₈ 580.8 ± 3.3 mm Hg) and trained twice daily at < 1200 m. For study III, all 23 field hockey players performed a 14-d LHTL training camp under normobaric hypoxic conditions (> 14 h·day⁻¹ and ~198 h total hypoxic dose); thereby they trained at sea level and slept in normobaric hypoxic rooms, and simulated altitude was gradually increased from 2500 m (P,O₂ 108.3 mm Hg; F,O₂ 15.1%, P₈ 768.0 mm Hg) up to 3000 m (P,O₂ 101.7 ± 0.8 mm Hg; F,O₂ 14.2 ± 0.1%, P₈ 765.3 ± 1.5 mm Hg) during the 14 days. In addition, they performed six repeated-sprints training sessions during the 14-d training camp either in normoxia (F,O₂ 20.9%; n=12) or normobaric hypoxic conditions (3000 m; F,O₂ ~14.5%; n=11). In summary, according to the definition of Garvican-Lewis et al. (2016), the metrics for hypoxic dose (in km.h) between the LHTL groups were similar and differed within 6%, assuming that the present hypoxic doses were comparable (table 1).

***Table 1 near here***

### Hemoglobin mass

**Paragraph Number 8** In all athletes, Hbₘₐₜₜ was measured in duplicate using a slightly modified version (Steiner & Wehrlin, 2011) of the optimised carbon monoxide (CO)-rebreathing method described by Schmidt and Prommer (2005). For details, see Hauser et al. (2016) and Brocherie et al. (2015). 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) was calculated from duplicate measurements as the SD of the difference score divided by \( \sqrt{2} \) (Hopkins, 2000). In our mobile laboratories, the TEs ranged between 1.6% and 2.0%. Since duplicate measurements reduce the TE by a factor of 1/\( \sqrt{2} \) (Hopkins, 2000), the TEs for averaged duplicate Hbₘₐₜₜ measurements ranged between 1.1% and 1.4%. For each athlete, Hbₘₜₜ measures were performed by the same investigator throughout the studies.

### Data analysis

**Paragraph Number 9** Values are presented as means ± SD. All data were checked for normality (Shapiro-Wilk test). A sample size estimation for a power of 0.8 (80%), a significance level at \( P = 0.05 \) and a correlation coefficient of \( r = 0.4 \) was performed and resulted in a minimal number of 46 subjects. Linear regressions were used to determine the Pearson’s product-moment correlation coefficients (\( r \)) between initial absolute and relative Hbₘₜₜ and their respective percent changes in Hbₘₜₜ, as well as for percent changes between body weight and Hbₘₜₜ. The standard error (SE) of the slope of the linear regression was calculated by bootstrapping. Correlation size was interpreted using the correlation classification of Hopkins (Hopkins et al., 2009): trivial (\( r < 0.1 \)), small (0.1 < \( r < 0.3 \)),
Results

Relationship between initial Hbmass and Hbmass increase

There was no relationship between the absolute initial Hbmass (g) and the percentage increase in absolute Hbmass ($r = 0.02, P = 0.91$) (Figure 1 A). The linear regression equation for absolute Hbmass was $y = -0.0004x + 3.5$ and the SE of the slope was ± 0.003. A moderate negative correlation between the relative initial Hbmass (g·kg⁻¹) and the percentage increase in relative Hbmass ($r = -0.31, P = 0.02$) was observed (Figure 1 B). The linear regression equation for relative Hbmass was $y = -0.98x + 16.4$ and the SE of the slope was ± 0.348. When, including body weight changes (%) to the multiple linear regression model (initial Hbmass (g·kg⁻¹) × body weight changes (%)) the initial relative Hbmass (g·kg⁻¹) was no longer significantly associated with percentage increase in Hbmass (g·kg⁻¹) ($P = 0.4$).

Mean Hbmass response

Mean absolute Hbmass increased to the same extent in endurance ($916 ± 88$ to $951 ± 96$ g, +$3.8 ± 2.9\%$, $P < 0.001$) and team-sport ($920 ± 120$ to $957 ± 127$ g, +$4.0 ± 2.9\%$, $P < 0.001$) athletes ($P = 0.81$). Mean relative Hbmass increased equally in endurance ($13.1 ± 1.2$ to $13.6 ± 1.1$ g·kg⁻¹, +$4.1 ± 4.2\%$, $P < 0.001$) and team-sport ($11.9 ± 0.9$ to $12.3 ± 0.9$ g·kg⁻¹, +$4.0 ± 3.2\%$, $P < 0.001$) athletes ($P = 0.94$).

Body weight

The mean pre-body weight for endurance and team-sport athletes was $70.2 ± 6$ kg and $77.5 ± 9$ kg, respectively, while the mean post-body weight was $70.0 ± 6$ kg and $77.4 ± 8$ kg, respectively. The changes pre- to post-body weight did not differ between the groups ($P ≥ 0.53$). There was no relationship ($r = -0.006, P = 0.96$) between individual percent changes in body weight.
and absolute Hbmass (Figure 2 A). A large inverse relationship ($r = -0.64$, $P < 0.001$) occurred between individual percent changes in body weight and relative Hbmass (Figure 2 B). Further, the multiple linear regression model for percent changes in relative Hbmass ($\text{initial Hbmass} (\text{g·kg}^{-1}) \times$ body weight changes ($\%$)) showed that percent changes in body weight were significantly associated with percent changes in Hbmass ($\text{g·kg}^{-1}$) ($P < 0.001$).

***Figure 2 near here***

**Discussion**

*Paragraph Number 13* To our knowledge, the present study is the first to demonstrate trivial (absolute values) to moderate (relative values) relationships between initial Hbmass and percentage change in Hbmass following LHTL altitude training in male endurance and team-sport athletes using individual data. Mean absolute and relative Hbmass increased to the same extent in endurance and team-sport athletes following sport-specific LHTL interventions. Further, a large inverse relationship occurred between individual percent changes in body weight and relative Hbmass.

**Effect of absolute initial Hbmass on Hbmass response**

*Paragraph Number 14* The observed trivial relationship ($r = 0.02$) between absolute initial Hbmass and percentage changes in absolute Hbmass might suggest that absolute initial Hbmass in our athlete cohort had no impact in regard to further Hbmass improvements following LHTL. Thus far, no study has focused on this relationship using absolute Hbmass values, with the rationale that absolute Hbmass values are not an indicator for an individual’s maximal aerobic capacity (Gore et al., 1998; Lundby et al., 2012; Robach & Lundby, 2012). However, to precisely evaluate the sole effect of initial Hbmass on Hbmass response to altitude training both absolute and relative Hbmass values should be assessed to exclude the confounding factor ‘body weight changes’ during altitude training. Further, the average percentage increase in absolute Hbmass was of a similar magnitude in endurance and team-sport athletes (+3.8 vs. +4.0%). This increase is in accordance with LHTL studies of similar total hypoxic hours (230–240 h), showing a measurable mean absolute Hbmass increase in elite triathletes (+3.2%) (Humberstone-Gough et al., 2013) and semi-professional Australian Footballers (+6.7%) (Inness et al., 2016). Furthermore, to better fit the team sport’s physical requirements, some team-sport athletes in the present study performed a combination of LHTL and repeated-sprints training sessions in hypoxia, the so-called ‘live high–train low and high’ method (Brocherie et al., 2015). However, since mean Hbmass response did not differ between the two hypoxic groups (LHTL vs. LHTL and high), it seems that the additional hypoxic sprints had no beneficial effect on mean Hbmass response. Overall, in the present sample absolute initial Hbmass demonstrated no adverse effect for further absolute Hbmass improvement following LHTL.
Effect of relative initial Hb\text{mass} on Hb\text{mass} response

**Paragraph Number 15** We found a moderate inverse correlation between initial relative Hb\text{mass} and percentage increase in relative Hb\text{mass} ($r = -0.31$) following LHTL. Compared to the analysis of Robach and Lundby (2012) and a classic altitude training study on Australian footballers (McLean et al., 2013), the present correlation coefficient was much smaller than in those studies ($r = -0.51$ to $-0.86$). The above mentioned studies suggested that athletes starting with high relative Hb\text{mass} levels have smaller chances to further increase their relative Hb\text{mass} following altitude training, with the rationale that those athletes would already have maximized their relative Hb\text{mass} level by training at sea level (Robach & Lundby, 2012; McLean et al., 2013). However, in the present study it seems that the moderate inverse relationship between initial relative Hb\text{mass} and percent change in relative Hb\text{mass} could not be attributed to the physiological limit of an athlete.

**Paragraph Number 16** Changes in an individual’s body weight from pre- to post- intervention could explain the moderate relationship between initial relative Hb\text{mass} and its percentage Hb\text{mass} increase following LHTL. There was a large inverse relationship ($r = -0.64$) between individual percent changes in body weight and relative Hb\text{mass}, whereas no relationship between individual percent changes in body weight and absolute Hb\text{mass} occurred. Further, percent changes in body weight were significantly associated with percent changes in relative Hb\text{mass} ($P < 0.001$) in contrast to initial relative Hb\text{mass} ($P = 0.4$). This assumes that, primarily, individual changes in body weight from pre- to post LHTL camp led to the moderate relationship between initial relative Hb\text{mass} and percent change in Hb\text{mass} following LHTL camp. Whether the body weight changes were due to alterations in fat and/or muscle mass or because of the weekly fluctuation in body weight/fluid (Orsama et al., 2014) remains unclear. With a lack of significant relationship between individual changes in body weight and absolute Hb\text{mass}, it can be assumed that body weight alterations did not negatively influence absolute Hb\text{mass} response in the present study. Thus, we propose that lean body mass-adjusted relative Hb\text{mass} values would be a better unit for future comparisons.

**Paragraph Number 17** A further point that must be considered when assessing the relationship between change and initial values, is the statistical phenomenon ‘regression to the mean’ (Galton, 1886; Bland & Altman, 1994). Although in the present study there was no relationship between initial absolute Hb\text{mass} and percent changes in absolute Hb\text{mass}, the ‘regression to the mean’ effect could have still appeared. Further, since individual changes in body weight from pre- to post-LHTL camp occurred, it could also be possible that the ‘regression to the mean’ effect arose within the relationship between initial body weight and body weight changes. This makes the speculation that part of the inverse relationship between initial relative Hb\text{mass} and percent changes in relative Hb\text{mass} following LHTL camp could be due to the statistical phenomenon ‘regression to the mean’. However, this needs to be confirmed with a larger dataset, involving athletes of different performance levels and from various sport disciplines as well as using different altitude training paradigms with various characteristics (e.g., duration, altitude severity, hypobaric vs. normobaric hypoxia). Lastly, one should also keep in mind that the chosen metric for total ‘hypoxic dose’, i.e., ‘kilometre hours’ (Garvican Lewis et al., 2016), is still debated in the literature (Millet et al., 2016).
Conclusion

Our results indicate that trivial (absolute values) to moderate (relative values) relationships occurred between initial \( \text{Hb}_{\text{mass}} \) and \( \text{Hb}_{\text{mass}} \) increase following LHTL altitude training in endurance and team-sport athletes. This indicates that even athletes with higher initial \( \text{Hb}_{\text{mass}} \) can reasonably expect \( \text{Hb}_{\text{mass}} \) gains post-LHTL. Further, it seems that in the present study the moderate relationship between initial relative \( \text{Hb}_{\text{mass}} \) and percentage increase in relative \( \text{Hb}_{\text{mass}} \) following LHTL could be attributed to changes in body weight and possibly to the statistical phenomenon ‘regression to the mean’, rather than to a pure physiological effect.

Additional information

Conflict of Interest

The authors have no conflicts of interest.

Author Contributions

All authors performed the research and analysed or interpreted the data for the work. JPW, GPM, OG, LS and AH conceived and designed the research. AH, ST, JPW and GPM drafted the manuscript. All authors edited and revised the manuscript critically and approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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References


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**Table 1.** Characteristics of the altitude training interventions

<table>
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LHTL = live high-train low; RSH = repeated-sprints in hypoxia; NH = normobaric hypoxia; HH = hypobaric hypoxia.

**Figure legends**

**Figure 1.** (A) Linear regression between the individual’s initial absolute Hb\(_{\text{mass}}\) (g) and the individual’s absolute Hb\(_{\text{mass}}\) change (%) following LHTL. (B) Linear regression between the individual’s initial...
relative Hb$_{mass}$ (g/kg) and the individual’s relative Hb$_{mass}$ change (%) following LHTL. Regression slope (solid line) and 95% confidence limits (dashed lines) are shown. n = 58. LHTL = live high–train low, RSH = repeated-sprints in hypoxia, NH = normobaric hypoxia, HH = hypobaric hypoxia.

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Figure 2. Linear regression between individual body weight change (%) and (A) individual absolute Hb\textsubscript{mass} change (%) and (B) individual relative Hb\textsubscript{mass} change (%) following LHTL. Regression slope (solid line) and 95% confidence limits (dashed lines) are shown. n = 58.