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Altitude training and individual hemoglobin mass response in athletes

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Thèse de doctorat ès sciences de la vie (PhD)

présentée à la

Faculté de biologie et de médecine
de l'Université de Lausanne

par

Anna Hauser

Master en sciences du sport - Haute école fédérale de sport de Macolin HEFSM

Jury

Prof. Martial Saugy, Président
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Dr. Jon Wehrlin, Co-directeur
Prof. Walter Schmidt, expert
Prof. Christopher Gore, expert

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Lausanne, le 17 août 2017

pour le Doyen
de la Faculté de biologie et de médecine

Prof. Martial Saugy

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List of publications

PhD related (included in the thesis)

1. **Hauser A**, Schmitt L, Troesch S, Saugy JJ, Cejuela-Anta R, Faiss R, Robinson N, Wehrlin JP, Millet GP. Similar hemoglobin mass response in hypobaric and normobaric hypoxia in athletes. *Med Sci Sports Exerc.* 2016;48(4):734-41.

2. Wehrlin JP, **Hauser A**, Schmitt L, Troesch S, Saugy JJ, Cejula-Anta R, Faiss R, Robinson N, Millet GP. Response. *Med Sci Sports Exerc*. 2016;48(7):1426-7.

3. **Hauser A**, Troesch S, Saugy JJ, Schmitt L, Cejuela-Anta R, Faiss R, Steiner T, Robinson N, Millet GP, Wehrlin JP. Individual hemoglobin mass response to normobaric and hypobaric "live high-train low": A one-year crossover study. *J Appl Physiol (1985)*. 2017. doi: 10.1152/ japplphysiol.00932.2016 [Epub ahead of print].

4. **Hauser A**, Troesch S, Steiner T, Brocherie F, Girard O, Saugy JJ, Schmitt L, Millet GP, Wehrlin JP. Do athletes with already high initial hemoglobin mass benefit from altitude training? *Manuscript.*

PhD related (not included in the thesis)

5. 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*. 2014;9(12):e114418.

6. Brocherie F, Millet GP, **Hauser A**, Steiner T, Rysman J, Wehrlin JP, Girard O. "Live High-Train Low and High" hypoxic training improves team-sport performance. *Med Sci Sports Exerc.* 2015;47(10):2140-9.

7. Brocherie F, Millet GP, **Hauser A**, Steiner T, Wehrlin JP, Rysman J, Girard O. Association of hematological variables with team-sport specific fitness performance. *PLoS One.* 2015;10(12):e0144446.

8. 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*. 2016;7:138.

Not PhD related

9. **Hauser A**, Zinner C, Born DP, Wehrlin JP, Sperlich B. Does hyperoxic recovery during cross-country skiing team sprints enhance performance? *Med Sci Sports Exerc*. 2014;46(4):787-94.

10. Sperlich B, Born DP, Zinner C, **Hauser A** & Holmberg HC. Does upper-body compression improve 3 x 3-min double-poling sprint performance? *Int J Sports Physiol Perform*. 2014;9(1):48-57.

11. Zinner C, **Hauser A**, Born DP, Wehrlin JP, Holmberg HC, Sperlich B. Influence of hypoxic interval training and hyperoxic recovery on muscle activation and oxygenation in connection with double-poling exercise. *PLoS One*. 2015;10(10):e0140616.

12. Wehrlin JP, Troesch S, **Hauser A**, Steiner T. Commentaries on viewpoint: Time for a new metric for hypoxic dose? *J Appl Physiol (1985)*. 2016;121(1):356-8.

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Abstract

Altitude training is frequently used by elite athletes to obtain potential benefits in sea-level performance. One of the main recognized physiological adaptations leading to enhanced sea-level performance is a hypoxia-induced increase in hemoglobin mass (Hb_{mass}). During the last decades, several types of altitude training have been developed, which can be performed under either hypobaric hypoxia (HH, natural altitude) or normobaric hypoxia (NH, simulated altitude). Whether NH and HH can be used interchangeably for altitude training is still unclear. Moreover, especially from an athlete's perspective, the individual adaptations to and the interchangeability of NH and HH are of interest.

Therefore, to compare individual and mean Hb_{mass} responses during an 18-day live high–train low (LHTL) altitude training camp in either NH or HH, we designed a randomized matched controlled (n = 28) study and a crossover (n = 15) study with endurance athletes. To more precisely quantify the individual Hb_{mass} response to altitude training, we implemented error-reducing duplicate Hb_{mass} measures. Furthermore, since altitude training is primarily used by elite endurance athletes that typically present elevated Hb_{mass} values, we tested the hypothesis that athletes with a high initial Hb_{mass} starting an altitude sojourn will have a limited ability to further increase their post-altitude Hb_{mass} in male endurance and team-sport athletes (n = 58).

The present thesis indicates that hypobaric and normobaric LHTL camps evoke a similar mean increase in Hb_{mass} as well as similar performance changes following an 18-day LHTL camp, suggesting that both hypoxic conditions can be used equally for an LHTL training camp. Among the mean Hb_{mass} changes, there was a notable inter-individual variation that could not be explained by the variation in the initial Hb_{mass}. This emphasizes the importance of individual Hb_{mass} response evaluation for altitude training in athletes.

Résumé (Abstract in French)

L'entrainement en altitude est couramment utilisé par les sportifs d'élites afin d'obtenir des bénéfices améliorant la performance au niveau de la mer. Le mécanisme-clef réside dans l'augmentation de la masse totale en hémoglobine (Hb_{mass}) induite par l'hypoxie. Ces dernières décennies, plusieurs méthodes d'entrainement en altitude ont été développées, soit en hypoxie hypobare (HH, altitude réelle) soit en hypoxie normobare (NH, altitude simulée). La question de savoir si HH et NH sont interchangeables n'est pas encore complètement résolue. En particulier, les effets de NH vs. HH sur les réponses individuelles de Hb_{mass} sont encore mal connus.

Par conséquent, afin de comparer les réponses de Hb_{mass} au cours d'un stage de 18 jours "vivre en haut et s'entrainer en bas, LHTL" soit en NH ou en HH, nous avons réalisé un protocole randomisé contrôlé (n = 28) puis un protocole croisé (n = 15) avec des athlètes d'endurance. Afin de quantifier plus précisément les réponses individuelles de Hb_{mass} et de réduire les erreurs, les mesures ont été dupliquées. De plus, comme l'entrainement en altitude est utilisé à ce jour principalement par les athlètes d'endurance ayant un niveau initial élevé de Hb_{mass}, nous avons testé l'hypothèse que les athlètes ayant une haute valeur de Hb_{mass} ont une augmentation limitée de celle-ci suite à un stage en altitude, que ce soit avec des sportifs d'endurance ou de sport collectif (n = 58).

Le travail de doctorat réalisé montre que LHTL induit la même augmentation de Hb_{mass} en hypoxie normobare et hypobare, ainsi qu'une amélioration similaire de la performance aérobie. Aussi, les deux conditions hypoxiques (HH et NH) peuvent être utilisées pour des stages de type LHTL. De plus, une variabilité importante interindividuelle des réponses de Hb_{mass} a été rapportée, qui ne dépendent pas du niveau initial de Hb_{mass} des sujets. Ceci met en évidence la nécessité de mesurer ces réponses individuelles avec précision.

Index of abbreviations

AMS
acute mountain sickness

βm
buffer capacity

CHT
continuous hypoxic training

CL
confidence limit

CO
carbon monoxide

CO_2
carbon dioxide

CON
control group

CV
coefficient of variation

d
cohen's d (effect size)

F_iO_2
inspired fraction of oxygen

F_iN_2
inspired fraction of nitrogen

Ftn
serum ferritin

EAA
equivalent air altitude model

ECO
objective load scale

EMG
electromyographic signals

EPO
erythropoietin

Hb
hemoglobin

Hb_{mass}
hemoglobin mass

Hct
hematocrit

HH
hypobaric hypoxia

HIF-1
hypoxia-inducible factor 1

HIF-1α
hypoxia-inducible factor 1 alpha

HIF-1β
hypoxia-inducible factor 1 beta

HR
heart rate

LHTH
live high-train high

LHTL
live high-train low

LHTLH
live high-train low and high

LLTH
live low-train high

Mb
myoglobin

mmHg
millimeter of mercury

N_2
nitrogen

NH
normobaric hypoxia

NO
nitric oxide

O_2
oxygen

OFF Score
Hb ($g \cdot L^{-1}$) - 60√(reticulocytes in %)

P
significance level

P_B
barometric pressure

PO_2
partial pressure of oxygen

P_aO_2
arterial pressure of oxygen

P_iO_2
partial pressure of inspired oxygen

PCO_2
partial pressure of carbon dioxide

Post
after training

P_{max}
maximal power

Pre
before training

r
correlation coefficient

RCV
red cell volume

RBC
red blood cells

Ret
reticulocyte

RSH
repeated sprint training in hypoxia

S_aO_2
arterial oxygen saturation

S_pO_2
pulse oxygen saturation

SE
standard error

SD
standard deviation

SD_{diff}
standard deviation of the difference scores

SWC
smallest worthwhile change

TE
typical error

VEGF
vascular endothelial growth factor

$\dot{V}O_{2max}$
maximal oxygen uptake

%HbCO
carboxyhemoglobin

^{51}Cr
chromium 51

Chapter 1

Introduction

1. Introduction

The general introduction provides an overview of the current altitude training methods, the erythropoietic response to altitude exposure, and the measurement principles of hemoglobin mass (Hb_{mass}). The second part of the introduction, "hemoglobin mass response to normobaric and hypobaric hypoxia", is more specific and introduces the topic of the present thesis.

1.1 General introduction

1.1.1 Altitude training methods

Athletes frequently use different altitude training methods for potential benefits in sea-level performance (7, 94, 107). The main rationale behind altitude training is that the body responds to a hypoxic environment (lower oxygen availability in the air and within the body) by an enhanced red cell production, resulting in an improved oxygen-carrying capacity, which in turn should produce in a better aerobic performance at sea level (12). During the last decades, various altitude or hypoxic training methods for athletes have been developed (67, 104). The different methods include the traditional "live high–train high" (LHTH), the "live high–train low" (LHTL), and the "live low–train high" (LLTH) approaches (figure 1).

The traditional LHTH method, where athletes live and train at moderate altitude (1800–2500 m) for 2 to 4 weeks (67), was further modified into the LHTL method in the 1990s by Levine and Stray-Gundersen (56, 57). In contrast to LHTH, in the LHTL method, the athletes live at moderate altitude (2500 m) but train at low altitude or near sea level; this allows them to maintain an exercise intensity comparable to sea level as well as to obtain the physiological benefits of altitude acclimatization (54, 107). The first implementation of the LHTL method was performed at natural altitude, where the athletes lived for 28 days at 2500 m and trained at

1250 m (57). Since there are only few suitable places in the world to perform LHTL and LHTL is generally associated with a time-consuming travel effort from high to low altitudes, hypoxic devices have been developed to simulate an altitude environment at sea level (82, 104). Common types of altitude simulation are nitrogen dilution (e.g., normobaric hypoxic rooms or apartments), oxygen filtration (e.g., normobaric hypoxic rooms or tents) or pressure reduction (e.g., hypobaric hypoxic chambers) (105). In recent years, the use of an artificial LHTL method has become more and more popular in athletes, since it provides a more logistically convenient environment and a time-efficient way of training (94). Overall, there is a broad consensus that the success of altitude training relies on an adequate hypoxic "dose", corresponding to an altitude of >2000 m for >12 h·day^{-1} and a period of >21 days (59, 82, 94, 107), i.e., approximately 300 total hypoxic hours (13).

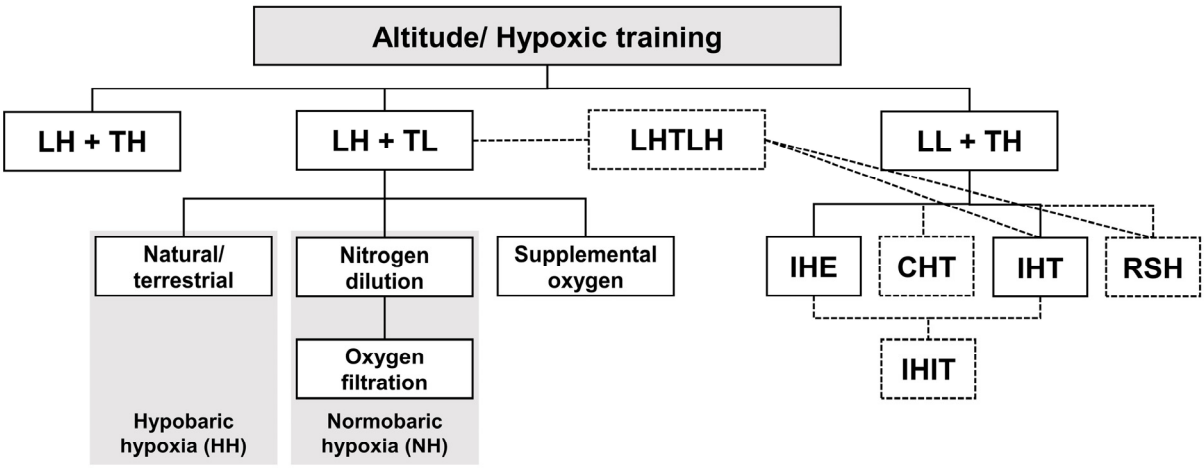

Figure 1. Different altitude/-hypoxic training methods. Modified from Wilber (105) and expanded by Millet et al. (67) and (65), dotted lines. LH = live high; TH = train high; TL = train low; LL = live low; LHTLH = live high–train low and high; IHE = intermittent hypoxic exposure; CHT = continuous hypoxic training; IHT = intermittent hypoxic training; RSH = repeated sprint training in hypoxia; IHIT = intermittent hypoxic exposure during interval training.

Another altitude training method is LLTH, where athletes live at or near sea level and are exposed to short or longer bouts of hypoxia (5–180 min) that are repeated over several days or weeks (67). LLTH can be performed by the athletes at rest (intermittent hypoxic exposures

[IHE]) or during training sessions (intermittent hypoxic training [IHT]) (105). This altitude training method was further divided by Millet et al. (65, 67) into four sub-categories: IHE, continuous hypoxic training (CHT, >30 min and low intensity), IHT, and repeated sprint training in hypoxia (RSH). In contrast to the prolonged altitude training methods (LHTH and LHTL), the intermittent hypoxic methods can be more easily integrated into an athlete's training schedule, due to less travel and time effort (44). To date, altitude training has been mainly used by endurance athletes (94), but it has become increasingly popular in team sports during the last few years (5). Thus, combinations of altitude or hypoxic methods have been introduced, such as the "live high–train low and high" (LHTLH = LHTL + IHT/RSH) method or the intermittent hypoxic interval training (IHIT = IHE + IHT) (8, 65, 67), in order to better fit the team sport's physical requirements (a combination of aerobic and anaerobic adaptations). A recent meta-analysis (7) calculated the overall efficacy of the different altitude methods and showed that the natural LHTL method was the best protocol to obtain advantageous endurance performance adaptations (1–4%), in contrast to artificial LHTL, LHTH, and LLTH in semi-elite and elite athletes.

1.1.2. Erythropoietic responses to hypoxia

Hypoxia can be defined as combinations of barometric pressure (P_B) and an inspired fraction of oxygen (F_iO_2) that result in an inspired partial pressure of oxygen (P_iO_2) lower than a normoxic value of 150 mmHg (15). Hypoxic conditions occur with increasing altitude (reduction in P_B, also referred to as "hypobaric hypoxia" [HH]) or in low–oxygen environments by reducing the oxygen portion in ambient air (reduction in F_iO_2, also referred to as "normobaric hypoxia" [NH]) (20). Exposure to hypoxia leads to a decrease in P_iO_2 and a subsequent reduction in arterial oxygen pressure (P_aO_2) and arterial oxygen saturation (S_aO_2) (45). This oxygen deficiency in the blood and muscles is also called "hypoxemia".

The human body responds to hypoxia with hematological and non-hematological adaptations, which have the potential to improve athletic performance (figure 2). Thus far, there is strong evidence that the primary regulator of hypoxia-mediated cellular adaptations is hypoxia-inducible factor 1 (HIF-1) (20), a transcription factor that regulates oxygen homeostasis (91). The α-subunit of HIF-1 (HIF-1α) is highly oxygen sensitive; under normoxia, it is rapidly degraded (half-life ~5min), while under hypoxic conditions, it stabilizes, translocates to the nucleus, and dimerizes with the hypoxia-inducible factor 1β (HIF-1β, an oxygen-independent subunit) to form the stable HIF-1 complex (53). HIF-1 becomes transcriptionally active and drives the expression of target genes involved in erythropoiesis (erythropoietin [EPO]), iron metabolism (e.g., transferrin and transferrin receptor), angiogenesis (e.g., vascular endothelial growth factor [VEGF]), vascular tone (e.g., nitric oxide [NO] synthase) or glucose metabolism (e.g., glycolytic enzymes) (53). Overall, the adaptations to hypoxia driven by HIF-1 lead to enhanced oxygen transport capacity and/-or improved oxygen extraction/-utilization at the cellular level (60).

One of the main recognized physiological adaptations leading to enhanced aerobic performance is a hypoxia-induced increase in erythropoiesis (11, 57, 103). Under hypoxia, erythropoiesis is mainly triggered by an enhanced stimulation of the EPO hormone in the kidneys (52). After arrival at altitude, EPO increases within hours and peaks after 1 to 2 days of hypoxic exposure, before it declines toward baseline (52, 75). The increased EPO levels lead to an enhanced production of red blood cells (RBC) in the bone marrow, resulting in an elevated Hb_{mass} and thus an improved oxygen transport capacity of the blood, which in turn should enhance exercise performance (58, 96, 102, 107). In the original paper by Levine and Stray-Gundersen that presents the LHTL training model (57), the results demonstrated that 4 weeks of LHTL (>20 $h \cdot day^{-1}$) at 2500 m altitude led to an increase in RBC and maximal oxygen consumption ($\dot{V}O_{2max}$), and improved running performance (5000 m time trial) in comparison to a control

group. In a subsequent article by the same research group, the authors showed retrospectively that those athletes who had a performance enhancement after LHTL (greater than the mean change) also demonstrated an increased plasma EPO concentration after 30 hours at altitude, which was elevated up to 14 days later when compared to those athletes who had no performance increase (11). The authors concluded that an improved erythropoietic response after an LHTL is the primary mediator for positive effects on performance at sea level (58).

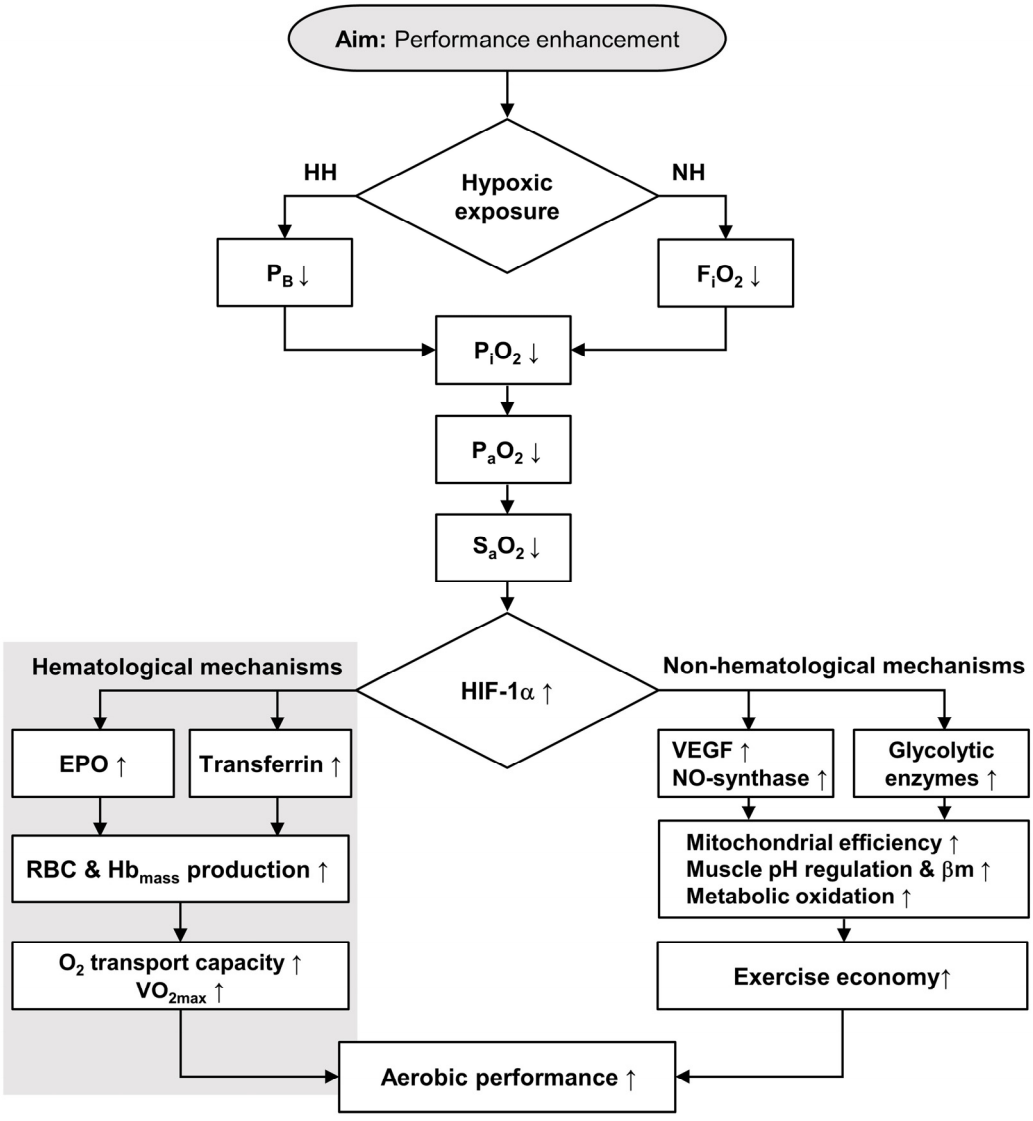

Figure 2. Schematic representation of the hematological and non-hematological adaptations following hypoxic exposure. See text for details. HH = hypobaric hypoxia; NH = normobaric hypoxia; P_B = barometric pressure; F_iO_2 = inspired fraction of oxygen; P_iO_2 = inspired pressure of oxygen; P_aO_2 = arterial pressure of oxygen; S_aO_2 = arterial oxygen saturation; HIF-1 = hypoxia-inducible factor 1; EPO = erythropoietin; RBC = red blood cells; Hb_{mass} = hemoglobin mass; $\dot{V}O_{2max}$ = maximal oxygen uptake; VEGF = vascular endothelial growth factor; NO = nitric oxide; βm=buffer capacity.

Recently, a meta-analysis of altitude training studies (40) has calculated that an increase in Hb_{mass} of approximately 1.1% per 100 h of hypoxic exposure at ≥2100 m can be expected. Furthermore, the linear relationship between Hb_{mass} and $\dot{V}O_{2max}$ under normoxic conditions is well established, and it has been shown that a 1 g increase in Hb_{mass} results in a ~4 $mL \cdot min^{-1}$ rise in $\dot{V}O_{2max}$ (90). However, not all altitude training studies have demonstrated an increase in Hb_{mass} and/or red cell volume (RCV) (3, 18, 77). Therefore, it has to be considered that performance enhancement after altitude training may not solely depend on erythropoietic adaptations but also on non-hematological adaptations, such as enhanced mitochondrial efficacy, improved muscle pH regulation and muscle buffering capacity (34). In conclusion, it can be noted that hypoxic exposure causes a multitude of responses in the human body (figure 2), but it seems possible that the increase in Hb_{mass} post-altitude is the central factor leading to enhanced exercise performance.

1.1.3. Measurement of hemoglobin mass (Hb_{mass})

The determination of Hb_{mass} in exercise science has become increasingly important in recent years. Hb_{mass} measurements help researchers to evaluate the efficiency of training interventions (36, 89), especially altitude training methods (40), and the impact of iron supplementation (25). These measurements also aid in the detection of hematological manipulations (i.e., blood doping) in the antidoping field (72). The methods for the determination of Hb_{mass} are based on the dilution principle, whereby tracers are injected into the blood circulation. Traditionally, radioactive tracers such as chromium 51 (^{51}Cr) have been used and these are considered to be the gold standard (38, 88). However, methods with radioactive tracers are time- and cost-intense and associated with health concerns (38). As a result, a less-harmful method with Evans blue dye gained popularity in clinical settings (32), although its validity and reliability remain questionable (38). With the development of new multi-wavelength spectrophotometers (22) and the ability to measure carboxyhemoglobin (%HbCO) accurately and precisely (23), the use of

carbon monoxide (CO) as a tracer for determining Hb_{mass} has been revived. The binding of CO to hemoglobin (Hb) has a 200–300 times higher affinity than that of oxygen (81), and CO is therefore a suitable tracer for labelling Hb and much less harmful than the radioactive tracers.

The so-called CO rebreathing method which uses CO to label Hb, was first described by Grehant and Quinquard in 1882 and then gradually modified and improved by Thomsen et al. (97), Burge and Skinner (10), Schmidt and Prommer (88) and Prommer and Schmidt (71). Today, it is one of the most widely used methods for determining the Hb_{mass}, since it is minimally invasive, not harmful (88), and enables quantification of the total Hb_{mass} independent of plasma volume (83). Furthermore, the method allows for reliable Hb_{mass} determinations from capillary blood samples; compared to venepuncture blood sampling, this method is less invasive and demands little technical effort (2). A recent meta-analysis of the common methods (38) demonstrated that the CO rebreathing method has a low 'one-day' measurement error of 2.2% (expressed as a coefficient of variation [CV]) similar to the ^{51}Cr labelling method (CV 2.8%), whereas the Evans blue dye method showed a CV of 6.7%. The CO rebreathing method is considered to be valid and reliable to determine Hb_{mass} in routine settings, while the Evans blue dye method should be used with caution and only in clinical applications (38).

Schmidt and Prommer (88) further refined the CO rebreathing method by reducing CO rebreathing time (from ten to two minutes) and the number of blood samples (from eight to four, with capillary blood) as well as developing a new portable rebreathing spirometer. This method is also known as the "optimized CO rebreathing method" and is one of the most accurate techniques to determine total Hb_{mass} with a mean 'one-day' typical error of approximately 2% (40) that enables the identification of small changes in Hb_{mass}. CO rebreathing methods have been criticized for overestimating Hb_{mass} due to the loss of CO to extravascular compartments, such as myoglobin (Mb) (87). Prommer and Schmidt (71) showed that only a small amount of CO is lost from the vascular space (0.24 $mL \cdot min^{-1}$) in the optimized

CO rebreathing method, resulting in an overestimation in Hb_{mass} of 2%. Thus, the authors recommended the inclusion of a correction for CO flux to Mb (0.3% of administered CO per minute) into the calculation for total Hb_{mass}. Nevertheless, cautious use of the optimized CO rebreathing method is recommended, since multiple factors can influence the measurement error, such as the dose of administered CO (99), replicate measures of %HbCO (1), and/or CO leaks (83). In summary, the optimized CO rebreathing method allows valid and reliable determination of Hb_{mass} in an efficient and athlete-friendly routine procedure.

The determination of Hb_{mass} using the optimized CO rebreathing method is based on the change in %HbCO (Δ%HbCO) from before to after rebreathing a known volume of CO (88). Baseline %HbCO concentration is determined in the capillary blood, before a given individual CO bolus along with oxygen is rebreathed for two minutes within a closed circuit. It is assumed during the optimized CO rebreathing method that the CO should be equally distributed throughout the vascular system after six to eight minutes (9, 88). Therefore, at six and eight minutes after the inhalation of the bolus, capillary %HbCO is determined again and averaged to get the seven-minute post-%HbCO value. In addition, the amount of CO that has not been taken up during the rebreathing period must be accounted for, such as the remaining CO in the spirometer and the CO lost via exhalation and/or diffusion to Mb. The total Hb_{mass} is calculated using the formula below, described in detail by Schmidt and Prommer (88), and completed with a factor for CO flux to Mb presented by Prommer und Schmidt (71):

$$Hb_{mass}\ (g) = k \times MCO \times 100/(\Delta\%HbCO \times 1.39)$$

$k = P_B/(760 \times (1 + (0.00369 \times T)))$, P_B in mbar and T is temperature in ºC; $MCO = CO_{admin} - (CO_{system} + CO_{exhalation}) - VCO_{Mb}$, CO_{admin} is the CO volume administered into the system in mL, $CO_{system} + CO_{exhalation}$ is the CO volume not bound to hemoglobin in mL, VCO_{Mb} is the CO volume diffused to Mb (0.3% × min^{-1} of administered CO); Δ%HbCO = difference between baseline %HbCO and %HbCO after rebreathing; 1.39 = Hüfner's number (1 g Hb binds 1.39 mL CO).

The CO dose administered during the optimized CO rebreathing method can be classified as low and safe (88). The CO dose can elicit Δ%HbCO ranging from 3.5% to 8.2% (99), which is below the CO toxicity level (>15 %HbCO) and thus not harmful to health. The half-life of CO in the bloodstream after completing the optimized CO rebreathing method is approximately 2 hours, and baseline %HbCO can be reached after six hours (88). However, since the inhalation of CO temporarily affects an athlete's performance ($\dot{V}O_{2max}$ decrease by 3%), a time lag of >12 hours between measurements and competition is recommended (88). Taking these conditions into account, the optimized CO rebreathing method allows for the evaluation of Hb_{mass} adaptations to altitude training.

1.2. Hemoglobin mass response to normobaric and hypobaric hypoxia

1.2.1. Normobaric versus hypobaric hypoxia

It is still debated whether NH and HH cause similar performance and physiological responses and thus can be used interchangeably (16, 66). The relevant difference between these two types of hypoxia is that NH is achieved by a reduction in F_iO_2 (<20.9%), and HH is achieved by a decline in P_B (<760 mmHg) while inducing the same P_iO_2 (<150 mmHg) (15, 86). Thus far, it has been assumed that a decrease in P_iO_2, independently caused by a decline in P_B or F_iO_2, will evoke the same physiological responses (15). This assumption is called the "equivalent air altitude" (EAA) model, which is an isoP_iO_2 model wherein P_iO_2 is calculated by subtracting the partial pressure of water vapor (47 mmHg at 37°C) from P_B and multiplying by F_iO_2: $P_iO_2 = (P_B–47) \times F_iO_2$ (15). However, it has been claimed that the EAA model is inaccurate and that HH and NH are not equal even for the same P_iO_2 (14, 15).

A recent review by Coppel et al. (16) of the physiological effects of HH versus NH and a series of point-counterpoint discussions (66) revived the debate about Conkin's critique of the EAA

model. Research on the direct comparison between HH and NH has focused only on short-term exposure, between 5 minutes and 26 hours (16, 84). Studies reported significant differences between HH and NH on physiological and performance responses when using altitudes from 1700–7620 m (figure 3). The majority of the research investigated parameters related to ventilation and oxygenation. A reduced minute ventilation in HH was seen, accompanied with smaller tidal volumes, whereas inconsistent results for breathing frequency were reported (19, 61, 86). Therefore, it has been suggested that HH induces greater dead space ventilation, possibly associated with the reduced P_B (61, 86). Furthermore, HH resulted in lower S_aO_2 and pulse oxygen saturation (S_pO_2) at rest (86) and during a cycling time trial (84). It is argued that under HH, the P_B changes might modify fluid circulation and alveoli-capillary membrane flux, leading to greater pulmonary vasoconstriction, and modify oxygen diffusion by decreasing the alveoli-capillary pressure gradient (15, 55). Under hypoxic conditions, oxidative stress is increased; recently, Faiss et al. (19) have shown that 24 hours of exposure in HH (3000 m, P_B 530 mmHg) exaggerated certain markers of oxidative stress more than in NH (F_iO_2 14.7%). In this study, they also demonstrated an impaired NO bioavailability and lower plasma pH level in HH compared to NH. The authors concluded that the different response in NO bioavailability and oxidative stress could explain the lower ventilatory response in HH. Lastly, acute mountain sickness (AMS) symptoms were higher (62) and postural control was decreased (17) in HH compared to NH. Taken together, these differences suggest that HH is a more stressful stimulus to the human body than NH, but the performance parameter is the most important from an athlete's perspective.

Only two studies have compared endurance performance (cycling time trial) after short-term exposure (4 to 26 h) to HH versus NH (3450 m and 4300 m) (4, 84). Both studies demonstrated that endurance performance impairment was greater in HH. Furthermore, the study by Saugy et al. (84) found lower electromyographic signals (EMG) in the vastus lateralis in HH versus

NH. In contrast, HH improved power and velocity performance (P_{max} and 1 RM) to a greater extent when performing a force-velocity curve on a bench press (21). Nevertheless, the practical meaning of these differences for an athlete's training is still unclear, since no studies exist that present a direct comparison of long-term exposure to NH and HH. Specifically, the impact of NH versus HH on Hb_{mass} changes is unknown. To date, no study has directly compared LHTL camps in NH versus HH in terms of the effects on Hb_{mass} and performance responses in endurance athletes. Therefore, we designed a randomized matched controlled study (article I) and a same-subject crossover study (article II) over a period of one year.

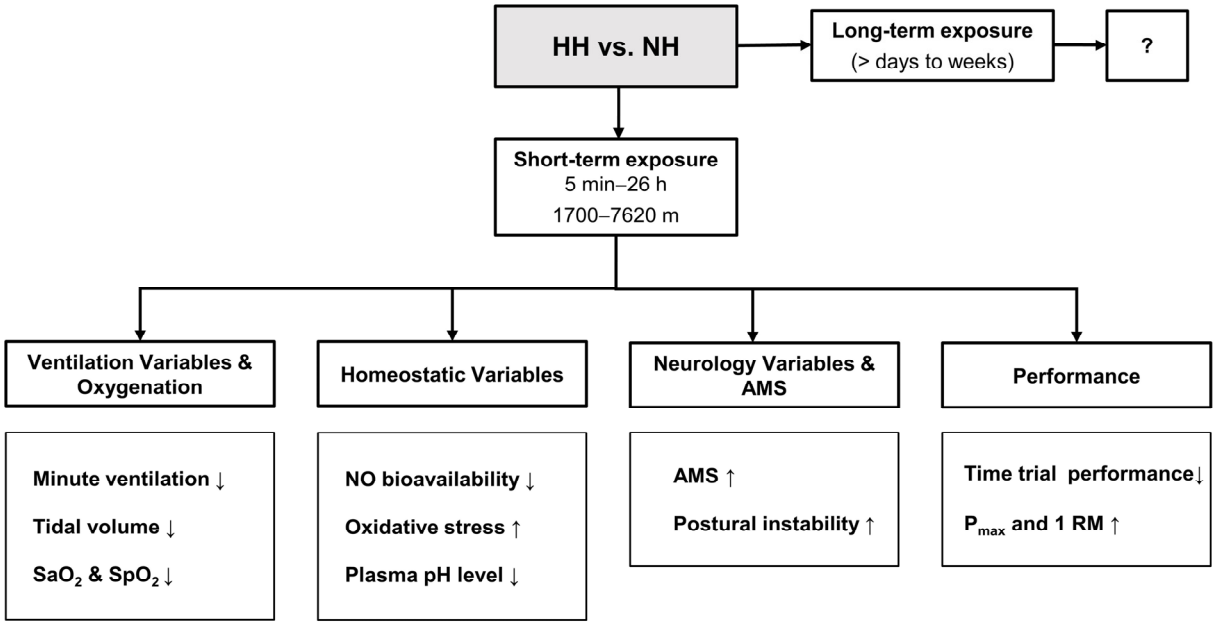

Figure 3. Summary of main physiological and performance differences of hypobaric hypoxia (HH) versus normobaric hypoxia (NH) after short-term exposure. P_iO_2 = inspired pressure of oxygen; S_aO_2 = arterial oxygen saturation; S_pO_2 = pulse oxygen saturation; NO = nitric oxide; AMS = acute mountain sickness, P_{max} = maximum power; 1 RM = one-repetition maximum.

1.2.2. Individual hemoglobin mass response to altitude training

Individual variability in an athlete's response to altitude training has been previously discussed (11, 24, 57). Chapman et al. (11) demonstrated that after 4 weeks LHTL (>20 $h \cdot day^{-1}$) at 2500 m altitude, a remarkable inter-individual variability was evident in the effect of the altitude stay

on sea-level running performance. Retrospectively, the authors showed that those athletes who had an increased sea-level performance also experienced an enhanced erythropoietic response during altitude training (11). It was concluded that the variability in performance after an altitude training camp might be due to an individual's acute increase in EPO, sufficient to evoke a gain in RCV. Further studies (24, 28, 70, 93, 100) have indicated wide individual variations in Hb_{mass} response to different altitude training methods, although the correlation between the acute EPO increase during altitude and the change in Hb_{mass} after altitude could not always be confirmed (24, 100).

The reason for the inter-individual variability in Hb_{mass} response to altitude training is still uncertain and may be related to several factors (see table 1). In addition to the aforementioned individual EPO response to altitude exposure (11, 31), it is suggested that genetic factors (i.e., the transcriptional mechanism of EPO gene expression [HIF-1]) may be responsible (31, 51, 107). In this context, another hypothesis is the occurrence of a mild neocytolysis (the hemolysis of young RBC) (74) in some athletes after descending from altitude (24). Others have proposed that different baseline conditions of the athletes, such as the individual's fitness level, health conditions (26, 79, 100), or insufficient iron stores (30, 95), could be responsible for the variation in Hb_{mass} response. Subject variables during altitude training, such as psychosociological concerns (e.g., leaving family or training environment), fatigue recovery, or stress level might also impair individual's Hb_{mass} adaptations (26, 33, 46, 79). Furthermore, studies demonstrated that body weight loss or health conditions (inflammation, illness, or injury) during altitude camps can suppress the erythropoietic response (26, 41, 63, 100). It is supposed that a catabolic state (a mismatch between energy intake and energy expenditure) or an increase in proinflammatory cytokines may impair erythropoiesis (63). In this regard, an iron supplementation strategy during altitude training (the maintenance of iron balance and enhancement of iron absorption, which in turn supports erythropoiesis) is also discussed (30,

43). Recently, it was demonstrated that athletes who used a daily iron supplement during moderate altitude exposure (mean: 3000 m and 21 days) increased their Hb_{mass} to a greater extent (~3–4%) than athletes who did not (~1.8%), particularly in athletes with low initial ferritin values. Lastly, it has also been suggested that the variability in individual Hb_{mass} response relies on the individual's initial Hb_{mass} level before embarking upon the altitude training camp; this argument proposes that athletes with a high initial Hb_{mass} have a limited ability to further increase their Hb_{mass} after altitude training (35, 63, 76).

Table 1 Summary of factors that might drive individual variability in hemoglobin mass (Hb_{mass}) response to altitude training.

Factors before altitude training	Factors during altitude training
▪ Genetic predisposition or determinants (31, 51, 107)	▪ Increase in EPO response to hypoxia (11, 31)
▪ Individual's baseline Hb_{mass} ($g \cdot kg^{-1}$) (35, 63, 76)	▪ Fatigue recovery or stress level (26, 46, 79)
▪ Individual's fitness level or health (26, 79, 100)	▪ Health condition or body weight loss (26, 41, 63, 100)
▪ Individual's initial ferritin level (30, 95)	▪ Ferritin status and supplementation (30, 43)
	▪ Psychosociological concerns (33)

Given the diverse sources of inter-individual Hb_{mass} variation in the response to altitude training, the individual evaluation of altitude training camps should be considered. This is particularly applicable for elite athletes, who are trying to maximize their individual performance and for whom even a small increase in Hb_{mass} (e.g., 1%) could be worthwhile for performance enhancement (27). Thus, it is important to improve the accuracy of individual

Hb_{mass} measures for altitude training. The optimized CO rebreathing method is a reliable tool for determining Hb_{mass} in athletes, with a measurement error of approximately 2% (40). The measurement error can be further minimized by averaging duplicate Hb_{mass} measurements in a close time series (time lag < 24 h) (28, 68), which reduces the measurement error by a factor of √2 (30%) (47). Consequently, to more precisely identify individual Hb_{mass} responses to altitude training camps, duplicate Hb_{mass} measurements before and after an altitude intervention are recommended (28, 68).

As the accuracy of Hb_{mass} measures to altitude training, the quantification of individual Hb_{mass} responses to altitude training per se is an important aspect. Studies have generally reported only the range of individual Hb_{mass} values after an altitude training intervention (24, 28, 70, 93, 100). Since the measured variation in Hb_{mass} responses includes both the biological and technical variations (1), the biological variation can be quantified by withdrawing the technical variation from the total variation. One possibility to quantify individual biological Hb_{mass} variation in response to an intervention free of technical noise is the calculation of "individual responsiveness" according to Hopkins (47). The individual responsiveness can be expressed as the SD from the mean change and is calculated as the square root of the difference between the variance of the changes in the intervention group $(SD_{diff\ exp.})^2$ and the variance of the changes in the control group $(SD_{diff\ con.})^2$. It can be also calculated via TE: $\sqrt{((SD_{diff\ exp.})^2 - (TE \cdot \sqrt{2})^2)}$ (47). Few extant altitude training studies (63, 64, 69, 78-80) have quantified individual Hb_{mass} responses to altitude interventions (details see, table 2). In summary, the abovementioned studies demonstrated an individual Hb_{mass} responsiveness (i.e., individual variation free of technical noise) of ±1.3% to ±2.6% in HH and ±1.4% to ±2.9% in NH.

As presented above, a wide variability in individual Hb_{mass} response to altitude training has been shown, and several factors might be responsible. To quantify the individual variation more

precisely and provide further insights into individual Hb_{mass} response to altitude training, we therefore implemented duplicate Hb_{mass} measures, quantified individual responsiveness according to Hopkins (47), and created a same-subject crossover design (articles I and II). Furthermore, since altitude training is primarily used by elite endurance athletes, who typically present elevated Hb_{mass} values, we tested the hypothesis that athletes embarking on an LHTL camp with a high initial Hb_{mass} have a limited ability to further increase their Hb_{mass} post-intervention with a direct comparison study design using individual data and matched hypoxic dose (article III).

1.2.3. Aims of the thesis

Article I: To compare Hb_{mass} and performance changes during an 18-day LHTL altitude training camp between NH and HH using a randomized matched controlled study design.

Article II: To investigate whether Hb_{mass} responses differ between an 18-day LHTL altitude training camp in NH or HH using a same-subject crossover design. The study also focused on the quantification of individual Hb_{mass} responsiveness to NH and HH.

Article III: To examine the relationship between initial Hb_{mass} prior to LHTL (absolute and relative values) and percentage Hb_{mass} increase following LHTL camps with comparable hypoxic doses in male endurance and team-sport athletes.

Table 2 Summary of current research calculating individual hemoglobin mass response to altitude training, according to Hopkins (47).

Author	*n*	Sex	Sport	Level	Training mode	Hypoxic mode	Duration (day)	Altitude (meter)	Hypoxic dose (hours)	Hypoxic dose (km.h)	Typical error (%)	Mean Hb_{mass} change (%)	Individual response*
Troesch et al. (98)	7	m	Running	Elite	LHTH	HH	20	1800	480	864	1.8	+1.6	**±1.5%**
McLean et al. (63, 64)	21 23	m	Australian Football	National	LHTH LHTH	HH HH	19 18	2100 2130	456 432	958 920	2.6	+3.6 +4.0	**±1.3%** **±2.2%**
Neya et al. (69)	7	m	Running	Collegiate	LHTL+ TM	NH HH	21	3000 <1800	210	630	2.4	+2.1	**±2.9%**
Robertson et al. (78)	9	m	Swimming	Elite	LHTL LHTL+ LMTM	NH NH + HH	2 x 10 2 x 10	2600 2600 1350	~190 ~95 240	494 571	1.2	+0.9 (mean) LHTL 1+3: <0.7 LHTL 2+4: <2.3	**±2.6%**
Robertson et al. (79)	8	m, f	Running	Highly trained	2 x LHTL	NH	21	3000	294	882	2.0	+2.8 (camp 1) +2.7 (camp 2)	**±2.4%** **±1.4%**
Robertson et al. (80)	8	m, f	Running	Well trained	LHTL+ TH	NH NH	21	3000 2200	294 ~14	913	1.8	+3.6	**±2%**

*i.e. the individual variation in the response to an intervention free of typical error.

Hb_{mass} = hemoglobin mass, HH = hypobaric hypoxia, NH = normobaric hypoxia, LHTH = live high–train high, LHTL = live high–train low, LMTM = live moderate–train moderate, TM = train moderate, TH = train high

Chapter 2

Summary of experimental results

2. Summary of experimental results

2.1 Article I: Hemoglobin mass response in normobaric versus hypobaric hypoxia

Using a randomized matched controlled study design, we compared Hb_{mass} response after the same hypoxic dose (approximately 230 h) and after the same duration (18 days) for groups in normobaric (NH; n = 10) and hypobaric (HH; n = 11) LHTL training camps as well as a control group (CON; n = 7), for the first time.

Hb_{mass} increased similarly in the HH (+4.4%, $P < 0.001$ at day 13; +4.5%, $P < 0.001$ at day 18) and NH (+4.1%, $P < 0.001$) groups compared to CON (+1.9%, $P = 0.08$). The likelihood of $\%Hb_{mass}$ changes in the altitude groups was likely beneficial compared to CON (>79% positive), with an unclear effect (>50% trivial) between the HH and NH groups after the same hypoxic dose and after 18 days. There was a wide variability in individual Hb_{mass} responses in the HH (–0.1 to +10.6%) and NH (–1.4 to +7.7%) groups. Lastly, after hypoxic exposure, a decrease in EPO was observed in the HH ($d = 1.9$, $P < 0.001$, –39.4%) and NH ($d = 1.6$, $P < 0.001$, –51.3%) groups compared to the CON ($d = 0.3$, $P = 0.48$, –8.4%) group.

Post-test running time decreased in the HH (-3.9%, $P < 0.001$), NH (-3.3%, $P < 0.001$), and CON (–2.1%, $P = 0.03$) groups, whereas cycling performance changed non-significantly in the HH and NH (+2.4%, $P > 0.08$) groups and remained unchanged in CON (+0.2%, $P = 0.89$). The performance gains in the altitude groups were likely higher compared to the CON group (>64% positive), with an unclear effect (>39% trivial) between the HH and NH groups. There was a large correlation between the percent changes in relative Hb_{mass} ($g \cdot kg^{-1}$) and 3-km running time from pre- to post-test in the altitude groups ($r = -0.64$, $P < 0.001$).

2.2 Article II: Individual Hb_{mass} responses in normobaric and hypobaric LHTL

To have a higher statistical power and to compare mean and individual Hb_{mass} responses following 18 days in normobaric and hypobaric LHTL, a same-subject crossover study design (n = 15) was applied.

After 18 days, Hb_{mass} increased similarly in the HH (916 to 957 g, 4.5 ± 2.2%, $P < 0.001$) and NH (918 to 953 g, 3.8 ± 2.6%, $P < 0.001$) groups, and changes did not differ between the two groups ($P = 0.42$). The chance of the percent Hb_{mass} changes being greater in the HH group compared to the NH group was 36%. The percent changes in individual Hb_{mass} ranged from +0.4% to +8.7% in the HH group and from –1.4% to +7.7% in the NH group after an 18-day LHTL camp. The 95% confidence limits (CLs) for individual percent Hb_{mass} changes was ± 3.9%, and the upper CL was exceeded by eight out of 15 athletes in HH and seven out of 15 athletes in NH. Individual responsiveness (i.e., individual variation in response to an intervention free of technical noise) was ±0.9% in the HH group and ±1.7% in the NH group. There was a correlation between intra-individual delta Hb_{mass} changes (by percentage) in HH and NH ($r = 0.52$, $P = 0.048$).

Lastly, all athletes had initial ferritin levels of >30 $\mu g \cdot L^{-1}$ and were within the cut-off limits for the OFF scores (< 125.3, (39)) in the HH and NH groups both pre-test (91.7 ± 5.4 vs. 94.6 ± 14.1, respectively) and post-test (97.2 ± 6.3 vs. 97.9 ± 5.1, respectively). 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 training camps of the crossover study ($P = 0.54$).

2.3 Article III: Relationship between initial Hb_{mass} and Hb_{mass} response following LHTL in athletes

To test the hypothesis that athletes embarking on an LHTL camp with a high initial Hb_{mass} have a limited ability to further increase their Hb_{mass} post-intervention, a data set of 58 male athletes was analyzed. This is the first study to evaluate this hypothesis by using individual data and strict methodological control (duplicate Hb_{mass} measures and matched hypoxic dose).

While there was no relationship ($r = 0.02$, $P = 0.91$) between absolute initial Hb_{mass} (g) and percent absolute Hb_{mass} increase, a moderate relationship ($r = -0.31$, $P = 0.02$) was detected between initial relative Hb_{mass} ($g \cdot kg^{-1}$) and percent relative Hb_{mass} increase. Mean absolute and relative Hb_{mass} increased to a similar extent ($P \geq 0.81$) in athletes partaking 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 $g \cdot kg^{-1}$, +4.1%, $P < 0.001$, respectively) and team (from 920 ± 120 to 957 ± 127 g, +4.0%, $P < 0.001$ and from 11.9 ± 0.9 to 12.3 ± 0.9 $g \cdot kg^{-1}$, +4.0%, $P < 0.001$, respectively) sports following LHTL. Furthermore, there was no relationship ($r = -0.006$, $P = 0.96$) between individual percent changes in body weight and absolute Hb_{mass}. A large inverse relationship ($r = -0.64$, $P = 0.002$) occurred between individual percent changes in body weight and relative Hb_{mass}.

Chapter 3

Discussion and perspectives

3 Discussion and perspectives

3.1 Discussion

3.1.1. Hemoglobin mass responses in normobaric versus hypobaric LHTL

The present thesis provides new insights about Hb_{mass} responses to hypobaric versus normobaric LHTL in endurance athletes with a special focus on inter-individual variability in Hb_{mass} response to altitude exposure. There has been no previous direct comparison of Hb_{mass} response to NH and HH using a randomized matched controlled study design (article I) and a crossover study design (article II). The main findings indicate that HH and NH evoked a similar mean increase in Hb_{mass} following an 18-day LHTL camp, despite a larger hypoxic dose in HH compared to NH.

The observed mean Hb_{mass} increases of 4.4% and 4.5% in HH and 4.1% and 3.8% in NH after 18 days of LHTL in articles I and II, respectively, were of greater magnitude than in LHTL studies with similar total hypoxic hours (13, 49, 77). Furthermore, despite greater total hours of hypoxic exposure in HH (approximately 315 h) compared to NH (approximately 230 h), the mean Hb_{mass} response was similar. Recently, a meta-analysis by Gore at al. (40) estimated that Hb_{mass} increases by ~1% per 100 h of NH or HH exposure (>2000 m), which would mean lower averaged Hb_{mass} responses (~1% to 2%) in NH but also in HH. However, Hb_{mass} response to altitude depends on inter-individual variability, and in the specified meta-analysis, the calculated "upper 95% individual response limits" for ~230 h and ~315 h were around 5% and 6%, respectively, suggesting that group composition can noticeably influence the mean Hb_{mass} response to altitude exposure. The nature of natural altitude means that athletes in HH accumulated hypoxic hours much faster than in NH (17 $h \cdot day^{-1}$ vs 13 $h \cdot day^{-1}$). Therefore, to compare matched hypoxic hours in HH and NH (approximately 230 h), an additional duplicate

Hb_{mass} measurement was performed during the second training camp at day 13 in the HH group (hypoxic dose matched to 18-day NH, article I). In addition, a similar mean Hb_{mass} response between the HH and NH groups was found for matched hypoxic hours. More interesting, there was no additional increase from day 13 to day 18 in the HH groups despite a larger number of hypoxic hours after an 18-day LHTL camp (230 vs. 315 h). This lack of increase could be attributed to individual variability in the time course of Hb_{mass} response to altitude (13, 28) or still be due to measurement error. Overall, the detected Hb_{mass} increase after 230 h and after 13 days is in line with previous studies (28, 29, 69, 100) that show measurable Hb_{mass} increases (2.1% to 3.7%) within a shorter time period (11–13 d) or lower total hypoxic exposure (<210 h) than previously recommended (>12 h·day^{-1} for at least 21 days) (82). Therefore, the present thesis assumes that Hb_{mass} adaptations can be achieved with fewer hypoxic hours in some subjects.

Whether a hypoxia-induced increase in Hb_{mass} is the primary physiological mechanism leading to enhanced sea-level performance following LHTL is still under discussion (37, 50, 58, 106). The present thesis demonstrated a correlation between the percent changes in relative Hb_{mass} (g·kg^{-1}) and 3-km running time for the HH and NH groups ($r = -0.64$, $P = 0.002$), suggesting that the enhancement of endurance performance was primarily mediated by an increase in Hb_{mass}. However, due to the multifactorial adaptation mechanisms in hypoxia, the enhancement in endurance performance cannot be attributed solely to one parameter. Thus, non-hematological mechanisms, such as improved mitochondrial efficiency and/or muscle pH regulation, might also contribute to enhanced sea-level performance following altitude exposure (34).

Thus far, short-term exposure (<26 h) to HH seems to be a more stressful stimulus to the human body than NH (16, 66). The present thesis indicated that long-term exposure (i.e., 18-day LHTL camp) at 2250 m to HH and NH induced similar Hb_{mass} responses using a randomized matched

controlled study design and a crossover study design. Furthermore, running and cycling performance changes as well as blood parameters did not differ between the HH and NH groups after the 18-day LHTL training camp (figure 4). To date, only a meta-analysis (7) has indirectly compared the effects of hypobaric versus normobaric LHTL on endurance performance, demonstrating that hypobaric LHTL was more beneficial compared to normobaric LHTL (+4.2% vs. +1.4%) in sub-elite athletes. Nevertheless, the present data indicate that chronic LHTL exposure to HH or NH over a period of >13 days at moderate altitude results in no substantial differences in performance and physiological responses, suggesting that HH and NH can be used interchangeably for LHTL camps in endurance athletes.

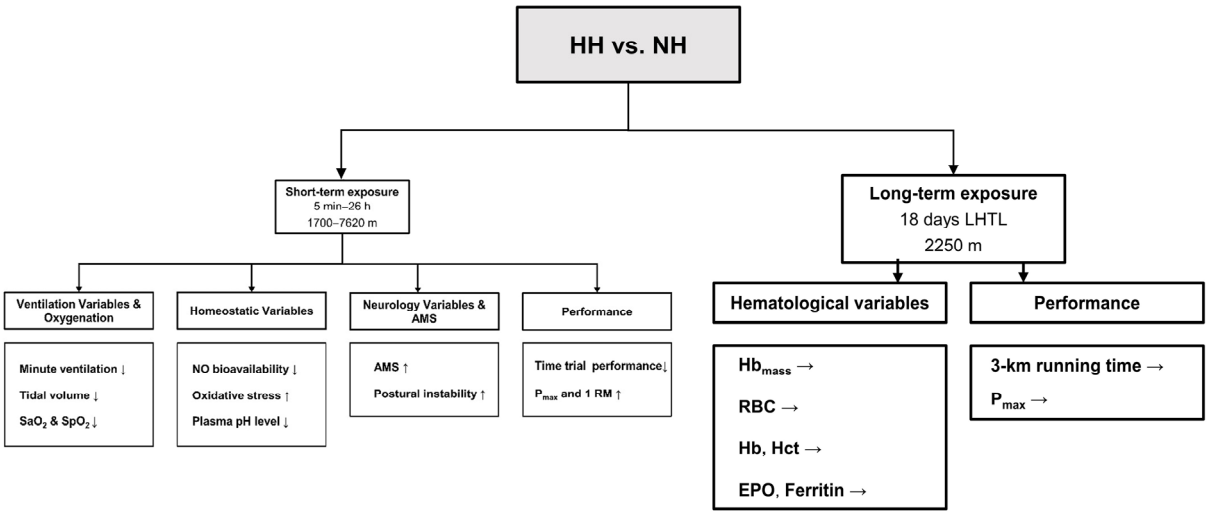

Figure 4. Extension of figure 3 (see chapter 1.2.1) – Summary of main physiological and performance differences of hypobaric hypoxia (HH) versus normobaric hypoxia (NH) after short- and long-term exposure. LHTL = live high–train low; Hb_{mass} = hemoglobin mass; RBC = red blood cells; Hb = hemoglobin; Hct = hematocrit; EPO = erythropoietin; P_{max} = maximal power

3.1.2. Individual hemoglobin mass response to altitude training

The present thesis confirmed the previously recognized variability in individual Hb_{mass} response to altitude training in athletes (11, 24, 28, 100). To enhance the methodological control in all studies, duplicate Hb_{mass} measures were applied, and a same-subject crossover design (article

II) was used in one study. Furthermore, to quantify individual responses or to detect significant individual effects, statistical approaches were applied according to Hopkins (47, 48). Overall, a notable inter-individual variability in Hb_{mass} response to HH and NH in endurance and team-sport athletes was shown, which was not attributed to the initial Hb_{mass} value or ferritin stores prior altitude exposure.

The absolute range in percent changes in individual Hb_{mass} responses after 18 days of LHTL was –0.1 to +10.6% for the HH groups and–1.4 to +7.7% for the NH groups (articles I and II). To detect significant individual effects, the 95% CLs for percent changes of Hb_{mass} were derived from the present TE of the Hb_{mass} measurement (95% CL = ± 1.96 TE · √2 · 1/√2) (47). The upper 95% CL (± 3.7% and ± 3.9%) for $\%Hb_{mass}$ was exceeded by more than half the athletes in the HH and the NH groups. Because we applied duplicate Hb_{mass} measures at each time point, which enhances the measurement precision, it can be assumed that those athletes who exceeded the upper CL were very likely "Hb_{mass} responders" to 18-days of LHTL training at 2250 m in either the HH or NH groups. In addition to the statistical significance, all athletes demonstrated normal initial ferritin values (Ftn >30 $\mu g \cdot L^{-1}$) (42) without doping abuse (doping control scores within normal ranges (85)) or different daily training loads during the altitude stay, which further supported the beneficial effect of an 18-day LHTL camp on Hb_{mass}. Since the absolute range of percent changes in individual Hb_{mass} is limited to quantify the extent of individual responsiveness, we used the Hopkins approach (47), where the magnitude of individual responses can be expressed as the SD from the mean change. In the present thesis, the magnitude of individual responsiveness was ±0.9% in the HH group and ±1.7% in NH group after an 18-day LHTL camp (article II), which was slightly lower than in other studies that report individual Hb_{mass} responsiveness of ±1.3% to ±2.6% in HH (63, 78) and ±1.4% to ±2.9% in NH (69, 79). For details, see table 2. These studies were primarily based on single measures of Hb_{mass} with the optimized CO rebreathing method, suggesting that duplicate measures of

Hb_{mass}, which reduces the TE by a factor of $1/\sqrt{2}$ (47), diminish the inter-individual variability in Hb_{mass} response to altitude training. Whether individual Hb_{mass} responses to altitude training are reproducible is still unclear. In the crossover study design (article II) many of the athletes demonstrated a reproducible Hb_{mass} response after the second LHTL camp ($r = 0.52$, $P = 0.048$), suggesting that those athletes who responded once to altitude training will likely respond another time regardless of the type of hypoxia. This result is not in line with previously published studies that focus on the reproducibility of Hb_{mass} responses in athletes to altitude training camps (63, 100) and that demonstrate reproducible mean percent Hb_{mass} changes but only a small trend toward reproducible individual Hb_{mass} changes. Therefore, further research on the reproducibility and quantification of the magnitude of individual Hb_{mass} responses is needed.

The reason for individual variability in Hb_{mass} response to altitude training is still unknown and may be related to several factors (see table 1). It has been suggested that initial low iron stores may impair Hb_{mass} production and thus reduce the effectiveness of altitude training (95). Articles I and II demonstrated a small ($r = 0.3$, $P = 0.095$) and inverse ($r = -0.30$, $P = 0.10$) correlation between the pre-altitude ferritin level and Hb_{mass} (in g) changes, suggesting that in the present thesis, pre-altitude ferritin levels did not influence individual variability in Hb_{mass} responses. However, the possibility cannot be ruled out that higher pre-altitude ferritin levels or individualized iron supplementation during altitude training would have supported Hb_{mass} production (30). A further reason for this individual variation in Hb_{mass} response could be an individual's baseline Hb_{mass} level prior to altitude training. It is supposed that athletes starting with high initial Hb_{mass} levels have a limited ability to further increase their Hb_{mass} following altitude training because those athletes would already have maximized their Hb_{mass} level by training at sea level (63, 76). Since altitude training is primarily used by elite endurance athletes who typically already have elevated Hb_{mass} values, this hypothesis is important to the relevance

of altitude training in elite athletes. A recent analysis of nine LHTL studies in endurance athletes (76) and a classic altitude training study on Australian footballers (63) demonstrated significant correlations ($r = -0.86$, $P < 0.01$ and $r = -0.51$, $P = 0.04$) between initial relative Hb_{mass} and post-altitude percentage increase in relative Hb_{mass}. By contrast, the present thesis (article III) showed a trivial ($r = 0.02$, absolute values) and moderate ($r = -0.31$, relative values) correlation between initial Hb_{mass} and percentage Hb_{mass} increase following LHTL in endurance and team-sport athletes. Because we used strict methodological control (duplicate Hb_{mass} measures, matched hypoxic dose, and different athlete populations), it can be concluded that even athletes with elevated pre-altitude Hb_{mass} levels can benefit from LHTL camps. The moderate relationship between initial relative Hb_{mass} and percent change in Hb_{mass} following the LHTL camp could be attributed to the individual changes in body weight before and after the LHTL camp because there was an inverse relationship ($r = -0.64$) between individual percent changes in body weight and relative Hb_{mass}, whereas no relationship between individual percent changes in body weight and absolute Hb_{mass} appeared. Therefore, both absolute and relative Hb_{mass} values should be evaluated after altitude camps to assess the sole effect of initial Hb_{mass} on Hb_{mass} response to altitude and exclude the confounding factor "body weight changes". Furthermore, for future comparisons, it seems that relative Hb_{mass} values adjusted for lean body mass would be a better unit.

3.1.3. Limitations

In articles I and II, it was important to compare two common real-world LHTL training practices performed by endurance athletes (e.g., daily hypoxic exposure under NH and HH, with the same training loads and recovery time). Therefore, the stated total (315 h vs. 230 h) and daily (17 h vs. 13 h) hypoxic exposures were higher in the HH groups compared to the NH groups, which is in accordance with previously published LHTL studies (7, 40). The difference in daily hypoxic exposures can be explained by a faster accumulation of hypoxic hours in HH

than in NH. Under HH conditions, athletes lived continuously in a hypoxic environment except during the training times, whereas under NH conditions, athletes accumulated hypoxic hours only during their stays in the hypoxic rooms (sleep and recovery times). To directly compare the same hypoxic hours (approximately 230 h) between the two conditions, we performed an additional Hb_{mass} measurement in the HH group at day 13 of the second training camp (see article II crossover design). However, the possibility that the unequal nature of the daily hypoxic dose in HH and NH could have influenced the Hb_{mass} responses cannot be excluded.

It was argued that endurance training rather than the hypoxic stimulus was responsible for the increase in Hb_{mass} response in the LHTL groups (92), since the rate of Hb_{mass} in the HH group at day 13 and in the NH group at day 18 was too high compared to typical altitude training responses (73). However, individual variability in Hb_{mass} response to altitude training exists, so the group compositions could have notably influenced the mean Hb_{mass} responses (101). Furthermore, all measured mean Hb_{mass} responses (3.8–4.5%) for the present thesis were within the upper 95% CL calculated by the meta-analysis by Gore (40). Nevertheless, even with duplicate Hb_{mass} measurements, there is still the possibility of random noise (40).

Altitude training studies with athlete populations often have small sample sizes (~10 subjects) and thus have a low statistical power, as in articles I and II. Therefore, in addition to the conventional statistical approach, we used a contemporary statistical approach recommended by Hopkins (11), which defines the chances that the "true" value of an effect is practically relevant. Lastly, in article III, despite a large sample size (n = 58), it seems that part of the inverse relationship between initial relative Hb_{mass} and percent changes in relative Hb_{mass} after the LHTL camp could be attributed to the statistical phenomenon "regression to the mean". Thus, when evaluating the relationship between changes and initial values, the statistical phenomon regression to the mean must be taken into account (6).

3.2 Overall conclusion and perspectives

The present thesis indicated that hypobaric and normobaric LHTL evoked similar mean increases in Hb_{mass} as well as similar performance changes after an 18-day LHTL camp. Among the Hb_{mass} changes, there was a notable variation in individual Hb_{mass} response, which tended to be reproducible. The individual variability in Hb_{mass} response to LHTL training camps was not reliant on the individual's initial Hb_{mass} level before embarking upon the altitude training camp. It was found that the moderate relationship between initial relative Hb_{mass} and percent change in relative Hb_{mass} following LHTL in the present thesis 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. Lastly, the present thesis indicated that Hb_{mass} adaptations can be achieved with fewer hypoxic hours in some subjects than was previously hypothesized.

From a practical point of view, it can be assumed that both hypoxic conditions can be used interchangeably for a LHTL training camp to obtain beneficial effects on Hb_{mass} and endurance performance. However, both hypoxic conditions do have specific benefits and drawbacks. While an athlete under HH accumulates hypoxic hours much faster, HH is logistically more difficult (e.g., the travel effort from high to low altitude). In contrast, NH is more convenient and enables the individualization of the hypoxic stimulus for the athletes, but they require more time to accumulate sufficient hypoxic doses and living in hypoxic rooms or tents might be more uncomfortable. The variability in individual Hb_{mass} response to altitude training in the present thesis emphasizes the importance of individual Hb_{mass} evaluation for altitude training in athletes. To enhance Hb_{mass} measurement precision, we recommend error-reducing duplicate Hb_{mass} measures directly before and after an altitude training camp (time lag <24 h between the two measurements). Overall, altitude training for elite athletes should be carefully planned and performed (i.e., sufficient iron status both before and during altitude, acclimatization time,

positive ratio of stress and recovery, and sufficient nutrition intake) to expect positive effects on Hb_{mass} and performance.

As the present thesis and the reported studies used small sample sizes and different athlete populations and applied different altitude protocols, further research on reproducibility and quantification of individual Hb_{mass} response to HH and NH is needed. Lastly, future studies should also focus on genetic parameters to better understand the individual variability in Hb_{mass} responses to altitude training.

Chapter 4

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Chapter 5

Article I

Similar hemoglobin mass response in hypobaric and normobaric hypoxia in athletes.

5 Article I: Similar hemoglobin mass response in hypobaric and normobaric hypoxia in athletes

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ABSTRACT

Purpose: To compare hemoglobin mass (Hb_{mass}) changes during an 18-day live high-train low (LHTL) altitude training camp in normobaric hypoxia (NH) and hypobaric hypoxia (HH). **Methods:** Twenty-eight well-trained male triathletes were split into three groups (NH: n = 10, HH: n = 11, control (CON): n = 7) and participated in an 18-day LHTL camp. NH and HH slept at 2250 m while CON slept and all groups trained at altitudes <1200 m. Hb_{mass} was measured in duplicate with the optimized CO rebreathing method before (pre-), immediately after (post-) (hypoxic dose: 316 vs. 238 h for HH and NH), and at day 13 in HH (230 h, hypoxic dose matched to 18-day NH). Running (3-km run) and cycling (incremental cycling test) performances were measured pre- and post. **Results:** Hb_{mass} increased similar in HH (+4.4%, $P < 0.001$ at day 13; +4.5%, $P < 0.001$ at day 18) and NH (+4.1%, $P < 0.001$) compared to CON (+1.9%, $P = 0.08$). There was a wide variability in individual Hb_{mass} responses in HH (–0.1 to +10.6%) and NH (–1.4 to +7.7%). Post-running time decreased in HH (-3.9%, $P < 0.001$), NH (-3.3%, $P < 0.001$), and CON (–2.1%, $P = 0.03$), whereas cycling performance changed non-significantly in HH and NH (+2.4%, $P > 0.08$) and remained unchanged in CON (+0.2%, $P = 0.89$). **Conclusion:** HH and NH evoked similar Hb_{mass} increases for the same hypoxic dose and after 18-day LHTL. The wide variability in individual Hb_{mass} responses in HH and NH emphasize the importance of individual Hb_{mass} evaluation of altitude training.

Introduction

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

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

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

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

Methods

Subjects

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

Study Design

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

Hypoxic Exposure

The HH group lived at Fiescheralp, Switzerland (2250 m, inspired oxygen pressure (P_iO_2) 111.7 ± 0.7 mm Hg; inspired oxygen fraction (F_iO_2) 20.9% ± 0.0, barometric pressure (P_B) 580.8 ± 3.3 mm Hg) and traveled by cable car twice daily to the valley (altitude <1200 m) for training. The daily hypoxic dose in the HH group amounted 17.4 ± 1.2 h. At day 13 during the training camp, the total hypoxic dose in the HH group was 229.5 ± 1.3 h, and after 18 days, the dose was 316.4 ± 2.3 h. The NH group lived in Prémanon, France (1150 m) and was exposed to normobaric hypoxia equivalent to 2250 m in hypoxic rooms (medium size: 15 ± 1 m^2).

Normobaric hypoxia was obtained by extracting oxygen from ambient air in hypoxic rooms (P_iO_2 112.7 ± 0.1 mm Hg; F_iO_2 18.1% ± 0.1; P_B 668.2 ± 2.5 mm Hg). In each hypoxic room, the gas composition was continuously monitored with oxygen and carbon dioxide analyzers (FIELDBROOK Ltd, London, UK), which were connected to a central monitoring station under the control of an experienced physiologist. The NH group in Prémanon left the hypoxic rooms on average 5–6 times per day to eat and train. The daily hypoxic dose in the NH group was 13.1 ± 1.6 h, and the total hypoxic dose after 18 days in the NH group amounted 238.2 ± 10.6 h. For both groups, the time spent in hypoxia was monitored daily and recorded manually.

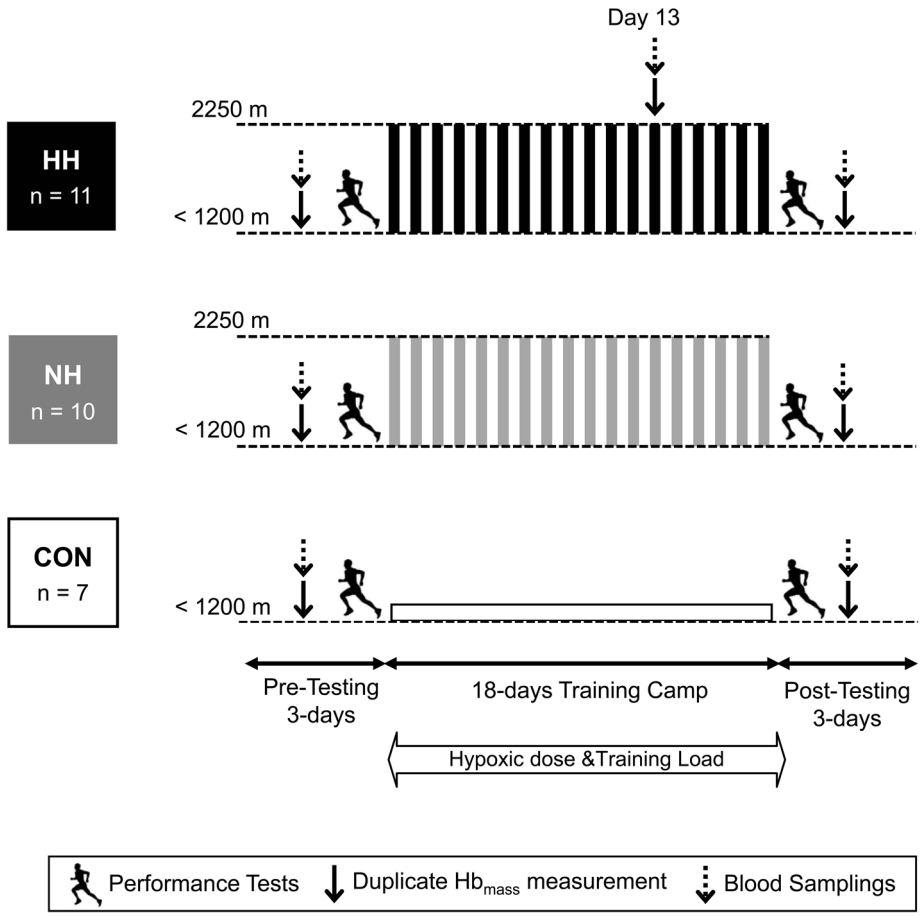

Figure 1. Illustration of the study design in hypobaric hypoxia (HH), normobaric hypoxia (NH), or normoxia (CON).

Training Load

All training sessions during the training camp were supervised with the volume and intensity matched for all groups by two experienced certified coaches. The HH and NH group trained separately, since they were located at two different places. The CON group lived nearby the NH group and trained most of the time together with the NH group. The training consisted of cycling, running, and swimming. Training load quantification was performed using the Objective Load Scale (ECOs) (4), which was specially developed for training load quantification in triathlon. 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.

Running and Cycling Performance

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

Hemoglobin Mass

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

Blood Samples

On the first morning in pre- and post-testing, 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 determine red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), and reticulocyte percentage (Ret), blood was analyzed via fluorescent flow cytometry

and hydrodynamic focusing (XT-2000i, Sysmex Europe, Norderstedt, Germany). The coefficient of variation (CV), which was determined using internal quality controls, was below 1.5% for Hb and 15% for Ret. Plasma EPO was measured using a standard procedure with an enzyme-linked immunosorbent assay (ELISA) kit (Stemcell Technologies, Grenoble, France). CVs determined with three internal quality controls (levels: low, medium, and high) were below 15%. Additionally, serum ferritin concentration (Ftn) was quantified using standard laboratory procedures (Dimension EXL, Siemens Healthcare Diagnostics SA, Zürich, Switzerland). To exclude the potential risk of misuse of recombinant human erythropoietin, all athletes were tested for doping by an accredited laboratory (Swiss Laboratory for Doping Analyses, Lausanne, Switzerland) according to the standards of the Athlete Biological Passport (31). All plasma samples were analyzed in duplicate, and the mean values were used for this study.

Statistical Analyses

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

To quantify the likelihood that the true mean of percent changes in Hb_{mass} and performance parameters was relevant (i.e., more extreme than the smallest worthwhile change (SWC) of Hb_{mass} and performance, set to ± 1%), a contemporary statistical approach was used (18). The magnitude of the change in the mean and the spreads of the 90% confidence limits (CL) were used to classify the effects (positive, trivial, or negative) (19). The magnitude of the change was determined with the following descriptors (1): <1%, almost certainly not; 1–5%, very unlikely; 5–25%, unlikely or probably not; 25–75%, possibly or may be; 75–95%, likely or probably; 95–99%, very likely; >99%, almost certainly. The magnitude of change was termed "unclear" if the CL overlapped the positive and negative SWC thresholds. To detect significant individual effects, the 95% CL for percent changes of Hb_{mass} were derived from the present TE of the Hb_{mass} measurement (95% CL = ± 1.96 TE √2 1/√2) (17).

Results

Hemoglobin Mass

After the same hypoxic dose, the absolute Hb_{mass} of the HH (d = 0.5, P < 0.001, +4.4%) and NH (d = 0.5, P < 0.001, +4.1%) groups increased to the same extent (Table 1). Similar increases were also observed for the relative Hb_{mass} values in the HH (d = 0.6, P < 0.001, +4.3%) and NH (d = 0.4, P < 0.001, +3.8%) groups. After 18 days, Hb_{mass} was not further increased in the HH group either for absolute (d = 0.5, P < 0.001, +4.5%) or relative (d = 0.6, P < 0.001, +4.5%) values. No significant change in the CON group was observed either for absolute (d = 0.1, P = 0.08, +1.9%) or relative (d = 0.2, P = 0.46, +1.0%) values. Absolute and relative Hb_{mass} changes did not differ between the groups with the same hypoxic dose (P > 0.75), as well as after 18

days (P > 0.12). The likelihood of %Hb_{mass} changes in the altitude groups was likely beneficial compared to CON (>79% positive), with an unclear effect (>50% trivial) between the HH and NH groups after the same hypoxic dose and after 18 days (Table 2). Individual absolute Hb_{mass} responses ranged from –0.1 to +10.6% in the HH group, from –1.4 to +7.7% in the NH group and from -3.3 to + 6.0% in the CON group. The 95% CL for %Hb_{mass} changes were ± 3.7% and the upper CL was exceeded by most of the subjects in the altitude groups (Figure 2).

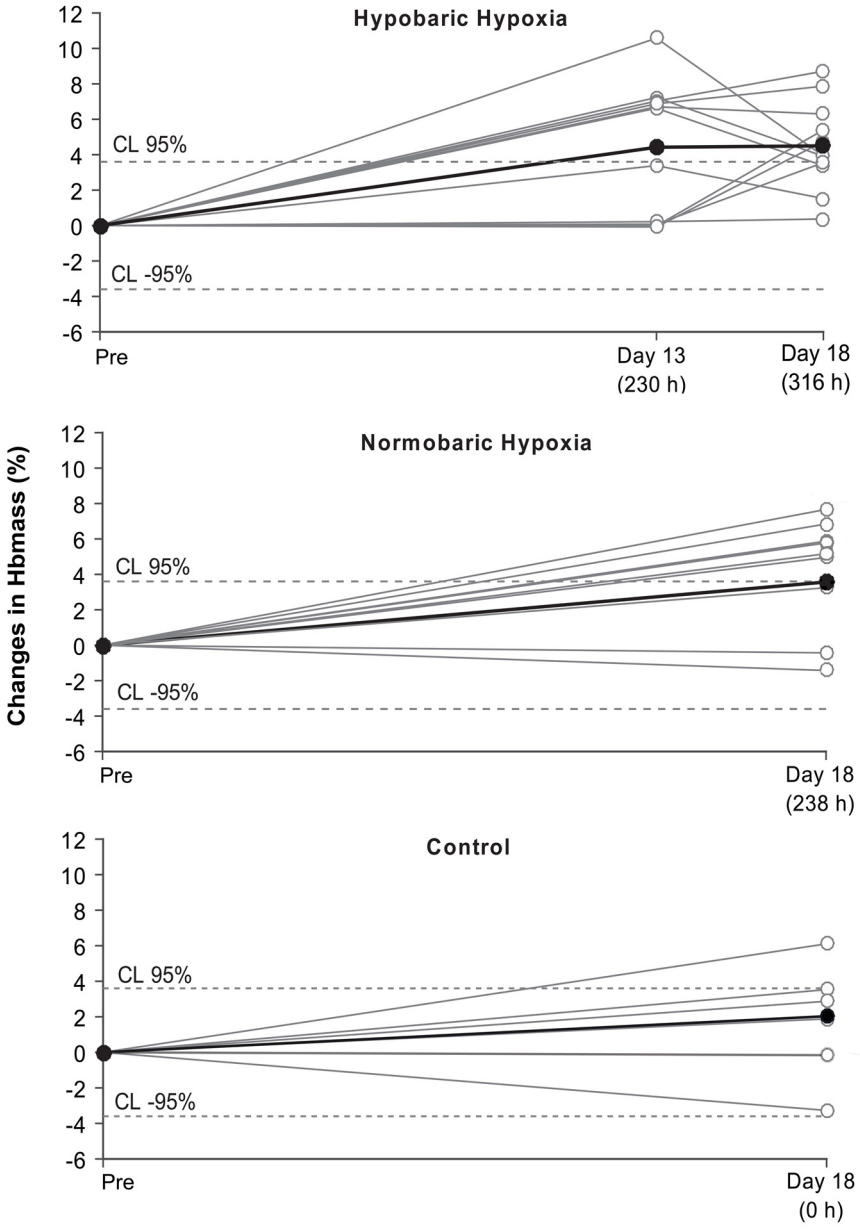

Figure 2. Percent changes in hemoglobin mass of each athlete (open circle) and mean changes of each group (filled circle) after 18-day LHTL and after the same hypoxic exposure (230 h). The 95% confidence limits (95% CLs) are indicated by dotted lines.

Table 1 Hemoglobin mass (Hb_{mass}) and hematological parameters before (Pre) and after (Post) the 18-d LHTL training camp for hypobaric hypoxia (HH), normobaric hypoxia (NH) and control (CON). As well for the similar hypoxic dose (230 h and 238 h) in HH and NH.

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

RBC = red blood cells, Hb = hemoglobin concentration, Hct = hematocrit, Ret = reticulocytes, Ftn = serum ferritin concentration. Data are mean ± SD, *significant difference between different levels of time ($P < 0.05$).

Performance

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

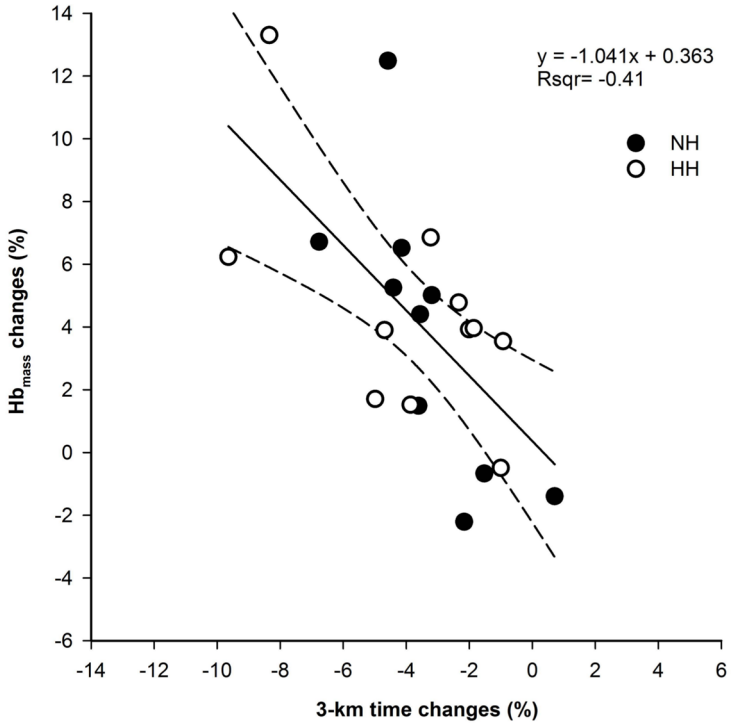

Figure 3. Linear regression (and 95% CL) for percent changes from pre- to post-intervention in hypobaric hypoxia (HH) and normobaric hypoxia (NH) between relative Hb_{mass} and 3-km running time. Regression slope (solid line) and 95% CL (dashed lines) are shown.

Table 2 Differences in hemoglobin mass (Hb_{mass}) and performance improvements after 18-d LHTL camp between hypobaric hypoxia (HH), normobaric hypoxia (NH) and control (CON).

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

P_{max} = maximal power output, ΔMean = differences in mean, CL = confidence limits. [1] With references to a smallest worthwhile change of 1% for performance and Hb_{mass}. Group comparison was calculated first group minus second group.

Blood Parameters

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

Training Load and Body Weight

No differences were found in daily training loads between the groups (213.6 ± 29 vs. 205.2 ± 16 vs. 155.4 ± 71 ECOs for the NH, HH, and CON groups, respectively) during the training camp (P = 0.21). Body weight did not differ (P = 0.76) between the groups. Pre-body weight was 68.6 ± 6.5, 70.4 ± 4.8, and 72.1 ± 8.2 kg, and post-body weight was 68.6 ± 5.6, 70.6 ± 4.9, and 72.7 ± 8.5 kg for the HH, NH, and CON groups, respectively.

Discussion

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

Mean Hb_{mass} Responses

The altitude groups demonstrated a similar group mean increase in Hb_{mass} after the same hypoxic dose (+4.4% vs. +4.1%) to LHTL at 2250 m. The Hb_{mass} increase was of similar magnitude to that observed by other LHTL studies (12, 15). It is well accepted that an adequate hypoxic dose of >12 h/day at sufficient altitude for >21 days (25, 27), i.e., approximately 300

h (7) is recommended to substantially increase Hb_{mass}. However, in the current study, both altitude groups enhanced their Hb_{mass} by approximately 4% after approximately 230 h of hypoxic exposure at 2250 m, which is in accordance with other studies (12, 26). These studies also showed a measurable increase in Hb_{mass} (3.0 - 3.5%) after 210 h of normobaric hypoxic exposure at 3000 m (26) and after 236 h of hypobaric hypoxia at 2760 m (12). Furthermore, due to the nature of natural altitude, the HH group accumulated hypoxic hours much faster than the NH group (17 $h \cdot day^{-1}$ vs. 13 $h \cdot day^{-1}$) and achieved a similar hypoxic dose (approximately 230 h) after 13 days of altitude training compared to the NH group (18 days), with no additional group mean Hb_{mass} increase in HH (+4.4% vs. +4.5%) by day 18 (316 h). This suggests that approximately 230 h of hypoxic exposure at 2250 m in either HH or NH is sufficient to increase Hb_{mass} in endurance athletes and that these erythropoietic adaptations were feasible within a shorter duration of hypoxic exposure than commonly recommended (26). Otherwise, altitude studies have shown that Hb_{mass} increases at a mean rate of 1.1%/ 100 h of exposure (14), expecting a further Hb_{mass} increase of ~1% from day 13 to day 18 in the HH group. However, there is a wide individual variability in the time course of Hb_{mass} response to altitude training (7, 12), which was also present in the HH group from day 13 to day 18 (Figure 2). Some of the athletes could further increase their Hb_{mass} from day 13 to day 18 (+0.9 to +5.4%), whereas in others Hb_{mass} decreased from day 13 to day 18 (-1.8 to -6.0%). Furthermore, even using duplicate Hb_{mass} measurements it is still difficult to certainly detect Hb_{mass} changes smaller than the TE (1.3%). Therefore, it might be possible that the lack of increase in Hb_{mass} from day 13 to day 18 in HH is due to individual variation in the time course of Hb_{mass} responses and due to measurement error. Last, the $\%Hb_{mass}$ changes in both altitude groups were likely beneficial (>79% positive) in comparison to the CON group, indicating that LHTL either in HH or NH is advantageous for Hb_{mass} increase compared to sea-level training. However, the difference

between Hb_{mass} response in the NH and HH groups was unclear with a tendency to be trivial after the same hypoxic dose (50%) and after 18 days of LHTL (57%) (Table 2).

Individual Hb_{mass} Responses

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

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

Performance

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

LHTL in HH versus NH in performance responses. Therefore, a cross-over study with the same athletes exposed to HH and NH is needed to confirm the present results.

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

Blood Parameters

The majority of the hematological parameters were similar between the HH and NH groups before and after the 18-day LHTL training camp. EPO was lower in both groups after the LHTL training camp compared to the CON group, which is in line with previous findings (5, 7, 40), showing that EPO increases at the beginning of altitude exposure and peaks within 2–3 days before beginning to decrease toward sea-level values. It has been suggested that low iron stores (Ftn <30 $\mu g \cdot L^{-1}$) interfere with Hb_{mass} responses to hypoxic exposure and may reduce the effectiveness of altitude training (38). In the present study, the ferritin levels were above >30 µg·L–1 in all athletes and only a small correlation between the initial ferritin level and the

Hb_{mass} responses (r = 0.3, P = 0.095) was detected. However, one cannot rule out that low ferritin levels may limit Hb_{mass} responses to altitude training.

Study Limitations

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

to HH and NH, a cross-over design with a similar hypoxic dose of NH and HH would be needed. However, due to different periods of the athlete's training (e.g., competition period, off-season, tapering or peaking), a cross-over design with athletes is only feasible if the interventions take place at the same time point of the season.

Conclusion

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

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Chapter 6

Response

Hemoglobin mass expansion during 13 days of altitude training: altitude or training?

6 Response: Hemoglobin mass expansion during 13 days of altitude training: altitude or training?

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Dear Editor-in-Chief,

We thank Dr. Siebenmann for the interest in our study. He wrote, that one should be careful to interpret the increase in hemoglobin mass (Hb_{mass}) unambiguously as a response to altitude. We agree with this point, as several studies have shown that Hb_{mass} can be slightly increased after a training camp, especially when subjects are not at top-elite level (7). Our current results support this, as there was a tendency ($P = 0.08$) but not a significant effect of Hb_{mass} to be 1.9% "elevated" after 18 d endurance training in our control group (4). However, our main goal was not to distinguish between the altitude and training effect, but between the effect of normobaric and hypobaric "live high–train low" (LHTL). Further, Dr. Siebenmann expressed the opinion that three aspects of the hematological results differ from those typically observed at altitude.

First, he noted a "relatively high" increase in Hb_{mass} (4.4%) already at day 13 (230 h) in the HH group (2250 m). In our opinion, these results do not differ from typical LHTL altitude training studies that have indicated similar increases in Hb_{mass} after 14 d. For example, Garvican-Lewis et al. (2) reported Hb_{mass} increases between 3.7% and 4.5% after LHTL blocks lasting as short as 9 to 11 d. Gore et al. (3) suggest in their comprehensive meta-analysis that an increase of 1.1%/100 h of adequate altitude exposure can be expected. This would result in a 2.5% increase after 230 h; however, the "upper 95% response limit" for 230 h in this study was around 5%. Interestingly, Dr. Siebenmann himself reported increased Hb_{mass} (+3.5%) already at day 12 at altitude (8). Our individual Hb_{mass} increase after 13 d varied between 0% and +10%. It can therefore be reasoned, that group composition (i.e. Hb_{mass}-responder vs Hb_{mass}-nonresponder) can noticeably influence the mean Hb_{mass} response.

Second, Dr. Siebenmann pointed out that erythropoietin was not elevated at day 13. In our opinion, our results also align well with other studies, where erythropoietin values increase during the first days at altitude and then return to pre values after around 10–14 d. For example,

Chapman et al. (1) demonstrated in four different LHTL altitude training groups (living altitudes 1780, 2085, 2454, and 2800 m) that erythropoietin returned to pre altitude levels at 7, 14 and 21 d in all the four LHTL groups.

Third, Dr. Siebenmann noted the slightly reduced hematocrit values compared with baseline. It is well known that plasma volume is easily changed by a variety of short-term factors (6). As already indicated (4), we think that these data should not be overinterpreted, since hematocrit values at pretests were potentially elevated due to the long travel and suboptimal fluid intake.

In summary, we emphasize, that all Hb_{mass} measurements were made in duplicate (5) and with a high level of documented reproducibility (4). Furthermore, we wish to reinforce, that our hematological values compare favorably with those typically observed within LHTL studies. Finally, an interesting aspect for future research is the varied individual Hb_{mass} responses detected in triathletes.

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Chapter 7

Article II

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

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

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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.

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

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.

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

Methods

Subjects

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

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 however only with those athletes, 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.

Hypoxic exposure

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.

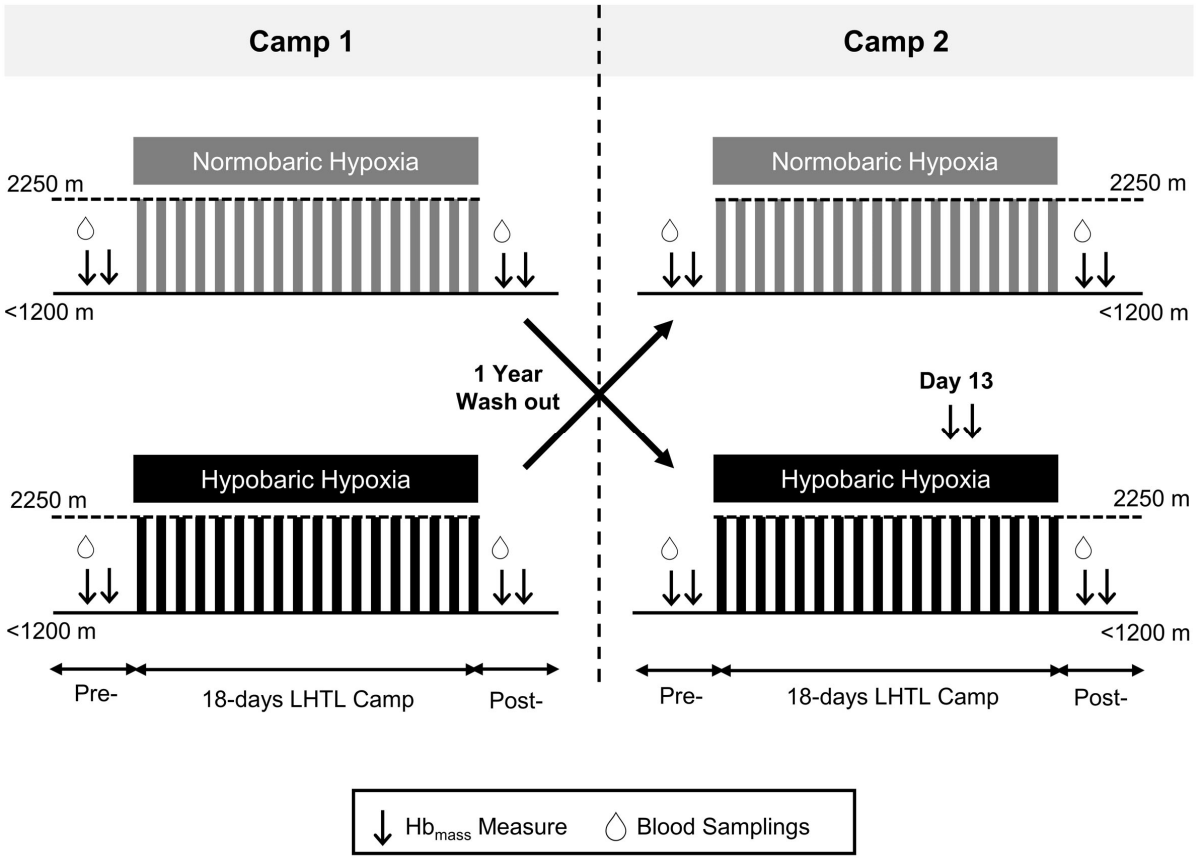

Figure 1. Illustration of the study design (n =15).

Training load

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

Hb_{mass} 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). Hb_{mass} 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 Hb_{mass} 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 Hb_{mass} 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 Hb_{mass} 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

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 $\mu g \cdot L^{-1}$), 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 ($g \cdot L^{-1}$) - $60\sqrt{}$(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

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).

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 ± 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 ($(TE \cdot \sqrt{2})^2$). 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% CL = $\pm 1.96 \cdot TE \cdot \sqrt{2} \cdot 1/\sqrt{2}$; (17)).

Results

Mean Hb_{mass} responses

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).

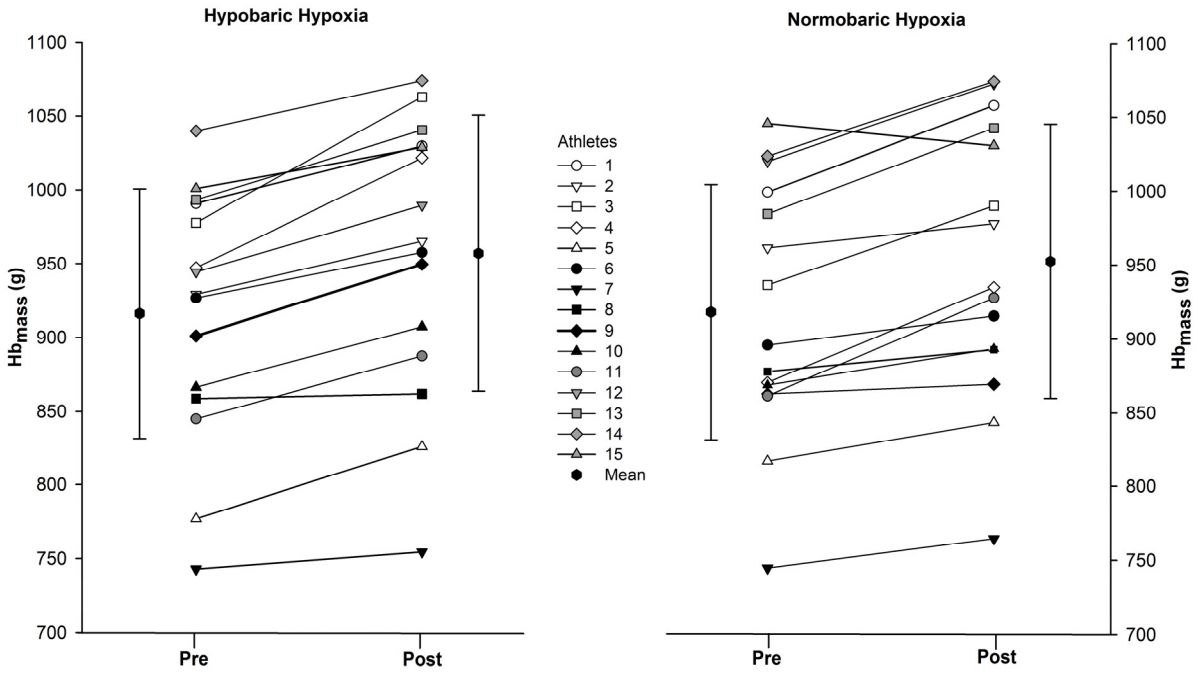

Figure 2. Individual Hbmass (g) for before (Pre) and after (Post) 18 d of LHTL in either hypobaric or normobaric hypoxia, n = 15.

Table 1 Likelihoods of magnitudes of hemoglobin mass (Hb_{mass}) changes between hypobaric hypoxia (HH) and normobaric hypoxia (NH) after 18-d 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-d 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.

Individual Hb_{mass} responses

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 ($r = 0.52$, $P = 0.048$).

Ferritin and OFF score

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.

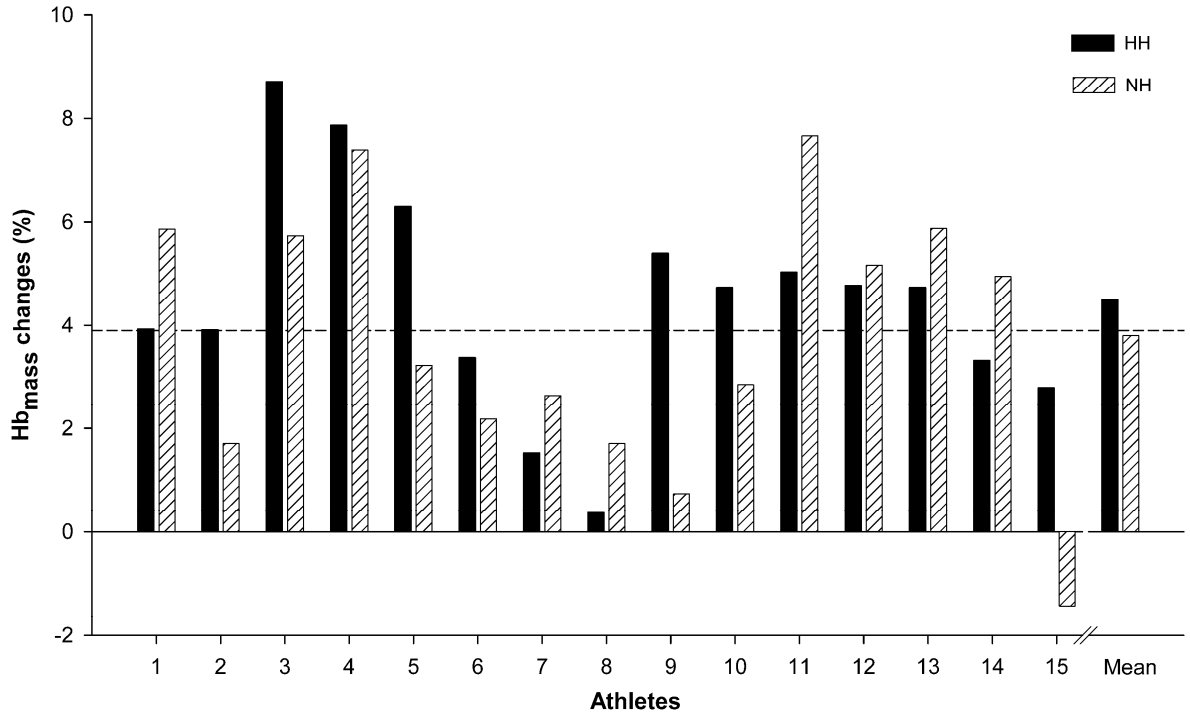

Figure 3. Individual hemoglobin mass (Hbmass) changes (%) after 18 d of LHTL in hypobaric hypoxia (HH, 312 h) or in normobaric hypoxia (NH, 225 h). The 95% limits (95% CLs) are indicated by dotted lines.

Training load and body weight

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

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

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 $h \cdot day^{-1}$ at natural or simulated altitude > 2000 m for > 21 d; that is, approximately 300 h is required to substantially increase Hb_{mass} (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 Hb_{mass} 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 Hb_{mass} to moderate altitude (2500–3000 m). The data from these studies showed measurable Hb_{mass} 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 Hb_{mass} to different altitude protocols in different athlete populations.

An hypoxia-induced increase in Hb_{mass} seems to be one of the main physiological mechanisms leading to improved sea-level endurance performance after altitude training (14, 22, 23, 42). Hb_{mass} is closely related to maximal oxygen 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 $\dot{V}O_{2max}$ under normoxic conditions (37). Further, Hb_{mass} 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 Hb_{mass} data (in $g \cdot kg^{-1}$) 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 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.

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

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).

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

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

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.

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Chapter 8

Article III

Do athletes with already high initial haemoglobin mass benefit from altitude training?

8 Article III: Do athletes with already high initial haemoglobin mass benefit from altitude training?

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Abstract

Purpose: It has been proposed that athletes with high initial values of hemoglobin mass (Hb_{mass}) will have a lower Hb_{mass} increase in response to 'live high-train low' (LHTL) altitude training. To verify this assumption, the relationship between initial absolute and relative Hb_{mass} values and their respective Hb_{mass} increase following LHTL in male endurance and team-sport athletes was investigated. **Methods:** 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_{mass} was measured in duplicate pre- and post-LHTL with the carbon monoxide rebreathing method. **Results:** While there was no relationship ($r = 0.02$, $P = 0.91$) between absolute initial Hb_{mass} (g) and percent absolute Hb_{mass} increase, a moderate relationship ($r = -0.31$, $P = 0.02$) between initial relative Hb_{mass} ($g \cdot kg^{-1}$) and percent relative Hb_{mass} increase was detected. Mean absolute and relative Hb_{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 $g \cdot kg^{-1}$, +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 $g \cdot kg^{-1}$, +4.0%, $P < 0.001$) athletes following LHTL. **Conclusion:** The direct comparison study using individual data of endurance and team-sport athletes and strict methodological control (duplicate Hb_{mass}-measures, matched-hypoxic dose) indicated that even athletes with higher initial Hb_{mass} can reasonably expect Hb_{mass} gain post-LHTL.

Introduction

Many elite endurance athletes perform altitude training with the aim to enhance their oxygen-carrying capacity and eventually their sea-level performance (2, 24, 34). 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 (3, 23). Compared to endurance athletes, team-sport athletes are generally characterised by a lower maximal aerobic capacity (11) and possess lower hemoglobin mass (Hb_{mass}) (15, 31). The potential main benefit derived from the popular 'live high–train low' (LHTL) altitude training intervention seems to rely on an increase in Hb_{mass} (13, 20, 33). In a particular sport (e.g. endurance or team sports), considerable individual variation in Hb_{mass} response to altitude training has been reported (6, 8, 9, 14, 22, 29, 32). Although sources of this variability still remain unclear, aspects such as erythropoietic response to hypoxia (5, 6), genetic predisposition (35), residual fatigue and training history (7) and/or intra-individual conditions (32) likely play a role.

Another suggested reason for this variability relies on the individual's initial Hb_{mass} level before embarking upon the altitude training camp. Hence, it has been proposed that athletes with already high initial Hb_{mass} have a limited ability to further increase (i.e. ceiling effect) their Hb_{mass} following altitude training (12, 22, 26). A recent analysis of nine LHTL studies (26) demonstrated a high correlation ($r = 0.86$, $P < 0.01$) between initial relative (expressed in $g \cdot kg^{-1}$) Hb_{mass} and post-intervention percentage increase in relative Hb_{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_{mass}, with different accuracy/reliability levels, have been used (33). Furthermore, these studies examined different genders (male and female), 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_{mass} response to altitude training exists (6, 8, 14, 22).

Since LHTL is primarily used by elite endurance athletes typically presenting elevated Hb_{mass} values compared to team-sport athletes, the hypothesis that athletes embarking on an LHTL camp with already high initial Hb_{mass} have a limited ability to further increase their Hb_{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 was to examine the relationship between individual initial Hb_{mass} prior to LHTL (absolute and relative values) and percentage Hb_{mass} increase following a LHTL camp with comparable hypoxic doses in male endurance and team-sport athletes.

Methods

Study design

Data from three altitude training interventions (studies I, II and III), with similar hypoxic doses (200–230 h) and identical Hb_{mass} measurement procedures, were re-analysed to determine the nature of the association of individual Hb_{mass} increase the individual initial absolute and relative Hb_{mass}. The details of the experimental design of the three altitude training interventions have been published elsewhere (see Study I (27), Study II (14) and Study III (4)).

Participants

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, endurance training experience ≥ 5 years) were recruited. These studies were 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). 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. This study was approved by the Anti-Doping Lab Qatar institutional review board (SCH-ADL-070; Doha, Qatar). A total of 58 athletes were included in the final study sample. Inclusion criteria were as follows: completion of an LHTL altitude training camp with all Hb_{mass} measures done in duplicate by the same investigator prior to and after the intervention. All procedures were conducted in accordance with the Declaration of Helsinki guidelines, and all athletes provided written informed consent to participate in the respective studies.

Altitude interventions

For studies I and II (normobaric groups), 24 endurance athletes performed an 18-d LHTL altitude training camp under normobaric hypoxic conditions (~12.5 $h \cdot day^{-1}$ 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 in hypoxic rooms (1150 m, P_iO_2 111.9 ± 0.6 mm Hg; F_iO_2 18.1 ± 0.1%; P_B 666.6 ± 3.6 mm Hg). For study II (hypobaric group), since normobaric and hypobaric hypoxia induces similar Hb_{mass} and endurance performance responses after LHTL altitude training(14, 27), 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 $h \cdot day^{-1}$). Those athletes lived at 2250 m (P_iO_2 111.7 ± 0.7 mm Hg; F_iO_2 20.9%, P_B

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 $h \cdot day^{-1}$ 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 (F_iO_2 15.1%, P_B 768.0 mm Hg, P_iO_2 108.3 mm Hg) up to 3000 m (F_iO_2 14.2 ± 0.1%, P_B 765.3 ± 1.5 mm Hg, P_iO_2 101.7 ± 0.8 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_iO_2 20.9%; n=12) or normobaric hypoxic conditions (3000 m, F_iO_2 ~14.5%; n=11). In summary, according to the definition of Garvican-Lewis et al. (10), 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.

Hemoglobin mass

In all athletes, Hb_{mass} was measured in duplicate using a slightly modified version (30) of the optimised carbon monoxide (CO)-rebreathing method described by Schmidt and Prommer (28). For details, see Hauser et al. (14) and Brocherie et al. (4). 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 √2 (16). In our mobile laboratories, the TEs ranged between 1.6% and 2.0%. Since duplicate measurements reduce the TE by a factor of 1/√2 (16), the TEs for averaged duplicate Hb_{mass} measurements ranged between 1.1% and 1.4%. For each athlete, Hb_{mass} measures were performed by the same investigator throughout the studies.

Data analysis

Values are presented as means ± standard deviation (SD). All data were checked for normality (Shapiro-Wilk test). Linear regressions were used to determine the Pearson's product-moment correlation coefficients (r) between initial absolute and relative Hb_{mass} and their respective percent changes in Hb_{mass}, as well as for percent changes between body weight and Hb_{mass}. 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 (17): trivial ($r < 0.1$), small ($0.1 < r < 0.3$), moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large ($0.7 < r < 0.9$), nearly perfect ($r > 0.9$) and perfect ($r = 1.0$). Paired *t*-tests were conducted to compare pre- and post-values in Hb_{mass} and body weight within the athlete group. An unpaired t-test was performed to compare percent changes between endurance and team-sport athletes. The level of significance was set at $P < 0.05$. All analyses were processed using Sigmaplot 11.0 (Systat Software, San Jose, CA) and the statistical software package R (Vienna, Austria).

Results

Relationship between initial Hb_{mass} and Hb_{mass} increase

There was no relationship between the absolute initial Hb_{mass} (g) and the percentage increase in absolute Hb_{mass} ($r = 0.02$, $P = 0.91$) (Figure 1 A). The linear regression for absolute Hb_{mass} was $y = -0.0004x + 3.5$ and the SE of the slope was ± 0.003. A moderate negative correlation between the relative initial Hb_{mass} ($g \cdot kg^{-1}$) and the percentage increase in relative Hb_{mass} ($r = -0.31$, $P = 0.02$) was observed (Figure 1 B). The linear regression for relative Hb_{mass} was $y = -0.98x + 16.4$ and the SE of the slope was ± 0.348.

Mean Hb_{mass} response

Mean absolute Hb_{mass} 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 Hb_{mass} increased equally in endurance (13.1 ± 1.2 to 13.6 ± 1.1 $g \cdot kg^{1}$, +4.1 ± 4.2%, $P < 0.001$) and team-sport (11.9 ± 0.9 to 12.3 ± 0.9 $g \cdot kg^{-1}$, +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 ($P \geq 0.53$). There was no relationship ($r = -0.006$, $P = 0.96$) between individual percent changes in body weight and absolute Hb_{mass} (Figure 2 A). A large inverse relationship ($r = -0.64$, $P < 0.001$) occurred between individual percent changes in body weight and relative Hb_{mass} (Figure 2 B).

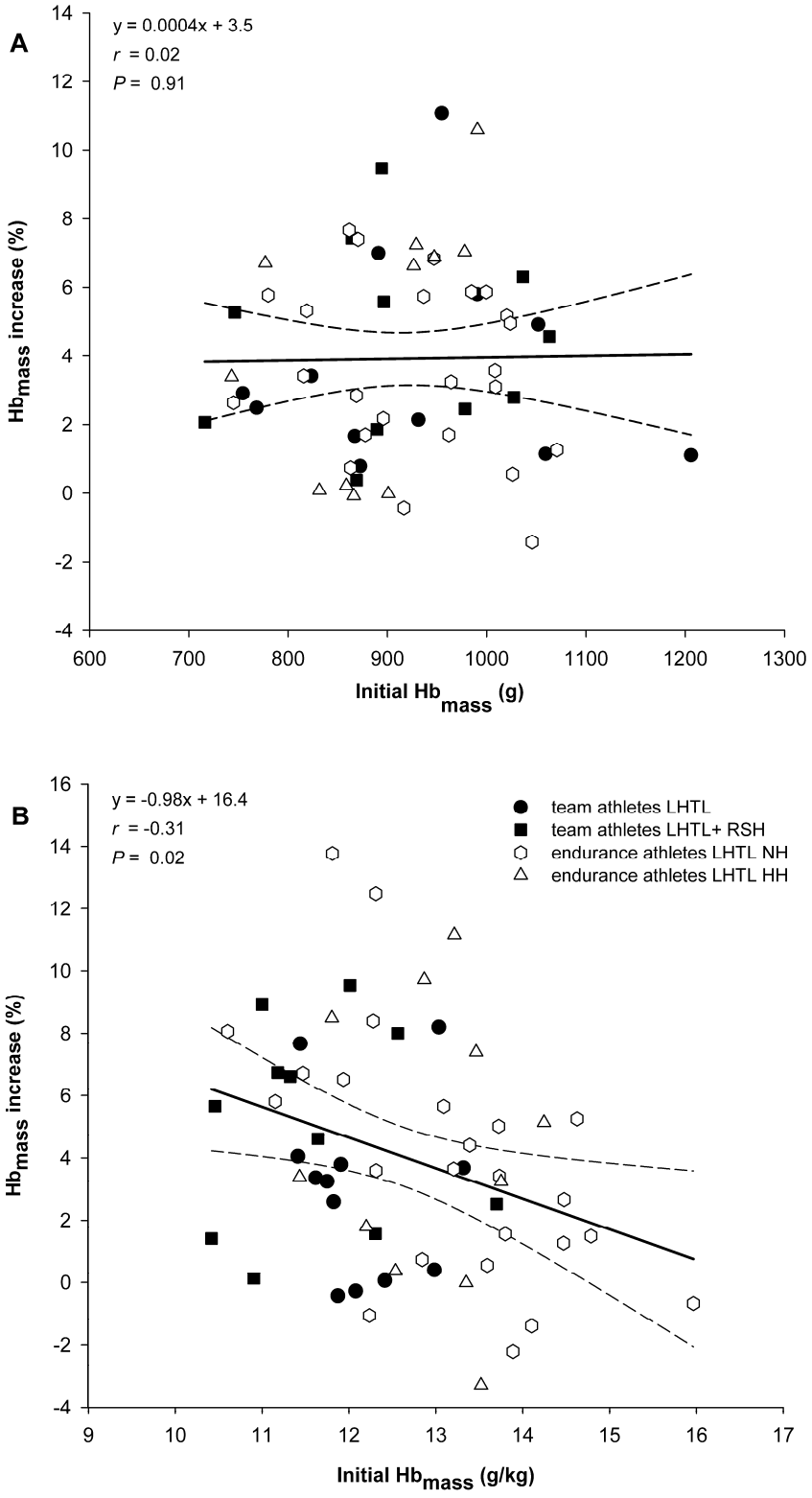

Figure 1. (A) Linear regression between the individual's initial absolute Hb_{mass} (g) and the individual's absolute Hb_{mass} change (%) following LHTL. (B) Linear regression between the individual's initial relative Hb_{mass} (g/kg) and the individual's relative Hb_{mass} change (%) following LHTL. 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.

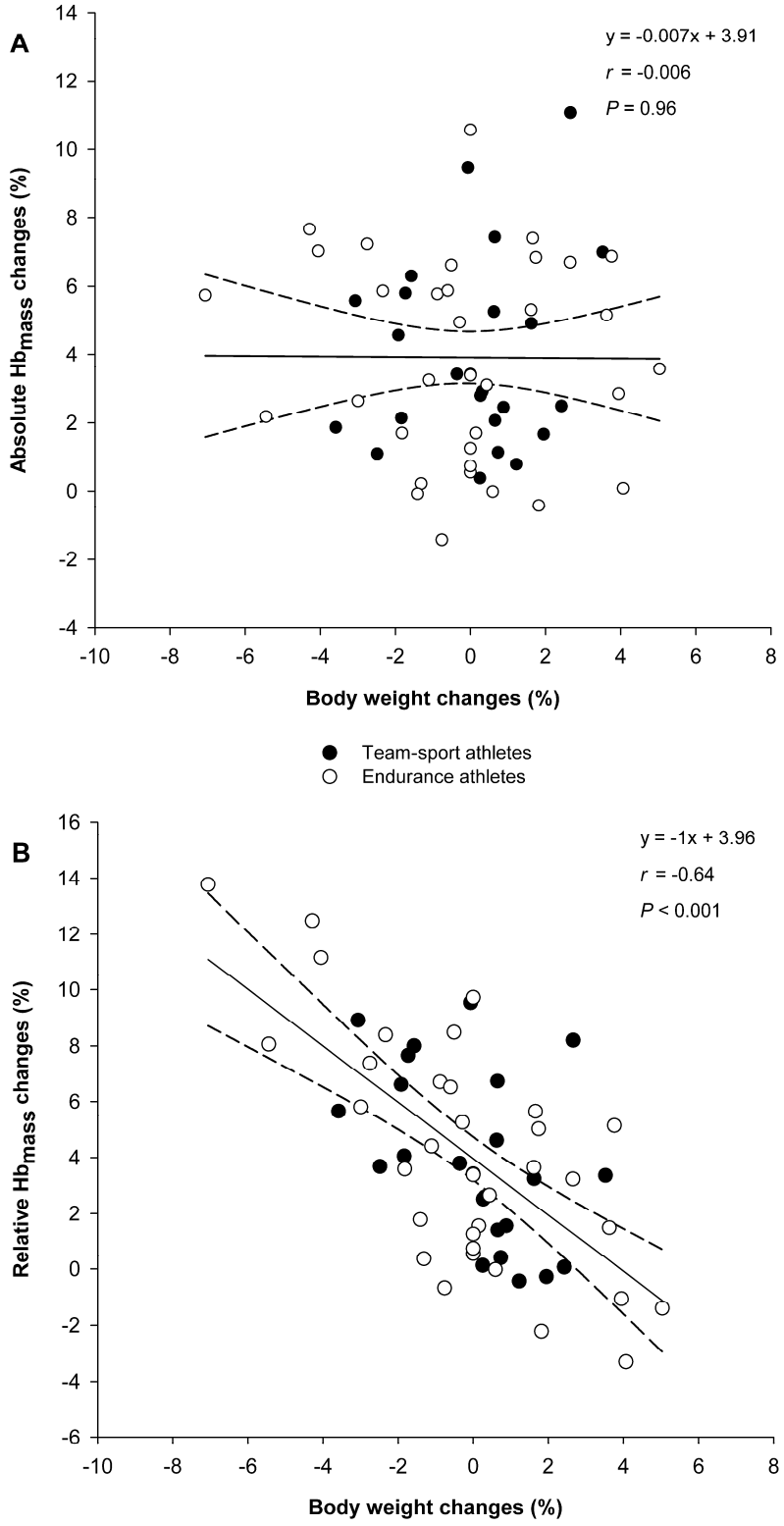

Figure 2. Linear regression between individual body weight change (%) and (A) individual absolute Hb_{mass} change (%) and (B) individual relative Hb_{mass} change (%) following LHTL. Regression slope (solid line) and 95% confidence limits (dashed lines) are shown. n = 58.

Discussion

The present study demonstrated trivial (absolute values) to moderate (relative values) relationships between initial Hb_{mass} and percentage change in Hb_{mass} following LHTL altitude training in endurance and team-sport athletes. Mean absolute and relative Hb_{mass} 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 Hb_{mass}.

Effect of absolute initial Hb_{mass} on Hb_{mass} response

The observed trivial relationship ($r = 0.02$) between absolute initial Hb_{mass} and percentage changes in absolute Hb_{mass} might suggest that absolute initial Hb_{mass} in our athlete cohort had no impact in regard to further Hb_{mass} improvements following LHTL. Thus far, no study has focused on this relationship using absolute Hb_{mass} values, with the rationale that absolute Hb_{mass} values are not an indicator for an individual's physiological limit (12, 21, 26). However, to precisely evaluate the sole effect of initial Hb_{mass} on Hb_{mass} response to altitude training both absolute and relative Hb_{mass} values should be assessed to exclude the cofounding factor 'body weight changes' during altitude training. Further, the average percentage increase in absolute Hb_{mass} 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 Hb_{mass} increase in elite triathletes (+3.2%) (18) and semi-professional Australian Footballers (+6.7%) (19). Thus, in the present sample absolute initial Hb_{mass} demonstrated no adverse effect for further Hb_{mass} improvement following LHTL.

Effect of relative initial Hb_{mass} on Hb_{mass} response

We found a moderate inverse correlation between initial relative Hb_{mass} and percentage increase in relative Hb_{mass} (r = -0.31) following LHTL. Compared to the analysis of Robach and Lundby (26) and a classic altitude training study on Australian footballers (22), 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_{mass} levels have smaller chances to further increase their relative Hb_{mass} following altitude training, with the rationale that those athletes would already have maximised their Hb_{mass} level by training at sea level (22, 26). However, in the present study it seems that the moderate inverse relationship between initial relative Hb_{mass} and percent change in relative Hb_{mass} could not be attributed to the physiological limit of an athlete.

Changes in an individual's body weight from pre- to post- intervention could explain the moderate relationship between initial relative Hb_{mass} and its percentage Hb_{mass} increase following LHTL. There was a large inverse relationship (r = -0.64) between individual percent changes in body weight and relative Hb_{mass}, whereas no relationship between individual percent changes in body weight and absolute Hb_{mass} occurred. This assumes that, primarily, individual changes in body weight from pre- to post LHTL camp led to the moderate relationship between initial relative Hb_{mass} and percent change in Hb_{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 (25) remains unclear. With a lack of significant relationship between individual changes in body weight and absolute Hb_{mass}, it can be assumed that body weight alterations did not negatively influence Hb_{mass} response in the present study. Thus, we propose that lean body mass- adjusted relative Hb_{mass} values would be a better unit for future comparisons.

A further point that must be considered when assessing the relationship between change and initial values, is the statistical phenomon 'regression to the mean' (1). Although in the present study there was no relationship between initial absolute Hb_{mass} and percent changes in absolute Hb_{mass}, the 'regression to the mean' effect could have still appeared. Further, since in the present study 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_{mass} and percent changes in relative Hb_{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 (duration, altitude severity, type of hypoxia, etc.).

Conclusion

Our results indicate that trivial (absolute values) to moderate (relative values) relationships occurred between initial Hb_{mass} and Hb_{mass} increase following LHTL altitude training in endurance and team-sport athletes. This indicates that even athletes with higher initial Hb_{mass} can reasonably expect Hb_{mass} gains post-LHTL. Further, it seems that in the present study the moderate relationship between initial relative Hb_{mass} and percentage increase in relative Hb_{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.

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Conflict of Interest

The authors have no conflicts of interest, source of funding, or financial ties to disclose and no current or past relationship with companies or manufacturers that could benefit from the results of the present study. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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