Skattebo, Ø., Hallén, J. (2022). Individual variations in pre-altitude hemoglobin mass influence hemoglobin mass responses to repeated altitude sojourns. *Scandinavian Journal of Medicine & Science in Sports, 32*(10), 1493-1501. http://dx.doi.org/10.1111/sms.14218

Individual variations in pre-altitude hemoglobin mass influence hemoglobin mass responses to repeated altitude sojourns

Øyvind Skattebo[1] & Jostein Hallén[1]

[1] Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway

Address for correspondence:

Øyvind Skattebo

Norwegian School of Sport Sciences

Post box 4014 Ullevål Stadion, 0806 Oslo, Norway

E-mail: oyvind.skattebo@nih.no

Phone number: +47 23 26 23 09

ORCID:

Øyvind Skattebo: 0000-0003-0771-9715

Jostein Hallén: 0000-0002-6646-0734

Abstract

Introduction: Previous studies have shown variable within-subject hemoglobin mass (Hb_{mass}) responses to altitude training. We investigated whether Hb_{mass} responses depend on individual variations in pre-altitude Hb_{mass} during repeated altitude sojourns.

Methods: Nine elite endurance athletes carried out 3-5 altitude sojourns over 17±10 months (mean±95% confidence interval), at an altitude of 1976±62 m, for 21±1 days, and a total hypoxic dose of 989±46 km · hours, with Hb_{mass} assessed before and after each sojourn (carbon monoxide rebreathing). The individual mean baseline was calculated as the mean of all pre-altitude Hb_{mass} values for an athlete, and it was investigated whether the percent deviation from the individual mean baseline affected the altitude-induced Hb_{mass} response.

Results: On average, Hb_{mass} increased by 3.4±1.1% ($P<0.001$) from pre- to post-altitude. The intra-individual changes in Hb_{mass} were highly inconsistent (coefficient of variation, CV: 88%), and we found no relationship between Hb_{mass} changes in successive altitude sojourns ($r=0.01$; $P=0.735$). However, the percent increase in Hb_{mass} was highly correlated with the pre-altitude Hb_{mass}, expressed as the percent deviation from the individual mean baseline ($y=-0.7x+3.4$; $r=0.75$; $P<0.001$). Linear mixed-model analysis confirmed a -0.6±0.2% smaller increase in Hb_{mass} for each 1% higher pre-altitude Hb_{mass} than the individual mean baseline ($P<0.001$) after adjusting for the covariates hypoxic dose ($P=0.032$) and the relative Hb_{mass} (g · kg^{-1} body weight; $P=0.031$).

Conclusion: Individual variations in pre-altitude Hb_{mass} significantly influence the athletes' Hb_{mass} responses to repeated altitude sojourns, with a potentiated response after traveling to altitude with a low pre-altitude Hb_{mass}.

Keywords: altitude training, blood volume, erythropoiesis, endurance athletes, hemoglobin mass, hypoxia, individual response, maximal oxygen uptake

Introduction

Endurance athletes use sojourns at moderate altitude with the aim of increasing their total hemoglobin mass (Hb_{mass}) and ultimately their maximal oxygen uptake ($\dot{V}O_{2max}$) and sea-level endurance performance. It is well established that the reduction in partial pressure of O_2 with increasing altitude stimulates erythropoiesis, and a 3-week stay at 2000-2500 m above sea level increases the Hb_{mass} by ~3-5%.[1-3] Although average increases in Hb_{mass} are commonly observed, the responses are highly variable between individuals at a specific camp and within individuals when the altitude sojourns are repeated.[4-8] What causes the intra-individual variability in Hb_{mass} response to repeated altitude sojourns is currently unknown.

Oscillations in Hb_{mass} occur during periods of living and training at low altitudes due to biological variations and technical errors associated with the measurement of Hb_{mass}.[9,10] Intra-individual biological variations may be attributed to factors such as changes in training load,[10] energy intake,[8] disease, or injury-related immobility,[11,12] in addition to the impact of living and training at moderate to high altitude.[1,2] Several studies have reported that athletes with the largest pre-altitude relative Hb_{mass} (i.e., $g \cdot kg^{-1}$ body weight) increase their Hb_{mass} less than those athletes with the lowest values,[8,13] which may be caused by optimized sea-level training providing a limited opportunity to elevate the Hb_{mass} further using other stimuli. It is conceivable that this pattern may also exist within an individual, meaning that an athlete may be more prone to increase his or her Hb_{mass} if undertaking a sojourn at moderate altitude close to the nadir of the individual Hb_{mass} oscillation (i.e., at a typical low pre-altitude Hb_{mass} for this athlete). This may explain why athletes increase their Hb_{mass} during some but not all altitude sojourns.

The present study retrospectively analyzed whether intra-individual variations in pre-altitude Hb_{mass} explain the Hb_{mass} response to repeated altitude sojourns in elite endurance athletes who had carried out three or more altitude sojourns. Specifically, we explored

whether the percent change in Hb_{mass} is associated with the pre-altitude Hb_{mass} when expressed as the percent deviation from the individual mean baseline (i.e., the mean of all pre-altitude Hb_{mass} values for an athlete).

Materials and methods

Study design

Hb_{mass}, Hb concentration ([Hb]), and hematocrit values from athletes conducting multiple altitude sojourns were collected retrospectively from laboratory files. The inclusion criteria were 1) being a national team athlete in an endurance sport, and 2) carrying out three or more altitude sojourns (living height > 1800 m above sea level) with Hb_{mass} assessed at the Norwegian School of Sport Sciences' laboratories before the sojourn and directly after the descent from altitude (the day of descent or the day after). The subjects' highest reported $\dot{V}O_{2max}$ values during the data collection period were used for descriptive purposes.

Subjects

Nine Norwegian national team endurance athletes (6♂ and 3♀) at high international levels in their respective sports (swimming: n = 1; rowing: n = 2; speed skating: n = 6) met the inclusion criteria and was asked to participate, and all gave their written informed consent that their data could be included in this analysis (mean ± 95% confidence interval (CI); age: 23 ± 5 years; height: 1.87 ± 0.06 (♂) and 1.72 ± 0.06 (♀) meter; body weight: 81.9 ± 9.8 (♂) and 71.2 ± 7.2 (♀) kg; $\dot{V}O_{2max}$: 75.4±5.2 (♂) and 61.3±1.6 (♀) ml · kg^{-1} ·min^{-1}). The cohort included six medalists in World Championships in their respective sports, of whom four were also Olympic medalists. All athletes resided below 250 m above sea level, excluding trips and training camps.

Ethical approval

The Ethics Committee of the Norwegian School of Sport Sciences approved this study (143-180620).

Altitude camps

The athletes carried out 34 altitude sojourns (3-5 sojourns per athlete) over 17 ± 10 months (range: 9-51 months; 8 out of 9 athletes conducted the sojourns within 15 months) in 12 different camps arranged by the Norwegian national teams or by the Norwegian Olympic Sports Center. In Table 1, the characteristics of the camps are described, including the location, living altitude (1976 ± 62 m), duration (20.8 ± 0.5 days), hypoxic dose (989 ± 46 km · hours; calculated as the living altitude multiplied by the hours at altitude), and whether the training was carried out at moderate/high altitude (live high, train high: LH-TH), at low altitude (live high, train low: LH-TL) or a mix of training at low and moderate/high altitude (LH-Tmix). Only completed sojourns without illness are included. Data on training and nutritional intake were not collected during the altitude sojourns and are not included in this report and statistical analysis.

<<Table 1 here>>

Measurements and procedures

Hemoglobin mass

Hb_{mass} was measured by the same experimenter before and after the altitude camps using a carbon monoxide (CO) rebreathing method.[14,15] First, the subjects rested seated for 10 min, followed by capillary blood sampling in two 125-µL pre-heparinized tubes (Clinitubes; Radiometer, Copenhagen, Denmark) from a pre-heated fingertip. The subjects inhaled a bolus

(men: 1.2 mL per kg body weight; women: 1.0 mL per kg body weight) of 99.97% chemically pure CO (AGA, Oslo, Norway) administered via a 100-mL plastic syringe (Omnifix; Braun, Kronberg im Taunus, Germany) attached to a spirometer (Blood tec GmbH, Germany). In this closed circuit, the CO was rebreathed for 2 min together with 3 L of pure O_2 (AGA) while checking for leakages using a CO analyzer (Draeger, Lübeck, Germany). Two capillary blood samples were collected, 6 and 8 min after the administration of CO, and analyzed in duplicate for percentage of carboxyhemoglobin using a hemoximeter (ABL80 CO-OX FLEX; Radiometer, Copenhagen, Denmark). The CO not absorbed by the body was calculated by multiplying the CO concentration in the rebreathing bag by the bag volume and the subject's estimated residual lung volume.[16] The CO exhaled between the time-point of disconnecting from the spirometer and the blood sampling was estimated by multiplying the difference in end-tidal CO concentration before and after rebreathing by the estimated alveolar ventilation.[17] Hb_{mass} was calculated by dilution of CO in the blood[14] with correction for loss of CO to myoglobin (0.3% of the administered CO per minute).[15] The coefficient of variation of duplicate Hb_{mass} determinations (the standard deviation of the difference scores/$\sqrt{2}$) conducted by Ø.S. in our lab is typically 1.1-1.6 %,[18,19] and was calculated to be 1.8% in the present study based on a subset of the measurements (duplicates were obtained in 28 out of 68 measurements). EDTA blood was obtained from an antecubital vein and analyzed for [Hb] and hematocrit using a Sysmex XN-9000 (Sysmex, Kobe, Japan). The mean corpuscular Hb concentration (MCHC) was calculated from [Hb] and hematocrit and intravascular volumes (BV; RBCV; plasma volume, PV) were derived using an F-cell ratio of 0.91.[20]

Maximal oxygen uptake

$\dot{V}O_{2max}$ was measured during cycling (Excalibur Sport; Lode B.V., Groningen, The Netherlands) or treadmill running (Woodway GmbH, Weil am Rhein, Germany) using open-circuit indirect calorimetry with a mixing chamber (Oxycon Pro; Jaeger Instrument, Friedberg, Germany). The equipment and calibration routines have been described previously.[21]

Statistical analyses

Data are presented as mean ± 95% CI. Changes from pre- to post-altitude were investigated using a paired sample t-test. The association between two variables was assessed using simple linear regression (Pearson's r). The inter-individual (between-subjects) coefficient of variation (CV_{inter}) was calculated from the athletes' mean increase in Hb_{mass} ($CV_{inter} = 100 \cdot SD_{inter} / Mean_{inter}$), and the intra-individual (within-subject) CV (CV_{intra}) was calculated as the mean of each athlete's individual CV (each athlete's CV was calculated as follows: $100 \cdot SD_{intra} / Mean_{intra}$). To account for repeated data (multiple altitude sojourns for each subject) and possible covariates, a linear mixed model was constructed with the log-transformed (natural logarithm) change in Hb_{mass} as the dependent variable. The model included an intercept and the fixed effects in the model were the percent deviation from the individual mean baseline in pre-altitude Hb_{mass} (numeric linear), relative Hb_{mass} (i.e., $g \cdot kg^{-1}$ body weight, calculated as the difference from the sex-specific mean; numeric linear), and hypoxic dose (km · hours; numeric linear). Initially, the influences of sex (two categories: male, female) and type of altitude sojourn (three categories: LH-TL, LH-TH, and LH-Tmix) were investigated in separate models but did not influence the change in Hb_{mass} from pre- to post-altitude. Therefore, data were pooled by these covariates being excluded from the final model. A repeated statement was specified for subject identity to account for within-subject correlated errors. The intercepts and covariates were back-transformed and expressed as

percentages with 95% CI. The alpha-level was set to < 0.05. GraphPad Prism 9 (v.9.0.0; GraphPad Software, CA, USA) and SAS OnDemand for Academics (SAS Studio 3.8, SAS Institute Inc., Cary, NC) were used for statistical analysis.

Results

Hematological effects of the altitude sojourns

From pre- to post-altitude, Hb_{mass} increased on average by 3.4 ± 1.1% (33 ± 11 g; P < 0.001; Table 2). MCHC also increased (2.4 ± 2.1%; P = 0.029), leading to an unchanged RBCV (1.6 ± 2.7%; P = 0.275). The PV (-0.1 ± 4.0%; P = 0.667) and BV (0.4 ± 2.1%; P = 0.865) were unaltered. Using simple linear regression, the percent change in Hb_{mass} was not associated with the living altitude (r = 0.09; P = 0.599), the total hours at altitude (r = -0.11; P = 0.537), and the hypoxic dose, expressed as km · hours (r = 0.02; P = 0.926). However, the percent change in Hb_{mass} correlated significantly with the pre-altitude relative Hb_{mass}, expressed as the difference from the sex-specific mean (♀ = 10.9 g · kg^{-1} and ♂ = 13.7 g · kg^{-1}; r = -0.50; P = 0.003).

<<Table 2 here>>

Individual responses in Hb_{mass}

The individual changes in Hb_{mass} in the 34 altitude sojourns ranged from an 11.0% increase to a 2.2% reduction, while the athletes' mean responses ranged from 1.6-4.5% (CV_{inter}: 27%; Fig. 1A). The athletes' individual changes in Hb_{mass} were highly inconsistent (CV_{intra}: 88%; Fig. 1), and there was no relationship between the Hb_{mass} changes in successive altitude sojourns (r = 0.01; P = 0.735; Fig. 1B). Together, this indicates that there are no responders and non-responders in this group of athletes. However, the pre-altitude Hb_{mass}, expressed as

the percent deviation from the individual mean baseline, was highly correlated with the percent change in Hb_{mass} from pre- to post-altitude ($r = 0.75$; $P < 0.001$; $y = -0.7x + 3.4$; Fig. 2A). Calculated post-hoc, the achieved statistical power for this regression was 1.00 (G*Power ver. 3.1.9.4; University of Kiel, Germany). The same direction of regression was found, on an individual basis, in eight out of nine athletes, for whom six out of nine plots showed an r-value >0.90. From this analysis, these athletes could expect an average 3.4% increase in Hb_{mass} after a sojourn at moderate altitude if the pre-altitude Hb_{mass} is close to their individual mean baseline. For each percent deviation from this mean baseline, they could expect an additional 0.7% increase (lower baseline) or a -0.7% lower increase (higher baseline). These data are also expressed in gram in the Supplementary material. Across sojourns, the Hb_{mass} varied significantly less post-altitude than pre-altitude within the individuals (Fig. 2B; $P = 0.018$).

<<Fig. 1-2 here>>

Linear mixed model

The intercept in the linear mixed model was 3.4 ± 0.2% ($P < 0.001$). A 1% higher pre-altitude Hb_{mass} than the individual mean baseline was associated with a -0.6 ± 0.2% ($P < 0.001$) smaller increase in Hb_{mass}, close to the slope given by the simple linear regression (i.e., -0.7%). Significant associations were found between the percent change in Hb_{mass} and the covariates hypoxic dose ($P = 0.032$; Table 3) and the relative Hb_{mass} ($g \cdot kg^{-1}$ body weight) expressed as the difference from the sex-specific mean ($P = 0.031$; Table 3).

<<Table 3 here>>

Discussion

The present investigation suggests that the pre-altitude Hb_{mass} influences the Hb_{mass} response to repeated altitude sojourns. We found a pronounced increase in the athletes' Hb_{mass} when travelling to altitude with a low Hb_{mass} and a minor (or no) increase when the Hb_{mass} was already high before ascending to altitude compared to the individual mean baseline. Across sojourns, the variations in the individuals' Hb_{mass} were smaller post-altitude than pre-altitude (CV: 1.9% vs. 3.5%), and this may indicate that the athletes approached an individual physiological ceiling of Hb_{mass} at each altitude sojourn.

Hematological effects of altitude sojourns

The athletes increased their Hb_{mass}, on average, by 3.4% after training and living at moderate altitude, which is similar to findings for elite endurance athletes in previous investigations using a similar hypoxic dose (~900-1200 km · hours: 2.7 – 5.3%),[1,3,6,18] and is close to that predicted by using the exponential model for hypoxic dose (km · hours) proposed by Garvican-Lewis et al. (predicted for 989 km · hours ≈ 3.8% increase in Hb_{mass}).[22] Hb_{mass} increased partly due to increased concentration of Hb within the RBCs (i.e., an increased MCHC by 2.4 ± 2.1%) since we did not observe any significant change in the calculated RBCV. Increased MCHC after altitude sojourns has previously been observed,[7] but the MCHC values are rarely reported. Estimated from reported mean values of [Hb] and hematocrit (or Hb_{mass} and RBCV), MCHC has both increased (0.5 to 0.7 g · dL^{-1})[4,18,23] and remained unchanged (-0.1 to 0.2)[1,5,23] after sojourns at moderate altitude.

Inter- and intra-individual variability in Hb_{mass} responses

Although the Hb_{mass} responses to the 34 altitude sojourns varied considerably (+11% to -2%), the athletes' mean increases over 3-5 altitude sojourns were relatively similar (range: 1.6-4.5%). Moreover, we observed substantial intra-individual variations in the altitude-induced

change in Hb_{mass} (CV_{intra}: 88%), strengthening that responders and non-responders are not a "fixed trait". This is in line with most previous investigations that have found substantial intra-individual variations in Hb_{mass} responses over 2-5 altitude sojourns,[5-8] showing that it is not possible to predict future Hb_{mass} responses based on only one altitude sojourn.[4-8]

The impact of pre-altitude Hb_{mass}

We propose a hypothesis for the large intra-individual variability in the Hb_{mass} response to altitude: intra-individual variations in pre-altitude Hb_{mass} substantially impact the Hb_{mass} response to repeated altitude sojourns. We observed a robust relationship between the change in Hb_{mass} and the pre-altitude Hb_{mass} expressed as the deviation from the individual mean baseline. This pattern is likely due to several related factors, such as 1) when an athlete has a "low" pre-altitude Hb_{mass}, the Hb_{mass} will likely increase until the next measurement due to biological oscillations and training, independent of hypoxic stimuli. Therefore, the increase in Hb_{mass} during the altitude sojourn will be the summed effect of the biological oscillation and the altitude-induced erythropoiesis; and 2) this pattern can partly be caused by measurement error in the CO rebreathing method and an effect of statistical regression to the mean.

Although the Hb_{mass} is relatively stable while living and training at low altitude, some oscillations occur.[9,10,24] For instance, in competitive cyclists, the seasonal CV in Hb_{mass} was 3.3%.[10] Moreover, the maximal intra-individual oscillation (the difference between the highest and lowest Hb_{mass} value over a year) was, on average, 4.6%, with the highest individual value being ~7% in a group of 15 elite triathletes and cyclists.[24] These oscillations are larger than the measurement error of the CO rebreathing method, and it is conceivable that the erythropoietic response will be more pronounced if the athlete ascends to altitude with a low pre-altitude Hb_{mass}. This pattern was found in previous inter-individual comparisons and was confirmed in our mixed-model analysis, with those athletes having the

highest pre-altitude relative Hb_{mass} (i.e., gram · kg^{-1} body weight) increasing their Hb_{mass} the least,[3,8,13,25] and vice versa. Even more interesting, this pattern was also found within individuals in the present study, indicating a clear impact of baseline Hb_{mass} on its change during an altitude sojourn. What causes the individual variations in pre-altitude Hb_{mass} is not known, but is likely a combination of several factors such as changes in training load,[10] energy intake,[8] and illness[11,12]. Regardless of the basis for the pre-altitude Hb_{mass} variations, the potential for further increase when employing hypoxic stimuli will be affected.

The above analysis, and the fact that the Hb_{mass} varied less between sojourns post-altitude than pre-altitude, may also indicate an upper physiological ceiling of Hb_{mass} (i.e., that the athletes approached the same upper limit at the end of each altitude sojourn). A physiological ceiling of Hb_{mass} may be specific for a given erythropoietic stimulus since recombinant erythropoietin administration[26] and a higher hypoxic dose[25] may lead to a larger increase in Hb_{mass} than observed in the present study. Therefore, such a ceiling may be dynamic and represent a fine-tuned balance between the erythropoietic stimuli and other physiological mechanisms such as blood pressure regulation, and may relate to the concept of symmorphosis.[13] However, the current data cannot pinpoint the exact physiological mechanism governing such a potential ceiling, and this needs to be addressed in future studies.

Considering the impact of measurement error, if the Hb_{mass} is underestimated on the first assessment, it may, by chance, be closer to the true mean for this subject at the next assessment due to random error and scattering around the mean. Hence, if the Hb_{mass} is underestimated pre-altitude, the observed change in Hb_{mass} may be a combination of the pre-altitude underestimation and the true altitude-induced erythropoiesis. The probability of this explanation will increase with the measurement error in Hb_{mass} since this will impact the scattering around the mean. A relatively low measurement error in the CO rebreathing

method was reported in the present study (1.8%), but we only had duplicate pre- and post-measurements in 41% of the sojourns. Therefore, this pattern must be investigated in future studies employing repeated altitude sojourns, preferably in the same location, with similar hypoxic doses, and with the Hb_{mass} being assessed in duplicate or triplicate to increase the signal-to-noise ratio. The altitude-induced Hb_{mass} response pattern should be compared to the intra-individual Hb_{mass} oscillations during periods free of altitude exposure to decipher the isolated effect of altitude sojourns and natural biological oscillations.

Hypoxic dose

Although we found no bivariate relationship between hypoxic dose (km · hours) and the change in Hb_{mass}, a significant relationship arose in the mixed model analysis after adjusting for covariates. In this analysis, an increase of 100 km · hours was associated with a 0.4%-point increase in Hb_{mass}, similar to previous investigations,[3,22] and highlights that the hypoxic dose influences the erythropoietic response. Whether other environmental factors also influenced the outcome is unclear, as neither temperature, humidity, etc., was incorporated in the statistical model.

Conclusions

Variations in pre-altitude Hb_{mass} significantly influence Hb_{mass} responses to repeated altitude sojourns. For each percent deviation from the individual mean baseline in Hb_{mass}, an athlete can expect an additional 0.7% increase (lower pre-altitude Hb_{mass}) or a 0.7% smaller increase (higher pre-altitude Hb_{mass}) in Hb_{mass} than average (~3-4%).

Perspectives

The inter-individual variation in the Hb_{mass} response to altitude is smaller than initially thought, as evident when the response over several sojourns per athlete, instead of only one, is considered. Hence, the current knowledge indicates no particular responders and non-responders to altitude sojourns. Whether elite endurance athletes increase their Hb_{mass} during a specific sojourn depends on numerous factors, including the athlete's health and nutritional status, the characteristics of the altitude camp, and, as shown here, the athlete's pre-altitude Hb_{mass}. Therefore, the pre-altitude Hb_{mass} and its deviation from the individual mean baseline should be incorporated in the evaluation of an altitude camp's effectiveness. If an athlete's primary goal is to increase the Hb_{mass} when ascending to altitude, he or she may consider to skip an altitude camp if the pre-altitude Hb_{mass} is already high compared to the individual mean baseline, as a further increase is likely minimal. This is especially relevant if the training facilities are not optimal at the altitude destination. Future studies should assess the frequency and amplitude of Hb_{mass} oscillations in periods involving and not involving altitude sojourns to separate the isolated effect of altitude from biological variations originating elsewhere.

Additional information

Acknowledgements

The authors would like to thank the volunteers for their participation and cooperation during the study. Hege W. Landgraff is thanked for technical help during data collection.

Author Contributions

Conception and design of the experiment: Ø.S., J.H.

Data collection: Ø.S.

Analysis of data: Ø.S.

Interpretation of data: Ø.S., J.H.

Writing the first draft: Ø.S.

Revising the manuscript: Ø.S., J.H.

All authors have read and approved the final version of the manuscript.

Conflict of interests

The authors declare that they have no conflicts of interest regarding the publication of this paper. There are no financial conflicts of interest to disclose. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Sources of funding

Internal funding from the Norwegian School of Sport Sciences supported this investigation.

Data availability statement

The data supporting this study's findings are available from the corresponding author on reasonable request. The data are not publicly available due to privacy or ethical restrictions.

References

1. Wehrlin JP, Zuest P, Hallen J, Marti B. Live high-train low for 24 days increases hemoglobin mass and red cell volume in elite endurance athletes. *J Appl Physiol (1985).* 2006;100:1938-1945.

2. Garvican L, Martin D, Quod M, Stephens B, Sassi A, Gore C. Time course of the hemoglobin mass response to natural altitude training in elite endurance cyclists. *Scand J Med Sci Sports.* 2012;22:95-103.

3. Koivisto-Mork AE, Svendsen IS, Skattebo O, Hallen J, Paulsen G. Impact of baseline serum ferritin and supplemental iron on altitude-induced hemoglobin mass response in elite athletes. *Scand J Med Sci Sports.* 2021;31:1764-1773.

4. Chapman RF, Stray-Gundersen J, Levine BD. Individual variation in response to altitude training. *J Appl Physiol (1985).* 1998;85:1448-1456.

5. Nummela A, Eronen T, Koponen A, Tikkanen H, Peltonen JE. Variability in hemoglobin mass response to altitude training camps. *Scand J Med Sci Sports.* 2021;31:44-51.

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

7. Wachsmuth NB, Völzke C, Prommer N, et al. The effects of classic altitude training on hemoglobin mass in swimmers. *Eur J Appl Physiol.* 2013;113:1199-1211.

8. McLean BD, Buttifant D, Gore CJ, White K, Kemp J. Year-to-year variability in haemoglobin mass response to two altitude training camps. *Br J Sports Med.* 2013;47:i51-i58.

9. Eastwood A, Hopkins WG, Bourdon PC, Withers RT, Gore CJ. Stability of hemoglobin mass over 100 days in active men. *J Appl Physiol (1985).* 2008;104:982-985.

10. Garvican LA, Martin DT, McDonald W, Gore CJ. Seasonal variation of haemoglobin mass in internationally competitive female road cyclists. *Eur J Appl Physiol.* 2010;109:221-231.

11. Gough CE, Sharpe K, Garvican LA, Anson JM, Saunders PU, Gore CJ. The effects of injury and illness on haemoglobin mass. *Int J Sports Med.* 2013;34:763-769.

12. Schumacher YO, Ahlgrim C, Ruthardt S, Pottgiesser T. Hemoglobin mass in an elite endurance athlete before, during, and after injury-related immobility. *Clin J Sport Med.* 2008;18:172-173.

13. Robach P, Lundby C. Is live high-train low altitude training relevant for elite athletes with already high total hemoglobin mass? *Scand J Med Sci Sports.* 2012;22:303-305.

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

15. Prommer N, Schmidt W. Loss of CO from the intravascular bed and its impact on the optimised CO-rebreathing method. *Eur J Appl Physiol.* 2007;100:383-391.

16. Miller WC, Swensen T, Wallace JP. Derivation of prediction equations for RV in overweight men and women. *Med Sci Sports Exerc.* 1998;30:322-327.

17. West JB. *Respiratory physiology: the essentials.* 8th ed. Philadelphia, PA: Wolters Kluwer/Lippincott Williams & Wilkins; 2008.

18. Koivisto AE, Paulsen G, Paur I, et al. Antioxidant-rich foods and response to altitude training: A randomized controlled trial in elite endurance athletes. *Scand J Med Sci Sports.* 2018;28:1982-1995.

19. Skattebo Ø, Bjerring AW, Auensen M, et al. Blood volume expansion does not explain the increase in peak oxygen uptake induced by 10 weeks of endurance training. *Eur J Appl Physiol.* 2020;120:985-999.

20. Chaplin H, Jr., Mollison PL, Vetter H. The body/venous hematocrit ratio: its constancy over a wide hematocrit range. *J Clin Invest.* 1953;32:1309-1316.

21. Skattebo Ø, Johansen ES, Capelli C, Hallén J. Effects of 150- and 450-mL Acute Blood Losses on Maximal Oxygen Uptake and Exercise Capacity. *Med Sci Sports Exerc.* 2021;53:1729-1738.

22. Garvican-Lewis LA, Sharpe K, Gore CJ. Time for a new metric for hypoxic dose? *J Appl Physiol (1985).* 2016;121:352-355.

23. Hauser A, Schmitt L, Troesch S, et al. Similar Hemoglobin Mass Response in Hypobaric and Normobaric Hypoxia in Athletes. *Med Sci Sports Exerc.* 2016;48:734-741.

24. Prommer N, Sottas PE, Schoch C, Schumacher YO, Schmidt W. Total hemoglobin mass--a new parameter to detect blood doping? *Med Sci Sports Exerc.* 2008;40:2112-2118.

25. Rasmussen P, Siebenmann C, Diaz V, Lundby C. Red cell volume expansion at altitude: a meta-analysis and Monte Carlo simulation. *Med Sci Sports Exerc.* 2013;45:1767-1772.

26. Lundby C, Thomsen JJ, Boushel R, et al. Erythropoietin treatment elevates haemoglobin concentration by increasing red cell volume and depressing plasma volume. *J Physiol.* 2007;578:309-314.

Figure 1

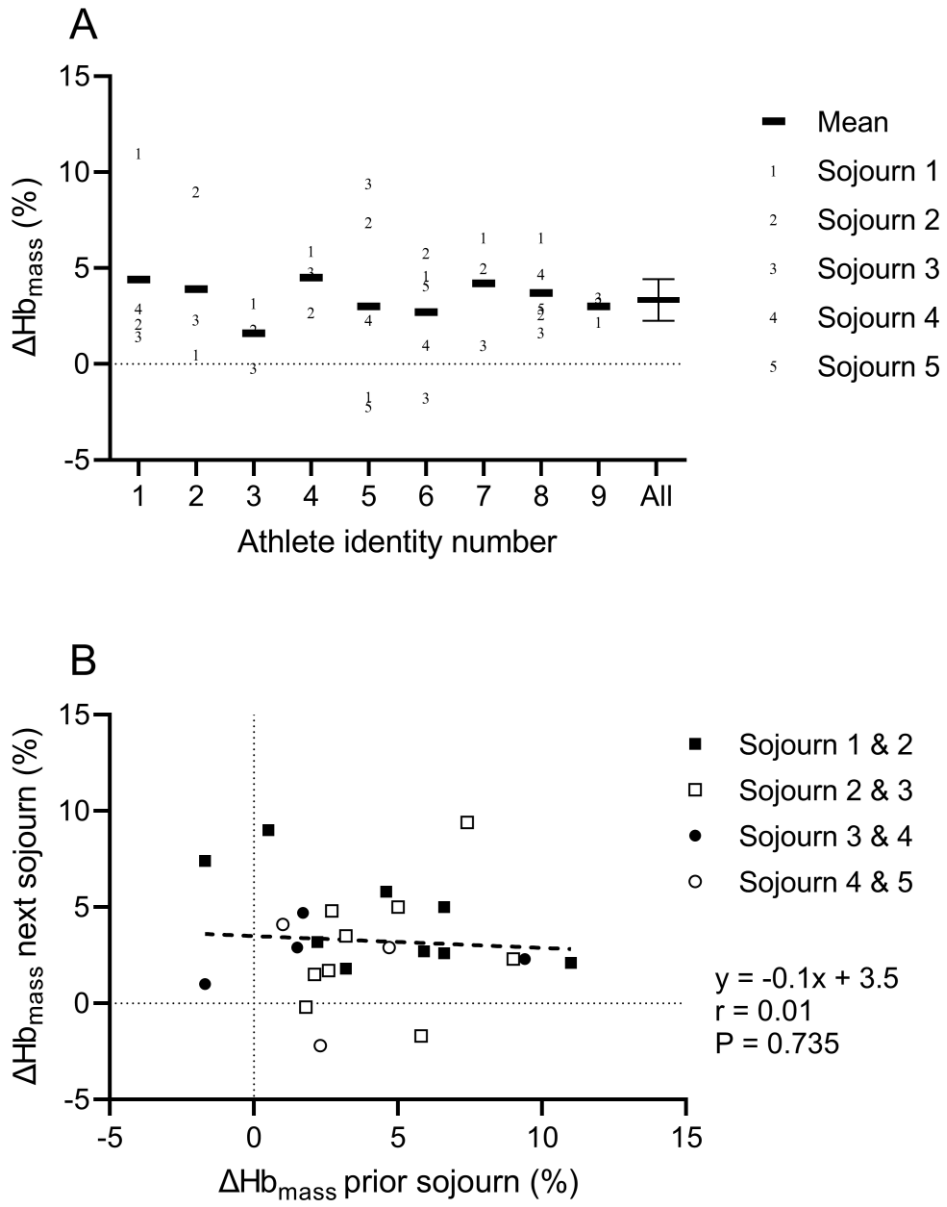


Fig. 1: A, The athletes' individual changes in hemoglobin mass (Hb_{mass}) from before to after each of their 3-5 altitude sojourns. The mean response for all 34 altitude sojourns is presented with its 95% confidence interval. **B,** The relationship between the percent changes in Hb_{mass} from before to after successive altitude sojourns (n = 25).

Figure 2

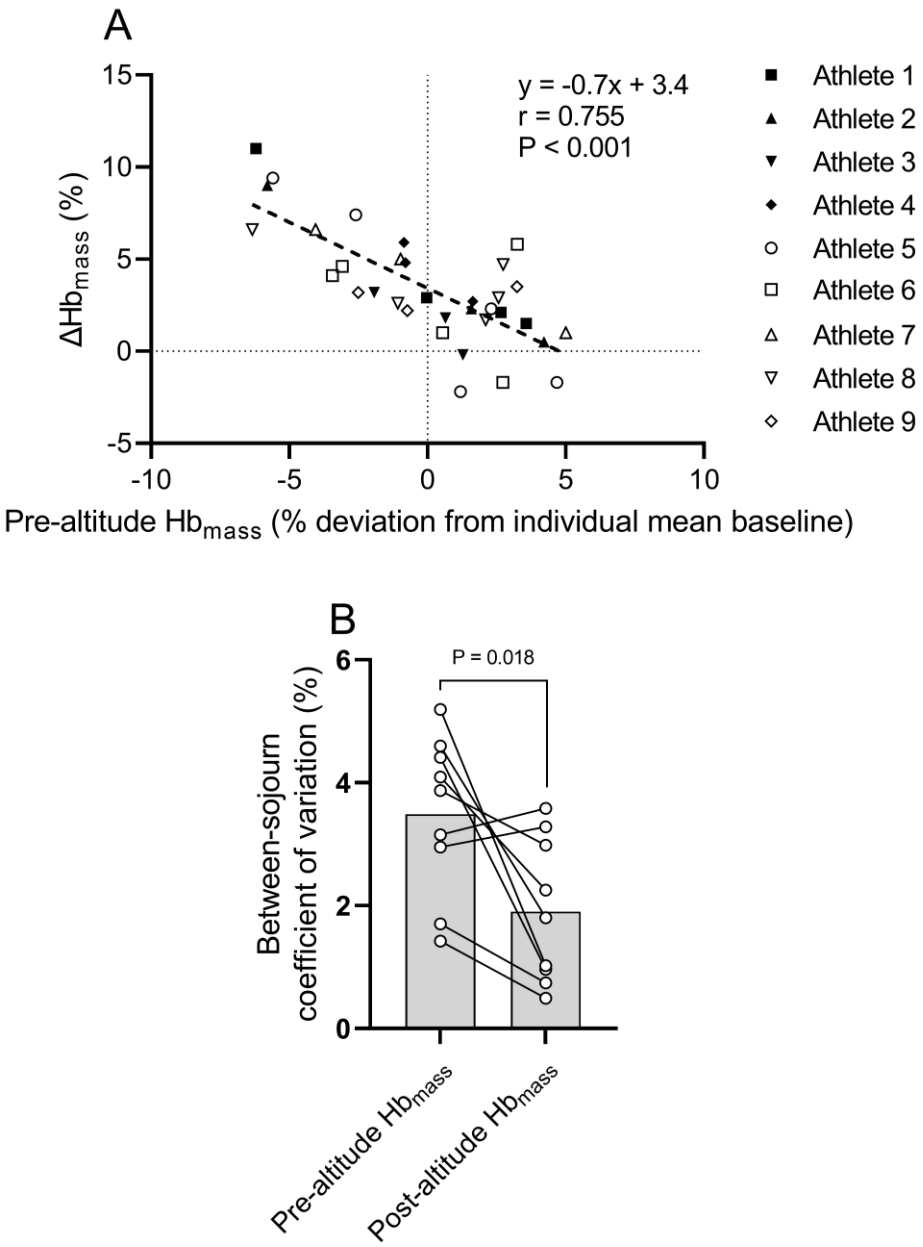


Fig. 2: A, The relationship between the percent change in hemoglobin mass (Hb_{mass}) from before to after an altitude sojourn and the pre-altitude Hb_{mass}, expressed as the percent deviation from the individual mean baseline (n = 34). **B**, Individual (points and lines) and mean (bars) between sojourns coefficient of variation in pre- and post-altitude Hb_{mass}.

Table 1. Characteristics of the altitude camps.

	Camp 1	**Camp 2**	**Camp 3**	**Camp 4**	**Camp 5**	**Camp 6**	**Camp 7**	**Camp 8**	**Camp 9**	**Camp 10**	**Camp 11**	**Camp 12**
Year(month: 1-12)	2011(6)	2011(8)	2012(1)	2012(6)	2012(8)	2015(8)	2015(10)	2016(8)	2016(5)	2016(6)	2016(6)	2016(10)
Camp location	Font Romeu	St. Moritz/ Kühtai	Kühtai	Font Romeu	Font Romeu	Park City, Utah	Sierra Nevada	Seiser Alm	Sierra Nevada	Sierra Nevada	Livigno	Sierra Nevada
Altitude	1850	1822/ 2017	2017	1850	1850	2100	2320	1900	2320	2320	1816	2320
Days	20	19(12+7)	20	21	22	21	23	18	21	22	22	22
Hypoxic dose (km · hours)	888	860	968	932	977	1058	1281	821	1169	1225	959	1225
Type	LH-Tmix	LH-Tmix	LH-TL	LH-Tmix	LH-Tmix	LH-TL	LH-TH/Tmix	LH-TH	LH-TH	LH-TH	LH-Tmix	LH-TH
Included athletes at each camp (n)	6	4	3	5	5	1	3	2	1	1	2	1

LH-TH, live high and train at high altitude; LH-TL, live high and train at low altitude; LH-Tmix, live high and training at both low and high altitude.

Table 2. Hematological measurements and body weight obtained pre- and post-altitude

	Pre-Altitude (mean ± 95% CI)	Post-Altitude (mean ± 95% CI)	P-value
Hemoglobin mass (g)	996 ± 71	1029 ± 72	< 0.001
Relative hemoglobin mass ($g \cdot kg^{-1}$)	12.8 ± 0.5	13.2 ± 0.5	< 0.001
Blood volume (mL)	6923 ± 438	6935 ± 418	0.865
Red blood cell volume (mL)	2903 ± 226	2947 ± 233	0.275
Plasma volume (mL)	4020 ± 259	3988 ± 227	0.667
Hemoglobin concentration ($g \cdot dL^{-1}$)	14.8 ± 0.5	15.3 ± 0.5	< 0.001
Hematocrit (%)	46.0 ± 1.7	46.5 ± 1.6	0.465
MCHC ($g \cdot dL^{-1}$)	32.3 ± 0.7	33.1 ± 0.6	0.029
Body weight (kg)	77.4 ± 3.1	77.5 ± 3.2	0.508

N = 34 (data pairs) for hemoglobin mass and body weight, and n = 26 for the remaining variables due to lacking hemoglobin concentration and hematocrit values. MCHC, mean corpuscular hemoglobin concentration. Pre- and post-altitude data were compared using a paired sample t-test.

Table 3. Linear mixed model analyzing the change in hemoglobin mass from pre- to post-altitude (%).

Model parameter	Estimate (mean ± 95% CI)
Intercept	3.4 ± 0.2
Pre-altitude Hb_{mass} (deviation from individual mean baseline (+1%)	-0.6 ± 0.2
Relative Hb_{mass} (+1 g · kg^{-1} body weight)	-0.8 ± 0.5
Hypoxic dose (+100 km · hours)	0.4 ± 0.3

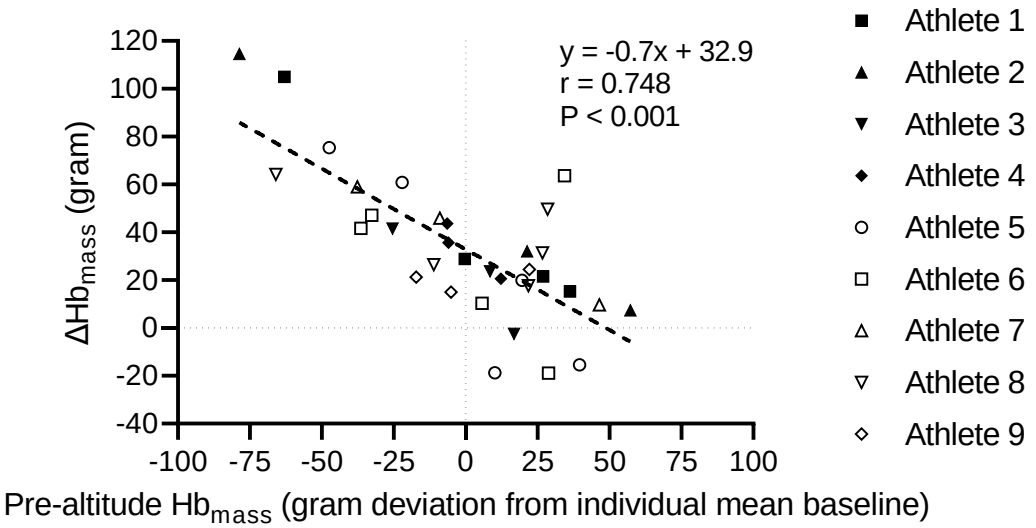
Linear mixed model with the log-transformed change in Hb_{mass} as the dependent variable and the percent deviation from the individual mean baseline in pre-altitude Hb_{mass} (numeric linear), relative Hb_{mass} (i.e., g · kg^{-1} body weight, calculated as the difference from the sex-specific mean; numeric linear), and hypoxic dose (numeric linear) as fixed effects. A repeated statement was specified for subject identity to account for within-subject correlated errors. The intercept was calculated at mean hypoxic dose (989 km · hrs) and mean relative Hb_{mass} (10.9 and 13.7 g · kg^{-1} body weight for women and men, respectively). The effects were back-transformed after modelling and expressed as percentages. N=34.

Supplements

Individual variations in pre-altitude hemoglobin mass influence hemoglobin mass responses to repeated altitude sojourns

Øyvind Skattebo & Jostein Hallén

Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway



Supplemental Fig. 1: The relationship between the change in hemoglobin mass (Hb_{mass}; gram) from before to after an altitude sojourn and the pre-altitude Hb_{mass}, expressed as the gram deviation from the individual mean baseline (n = 34).