

DR ARI TAPANI NUMMELA (Orcid ID : 0000-0002-7965-3092)

Article type : Original Article

TITLE: VARIABILITY IN HEMOGLOBIN MASS RESPONSE TO ALTITUDE TRAINING CAMPS

RUNNING TITLE: HEMOGLOBIN MASS RESPONSE TO ALTITUDE TRAINING

AUTHORS:

Ari Nummela PhD^{1,4}, Timo Eronen MD^{2,4}, Anne Koponen MSc^{2,3}, Heikki Tikkanen MD, PhD^{2,4}, Juha Peltonen PhD^{2,3}

¹KIHU – Research Institute for Olympic Sports, Jyväskylä, Finland

²Department of Sports and Exercise Medicine, Clinicum, University of Helsinki, Helsinki, Finland ³Clinic for Sports and Exercise Medicine, Foundation for Sports and Exercise Medicine, Helsinki, Finland ⁴University of Eastern Finland, Kuopio, Finland

CORRESPONDING AUTHOR:

Name: Ari Nummela Institute: KIHU – Research Institute for Olympic Sports, Jyväskylä, Finland Address: Rautpohjankatu 6, 40700 Jyväskylä, Finland Phone: +358 40 543 9217 E-mail: ari.nummela@kihu.fi

ACKNOWLEDGEMENTS: This study has received financial support from the Finnish Olympic Committee and Foundation of Finnish Sports Research.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the <u>Version of Record</u>. Please cite this article as <u>doi:</u> <u>10.1111/SMS.13804</u>

This article is protected by copyright. All rights reserved

The authors have no conflict of interest to declare.

ABSTRACT

The present study investigated if athletes can be classified as responders or non-responders based on their individual change in total hemoglobin mass (tHb-mass) following altitude training while also identifying the potential factors that may affect responsiveness to altitude exposure. Measurements were completed with 59 elite endurance athletes who participated in national team altitude training camps. Fifteen athletes participated the altitude training camp at least twice. Total Hb-mass using a CO rebreathing method and other blood markers were measured before and after a total of 82 altitude training camps (1350-2500 m) in 59 athletes. In 46 (56 %) altitude training camps tHb-mass increased. The amount of positive responses increased to 65 % when only camps above 2000 m were considered. From the fifteen athletes who participated in altitude training camps at least twice, 27 % always had positive tHb-mass responses, 13 % only negative responses and 60 % both positive and negative responses. Logistic regression analysis showed that altitude was the most significant factor explaining positive tHbmass response. Furthermore, male athletes had greater tHb-mass response than female athletes. In endurance athletes, tHb-mass is likely to increase after altitude training given that hypoxic stimulus is appropriate. However, great inter- and intraindividual variability in tHb-mass response does not support classification of an athlete permanently as a responder or non-responder. This variability warrants efforts to control numerous factors affecting an athlete's response to each altitude training camp.

Key words: hypoxic dose, elite athletes, endurance training, serum ferritin

1 INTRODUCTION

Altitude training is frequently used by competitive endurance athletes because athletes and coaches believe that altitude training may provide athletes an improvement in performance not achievable at sea level. The main positive effect of altitude training for improved sea-level or altitude performance is an expected increase in total haemoglobin mass (tHb-mass) and thereafter an increase in VO_{2max}. A 1 g change in tHb-mass is associated with a change of approximately 4 ml·min⁻¹ in VO_{2max}.¹

Although the mean improvement in tHb-mass response with altitude training is clear, the individual response seems to display a great variability. To classify an athlete as a responder or non-responder as a fixed trait, an athlete should respond consistently to the same training stimulus at a given altitude. Only a few studies have investigated the repeatability of responses to altitude. Robertson et al.² studied highly trained runners during two three-week live high train low (LHTL) blocks and reported reproducible group mean increases for VO_{2max} and Hb-mass but not in time trial performance. Chapman et al.³ retrospectively classified a group of 39 distance runners as responders or non-responders, based on their change in 5000

m time trial performance following four weeks of LHTL. In the study of McLean et al.⁴, they observed that two preseason altitude camps yielded a similar mean increase in tHb-mass of elite Australian football players, but the same players did not change their Hb-mass consistently from year to year indicating that responders and non-responders to altitude in terms of tHb-mass may not appear to be a fixed trait.

The increase in tHb-mass as a result of hypoxia exposure is not a simple and straightforward process. There are numerous factors, which affect the tHb-mass response to hypoxia exposure. The optimal hypoxic dose for improvement of sea level performance has been investigated since the 1990's.^{5,6,7,8,9,10,11,12} The key issues are the altitude or PO₂ and the time of exposure. When altitude is too low or hypoxia exposure is too short, the stimulus is insufficient to achieve any worthwhile physiological adaptation. Likewise, when altitude is too high, it may disturb recovery and thereby blunt any beneficial effects. In addition to hypoxic dose, dehydration^{13,14,15}, pre-altitude Hb-mass^{16,17}, iron stores^{18,19}, inflammation^{20,21} and sex^{20,21,22} are potential factors that may affect intra-individual variability in tHb-mass responses.

Since there seems to be a lack of knowledge about the repeatability of responses to altitude, the primary aim of this study was to investigate whether or not athletes can be classified to responders and nonresponders by their individual change in total Hb-mass during altitude training and whether the classification is fixed so that responders always benefit from altitude training and non-responders do not. A secondary aim was to identify some potential factors (i.e. iron status, altitude dose, and preintervention Hb-mass) that may affect responsiveness to altitude exposure.

2 MATERIALS AND METHODS

2.1 Subjects

A total of 59 Finnish national team endurance athletes (27 men and 32 women) participated in this study. There were five cross country skiers, 13 distance runners, three racewalkers, one canoeist, three rowers, five orienteers and 27 swimmers. The baseline characteristics of participating athletes are shown in Table 1. The total number of pre- and post-measurements in the present study was 82. Single altitude training camp measurements were obtained from 45 endurance athletes and two or more altitude training camps were measured from 15 athletes (Table 2). All subjects gave their written informed consent to participate and measurements were approved by the Ethical Committee of the University of Jyväskylä.

2.2 Altitude training camp

The study was performed from 2009 to 2015 as a part of the national teams' altitude training programs. The athletes and national team coaches were asked to follow specific instructions for altitude training that were given by the exercise physiologists of the present research group. The altitude of the training camp was instructed to be from 2000 m to 2500 m and whenever it was possible, the living high training high and low (LHTHL) concept was recommended.²³ Iron supplementation was recommended, but it was not controlled who followed this recommended.¹⁴ Iron supplementation was recommended, but it was not controlled who followed this recommendation and who did not. The duration of the training camp was instructed to be at least 21 days. Ultimately, the altitude of the training camps varied from 1350 m to 2500 m and the duration from 16 to 42 days. Hypoxia dose was calculated by multiplying the altitude in kilometers by the hypoxia exposure time in hours and it varied from 730 km·h to 1789 km·h.²⁴ Athletes were discouraged from participating in the altitude training camp if (1) their iron stores were low (S-Ferritin < 30 µg·l⁻¹); (2) they were ill or had inflammation (S-hs-CRP > 3 mg·l⁻¹); or (3) they had symptoms of over-reaching just before the altitude training camp. Data was collected for analysis from all of the athletes whether they had followed the instructions or not.

2.3 Hematological measures

Blood samples were taken from an antecubital vein to determine basic blood count 1-5 days before as well as 1-3 days after the altitude training camp. Serum ferritin (S-Ferrit) and transferrin receptors (S-TfR) were also assessed before and after the altitude training camp to identify iron-deficient athletes and changes in iron stores during altitude exposure. In order to detect possible inflammation, serum high-sensitive C-reactive protein (S-hs-CRP) was measured before and after the altitude training camp.

The optimized carbon monoxide (CO) rebreathing method^{25,26} was used to calculate tHb-mass, erythrocyte volume (EV), plasma volume (PV) and blood volume (BV) from all athletes 1-5 days before and 1-3 days after the altitude exposure. Briefly, the athletes rebreathed 99 % CO equivalent to 0.8 (women) or 1.0 ml·kg⁻¹ (men) of body mass through a glass spirometer balanced in oxygen (SpiCO, Blood tec GbR, Bayreuth, Germany) for 2 min. Hb and the fraction of carboxyhemoglobin (%HbCO) in fingertip capillary blood was measured using the ABL725 blood gas analyzer (Radiometer, Copenhagen, Denmark), and hematocrit (Hct) after centrifuging before and 7 min after administration of the CO dose. The measured values were also normalized for body mass. The typical error²⁷ of the tHb-mass measurement in our laboratory obtained by duplicate tests (n = 23) was 1.7 % (95 % confidence limit 1.3-2.4 %) for relative tHb-mass.²⁶

2.4 Statistical analyses

Statistical analysis was completed with IBM SPSS Statistics 26. The results are shown as mean ± standard deviation (SD). One-Way ANOVA, unpaired and paired t-tests were made to analyze significance differences between groups and pre- and post-measurements. An athlete was classified as a responder once the change in tHb-mass increased more than 1.7 % (Hbm+) following altitude training and others were classified as non-responder (Hbm-). As an increase > 1.7 % exceeds the typical error of tHb-mass measurement in our laboratory, it allows us to consider these athletes as "true" responders. Multiple logistic regression analysis was used to explore the factors that are significant for an increase in relative tHb-mass during the altitude training camp. In the regression analysis all 82 training camps are included. In the analysis, the change in relative tHb-mass is a binomial nominal variable; it only has two values, whether the increase is more or less than 1.7 %. The independent variables chosen in the analysis were altitude, time of exposure, pre-tHb-mass, pre-S-Ferrit and pre-S-hs-CRP. Pearson correlation coefficients were used to calculate the relationships between relative tHb-mass and other factors. Differences and correlations are considered statistically significant on p-values lower than 0.05.

3 RESULTS

3.1 Altitude training responses

The present data shows that in 56 % (46 of 82) of altitude training camps athlete's tHb-mass increased more than 1.7 % (Hbm+) and in 44 % (36 of 82) of altitude training camps no change or a decrease (Hbm-) was observed. The pre- and post-tHb-mass values of all training camps were plotted in Fig 1. When only the training camps above 2000 m were considered (n = 52) the percentage of positive tHb-mass response increased to 65 %. It further increased to 69 %, when pre-S-Ferritin values below 30 μ g·l⁻¹ and pre-S-hs-CRP values above 3.0 mg·l⁻¹ were excluded (n = 7) from the data (Table 3). In this sample of 45 training camps, in the camps with positive responses, the increase in tHb-mass was 4.6 ± 2.8 % (g) and 5.0 ± 2.3 % (g·kg⁻¹ BW). S-Ferrit decreased 22.6 ± 21.9 % during the training camps in Hbm+, whereas an increase in S-Ferrit (11.1 ± 46.3 %) was observed in Hbm- (p < 0.05).

3.2 Repeatability of tHb-mass responses

In athletes with multiple hypoxic exposures (n = 15), 60 % (9 athletes) got variable responses (both positive and negative responses) of altitude on tHb-mass, 27 % (4 athletes) got only positive responses and 13 % (2 athletes) got only negative responses (Fig 2). The correlation coefficient between the changes in tHb-mass ($g\cdot kg^{-1}$ BW) in two successive altitude training camp was not significant (Fig 3).

3.3 Effect of various variables on Hb-mass response

Multiple logistic regression analysis was used to explore the factors that are significant for an increase in relative tHb-mass during the altitude training camp. The data of all 82 training camps were included in the regression analysis. The variables entered in the analysis included altitude, time of exposure, pre-tHb-mass, pre-S-Ferrit and pre-S-hs-CRP. The results showed that the altitude is the only significant factor explaining relative tHb-mass increase during the altitude training camp (Table 4). In male athletes' the change in tHb-mass was significantly higher than in female athletes both in absolute (male athletes: from 973 ± 103 g to 1004 ± 99 g, female athletes: from 649 ± 116 g to 655 ± 113 g, p = 0.001) and body weight normalized values (male athletes: from 13.1 ± 1.2 g·kg⁻¹ to 13.6 ± 1.1 g·kg⁻¹, female athletes: from 10.7 ± 1.2 g·kg⁻¹ to 10.8 ± 1.1 g·kg⁻¹, p < 0.004). In the present data, 12 athletes had low pre-S-Ferrit (< 30 µg·l⁻¹) values and 11 of them were female and only one male. From those 11 female athletes six had negative tHb-mass responses following altitude training camp.

4 **DISCUSSION**

In the present study most of the altitude training camps produced positive tHb-mass response. From the athletes (n = 15) who had at least two altitude training camps 27 % had only positive Hb-mass responses, 13 % only negative responses and 60 % of the athletes had both positive and negative tHb-mass responses to altitude exposures. The wide variability in tHb-mass responses during the altitude training camp within each athlete suggest that responding or not responding to altitude training is not a fixed and classifiable trait.

To identify some potential factors explaining inter- and intra-individual variability in responsiveness to altitude exposure, multiple logistic regression analysis was used. There are several possible explanations as to why the athletes did not have positive Hb-mass responses to the altitude training camps. The independent variables chosen in the regression analysis were altitude, time of exposure, pre-tHb-mass, pre-S-Ferrit and pre-S-hs-CRP. The analysis revealed that altitude is the most significant variables to explain the increase in tHb-mass during an altitude training camp. Furthermore, the present results showed that there was a significant sex difference in tHb-mass response. Male athletes had greater tHb-mass response than female athletes.

The present data shows that tHb-mass was increased in 56 % of all altitude training camps. When the coaches and athletes followed the instructions of the research group in terms of the suggested altitude (2000-2500 m), iron deficiency (pre-S-Ferritin >30 μ g·l⁻¹) and inflammation (pre-S-hs-CRP <3 mg·l⁻¹), a positive response in tHb-mass (more than 1.7 % increase) was observed in 69 % of the altitude training camps. Similar percentages of positive response in tHb-mass have been observed in the study of MacLean

et al.⁴, whereas in a study with competitive swimmers (Wachsmuth et al.²⁰) negative responses were only observed in sick athletes, with one exception. The results of the present study show that great individual variation occurs in tHb-mass response to altitude training in endurance athletes. Although the average increase in tHb-mass was 17.6 g (2.1 %), the response varied from -84.3 to +91.0 g. This is supported by an earlier study, which showed a great individual variability in the altitude-induced EPO response.³

4.1 Repeatability of tHb-mass responses

Robertson et al.² were the first researchers to demonstrate that three weeks of simulated LHTL exposure elicited reproducible mean increases in tHb-mass (2.8 % and 2.7 %), but there was a moderate yet unclear negative correlation for changes in tHb-mass from one exposure to the next. In the present study, similar increases in tHb-mass were observed for 15 athletes in the first (1.8 %) and second (2.6 %) altitude training camps, but great individual variability was observed (Fig 3). Moreover, the correlation coefficient between the changes in relative Hb-mass (g·kg⁻¹ BW) in two successive altitude training camp was not significant (Fig 3), suggesting high variability in individual responsiveness between exposures. Similarly, Wachsmuth et al.²⁰ observed a weak correlation (r = 0.379, p = 0.160) between tHb-mass responses following two altitude training camps in elite swimmers, despite a highly reproducible initial EPO responses to altitude after two days (r = 0.905, p < 0.01). The results in the present and previous studies^{2,4,20,28} demonstrated that the altitude tHb-mass response varies due to inter-individual as well as intra-individual conditions. The physiological explanation for the intra-individual variability may be different baseline conditions including differences in preceding training days, training status, recovery, or health. For example, an increase in tHb-mass (first camp) can be decreased without appropriate monitoring at the second camp (perhaps due to a too strenuous training period at altitude, a loss of body weight, a short period of sickness, etc.). Thus, a "responder" and "non-responder" to altitude training in terms of tHb-mass does not appear to be a fixed trait.²⁸ Overall, the variability in individual tHb-mass response to hypoxia detected in the present and previous studies emphasizes the importance of evaluating the individual tHb-mass response of an athlete to each altitude training camps.

4.2 Hypoxic dose: altitude and exposure time

Several factors may affect intra-individual variability of tHb-mass response to hypoxia including altitude and time of exposure. The optimal hypoxic dose for tHb-mass and sea level performance has been debated.²⁴ Too long or too extreme exposure to altitude may compromise positive responses, whereas exposure that is too short or at too low an altitude may be insufficient to stimulate any worthwhile physiological adaptation. Rusko et al.⁸ suggested a minimum exposure for athletes of 12 h·day⁻¹ for at least three weeks at altitudes of 2000-2500 m. Garvican et al.²⁹, however, presented that tHb-mass increases, on average, 1 % per 100 h of exposure, indicating that two weeks of hypoxic exposure might be enough to elicit some erythropoietic adaptation. Based on the Gore et al.¹² meta-analysis of studies from a range of altitudes from 1300 m to 3600 m and an extended data set, Garvican-Lewis et al.²⁴ proposed a model where hypoxic dose is termed "kilometer hours" and defined as km·h, in which km indicates the altitude in kilometers and h indicates total hours of exposure.

Previous literature has shown that there is not a linear relationship between the hypoxic dose (km·h) and tHb-mass response. Chapman et al.⁷ did not show any hypoxic dose-related response in Hb-mass after four weeks LHTL between 1780 m, 2085 m, 2454 m, and 2800 m. Their results suggest that increasing the altitude above currently recognized optimal levels may not provide any benefit. The data from the present study show that altitude significantly affects tHb-mass response in the logistic regression analysis, but non-significant correlation exists between tHb-mass response and altitude (r = 0.218, p = 0.318) as well as hypoxic dose (r = 0.141, p = 0.520). As a high hypoxic dose as 1400 km·h was not enough to induce any changes in tHb-mass in some individuals. The possible reason for the negative effect of altitude on tHb-mass response may be explained by the regression model and the relationships between the variables in the model. The present results suggest that not only altitude and hypoxic dose explain tHb-mass responses, but also various other factors including training load and recovery influence on tHb-mass changes. This reasoning is supported by a study with climbers³⁰, where an extreme hypoxic dose did not guarantee an increase in tHb-mass under strenuous physical stress. Thus, the present and previous results suggest that there is a need for careful evaluation of all factors influencing athletes' responses rather than blindly believing that hypoxic dose is the dominant factor in altitude training.

4.3 Effect of iron stores and inflammation

In addition to hypoxic dose, pre-altitude Hb-mass and EPO responses, there are additional factors that may affect the increase in tHb-mass during an altitude training camp. In the present study, iron stores and inflammations were evaluated by S-Ferrit and S-hs-CRP before and after each altitude training camp. The correlation and regression analysis showed that there was not a relationship between pre-altitude S-Ferrit and S-hs-CRP with the changes in tHb-mass suggesting that pre-altitude high S-Ferrit and low S-hs-CRP values do not guarantee tHb-mass increase in the altitude training camp. However, low S-Ferrit values may prevent increase in tHb-mass during the altitude training camp since in the present study three times more athletes in Hbm- compared to Hbm+ had pre-S-Ferritin values below 30 µg·l⁻¹. Interestingly, S-Ferrit decreased in Hbm+ but not in Hbm- suggesting that, for some reason, Hbm- could not use their iron stores for hemoglobin reconstitution.

Garvican et al.¹⁹ presented a case study of an iron-deficient and anemic female endurance runner whose tHb-mass increased by 49 % following iron supplementation. The study illustrates that tHb-mass is readily responsive to iron supplementation in a severely iron-deficient and anemic athlete. The importance of iron stores was also proven in the study of Gough et al.³¹, who observed that a 4.2 % increase in Hb-mass following iron supplementation was the largest of the estimated effects of any factor examined in their study. In the study of Garvican-Lewis et al.¹⁸, they concluded that iron supplementation is necessary for optimal EPO adaptation to altitude exposure. They also suggest that intravenous iron supplementation. This result, however, was inconsistent with the result of Friedmann et al.³² who observed no benefit of oral iron supplementation for tHb-mass in athletes with low ferritin compared with a matched placebo group.

There are few studies that have indicated that health status or illness is associated with changes in Hbmass in athletes. Wachsmuth et al.²⁰ showed a 7.2 % increase in Hb-mass following 3-4 weeks of training at 2320 m in swimmers, whereas no increase was observed in sick/injured athletes (n = 8). In a more recent study of Heikura et al.²¹, healthy athletes were able to increase their Hb-mass more than athletes who became sick during the training camp (+5.4 % vs -0.5 %). In the present study, pre-S-hs-CRP did not explain Hb-mass changes during the altitude training camp. It may be that inflammation itself is not responsible for the reduced tHb-mass response at altitude, but that training interruptions and reduced training is. Gough et al.³³ showed that reduced training and surgery led to a 2.3 % and 2.7 % decrease in Hb-mass, respectively. Furthermore, despite suffering minor injuries during the altitude training camp, two female athletes did not show any remarkable effects on Hb-mass response, with an average Hb-mass increase of ~10 %.²¹

4.4 Effect of sex

Previous studies have failed to find a difference in Hb-mass response to altitude training between sexes, but this has not been systematically studied and smaller numbers of female than male athletes are generally involved in altitude training studies.^{20,22} In the present study, male athletes had significantly higher increases in Hb-mass than female athletes (3.6 vs 0.7 %, respectively). Sex difference can be at least partly explained by the fact that from 12 low pre-S-Ferrit (< 30 μ g·l⁻¹) values 11 were those of female athletes and only one was from a male athlete. From those 11 female athletes six had negative tHb-mass responses suggesting that iron deficiency could be one reason for this negative result from altitude training camp. The different response between sexes in the present study was contrary to the finding of Heikura et al.²¹, who observed that female athletes improved their tHb-mass more than their male

counterparts (6.2 % vs 3.2 %). The conflicting result between the present and Heikura et al.²¹ results cannot be explained by the results of the present study. One possible explanation could be that the prealtitude levels of relative tHb-mass were higher in the study of Heikura et al.²¹ than in the present study (+1.5 g·kg⁻¹ or +11.6 % in men and +1.1 g·kg⁻¹ or +10.0 % in women). However, the effect of sex on Hbmass responses following altitude training camp warrants further studies.

5 PERSPECTIVES

These results represent one of the largest follow-up studies to date regarding the effects of various factors on tHb-mass response to altitude training in national team endurance athletes. The increase in tHb-mass during an altitude training camp is not a straightforward process, since there are numerous factors which affect the tHb-mass response to hypoxia exposure. The regression model and analysis in the present study showed that the key issue is the altitude. As the likelihood of positive tHb-mass response increased at altitudes above 2000 m, training camps with a duration approximately from 2.5 to 6 weeks should meet this altitude criteria.

These results also suggest that great inter- and intra-individual variability existed in tHb-mass responses. The tHb-mass response to hypoxia exposure is not a black and white issue, since numerous factors may change positive response to negative or vice versa. It is rewarding to use time for planning properly the altitude training camp and try to consider all possible factors which may affect positive tHb-mass response and endurance performance improvement. Before, during and after the altitude training camp it is also rewarding to use time and resources to monitor physiological adaptation to changes in the oxygen content of the breathing air as well as training load and recovery.

REFERENCES

- 1. Schmidt W, Prommer N. Impact of alterations in total hemoglobin mass on VO_{2max}. *Exerc Sport Sci Rev.* 2010;38:68–75.
- Robertson EY, Saunders PU, Pyne DB, Aughley RJ, Anson JM, Gore CJ. Reproducibility of performance changes to simulated live high/train low altitude. *Med Sci Sports Exerc*. 2010;42:394-401.
- 3. Chapman RF, Stray-Gundersen J, Levine BD. Individual variation in response to altitude training. *J Appl Physiol (1985)*. 1998;85(4):1448–1456.
- 4. McLean B, Buttifant D, Gore C, White K, Kemp J. Year-to-year variability in haemoglobin mass response to two altitude training camps. *Br J Sports Med*. 2013;47:51-58.

- 5. Wilber RL, Stray-Gundersen J, Levine BD. Effect of hypoxic "dose" on physiological responses and sea-level performance. *Med Sci Sports Exerc*. 2007;39(9):1590-1599.
- Levine BD, Stray-Gundersen J. Dose-response of altitude training: how much altitude is enough?
 In: *Hypoxia and Exercise*. New York, NY: Springer; 2006, 233-247.
- Chapman RF, Karlsen T, Resaland GK, Ge R-L, Harber MP, Witkowski S, Stray-Gundersen J, Levine BD. Defining the "dose" of altitude training: how high to live for optimal sea level performance enhancement. *J Appl Physiol*. 2014;116:595-603.
- 8. Rusko H. New aspects of altitude training. *Am J Sports Med*. 1996;24(6):48-52.
- Rusko HK, Tikkanen HO, Peltonen JE. Altitude and endurance training. J Sports Sci. 2004;22:928– 944.
- Mazzeo RS. Physiological responses to exercise at altitude. An update. *Sports Med*. 1008;38(1):1 8.
- Bonetti DL, Hopkins WG. Sea-level exercise performance following adaptation to hypoxia. *Sports Med.* 2009;39(2):107-127.
- 12. Gore CJ, Sharpe K, Garvican-Lewis LA, Saunders PU, Humberstone CE, Robertson EY, Wachsmuth NB, Clark SA, McLean BD, Friedmann-Bette B, Neya M, Pottgiesser T, Schumacher YO, Schmidt WF. Altitude training and haemoglobin mass from the optimised carbon monoxide rebreathing method determined by a meta-analysis. *Br J Sports Med*. 2013;47:31-39.
- 13. Kayser B. Nutrition and energetics of exercise at altitude. *Sports Med.* 1994;17:309-323.
- 14. Laciga P, Koller EA. Respiratory, circulatory, and ECG changes during acute exposure to altitude. *J App Physiol*. 1976;41:159-167.
- 15. Hogan RP, Kotchen TA, Boyd AE, Hartley LH. Effect of altitude on renin-aldosterone system and metabolism of water and electrolytes. *J Appl Physiol*. 1973;35:385-390.
- 16. Lundby C, Millet GP, Calbet JA, Bärtsch P, Subudhi AW. Does 'altitude training' increase exercise performance in elite athletes? *Br J Sports Med*. 2012;46:792-795.
- Gore CJ, Craig N, Hahn A, Rice A, Bourdon P, Lawrence S, Waish C, Stanef T, Barnes P, Parisotto R, Martin D, Pyne D. Altitude training at 2,690 m does not increase total haemoglobin mass or sea level VO2max in world champion track cyclist. *J Sci Med Sport*. 1998;1:156–170.
- Garvican-Lewis LA, Vuong VL, Govus AD, Peeling P, Jung G, Nemeth E, Hughes D, Lovell G, Eichner D, Gore CJ. Intravenous iron does not augment the hemoglobin mass response to simulated hypoxia. Med Sci Sports Exerc. 2018;50:1669-1678.

- Garvican LA, Lobigs L, Telford RD, Fallon KE, Gore CJ. Haemoglobin mass in an anaemic female endurance runner before and after iron supplementation. *Int J Sports Physiol Perform*. 2011;6:137-140.
- 20. Wachsmuth NB, Volzke C, Prommer N, Schmidt-Trucksäss A, Frese F, Spahl O, Eastwood A, Stray-Gundersen J, Scmidt W. The effects of classic altitude training on hemoglobin mass in swimmers. *Eur J Appl Physiol*. 2013;113:1199-211.
- 21. Heikura IA, Burke LM, Bergland D, Uusitalo ALT, Mero AM, Stellingwerff T. Impact of energy availability, health, and sex on hemoglobin-mass responses following Live-High-Train-Low altitude training in elite female and male distance athletes. *Int J Sports Physiol Perf*. 2018;13:1090-1096.
- 22. Heinicke K, Heinicke I, Schmidt W, Wolfarth B. A three-week traditional altitude training increases hemoglobin mass and red cell volume in elite biathlon athletes. *Int J Sports Med*. 2005;26:350-355.
- 23. Millet GP, Roles B, Schmitt L, Woorons X, Richalet JP. Combining hypoxic methods for peak performance. *Sports Med.* 2010;40(1):1-25.
- Garvican-Lewis LA, Sharpe K, Gore CJ. Time for a new metric for hypoxic dose? J Appl Physiol. 2016;121:352-355.
- 25. Schmidt W, Prommer N. The optimised CO-rebreathing method: a new tool to determine total haemoglobin mass routinely. *Eur J Appl Physiol*. 2005;95(5-6):486–495.
- 26. Koponen A, Peltonen J, Päivinen M, Aho J, Hägglund H, Uusitalo A, Lindholm H, Tikkanen H. Low total haemoglobin mass, blood volume and aerobic capacity in men with type 1 diabetes. *Eur J Appl Physiol*. 2013;113(5):1181-1188
- 27. Hopkins WG. Measures of reliability in sports medicine and science. Sports Med. 2000;30(1):1–15.
- 28. Hauser A, Troesch S, Saugy JJ, Schmitt L, Cejuela-Anta R, Faiss R, Steiner T, Robinson N, Millet GP, Wehrlin J. Individual hemoglobin mass response to normobaric and hypobaric "live high–train low": A one-year crossover study. *J Appl Physiol*. 2017;123:387-393.
- 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.
- 30. Cheung SS, Mutanen NE, Karinen HM, Koponen AS, Kyröläinen H, Tikkanen HO, Peltonen JE. Ventilatory chemosensitivity, cerebral and muscle oxygenation, and total hemoglobin mass before and after a 72-day Mt. Everest expedition. High Alt Med Biol. 2014;15:331-340.
- 31. 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.

32. Friedmann B, Weller E, Mairbaurl H, Bartsch P. Effects of iron repletion on blood volume and performance capacity in young athletes. *Med Sci Sports Exerc*. 2001;33:741-746.

LEGENDS FOR FIGURES

Fig 1. The pre- and post-tHb-mass values ($g \cdot kg^{-1}$) of all training camps (n = 82). The first training camp is marked as black circle, the second as orange circle and $3^{rd}-5^{th}$ as grey circle. Solid line represents the line of unity and dashed lines the typical error of Hb-mass measurement (±1.7 %).

Fig 2. Change in tHb-mass (%) in fifteen male (M) and female (F) athletes who had at least two altitude training camps. M1 to M4 had only positive responses, F1 to F6 had both positive and negative responses and M8 to F7 had only negative responses.

Fig 3. Change in tHb-mass ($g \cdot kg^{-1}$) in two successive training camp (n = 23).

Table 1. Basic characteristics of athletes participating in this study.

		Age	Body mass	Height	BMI	
		(years)	(kg)	(m)	(kg·m⁻²)	
	Men (n = 27)	$\textbf{23.2} \pm \textbf{5.0}$	75.5 ± 8.5	1.83 ± 0.06	$\textbf{22.5} \pm \textbf{1.7}$	
	Women (n = 32)	$\textbf{23.4} \pm \textbf{5.9}$	$\textbf{60.8} \pm \textbf{10.1}$	1.70 ± 0.07	$\textbf{21.0} \pm \textbf{2.2}$	
	All (n = 59)	23.3 ± 5.3	$\textbf{67.6} \pm \textbf{11.9}$	1.76 ± 0.09	21.7 ± 2.1	

Table 2. Number of pre-and post-measurements in the present study.

	Women	Men
All training camps	43	39
One training camp	25	19
Two training camps	3	6
Three training camps	4	1
Five training camps	0	1

Table 3. Group differences and comparison of pre- and post-measurements of athletes with positive (Hbm+) and negative (Hbm-) tHb-mass responses in altitude training camps. Only the training camps above 2000 m and pre-S-Ferritin values above 30 μ g·l⁻¹ and pre-S-hs-CRP values below 3.0 mg·l⁻¹ were included in the data. Abbreviations: PV = plasma volume, BV = blood volume, Hb = hemoglobin, Hct = hematocrit, S-TfR = serum transferrin receptors, S-Ferrit = serum ferritin, S-hs-CRP = serum high-sensitive C-reactive protein.

	Hbm+ ((n = 31)	Hbm- (r	ANOVA	
Men/Women	20/11		9/5		
Altitude (m)	2248 ± 126		2164 ± 138		p = 0.049
Hypoxia dose (km·h)	1330 ± 167		1406 ± 189		p = 0.182
Age (years)	21.8 ± 4.9		24.7 ± 3.8		p = 0.059
Height (m)	1.79 ± 0.09		1.79 ± 0.07		p = 0.983
Body mass (kg)	71.0 ± 11.7	70.8 ± 11.7	66.5 ± 6.9	67.0 ± 7.1	p = 0.101
Hb-mass (g)	836 ± 207	873 ± 214	866 ± 172	859 ± 173	p < 0.001
Hb-mass (g·kg⁻¹)	11.6 ± 1.8	12.2 ± 1.9	12.9 ± 1.8	12.7 ± 1.8	p < 0.001
PV (ml)	3939 ± 775	3777 ± 716	3971 ± 650 3878 ± 585		p = 0.403
BV (ml)	6403 ± 1366	6367 ± 1300	6533 ± 1142	6410 ± 1080	p = 0.338
Hb (g·l⁻¹)	14.2 ± 0.9	15.0 ± 1.0	14.5 ± 0.8	14.7 ± 0.9	p = 0.005
Hct (%)	41.9 ± 2.9	44.4 ± 2.8	42.9 ± 1.8	43.1 ± 2.3	p < 0.001
S-TfR (mg·l⁻¹)	3.0 ± 0.5	3.7 ± 0.9	3.1 ± 0.7	3.3 ± 0.6	p = 0.028
S-Ferrit (µg·l ⁻¹)	71.4 ± 28.3	54.6 ± 23.9	75.0 ± 37.0	65.9 ± 22.8	p = 0.041
S-hs-CRP (mg·l ⁻¹)	0.6 ± 0.7	0.4 ± 0.3	0.3 ± 0.3	0.3 ± 0.2	p = 0.286

Table 4. Multiple logistic regression analysis for the selected variables to explain tHb-mass/kg BW change during the altitude training camp. Abbreviations: pre = before altitude training camp measured values.

		В	S.E.	Exp(B)	95% C.I. fo	or EXP(B)	Sig.
	Constant	-4.377	3.142	0.013			0.164
	Altitude	2.572	1.155	13.094	1.361	125.995	0.026
	Pre-tHb-mass/kg	-0.009	0.169	0.991	0.712	1.38	0.959
	Pre-Ferrit	0.005	0.007	1.005	0.992	1.018	0.475
	Pre-CRP	-0.072	0.089	0.930	0.782	1.107	0.416
	Time of exposure	-0.034	0.057	0.966	0.864	1.08	0.548



sms_13804_f1.tif



sms_13804_f2.tif

This article is protected by copyright. All rights reserved

2.7



sms_13804_f3.tif

This article is protected by copyright. All rights reserved

ACCE