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Title:

Impact of energy availability, health and sex on hemoglobin mass responses following live high - train high altitude training in elite female and male distance athletes

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ABSTRACT

Background: We investigated the effects of sex, energy availability (EA), and health status on the change in hemoglobin mass (ΔHbmass) in elite endurance athletes over ~3 to 4 weeks of Live-High/Train-High altitude training (Flagstaff, AZ, 2135m; n=27 females; n=21 males; 27% 2016 Olympians). **Methods:** Pre- and post-camp Hbmass (optimized CO re-breathing method) and iron status were measured, EA was estimated via food and training logs and Low Energy Availability in Females Questionnaire (LEAF-Q) and a general injury/illness questionnaire was completed. Hypoxic exposure (hours) was calculated with low (<500h), moderate (500-600h) and high (>600h) groupings. **Results:** Absolute and relative percentage ΔHbmass (%ΔHbmass) was significantly greater in females (6.2±4.0%, p<0.001) than in males (3.2±3.3%, p=0.008). (%ΔHbmass) showed a dose-response with hypoxic exposure (3.1±3.8 vs 4.9±3.8 vs 6.8±3.7%; p=0.013). $\text{Hbmass}_{\text{pre}}$ was significantly higher in eumenorrheic vs amenorrheic females (12.2±1.0 vs 11.3±0.5 g/kg; p=0.004). Although statistically under-powered, %ΔHbmass was significantly less in sick (n=4, -0.5±0.4%) versus healthy (n=44 athletes; 5.4±3.8%; p<0.001). There were no significant correlations between self-reported iron intake, sex hormones or EA on Hbmass outcomes. However, there was a trend for a negative correlation between LEAF-Q score and %ΔHbmass (*r*=–.353, p=0.07). **Conclusion:** Our findings confirm the importance of baseline Hbmass and exposure to hypoxia on increases in Hbmass during altitude training, while emphasizing the importance of athlete health and indices of EA on an optimal baseline Hbmass and hematological response to hypoxia.

Key words: word-class athletes, athlete health, adaptations to altitude, altitude training, hemoglobin mass

INTRODUCTION

Many high performance endurance athletes undertake specialized forms of altitude training. The lack of agreement regarding the effects of altitude training on hematology and performance is partially explained by various differences in the methodology of altitude training studies[1]. For example, there are different modalities of altitude exposure, with several common options being Live High-Train High (LHTH) or Live High-Train Low (LHTL) with hypobaric or normobaric hypoxia, or intermittent hypoxic exposure at rest (IHE) or during training (IHT)[2]. Nevertheless, irrespective of changes in performance, a change in hemoglobin mass (Hbmass) is considered an objective and relatively easily measured outcome of altitude exposure within a standardized altitude training protocol, with typical increase of 2–5% following a block of altitude training being reported [3-7]. However, the mechanisms associated with optimizing Hbmass increases are multifactorial and include the type of altitude modality, the duration and level of exposure (also termed hypoxic dose[8]) and possibly the initial Hbmass level[9]. Indeed, there is consistent evidence of a progressive increase in Hbmass with three weeks of altitude training[3,7,10], with supportive factors including the adequacy of baseline ferritin concentrations and doses of iron supplementation[11,12]. Meanwhile, only two studies have reported that injury and/or illness tends to negatively affect Hbmass changes[6,13]. One aspect of athlete health is optimal energy availability (EA), which is defined as the dietary energy available to support body function once the energy cost of exercise has been deducted from daily energy intake[14]. Low EA has detrimental effects on many areas of health and training adaptation, including impairment of menstrual status, protein synthesis and iron status and an increased risk of illness and injury[15]. However, to our knowledge no study has investigated the effects of symptoms of low EA on altitude-induced hematological adaptations.

There are conflicting findings in the literature regarding factors which alter the Hbmass response to altitude. For example, Wachsmuth *et al*.[6] found no sex based differences in 45 elite swimmers in the relative Hbmass response with 3-4 weeks of LHTH over multiple camps; however the absolute change was higher in males, which they hypothesized to be due to higher baseline values. Conversely, in a meta-analysis Rasmussen et al. calculated lower Hbmass changes in athletes with high baseline values following various altitude training protocols[9]. In contrast, Heinicke *et al.*[16] investigated the effects of 3-weeks of LHTH altitude training at 2050m on Hbmass in 6 male and 4 female word class biathletes and reported that Hbmass improved by ~9% in both males and females despite differences in baseline levels and very low subject numbers. Athlete calibre is another factor that may affect the hematological adaptations to altitude. Indeed, while some studies have shown increased Hbmass in elite athletes[6,17], others[18,19] have failed to do so, leading some experts to question the usefulness of altitude training in athletes with already high Hbmass levels[20]. Obviously, the impact of baseline Hbmass values, which are greater in males than females and in elite versus non-elite, and the subsequent hypoxic induced changes in Hbmass, is far from being completely understood. Finally, the beneficial effects of altitude training on other body systems such as angiogenesis and increased buffering capacity[21] are often forgotten. Indeed, even if no hematological improvements are seen after altitude training, an athlete may have benefited from the camp via improvements in non-hematological outcomes.

Due to lack of studies on the effects of EA and hormonal health (i.e. reproductive, metabolic and anabolic hormones) on the Hbmass response at altitude, and due to contrasting results regarding other factors that may influence this response in males vs. females and elite athletes, our aim was to investigate the changes in Hbmass following LHTH altitude training in one of the largest to date single cohort of elite female and male endurance athletes (27% Olympians) over a single training camp. Specifically, we aimed to confirm previous findings on the effects of length of exposure to hypoxia on change in Hbmass. However, we also wanted to investigate whether additional factors including sex, pre-camp Hbmass, health status (illness/injuries), EA sex hormone concentrations and bone health would affect Hbmass changes. Our hypothesis was that the magnitude of increase in Hbmass would depend primarily on hypoxic exposure, and possibly also on pre-camp Hbmass levels and health status.

METHODS

Participants

World-class middle- and long-distance runners and racewalkers (females, n=27; males, n=21) were recruited. The inclusion criteria was 18–40 years of age and having an IAAF score (International Association of Athletics Federations Scoring Tables 2011 [22]) of at least 1050 points (corresponds to 13:45.20min and 16:00.04min in the 5000m in males and females, respectively) scored within the preceding two years prior to study (baseline IAAF score). The study protocol was approved by the Ethics Committee of University of Jyväskylä and conducted according to the Declaration of Helsinki. All participants were enrolled in, and regularly screened by, anti-doping monitoring programs. No participants have ever served any anti-doping rule violation, and thus, to the best of our knowledge, were not involved with the use of any prohibited substances.

Study design

In a non-blinded longitudinal study design, we investigated pre- ($\text{Hbmass}_{\text{pre}}$) and post-altitude ($\text{Hbmass}_{\text{post}}$) Hbmass, iron and health status (sex hormones, bone mineral density

(BMD), injury/illness frequency) during a pre-competition LHTH altitude training camp in Flagstaff, AZ (2135m altitude; spring 2016). The measurements included baseline fasted blood samples, body composition and BMD measurements via Dual-energy X-ray Absorptiometry (DXA), followed by 7-day food and training logs on the second week of the camp. Female athletes filled out a validated Low Energy Availability in Females Questionnaire (LEAF-Q[23]).

Hemoglobin mass

Total Hbmass was measured with the adapted two-minute carbon monoxide (CO) rebreathing protocol[24]. In brief, subjects rebreathed a dose of CO based on body mass (BM) (1.25 ml/kg BM for males and 1.00 ml/kg BM for females) and ~4 L pure oxygen for 2 minutes via closed circuit spirometer. A nose clip was worn and a portable CO meter (FLUKE CO-220, Everett, Washington) was used to detect possible CO leakage via the nose, mouthpiece and spirometer throughout the 2 minutes of CO rebreathing. Determination of %HbCO was measured for baseline and 6 and 8 minutes after rebreathing from capillary fingertip blood samples tested with OSM3 hemoximeter (Radiometer, Copenhagen, Denmark). Hbmass was calculated from the mean change in %HbCO before and after CO rebreathing. Measurements were conducted pre- (within ~48-72h of arrival) and post-camp (within ~48-72 h from departure) by the same technician at Hypo2 High Performance Sport Center in Flagstaff, AZ. The typical error reported for the measurement done at Hypo2 is 1.9%. Throughout this manuscript, Hbmass values are reported as absolute (absolute total Hbmass), relative (Hbmass relative to BM) and percentage (percentage change in Hbmass, %ΔHbmass).

Hematology and anthropometry

Resting overnight fasted venous blood samples were collected at the beginning and at the end of the camp. Venous blood was collected into 8.5 mL SST gel tubes (BD Vacutainer, Franklin Lakes, NJ, USA) and centrifuged at 3400rpm for 10min using a Mini E Horizon centrifuge (Drucker Company, Philipsburg, PA, USA). The fasted samples were analyzed for serum iron, ferritin, testosterone and estradiol and measured via electrochemiluminescence immunoassay (ECLIA) method. Body composition and BMD was measured in a fasted state by a trained technician with DXA (GE Lunar DPX-IQ).

Dietary intakes and training characteristics

To avoid the possible effects of the initial altitude acclimatization on training and eating habits the athletes were asked to keep food and training logs concurrently on the second week of the altitude training camp. The principal investigator met each athlete to provide detailed instructions on how to record all food and fluid intake accurately. The participants were asked to record the time of all meals and training sessions, the type of food (brand names, flavors, etc.) and amounts. Participants were provided with kitchen scales and measurement cups to facilitate the recording process. If the participants ate out, they were asked to provide photos of meals with verbal description to facilitate cross-checking. Athletes were free to supplement with iron according to self-chosen protocols (e.g. brand and dosage) during the camp, however details of this were recorded.

The participants were asked to record training for seven days including total distance, time and session rating of perceived exertion (sRPE[25]). The use of sRPE is validated to reflect training load, and sRPE values of <4 (zone 1), 4–7 (zone 2) and >7 (zone 3) have been shown to correspond well to the heart rate and blood lactate values[26].

Analysis of nutrient intake, energy expenditure and energy availability

The principal investigator analyzed all dietary records with ESHA Food Processor (Oregon, US, 2016). EA was estimated from food and training diaries as energy intake minus EEE and expressed in kcal·kg^{-1} FFM·day^{-1}[14]. Detailed information on methodology used and outcomes of these analyses is reported elsewhere[27].

Statistical analysis

Statistical analyses were conducted using SPSS Statistics 22 (INM, Armonk, New York, USA) with data normality assessed via Shapiro-Wilk. Data were analysed for all athletes pooled, and with comparisons for sex and for female menstrual status (eumenorrheic vs. amenorrheic, defined as the absence of ≥three consecutive menses). Hypoxic dose[8] at 2135m was stratified into low (LOW: <1200 km.h; <23 days; n=27), moderate (MOD: 1200–1400 km.h; 23–27 days; n=13) and high (HIGH: >1400 km.h; >27 days; n=8) groups. For further comparison, athletes were also categorized based on hours of exposure[3] as follows: low (<500 hours; n=18), moderate (500-600 hours; n=14) and high (>600 hours; n=16). Analysis of Covariance (ANCOVA) was used to test the differences in the change in Hbmass with different hypoxic doses when controlling for Hbmass$_{pre}$. Athletes were divided into healthy and sick (with an illness being defined as anything that caused overall decrease in training/alteration to an athletes' training program, but excluded minor routine injuries where training could be modified or training load was not reduced due to cross-training) groups for further analysis. Baseline IAAF scores were compared to the best race performance (IAAF score) within three weeks of descent from altitude (Post IAAF score).

Differences in pre-camp body composition, Hbmass, iron status, EA, sex hormones and BMD between sexes, amenorrheic vs eumenorrheic females, and healthy vs sick athletes were analyzed with Student's t-test (parametric data) or Mann-Whitney U-test (nonparametric data). Changes in pre- to post-iron status, Hbmass and IAAF score were analyzed with Student's paired t-test (parametric data) or Wilcoxon signed rank test (nonparametric). Correlations were analyzed using Pearson's correlation coefficient (parametric) or Spearman's test (nonparametric). Data are presented as means±standard deviations (SD). Statistical significance was set at $p \leq 0.05$.

RESULTS

Table 1 summarizes athlete characteristics, dietary and training data, iron status parameters and raw Hbmass changes during the altitude camp. Pre- and post-camp Hbmass were higher in males than in females. %ΔHbmass (g/kg) was 4.9±4.0% ($p<0.001$) in all athletes pooled, with significantly higher percentage ($p=0.008$) and absolute ($p=0.033$) increases in relative Hbmass values in females vs males. Relative $\text{Hbmass}_{\text{pre}}$ was significantly higher in eumenorrheic (n=20) vs amenorrheic (n=7) females (12.2±1.0 vs 11.3±0.5 g/kg; $p=0.004$). LOW hypoxic dose (+3.7±3.9%; 1013±137km.h) increased relative Hbmass significantly less than MOD (+7.3±3.4%; 1320±70km.h; $p=0.018$) and, although not statistically significant, less than HIGH (4.8±3.6%; 1563±95km.h) groups. In contrast, when hypoxic dose was characterized as hours of exposure, there was a trend for higher response with increasing hours of exposure, and a significant difference between low and high in all athletes pooled ($F(2,48)=8.192$, $p=0.017$). However, when females and males were analysed separately, only males showed a difference in the %ΔHbmass response based on hours of exposure ($F(2,20)=10.21$, $p=0.001$; Figure 2). Also, ANCOVA showed that there was a significant difference in the relative Hbmass response between hours of exposure groups when controlling for Hbmass_{pre} (F(2, 44)=4.413, p=0.018, partial eta squared=0.167). In addition, there was a strong relationship between HBmass_{pre} and Hbmass_{post} (partial eta squared=0.928)

The relative Hbmass_{pre} negatively and significantly correlated with the %ΔHbmass (females r=-.406, p=0.035; males r=-.470, p=0.032; Figure 1). In addition, hypoxic dose as km.h (*r*=.333, p=0.021) and as hours of exposure (*r*=.374, p=0.009) positively correlated with %ΔHbmass. Relative Hbmass increased significantly more in healthy athletes (n=44, 26 females and 18 males) compared to those who suffered from illness (n=4, 1 females and 3 males) during the camp (Figure 3; p<0.001). Two females (%ΔHbmass +7.1 and +14.6%) suffered mild injuries but continued cross-training to maintain training load during the camp and thus, were not considered as having an illness. When these athletes were included in the analysis as "sick/injured", the difference in the %ΔHbmass between sick and healthy athletes became non-significant (3.3±6.3 vs 5.1±3.6%, respectively; p=0.11). No correlations were found between baseline IAAF score, change in IAAF from baseline to post-camp or EA and %ΔHbmass. In females, LEAF-Q score showed a strong trend for a negative correlation with %ΔHbmass (g/kg; *r*=–.353, p=0.07). There were no correlations or effect of self-reported iron supplementation protocols, baseline ferritin levels, sex hormones (data in our companion paper[27]), body composition parameters or BMD (data in our companion paper[27]) on Hbmass outcomes.

DISCUSSION

This is one of the largest studies to date to investigate the contribution of hours of exposure to hypoxia, Hbmass_{pre} and aspects of health status (e.g. outcomes of EA and illness incidence at altitude) to the Hbmass response to altitude training in a single camp and single cohort of male and female world-class endurance athletes (27% Olympians). Furthermore, our large subject pool allowed for sufficient statistical power to allow a comparison of sex-based differences in responses. Our main findings were that Hbmass increased significantly in both female and males, with significantly greater relative and percentage increases in females. In addition, $\text{Hbmass}_{\text{pre}}$ was higher in eumenorrheic compared to amenorrheic females, and the increase in Hbmass was more prominent in athletes who remained healthy throughout. Finally, in line with previous studies, we found superior increases in Hbmass with greater hypoxic exposures and in those with lower initial $\text{Hbmass}_{\text{pre}}$ values.

Our investigation further expands the current literature on altitude training in elite athletes in which studies are commonly characterised by the collection of data over multiple time periods[6], with varying altitude exposures[28] and/or use of simulated hypoxia[11], or in the absence of measures of changes in Hbmass[29-33]. Unlike a few previous studies ([18,19]) that have failed to find an increase in Hbmass in elite athletes, and contrary to speculations on whether elite athletes with already high Hbmass benefit from altitude training[20], we found significant Hbmass increases in our group of world-class distance athletes. This is in line with a recent study by Hauser et al.[34], who showed increases in Hbmass after 200-230 hours of exposure to a LHTL protocol in male endurance and team sport athletes. Indeed, despite a moderate inverse relationship between baseline Hbmass and change in Hbmass, even athletes with high initial Hbmass levels (13.1g/kg in endurance athletes) showed ~4% increases following exposure to hypoxia[34]. Interestingly, despite similarities in the calibre of our female and male athletes, as shown by their identical baseline IAAF scores, females were more successful in improving their Hbmass over the camp (6.2 vs 3.2%; Table 1). While previous studies have failed to find a difference in Hbmass response to altitude between sexes[6,16], these have generally involved smaller numbers of female-to-male comparisons[5,13,16,35,36] or have investigated only females[4,10] or males[18,19,34,37,38]. The findings of the current study could be explained by the fact that males had significantly higher relative $\text{Hbmass}_{\text{pre}}$ levels (14.4 vs 12.0 g/kg; Table 1), although this is just speculation. Nevertheless, we found a negative relationship between $\text{Hbmass}_{\text{pre}}$ and change in Hbmass (ΔHbmass; Figure 1); previous investigations have also suggested that initial Hbmass play a role in the magnitude of the hematological adaptations at altitude[9,20,34], although not all studies support this finding[6].

The magnitude and length of exposure to hypoxia are crucial for altitude-induced hematological adaptations. Based on several studies, an increase of 1% per week[10] or 1% per 100 hours of exposure[3] can be expected, although an exponential model of hypoxic dose has also been proposed by Garvican-Lewis and colleagues[8]. Indeed, we found increases of 3.7, 7.3, and 4.8% at low (1013 km.h), moderate (1320 km.h) and high (1563 km.h) hypoxic doses, respectively, which resulted in a significant positive correlation between hypoxic dose and ΔHbmass. Interestingly, comparison of changes in Hbmass with differing hours of exposure showed greater increases in Hbmass with increasing hours of exposure (3.6 vs 4.0 vs 6.2% with <500, 500-600, and >600 hours of exposure; Figure 2), which is in line with a meta-analysis showing that even shorter exposures of LHTH or LHTL are able to increase Hbmass ~3% given the athlete is free from injury/illness and has adequate iron supplementation[39]. Considering the same elevation for each athlete in the current study, perhaps the difference in findings between hypoxic dose and hours of exposure comparisons can be explained with different cut-off points that resulted in different categorization of athletes into low, moderate and high groups. Alternatively, our analysis between hypoxic dose groups may have been under-powered (n=8 in high hypoxic dose group). However, although exposure to hypoxia is important, our findings suggest that initial Hbmass levels (Figure 1) appear to have an even greater effect on the magnitude of hematological adaptations following altitude training.

There have been previous indications that athlete health is associated with changes in erythropoiesis in athletes. Gough *et al.*[13] tracked changes in Hbmass in 15 athletes over lengthy periods (162±198 days) of training interruptions due to illness and injury, showing that reduced training and surgery (n=3) led to 2.3 and 2.7% decreases in Hbmass, respectively. Furthermore, Wachsmuth *et al*.[6] showed a 7.2% increase in Hbmass following 3-4 weeks of LHTH training at 2320m in swimmers, while no increase was observed in ill/injured athletes (n=8). The results of our study show several new insights into the importance of health status in optimizing the response to altitude training. Principally, healthy athletes were able to increase Hbmass significantly more than athletes who became sick during the training camp (+5.4 vs -0.5%; Figure 3), which confirms the findings of previous research. While we acknowledge that our sample size of injured athletes was small and thus may have reduced the statistical power, as mentioned earlier, this finding is in line with previous studies showing an impaired response to hypoxia in athletes who were not healthy[6,13]. Interestingly, despite suffering minor injuries during the camp, two females who managed to maintain their training loads via cross-training did not show Hbmass erosion, with an average Hbmass increase of ~10%. This novel finding suggests that athletes suffering from minor injuries (where serious inflammation may not present) may still be able to benefit from altitude training where training volume is not compromised (via inclusion of cross-training) and where non-steroid anti-inflammatory drugs are not used (may compromise response). This is aligned with Gough *et al*.[13] who also showed training reductions causing decreases in Hbmass. However, these findings should be interpreted with caution as we only had a very small number of athletes who developed illnesses during the camp.

We were also interested to look at the effect of low EA (based on food and training records as well as physiological outcomes[27]) on adaptations to altitude training, since it has previously been shown to impair health and performance[15], including processes such as the protein synthetic response to exercise[40] that are likely to be important in hemapoiesis. Our estimations from food and training logs captured during the mid-period of the altitude training camp identified a range of EA scores among both male and female cohorts spanning healthy (~45 kcal.kg $BM^{-1}.d^{-1}$) to low (<30 kcal.kg $BM^{-1}.d^{-1}$)[14]. However, we failed to identify a correlation between these estimates and Hbmass changes. This is not entirely surprising since these EA calculations are based on self-reports from a single time period of 1 week which are fraught with methodological issues, as well as not necessarily representative of earlier behaviors which may have caused chronic metabolic and hormonal perturbations[27]. Indeed, it is likely that athletes' eating and exercise activities during the camp were different to their habitual practices due to deliberate alterations in nutrition practices and training program to accommodate the special needs of altitude training, as well as secondary changes due to a new food environment and daily routine. These changes may have altered both the magnitude and direction of habitual EA compared to the optimal levels. Surprisingly and contrary to our hypothesis, we failed to find correlations or effects of sex hormones or BMD on Hbmass outcomes. Indeed, we assumed that low sex hormone or BMD status would negatively affect ΔHbmass, however this was not the case. Nevertheless, other data collected in our study which identified a high risk of chronic low EA was correlated with Hbmass responses to the altitude training. We found significantly lower $Hbmass_{pre}$ in amenorrheic vs eumenorrheic females (amenorrhea signals of chronic low EA[15]) and a trend for higher increases in Hbmass with lower LEAF-Q scores (LEAF-Q scores of >8 are likely to be indicative of low EA in females[23]), which indicates that menstrual dysfunction, an indicator of long-term low EA, may influence these adaptations or their magnitude. However, despite this trend, this association between LEAF-Q and Hbmass changes at altitude requires further validation.

Strengths and limitations. The major strength of the current study is that it was conducted during the preparation period for the 2016 Olympic Games and thus, unlike several other previous studies, reflects the true training characteristics and altitude camp outcomes of elite athlete in preparation for a major competition. The sample size for the current study is one of the largest to date reported in the literature in an elite athlete population, with a single camp and a single time period protocol allowing us to detect differences that might not otherwise be detectable when using other forms of data collection. Furthermore, to our knowledge, we have highest numbers of female-to-male comparisons within these conditions. Finally, our study adds to the growing literature of the likely detrimental effects of low EA and/or menstrual dysfunction on athlete health. However, there are several limitations. First, due to the involvement of truly world-class athletes in preparation in the Olympic year, we were not able to standardise factors such as duration of altitude exposure and use or dose of iron supplementation (although all were recommended to take iron between 100 to 200mg/day). In addition, we were not able to include performance tests to provide physiological characteristics of the athletes. Therefore, it is impossible to estimate the effects of altitude exposure or changes in Hbmass on performance outcomes in these athletes. In addition, the dietary and training information was collected from a single week of the camp and may not represent habitual practices and/or the practices over the entire camp. However, since the reliability and accuracy of food records decreases with increasing recording periods, and since elite athletes tend to keep their dietary intakes relatively stable over a microcycle (*personal observations*), we believe this time period was sufficient to yield an idea of the dietary patterns of these athletes. Given the study design, and the fact that altitude training tends to enhance performance, we were unable to add a sea-level control group. Finally, we acknowledge that comparing the results of the current study to the findings of previous altitude training literature, where a different study population (calibre and sport), different altitude exposure (length and elevation) and different protocol (LHTH, LHTL, IHE, IHT in normobaric or hypobaric hypoxia) make it challenging to make direct comparisons across studies. Nonetheless, we believe that our study adds novel information to the existing literature on altitude training.

Conclusions

These data represent one of the largest investigations to date of the effects of various factors on the Hbmass response to LHTH altitude training in world-class endurance athletes, including a robust comparison of responses in males versus females, during a pre-season preparation camp before the 2016 Olympic Games. We showed that females have significantly lower $\text{Hbmass}_{\text{pre}}$ than males, with a negative correlation between $\text{Hbmass}_{\text{pre}}$ and change in Hbmass over the camp. However, we would like to highlight the fact that despite previous expert opinions on the lack of effectiveness of altitude training in elite athletes, our cohort of world-class athletes were able to benefit from the hematological effects of the altitude camp despite being elite and possessing high initial Hbmass levels. Furthermore, our findings emphasize and confirm the previous findings on the importance of athlete health in the optimal hematological response to altitude exposure. Indeed, to our knowledge, we are the first to show that menstrual function is correlated with baseline Hbmass levels and that a higher risk score for low EA in females shows a trend to correlate with less favorable changes in Hbmass following altitude training. We also found a significant difference in the Hbmass response to altitude, where healthy athletes were able to increase Hbmass on average by 5.4% compared to an average decrease of -0.5% in those who were sick during the camp, although it should be emphasized that out small sample size (n=4) of sick athletes may have reduced the statistical power. Finally, we confirm previous findings of the importance of sufficient exposure to hypoxia on hematological adaptations to altitude, where increasing hours of exposure seem to provide increasing hematological benefits independent of initial Hbmass levels.

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CONFLICTS OF INTEREST

The authors and funding agents do not have any conflicts of interests.

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FIGURE LEGENDS

Figure 1. Correlation between pre-camp hemoglobin mass ($Hbmass_{pre}$) and the relative change in Hbmass (%ΔHbmass) in females (A) and males (B). *Open circles*, low hypoxic dose group (<1200km.h); *open triangles,* moderate hypoxic dose group (1200-1400 km.h); *open squares*, high hypoxic dose group (>1400 km.h).

Figure 2. Differences in the percentage hemoglobin mass response (%ΔHbmass) to altitude in low (LOW: <500 hours of exposure, corresponds to <21 days at 2135 m), moderate (MOD: 500-600 hours of exposure, corresponds to 21-25 days at 2135 m) and high (HIGH: >600hours of exposure, corresponds to >25 days at 2135 m) hypoxic exposure groups in females and males. * $p < 0.05$, ** $p < 0.01$ significant difference between groups.

Figure 3. The magnitude of percentage change in hemoglobin mass (%ΔHbmass) in athletes who were not sick or injured during the altitude camp (healthy athletes; *white bar*) and athletes who were sick or injured during the camp (*black bar*). *** $p < 0.001$ significant difference between groups

TABLES

Table 1. Athlete characteristics, dietary and training data, iron status parameters and Hbmass outcomes in elite female and male distance athletes. Values are means ± SD.

	Females (n=23)	Males (n=15)
Athlete characteristics		
Age (yr)	26.0 ± 3.2	27.2 ± 4.1
Height (m) ***	1.68 ± 0.05	1.80 ± 0.06
Weight pre (kg) ***	54.1 ± 4.5	68.0 ± 5.9
Weight post (kg) ***	53.7 ± 4.5	68.2 ± 5.8
Body fat (%) ***	11.7 ± 2.7	6.7 ± 1.2
Baseline IAAF score	1113 ± 39	1109 ± 45
Post IAAF score $^{\# \$\$}$	1090 ± 39	1072 ± 67
Altitude camp activities		
EA (kcal/kg FFM/day)	33 ± 7	36 ± 6
Iron supplement (mg elemental iron)	110 ± 61	142 ± 68
Dietary iron ($mg.d^{-1}$) **	16.6 ± 5.1	24.7 ± 8.6
Running ($km \cdot wk^{-1}$) *	94 ± 27	114 ± 30
TRIMP (AU)	1998 ± 601	2363 ± 1424
Hypoxic dose ($km.h^{-1}$) *	1180 ± 193	1038 ± 235
Iron status parameters		
Pre serum iron	121 ± 42	112 ± 31
Post serum iron	134 ± 44	113 ± 58
Pre serum ferritin	87 ± 50	106 ± 37
Post serum ferritin $^{\#\#\#}$	83 ± 45	82 ± 24
Hbmass parameters		
$Hbmass_{pre}$ (g) ***	646 ± 57	979 ± 103
$Hbmass_{post}$ (g) *** $^{\#\#\# \$\$\$}$	681 ± 67	1013 ± 109
$Hbmass_{pre}$ (g/kg) ***	12.0 ± 1.0	14.4 ± 1.1
$Hbmass_{post}$ (g/kg) *** $^{\#\#\# \$\$\$}$	12.7 ± 0.9	14.9 ± 1.0
ΔHbmass (g)	36 ± 25	34 ± 28
ΔHbmass (g/kg) *	0.7 ± 0.5	0.4 ± 0.4
%ΔHbmass (g) *	5.5 ± 3.8	3.4 ± 3.0
%ΔHbmass (g/kg) **	6.2 ± 4.0	3.2 ± 3.3

Baseline IAAF score, IAAF score (International Association of Athletics Federations scoring table 2011) prior to the camp; Post IAAF score, race IAAF score in the three-week post-camp period; EA, energy availability; TRIMP, training impulse; AU, arbitrary unit; Hbmass, hemoglobin mass; ∆Hbmass, absolute change in Hbmass; %∆Hbmass, relative change in Hbmass. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ significant difference between females and males; $^{\$}$ $p<0.05$, $^{\$\$}$ $p<0.01$, $^{\$\$\$}$ $p<0.001$ significant difference from pre to post in females; $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$, $^{\#\#\#}$ $p<0.001$ significant difference from pre to post in males

FIGURES

Figure 1.

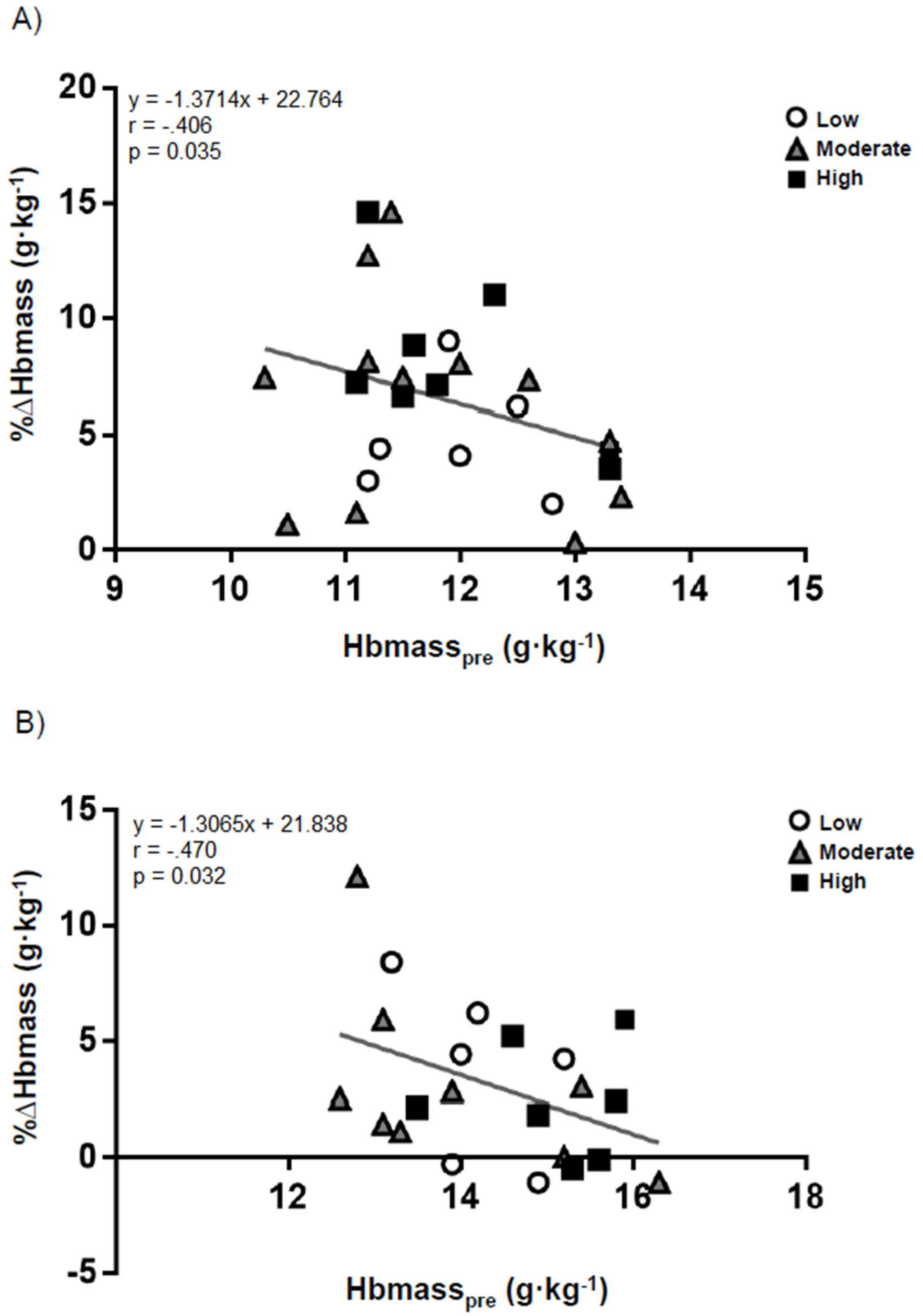

Figure 2.

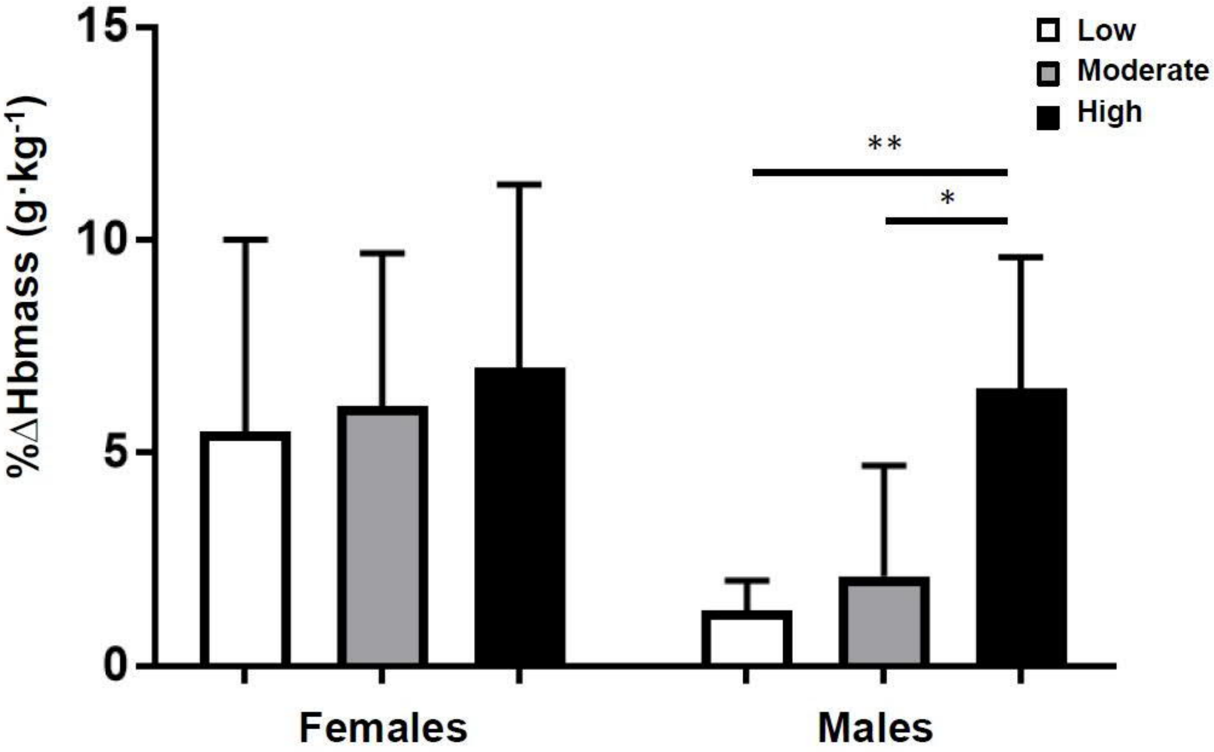

Figure 3.