This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.

Author(s): Heikura, Ida A.; Burke, Louise M.; Bergland, Dan; Uusitalo, Arja L. T.; Mero, Antti; Stellingwerff, Trent

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

Year: 2018

Version: Accepted version (Final draft)

Copyright: © Human Kinetics, 2018.

Rights: In Copyright

Rights url: <http://rightsstatements.org/page/InC/1.0/?language=en>

Please cite the original version:

Heikura, I. A., Burke, L. M., Bergland, D., Uusitalo, A. L. T., Mero, A., & Stellingwerff, T. (2018). 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. *International Journal of Sports Physiology and Performance*, 13(8), 1090-1096.
<https://doi.org/10.1123/ijsp.2017-0547>

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

Submission type: Original investigation

Authors:

Ida A. Heikura^{1,2}, Louise M. Burke^{1,2}, Dan Bergland³, Arja L. T. Uusitalo^{4,5}, Antti A. Mero⁶, and Trent Stellingwerff⁷

Institutions:

¹ Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, Australia 3000

² Sports Nutrition, Australian Institute of Sport, Canberra, Australia 2616

³ Hypo2 High Performance Sport Center, Flagstaff (AZ), USA

⁴ Department of Clinical Physiology and Nuclear Medicine, HUS Medical Imaging Center, Helsinki

⁵ University of Central Hospital and University of Helsinki, Finland

⁶ Biology of Physical Activity, University of Jyväskylä, Finland

⁷ Canadian Sport Institute Pacific, Victoria, Canada

Address for correspondence:

Ida Heikura, M.Sc.

Address: Level 5, 215 Spring Street, Melbourne, VIC 3000, Australia

Telephone: +61 422 863 010

E-mail: ida.heikura@myacu.edu.au

Running head: Health status influences Hbmass response

Word count: 4171 words

Abstract count: 231 words

Number of references: 40

Number of figures and tables: 1 table and 3 figures

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 ($\%\Delta\text{Hbmass}$) was significantly greater in females ($6.2\pm4.0\%$, $p<0.001$) than in males ($3.2\pm3.3\%$, $p=0.008$). ($\%\Delta\text{Hbmass}$) showed a dose-response with hypoxic exposure (3.1 ± 3.8 vs 4.9 ± 3.8 vs $6.8\pm3.7\%$; $p=0.013$). $\text{Hbmass}_{\text{pre}}$ 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, $\%\Delta\text{Hbmass}$ was significantly less in sick (n=4, $-0.5\pm0.4\%$) versus healthy (n=44 athletes; $5.4\pm3.8\%$; $p<0.001$). 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 $\%\Delta\text{Hbmass}$ ($r=-.353$, $p=0.07$). **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

60 Many high performance endurance athletes undertake specialized forms of altitude training.
61 The lack of agreement regarding the effects of altitude training on hematology and performance
62 is partially explained by various differences in the methodology of altitude training studies¹. For
63 example, there are different modalities of altitude exposure, with several common options being
64 Live High-Train High (LHTH) or Live High-Train Low (LHTL) with hypobaric or normobaric
65 hypoxia, or intermittent hypoxic exposure at rest (IHE) or during training (IHT)². Nevertheless,
66 irrespective of changes in performance, a change in hemoglobin mass (Hbmass) is considered an
67 objective and relatively easily measured outcome of altitude exposure within a standardized
68 altitude training protocol, with typical increase of 2–5% following a block of altitude training
69 being reported³⁻⁷. However, the mechanisms associated with optimizing Hbmass increases are
70 multifactorial and include the type of altitude modality, the duration and level of exposure (also
71 termed hypoxic dose⁸) and possibly the initial Hbmass level⁹. Indeed, there is consistent
72 evidence of a progressive increase in Hbmass with three weeks of altitude training^{3,7,10}, with
73 supportive factors including the adequacy of baseline ferritin concentrations and doses of iron
74 supplementation^{11,12}. Meanwhile, only two studies have reported that injury and/or illness tends
75 to negatively affect Hbmass changes^{6,13}. One aspect of athlete health is optimal energy
76 availability (EA), which is defined as the dietary energy available to support body function once
77 the energy cost of exercise has been deducted from daily energy intake¹⁴. Low EA has
78 detrimental effects on many areas of health and training adaptation, including impairment of
79 menstrual status, protein synthesis and iron status and an increased risk of illness and injury¹⁵.
80 However, to our knowledge no study has investigated the effects of symptoms of low EA on
81 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 protocols⁹. 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 world 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 levels²⁰. 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 capacity²¹ 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 2011²²) 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- (Hbmass_{pre}) and post-altitude (Hbmass_{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-Q²³).

Hemoglobin mass

Total Hbmass was measured with the adapted two-minute carbon monoxide (CO) rebreathing protocol²⁴. 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, % Δ Hbmass).

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 (sRPE²⁵). 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 values²⁶.

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 $\text{kcal} \cdot \text{kg}^{-1} \text{ FFM} \cdot \text{day}^{-1}$ ¹⁴. Detailed information on methodology used and outcomes of these analyses is reported elsewhere²⁷.

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 $\geq$ three consecutive menses). Hypoxic dose⁸ at 2135m was stratified into low (LOW: $<1200 \text{ km.h}$; $<23 \text{ days}$; $n=27$), moderate (MOD: $1200\text{--}1400 \text{ km.h}$; $23\text{--}27 \text{ days}$; $n=13$) and high (HIGH: $>1400 \text{ km.h}$; $>27 \text{ days}$; $n=8$) groups. For further comparison, athletes were also categorized based on hours of exposure³ as follows: low ($<500 \text{ hours}$; $n=18$), moderate ($500\text{--}600 \text{ hours}$; $n=14$) and high ($>600 \text{ hours}$; $n=16$). Analysis of Covariance (ANCOVA) was used to test the differences in the change in Hbmass with different hypoxic doses when controlling for $\text{Hbmass}_{\text{pre}}$. 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 $\pm$ 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. $\% \Delta \text{Hbmass}$ (g/kg) was $4.9 \pm 4.0\%$ ($p < 0.001$) 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 ($+3.7 \pm 3.9\%$; $1013 \pm 137 \text{ km.h}$) increased relative Hbmass significantly less than MOD ($+7.3 \pm 3.4\%$; $1320 \pm 70 \text{ km.h}$; $p = 0.018$) and, although not statistically significant, less than HIGH ($4.8 \pm 3.6\%$; $1563 \pm 95 \text{ km.h}$) 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 $\% \Delta \text{Hbmass}$ 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 % Δ Hbmass (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 % Δ Hbmass. 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 (% Δ Hbmass +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 % Δ Hbmass between sick and healthy athletes became non-significant (3.3 ± 6.3 vs $5.1\pm3.6\%$, respectively; $p=0.11$). No correlations were found between baseline IAAF score, change in IAAF from baseline to post-camp or EA and % Δ Hbmass. In females, LEAF-Q score showed a strong trend for a negative correlation with % Δ Hbmass (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 paper²⁷), body composition parameters or BMD (data in our companion paper²⁷) 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, Hbmass_{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 Hbmass_{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 periods⁶, with varying altitude exposures²⁸ and/or use of simulated hypoxia¹¹, or in the absence of measures of changes in Hbmass²⁹⁻³³. 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 training²⁰, 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 hypoxia³⁴. 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 Hbmass_{pre} levels (14.4 vs 12.0 g/kg; Table 1), although this is just speculation. Nevertheless, we found a negative relationship between Hbmass_{pre} and change in Hbmass (Δ Hbmass; 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 finding⁶.

The magnitude and length of exposure to hypoxia are crucial for altitude-induced hematological adaptations. Based on several studies, an increase of 1% per week¹⁰ or 1% per 100 hours of exposure³ can be expected, although an exponential model of hypoxic dose has also been proposed by Garvican-Lewis and colleagues⁸. 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 Δ Hbmass. 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 supplementation³⁹. 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 outcomes²⁷) on adaptations to altitude training, since it has previously been shown to impair health and performance¹⁵, including processes such as the protein synthetic response to exercise⁴⁰ 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 ($\sim 45 \text{ kcal.kg BM}^{-1}.\text{d}^{-1}$) to low ($<30 \text{ kcal.kg BM}^{-1}.\text{d}^{-1}$)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 perturbations²⁷. 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 $\text{Hbmass}_{\text{pre}}$ in amenorrheic vs eumenorrheic females (amenorrhea signals of chronic low EA¹⁵)

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 females²³), 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 Hbmass_{pre} than males, with a negative correlation between Hbmass_{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 our 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.

ACKNOWLEDGEMENTS

The study was designed by IH, TS, AM, AU and LB; data were collected and analyzed by IH, TS, DB and LB; data interpretation and manuscript preparation were undertaken by IH, TS, AM, AU, DB and LB. All authors approved the final version of the paper. This study was financially supported by grants from the Canadian Sport Institute, Australian Catholic University/Australian Institute of Sport, Finnish Sport Institute Foundation, and Finnish Sport Research Foundation. The authors wish to thank Hypo2 High Performance Sport Center and Northern Arizona Orthopaedics and Prof. Jennifer Peat for technical analysis and statistical assistance. Finally, the authors wish to thank all the participants for volunteering for the study.

CONFLICTS OF INTEREST

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

REFERENCES

1. Lundby C, Robach P. Does 'altitude training' increase exercise performance in elite athletes? *Exp Physiol*. 2016;101(7):783-788.
2. Millet GP, Roels B, Schmitt L, Woorons X, Richalet JP. Combining hypoxic methods for peak performance. *Sports Med*. 2010;40(1):1-25.
3. 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(1):95-103.
4. Pottgiesser T, Garvican LA, Martin DT, Featonby JM, Gore CJ, Schumacher YO. Short-term hematological effects upon completion of a four-week simulated altitude camp. *Int J Sports Physiol Perform*. 2012;7(1):79-83.
5. 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(6):1938-1945.
6. Wachsmuth NB, Volzke C, Prommer N, et al. The effects of classic altitude training on hemoglobin mass in swimmers. *Eur J Appl Physiol*. 2013;113(5):1199-1211.
7. Garvican-Lewis LA, Halliday I, Abbiss CR, Saunders PU, Gore CJ. Altitude exposure at 1800 m increases haemoglobin mass in distance runners. *J Sports Sci Med*. 2015;14(2):413-417.
8. Garvican-Lewis LA, Sharpe K, Gore CJ. Time for a new metric for hypoxic dose? *J Appl Physiol (1985)*. 2016;121(1):352-355.

- 416 9. Rasmussen P, Siebenmann C, Diaz V, Lundby C. Red cell volume expansion at altitude: A
417 meta-analysis and monte carlo simulation. *Med Sci Sports Exerc.* 2013;45(9):1767-1772.
- 418 10. Clark SA, Quod MJ, Clark MA, Martin DT, Saunders PU, Gore CJ. Time course of
419 haemoglobin mass during 21 days live high:Train low simulated altitude. *Eur J Appl Physiol.*
420 2009;106(3):399-406.
- 421 11. Govus AD, Garvican-Lewis LA, Abbiss CR, Peeling P, Gore CJ. Pre-altitude serum ferritin
422 levels and daily oral iron supplement dose mediate iron parameter and hemoglobin mass
423 responses to altitude exposure. *PLoS One.* 2015;10(8):e0135120.
- 424 12. Garvican-Lewis LA, Govus AD, Peeling P, Abbiss CR, Gore CJ. Iron supplementation and
425 altitude: Decision making using a regression tree. *J Sports Sci Med.* 2016;15(1):204-205.
- 426 13. Gough CE, Sharpe K, Garvican LA, Anson JM, Saunders PU, Gore CJ. The effects of injury
427 and illness on haemoglobin mass. *Int J Sports Med.* 2013;34(9):763-769.
- 428 14. Loucks AB, Kiens B, Wright HH. Energy availability in athletes. *J Sports Sci.* 2011;29 Suppl
429 1:S7-15.
- 430 15. Mountjoy M, Sundgot-Borgen J, Burke L, et al. The IOC consensus statement: Beyond the
431 female athlete Triad—Relative energy deficiency in sport (RED-S). *British Journal of Sports*
432 *Medicine.* 2014;48(7):491-497.
- 433 16. Heinicke K, Heinicke I, Schmidt W, Wolfarth B. A three-week traditional altitude training
434 increases hemoglobin mass and red cell volume in elite biathlon athletes. *Int J Sports Med.*
435 2005;26(5):350-355.

- 436 17. Garvican LA, Pottgiesser T, Martin DT, Schumacher YO, Barras M, Gore CJ. The
437 contribution of haemoglobin mass to increases in cycling performance induced by simulated
438 LHTL. *Eur J Appl Physiol.* 2011;111(6):1089-1101.
- 439 18. Gore CJ, Hahn A, Rice A, et al. Altitude training at 2690m does not increase total
440 haemoglobin mass or sea level VO₂max in world champion track cyclists. *J Sci Med Sport.*
441 1998;1(3):156-170.
- 442 19. Pottgiesser T, Ahlgrim C, Ruthardt S, Dickhuth HH, Schumacher YO. Hemoglobin mass
443 after 21 days of conventional altitude training at 1816 m. *J Sci Med Sport.* 2009;12(6):673-675.
- 444 20. Robach P, Lundby C. Is live high-train low altitude training relevant for elite athletes with
445 already high total hemoglobin mass? *Scand J Med Sci Sports.* 2012;22(3):303-305.
- 446 21. Gore CJ, Clark SA, Saunders PU. Nonhematological mechanisms of improved sea-level
447 performance after hypoxic exposure. *Med Sci Sports Exerc.* 2007;39(9):1600-1609.
- 448 22. IAAF Scoring Tables 2011. Accessed on 31 Jan 2016. Retrieved from
449 <https://www.iaaf.org/about-iaaf/documents/technical>
- 450 23. Melin A, Tornberg AB, Skouby S, et al. The LEAF questionnaire: A screening tool for the
451 identification of female athletes at risk for the female athlete triad. *Br J Sports Med.*
452 2014;48(7):540-545.
- 453 24. Schmidt W, Prommer N. The optimised CO-rebreathing method: A new tool to determine
454 total haemoglobin mass routinely. *Eur J Appl Physiol.* 2005;95(5-6):486-495.

- 455 25. Foster C. Monitoring training in athletes with reference to overtraining syndrome. *Med Sci*
456 *Sports Exerc.* 1998;30(7):1164-1168.
- 457 26. Seiler KS, Kjellerud GO. Quantifying training intensity distribution in elite endurance
458 athletes: Is there evidence for an "optimal" distribution? *Scand J Med Sci Sports.* 2006;16(1):49-
459 56.
- 460 27. Heikura IA, Uusitalo AL, Stellingwerff T, Berglund D, Mero AA, Burke LM. Low energy
461 availability is difficult to assess but outcomes have large impact on bone injury rates in elite
462 distance athletes. *Int J Sport Nutr Exerc Metab* In press
- 463 28. Rodriguez FA, Iglesias X, Ferlic B, et al. Altitude training in elite swimmers for sea level
464 performance (altitude project). *Med Sci Sports Exerc.* 2015;47(9):1965-1978.
- 465 29. Woods AL, Garvican-Lewis LA, Rice A, Thompson KG. 12 days of altitude exposure at
466 1800 M does not increase resting metabolic rate in elite rowers. *Appl Physiol Nutr Metab.*
467 2017;42(6):672-676.
- 468 30. Stray-Gundersen J, Chapman RF, Levine BD. "Living high-training low" altitude training
469 improves sea level performance in male and female elite runners. *J Appl Physiol (1985).*
470 2001;91(3):1113-1120.
- 471 31. Chapman RF, Karlsen T, Resaland GK, et al. Defining the "dose" of altitude training: How
472 high to live for optimal sea level performance enhancement. *J Appl Physiol (1985).*
473 2014;116(6):595-603.

- 474 32. Sharma AP, Saunders PU, Garvican-Lewis LA, et al. The effect of training at 2100-m
475 altitude on running speed and session rating of perceived exertion at different intensities in elite
476 middle-distance runners. *Int J Sports Physiol Perform.* 2017;12(Suppl 2):S2147-S2152.
- 477 33. Sperlich B, Achtzehn S, de Marees M, von Papen H, Mester J. Load management in elite
478 german distance runners during 3-weeks of high-altitude training. *Physiol Rep.*
479 2016;4(12):10.14814/phy2.12845.
- 480 34. Hauser A, Troesch S, Steiner T, Brocherie F, Girard O, Saugy JJ, Schmitt GP, Wehrlin JJP.
481 Do male athletes with already high initial haemoglobin mass benefit from 'live high-train low'
482 altitude training? *Exp Physiol.* 2017;Oct11 doi: 10.1113/EP086590. [Epub ahead of print]
- 483 35. Woods AL, Sharma AP, Garvican-Lewis LA, Saunders PU, Rice AJ, Thompson KG. Four
484 weeks of classical altitude training increases resting metabolic rate in highly trained middle-
485 distance runners. *Int J Sport Nutr Exerc Metab.* 2017;27(1):83-90.
- 486 36. Gough CE, Saunders PU, Fowlie J, et al. Influence of altitude training modality on
487 performance and total haemoglobin mass in elite swimmers. *Eur J Appl Physiol.*
488 2012;112(9):3275-3285.
- 489 37. Garvican-Lewis LA, Schumacher YO, Clark SA, et al. Stage racing at altitude induces
490 hemodilution despite an increase in hemoglobin mass. *J Appl Physiol (1985).* 2014;117(5):463-
491 472.

492 38. McLean BD, Buttifant D, Gore CJ, White K, Kemp J. Year-to-year variability in
 493 haemoglobin mass response to two altitude training camps. *Br J Sports Med.* 2013;47 Suppl
 494 1:i51-8.

495 39. Gore CJ, Sharpe K, Garvican-Lewis LA, Saunders PU, Humberstone CE, Robertson EY,
 496 Wachsmuth NB, Clark SA, McLean BD, Friedmann-Bette B, Neya M, Pottgiesser T,
 497 Schumacher YO, Schmidt WF. Altitude training and haemoglobin mass from the optimised
 498 carbon monoxide rebreathing method determined by a meta-analysis. *Br J Sports Med.*
 499 2013;47(S1):i31-39.

500 40. Areta JL, Burke LM, Camera DM, et al. Reduced resting skeletal muscle protein synthesis is
 501 rescued by resistance exercise and protein ingestion following short-term energy deficit. *Am J*
 502 *Physiol Endocrinol Metab.* 2014;306(8):E989-97.

503

FIGURE LEGENDS

Figure 1. Correlation between pre-camp hemoglobin mass ($Hbmass_{pre}$) and the relative change in Hbmass ($\% \Delta Hbmass$) 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 ($\% \Delta Hbmass$) 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 ($\% \Delta Hbmass$) 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 $\pm$ SD.

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