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Impact of baseline serum ferritin and supplemental iron on altitude-induced hemoglobin mass response in elite athletes

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Abstract

The present study explored the impact of pre-altitude serum (s)-ferritin and iron supplementation on changes in hemoglobin mass (ΔHbmass) following altitude training. Measures of Hbmass and s-ferritin from 107 altitude sojourns (9-28 days at 1800-2500 m) with world-class endurance athletes (males n=41, females n=25) were analyzed together with iron supplementation and self-reported illness. Altitude sojourns with a hypoxic dose [median (range)] of 1169 (912) km·hrs increased Hbmass (mean ± SD) 36 ± 38 g (3.7 ± 3.7%, $p<0.001$) and decreased s-ferritin -11 (190) $\mu g \cdot L^{-1}$ ($p=0.001$). Iron supplements [27 (191) $mg \cdot day^{-1}$] were used at 45 sojourns (42%), while only 11 sojourns (10%) were commenced with s-ferritin <35 µg/L. Hbmass increased by 4.6 ± 3.7%, 3.4 ± 3.3%, 4.2 ± 4.3% and 2.9 ± 3.4% with pre-altitude s-ferritin ≤ 35 $\mu g \cdot L^{-1}$, 36-50 $\mu g \cdot L^{-1}$, 51-100 $\mu g \cdot L^{-1}$ and >100 $\mu g \cdot L^{-1}$, respectively, with no group difference ($p=0.400$). Hbmass increased by 4.1 ± 3.9%, 3.0 ± 3.0% and 3.7 ± 4.7% without, ≤ 50 $mg \cdot day^{-1}$ or >50 $mg \cdot day^{-1}$ supplemental iron, respectively ($p=0.399$). Linear mixed model analysis revealed no interaction between pre-altitude s-ferritin and iron supplementation on ΔHbmass ($p=0.906$). However, each 100 km·hrs increase in hypoxic dose augmented ΔHbmass by an additional 0.4% (95% CI: 0.1-0.7%; $p=0.012$), while each 1 $g \cdot kg^{-1}$ higher pre-altitude Hbmass reduced ΔHbmass by -1% (-1.6 to -0.5; $p<0.001$), and illness lowered ΔHbmass by -5.7% (-8.3 to -3.1%; $p<0.001$).

In conclusion, pre-altitude s-ferritin or iron supplementation were not related to the altitude-induced increase in Hbmass (3.7%) in world-class endurance athletes with clinically normal iron stores.

Key words: hypobaric hypoxia, erythropoiesis, hemoglobin mass, iron, ferritin cut-off, elite athletes

Introduction

Periodic training at moderate altitude is commonly practiced by endurance athletes to enhance hemoglobin mass (Hbmass), and thereby improve the oxygen-carrying capacity of the blood, either in preparation for competitions at altitude and/or to improve endurance performance at sea level [1]. However, Hbmass responses to altitude training are notoriously heterogeneous, as some athletes demonstrate large effects while others have none [2]. Among several factors, adequate iron availability appears necessary for a beneficial Hbmass response [3].

The hypoxia-induced increase in erythropoiesis augments the iron demand, since each gram of hemoglobin (Hb) contains ~3.4 mg of iron [4]. Iron demand seems to increase three- to fivefold at altitude [5]. This additional iron can be drawn from the body's iron stores (s-ferritin) or obtained from dietary iron or iron supplements, or a combination of these. Iron injections, although highly efficient for rapid improvements in iron status [6] or in the presence of illness in the gastrointestinal tract [7], do not seem to be superior to oral iron supplements to support altitude-induced increase in Hbmass [8], and are not usually recommended for athletes due to general "no needle" policy in many international sports federations.

A typical increase in total Hbmass of 30-50 g following a successful altitude sojourn [9] theoretically requires 100-170 mg additional iron. If dietary iron was the only iron source for the enhanced Hb production, this would require 30-51 mg iron from mixed dietary sources [14-18% iron bioavailability [10]] daily during a three-week altitude sojourn. This is higher than the usual iron intake of endurance athletes [12-20 mg iron per day [11]], suggesting that iron supplementation at altitude is warranted. However, iron absorption at altitude is enhanced via a reduction in the major iron regulatory peptide, hepcidin [12]. Indeed, iron absorption in hypoxia seems to be three times higher than in normoxia, at least in rodents [13]. Furthermore, athletes appear to increase their dietary iron intake at altitude, but they only manage to cover approximately half of the calculated iron demand [14]. Consequently, iron stores and internal iron recirculation most likely also contribute to the bone marrow requirements for erythropoiesis, as demonstrated by a reduced s-ferritin following altitude exposure [15,16]. Yet, the relative contribution of iron from different origins (s-ferritin vs dietary iron vs supplements) during accelerated erythropoiesis is unknown. Some authors have suggested that the incorporation of iron to the erythrocytes might be more efficient from iron supplements than stored iron [17], while others speculate that the iron kinetics is quicker from stored iron, which does not need to traverse the intestine first [18].

Furthermore, the cut-off value for pre-altitude s-ferritin for when iron supplementation should be recommended and which supplementation strategy is most efficient are still unclear [3,19].

Previous studies suggest that poor iron status, defined as s-ferritin < 20 µg·L^{-1} for females and <30 µg·L^{-1} for males, compromises Hbmass response to altitude exposure [18]. However, substantial Hbmass increases can be achieved even with low s-ferritin, given adequate iron supplementation is implemented at altitude [17]. Accordingly, recent dietary recommendations for altitude training suggest that iron supplementation should be considered for all athletes [3,17,20].

There is a shortage of studies investigating the association between s-ferritin and Hbmass responses to terrestrial altitude training in a true elite athlete population with iron supplement doses lower than 100 mg·day^{-1}. Thus, we aimed to investigate the influence of pre-altitude s-ferritin, with and without iron supplementation, on changes in Hbmass following altitude training (1800-2500 m) in world-class endurance athletes.

Methods

Compliance with Ethical Standards

This study was approved by the Norwegian Regional Ethics Committee (REK number 142504) and all participants provided written informed consent after receiving comprehensive oral and written information about the study. The data was treated and stored in accordance with the Norwegian center for research data (notification form 782326).

Overview of study design

This is a retrospective observational study. De-identified hematological data from 66 elite endurance athletes were extracted from the Norwegian Olympic Sport Center's medical database and laboratory reports between October 2015 and March 2020. This cohort includes some data from athletes (n=31) that participated in our previous study investigating the impact of antioxidant-rich foods on altitude training [9]. Information about the highest measured VO_{2max} and use of iron supplements at altitude were collected retrospectively, where this information was not recorded in laboratory files.

Study subjects

All national team athletes who had measured their Hbmass before and after an altitude sojourn between October 2015 to March 2020 at Norwegian Olympic Sports Centre or at Norwegian School of Sports Sciences were invited to participate in the study. Overall, 66 of 68 invited athletes from eight different endurance sports (n=41 males, n=25 females) gave their informed consent to analyze their data (**Table 1**). More than one third of the athletes in this cohort (n=25) had won a medal in either Olympic Games or World Championships.

Altitude sojourns

The 107 individual altitude sojourns included in this study were conducted at eight different sites: Sierra Nevada, Spain (2320 m), Lesotho, South Africa (2000 m), Val Senales, Italy (2000 m), Aspen, USA (2500 m), Livigno, Italy (1900 m), Font Romeau, France (1800 m), Seiser Alm, Italy (1850 m), Park City, USA (2130 m) and Teide, Tenerife, Spain (2100 m) with a median(range) elevation of 2320 (700) m above sea level. The duration of altitude sojourns was 21(19) days, providing a hypoxic dose of 1169 (912) hrs·km. Three athletes (4%) in the cohort were registered with 5 altitude sojourns, four athletes (6%) participated in four altitude sojourns, three athletes (4%) in three altitude sojourns, 12 athletes (18%) in two altitude sojourns, while the rest of the athletes (68%) took part in one altitude sojourn. Most of the altitude sojourns were conducted during the athletes' general preparation phase. Only 6 (6%) altitude sojourns were reported with illness. Forty-five (42%) of the athletes reported

use of iron supplements at altitude, with a median (range) dose of 27(191) mg·day^{-1}, with no difference between the sexes (p=0.374).

Total hemoglobin mass (Hbmass)

Hbmass assessments at Norwegian School of Sport Sciences were measured in duplicate using a slightly modified version of the optimized carbon monoxide (CO) rebreathing method [21]. The detailed protocol and equipment is outlined in Skattebo *et al* [22]. Hbmass assessments at the Norwegian Olympic Sports Center were conducted using an automated version of the CO rebreathing method (OpCO; Detalo Instruments, Denmark), as previously described [23]. The assessments were conducted within three days following descend from altitude, except for 13 occasions where the athletes were tested on day 13 due to logistical challenges. Previous studies have shown that Hbmass remains elevated for up 10-14 days [15,24]. The coefficient of variance (CV) for Hbmass assesment was 1.62% at Norwegian School of Sport Sciences determined by duplicate measures pre-and post-altitude for 31 altitude sojourns, as previously reported [9]. While CV for Hbmass assesment at Norwegian Olympic Sports Center determined by 10 duplicate measures pre-and post-altitude independent of the current study was 1.30%.

Iron parameters

Blood samples before and after altitude sojourns were collected from a peripheral vein into an EDTA-treated and a serum separation tube (SST). The EDTA sample was analyzed for Hb concentration ([Hb]) using Sysmex XN-9000 (Sysmex, Japan). The SST was placed at room temperature for coagulation (~20° C) for 30 minutes, centrifuged for 10 minutes at 1500 G, and placed at + 4°C until analyzed for s-ferritin with immunoturbimetric assay (Advia Chemistry XPT; Siemens, Healthineers, Erlangen, Germany). The analytical CV for the assay is 2.5%.

Statistical analysis

Normally distributed data are presented as mean ± standard deviation (SD), non-normally distributed data as median and range, and categorical data as ranks and percentages. A paired T-test was used to determine whether there were changes in any of the variables from before to after the altitude sojourns. A two samples T-test was used to investigate sex differences in altitude-induced changes in Hbmass, s-ferritin and [Hb]. The effect of baseline s-ferritin and iron supplementation dose on the change in Hbmass were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Baseline ferritin was stratified into three categories (≤ 35 µg·L^{-1}, 36-100 µg·L^{-1} and >100 µg·L^{-1}) and iron

supplement dose was categorized accordingly: none, low (≤ 50 mg·day^{-1}), moderate/high (> 50 mg·day^{-1}). The strength and direction of the relationship between two variables were assessed using Pearson (r) or Spearman (rho) correlation. Partial correlation was used to assess the association between Hbmass change and iron dose controlling for baseline s-ferritin. To account for the combination of single and repeated data (athletes with multiple altitude sojourns), a linear mixed model was constructed with the log-transformed (natural logarithm) change in Hbmass as the dependent variable. The fixed effects in the model were hypoxic dose (km·hrs, numeric linear), self-reported health status at altitude (two categories: healthy, ill), pre-altitude Hbmass (g·kg body^{-1} weight (BW)), calculated as the difference from the sex-specific mean (numeric linear), and the interaction between pre-altitude s-ferritin (two categories: <35 µg·L^{-1}, ≥35 µg·L^{-1}) and iron supplementation (2 categories: yes, no). To account for repeated data, the model included a repeated statement for subject ID and the altitude sojourn's numeric time to account for within-subject correlated errors. Spatial power was chosen as the covariance structure to specify a decreasing correlation with the Euclidean distance (the time variable) between sojourns. Estimated marginal means and their 95% confidence intervals (CI) were calculated for the four groups indicated by the interaction term (pre-altitude s-ferritin × iron supplementation) at mean hypoxic dose, sex-specific mean pre-altitude Hbmass (g·kg^{-1} BW), and for no reported illness during the altitude sojourns, followed by multiple comparisons using the Tukey-Kramer method. Estimates were back-transformed and expressed as percent. All statistical analysis was performed with IBM SPSS Statistics 26.0 (IBM, Armonk, NY), except the linear mixed model that was carried out in SAS University Edition (SAS Studio 3.8, SAS Institute Inc., Cary, NC). P-values were considered significant at < 0.05. The number of participants (n) is provided for each individual study component.

The magnitude of the change, i.e. effect size (ES), in pre-altitude versus post-altitude values for [Hb], s-ferritin and absolute and relative Hbmass were determined using Cohen’s d or Rosenthal’s r (for non-parametric variables).

Results

Hbmass response to altitude training

Altitude sojourns significantly increased Hbmass (mean ± SD) by 36 ± 38 g (3.7 ± 3.7%, ES=0.9, p<0.001) (**Table 2)**. There was a significant association between ΔHbmass (%) and the hypoxic dose expressed as km·hrs (r=0.401, n=107, p<0.001) (**Figure 1**). In contrast, the association between ΔHbmass and pre-altitude Hbmass was significant only when adjusted for body weight (r=-0.315, n=107, p=0.001), as illustrated in Figure 1. Seventy-seven (73 %) of the altitude sojourns led to a higher ΔHbmass (%) than the CV for the CO-rebreathing method in our labs.

Blood hemoglobin concentration and s-ferritin responses to altitude sojourns

The [Hb] increased by 0.7 ± 0.7 $g \cdot dL^{-1}$ (6.8%, ES=1.0, p<0.001) following altitude training sojourn, with no sex difference (Table 2). The concentration of s-ferritin decreased by [median (range)] -11 (190) $\mu g \cdot L^{-1}$ (ES=0.5, p=0.002, Table 2), with no sex difference. Of note, only one altitude sojourn was commenced with baseline s-ferritin < 20 $\mu g \cdot L^{-1}$.

Associations between pre-altitude s-ferritin, iron supplementation and ΔHbmass

Pre-altitude s-ferritin was significantly lower among the athletes who used iron supplements at altitude (74.5 ± 37.1 $\mu g \cdot L^{-1}$) as compared to those who did not (97.5 ± 51.8 $\mu g \cdot L^{-1}$, p=0.016). Also, there was a significant inverse association between pre-altitude s-ferritin and the daily iron supplement dose (r=-0.300, n=102, p=0.002). S-ferritin decreased by -15 ± 44% among the non-supplemented athletes (p<0.001) while it remained unchanged among the iron supplemented athletes (9 ± 28%, p=0.826), and Δs-ferritin was significantly different between the supplemented and non-supplemented athletes (p=0.003). There was no difference in ΔHbmass between the groups stratified by daily iron supplement dose [none, low (≤50 mg), medium/high (>50 mg)] ($F_{(2,104)}$=0.927, p=0.399, **Figure 2A**). Partial correlation did not reveal any association between baseline s-ferritin and ΔHbmass when adjusting for iron supplementation. The ΔHbmass was not associated with baseline s-ferritin (r=-0.100, n=102, p=0.319). Furthermore, exploring the difference in ΔHbmass based on baseline s-ferritin [<35 $\mu g \cdot L^{-1}$ (n=11), 36-50 $\mu g \cdot L^{-1}$ (n=12), 51-100 $\mu g \cdot L^{-1}$ (n=42) and >100 $\mu g \cdot L^{-1}$ (n=37)] showed no difference between the categories ($F_{(3,98)}$=0.992, p=0.400, **Figure 2B**). See details about iron supplement use and daily dose at the various pre-altitude s-ferritin categories in **Supplemental Table 1**.

Linear mixed model did not show any effects of iron supplementation (supplemented vs. non-supplemented) on ΔHbmass (%) for either those commencing the altitude sojourn with low baseline s-ferritin (≤ 35 $\mu g \cdot L^{-1}$; p=0.918) or moderate/high baseline s-ferritin (> 35 $\mu g \cdot L^{-1}$;

p=1.0), as shown in **Table 3**. However, as a secondary finding, the linear mixed model revealed a significant association between ΔHbmass (%) and hypoxic dose, baseline relative Hbmass and self-reported illness. Each 100 km·hrs increase in hypoxic dose was associated with 0.4% (95% CI : 0.1 to 0.7%; p=0.012) additional increase in ΔHbmass, a 1 $g \cdot kg^{-1}$ BW higher pre-altitude Hbmass reduced the ΔHbmass by -1.0% (95% CI : -1.6 to -0.5%; p<0.001) while illness at altitude was associated with a -5.7% (-8.32 to -3.1%; p<0.001) reduction in the ΔHbmass compared to altitude sojourns associated with no illness (i.e. a -1.4 ± 2.8% reduction compared with baseline).

Discussion

The main finding of the present study was that the altitude-induced increase in Hbmass was *not* associated with pre-altitude s-ferritin concentration or iron supplementation in a cohort of world-class endurance athletes with clinically normal iron status, although the fall in s-ferritin was prevented with the use of iron supplements. A secondary finding showed that ΔHbmass was positively associated with the hypoxic dose but negatively associated with relative Hbmass at baseline and self-reported illness at altitude.

Hbmass response to training at moderate altitude

The 3.7% increase in total Hbmass observed in the current study is more than four-fold of the calculated smallest worthwhile change [0.2SD; [25]] and larger than the typical error of measurement [CV: 1.3-1.6% [9,15]]. The observed Hbmass response is in line with previous studies of well-trained athletes exposed to a similar hypoxic dose (~1170 km·hrs): 3.1- 4.4% [26,27].

Impact of pre-altitude serum ferritin

We found no association between baseline s-ferritin and change in Hbmass, nor a significantly different ΔHbmass for those with baseline s-ferritin ≤ 35 $\mu g \cdot L^{-1}$ as compared to >35 $\mu g \cdot L^{-1}$. This is in line with previous studies showing no relationship between pre-altitude s-ferritin and the altitude-induced Hbmass response [8,28,29]. However, contrary to these findings, two previous studies have shown an impaired erythropoietic response to altitude in athletes with iron insufficiency (s-ferritin; <20 $\mu g \cdot L^{-1}$ for females and <30 $\mu g \cdot L^{-1}$ for males) [18,30]. Notably, in the current study, only one sojourn was commenced with pre-altitude s-ferritin < 20 $\mu g \cdot L^{-1}$, demonstrating a low prevalence of iron insufficiency in our cohort, possibly explaining this discrepancy. Our results demonstrate that baseline s-ferritin appears to be a poor predictor of ΔHbmass following altitude training in athletes with clinically normal iron stores, as suggested in a recent review [19]. However, it should be noted that low pre-altitude s-ferritin does not seem to be a limiting factor for altitude-induced Hbmass increase given adequate iron supplementation is provided [17,26]. Noteworthy, it is possible that alterations in blood volume may obscure the real changes in s-ferritin, especially in an altitude training context, given it is measured as a concentration variable. Thus, if possible, perhaps total s-ferritin (μg) should be the selected variable for the assessment of body iron stores (instead of s-ferritin concentration), just as total Hbmass is preferred to [Hb] for assessing total circulating Hb.

Impact of iron supplementation

We observed that s-ferritin fell only among the non-supplemented athletes. Similar findings have been reported by others, although with higher iron supplement doses [26]. Govus *et al* [26] reported that 210 mg of elemental iron was needed to increase s-ferritin following hypoxic exposure, while 105 mg merely limited the s-ferritin reduction. Thus, our study provides further support that iron supplementation is important for preventing a fall in s-ferritin at altitude, which may be especially important for athletes who plan multiple, closely spaced, altitude training sojourns. However, the present findings may also suggest that initiating an altitude sojourn with low vs high s-ferritin merely reflects the iron absorption efficacy, i.e., the impact that low iron status has on hepcidin and hence iron absoprtion [31,32].

The prevalence of iron supplement use in the present study was moderate (42%) with a relatively low daily dose (median: 27 mg elemental iron). By comparison, another study found that 92% of endurance athletes used iron supplements during hypoxic exposure, with an average daily dose of 119 mg elemental iron [26]. Indeed, iron supplements are both widely used and recommended for athletes training at altitude [3,33], although the recommendations in the literature as when to supplement (s-ferritin cut-offs for initiating iron supplementation) vary between <30 $\mu g \cdot L^{-1}$ and < 100 $\mu g \cdot L^{-1}$ [15,26,33,34]. None of the mentioned cut-offs are based on a specific study examining precisely this issue. The literature merely states that a higher s-ferritin threshold is *likely* required for athletes who train at altitude to compensate for the hypoxia-induced increase in erythroid iron demand [26]. Indeed, a recent review discussing the role of iron for the female athlete concluded that whether or not iron treatment is appropriate for iron-deficient non-anemia (s-ferritin < 35 $\mu g \cdot L^{-1}$ with all other hematology being normal) still remains unsolved [35].

Interestingly, we did not find any difference in ΔHbmass between athletes who used iron supplements and those who did not, or any association with iron supplement dose and ΔHbmass. This suggests that iron supplementation was not mandatory for the observed Hbmass increase. Another study that questioned the necessity of iron supplementation for subjects with clinically normal iron stores showed that 16 days at 5260 m led to a large Hbmass increase (7.6%) without any iron supplementation [28]. Also, the hematological effects of training in terrestrial altitude (1800 m) in elite boxers showed, surprisingly, a reduction in Hbmass despite daily high dose iron supplementation (200 mg elemental iron) [36]. These findings are, however, in contrast with previous studies that show that athletes who did not supplement with iron at altitude had only minimal Hbmass increases (1.2%), whereas athletes that supplemented with 105 and 210 mg of elemental iron per day increased Hbmass by 3.3% and 4.0% [17,26]. A possible explanation for this discrepancy could be methodological

differences, with a mix of altitude training methods and substantial variations in elevation (1350 – 5260 m). Perhaps, the lack of improvement in Hbmass in non-supplemented athletes, despite plenty of iron in the body stores [8,26], might be attributed to different hypoxic stimuli (i.e. intermittent hypoxia with 14 hrs a day at 3000 m) that may require a different supplementation to improve iron availability than longer hypoxic exposures at lower elevations, even if the total hypoxic dose is similar.

The iron supplementation in the above-mentioned studies utilized high dosages (105 or 210 mg elemental iron per day) [17,26,36], while the daily iron dosages used by athletes in our study were lower and had a larger range (9-200 mg elemental). Indeed, to our knowledge, only one study has used iron supplement dose <100 mg elemental iron per day (i.e., 51 mg) at altitude [37], showing an impressive 9.2% increase in Hbmass in middle-distance runners following nightly hypoxic exposure for 18 days [37]. Perhaps, the lower iron dosages were absorbed more efficiently, since the fractional iron absorption decreases with increasing dosages (>40 mg elemental iron per day) [31]. Additionally, it may limit the potential negative effects of high dose iron, such as reduced absorption of other divalent trace elements, increased pro-oxidative potential, and alterations in the gut microflora [38]. Also, while it is well-known that iron has poor bioavailability (14-18% from mixed diets), it might be less recognized that the bioavailability of supplemental iron is also poor (2-20%) [10]. Hence, the diversity in the chemical form of iron supplements might also explain some of the discrepancies between the aforementioned studies.

Impact of hypoxic dose, pre-altitude relative Hbmass and illness

We found a positive association between the hypoxic dose and ΔHbmass, suggesting a 0.4% increase for every additional 100 km·hrs at 1800-2500 m of altitude. This finding is in accordance with previous findings that clearly associate the hypoxic dose with the erythropoietic drive and subsequent rise in Hbmass [39]. However, there was a large spread in the total hypoxic dose in the altitude sojourns in the current study; thus potentially, some of the sojourns may have had too low hypoxic dose to stimulate a robust Hbmass response. Also, there is debate as to whether elite athletes with already high baseline Hbmass can expect large hematological improvements [40,41]. Our finding that each 1 g·kg^{-1} BW higher pre-altitude Hbmass was associated with a 1% lower increase in Hbmass is supported by previous data, showing that the altitude-induced ΔHbmass is greatest in subjects with initially low Hbmass values [40]. However, our results suggest that even world-class endurance athletes with high baseline Hbmass (13.8 ± 1.2 and 11.7 ± 1.2 g·kg^{-1} for males and females, respectively) can

achieve a meaningful improvement, although the magnitude of the increase is moderated by baseline Hbmass.

We found a negative effect of illness at altitude on ΔHbmass. While healthy athletes increased their Hbmass by ~4%, athletes who get ill at altitude may expect a small reduction of 1-2% in their ΔHbmass. However, these findings should be interpreted with caution as we only had a small number of athletes who got ill during the altitude sojourns. Yet, in several other studies, the lack of altitude-induced hematological response in well-trained athletes has been explained by illness/infection [15,29,42]. The underlying mechanism for the suppressed erythropoietic response during an infection may be explained by the augmented production of leukocytes at the cost of erythrocytes, which takes place during the differentiation of hematopoietic stem cells in the bone marrow [43]. Moreover, increased concentration of the inflammatory cytokine IL-6 in response to infection may increase circulating hepcidin that negatively affects the iron absorption and recirculation [12], which in turn reduces iron availability that is essential for erythrocyte maturation [32].

The underlying physiology regulating the response to hypoxia is complex and might explain much of the conflicting results in the literature regarding iron, athletes and altitude. At the center of the adaptive responses to hypoxia is the hypoxia-inducible factor (HIF), which is regulated by both iron and oxygen availability in a tight interplay [44]. While HIF regulates several genes related to hypoxia, including erythropoietin (EPO), the EPO-controlled erythroferrone hormone reduces the expression of the iron regulatory peptide hepcidin [44]. Low iron stores and hypoxia downregulate hepcidin synthesis and enhance iron absorption and recirculation, while post-exercise inflammatory response and iron overload increase hepcidin and reduce iron availability [12,45]. Future studies should prospectively explore the impact of commencing natural and simulated altitude training blocks with low s-ferritin but normal [Hb] (iron deplete non-anemic athletes) with concomitant iron supplementation protocol to maximize iron availability (e.g. one large dose every other day [31], 3-6 hrs apart from training [45]). Furthermore, the supplemental iron should be "labeled" (iron isotopes) to provide further insight about the destination(s) of the ingested iron.

Limitations

This study is limited by its retrospective nature. The data set includes a wide range of different iron dosages that were self-selected instead of randomly allocated, limiting firm causal conclusions. We lack information about the timing of the iron supplement intake, which might affect the iron bioavailability and hence the hematological variables. Also, we lack information about the athletes' dietary intake and training. Thus, potential influence of

low energy availability, dietary iron intake, and alterations in training load on Hbmass response cannot be analysed. Furthermore, we only had a limited number of athletes at the lower end of the s-ferritin scale (<35 $\mu g \cdot L^{-1}$), thus our results cannot be extrapolated to athletes with clinically low pre-altitude iron stores. Finally, a lack of a sea level control group is a limitation of the study, especially in evaluating the effect of altitude *per se*.

Conclusions

The altitude-induced increase in Hbmass was not associated with baseline s-ferritin in world-class endurance athletes with clinically normal iron stores. Iron supplementation, that was utilised at nearly half of the altitude sojourns, was not associated with ΔHbmass. However, the use of iron supplements hindered the altitude-induced reduction in s-ferritin.

Perspective

Iron availability is one of numerous factors that can modulate the adaptive response to altitude training. The present study found no association between baseline s-ferritin and altitude-induced Hbmass response, suggesting that the cut-off (35 $\mu g \cdot L^{-1}$) for "adequate" pre-altitude iron stores may be lower than previously thought. Athletes with pre-altitude s-ferritin at the lower end of the clinically normal range (20-35 $\mu g \cdot L^{-1}$) may expect similar Hbmass responses to athletes with higher pre-altitude iron stores. Iron supplementation was found to blunt the fall in s-ferritin, which is highly relevant for athletes who have several altitude sojourns in close proximity. However, no association between iron supplementation and altitude-induced Hbmass response was found, challenging the necessity of daily high-dose iron supplements, given their potential negative effects[35]. Based on existing literature and the present study, we suggest an individualized holistic approach when tailoring iron supplementation protocols for athletes at altitude which takes into consideration the athletes' current and past hematological iron status (s-ferritin) and dietary intake (amount and type of dietary iron and iron absorption enhancers/inhibitors) and plans for future altitude sojourns, and considers the use of lower daily iron doses instead of applying standard 100-200 mg elemental iron per day for all athletes.

Conflict of interest

The authors have no conflict of interest to declare.

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Contributions

The study was conceived by AEKM, and the data was collected by AEKM, IS, ØS and GP. AEKM and ØS performed statistical analysis and AEKM, IS, ØS, JS and GP interpreted the results. AEK and GP wrote the manuscript, and all co-authors commented and approved the final version of it.

References

1. Mujika I, Sharma AP, Stellingwerff T. Contemporary Periodization of Altitude Training for Elite Endurance Athletes: A Narrative Review. *Sports Med.* 2019;49(11):1651-1669.
2. Nummela A, Eronen T, Koponen A, Tikkanen H, Peltonen J. Variability in hemoglobin mass response to altitude training camps. *Scand J Med Sci Sports.* 2020.
3. Stellingwerff T, Peeling P, Garvican-Lewis LA, et al. Nutrition and Altitude: Strategies to Enhance Adaptation, Improve Performance and Maintain Health: A Narrative Review. *Sports Med.* 2019;49(Suppl 2):169-184.
4. al-Momen AK, al-Meshari A, al-Nuaim L, et al. Intravenous iron sucrose complex in the treatment of iron deficiency anemia during pregnancy. *Eur J Obstet Gynecol Reprod Biol.* 1996;69(2):121-124.
5. Reynafarje C, Lozano R, Valdivieso J. The polycythemia of high altitudes: iron metabolism and related aspects. *Blood.* 1959;14(4):433-455.
6. Garvican LA, Saunders PU, Cardoso T, et al. Intravenous iron supplementation in distance runners with low or suboptimal ferritin. *Med Sci Sports Exerc.* 2014;46(2):376-385.
7. Bonovas S, Fiorino G, Allocca M, et al. Intravenous Versus Oral Iron for the Treatment of Anemia in Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Medicine (Baltimore).* 2016;95(2):e2308.
8. Garvican-Lewis LA, Vuong VL, Govus AD, et al. Intravenous Iron Does Not Augment the Hemoglobin Mass Response to Simulated Hypoxia. *Med Sci Sports Exerc.* 2018;50(8):1669-1678.
9. Koivisto AE, Paulsen G, Paur I, et al. Antioxidant-rich foods and response to altitude training: A randomized controlled trial in elite endurance athletes. *Scand J Med Sci Sports.* 2018;28(9):1982-1995.
10. Hurrell R, Egli I. Iron bioavailability and dietary reference values. *Am J Clin Nutr.* 2010;91(5):1461S-1467S.
11. Wardenaar F, Brinkmans N, Ceelen I, et al. Micronutrient Intakes in 553 Dutch Elite and Sub-Elite Athletes: Prevalence of Low and High Intakes in Users and Non-Users of Nutritional Supplements. *Nutrients.* 2017;9(2).
12. Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. *Annu Rev Nutr.* 2006;26:323-342.
13. Leung PS, Srai SK, Mascarenhas M, Churchill LJ, Debnam ES. Increased duodenal iron uptake and transfer in a rat model of chronic hypoxia is accompanied by reduced hepcidin expression. *Gut.* 2005;54(10):1391-1395.

14. Koivisto-Mørk AE, Paur I, Paulsen G, et al. Dietary Adjustments to Altitude Training in Elite Endurance Athletes; Impact of a Randomized Clinical Trial With Antioxidant-Rich Foods. *Frontiers in Sports and Active Living.* 2020;2(106).
15. 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.
16. 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(5):350-355.
17. Garvican-Lewis LA, Govus AD, Peeling P, Abbiss CR, Gore CJ. Iron Supplementation and Altitude: Decision Making Using a Regression Tree. *J Sports Sci Med.* 2016;15(1):204-205.
18. Okazaki K, Stray-Gundersen J, Chapman RF, Levine BD. Iron insufficiency diminishes the erythropoietic response to moderate altitude exposure. *J Appl Physiol (1985).* 2019;127(6):1569-1578.
19. Sim M, Garvican-Lewis LA, Cox GR, et al. Iron considerations for the athlete: a narrative review. *Eur J Appl Physiol.* 2019;119(7):1463-1478.
20. Hall R, Peeling P, Nemeth E, Bergland D, McCluskey WTP, Stellingwerff T. Single versus Split Dose of Iron Optimizes Haemoglobin Mass Gains at 2106 m Altitude. *Med Sci Sports Exerc.* 2018.
21. 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.
22. Skattebo O, Bjerring AW, Auensen M, et al. Blood volume expansion does not explain the increase in peak oxygen uptake induced by 10 weeks of endurance training. *Eur J Appl Physiol.* 2020;120(5):985-999.
23. Siebenmann C, Keiser S, Robach P, Lundby C. CORP: The assessment of total hemoglobin mass by carbon monoxide rebreathing. *J Appl Physiol (1985).* 2017;123(3):645-654.
24. Prommer N, Thoma S, Quecke L, et al. Total hemoglobin mass and blood volume of elite Kenyan runners. *Med Sci Sports Exerc.* 2010;42(4):791-797.
25. Hopkins WG. How to Interpret Changes in an Athletic Performance Test. *Sportscience.* 2004;8:1-7.
26. Govus AD, Garvican-Lewis LA, Abbiss CR, Peeling P, Gore CJ. Pre-Altitude Serum Ferritin Levels and Daily Oral Iron Supplement Dose Mediate Iron Parameter and Hemoglobin Mass Responses to Altitude Exposure. *PLoS One.* 2015;10(8):e0135120.
27. Hauser A, Troesch S, Steiner T, et al. Do male athletes with already high initial haemoglobin mass benefit from 'live high-train low' altitude training? *Exp Physiol.* 2018;103(1):68-76.

28. Ryan BJ, Wachsmuth NB, Schmidt WF, et al. AltitudeOmics: rapid hemoglobin mass alterations with early acclimatization to and de-acclimatization from 5260 m in healthy humans. *PLoS One.* 2014;9(10):e108788.
29. Heikura IA, Burke LM, Bergland D, Uusitalo ALT, Mero AA, Stellingwerff T. 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. *Int J Sports Physiol Perform.* 2018;13(8):1090-1096.
30. Stray-Gundersen J, Alexander AC, Hochstein A, deLemos D, Levine BD. Failure of red cell volume to increase to altitude exposure in iron deficient runners. *Medicine and science in sports and exercise.* 1992;24:S90.
31. Moretti D, Goede JS, Zeder C, et al. Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women. *Blood.* 2015;126(17):1981-1989.
32. Muckenthaler MU, Mairbaurl H, Gassmann M. Iron metabolism in high-altitude residents. *J Appl Physiol (1985).* 2020;129(4):920-925.
33. Constantini K, Wilhite DP, Chapman RF. A Clinician Guide to Altitude Training for Optimal Endurance Exercise Performance at Sea Level. *High Alt Med Biol.* 2017;18(2):93-101.
34. Bergeron MF, Bahr R, Bartsch P, et al. International Olympic Committee consensus statement on thermoregulatory and altitude challenges for high-level athletes. *Br J Sports Med.* 2012;46(11):770-779.
35. Pedlar CR, Brugnara C, Bruinvels G, Burden R. Iron balance and iron supplementation for the female athlete: A practical approach. *Eur J Sport Sci.* 2018;18(2):295-305.
36. Friedmann B, Jost J, Rating T, et al. Effects of iron supplementation on total body hemoglobin during endurance training at moderate altitude. *Int J Sports Med.* 1999;20(2):78-85.
37. Brugniaux JV, Schmitt L, Robach P, et al. Eighteen days of "living high, training low" stimulate erythropoiesis and enhance aerobic performance in elite middle-distance runners. *J Appl Physiol (1985).* 2006;100(1):203-211.
38. Fisher AE, Naughton DP. Iron supplements: the quick fix with long-term consequences. *Nutr J.* 2004;3:2.
39. Rasmussen P, Siebenmann C, Diaz V, Lundby C. Red cell volume expansion at altitude: a meta-analysis and Monte Carlo simulation. *Med Sci Sports Exerc.* 2013;45(9):1767-1772.
40. Robach P, Lundby C. Is live high-train low altitude training relevant for elite athletes with already high total hemoglobin mass? *Scand J Med Sci Sports.* 2012;22(3):303-305.

41. Millet GP, Chapman RF, Girard O, Brocherie F. Is live high-train low altitude training relevant for elite athletes? Flawed analysis from inaccurate data. *Br J Sports Med.* 2019;53(15):923-925.
42. Gore CJ, Hahn A, Rice A, et al. Altitude training at 2690m does not increase total haemoglobin mass or sea level VO2max in world champion track cyclists. *J Sci Med Sport.* 1998;1(3):156-170.
43. Oduro KA, Jr., Liu F, Tan Q, et al. Myeloid skewing in murine autoimmune arthritis occurs in hematopoietic stem and primitive progenitor cells. *Blood.* 2012;120(11):2203-2213.
44. Gassmann M, Muckenthaler MU. Adaptation of iron requirement to hypoxic conditions at high altitude. *J Appl Physiol (1985).* 2015;119(12):1432-1440.
45. Peeling P, Sim M, Badenhorst CE, et al. Iron status and the acute post-exercise hepcidin response in athletes. *PLoS One.* 2014;9(3):e93002.

Table 1. Description of the study subjects.

	All (n=66)
Age (yrs)	24 ± 5
Weight (kg)	75.2 ± 11.6
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)[1]	
Males	75.9 ± 7.6
Females	71.5 ± 7.0
VO_{2max} ($L \cdot min^{-1}$)[1]	
Males	6.2 ± 0.6
Females	4.2 ± 0.7
Sports	
Cross-country skiing	18 (27 %)
Swimming	15 (23 %)
Rowing	14 (21 %)
Kayaking	6 (9 %)
Triathlon	5 (8 %)
Nordic combined	3 (5 %)
Road cycling	3 (4 %)
Athletics	2 (3 %)
Sex	
Males	41 (62 %)
Females	25 (38 %)
Able-bodied/disabled athletes	
Able-bodied athletes	62 (94%)
Athletes with disability	4 (6 %)
Olympian	
Yes	22 (33 %)
No	44 (67 %)
Medalist in Olympic Games or World Championships	
Yes	23 (35 %)
No	43 (65 %)

Values are presented as mean ± SD or count (%).

[1] n=47, excludes swimmers since we don't have VO_{2max} data on them

Table 2. Hemoglobin mass, s-ferritin and hemoglobin concentration in all, females and males before (Pre-altitude) and after (Post-altitude) the training sojourns.

	All (n = 107)				**Males[1]** (n = 75)			**Females[2]** (n = 33)			Between sex	
	Pre-altitude	Post-altitude	p_{paired}	*ES*	Pre-altitude	Post-altitude	p_{paired}	Pre-altitude	Post-altitude	p_{paired}	p_{change}	*ES*
Hbmass (g)	1023 ± 220	1059 ± 221	<0.001**	0.9	1140 ± 141	1181 ± 126	<0.001**	754 ± 105	778 ± 109	<0.001**	0.029*	0.5
Relative Hbmass ($g \cdot kg^{-1}$ BW)	13.2 ± 1.5	13.7 ± 1.4	<0.001**	0.9	13.8 ± 1.2	14.3 ± 1.1	<0.001**	11.7 ± 1.2	12.2 ± 1.1	<0.001**	0.700	0.0
s-ferritin ($\mu g \cdot L^{-1}$)	83 (305)	65 (277)	0.001*	0.5^{r}	102 (305)	73 (275)	<0.001**	49 (99)	40 (137)	0.972	0.724	0.8^{r}
[Hb] ($g \cdot dL^{-1}$)	14.7 ± 1.0	15.7 ± 1.0	<0.001**	1.0	15.1 ± 0.7	15.9 ± 0.8	<0.001**	13.6 ± 0.7	14.5 ± 0.6	0.001*	0.866	0.1

Values are presented as mean ± SD or median (range) for non-normally distributed data.

[1]All recorded observations for each individual, including eighteen male athletes with more than one altitude sojourn.

[2]All recorded observations for each individual, including four female athletes with more than one altitude sojourn.

The p-value, p_{paired} is obtained from comparing the change from Pre-altitude to Post-altitude within the groups using paired t-test or Wilcoxon tests depending on the normality of the data.

The p-value, p_{change} is obtained from comparing the change (from Pre-altitude to Altitude) between the sex using t-test or Wilcoxon-MW tests depending on the normality of the data.

** p-value < 0.05, ** p-value < 0.001. ES=effect size expressed as Cohen´s (d) for parametric tests or Rosenthals (r) for non-parametric tests.*

Table 3. Estimated marginal means with 95% confidence intervals (CI) for the changes in Hbmass (ΔHbmass %).

		ΔHbmass (%)	
Pre-altitude ferritin	**Use of iron supplements**	**Estimate**	**95% CI**
Low	No	3.8	(1.0 to 6.7)
($\leq$ 35 µg·L^{-1})	Yes	5.1	(2.5 to 7.7)
Moderate/High	No	4.1	(3.3 to 5.0)
(> 35 µg·L^{-1})	Yes	4.1	(3.0 to 5.3)
Modifying factors	Hypoxic dose (+100 km·hrs)	0.4	(0.1 to 0.7)
	Baseline Hbmass (+1 g·kg^{-1})	-1.0	(-1.6 to -0.5)
	Illness during the altitude sojourn	-5.7	(-8.3 to -3.1)

Linear mixed model with the log-transformed change in Hbmass as the dependent variable and hypoxic dose (numeric linear), baseline relative Hbmass (the difference from the sex-specific mean; numeric linear), health status (2 categories) and the interaction between pre-altitude s-ferritin (2 categories) and iron supplementation (2 categories) as fixed effects. A repeated statement specified for subject ID and the numeric time of the altitude sojourn was used to account for within-subject correlated errors. The estimates are calculated at mean hypoxic dose (1049 km·hrs), mean relative Hbmass (11.9 and 13.8 g·kg^{-1} body weight for females and males, respectively) and with no experienced illness during the altitude sojourn. The effects were backtransformed and expressed as percentages. The model (n=102) excludes five altitude sojourns because of missing pre-altitude s-ferritin values.

Figure legends

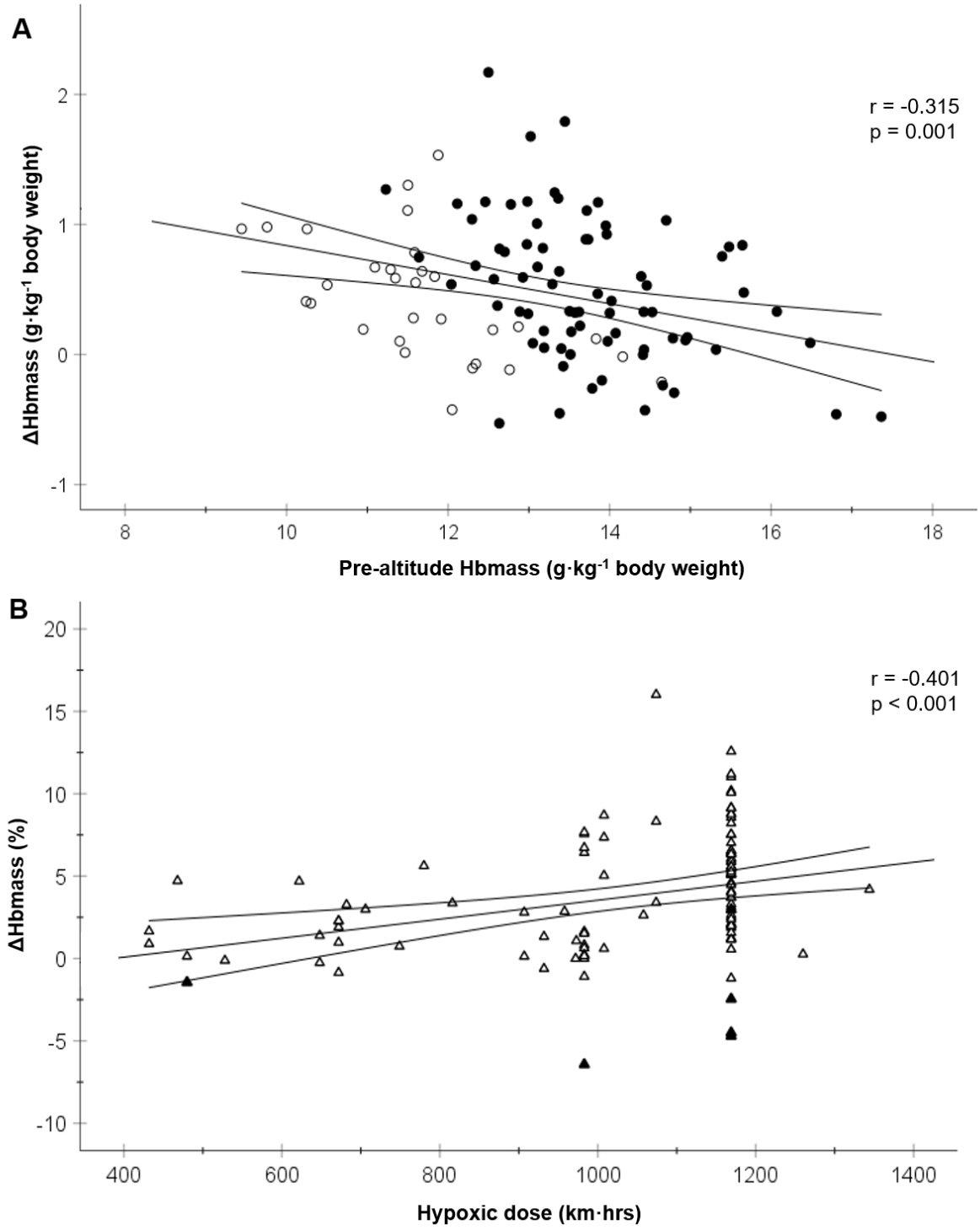

Fig 1. **Regression line with 95% confidence intervals for the association between change in Hbmass (g·kg^{-1} body weight) following altitude sojourns and pre-altitude Hbmass (g·kg^{-1} body weight) (A) and change in Hbmass (%) and the hypoxic dose (km·hrs) (B).** Closed circles represent males and open circles represent females (A).

Closed triangles represent subjects with illness and open triangles without illness at altitude (B). P-value is obtained for Pearson correlation coefficient.

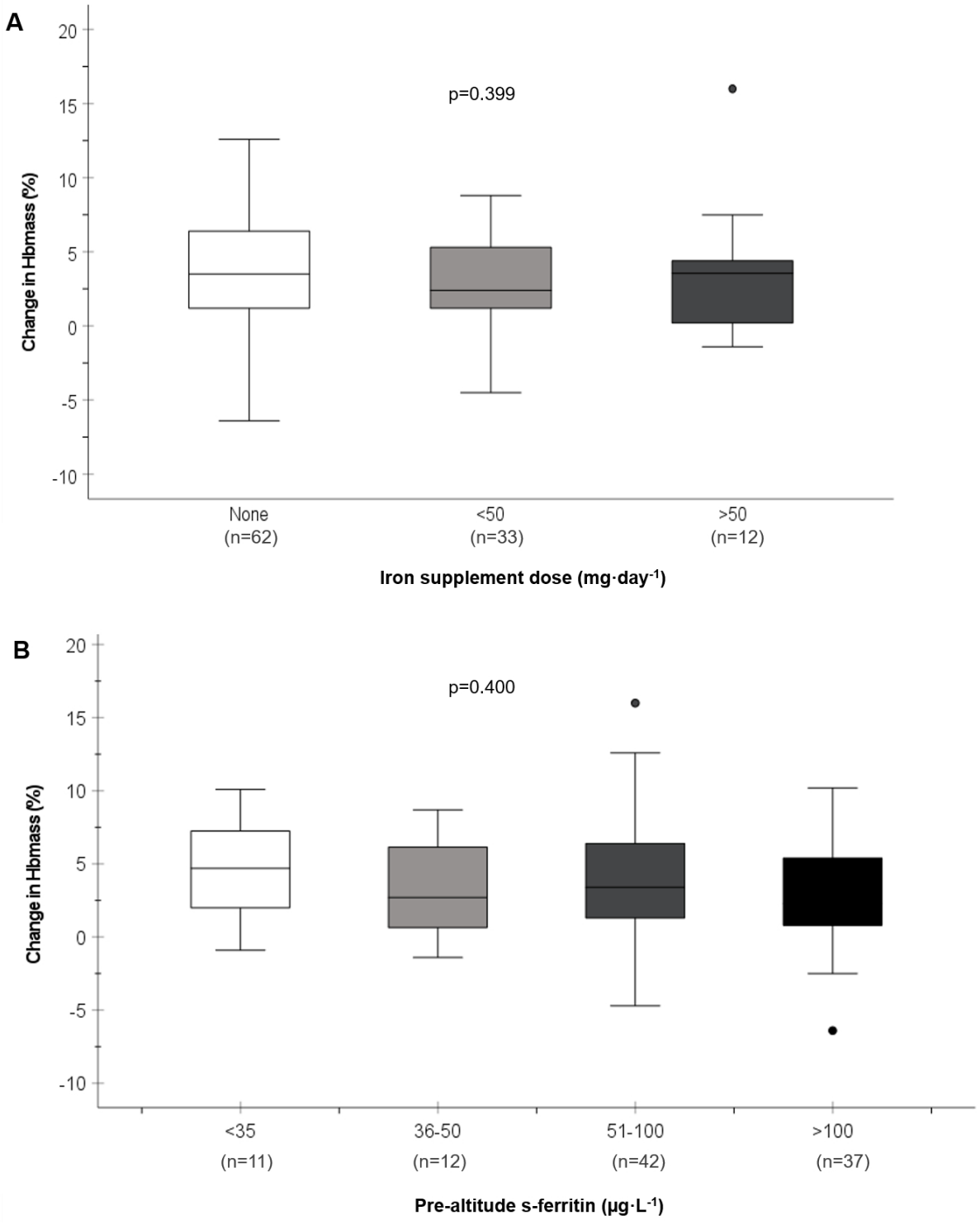

Fig 2. **Change in Hbmass (%) following altitude sojourns with three iron supplementation categories at altitude (none, ≤ 50mg/day or <50 mg·day^{-1}) (A), and commenced with <35, 36-50, 51-100 or >100 µg·L^{-1} in s-ferritin (B).** The boxplots

represent median, first and third quartile (interquartile range), minimum, maximum and outliers for each catogory. P-value is obtained from one-way ANOVA.