RESEARCH PAPER

EFFECT OF IRON SUPPLEMENTATION DURING HIGH ALTITUDE TRAINING ON HAEMOGLOBIN AND IRON STATUS OF SRI LANKAN MIDDLE- AND LONG-DISTANCE ATHLETES

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Abstract

Background: Literature reports significant disparities in the haematological response to altitude training among endurance athletes. The role of iron in determining the haematological response to altitude training is under-investigated.

Objective: This study compared haematological parameters between Sri Lankan endurance athletes exposed to hypoxic and normoxic conditions, with and without iron supplementation.

Method: Sri Lanka Army long and middle-distance male athletes were studied under four conditions; low altitude non-supplemented [LOW: n=14] and supplemented [LOW-S: n=7], high altitude non-supplemented [HIGH: n=6] and supplemented [HIGH-S: n=7]. High altitude groups lived at 2200 m and trained at 1800 m. Low altitude groups lived and trained at 40 or 120 m. All athletes underwent endurance training for five weeks. Pre and post intervention blood samples were obtained to determine haematological parameters.

Results: A significant increase in haemoglobin concentration (0.67 g/dl) was observed in the two high altitude groups after five weeks of training (p=0.004). Serum ferritin decreased by 28.4% (p=0.05) and red cell distribution width increased (p=0.04) in HIGH while ferritin increased by 26.5% (p=0.08) and red cell distribution width decreased (p=0.01) in HIGH-S. No changes were observed in the low altitude groups.

Conclusion: A substantial haematological response is observed when Sri Lankan endurance athletes are exposed to an adequate hypoxic dose. However, non-iron supplemented athletes are at a tendency to develop iron deficiency whilst supplemented athletes may accumulate iron even with previously recommended levels of pre-altitude ferritin.

Keywords: hypoxia, erythropoiesis, endurance athlete, ferritin, altitude

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Introduction

Many endurance athletes engage in altitude training protocols with the intention of improving their sea level performance.[1] Beneficial effects of altitude training on sea level performance are mainly attributed to the haematological adaptations that occur in response to exposure to hypoxia.[2] There is also another school of thought that non-haematological mechanisms such as improved mitochondrial efficiency and muscle buffer capacity contribute to improved sea level performance after altitude training.[3] While it is likely that several factors are responsible for the development of favourable results, it has also been observed that there is a significant individual variation in the haematological and performance related response to hypoxia. [4,5] A blunted erythropoietic response, [6] ventilatory limitations, [5] genetic factors [7,8] and ' the hypoxic dose' exposed to [9] are some factors that have been implicated for this variation.

Iron is a major substrate required in the synthesis of haem as well as mitochondrial iron-dependent proteins. [10] The exact role of iron in determining the haematological and non-haematological response to hypoxia has not been investigated in detail thus far. Some groups have shown that iron supplementation during altitude training improves haemoglobin (Hb) mass [11,12] whilst others have shown that Hb mass does not increase with supplementation at altitude. [13-15] The contradictory nature of previous literature creates an obstacle in coming to a consensus regarding the ideal iron status and need for supplementation during altitude training.

Though there are defined cut off Hb and serum ferritin levels beyond which iron supplementation is recommended in athletes undergoing training at sea level, [16] currently there are no clear guidelines on iron supplementation practices during altitude training. In the presence of the controversial findings regarding the need for supplementation at altitude and the absence of proper recommendations, the role of iron during high altitude training is an area that requires further investigation. Moreover, no studies on the haematological response to hypoxia have been performed to date on Sri Lankan endurance athletes. Hence, the aim of the present study was to compare haematological parameters between Sri Lankan endurance athletes living and training in hypoxic conditions and normoxic conditions, with and without iron supplementation.

Materials and Methods

All participants were Sri Lanka Army male middle and long-distance runners (800 m or longer distance runners) who were training to compete at national level and inter-defence services competitions. None of them were residents of altitudes above 1500 m and none had exposure to an altitude above 1500 m during the previous four weeks. The study protocol complied with the code of ethics of the World Medical Association Declaration of Helsinki.[17] Ethical approval was obtained from the Institutional ethical review committee of the Faculty of Medicine, University of Peradeniya, Sri Lanka. Informed written consent was obtained from each participant prior to starting the study.

Sixty-four athletes were recruited for the study. Half were assigned to live and train at low altitude at either Mulaitivu, Sri Lanka (altitude 40 m above sea level) or Kuruwita, Sri Lanka (altitude 120 m above sea level) for an average duration of five weeks. Half were assigned to live at 2200 m at Shanthipura, the village with the highest altitude in Sri Lanka, and train at 1800 m at Nuwaraeliya for five weeks.

Sixteen athletes who lived and trained at low altitude and eighteen at high altitude received micronutrient supplements at a dose of one tablet daily for the duration of the study (LOW-S and HIGH-S respectively). One tablet consisted of 360mg ferrous fumarate (equivalent to 110mg elemental iron), and 400µg folic acid, cyanocobalamin 15µg, cholecalciferol 400IU, calcium carbonate 200µg and ascorbic acid 75mg (Anemidox® MERCK LIMITED, India). Sixteen low altitude athletes and fourteen high altitude athletes did not receive supplements (LOW and HIGH groups respectively).

Five millilitres of venous blood was obtained from each athlete to determine haematological parameters. Hb concentration, haematocrit (Hct), red cell count (RCC) and coefficient of variation of the red cell distribution width (RDW-CV) were determined using a BC-6800 auto haematology analyser (Mindray, China) within 4-6 hours of sample collection. Serum ferritin (FTN) levels was obtained by an immunoenzymatic florescent method using the mini VIDAS® immunoanalyser (bioMerieux, France).

Baseline height (cm) and weight (kg) were measured using standard protocols and body mass index (BMI) was calculated in all athletes. Athletes performed a submaximal bicycle ergometer test on a Monark 828E cycle ergometer (Monark, Vansbro, Sweden) and baseline VO_2max was calculated using standard nomograms.[18]

Measurements and blood samples were obtained at the Exercise and Sports Science Laboratory at Faculty of Medicine, University of Peradeniya, Sri Lanka (altitude 500 m above sea level) on the day prior to commencing (PRE) and between two to twelve days (mean time 7 days) after ending the interventional period (POST). Athletes were requested not to engage in strenuous physical activity during the 24 hours prior to testing. All blood samples and measurements were taken during morning hours of the day.

Data analysis was performed using SPSS statistical software version 23 (SPSS Inc., Chicago, IL, USA). All continuous data are presented as mean ± standard deviation. Data were tested for normality using Shapiro-Wilks test and homogeneity of variance using Levine's test for equality of variances. Differences between baseline measures were assessed by performing one-way ANOVA. Since samples sizes are unequal, Tukey-Kramer post hoc test was used for pairwise comparisons when one-way ANOVA results showed a significant difference between groups. [19-20] The response of each of the dependent variables over time, to the two independent variables (altitude and supplementation) was assessed using a three-way mixed design ANOVA. When there were significant interactions, follow up two-way mixed design ANOVA was performed after splitting the groups accordingly. Paired t-tests were performed when there was a significant interaction in the two-way ANOVA to determine whether there was a significant mean difference in any of the groups. A probability of $p \leq 0.05$ was taken as statistically significant.

Results

The final number of athletes included in the analysis of Hb concentration and red cell parameters in each group were fourteen, seven, six and seven for LOW, LOW-S, HIGH and HIGH-S respectively since several athletes failed to be present for the POST testing sessions due to injury or personal reasons and some were excluded because of failure to comply with the protocol. Figure 1 depicts the flow of participants through the study.

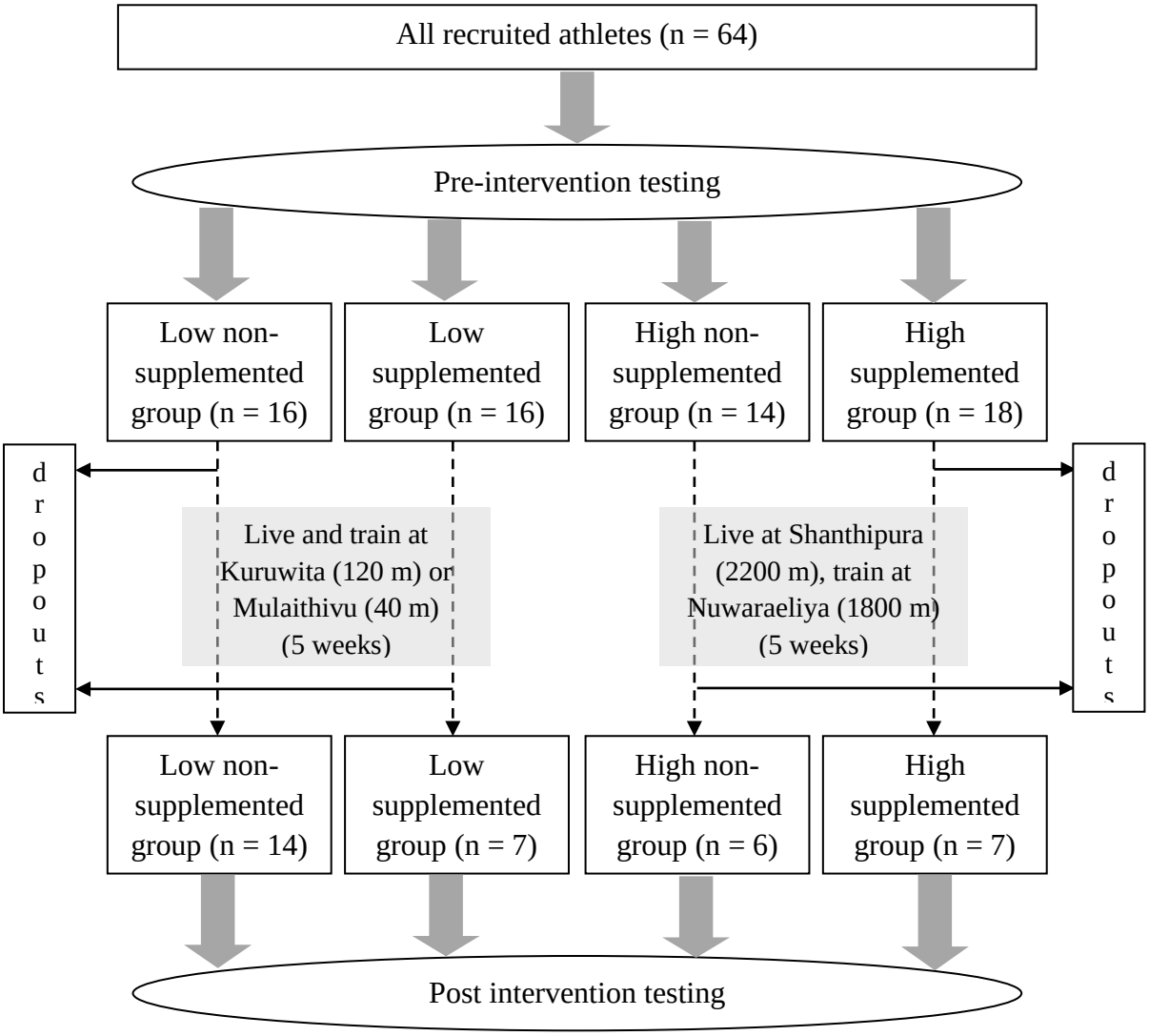

Figure 1: Flow of participants through the study (Indicating the numbers involved).

There was no significant difference in mean age, BMI, VO_2max or FTN between the four groups indicating that athletes of all four groups had similar baseline anthropometric measures, aerobic performance measures and iron stores (Table 1). One way ANOVA with post hoc pairwise comparisons revealed that mean PRE Hb was significantly lower in the LOW-S group by 1.38 g/dl (p=0.009), 2.23 g/dl (p<0.001) and 2.30 g/dl (p<0.001) compared to LOW, HIGH and HIGH-S groups respectively. RCC was significantly lower in LOW-S compared to LOW by 0. 40 × 10^9/L (p=0.012), HIGH by 0.63 × 10^9/L (p=0.001) and HIGH-S by 0.56 × 10^9/L (p=0.001). The Hct was lower in LOW-S by 5.1%, 7.7% and 5.8% compared to LOW, HIGH and HIGH-S (p<0.001 for all three comparisons) respectively. The RDW-CV was significantly higher in HIGH-S compared to the other three groups by 1.3% (p<0.001 for all three comparisons).

For Hb, a significant time-altitude two-way interaction (p=0.05) was observed. Follow up analysis after splitting the groups by altitude revealed that there was no significant time-supplementation interaction (p=0.90) in the two high altitude groups. A statistically significant time main effect (p=0.004) was observed. The absolute improvement in Hb was

Table 1. Comparison of baseline parameters between the 4 groups[1].

	LOW (n = 14)	LOW-S (n = 7)	HIGH (n = 6)	HIGH-S (n = 7)	One-way ANOVA	
					F	p value
Age (years)	25.5 ± 3.4	26.4 ± 4.8	25.0 ± 4.1	23.9 ± 5.0	0.472	0.7
BMI (Kg/m^2)	18.9 ± 1.3	18.9 ± 1.6	19.6 ± 1.2	18.9 ± 1.4	0.424	0.7
VO_2max (ml/kg/min)	57.6 ± 5.1	58.9 ± 7.2	57.2 ± 4.5	59.9 ± 7.8	0.308	0.8
Hb (g/dl)	14.4 ± 0.8	13.0 ± 0.6*	15.2 ± 1.1	15.3 ± 0.9	10.445	<0.001
Hct (%)	44.1 ± 2.3	39.1 ± 1.7*	46.7 ± 2.1	44.9 ± 2.5	14.578	<0.001
RCC (× 10^9/L)	4.9 ± 0.3	4.5 ± 0.2*	5.1 ± 0.2	5.0 ± 0.3	8.471	<0.001
RDW-CV (%)	12.8 ± 0.4	12.8 ± 0.5	12.7 ± 0.6	14.0 ± 0.5*	13.354	<0.001
Serum ferritin (ng/ml)	73.2 ± 38.8 (n = 13)	83.9 ± 27.8 (n = 7)	72.9 ± 30.2 (n = 5)	54.3 ± 22.6 (n = 6)	0.889	0.5

[1]Data are mean ± SD. LOW, low altitude non-supplemented group; LOW-S, low altitude supplemented group; HIGH, high altitude non-supplemented group; HIGH-S, high altitude supplemented group; BMI, body mass index; VO_2max, maximal oxygen consumption; Hb, haemoglobin; Hct, haematocrit; RCC, red cell count; R DW-CV, coefficient of variation of the red cell distribution width
* significantly different from the other 3 groups

similar in both high altitude groups, at 0.67 g/dl from PRE mean Hb. There was no significant interaction (p=0.1) or time main effect (p=0.4) for the low altitude groups (Figure 2).

There was a significant time main effect for RCC (p=0.012) but no significant interactions. No significant increase in RCC was observed in any group. There were no significant interactions or a time main effect for Hct.

A significant time-supplementation interaction (p<0.001) and time-supplementation-altitude interaction (p=0.001) was observed for RDW-CV. Follow up analysis after splitting groups by altitude revealed a significant time-supplementation interaction for the high-altitude groups (p=0.001). Paired t-test for each high altitude group revealed that there was a significant increase in RDW-CV in HIGH (mean difference = 0.53%, p=0.044) and a significant decrease in HIGH-S (mean difference = 0.91%, p=0.013).There was no significant interaction or time main effect for the low altitude groups (Figure 3).

A significant time-supplementation two-way interaction (p=0.006) as well as a significant time-supplementation-altitude three-way interaction (p=0.005) was observed for FTN. Follow up analysis revealed a significant time-supplementation interaction for the high altitude groups (p=0.008). Paired t-tests revealed that there was a trend for decrease in FTN in HIGH (mean difference = -20.7 ng/ml, p=0.056) and trend for increase in FTN in HIGH-S

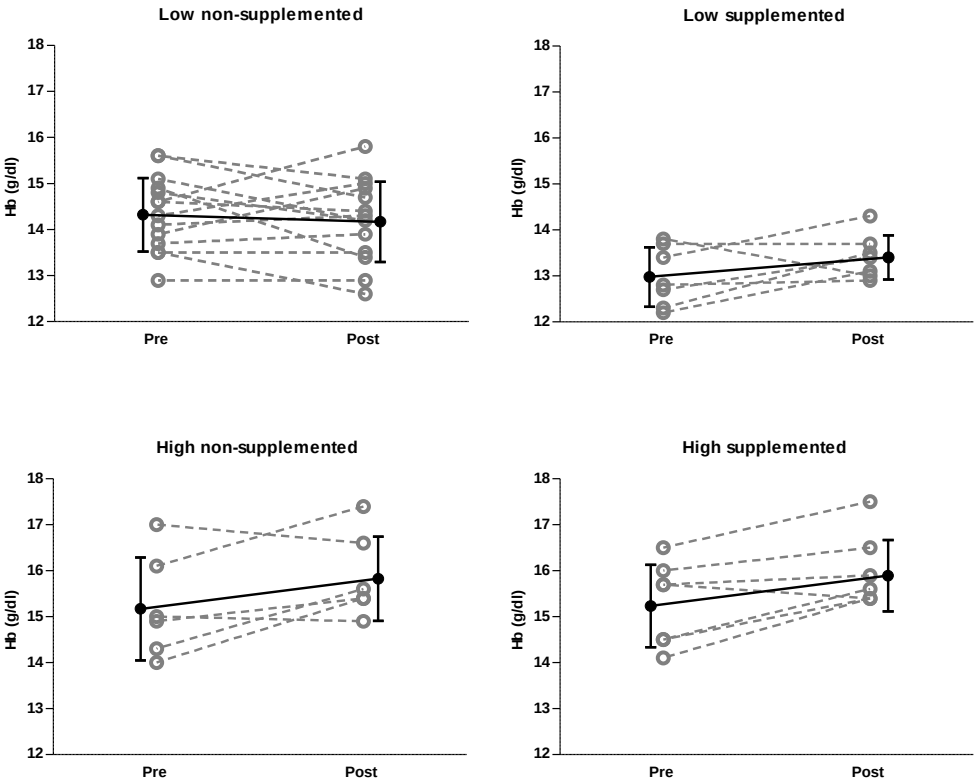

Figure 2: Change in haemoglobin concentration of each athlete (grey open circle with dashed line) and mean ± SD (black filled circle with continuous line) in the 4 groups.

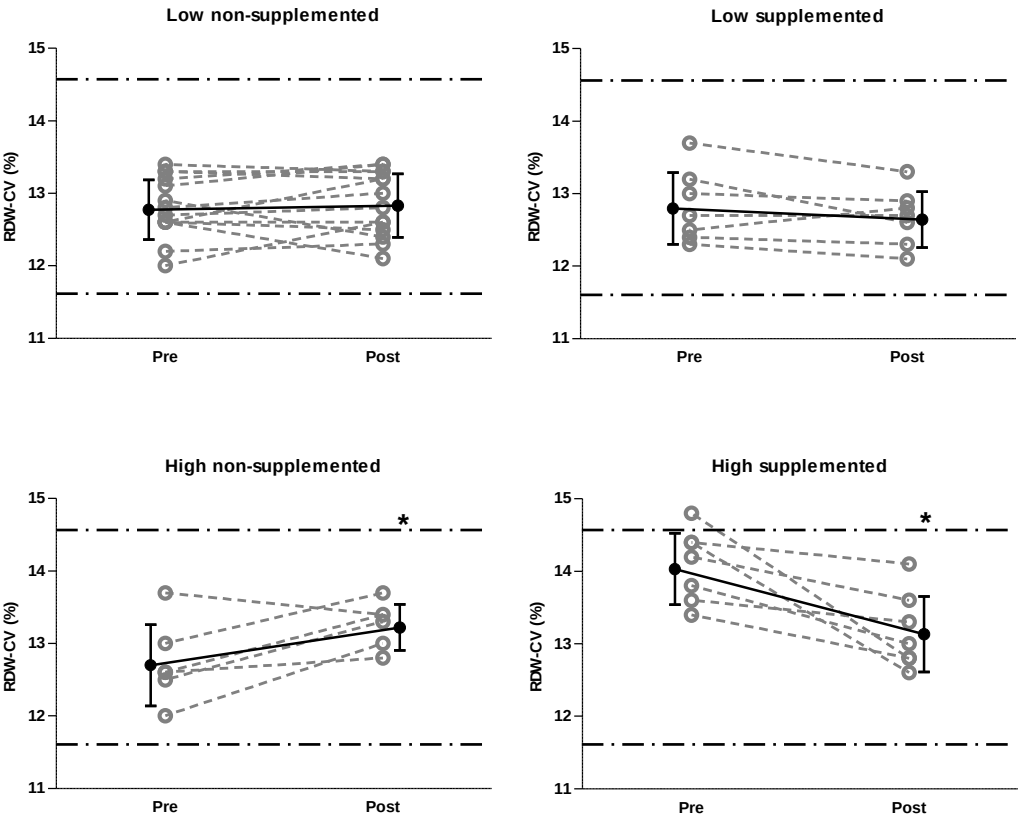

Figure 3. Change in RDW-CV of each athlete (grey open circle with dashed line) and mean ± SD (black filled circle with continuous line) in the 4 groups. Black broken lines indicate normal range for adult males.

*significantly different from pre-training mean.

(mean difference = 14.4 ng/ml, p=0.089). For the low altitude groups there was no significant interaction or main effect (Figure 4).

Discussion

This is the first study to investigate effects of a hypoxic exposure on Sri Lankan athletes. The results of this study demonstrate two main findings. Firstly, living and training at high altitude for an average duration of five weeks elicited a similar erythropoietic response in micronutrient supplemented as well as non-supplemented endurance athletes. Secondly, non-supplemented athletes showed changes in haematological parameters that indicated that they were at risk of developing iron deficiency despite having normal pre-training iron stores and, supplemented athletes showed an increase in iron stores with a dose of 110 mg elemental iron daily during altitude training.

An absolute increase in Hb concentration of 0.67 g/dl and a percentage increase of 4.5% were observed in both high altitude groups. In contrast, the athletes living and training at low altitude did not show a substantial change in Hb concentration irrespective of whether they were supplemented or not. These results point towards the fact that the increase in Hb concentration in the high-altitude groups was due to the hypoxic stimulus and not due to endurance training.

A recent meta-analysis by Lobigs and colleagues on influence of altitude exposure on biomarkers of erythropoiesis concluded that Hb concentration increases up to a maximum of 0.94 g/dl from baseline within the initial 1000 km.hr of hypoxic exposure.[21] The athletes in the present study spent an average of 19 hours

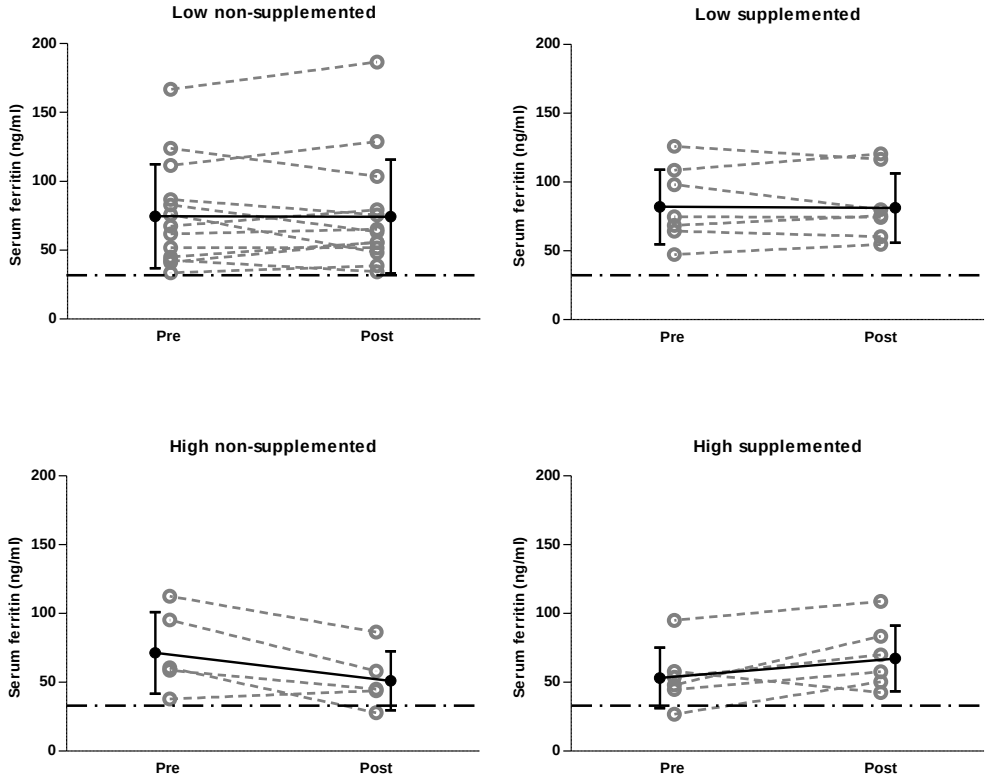

Figure 4. Change in serum ferritin concentration of each athlete (grey open circle with dashed line) and mean ± SD (black filled circle with continuous line) in the 4 groups. Black broken line indicates lower limit of normal range for adult males.

per day for 32 -35 days at 2200 m. Hence, the hypoxic dose the athletes of the present study were exposed to was between 1338 km.hr to 1463 km.hr. Previous literature advocates a hypoxic exposure of at least 22 hours per day above 2000 m for at least four weeks to obtain a notable erythropoietic response.[22,23] An extended stay of five weeks was selected for the present study with the intention of matching this hypoxic exposure since athletes in the present study had to spend more time travelling to the training site daily. The lower than expected increase in Hb concentration observed in the athletes of the present study could be because some athletes were tested after a delay of a few days post altitude, but within two weeks after descending, since they were unable to attend testing immediately. This may have resulted in their Hb concentration to drop from the value at the end of the training period.[21] Overall, though several previous studies have argued that there is no improvement in red cell parameters with altitude training, the results of the present study adds to the literature that a substantial erythropoietic response will be observed in endurance athletes provided the adequate hypoxic dose is met.

Interestingly, in the present study irrespective of whether athletes were supplemented with iron or not, the Hb concentration increased by a similar amount in both high altitude groups. To begin with, all athletes at high altitude had Hb concentrations of 14 g/dL or more and all except one athlete had serum ferritin levels within normal limits. The athlete who had a pre-training serum ferritin concentration below 30 ng/ml was however, in the supplemented group. Alterations in iron metabolism during chronic hypoxic exposure helps to increase the availability of iron for haem and non-haem protein synthesis by increasing iron mobilization from stores, increasing the rate of iron absorption from the gastrointestinal tract and up regulating iron transport proteins to improve transport mechanisms.[24,25] While these mechanisms mobilize adequate iron for erythropoiesis during the initial period of acclimatization to hypoxia, when iron stores deplete and iron demand by the bone marrow exceeds iron availability, erythropoietin production is inhibited to conserve iron stores. [26] Therefore, it could be inferred that athletes in both groups had adequate iron stores to be mobilized for erythropoiesis during the altitude stay.

Even though statistically not significant, the 26.5% increase in serum ferritin concentration in the high-supplemented athletes implies that consuming 110 mg of elemental iron daily during altitude training could exceed the rate of iron release for erythropoiesis from stores. These results are in contrast to findings of Govus and colleagues[11] where serum ferritin decreased by 13.8% in athletes supplemented with 105 mg who underwent training between 1350 – 3000 m for two–four weeks. The athletes in the study by Govus and colleagues trained at different sites ranging from 1350 m natural altitude to 3000 m simulated altitude. This difference in hypoxic stimulus may have given rise to the differing results in the two studies.

Contrary to the findings of the high altitude supplemented group, in the non-supplemented athletes the serum ferritin concentration decreased by 28.4% and the RDW-CV increased significantly. The changes in RDW-CV indicate that the non-supplemented athletes had greater heterogeneity in the sizes of their red cells which commonly results from nutritional deficiencies such as iron, folic acid or vitamin B_{12} in otherwise healthy individuals. One tablet of the micronutrient supplement that was provided for the supplemented groups consisted of folic acid, cyanocobalamin, cholecalciferol, calcium carbonate and ascorbic acid in addition to iron. Therefore, the

supplemented group may have had an adequate supply of these micronutrients and the non-supplemented athletes could have had a tendency to develop one or more deficiencies of iron, folate or vitamin B_{12}.

The reduction in serum ferritin in the non-supplemented athletes was complemented by an increase in Hb concentration by the same amount as the supplemented athletes. This is evidence that iron demand exceeded iron availability in these athletes during the hypoxic exposure thus exhausting the stores. The ferritin level decreased to below 30ng/ml in one athlete in the present study indicating a state of non-anaemic iron deficiency. These findings should not be considered as trivial since iron deficiency is known to impair endurance performance.[16, 27-29]

A recent recommendation has been made by the Swiss society of sports medicine to aim for a serum ferritin of 50ng/ml prior to altitude training.[30] The mean pre-intervention serum ferritin concentration of the high altitude non-supplemented athletes in the present study was 72.9 ± 30.2 ng/ml and the individual values were above 50 ng/ml in all except one athlete. However, the serum ferritin reduced in all athletes with ferritin level > 50 ng/ml. These results suggest that a ferritin level of 50 ng/ml prior to altitude training is insufficient to maintain normal iron balance.

A limitation of the present study is that Hb concentration was measured to determine the erythropoietic response whereas the gold standard is to measure the total circulating Hb mass. While Hb mass gives a more accurate indication of the amount of Hb in the body Hb concentration may be affected by changes in plasma volume.[31] Unfortunately, it was not possible to measure the Hb mass or plasma volume since the necessary equipment are not available in the country, as would be in most low resource countries. However, Lobigs and colleagues in their comprehensive meta-analysis have declared that while plasma volume changes affect Hb concentration during the first two days, a true increase in Hb mass is detected by measuring the Hb concentration after this initial period.[21] Hence, the observed changes in Hb concentration in the high altitude groups of the present study can be considered to be due to the true erythropoietic response to hypoxia.

The number of athletes in each group was relatively small for some groups, not equal and subjects were non-randomized since the intervention took place in the midst of their routine training schedules and it is practically and ethically not possible to separate the athletes training under a specific coach. This non-randomization, together with the small and uneven sample sizes may have had an effect on the statistically non-significant results that were observed in some parameters.

Conclusions

Hb concentration increases significantly in long- and middle-distance athletes undergoing altitude training, provided they are exposed to an adequate hypoxic stimulus and have adequate pre-altitude iron stores. However, non-iron supplemented athletes are at a risk of developing iron deficiency even in the presence of previously recommended pre-altitude levels of iron stores. Further, the present study provides some evidence that supplementing at altitude, even though for a short duration, without regular monitoring may result in iron accumulation. While it is necessary to conduct further studies to formulate much needed guidelines, the findings of this study provide important insight into the role of iron supplementation during altitude training. Overall, for the first time,

this study demonstrates the possible benefits that altitude training could have on Sri Lankan endurance athletes.

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Conflict of interest

Authors declare no conflicts of interest

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