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Research article

## Altitude Exposure at 1800 m Increases Haemoglobin Mass in Distance Runners

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### Abstract

The influence of low natural altitudes (< 2000 m) on erythropoietic adaptation is currently unclear, with current recommendations indicating that such low altitudes may be insufficient to stimulate significant increases in haemoglobin mass ( $Hb_{mass}$ ). As such, the purpose of this study was to determine the influence of 3 weeks of live high, train high exposure (LHTH) at low natural altitude (i.e. 1800 m) on  $Hb_{mass}$ , red blood cell count and iron profile. A total of 16 elite or well-trained runners were assigned into either a LHTH (n = 8) or CONTROL (n = 8) group. Venous blood samples were drawn prior to, at 2 weeks and at 3 weeks following exposure.  $Hb_{mass}$  was measured in duplicate prior to exposure and at 2 weeks and at 3 weeks following exposure via carbon monoxide rebreathing. The percentage change in  $Hb_{mass}$  from baseline was significantly greater in LHTH, when compared with the CONTROL group at 2 (3.1% vs 0.4%;  $p = 0.01$ ;) and 3 weeks (3.0% vs -1.1%;  $p < 0.02$ , respectively) following exposure. Haematocrit was greater in LHTH than CONTROL at 2 ( $p = 0.01$ ) and 3 weeks ( $p = 0.04$ ) following exposure. No significant interaction effect was observed for haemoglobin concentration ( $p = 0.06$ ), serum ferritin ( $p = 0.43$ ), transferrin ( $p = 0.52$ ) or reticulocyte percentage ( $p = 0.16$ ). The results of this study indicate that three week of natural classic (i.e. LHTH) low altitude exposure (1800 m) results in a significant increase in  $Hb_{mass}$  of elite distance runners, which is likely due to the continuous exposure to hypoxia.

**Key words:** LHTH, erythropoiesis, hypoxia, hypoxic dose.

### Introduction

Athletes are required to spend a prolonged period of time at moderate altitude (2000 – 3000 m (Bartsch et al., 2008)) in order to accumulate a sufficient total hypoxic “dose” to stimulate erythropoiesis (Garvican et al., 2012). Indeed, current guidelines for simulated altitude exposure suggest that athletes should spend  $\sim 14 \text{ h}\cdot\text{day}^{-1}$  at 3000 m for 3 weeks (totalling  $\sim 300$  hours of exposure) in order to observe a mean increase in haemoglobin mass ( $Hb_{mass}$ ) of 3 – 5% (Saunders et al., 2013). Likewise, a similar response may be achieved when 300 hours of exposure is accumulated at natural altitude (Garvican et al., 2012). Closer investigation of the time course of erythropoietic adaptation indicates that  $Hb_{mass}$  increases approximately 1% per 100 hours of exposure at simulated or natural altitude of 2300 to 3000 m (Clark et al., 2009; Garvican et al., 2012). However, achieving such altitude exposures is

often difficult for athletes due to limited availability of appropriate simulated or natural altitude, decreased living/sleeping and training quality (especially at altitudes above 3000 m), and interference with training or competition schedules (Neya et al., 2013).

To date, several models of hypoxic exposure have been established including; live high/train high (LHTH); live high/train low; live low/train high (i.e. intermittent hypoxic exposure during training) or intermittent hypoxic exposure at rest (McLean et al., 2014; Millet et al., 2013; Millet et al., 2010). The possible physiological and performance benefits of each of these modes of altitude exposure differ considerably with contributing factors including, normobaric vs hypobaric exposure (Saugy et al., 2014), the total elevation of exposure and the duration of exposure (Gore et al., 2013). Indeed, clear relationships exist between the dose of altitude exposure (measured as total duration (Bonetti and Hopkins, 2009; Gore et al., 2013), increases in  $Hb_{mass}$  and/or associated improvements in aerobic capacity (Schmidt and Prommer, 2010).

Natural low altitude venues attract a considerable number of athletes each year with the common belief that even ‘low’ altitude (< 2000 m) contributes favourably to athlete performance at sea-level (Gore et al., 2007). Supporting this, three weeks of classical (LHTH) altitude exposure at 1300 m and 1650 m interspersed with three weeks of sea level training has been shown to result in an increase in  $Hb_{mass}$  and erythropoietin concentration (Frese and Friedmann-Bette, 2010; Saunders et al., 2009). Likewise, four weeks of LHTH altitude exposure at 1900m has been reported to result an increase in left ventricular mass and improved aerobic capacity in six of seven elite skiers (Siebenmann et al., 2012). Conversely, current scientific recommendations would suggest that natural altitude exposure below (< 2000 m) is too low to stimulate significant erythropoietic benefit (Pottgiesser et al., 2009; Wilber, 2007). Despite this, little is known as to the minimal hypoxic dose required for erythropoiesis and thus the mechanism responsible for a possible increase in performance with low altitude is unclear. Indeed, current literature suggests that a minimum of two weeks of classic altitude exposure above 2100 m is required in order to observe improvements in  $Hb_{mass}$  (Gore et al., 2013).

It is plausible that improvements in performance following altitude exposure may be associated with altered training or the favourable belief that altitude training has been successful, rather than haematological altera-

tions. Indeed, it has been suggested that placebo effects and/or a better training environment (i.e. high-quality training camps, increased focus on training and recovery, less distractions, change of venue, and people to train with on a consistent basis) may be responsible for some of the improvements in performance observed within altitude exposure research (Saunders et al., 2009; 2010; Siebenmann et al., 2012). Clearly, further research is warranted in order to examine the possible haematological alterations of low altitude exposure in order to assess the effectiveness of such exposure. As such, the aim of the present study was to examine the effects of 3 weeks of live high, train high exposure (LHTH) at low natural altitude (i.e. Perisher Valley, 1800 m) on Hb<sub>mass</sub>, red blood cell count and iron profile in elite distance runners.

## Methods

### Participants

Sixteen elite or well-trained male and female distance runners were recruited from national and state sporting organisations and allocated into two groups (Table 1). These two groups comprised of participants that slept and trained at low altitudes (LHTH, n = 8) or remained near sea level for a period of 3 weeks (CONTROL, n = 8). All participants were provided with the procedures and risks associated with their participation in this study. Prior to data collection, written informed consent was obtained in accordance with the institution's Human Research Ethics Committee. All athletes were in a "pre-competition" phase meaning they were preparing to race in national and international track races following the winter build-up period. Therefore, all runners had significant prior aerobic conditioning, their training volume was high and included both threshold based interval sessions and some race specific interval sessions. Throughout the study, training intensity, duration and frequency were controlled by external coaches but athletes and coaches were asked not to substantially alter their training schedules during the study period.

**Table 1.** Characteristics and weekly training of participants in the live high, train high (LHTH) altitude exposure and control groups. Values are mean ( $\pm$ SD).

	Live High : Train High(n=8)	Control (n=8)
Male / Female	8 male	3 female, 5 male
Age (y)	22.0 (3.7)	25.9 (5.2)
Mass (kg)	68.0 (5.4)	62.8 (8.3)
Hb <sub>mass</sub> (g)	933 (131)	839 (196)
Relative Hb <sub>mass</sub> (g·kg <sup>-1</sup> )	13.6 (1.1)	13.2 (1.8)
Weekly TR Vol (km.wk <sup>-1</sup> )	86.2 (14.6)	100.2 (20.4)

The training volumes (TR Vol) are those completed during the intervention.

### Procedures

The LHTH group, lived at a natural altitude of 1800 m (Perisher Valley, New South Wales, Australia) and trained at altitudes of 1700 to 2200 m (Snowy Mountains, Australia). Twice a week, these athletes also descended to 1000 m to perform high intensity training sessions (~2h) on a synthetic athletics track or running trails. The CON-

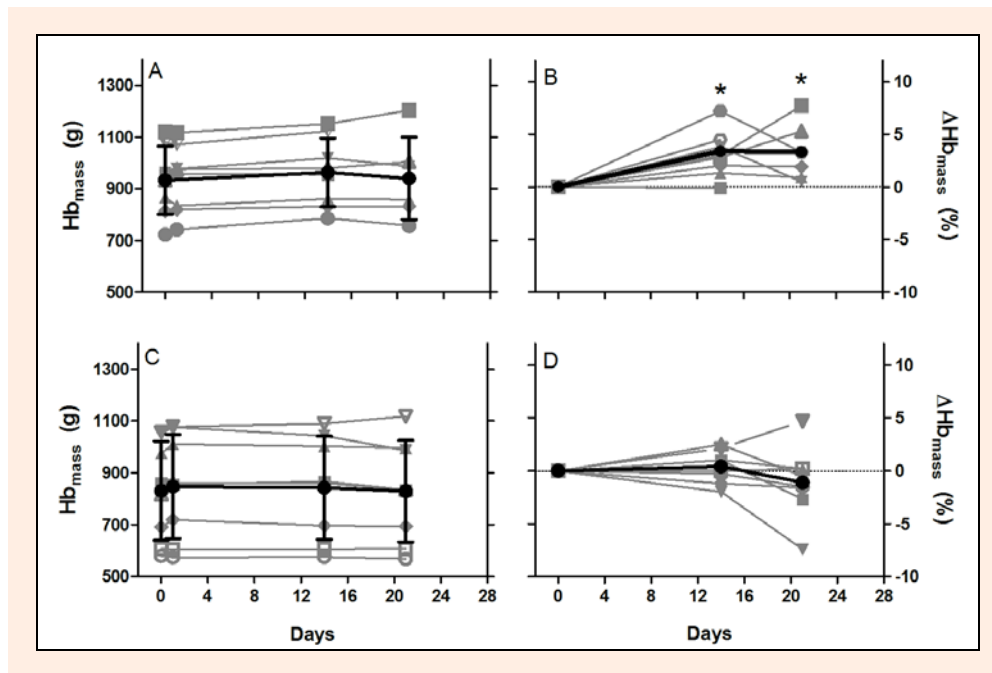
TROL group lived and trained near sea level for the duration of the study (Canberra, 600 m). All athletes were supplemented with oral iron (Ferro-Grad C, Abbott Laboratories, 105g elemental iron) on a daily basis to ensure any erythropoietic adaptations were not compromised by insufficient iron availability. At baseline, after two weeks and again at completion of the three-week intervention, haemoglobin mass (Hb<sub>mass</sub>) was assessed and a venous blood sample was taken from all participants.

Hb<sub>mass</sub> was measured using the 2-min carbon monoxide (CO) rebreathing method as described by Schmidt and Prommer (2005) with some modifications (Alexander et al., 2011; Garvican et al., 2010; Prommer and Schmidt, 2007). Briefly, participants rebreathed a CO bolus equivalent to 1.2 ml.kg<sup>-1</sup> of body weight for a period of two minutes. Capillary blood samples were obtained at the start of the test as well as at seven minutes post administration of the CO dose for determination of the percentage of bound carboxyhaemoglobin (%HbCO). Blood samples were measured a minimum of five times for %HbCO using an OSM3 hemoximeter (Radiometer, Copenhagen). The same analyser was used for all tests performed. Expired CO was determined using a Dräger Pac 7000 (Lübeck, Germany) CO sensor. Hb<sub>mass</sub> was calculated from the mean change in %HbCO before and after rebreathing. Hb<sub>mass</sub> was measured in duplicate at baseline and the typical error of measurement for Hb<sub>mass</sub>, calculated from these double baseline measures was 1.4% (90% confidence limits; 1.0 to 2.2).

Venous blood samples were drawn by trained phlebotomists from an antecubital forearm vein and analysed within 12 to 24 h. Samples collected at 1800 m were transported at 10°C and analysed in the same laboratory as all other samples. On each occasion, 2 ml of whole blood was collected into an EDTA vacutainer for measurement of haemoglobin concentration [Hb], haematocrit (Hct), and reticulocytes (%retics) (Sysmex XT-2000i; Sysmex Corporation, Japan). A further 4 ml was collected into a serum vacutainer to assess serum ferritin, soluble iron, transferrin and percent transferrin saturation (COBAS INTEGRA 400 plus, Roche Diagnostics, Switzerland). A total of 18 ml of whole blood was obtained over the course of the study, and thus likely to have had negligible effects on changes in Hb<sub>mass</sub> since this volume amounts to a loss of ~3 g of Hb.

### Statistical analysis

Participants' age, body mass and pre-exposure Hb<sub>mass</sub> were compared between groups using an independent sample t-test. Changes in serum ferritin, reticulocytes, transferrin, haemoglobin, haematocrit and the percent change in Hb<sub>mass</sub> were compared between Conditions and over Time using a mixed model two-way ANOVA. Where significant effects were observed Tukey post hoc tests were used. Assumptions of sphericity (Mauchly's test) were assessed. Where violations to assumptions of sphericity were observed, the degrees of freedom were corrected using Greenhouse-Geisser or Huynh-Feldt corrections where appropriate. The critical level of significance was set at  $p \leq 0.05$ .



**Figure 1.** Percentage change in  $Hb_{mass}$  from baseline following three weeks of live high, train high (LTHH; **A** and **C**) altitude exposure or training and living near sea-level (600m; **B** and **D**) group. Mean and standard deviation shown in black, with individual data shown in grey. \*  $p < 0.05$ , compared with CONTROL.

## Results

Age, body mass, as well as pre-exposure  $Hb_{mass}$  were not significantly different between LTHH and CONTROL (Table 1). All subjects in the CONTROL group completed the 3 weeks of prescribed training. In the LTHH group, 8 subjects completed 2 weeks but due to personal commitments only 5 subjects completed the entire 3 weeks of exposure. The increase in  $Hb_{mass}$  from baseline was significantly greater in LTHH, compared with CONTROL, at both 2 ( $P=0.01$ ) and 3 weeks ( $p = 0.02$ ) following exposure (Figure 1).

A significant interaction effect was observed for Condition and Time in Hct ( $p < 0.01$ ; Table 2). Post hoc comparisons revealed that Hct was greater in LTHH than CONTROL at 2 ( $p = 0.01$ ) and 3 weeks ( $p = 0.04$ ) of exposure (Table 2). Reticulocyte count was significantly greater in LTHH compared with CONTROL at 2 ( $p = 0.02$ ) but not at 3 weeks ( $p = 0.27$ ) of exposure. No significant interactions were observed for [Hb] ( $P=0.06$ ), serum ferritin ( $p = 0.43$ ), transferrin ( $p = 0.52$ ) or reticulocyte expressed as a percentage ( $p = 0.16$ ).

## Discussion

The present study found that 3 weeks of LTHH (i.e. classic) altitude exposure at just 1800 m was sufficient to induce a significant increase in  $Hb_{mass}$  in elite distance runners. This finding is of interest because it is generally considered that natural altitude exposure below 2000 m is insufficient to stimulate significant erythropoietic benefit (Pottgiesser et al., 2009; Wilber, 2007).

We observed a 3.0% increase in  $Hb_{mass}$  following three weeks of LTHH altitude exposure at 1800 m. This significant increase in  $Hb_{mass}$  was also associated with an increase in reticulocyte count and Hct, compared with the CONTROL group, within the first two weeks of the LTHH altitude exposure. Such an increase in  $Hb_{mass}$  is somewhat consistent with previous research showing that three weeks of classical altitude training at 1300 m and 1650 m interspersed with three weeks of sea level training resulted in an increased red blood cell volume,  $Hb_{mass}$  and serum EPO concentration (Frese and Friedmann-Bette, 2010). But, our results are contrary to those of Rasmussen et al. (2013), who concluded from a comprehensive

**Table 2.** Serum ferritin, reticulocytes, transferrin, haemoglobin and haematocrit of participants at baseline and following three weeks of live high, train high (LTHH) altitude exposure or training and living near sea-level (600m; CONTROL) group. Data are means ( $\pm$ SD).

	LTHH (1800 m)			Control (600 m)		
	Baseline	2 Weeks	3 Weeks	Baseline	2 Weeks	3 Weeks
Serum Ferritin ( $\mu$ g/L)	91.3 (60.0)	81.4 (42.0)	82.4 (37.5)	84.4 (43.4)	88.3 (41.5)	118.8 (79.6)
Reticulocytes ( $\times 10^9/L$ )	37.2 (10.8)	58.3 (17.3) *	52.5 (16.4)	38.2 (9.3)	40.7 (7.7)	43.7 (9.9)
Reticulocytes (%)	.94 (.25)	1.09 (.30)	1.01 (.33)	1.10 (.20)	1.13 (.22)	1.19 (.26)
Haemoglobin Concentration (g/dL)	15.4 (.9)	15.9 (.7)	15.6 (.9)	14.8 (1.2)	14.6 (1.0)	14.4 (1.3)
Transferrin (g/L)	2.81 (.37)	2.84 (.40)	2.7 (.56)	2.89 (.21)	2.87 (.15)	2.76 (.25)
Hematocrit (%)	44.2 (2.1)	45.1 (1.8) *	45.3 (2.3) *	42.4 (2.9)	41.7 (2.9)	41.1 (3.7)

\*  $p < 0.05$ , significantly different to CONTROL.

meta-analysis that exposure to natural altitude must exceed two weeks at 4000 m to observe a statically significant effect. On the other hand, our findings also support the recent conclusions surrounding the time course of erythropoietic adaptation of a 1% increase per 100 hours of exposure when exposed to either natural or simulated moderate altitudes (Clark et al., 2009; Garvican et al., 2012). A recent meta-analysis by Gore et al., (2013) examined Hb<sub>mass</sub> changes following a variety of hypoxic doses (including LHTH studies ranging from 10-28 days) and determined that during altitude exposure Hb<sub>mass</sub> increased at a rate of ~1.1% per 100 hours for both LHTH and live-high train-low altitude exposure; with the response at 2200 m natural altitude being just as effective as at 3000 m simulated altitude provided the total hours of exposure are matched. Notably, within the present study, the majority of the increase in Hb<sub>mass</sub> was observed within the first two weeks of altitude exposure (Figure 1). The meta-analysis of Gore et al. (2013), predicts an additional ~2% increase in Hb<sub>mass</sub> in the third week, which was not observed. Regardless, our current results identify 1800 m as a suitable threshold altitude for LHTH for increased red blood cell production and also reinforce the efficacy of LHTH since it provides continuous exposure to hypoxia.

Given that the present study examined low altitude exposure, it is also salient to examine the total hypoxic dose as calculated by hours of exposure multiplied by altitude height. Within this study the LHTL group accumulated 336 hours of exposure at 1800 m after 2 weeks and 504 h after 3 weeks, which equates to 604,800 AU and 907,200 AU, respectively. Interestingly, the recent study of Ryan et al. (2014) reported a 3.7% increase in Hb<sub>mass</sub> after 7 days at 5260 m, which equates to 883,680 AU, with the magnitude of increase in Hb<sub>mass</sub> somewhat comparable to the 3.0% increase for 907,200 AU observed in this study. It should be noted that this conceptual model for the determination of hypoxic dose assumes linear relationships between changes in Hb<sub>mass</sub> and increasing altitude height or duration of exposure. While this is unlikely, this model improves on previous models quantifying altitude dose which typically focus on the duration of exposure alone (Gore et al., 2013). Clearly, further research is needed in order to better understand the dose-response relationship of Hb<sub>mass</sub> at altitude, along with titrating the minimally effective altitude. Indeed, with training intensities even less compromised, it may be beneficial to examine Hb<sub>mass</sub> responses at even lower altitudes (1300-1600 m) (Frese and Friedmann-Bette, 2010) for athletes who only have access to such low lying altitudes venues.

### Limitations of the study

Although planned, performance data are unavailable for this study due to the timing of altitude exposure and athlete's specific training and racing schedules. Due to unforeseen circumstances some athletes could not and did not race as planned on their return to sea level while others engaged in a consecutive bout of altitude training a few weeks following this study and thus due to a number of confounding variables affecting individual results a

true measure of performance could not be formulated. However, our group has previously acknowledged the challenges of demonstrating that small changes in Hb<sub>mass</sub> translate in to a performance benefit (Gore, 2014).

### Conclusion

The results of this study have illustrated that it is possible for athletes to expect a ~ 3% increase in Hb<sub>mass</sub> after only 14 days of LHTH training at 1800 m. This lowers by ~200 m the threshold altitude considered sufficient to increase red blood cell production. Given the that accelerated red blood production seems to depend on the total dose of sufficient 'altitude' our results highlight that LHTH is the most time efficient modality for athletes and coaches to consider, because it provides 24 hour per day exposure to hypoxia, provided that sufficient total hours can be accumulated.

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### Key points

- Two and three weeks of LHTH altitude exposure (1800 m) results in a significant increase in Hb<sub>mass</sub>
- LHTH altitude exposure increased Hb<sub>mass</sub> by 3.1% after 2 weeks, and 3.0% after 3 weeks of exposure
- LHTH altitude exposure may be a practical method to increase Hb<sub>mass</sub> in well-trained athletes.

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