

THE EFFICACY OF HYPOXIC TRAINING
TECHNIQUES IN AUSTRALIAN
FOOTBALLERS

By

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STATEMENT OF AUTHORSHIP AND SOURCES

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Blake David McLean

24/07/2014

Date

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LIST OF PUBLICATIONS RELATED TO THIS THESIS

1. **McLean, B.D.**, D. Buttifant, C.J. Gore, K. White, C. Liess and J. Kemp (2013). Physiological and performance responses to a pre-season altitude training camp in elite team sport athletes. *International Journal of Sports Physiology Performance*. 8(4): pages 391-399.
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3. **McLean, B.D.**, C. Gore and J. Kemp (2014). Application of 'live low-train high' for enhancing normoxic exercise performance in team sport athletes – a systematic review. *Sports Medicine*. Online first 22 May.
4. Tofari, P.,* **B.D. McLean**,* J. Kemp & S. Cormack (Under review). A self-paced intermittent protocol on a non-motorised treadmill: A reliable alternative to assessing team sport running performance. *International Journal of Sports Physiology and Performance*.
* Authors made an equal contribution to the manuscript
5. **McLean, B.D.**, P. Tofari, C. Gore and J. Kemp. Changes in running performance following four weeks of interval hypoxic training in Australian Footballers: a single blind, placebo controlled study. Manuscript under preparation.

OTHER RELEVANT PUBLICATIONS

1. Gore, C., K. Sharpe, L. Garvican-Lewis, P. Saunders, C. Humberstone, E.Y. Robertson, N. Wachsmuth, S.A. Clark, **B.D. McLean**, B. Friedman-Bette, M. Neya, T. Pottgiesser, Y.O. Schumacher W. Schmidt, W (2013). Altitude training and haemoglobin mass from the optimised carbon monoxide re-breathing method – a meta-analysis. *British Journal of Sports Medicine*. 47(Supplement 1): pages i31-i39.

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ABSTRACT

Hypoxic training techniques have been gaining popularity in team sports, with a range of methods employed. However, before this work, there was a paucity of information on the responses of team sport athletes to hypoxic training. This work explores how team sport athletes respond to these interventions, complementing other recent work in this area. The primary focus of this thesis is on two hypoxic training techniques; Live High-Train High (LHTH) and Live Low-Train High (LLTH).

Study 1 (Chapter 3) in this thesis examined changes in running performance [2,000 m time-trial (TT)] and physiological responses [haemoglobin mass (Hb_{mass}) and intramuscular carnosine content] of 30 elite Australian Football (AF) players after a pre-season altitude camp. Participants completed 19 days of living and training at either moderate altitude [(~2,130 m) ALT; $n = 21$] or sea-level (CON; $n = 9$). TT performance and Hb_{mass} were assessed pre- (PRE) and post-intervention (POST₁) in both groups, and at four weeks after returning to sea-level (POST₂) in ALT only. Improvement in TT performance after altitude was *likely* 1.5 (± 4.8 ; 90% CL)% greater in ALT compared with CON. Improvements in TT were maintained at POST₂ in ALT. Hb_{mass} after altitude was *very likely* increased in ALT compared with CON ($2.8 \pm 3.5\%$). Hb_{mass} had returned to baseline at POST₂. Intramuscular carnosine did not change in either gastrocnemius or soleus from PRE to POST₁. In conclusion, Study 1 shows that a pre-season altitude camp improves TT performance and Hb_{mass} in elite AF players to a similar magnitude demonstrated by elite endurance athletes undertaking altitude training, and changes in running performance are maintained for four weeks, despite Hb_{mass} returning to baseline. These results suggest that LHTH camps may be a valuable intervention for the preparation of team sport athletes leading into the competitive season.

Study 2 (Chapter 4) aimed quantify the year-to-year variability of altitude-induced changes in Hb_{mass} in elite team sport athletes. Twelve athletes completed a 19- (ALT1) and 18-day (ALT2) moderate altitude (~2,100m) training camp separated by 12 months. An additional 20 subjects completed only one of the two training camps (ALT1 additional $N = 9$, ALT2 additional $N = 11$). Total Hb_{mass} was assessed before (PRE), at the end of (POST₁), and four weeks after each camp. Results show that POST₁ Hb_{mass} was *very likely* increased in both ALT1 ($3.6 \pm 1.6\%$; mean \pm ~90 CL) and ALT2 ($4.4 \pm 1.3\%$), with an individual responsiveness of 1.3% and 2.2%, respectively. There was a weak correlation between ALT1 and ALT2 ($R = 0.21$, $p = 0.59$) for change in Hb_{mass} , but a moderate inverse relationship

between change in Hb_{mass} and initial relative Hb_{mass} [g/kg ($R = -0.51$, $p = 0.04$)]. In conclusion, two pre-season, moderate altitude camps, separated by one year, yielded a similar (4%) mean increase in Hb_{mass} of elite footballers, with an individual responsiveness of approximately half the group mean effect, indicating that most players gained benefit. Nevertheless, individual athletes did not display consistent changes in their Hb_{mass} from year-to-year. Thus, a “responder” or “non-responder” to altitude for Hb_{mass} does not appear to be a fixed trait.

Despite the weak correlation between changes in Hb_{mass} from subsequent exposures reported in Study 2, there was evidence that one athlete exhibited consistently large erythropoietic responses to both ALT1 and ALT2. Therefore, Chapter 4.1 presents a case study of this individual’s data, suggesting that *responders* to altitude exposure may exist, but the phenomenon may be rarer than previously proposed.

A systematic review (Chapter 6) evaluated the normoxic performance outcomes reported in the available LLTH literature, with a particular focus on the influence of training intensity/modality and other methodological considerations when interpreting the efficacy of LLTH. A systematic search was conducted to capture LLTH studies, up to December 2013, with a matched normoxic (control) training group and the assessment of performance under normoxic conditions. Search results identified 40 papers that met the inclusion criteria, representing 31 separate studies. Within these 31 studies, four types of LLTH were identified: (1) continuous low-intensity training in hypoxia (CHT, $n=16$), (2) Interval hypoxic training (IHT, $n = 4$), (3) Repeated sprint training in hypoxia (RSH, $n=3$), and (4) Resistance training in hypoxia (RTH, $n=4$). Four studies also used a combination of CHT and IHT. The majority of studies reported no difference in normoxic performance between hypoxic and normoxic training groups. However, selection of training intensity was identified as a key factor in mediating normoxic performance outcomes. Improvements in normoxic performance appear most likely following high-intensity, short term and intermittent training (e.g. IHT, RSH).

Study 3 (Chapter 7) presents a novel ‘self-paced’ team sport running protocol and assesses its test-retest reliability. In this study, ten male team sport athletes completed five testing sessions (familiarisation + four reliability trials). The 30-min team sport protocol, performed on a curved non-motorised treadmill, consisted of three identical 10-min activity blocks, with visual and audible commands to direct locomotor activity; however, actual locomotor

speeds were self-selected by participants. Results show that peak and mean speed and distance variables assessed across the entire 30-min protocol exhibited a Coefficient of Variation (CV) < 5%. All peak and mean power variables exhibited a CV < 7.5%, except walking (CV 8.3-10.1%). In conclusion, this novel team sport running protocol displays good test-retest reliability across a range of speed, distance and power variables. Given its self-paced design, this type of protocol provides an ecologically valid alternative to common externally-paced protocols when assessing team sport running performance.

Study 4 (Chapter 8) aimed to assess changes in team sport running performance following four weeks of IHT. In a single-blinded, randomised controlled trial, subjects completed four weeks of either IHT or placebo (PLA) training. Participants completed Yo-Yo IR2 and the team sport running protocol (in Study 3) pre- and post-intervention. Data showed that four weeks of IHT in Australian Footballers resulted in: (i) smaller improvements in externally-paced, high-intensity running (Yo-Yo IR2) performance compared with training in normoxia; (ii) IHT and PLA participants exhibiting similar increases in high-intensity running distance during the 30-min self-paced team sport protocol; and (iii) IHT participants exhibiting greater improvements in the self-paced protocol for total distance and for distance covered during low-intensity activity. This suggests that hypoxic training may influence pacing strategies in team sport athletes.

In summary, a range of hypoxic techniques may be used to improve the physiological and running capacity of team sport athletes. This work highlights important practical considerations to adopt in order to optimise physiological and performance outcomes from these hypoxic training interventions.

LIST OF ABBREVIATIONS AND NOMENCLATURE

AF – Australian Football

bpm – beats per minute

CA – carbonic anhydrase

CO – carbon monoxide

COX – cytochrome oxidase complex

CS – citrate synthase

EPO – erythropoietin

F_IO₂ – fraction of inspired oxygen concentration

GLUT-4 – glucose transporter-4

GXT – graded exercise test to exhaustion

Hb_{mass} – haemoglobin mass

ΔHb_{mass} – change in Hb_{mass}

HR – heart rate

HR_{max} – maximum heart rate

HRR – heart rate reserve

La⁻ – Lactate

LDH – lactate dehydrogenase

LT – Lactate threshold

MRS – Magnetic resonance spectroscopy

MVC – maximal voluntary contraction

MVC₃ – 3 s maximal voluntary contraction

MVC₃₀ – 30 s maximal voluntary contraction

NIRS – near infrared spectroscopy

NMT – non-motorised treadmill

PFK – phosphofructokinase

PGC1α – peroxisome proliferator-activated receptor gamma coactivator 1α

PO₂ – partial pressure of oxygen

Reps – repetitions

Reps_{20%1RM} = Number of repetitions at 20% or one repetition maximum

RM – repetition maximum

RSA – repeated sprint ability

r-HuEPO – recombinant human erythropoietin

SpO₂ – saturation of peripheral oxygen

TFAM – mitochondrial transcription factor A

TT – time trial

TTE – Time to exhaustion

VO₂ – oxygen consumption

VO₂peak – peak oxygen consumption

VO₂max – maximal oxygen consumption

vVO₂max – velocity at VO₂max

WAnT – Wingate anaerobic test

W – Watts

Wmax – maximal watts achieved during graded exercise test to exhaustion

Yo-Yo IR1 – Yo-Yo intermittent recovery tests level 1

Yo-Yo IR2 – Yo-Yo intermittent recovery tests level 2

1RM – one repetition maximum

CHAPTER 1 – INTRODUCTION

Australian Football is an intermittent sport, characterized by periods of high-intensity exercise, interspersed with periods of low-intensity activity. Australian Football athletes are required to complete many high intensity efforts during match play (Mooney et al., 2011) over a prolonged duration (match time \approx 120 min), and training interventions therefore aim to improve both aerobic and anaerobic capacity of the athletes, whilst maintaining/improving other important physical (e.g. maximal strength and power) and technical (e.g. ball disposal efficiency) attributes important to this multi-disciplinary sport.

Endurance athletes have been using hypoxic training techniques for decades, in an attempt to improve aerobic endurance performance in normoxic environments. Recently, the use of hypoxic training techniques has been gaining popularity with team sport athletes (Billaut et al., 2012), with the goal of improving within-match running performance. However, very little information is available regarding the responses of team sport athletes to hypoxic training interventions. Although aerobic and anaerobic capacities are important in team sports, as in endurance athletes, the intermittent nature of team sports presents a very different challenge compared to the prolonged continuous performance in endurance sports. Therefore, it is important to investigate how team sport athletes respond to hypoxic training techniques and how these techniques impact on the physical attributes specific to team sport performance.

Within the spectrum of hypoxic training techniques, the two most commonly employed by team sport athletes are pre-season altitude training camps and Live Low-Train High (LLTH) hypoxic training (Billaut et al., 2012). However, there is limited information available on how either of these techniques influence team sport specific performance, with the majority of hypoxic training literature focusing on physiological and performance changes in endurance athletes. The effectiveness of hypoxic training interventions on performance outcomes is further complicated by the high variability of individual responsiveness to these techniques (Chapman et al., 1998; Robertson et al., 2010b). Indeed, it has previously been proposed that some individuals may be pre-disposed to an enhanced response to hypoxic exposure, possibly related to underlying genetic traits (Chapman et al., 1998).

Assessing team sport performance, *per se*, is inherently more challenging than assessing performance in acyclic endurance-based sports due to the multidisciplinary, intermittent and

skill-dominant nature of team sports. The development of physical performance tests that are more specific to the game demands of team sports may further progress the understanding of how training interventions, such as hypoxic training, may impact on physical capacity team sport athletes.

This work aimed to enhance the understanding of how Australian Football players respond to both pre-season altitude training camps and LLTH protocols. The specific aims of this research were:

1. Determine the physiological and performance responses to a pre-season altitude training camp in professional Australian Football players (Study 1 – see Chapter 3)
2. Investigate the individual variability of responses to an altitude training camp from year-to-year (Study 2 – see Chapter 4)
3. Systematically review the current LLTH literature, focusing on performance outcomes and methodological approaches which may be beneficial for team sport athletes (Chapter 6)
4. Develop an Australian Football specific running protocol as a tool to assess the efficacy of training interventions with team sport athletes (Study 3 – see Chapter 7)
5. Assess the efficacy of a four-week interval hypoxic training program for improving Australian Football specific running performance (Study 4 – see Chapter 8)

CHAPTER 2 – LITERATURE REVIEW

In 1968, the Olympic Games were held for the first time at moderate altitude (2,240 m) in Mexico City. Results from these Olympics suggested that competing at altitude made competition more difficult for endurance athletes, whilst times in running events 400m and under were estimated to be 1.7% faster than they would otherwise have been if run at sea-level (Ward-Smith, 1984). These differences in performance at altitude led many to investigate why these changes were occurring, and strategies to overcome these limitations. Moreover, endurance athletes competing at moderate-high altitudes began to implement acclimatisation camps in periods prior to performance at high altitude. Upon return to sea-level, after periods of acclimatisation, many athletes and coaches noted improvements in performance, whilst others experienced a worsening of performance; but often the training load had been increased and/or the focus on training had improved in a camp away from home (Stray-Gundersen and Levine, 2008). This led to the first studies examining responses to living and training at altitude for a number of weeks compared to living and training at sea-level (Levine and Stray-Gundersen, 1992b). This initial work revealed improvements in performance in all groups participating in the training camps and, therefore, it was concluded that organised training camps led to an improvement in endurance performance, regardless of the living or training altitude (Levine and Stray-Gundersen, 1992b). Levine and Stray-Gundersen then sought to improve the control design from this initial study, and remove possible confounding variables (Levine and Stray-Gundersen, 1997). The resulting study suggested that living at high altitude may augment endurance performance by increasing red cell volume (RCV), subsequently improving the athlete's ability to transport oxygen around the body, leading to improvements in endurance performance (Levine and Stray-Gundersen, 1997).

As a result of anecdotal observations and a growing body of scientific literature, ascending to moderate-high altitude is now common practice for endurance athletes prior to competition. It is commonly accepted that living in a hypoxic environment (created by altitude or artificial techniques) for greater than 16 hours a day for a period of approximately 3-4 weeks can augment the haemoglobin mass (Hb_{mass}) of an individual (Levine and Stray-Gundersen, 1997; Saunders et al., 2009; Gough et al., 2011). However, this finding is not universal (Gore et al., 1998; Siebenmann et al., 2012), and a number of other mechanisms have been suggested as responsible for increases in sea-level performance after prolonged exposure to hypoxic environments (Gore et al., 2007). While prolonged hypoxic exposures

may stimulate a range of physiological adaptations, there is no consensus on the exact mechanisms responsible for improvements in performance (Gore and Hopkins, 2005; Levine and Stray-Gundersen, 2005).

The traditional model of altitude training involves either living and training at high altitude [Live High-Train High (LHTH)] or a combination of living at altitude and descending to lower altitudes to complete some, or all, training [Live High-Train Low (LHTL)]. LHTL models may also be achieved by providing supplemental oxygen whilst at terrestrial altitude so that athletes are able to exercise with normal and/or enhanced oxygen levels whilst at altitude, or by the use of hypoxic living quarters at sea-level. The development of hypoxic living and training rooms at sea-level has led to a number of other hypoxic interventions, including intermittent hypoxic exposure (IHE) and Live Low-Train High (LLTH) techniques. During IHE and LLTH, athletes live at sea-level under normobaric, normoxic conditions and spend short durations in hypoxic rooms in an attempt to induce performance benefits. These hypoxic rooms may be created through normobaric techniques by oxygen filtration or by introducing higher concentrations of nitrogen (nitrogen dilution) into a sealed room. Hypobaric chambers may also be used to create hypoxic environments at sea-level. These hypoxic techniques may be used in isolation or in combination with other hypoxic techniques, as can be seen in Figure 2.1.

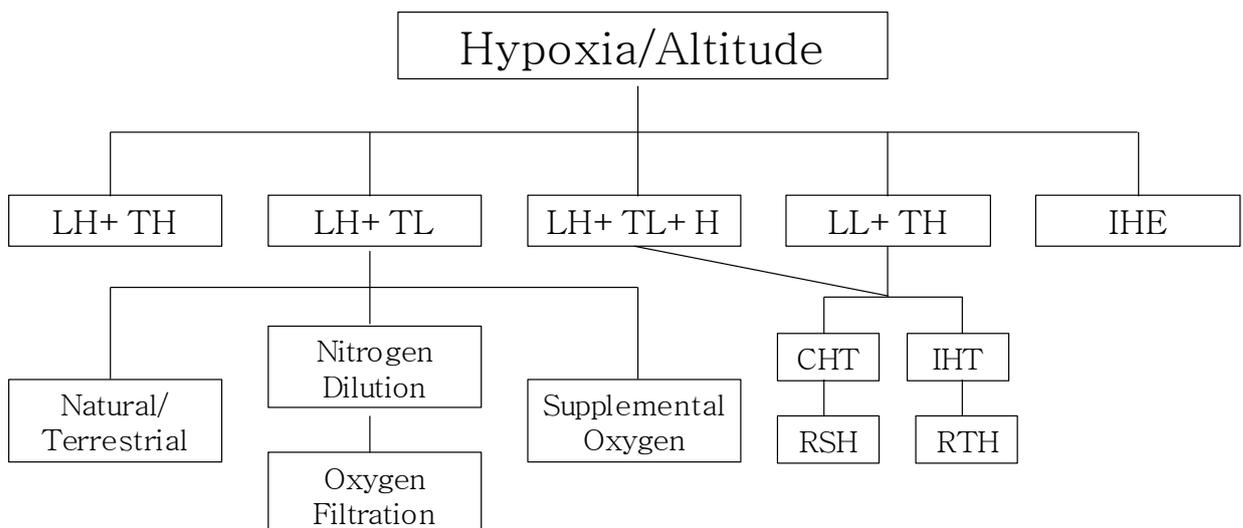


Figure 2.1 Summary of different hypoxic methods [adapted from (Millet et al., 2010)]

IHE = intermittent hypoxic exposure during rest; CHT = continuous hypoxic training; IHT = interval hypoxic training; RSH = repeated sprint in hypoxia; RTH = resistance training in

hypoxia; LH = live high; LHTLH = live high-train low and high; LL = live low; TH = train high; TL = train low; H = train high.

During hypoxic exposures, limited availability of oxygen in atmospheric air leads to a reduction in arterial oxygen saturation, which has little impact on oxygen consumption at rest, but becomes limiting to exercise performance as intensity increases (Clark et al., 2007). One of the earliest investigations examining exercise under hypoxic conditions found large increases in sub-maximal heart rate (HR; 116 bpm v 175 bpm) in subjects cycling at approximately 123 watts in hypobaric hypoxia equivalent to 4,550 m (Sutton, 1977). This increase in HR led Sutton (1977) to suggest that subjects were working between 30-50% and 70-90% of their sea-level VO_2 during normobaric and hypobaric conditions, respectively. However, earlier work (Pugh et al., 1964) and recent investigations (Clark et al., 2007) show no change in VO_2 between normoxic and hypoxic conditions at sub-maximal workloads, although there does appear to be a slight elevation in HR during sub-maximal workloads in hypoxic conditions (Clark et al., 2007), possibly related to increased sympathetic drive during stressful hypoxic exercise. Although sub-maximal oxygen consumption is not affected by hypoxic conditions, there is no doubt that maximal oxygen consumption ($\text{VO}_{2\text{max}}$) is reduced under hypoxia (Adams et al., 1975). Figure 2 shows reductions in $\text{VO}_{2\text{max}}$ across different altitudes, which are reduced by around 6-10% for every 1,000 m above sea-level.

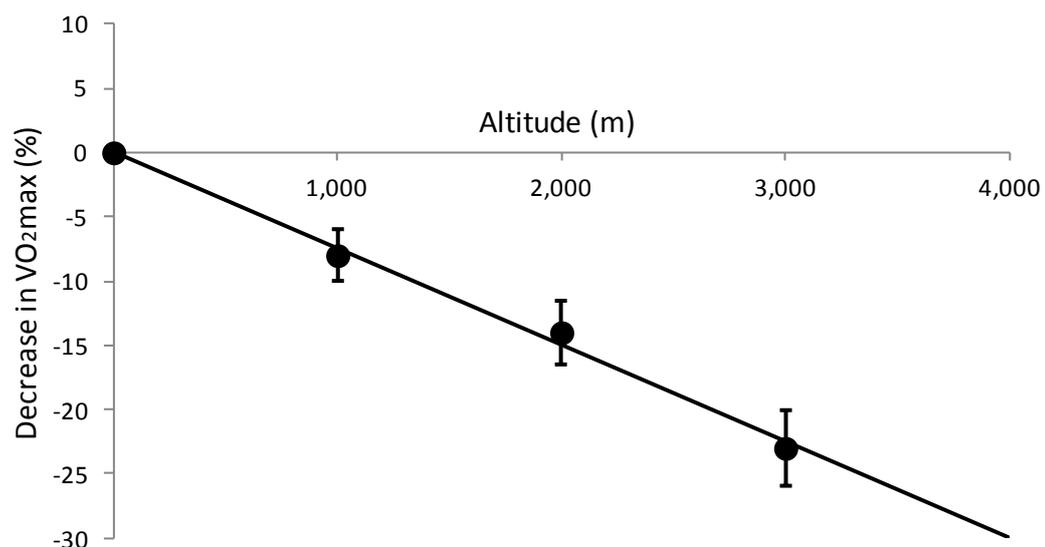


Figure 2.2 Percentage decrease in $\text{VO}_{2\text{max}}$ for every 1,000 m above sea-level [Reproduced from Clark et al. (2007)]

It is well established that VO_2max is impaired under hypoxic conditions (Clark et al., 2007). Reductions in VO_2max appear to be primarily related to reductions in arterial oxygen content, an idea that is supported by studies which demonstrate that individuals who are unable to maintain arterial oxygen saturation (SaO_2) during heavy exercise at sea-level are less able to maintain VO_2max (Lawler et al., 1988; Chapman et al., 1999) and running performance (Chapman et al., 2011) at moderate altitude. For example, Chapman et al. (1999) found a significant correlation ($r = -0.54$) between SaO_2 at VO_2max in normoxia and the decline in VO_2max from normoxia to mild hypoxia (18.7% O_2) in highly trained male endurance athletes. To more closely explore the relationship between SaO_2 during heavy exercise and the impairment in performance with acute altitude exposure, the same group examined 27 US national-class distance runners completing a 3,000 m time trial at sea-level and moderate altitude (2,100 m) (Chapman et al., 2011). These authors reported a significant correlation between SaO_2 during race pace exercise in normoxia and the reduction in 3,000 m running time ($r = -0.38$). This study also confirmed a strong relationship between SaO_2 and change in race pace VO_2 from normoxia to hypoxia ($r = -0.68$).

Impaired arterial oxygen saturation during high-intensity exercise is caused by limitations in pulmonary gas exchange, which is thought to be related to a number of factors, including diffusion limitations in the lung (creating a widened alveolar-arterial oxygen difference) and an inadequate hyperventilatory response to exercise [creating a reduced alveolar partial pressure of oxygen (PO_2)]. Traditionally, the primary factor behind arterial oxyhemoglobin desaturation in highly trained endurance athletes is believed to be a widened alveolar-arterial oxygen difference (Dempsey, 1987; Powers et al., 1989), possibly caused by extremely large cardiac outputs during high-intensity exercise, resulting in reduced transit times through the pulmonary circulation and thus, less time for pulmonary gas exchange. However, recent work has established a relationship between SaO_2 and ventilation/ VO_2 during high-intensity exercise in normoxia ($r = 0.62$, $P \leq 0.01$, $n = 27$) (Chapman et al., 2011). This result suggests that the athletes with the greatest ventilatory response to exercise are able to best defend exercise arterial oxygen saturation. In summary, highly trained endurance athletes may experience greater declines in VO_2max and performance under hypoxic conditions, and this may be primarily related to reduced pulmonary transit time (due to large cardiac outputs), but ventilatory responses to exercise likely also play a role in these reductions.

LHTH AND LHTL INTERVENTIONS

Physiological adaptations

Traditional LHTH and LHTL protocols involve prolonged periods of hypoxic exposure in an attempt to stimulate physiological adaptations that may enhance subsequent sea-level performance. At the onset of a sufficient hypoxic stimulus, the reduced PO₂ leads to an increase in the expression in hypoxia inducible factor (HIF) α subunits. HIF consists of an oxygen-regulated α subunit and a constitutive β subunit. Three α -chains have been identified, and HIF-1 α and HIF-2 α are considered the main functional proteins, with overlapping but partly distinct transcriptional specificities (Loboda et al., 2010). HIF-1 α is expressed in every tissue within the body, but is present in only small amounts during normoxic conditions due to its very short half life of approximately 5 min (Gore et al., 2007). However, during exposure to a hypoxic environment, the half life of HIF-1 α is increased to approximately 30 min, allowing it to accumulate within the cell, subsequently leading to the transcription of more than 100 target genes (Loboda et al., 2010). These downstream targets are involved in the regulation of angiogenesis/cell survival, erythropoiesis, glycolytic enzymes, glucose transporters, monocarboxylate transporters, pH regulation, vasodilation, enzymes involved dopamine synthesis, and accelerated ventilation (Sasaki et al., 2000). The stabilisation of HIF-2 α is also controlled by oxygen tension within the cell, and amounts of the protein increase with prolonged hypoxia (Holmquist-Mengelbier et al., 2006). HIF-2 α is the main HIF that regulates erythropoietin (EPO) production (Scortegagna et al., 2005). The up-regulation of the EPO gene (Wang et al., 1995) leads to erythropoiesis, which some argue is the primary physiological mechanisms leading to increases in endurance performance after LHTH and LHTL protocols (Levine and Stray-Gundersen, 1997).

EPO Cascade

At the onset of hypoxic exposure, expression of HIF-2 α is up-regulated, which stimulates an increase in EPO production, primarily within the kidneys, but also in the liver (Scortegagna et al., 2005). Sustained increases in EPO stimulate haematopoietic stem cells within the bone marrow to differentiate into erythroid precursor cells, reticulocytes and eventually mature erythrocytes (Sasaki et al., 2000). The use of pharmacological aids [e.g. recombinant human erythropoietin (r-HuEPO)] to induce erythrocythemia produces irrefutable endurance performance benefits (Simon, 1994). Indeed, 25 days of regular subcutaneous r-HuEPO injections (50 U/kg, approx. every 3 days) have been shown to induce a 12% increase in Hb_{mass} and an associated 7% increase in VO₂max in amateur endurance athletes (Parisotto et

al., 2000). Circulating EPO increases to approximately four times resting values 24 hours after r-HuEPO administration, and increases in EPO can be seen after hypoxic exposures as short as 90 min (Rodriguez et al., 2000). However, circulating EPO will only be approximately two-fold above resting levels after ascent to an altitude of 2,500 m (a common level of altitude/hypoxic exposure). Moreover, EPO has a very short half life of approximately 5.5 hours (Gore et al., 2007). Thus, elevated serum EPO levels are more sustained with repeated r-HuEPO injections than compared to living in hypoxic conditions (Hahn and Gore, 2001), with increases caused by a hypoxic stimulus returning to baseline very quickly upon exposure to normoxic conditions. Furthermore, following ascent to moderate-high altitude, circulating EPO will reach peak levels within 24-48 hours, before declining to near baseline levels after approximately one week at altitude (Hahn and Gore, 2001). As increases in EPO induced by hypoxic exposure are approximately half of that seen with r-HuEPO injections, and much less sustained, any increases in Hb_{mass} induced by prolonged hypoxic exposure are much more modest.

Timeline and magnitude of erythropoietic response

There is overwhelming consensus in the literature that prolonged hypoxic exposure (i.e. LHTH and LHTL) stimulates an increased production of red blood cells, given a sufficient ‘hypoxic dose’ (i.e. hr/day; number of days; degree of hypoxia) (Wilber et al., 2007; Gore et al., 2013; Rasmussen et al., 2013). However, there is less agreement as to the minimal ‘hypoxic dose’ necessary to induce detectable and meaningful increases in red blood cells following hypoxic exposure. Wilber et al. (2007) suggest that altitude training at 2,000–2,500 m for at least 22 hr/day and a minimum of 4 weeks is required to optimise erythropoietic benefits of prolonged hypoxic exposures, a contention supported by the findings of a recent meta-analysis (Rasmussen et al., 2013). After compiling the results of 66 hypoxic training studies, Rasmussen et al. (2013) suggest that altitude exposures < 3,000 m should generally last longer than 4 weeks to have a significant chance of increasing Hb_{mass} . This benefit may be accelerated if staying at higher elevations (e.g. at 4,000 m, benefits may be evident after ~ 2 weeks). This recommendation is based on a minimum threshold of a 5% increase in Hb_{mass} following altitude training, with the idea that this minimum increase is needed for increases in exercise performance (Robach and Lundby, 2012). However, detectable changes in Hb_{mass} (~3%) have recently been reported in periods as short as 11 days (Garvican et al., 2012a).

Physiological responses following LHTH or LHTL exposure

Traditionally, hypoxic exposure is thought to stimulate erythropoiesis, leading to an increased oxygen carrying capacity and, subsequently, improved VO_2max and endurance performance (Levine and Stray-Gundersen, 1997; Stray-Gundersen et al., 2001; Levine and Stray-Gundersen, 2005). High Hb_{mass} in elite athletes is well documented (Jelkmann and Lundby, 2011) and correlates well with exercise performance (Jacobs et al., 2011). There is also good consensus that a sufficient hypoxic stimulus (low enough PO_2 for long enough) will lead to augmented erythrocyte volume and red cell mass (Gore et al., 2007; Levine and Stray-Gundersen, 2007; Wilber et al., 2007). Thus, increases in Hb_{mass} are thought to be one of the primary mechanisms leading to increased exercise performance following prolonged hypoxic exposure. However, whether an increase in Hb_{mass} is the only/main contributing mechanism to improved endurance performance after hypoxic exposure remains highly contentious (2005; Gore and Hopkins, 2005; Levine and Stray-Gundersen, 2005). While some researchers strongly support the view that improvements in performance are due to augmented Hb_{mass} and subsequent increases in VO_2max (Levine and Stray-Gundersen, 2005), other researchers suggest that changes in muscle buffering capacity and movement efficiency also play a role in endurance performance changes following hypoxic exposures (Gore et al., 2001; Gore and Hopkins, 2005; Gore et al., 2007).

Levine and Stray-Gundersen (1997) were among the first to investigate the physiological effects of hypoxic training techniques. Through a series of studies, these authors witnessed increases in red cell volume, VO_2max and improved endurance performance after LHTL interventions (Stray-Gundersen and Levine, 1994; Levine and Stray-Gundersen, 1997; Stray-Gundersen et al., 2001). Increases in red cell volume may increase the oxygen carrying capacity of the blood and, thus, increase the arteriovenous oxygen difference during exercise, while increases in total blood volume (due to increased red cell volume) may produce improvements in maximal cardiac output (Gore and Hopkins, 2005). Both mechanisms may augment endurance performance if leading to a subsequent improvement in the fraction of VO_2max representing exercise intensity ($\text{VO}_{2\text{fracmax}}$) (Gore and Hopkins, 2005). Indeed, Chapman et al. (1998) suggest that those who have the greatest improvements in performance following hypoxic exposures are those individuals which have the largest and most sustained increases in EPO, leading to the largest increases in Hb_{mass} . However, there is also evidence to suggest that improvements in endurance performance are possible following hypoxic exposure even when improvements in red cell volume are not evident (Gore et al., 1998; Gore et al., 2001).

There are many studies that report decreased oxygen consumption at sub-maximal exercise intensities following hypoxic exposure (Hochachka et al., 1991; Green et al., 2000; Gore et al., 2001; Katayama et al., 2003; Katayama et al., 2004; Saunders et al., 2004b; Marconi et al., 2005; Schmitt et al., 2006; Neya et al., 2007), although this finding is not universal (Levine and Stray-Gundersen, 1997; Clark et al., 2004; Siebenmann et al., 2012). Improvements in movement efficiency would allow athletes to maintain higher velocities during competition at any given VO_2 , thereby leading to improved endurance performance (assuming that $\text{VO}_{2\text{fracmax}}$ remains unchanged) (Saunders et al., 2004a). Whilst haematological changes seem to be evident only after LHTH and LH TL protocols with sufficient hypoxic dose (Wilber et al., 2007), changes in exercise efficiency may be induced by shorter exposures as with LLTH protocols (Katayama et al., 2003; Katayama et al., 2004). Although many authors have been able to show changes in movement efficiency following hypoxic exposure, the mechanisms by which this occurs are currently unclear, but may be related to a decreased cost of ventilation, greater carbohydrate use for oxidative phosphorylation, and/or greater mitochondrial efficiency (Gore et al., 2007).

Along with changes in movement efficiency, a number of studies have shown improved muscle buffering capacity as a non-haematological mechanism associated with improved performance after hypoxic exposure (Mizuno et al., 1990; Saltin et al., 1995; Gore et al., 2001; Mizuno et al., 2008). Mizuno et al. (1990) reported improved muscle buffering capacity in the triceps brachii and gastrocnemius in well-trained cross country skiers following two weeks of altitude exposure. Improvements in buffering capacity of the gastrocnemius were also positively correlated with change in running time post-altitude ($r^2 = 0.83$, $P < 0.05$) (Mizuno et al., 1990). Saltin et al. (1995) subsequently showed improved muscle buffering capacity in six Scandinavian runners following two weeks at altitude, and postulated that increases in buffering capacity may be due to increased intramuscular carnosine content. More recently, Gore et al. (2001) showed an 18% increase in muscle buffering capacity after 23 days of LH TL with no change in sea-level controls; however, the same research group was unable to replicate the finding in a subsequent study (Clark et al., 2004). Conversely, Stray-Gundersen and Levine (1999b) reported decreases in tissue buffering capacity following four weeks at moderate altitude, and these authors strongly contend that improved muscle buffering capacity is not one of the mechanisms related to improved performance following hypoxic exposure.

Recently, a novel technique has been used to highlight possible non-haematological performance benefits of prolonged altitude exposure (Garvican et al., 2011). Garvican et al. (2011) exposed a group of eleven female cyclists to 26 nights of simulated (normobaric hypoxia) LHTL (16 hours per day at 3,000m) and clamped any erythropoietic response in six of these athletes via phlebotomy. While the athletes whose erythropoietic response was clamped showed no increase in VO_{2peak} (3.5% increase in VO_{2peak} in un-clamped group), this group did show a similar improvement (~4%) in 4-min all-out exercise performance as the un-clamped group. However, the un-clamped group showed a 40% larger improvement in a subsequent time to exhaustion ride at 100% peak power output (determined via a graded exercise test to exhaustion). This suggests that non-haematological adaptations may be equally, if not more, important than changes in Hb_{mass} and emphasises the role of Hb_{mass} in repeated high-intensity efforts.

Maintenance of physiological changes upon return to sea-level

As the goal of many altitude training camps is to improve subsequent performance at sea-level, the question of how long these induced physiological adaptations remain at sea-level becomes an important consideration. Although some non-haematological mechanisms may be related to improvements in performance following altitude exposure (Gore et al., 2007), the most understood physiological change after altitude exposure is the increase in Hb_{mass} . As the physiological cascade leading to this adaptation is also well understood, it is possible to measure its maintenance upon return to sea-level.

The initial increase in Hb_{mass} following exposure to hypoxic conditions is caused by the EPO cascade discussed earlier, while a somewhat opposing EPO cascade is observed upon return to sea-level. After returning to sea-level, EPO concentrations decline below baseline (pre-altitude) levels (Hahn and Gore, 2001). These low levels of circulating EPO facilitate neocytolysis, a process whereby young circulating red blood cells (neocytes) are subject to selective destruction (Rice and Alfrey, 2005). During this period, there is also altered behaviour of splenic endothelial cells, which become selectively more permeable to young red blood cells and permit increased phagocytosis of these cells by macrophages (Trial et al., 2001). Whilst athletes may increase haemoglobin mass at a rate of ~1.5% per week during 2-4 week altitude camps, the rate of decline in haemoglobin mass upon return to sea-level is approximately 2-3 fold higher due to EPO dropping below baseline levels (Pottgiesser et al., 2012). However, if serum EPO levels can be maintained upon returning

to sea-level, it is also possible to maintain increases in haemoglobin mass (Pottgiesser et al., 2012). Therefore, strategies that re-establish EPO levels may delay the decay in Hb_{mass} upon return to sea-level (Daniels, 1970). Short duration hypoxic exposures (3 h) are known to increase EPO levels and, while this increase is not sustained long enough to induce erythropoiesis, it may assist in delaying the effects of neocytolysis (Chapman et al., 2014).

In the absence of strategies to delay this decay, some studies have shown a rapid decline in Hb_{mass} upon returning to sea level (Garvican et al., 2012a). Indeed, following 4% increases in Hb_{mass} after 21 days of exposure at 2,760 m, Garvican et al. (2012a) reported a 1.5 % reduction in Hb_{mass} 3 days after returning to a sea-level environment. Interestingly, Hb_{mass} remained stable after this point, with Hb_{mass} still 2.3% above baseline 10 days after descent (Garvican et al., 2012a). In contrast, analysis of high-altitude natives descending to sea-level shows no immediate reductions in Hb_{mass} for up to 14 days, followed by a gradual decline in Hb_{mass} which plateaus after approximately 30 days (Prommer et al., 2010). This effect of neocytolysis is important for athletes to consider, as there is likely a limited timeframe in which altitude interventions influence physiological capacity upon returning to sea-level.

While the time course of decay of Hb_{mass} adaptations is an important consideration, the effect of ventilatory acclimatisation and alterations in neuromuscular function may also influence sea-level performance (Chapman et al., 2014). In response to prolonged hypoxic exposures, ventilation increases both at rest and during exercise in an attempt to offset the reduced PO_2 (Chapman et al., 1999). Following prolonged hypoxic exposure (LHTH or LH TL), these ventilatory adaptations often persist upon return to sea-level (Buskirk et al., 1967; Daniels and Oldridge, 1969; Levine and Stray-Gundersen, 1997), which results in a substantial increase in the work and cost of breathing during exercise, accounting for up to 15–20% of whole body VO_2 during maximal exercise (Aaron et al., 1992). This increased cost of breathing may be deleterious to maximal exercise capacity if the increase in ventilatory work is large enough to impair blood flow to the locomotor muscles (Chapman et al., 2014). In addition to these ventilatory responses, athletes living and training at moderate-high altitudes may experience alterations in neuromuscular function upon return to sea-level (Kayser et al., 1994). Indeed, athletes often report a loss of coordination at high running speeds (Wilber, 2007; Wilber et al., 2007), which likely results from altered running mechanics whilst training in a reduced atmospheric pressure. These biomechanical/neuromuscular considerations may be even greater for ball sport athletes training at altitude, as skill execution may also be affected by altered atmospheric pressures. Thus, the timing of optimal

performance upon return to sea-level is likely dependant on a number of factors, which includes the decay of any increases in Hb_{mass} , re-acclimatisation of ventilatory responses and restoration of neuromuscular function.

Performance outcomes following prolonged (LHTH + LHTL) hypoxic exposures

Assessing the impact of hypoxic training techniques on subsequent sea-level performance is perhaps more difficult than assessing physiological outcomes, because performance is multifaceted and dependant on many factors including fatigue, training status, and motivation (Robertson et al., 2010b). Drawing conclusions about expected changes in physical performance is also difficult because different hypoxic protocols have been reported in the literature - this includes differing degrees of hypoxic exposure, number of hours of exposure per day, and the number of days/weeks of continued hypoxic exposure. Nonetheless, the ultimate goal of most hypoxic training interventions is to augment performance at sea-level and, therefore, performance outcomes following such interventions should be carefully considered.

Live High-Train High (LHTH)

Traditional altitude camps involve ascending to a moderate terrestrial altitude of around 2,000-2,500 m for several weeks in an attempt to improve performance upon returning to sea-level. LHTH protocols are logistically easier to implement than some other common protocols (e.g. LHTL) as there is no need to transport athletes to multiple locations in order to manipulate the partial pressure of oxygen. LHTH protocols also provide the greatest 'hypoxic dose' in the shortest amount of time because athletes spend 24 hours a day in hypoxic conditions, and this may seem advantageous given responses to hypoxia are thought to be dose dependant (Levine and Stray-Gundersen, 2007). Indeed, greater increases in Hb_{mass} have been reported in some LHTH protocols compared to similar LHTL interventions (Eastwood et al., 2012), although this finding is not universal (Levine and Stray-Gundersen, 1997; Gough et al., 2011). Despite the possible benefit of a greater hypoxic dose, it is undisputed that maximal exercise capacity is compromised at high altitudes due to reduced oxygen delivery to the working muscles (Buskirk et al., 1967). As a result, the ability to perform high-intensity training during LHTH protocols is restricted, possibly leading to a detraining effect during prolonged LHTH camps.

Much of the LHTH literature has been conducted at altitudes below 2,000 m (Ingjer and Myhre, 1992; Jensen et al., 1993; Svedenhag et al., 1997; Bailey et al., 1998; Friedmann et al., 1999; Rusko et al., 1999; Saunders et al., 2004b), a height thought to be the approximate lower threshold for inducing physiological benefits (Wilber et al., 2007). Of the literature available where LHTH studies have been performed at or above 2,000 m, some have shown improved endurance performance post altitude (Miyashita et al., 1988; Martino et al., 1995; Burtscher et al., 1996; Gore et al., 1998) whilst others have shown no additional benefit (Levine and Stray-Gundersen, 1992a; Levine and Stray-Gundersen, 1997; Svedenhag et al., 1997). For example, Gore et al. (1998) showed a 4% improvement in work completed during a 4,000 m time trial in elite cyclists, however, no control group was included in this study. Martino et al. (1995) have also shown performance improvements in short duration swimming performance (100 m sprint) above those of control subjects after 21 days living and training at 2,800 m. In contrast, Levine and Stray-Gundersen failed to show any benefit of LHTH protocols on several occasions (Levine and Stray-Gundersen, 1992a; Levine and Stray-Gundersen, 1997). It is interesting to note that, in one of these studies (Levine and Stray-Gundersen, 1997), the control subjects, who trained at sea-level, demonstrated performance deterioration following the intervention. The authors suggest that this may be partially due to the performance tests being conducted in a hot environment, whilst all training was completed in cooler mountain regions, and that this heat de-acclimatisation may have been offset by the benefit of altitude in the LHTH and LHTL groups in this study (Levine and Stray-Gundersen, 1997). However, the authors also suggest that these reductions in performance could be related to ‘previously held expectations of a benefit from altitude training’ in the control group (Levine and Stray-Gundersen, 1997). Such placebo effects could also be present if the LHTH group (who did not improve performance during the intervention) believed that LHTL was a superior intervention than what they had received; however, knowledge of other interventions and pre-existing beliefs within this group was not discussed (Levine and Stray-Gundersen, 1997). While study outcomes in the LHTH literature are somewhat varied, a meta-analysis combining data from all available LHTH studies up to April 2007 suggested reasonable support for improved sea-level performance (Bonetti and Hopkins, 2009).

Live High-Train Low (LHTL)

To overcome the constraint of compromised exercise intensity (i.e. reduced exercise capacity during intervals, repeated efforts, etc.) whilst living in hypoxic conditions, Levine and Stray-Gundersen (1997) were the first to propose a LHTL model, involving living at altitudes high

enough to stimulate physiological adaptations, and travel to lower terrestrial altitudes to train at higher exercise intensities. Training at lower altitude would, thus, maintain high rates of oxygen flux, which may assist in preserving the muscle structure and function required for endurance success (Stray-Gundersen and Levine, 1999a; Levine and Stray-Gundersen, 2005). Since the introduction of this model, the majority of altitude training literature has focused on LHTL (as opposed to LHTH) protocols. The LHTL model can also be achieved by simulating “living” at altitude via the use of normobaric hypoxic rooms.

Interpretation of LHTL data are more difficult than comparing LHTH protocols because the time spent in hypoxic conditions varies between studies. Indeed, it is often thought that 12-16 hours per day of hypoxic exposure is needed to stimulate the beneficial physiological adaptations (Wilber et al., 2007). The majority of the literature examining LHTL protocols lasting greater than 15 days, with an exposure longer than 12 hours per day, report improvements in performance (Stray-Gundersen and Levine, 1994; Mattila and Rusko, 1996; Levine and Stray-Gundersen, 1997; Nummela and Rusko, 2000; Stray-Gundersen et al., 2001; Witkowski et al., 2001; Dehnert et al., 2002; Wehrlin et al., 2006), although some recent investigations using natural terrestrial altitude (Gough et al., 2011) and normobaric hypoxia (Siebenmann et al., 2012) have failed to find performance benefits. Improvements in performance have also been observed with shorter duration daily exposures (Hinckson and Hopkins, 2005), possibly related to a number of non-haematological physiological changes that may be beneficial for endurance performance, including improved movement efficiency and/or muscle buffering capacity (Gore et al., 2001).

A recent meta analysis of available LHTL studies suggested that, overall, LHTL protocols provide benefits to performance, and may be the most effective of all available hypoxic techniques for elite athletes (Bonetti and Hopkins, 2009). However, the presence of a placebo effect during LHTL and LHTH studies cannot be excluded (Bonetti and Hopkins, 2009), given that it is not possible to blind subjects and investigators from experimental conditions during terrestrial altitude training interventions. Bonetti and Hopkins (2009) have also suggested that nocebo effects may be present in some of the available LHTL literature, possibly leading to an overestimation of the benefit of LHTL in some cases. Recently, Siebenmann et al. (2012) conducted the first double-blinded, placebo-controlled LHTL study with the use of normobaric hypoxia (nitrogen dilution). These authors initially reported no change in endurance performance for the LHTL group over placebo controls, although the expected changes in Hb_{mass} , after hypoxic exposure for 16 hours per day for four weeks,

were not observed (Siebenmann et al., 2012). However, in a subsequent paper (Robach et al., 2012) from this same study, the authors reported that 5 of 10 LHTL subjects did increase Hb_{mass} on average by 4.6%, leading this group to suggest that some athletes respond to this type of hypoxic stimulus to a greater extent than others. The five LHTL ‘responders’ in this study also increased their maximal workload during an incremental exercise test, while no change was observed in non-responders or the placebo group (Robach et al., 2012). To assess the effect of changes in Hb_{mass} on performance, authors performed isovolumic haemodilution re-establish baseline Hb_{mass} values, and the aforementioned improvements in performance were reversed following this haemodilution (Robach et al., 2012). This investigation (Nordsborg et al., 2012; Robach et al., 2012; Siebenmann et al., 2012) is currently the only data available examining responses to a blinded LHTL training camp and these authors argue that the benefits of this LHTL intervention may be negligible for elite endurance athletes. However, some of the data presented suggests that some athletes ‘respond’ to a hypoxic stimulus to a greater extent than others, and those that do ‘respond’ may gain improvements in performance.

Since the introduction of the LHTL model by Levine and Stray-Gundersen (1997), LHTL protocols have been favoured by researchers, with little research focusing on classical LHTH interventions and how such protocols compare to LHTL. Indeed, this original study (Levine and Stray-Gundersen, 1997) is only one of two studies to directly compare the two protocols, showing greater benefits with LHTL. Although this study was well controlled, the absence of changes in performance in the LHTH group is interesting, considering a number of LHTH studies have been able to induce positive performance benefits (Miyashita et al., 1988; Burtscher et al., 1996; Gore et al., 1998). There is some evidence of a placebo effect in the control groups in this study and, thus, results should be interpreted with caution (Bonetti and Hopkins, 2009). Gough et al. (2011) is the only other group to directly compare LHTH and LHTL interventions. In contrast to Levine and Stray-Gundersen (1997), this group reported no improvement in swimming performance following either intervention and found race performance was slower in the LHTL group at seven days after exposure (Gough et al., 2011). Therefore, although the underlying theory supporting the use of LHTL over LHTH interventions is appealing, the available data remain equivocal.

LLTH INTERVENTIONS

LLTH techniques involve athletes living in normoxic conditions and performing some training under hypoxic conditions. Hypoxic exposures typically last < 3 h, 2-5 times per week and, therefore, do not provide a sufficient hypoxic stimulus (Wilber et al., 2007) to induce the haematological changes associated with LHTH and LHTL protocols. However, training in hypoxia may augment some non-haematological training adaptations which may subsequently enhance performance. The nature of adaptations stimulated by training in hypoxia, and any possible improvements in exercise capacity, is highly dependent on the type and intensity of the training completed – this includes continuous low-intensity training in hypoxia (CHT), interval hypoxic training (IHT), repeated sprint training in hypoxia (RSH), and resistance training in hypoxia (RTH) (see Chapter 6 for a detailed description of these techniques). The nature of tests used to measure performance (e.g. intensity, duration, continuous vs. intermittent) may also influence the observed changes in physical ‘performance’ following these hypoxic training interventions. As previously mentioned, maximal exercise capacity is limited in hypoxic conditions (Clark et al., 2007) and absolute training intensity is compromised, leading to a reduction in some physiological strain during hypoxic training sessions (Buchheit et al., 2012a). Therefore, some portion of high-intensity normoxic training is likely necessary in LLTH interventions in order to maximise possible performance benefits (McLean et al., 2014). A detailed discussion of the different LLTH modalities and the influence of these techniques on normoxic exercise performance are addressed in Chapter 6 (systematic review).

Physiological adaptations following LLTH

Training in moderate hypoxic environments effectively limits the amount of energy that can be produced oxidatively during exercise, and it is well established that this reduction in oxidative energy production leads to a reduced exercise performance in both endurance (Buchheit et al., 2012a) and team sport (Garvican et al., 2013) athletes. Although absolute exercise intensity is reduced under hypoxic conditions, the reduced oxygen availability may produce a greater peripheral physiological stimulus than training in normoxia, and this is thought to be the major contributor leading to possible increases in performance following LLTH interventions. Training in hypoxia up-regulates the activity of the transcriptional factor HIF-1 (Vogt et al., 2001), which has many downstream targets, including those involved in regulation of glycolytic enzymes, glucose transporters, monocarboxylate transporters and vasodilatory responses (Sasaki et al., 2000). Hypoxia-induced activation of

HIF-1 following LLTH is likely part of the signalling pathway leading to augmented expression of mRNA transcripts involved in carbohydrate metabolism, mitochondrial content (Terrados et al., 1990; Geiser et al., 2001; Vogt et al., 2001), oxidative stress defence and pH regulation (Zoll et al., 2006), although such findings are not universal following LLTH protocols (Messonnier et al., 2001). As discussed by Millet et al. (2010), the training intensity during LLTH sessions may be a key factor in mediating these peripheral adaptations. Indeed, Vogt et al. (2001) reported similar increases in HIF-1 α expression following six weeks of LLTH in both a high- and low-intensity training group; however, this increased expression translated into up-regulation of vascular endothelial growth factor (VEGF) and myoglobin mRNA in the high-intensity group only.

In support of this, six weeks of LLTH in elite level long distance runners has been shown to increase mRNA expression of glucose transporter-4 [GLUT-4 (+32%)], phosphofructokinase [PFK (+32%)], peroxisome proliferator-activated receptor gamma coactivator 1 α [PGC1 α (+60%)], citrate synthase [CS (+28%)], cytochrome oxidase 1 (+74%) and 4 (+36%), carbonic anhydrase-3 [CA-3 (+74%)], and manganese superoxide dismutase (+44%) (Zoll et al., 2006). Whilst data from mRNA transcripts must be interpreted with caution since the transcripts are protein precursors only, it is interesting to note that changes in MCT-1, CA-3 and GLUT-4 transcripts all correlated with time to exhaustion at VO₂max in the LLTH group only, suggesting a transfer of transcript expression to performance abilities. During this study, investigators found no change in oxidative enzyme activity [CS and cytochrome oxidase complex (COX)] or muscle oxidative capacity, but the control of mitochondrial respiration by cytosolic adenosine diphosphate was depressed (Ponsot et al., 2006). These authors contend that such adaptations represent better coupling between the energy utilization and production sites during exercise, promoting more efficient oxidative pathways and decreasing intracellular energetic perturbations (Ponsot et al., 2006). In contrast, Messonnier et al. (2001) reported training-induced increases in CS and lactate dehydrogenase (LDH) activity following 4 weeks of endurance training, but no additional increases in those subjects training in hypoxia.

Changes in deoxy-haemoglobin kinetics [measured via near infrared spectroscopy (NIRS)] may provide some insight into physiological adaptations following LLTH interventions (Kime et al., 2003). Using NIRS, Kime et al. (2003) reported higher muscle deoxyhaemoglobin and improved half-time to re-oxygenation following three weeks of LLTH,

compared with normoxic training, in a group of elite junior cyclists. These authors suggested that changes in muscle de-oxyhaemoglobin kinetics, representing greater O₂ extraction in the working muscle, may be due to increases in capillarisation (Mizuno et al., 1990; Desplanches et al., 1993; Geiser et al., 2001), elevated oxidative potential of the muscle [e.g. increased myoglobin (Terrados et al., 1990)], increases in mitochondrial content (Desplanches et al., 1993; Geiser et al., 2001) or other oxidative enzymes (Terrados et al., 1990).

Although LLTH interventions may enhance a number of peripheral physiological capacities, these findings are not universal (Messonnier et al., 2001) and some physiological capacities may be down-regulated (Green et al., 1999). Indeed, Green et al. (1999) reported ~14% reduction in Na⁺-K⁺-ATPase concentration following eight weeks of LLTH, whilst normoxic controls experienced a training-induced ~14% increase in Na⁺-K⁺-ATPase concentration. Both training groups also experienced increases in maximal CS activity, but these increases were greater in the hypoxic training group. These authors suggested that changes in Na⁺-K⁺-ATPase pump concentration and CS are regulated by differing physiological stimuli, with Na⁺-K⁺-ATPase pump concentration and CS activity primarily regulated by O₂ tension and cellular energy imbalance, respectively. Such findings further support the contention that some portion of high-intensity training should be completed in normoxia, even with the most aggressive LLTH programs.

Mechanisms leading to enhanced physiological capacity after near-maximal intensity LLTH interventions may differ from those resulting from low-intensity hypoxic training programs. Following four weeks of RSH training (2 sessions per week, involving 3 sets of 5 x 10 s all-out repeated sprints on a cycle ergometer), Faiss et al. (2013b) witnessed altered expression of a number of mRNA transcripts related to peripheral oxygen transport (myoglobin) and pH regulation (CA-3), but reported a concomitant down-regulation of genes implicated in mitochondrial biogenesis (TFAM and PGC1 α), suggesting a shift from aerobic to anaerobic glycolytic activity in the muscle. These results differ somewhat from the results in the IHT study of Zoll et al. (2006) who reported up-regulation of glycolytic related mRNA transcripts (CA-3, PFK) and genes related to mitochondrial biogenesis (TFAM and PGC1 α) and metabolism (COX). NIRS data collected by Faiss et al. (2013b) during repeated sprint ability tests also provide interesting insight into blood flow kinetics following a RSH intervention. These authors reported improved muscle blood perfusion (change in total

haemoglobin/myoglobin, used as a surrogate measure of blood volume within the muscle) during a repeated sprint ability test following the RSH intervention (Faiss et al., 2013b). This increased blood flow may have led to an improved removal of metabolites (Endo et al., 2005) during repeated sprints, possibly contributing to the increased number of sprints performed before exhaustion in the RSH group. Such changes in muscle blood flow could be augmented through nitric oxide mediated vasodilatation (Casey and Joyner, 2012), a mechanism which is thought to increase blood flow and vascular conductance primarily in fast twitch muscle fibres (Ferguson et al., 2013). The maximal exercise intensity prescribed during RSH is likely a key factor causing modified behaviour of primarily fast twitch muscle fibres, which may in part explain why other LLTH studies, with lower exercise intensities, have not produced additional performance benefits over normoxic-matched groups.

While peripheral adaptations may explain some of the possible benefits of LLTH protocols, central adaptations should not be overlooked. Indeed, one recent investigation reported a reduction in cerebral de-oxygenation during repeated sprint activities, following four weeks of RSH training (Galvin et al., 2013). Following this intervention, RSH subjects also experienced greater improvements in Yo-Yo intermittent recovery test level 1 (Yo-Yo IR1) performance compared with matched controls training in normoxia (Galvin et al., 2013). The authors suggested that these reductions in exercising cerebral de-oxygenation may explain some of the additional performance benefit in the RSH subjects, as changes in cerebral oxygenation may regulate central fatigue during exercise (Goodall et al., 2012).

Mechanisms related to improved performance after RTH likely differ from those observed following CHT, IHT and RSH. Increased hypertrophy and improvements in strength have been reported following a number of RTH studies (Nishimura et al., 2010; Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b). There is some suggestion that the increased metabolic stress during RTH may cause earlier fatigue of slow twitch muscle fibres, leading to a greater utilisation of fast twitch muscle fibres and increased hypertrophy in these motor units (Manimmanakorn et al., 2013a). Altered recruitment patterns under hypoxic conditions may also stimulate enhanced neuromuscular adaptations, evidenced by Nishimura et al. (2010) who reported greater improvements in muscular strength after three weeks of RTH compared with training in normoxic conditions, despite no significant change in muscle hypertrophy at this time. However, within the available RTH literature, a number of important methodological limitations have not been addressed. This includes matching of relative vs. absolute workloads between hypoxic and normoxic groups, training status of the

athletes/subjects and the prescription of the number of ‘repetitions’ for subjects throughout training protocols, as opposed to using a set load and asking participants to lift until failure (McLean et al., 2014). As discussed in detail in Chapter 6 (systematic review), these methodological issues preclude any definitive conclusions about the effectiveness of RTH for augmenting physiological and performance gains above traditional, maximal intensity, resistance training programs.

Performance responses to LLTH

Generalising performance outcomes following LLTH interventions is difficult due to the differing protocols/training stimuli used, different training intensities and durations, varying number of sessions per week (and thus overall hypoxic exposure per week), varying degrees of hypoxia during training, and the inclusion/exclusion of normoxic training in LLTH programs. As previously discussed, LLTH does not seem to provide a sufficient hypoxic dose to induce significant haematological changes (Millet et al., 2010) and, thus any improvements in performance are more likely associated with non-haematological adaptations that are highly dependent on the type of training completed in hypoxia. A comprehensive review of the current LLTH literature is included in “Chapter 6 - Application of ‘Live Low-Train High’ for enhancing normoxic exercise performance in team sport athletes – a systematic review.”

RESPONSES TO IHE INTERVENTIONS

IHE is defined as an exposure to hypoxia lasting from seconds to hours that is repeated over several days to weeks (Millet et al., 2010). Since only short periods of hypoxic exposure are needed to raise EPO levels (Rodriguez et al., 2000), some have hypothesised that IHE protocols may stimulate increases in serum EPO, leading to increased red blood cell production and, in turn, endurance performance (Rodriguez et al., 1999). Indeed, Rodriguez et al. (2000) reported increases in reticulocyte count (180%), red blood cells (7%), haemoglobin concentration (13%) and haematocrit (6%) following 90 min exposures, 3 d/wk for 3 wk. However, the same group failed to show similar haematological changes following IHE of 3 hours per day, 5 d/wk for 4 wk, despite significant increases in serum EPO after hypoxic exposures (Abellan et al., 2005). Similarly, other groups have reported no change in haematological parameters after similar IHE protocols (Frey et al., 2000; Ricart et al., 2000). Recent review articles and meta-analyses support the view that ≥ 10 hours per day

of hypoxic exposure, for a minimum of two weeks, is needed to induce significant haematological changes (Wilber et al., 2007; Gore et al., 2013; Rasmussen et al., 2013). Whilst there are some studies showing improved aerobic performance following IHE (Hellemans, 1999; Rodriguez et al., 2000), none have included a control group. Overall, IHE protocols do not appear to provide a sufficient hypoxic dose to stimulate beneficial physiological changes and, thus, are unlikely to lead to improved performance.

INFLUENCE OF DIFFERENT HYPOXIC STIMULI

The use of normobaric vs. hypobaric hypoxia

Hypoxic environments can be created under both normobaric [inspired fraction of oxygen ($F_{I}O_2$) < 20.9%; barometric pressure (PB) = 760 mmHg] and hypobaric ($F_{I}O_2$ = 20.9%; PB < 760 mmHg) conditions (Millet et al., 2012). Both methods ultimately lead to a decrease in the inspired partial pressure of oxygen ($P_{I}O_2$) which has long been thought to be the primary factor contributing to altered physiological responses to hypoxia (Millet et al., 2012). Direct comparisons of matched hypoxic training protocols in normobaric and hypobaric hypoxia are currently lacking in the literature. As recently highlighted by Millet et al. (2012), normobaric and hypobaric hypoxia may produce different responses in ventilation (Tucker et al., 1983; Loeppky et al., 1997; Savourey et al., 2003), fluid balance (Loeppky et al., 2005), acute mountain sickness (Roach et al., 1996; Schommer et al., 2010; Fulco et al., 2011), nitric oxide metabolism (Hemmingsson and Linnarsson, 2009; Kayser, 2009), and exercise performance (Bonetti and Hopkins, 2009). The contention that normobaric and hypobaric hypoxia lead to different performance responses is supported in a recent meta analysis (Bonetti and Hopkins, 2009), in which the authors concluded that hypobaric LHTL protocols lead to greater improvements in performance (mean improvements ~ 4.0%) compared with normobaric LHTL interventions (mean improvements ~ 0.6%) when completed by elite athletes. While these two techniques may influence the outcome of hypoxic training interventions, in practice, normobaric hypoxic environments are generally more accessible at sea-level, having wider commercial availability with less expensive equipment. Thus, results of normobaric LLTH studies may be of more interest to practitioners wishing to implement LLTH strategies. Conversely, when implementing LHTH or LHTL techniques living at altitude (i.e. in a hypobaric hypoxic environment) is often more accessible than elaborate normobaric hypoxic living quarters.

Optimal degree of hypoxia

There is likely a minimal threshold of hypoxia needed to stimulate physiological adaptations associated with improved performance. However, there has been little attention given to this topic within the hypoxic training literature. It may be that varying degrees of hypoxia and altitude are optimal for different hypoxic training techniques; for example, the detrimental effect of hypoxia on absolute training intensity is an important consideration for LLTH studies, but higher levels of hypoxia may be safe, and perhaps more beneficial, for techniques which are using the hypoxic stimulus at rest only (i.e. LHTL).

There is a strong consensus that a sufficient ‘hypoxic dose’ is needed during LHTH and LHTL to stimulate beneficial physiological adaptations, this includes the degree of hypoxia/height of altitude (Wilber et al., 2007; Gore et al., 2013; Rasmussen et al., 2013). However, recommendations on the optimal degree of hypoxia/altitude differ within the literature. Wilber et al. (2007) suggests that altitude training at 2,000–2,500 m for at least 22 hr/day and a minimum of 4 weeks is required to optimise benefits of prolonged hypoxic exposures. In their meta analysis, Rasmussen et al. (2013) also suggest that altitude exposures < 3,000 m will lead to increases in red cell volume, but only if exposures last longer than 4 weeks. Moreover, Rasmussen et al. (2013) propose that this benefit may be accelerated if staying at higher elevation – for example, at 4,000 m, benefits may be evident after ~ 2 weeks. In contrast, Garvican et al. (2012a) recently reported detectable increases in Hb_{mass} after 11 days of exposure at 2,760 m.

There is also a wide range of F_IO₂ prescribed within the LLTH literature [range = 11.7 - 16.1%; (McLean et al., 2014)]. As above, whilst there is a likely threshold of hypoxia needed to stimulate the enhanced physiological adaptations proposed with LLTH (Wilber et al., 2007), there are currently no investigations that systematically compare the effect of different F_IO₂ or normobaric/hypobaric techniques during extended training protocols. Practitioners may be interested in the least severe degree of hypoxia needed to induce the positive effects of LLTH, as training at this ‘minimum hypoxia’ may reduce limitations related to reductions in absolute training intensity (McLean et al., 2014), as well as reduce the risk of hypoxia related illness that is evident during extreme hypoxia (Bartsch and Saltin, 2008). Conversely, there may be a dose-response relationship between the degree of hypoxia and the extent of adaptations, but any such relationship is yet to be reported. However, a number of studies have investigated the effects of different degrees of hypoxia on a single repeated sprint training session (Bowtell et al., 2013; Goods et al., 2014). Goods et al. (2014)

reported that mean power output is maintained during an initial set of 9 x 4 s repeated sprints when training in 16.4 and 14.5% O₂, but reduced when training in 12.7% O₂. Mean power output was then reduced (compared to training in normoxia) during a second and third set at all degrees of hypoxia, although this occurred to a greater extent in the group training in 12.7% O₂. These authors therefore suggested that training in 12.7% O₂ is less suitable than more moderate hypoxia, as absolute training intensity is better preserved at less severe degrees of hypoxia. In contrast, Bowtell et al. (2013) suggest that the physiological demands (heart rate, minute ventilation, muscle deoxygenation) associated with repeated sprint training (10 x 6 s sprints) are incrementally greater as F_IO₂ is decreased to 13%, but these physiological responses are attenuated when F_IO₂ is decreased to 12%, due to significant reductions in exercise intensity. The interaction of physiological disturbance (potentially augmented by hypoxia) and negative effects of reduced absolute exercise intensity (e.g. reduced cardiovascular load) likely interact to produce adaptations to training in hypoxia. Therefore, prolonged training interventions that systematically compare the effect of different degrees of hypoxia are needed to determine what range of training F_IO₂ may be optimal.

CHAPTER 3 – STUDY 1: PHYSIOLOGICAL AND PERFORMANCE RESPONSES TO A PRE-SEASON ALTITUDE TRAINING CAMP IN ELITE TEAM SPORT ATHLETES

PUBLICATION STATEMENT

This work was submitted and accepted for publication in the International Journal of Sports Physiology and Performance, please see Appendix II:

McLean BD, Buttifant D, Gore CJ, White K, Liess C and Kemp J. (2013b) Physiological and performance responses to a pre-season altitude training camp in elite team sport athletes. *International Journal of Sports Physiology and Performance*. 8: 391-399.

ABSTRACT

PURPOSE. Little research exists investigating the physiological and performance effects of altitude training on team sport athletes. Therefore, this study examined changes in 2,000 m time-trial running performance (TT), haemoglobin mass (Hb_{mass}) and intramuscular carnosine content of elite Australian Football (AF) players after a pre-season altitude camp.

METHODS. Thirty elite AF players completed 19 days of living and training at either moderate altitude [(~2,130 m) ALT; n = 21] or sea-level (CON; n = 9). TT performance and Hb_{mass} were assessed pre- (PRE) and post-intervention (POST) in both groups, and at four weeks after returning to sea-level (POST2) in ALT only.

RESULTS. Improvement in TT performance after altitude was *likely* 1.5 ($\pm 4.8 - 90\%CL$)% greater in ALT compared with CON, with an individual responsiveness of 0.8%. Improvements in TT were maintained at POST2 in ALT. Hb_{mass} after altitude was *very likely* increased in ALT compared with CON ($2.8 \pm 3.5\%$) with an individual responsiveness of 1.3%. Hb_{mass} returned to baseline at POST2. Intramuscular carnosine did not change in either gastrocnemius or soleus from PRE to POST1.

CONCLUSIONS. A pre-season altitude camp improved TT performance and Hb_{mass} in elite AF players to a similar magnitude demonstrated by elite endurance athletes undertaking altitude training. The individual responsiveness of both TT and Hb_{mass} was approximately half the group mean effect, indicating that most players gained benefit. The maintenance of running performance for four weeks, despite Hb_{mass} returning to baseline, suggests that altitude training is a valuable preparation for AF players leading into the competitive season.

INTRODUCTION

High-intensity running performance is vital to success in team sports,(Mooney et al., 2011) requiring athletes to have a highly developed aerobic system together with a large anaerobic capacity.(Buchheit, 2012) Therefore, training modalities that increase an athlete's ability to perform high-intensity work should benefit overall team performance. A recent modality adopted by professional teams in an attempt to improve performance is altitude training, where athletes are exposed to a hypobaric, hypoxic environment. Altitude training has been reported to induce a range of physiological adaptations (Levine and Stray-Gundersen, 1997; Gore et al., 2007), and two of these responses; increases in oxygen-carrying capacity of the blood (Levine and Stray-Gundersen, 1997) and increases in muscle buffering capacity (Mizuno et al., 1990; Saltin et al., 1995), would be expected to improve high-intensity running ability. For improved oxygen transport, the primary physiological adaptation after altitude exposure is an increase in total haemoglobin mass (Hb_{mass})(Levine and Stray-Gundersen, 1997), and this can lead to increases in VO_2max (Schmidt and Prommer, 2008). With respect to increases in muscle buffering capacity following altitude exposure, the underpinning mechanism is unknown, but several authors propose that an increase in one of the intramuscular buffers, carnosine, is likely responsible (Saltin et al., 1995; Gore et al., 2001).

The live-high, train-high (LHTH) model, involving living and training at moderate altitudes in the range of 2000-3000 m (Bartsch and Saltin, 2008), is a common practice for hypobaric, hypoxic exposure. Endurance athletes have used this model for more than half a century, attempting to improve performance at sea-level. Similarly for professional team sports, the ultimate goal of a pre-season altitude camps is to elicit performance improvements and increase training capacity upon return to sea-level. However, there are no published data describing the physiological or performance responses after altitude exposure in elite team sport athletes. Given the unique physical attributes and performance demands of these athletes, it is important to determine how they respond to an altitude camp, and whether there are performance gains upon return to sea-level.

Therefore, the present study examined the physiological and performance changes in professional Australian Football (AF) players, following a 19-day altitude training camp. These data were compared to a control group of elite AF players undertaking similar training at sea-level. It was hypothesised that: 1) increases in Hb_{mass} and intramuscular carnosine content occur following altitude exposure; and 2) following a LHTH camp, athletes will

show greater improvements in running performance when compared to matched controls, completing similar training at sea-level.

METHODS

Subjects

Thirty elite AF players (mean \pm standard deviation; age 23 ± 3 y, stature 188.2 ± 8.0 cm, body mass 88.0 ± 9.0 kg, sum of 7 skinfolds = 44.9 ± 4.7 mm) were examined throughout an eight-week training block (see figure 3.1). Subjects were split into altitude (ALT) (n=21) and control (CON) (n=9) training groups. All training and testing took place during the Australian Football League (AFL) pre-season from November to January, following a six-week off-season break. All subjects provided written informed consent and this study was approved by the Human Research Ethics Committee at Australian Catholic University.

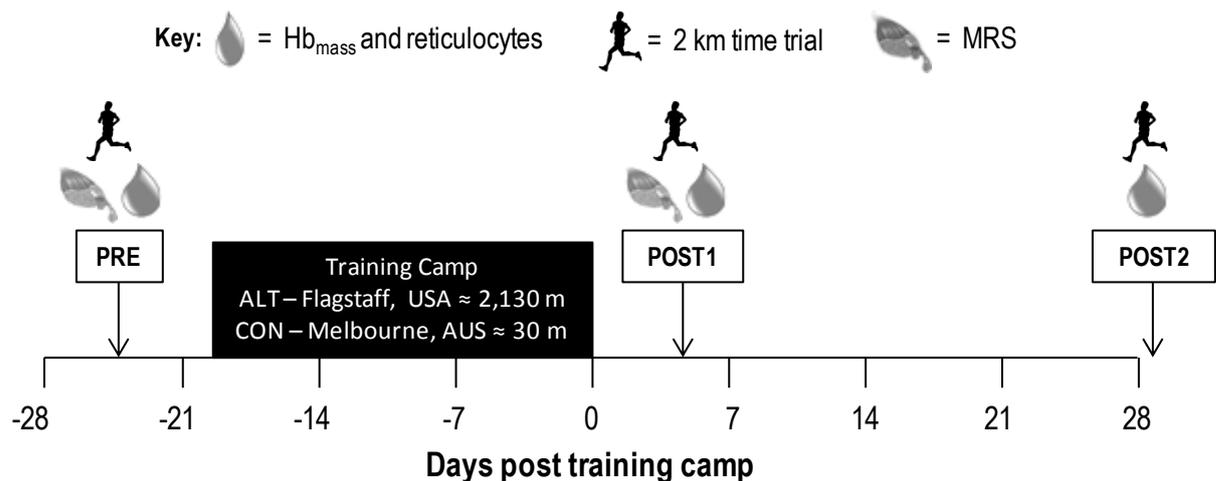


Figure 3.1. Schematic timeline of study design.

ALT group measured at PRE, POST1 and POST2. CON group measured and PRE and POST1. MRS = magnetic resonance spectroscopy.

Nutritional supplementation

Nutritional supplements were prescribed throughout the study by club dietitians. All subjects were supplemented with oral ferrous sulphate (325 mg/day) throughout the intervention period and athletes identified as having low serum ferritin pre-intervention (serum ferritin ≤ 30 $\mu\text{g/L}$; n = 2) were given a single, 2 mL ferrum H injection (equivalent to 100 mg of iron) prior to the altitude camp. No supplements containing β -alanine were prescribed and no subjects had used β -alanine within the preceding six-months. As β -alanine

is known to increase intramuscular carnosine concentration (Harris et al., 2006), players were asked to refrain from consuming any supplements containing β -alanine throughout the study and they reported 100% compliance.

Training

Altitude group training; During an eight-week training block, the ALT training group completed 19 days living and training at altitude, involving endurance and resistance exercise training, in Flagstaff, Arizona, USA (elevation \approx 2,130 m). *Control group training;* The CON group training in Melbourne, Australia (elevation \approx 30 m) completed similar training as the ALT group for the first four weeks of the intervention; thereafter training differed between groups. As such, data for the CON group are only reported through the first four weeks of the intervention period. All training was prescribed by team coaches and monitored via session rating of perceived exertion (RPE) (Foster et al., 2001). This method calculates a total load (arbitrary units [AU]) by multiplying the session-RPE (Borg's CR 10-scale) by the session duration.

Running Performance

To assess high-intensity running performance, subjects completed a 2,000 m running time-trial (TT) at the commencement (PRE) and four weeks into the training block (POST1). Additionally, TT performance was assessed again in ALT subjects eight weeks into the training block (POST2). TT performance was assessed on the same outdoor running track at sea-level in Melbourne, Australia, between 8:00–9:00 AM (temperature 18–26°C, humidity 50–80%) and measured via handheld stop watches. All players were familiar with the TT from previous years – most had completed 6-10 (minimum 3) similar time-trials in the preceding 1-3 years.

Haematological measures

All subjects were measured for Hb_{mass} using the optimised carbon monoxide re-breathing technique (Prommer and Schmidt, 2007) at PRE and POST1 (see Figure 3.1). Additionally, Hb_{mass} was measured again in ALT subjects at POST2. Briefly, subjects re-breathed a bolus of 99% CO equivalent to 1.0 mL/kg of body mass through a glass spirometer (BloodTec, Bayreuth, Germany) for two min. Percent carboxyhaemoglobin (%HbCO) in fingertip capillary blood was measured using an OSM3 hemoximeter (Radiometer, Copenhagen, Denmark) before and seven min after administration of the CO dose. Six repeat measures of

%HbCO were made for improved precision in Hb_{mass} estimation (Alexander et al., 2011). Venous blood samples were sent to a local hospital laboratory (St Vincent's Pathology, Fitzroy, Victoria) for assessment of reticulocyte count in both groups at PRE and POST1 and in the ALT group only at POST2. Preceding venous blood sample collection, subjects' ambulation was limited for 15 minutes, with the majority of this time spent sitting. The concentration of serum ferritin was also assessed at PRE to identify subjects who may be iron deficient.

Magnetic Resonance Spectroscopy

Magnetic resonance spectra were acquired in the soleus and gastrocnemius, of ALT only, at a field strength of 3T on a Philips Achieva system (Philips Medical Systems, Best, The Netherlands) using the point-resolved spectroscopy technique (Bottomley, 1987; Ozdemir et al., 2007) with a repetition time of 2000 ms and an echo time of 33 ms. Voxel sizes varied depending on the size of the muscle but were generally in the range of 10 mm x 20 mm x 40 mm. Care was taken to position the voxel in such a way as to avoid contributions from fasciae across the chemical shift range of the voxel. Voxels were shimmed to between 12 and 19 Hz; 256 water suppressed and 8 non-water suppressed averages were acquired at the same receiver gain. Spectral data analysis was carried out using jMRUI version 4.0 (Naressi et al., 2001) after zero-filling and line-broadening by 5Hz, metabolite peaks were fitted and expressed relative to the unsuppressed water signal. Peaks were assigned as follows: carnosine C2-H (8 ppm) and C4-H (7 ppm), residual water (around 4.7 ppm), phosphocreatine & creatine (3.05 & 3.95 ppm), containing metabolites (3.20 ppm). All analyses presented in this paper refer to the carnosine C2-H peak at 8 ppm and the carnitine and choline peak at 3.20 ppm. No relaxation time corrections were made.

Statistical Analysis

A contemporary analytical approach involving magnitude-based inferences (Hopkins et al., 2009) was used to detect small effects of practical importance. All data were log-transformed to account for non-uniformity error. The percentage changes in the mean TT and Hb_{mass} from pre-altitude to each time point after altitude were calculated. The differences within and between groups were assessed with dependent and independent t-tests for unequal variance (Hopkins et al., 2009). The magnitudes of changes were assessed in relation to the smallest worthwhile change (SWC) which was set to 2% for TT and Hb_{mass}, and a small effect size ($d = 0.2$) was used for all other variables. Analysis of overall training load revealed

differences between the CON (3229 ± 447 AU per week; mean \pm SD) and ALT (4249 ± 351) groups. As a result, training load was used as a covariate in the analysis of changes in TT and Hb_{mass}. The observed effects were reported as the mean change or difference \pm 90% confidence limits (CL). Effects were termed positive, trivial, or negative depending on the magnitude of the change relative to the SWC and were assigned a qualitative descriptor according to the likelihood of the change exceeding the SWC as follows: 50–74% “*possible*”, 75–94% “*likely*”, 95–99% “*very likely*”, >99% “*almost certainly*” (Batterham and Hopkins, 2006). Those effects where the 90% confidence interval overlapped simultaneously the substantially positive and the negative thresholds were deemed unclear. The individual response was also quantified for TT performance and Hb_{mass}. For each, the magnitude of individual responses were calculated from the square root of the difference in the variance of the change scores of the CON and ALT groups (Hopkins, 2003). A Pearson's correlation coefficient was used to examine the relationship between initial Hb_{mass} and change in Hb_{mass} at POST1 – however, two subjects reported illness during the study and were subsequently removed from the correlation analysis, as pro-inflammatory cytokines that are increased with infection are known to suppress EPO production (Jelkmann, 1998).

RESULTS

Training load

Training load, training duration and RPE are presented in Table 3.1. Throughout the first four weeks of the intervention period, overall training load was *almost certainly* $24.5 \pm 10\%$ higher in ALT compared with CON. Training duration was *very likely* $11.5 \pm 7.2\%$ higher and mean RPE was *almost certainly* $13.3 \pm 5.4\%$ higher in ALT compared with CON.

Table 3.1. Weekly training load, training duration and rate of perceived exertion (mean \pm SD) in ALT and CON groups, during the first four weeks of the intervention.

	ALT (N=21)	CON (N=9)	% Chances for ALT to be greater/similar/smaller than the SWC compared with CON
Training load (Arbitrary Units)			
Pre-intervention	2260 \pm 571	2553 \pm 952	25/8/67 (CON <i>possibly</i> higher)
Week 1	4765 \pm 685	2961 \pm 382	100/0/0 (ALT <i>almost certainly</i> higher)
Week 2	4962 \pm 313	3771 \pm 848	99/0/0 (ALT <i>very likely</i> higher)
Week 3	4536 \pm 240	3631 \pm 477	100/0/0 (ALT <i>almost certainly</i> higher)
Training duration (min)			
Pre-intervention	536 \pm 118	446 \pm 157	87/4/9 (ALT <i>likely</i> higher)
Week 1	702 \pm 95	562 \pm 59	100/0/0 (ALT <i>almost certainly</i> higher)
Week 2	748 \pm 41	663 \pm 115	92/4/4 (ALT <i>likely</i> higher)
Week 3	597 \pm 9	623 \pm 29	0/11/88 (CON <i>likely</i> higher)
Rate of Perceived Exertion			
Pre-intervention	4.84 \pm 0.58	5.38 \pm 0.68	1/4/95 (CON <i>likely</i> higher)
Week 1	6.97 \pm 0.26	5.01 \pm 0.75	100/0/0 (ALT <i>almost certainly</i> higher)
Week 2	6.35 \pm 0.13	5.38 \pm 0.48	100/0/0 (ALT <i>almost certainly</i> higher)
Week 3	6.49 \pm 0.38	5.64 \pm 0.65	99/1/0 (ALT <i>very likely</i> higher)

2,000 m time-trial performance

Time-trial performance was *possibly* faster in ALT (413 ± 14 s) compared to CON (422 ± 23 s) before the altitude training camp (Figure 3.2). The mean improvement in TT performance ($\pm 90\%$ CL) at POST1 was *likely* $2.1 \pm 2.1\%$ greater in ALT compared with CON. When training load was used as a covariate, change in TT was *possibly* $1.5 \pm 4.8\%$ greater in the ALT compared to CON at POST1. The individual variation in TT performance at POST1 was 0.9% without training load used as a covariate and 0.8% when load was used as a covariate. Thirty days post-descent (POST2), improvement in TT performance in ALT had been maintained, with the change from POST1 to POST2 *trivial* ($-0.8 \pm 0.9\%$).

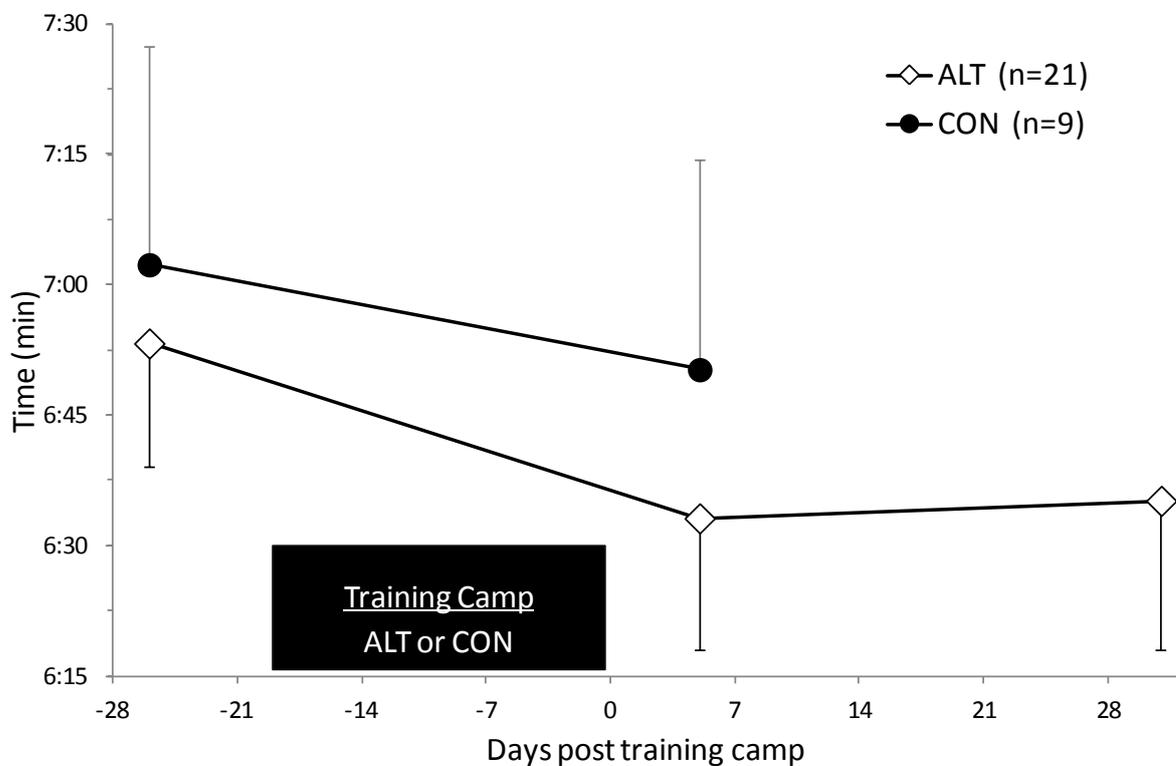


Figure 3.2. 2,000 m time-trial running performance at sea-level (mean \pm SD).

ALT = altitude training group, CON = sea-level control group.

Hb_{mass} and reticulocytes

Hb_{mass}: The mean (\pm SD) Hb_{mass} in the ALT and CON groups before the intervention were 992 ± 129 g and 980 ± 151 g, respectively. Percentage change in Hb_{mass} ($\% \Delta$ Hb_{mass}) from baseline is displayed in Figure 3.3. At POST1, mean $\% \Delta$ Hb_{mass} ($\pm 90\%$ CL) was *very likely* increased in ALT ($3.6 \pm 1.6\%$) whilst changes in CON were *trivial* ($0.5 \pm 2.4\%$). The changes in Hb_{mass} at POST1 were *likely* $2.8 \pm 3.5\%$ greater in ALT compared with CON. When training load was used as a covariate, the mean $\% \Delta$ Hb_{mass} was *possibly* $2.2 \pm 7.7\%$ higher in ALT compared to CON. The individual variation in Hb_{mass} at POST1 was 1.7% without training load used as a covariate and 1.3% (90% CL -4.2 – 4.8) when load was used as a covariate. Thirty days after the camp, Hb_{mass} was *not likely* different from PRE in ALT ($0.3 \pm 1.6\%$). A Pearson's correlation revealed a significant negative correlation ($R=-0.484$, $p=0.036$) between initial Hb_{mass} relative to body mass (RelHb_{mass}) and $\% \Delta$ Hb_{mass} post altitude exposure in ALT (two subjects who reported illness throughout the study removed from this analyses). *Reticulocytes*: Reticulocytes were *almost certainly* lower in ALT compared with CON at POST1 ($-26.2 \pm 8.6\%$) and were *almost certainly* lower than PRE at POST2 in ALT ($-64.5 \pm 12.6\%$).

Intramuscular metabolites

Carnosine: Intramuscular carnosine of ALT was *almost certainly* $35.5 \pm 15.0\%$ higher in the gastrocnemius (34.9 ± 8.6 AU) compared to soleus (22.4 ± 5.2 AU) at PRE. Changes in carnosine were *trivial* from PRE to POST1 in gastrocnemius ($3.9 \pm 9.0\%$) and *unclear* in soleus (-1.6 ± 13.1). *Carnitine & Choline*: The pooled carnitine and choline was *likely* $26.3 \pm 15.5\%$ lower in gastrocnemius (228 ± 60 AU) compared to soleus (283 ± 50 AU) at PRE. Pooled carnitine and choline was *likely* increased in soleus ($8.8 \pm 6.1\%$) but *trivial* in gastrocnemius from PRE to POST1 ($0.6 \pm 14.1\%$).

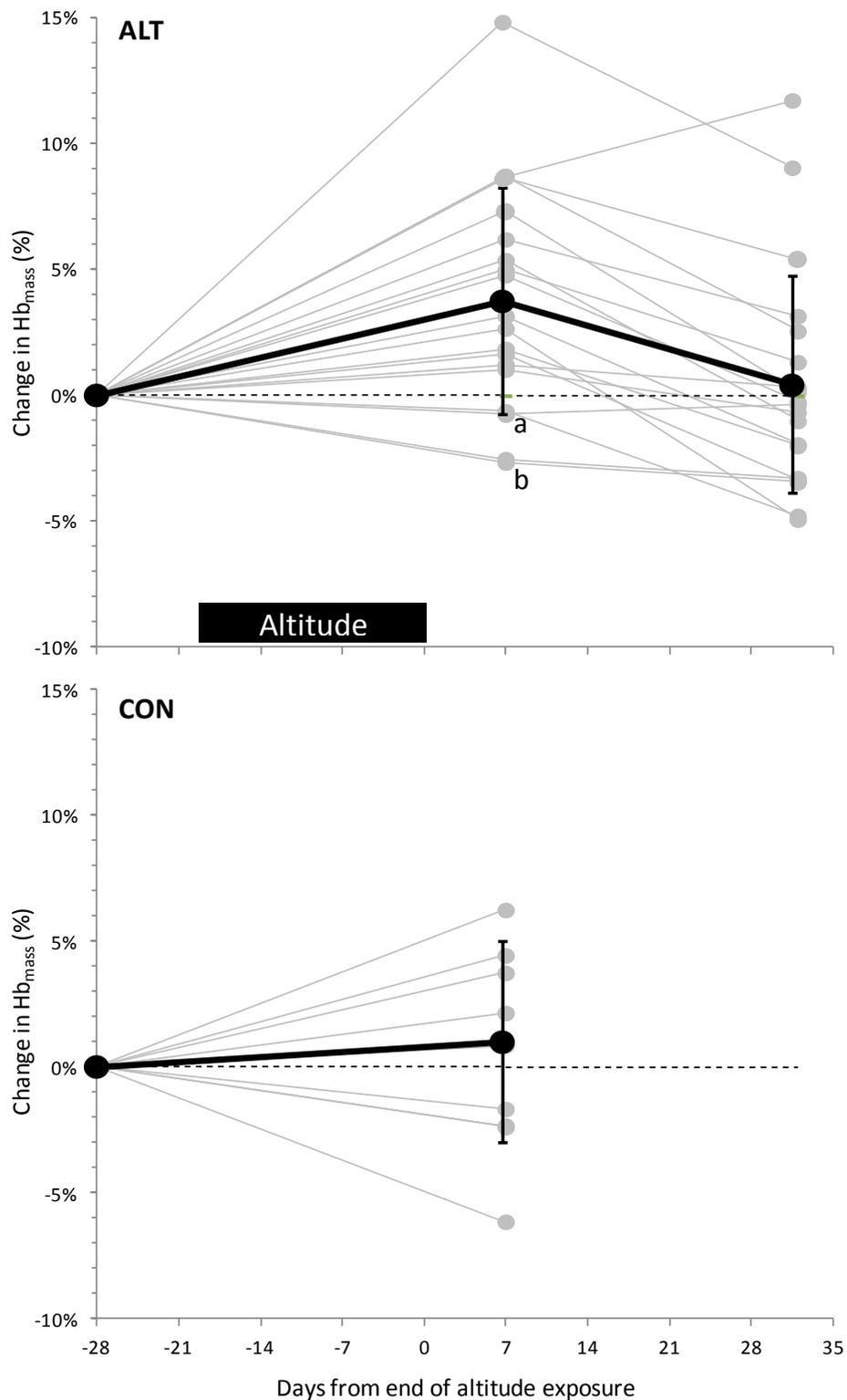


Figure 3.3. Changes in Hbmass after altitude exposure (n=21) or control conditions (n=9). Black circles and error bars show group change (%) as mean \pm SD and grey circles show individual responses. a & b depict responses of two subjects who reported illness before (b) and during (a) altitude exposure.

DISCUSSION

The main finding of the present study is that professional team sport athletes undertaking a LHTH pre-season training camp at moderate altitude had ~1.5% greater improvements in running performance than matched controls living and training at sea-level. These performance improvements were accompanied by ~3% increase in Hb_{mass} not observed in control subjects. Changes in Hb_{mass} returned to baseline four weeks post-altitude, however improvements in running performance were maintained. A novel finding of the present study is that there was no clear change in intramuscular carnosine concentration following altitude exposure, suggesting that changes in this intramuscular protein may not be responsible for previously reported changes in muscle buffering capacity (Saltin et al., 1995), or that the changes were too small for MRS measurements to identify.

Performance

The ~1.5% greater improvement in running performance in ALT, over that observed in CON subjects, in the current investigation is of similar magnitude to improvements seen in endurance athletes following altitude training interventions (Levine and Stray-Gundersen, 1997; Robertson et al., 2010a). However, the individual variability in this study was approximately half of the mean change in TT performance, which is less than previously reported in endurance athletes (Robertson et al., 2010a). This suggests that team sport athletes may experience more consistent improvements in performance than endurance athletes following an altitude intervention, which may be related to some endurance athletes approaching their physiological limits, with only small opportunities for improvements. It has previously been suggested that, following altitude exposure, athletes are able to train at higher intensities, thereby increasing the training stimuli and consequent improvements in performance (Rusko et al., 2004). As improvements in running performance were maintained for at least four weeks post-descent in our subjects, we propose that an altitude training camp may positively influence subsequent pre-season training in team sport athletes, thus improving preparation leading into the competitive season.

Haemoglobin mass

A change in the oxygen-carrying capacity of the blood is often proposed as the major physiological adaptation leading to improved performance following altitude exposure (Levine and Stray-Gundersen, 1997). In the present study, athletes achieved a mean increase in Hb_{mass} of 3.6%, which is similar to changes observed in endurance cyclists following 19

days residing at 2,760 m (Garvican et al., 2012a). Hb_{mass} returned to baseline 30 days post-descent, which is also similar to the results of Garvican et al. (2012a), suggesting that team sport athletes can achieve comparable improvements in Hb_{mass} following an altitude intervention as observed in endurance athletes, and that such changes follow a similar time course with ascent to altitude and descent to sea-level. The depression of reticulocytes upon return to sea-level in the ALT group provides additional evidence of accelerated erythropoiesis following altitude exposure. Pottgiesser et al. (2012) observed a ~20% depression of reticulocytes nine days after 26 nights spent at 3000 m, which is similar to the 26% depression we observed seven days post altitude.

Although it is commonly accepted that prolonged exposure to hypoxic conditions can elicit increases in Hb_{mass} , it is also acknowledged that high variability exists in this response between individuals (Chapman et al., 1998; Robertson et al., 2010a). Similar to previous findings (Robertson et al., 2010a), our data demonstrate high inter-individual variability, with individual responsiveness in Hb_{mass} approximately half the magnitude of the mean change in ALT. The causes of such variability between individuals are not well understood and may be related to a number of factors. The proinflammatory cytokine, interleukin 1 (IL-1), suppresses the release of erythropoietin (Jelkmann, 1998), therefore, athletes experiencing infections that lead to increases in IL-1 before or during an altitude camp may have a blunted erythropoietic response. This is supported by data from the present study in which two athletes reported illness, either before or during altitude exposure, and neither athlete demonstrated an increase in Hb_{mass} following the altitude training camp ($\Delta Hb_{mass} = -0.8$ and -2.7% , see Figure 3.3).

However, there is still large variability in the responses of our other subjects. Some have proposed that athletes starting with high initial Hb_{mass} may have a limited ability to stimulate further increases (Robach and Lundby, 2012). Robach and Lundby (2012) combined data from nine altitude training studies involving elite endurance athletes and found an inverse correlation between initial $RelHb_{mass}$ and change in Hb_{mass} ($R=0.86$, $p<0.01$). Our results also show a significant inverse correlation between initial $RelHb_{mass}$ and percentage change in Hb_{mass} following altitude exposure, but the magnitude was only about half that of Robach & Lundby.

Intramuscular metabolites

Whilst increased erythropoiesis appears to be an important adaptation with altitude exposure, it cannot completely account for increases in performance following such exposures, even in situations where the strongest relationships are observed (Levine and Stray-Gundersen, 1997). Nonhaematological adaptations may also play a role in improved performance (Gore et al., 2007), including increases in muscle buffering capacity (Mizuno et al., 1990; Saltin et al., 1995; Gore et al., 2001). However, such findings are not universal (Clark et al., 2004), and none of the aforementioned studies have been able to elucidate the mechanism responsible for this adaptation. Despite this, Saltin et al. (1995) proposed that changes in muscle buffering capacity following altitude training may be due to changes in intramuscular carnosine, a hypothesis further supported by others (Gore et al., 2001). The current study is the first to assess intramuscular carnosine levels before and after an altitude training intervention. However, we found no clear changes in carnosine content in the gastrocnemius or soleus following the altitude intervention. This finding may be due to the availability of β -alanine, which limits carnosine synthesis (Harris et al., 2006). If altitude exposure was to alter carnosine concentrations within skeletal muscle, such exposure would need to alter the availability of β -alanine, however there is no apparent mechanism for this to occur during altitude exposure. The present results, combined with the current understanding of factors limiting carnosine synthesis, would suggest that changes in carnosine are not responsible for previously observed changes in muscle buffering capacity, although muscle buffering capacity was not directly measured in the present study. An interesting finding in our data was an increase in the carnitine/choline peak, observed in soleus following the intervention period. The mechanism for such an increase is unclear, however, as pooled carnitine and choline increased only in soleus, and not in gastrocnemius, we propose that these increases are related to changes within the mitochondria, which is found in higher concentrations in soleus due to the greater proportion of type I muscle fibres (Edgerton et al., 1975). Carnitine acts as an acceptor for the acetyl groups, by forming acetylcarnitine, when the rate of acetyl CoA formation from glycolysis is high during high-intensity exercise (Jeppesen and Kiens, 2012). Therefore, we propose that possible increases in carnitine may be related to improved capacity for high-intensity exercise. Further research is needed to fully understand this finding, including whether this change was due to a training effect or altitude exposure, as the control group was not included in the MRS analysis in this study.

Limitations

One limitation in this study was the difference in training load observed between groups. However, although ALT subjects completed higher training loads than controls during the intervention period, covariate analyses suggest that improvements in performance and changes in Hb_{mass} are still greater in ALT subjects when controlling for training load. Furthermore, using RPE-based methods to assess training load during LHTH interventions may overestimate the mechanical and physiological training load. When training in hypoxic conditions, RPE is higher compared with similar training completed in normoxic conditions, even when training velocities and markers of physiological load (e.g. oxygen consumption and heart rate) are lower (Buchheit et al., 2012a). RPE was higher in the ALT subjects throughout the intervention period, and it is not possible to determine if this is merely due to perception of effort, or if the ALT group actually completed higher intensity training throughout this period. Although differences in RPE may account for some of the observed differences in training load, training duration was also ~12% higher in the ALT group.

Possible placebo, nocebo and training camp effects also cannot be eliminated from having an influence on the current results. However, while placebo and training camp effects may confound scientific results, these changes are nevertheless of interest to practitioners looking to achieve the best possible performance outcomes during pre-season period.

PRACTICAL APPLICATIONS

The current investigation was conducted in an ecologically valid environment with professional team sport athletes; therefore, the findings have strong practical relevance. Our results show improved running performance in team sports athletes completing a pre-season LHTH intervention. Altitude exposure also increased Hb_{mass}, and this benefit may be greatest in athletes starting with lower initial RelHb_{mass}. Such physiological changes may lead to improved quality of training upon returning to sea-level, and although we found performance benefit for a month, practitioners should not expect physiological changes to be maintained throughout the duration of a team sport season. Athletes experiencing illness leading into or during an altitude training camp are also unlikely to see erythropoietic benefit from the altitude exposure.

CONCLUSIONS

Team sport athletes completing 19-day altitude training camp experience greater improvements in running performance than athletes completing similar training at sea-level. Improvements in running performance are maintained for at least four weeks post-descent and may therefore lead to improved quality of training throughout this period, thereby improving preparation for competition. Therefore, we conclude that a pre-season altitude training camp is a worthwhile intervention for improving running performance in elite team sport athletes.

CHAPTER 4 – STUDY 2: YEAR-TO-YEAR VARIABILITY IN HAEMOGLOBIN MASS RESPONSE TO TWO ALTITUDE TRAINING CAMPS

PUBLICATION STATEMENT

This work was submitted and accepted for publication in the British Journal of Sports Medicine, please see Appendix II:

McLean BD, Buttifant D, Gore CJ, White K and Kemp J. (2013a) Year-to-year variability in haemoglobin mass response to two altitude training camps. British Journal of Sports Medicine. 47: i51-i58.

ABSTRACT

AIM. To quantify the year-to-year variability of altitude-induced changes in haemoglobin mass (Hb_{mass}) in elite team sport athletes.

METHODS. Twelve Australian-Footballers completed a 19- (ALT1) and 18-day (ALT2) moderate altitude (~2,100m), training camp separated by 12 months. An additional 20 subjects completed only one of the two training camps, (ALT1 additional N = 9, ALT2 additional N = 11). Total Hb_{mass} was assessed using carbon monoxide rebreathing before (PRE), after (POST₁), and four weeks after each camp. The typical error of Hb_{mass} for the pooled data of all 32 subjects was 2.6%. A contemporary statistics analysis was used with the smallest worthwhile change set to 2% for Hb_{mass} .

RESULTS. POST₁ Hb_{mass} was *very likely* increased in both ALT1 ($3.6 \pm 1.6\%$, n=19; mean \pm ~90 CL) and ALT2 ($4.4 \pm 1.3\%$, n=23) with an individual responsiveness of 1.3% and 2.2%, respectively. There was a small correlation between ALT1 and ALT2 ($R = 0.21$, $p = 0.59$) for change in Hb_{mass} , but a moderate inverse relationship between change in Hb_{mass} and initial relative Hb_{mass} [g/kg ($R = -0.51$, $p = 0.04$)].

CONCLUSIONS. Two pre-season moderate altitude camps one year apart yielded a similar (4%) mean increase in Hb_{mass} of elite footballers, with an individual responsiveness of approximately half the group mean effect, indicating that most players gained benefit. Nevertheless, the same individuals generally did not change their Hb_{mass} consistently from year-to-year. Thus a “responder” or “non-responder” to altitude for Hb_{mass} does not appear to be a fixed trait.

INTRODUCTION

High variability in physiological and performance responses exists between individuals following live high, train high (LHTH) and live high, train low (LHTL) altitude training. It has been proposed that some individuals respond better than others, possibly due to inherent genetic traits, and that ‘responders’ and ‘non-responders’ might explain the high variability in adaptations to altitude training (Chapman et al., 1998; Stray-Gundersen et al., 2001; Friedmann et al., 2005; Robertson et al., 2010b; Wachsmuth et al., 2013). Indeed, Chapman et al. (1998) retrospectively classified a group of distance runners as ‘responders’ or ‘non-responders’, based on their change in 5,000 m time trial performance, following 28 days of LHTL. These authors (Chapman et al., 1998) reported that athletes who improved their time trial also exhibited the greatest erythropoietin (EPO) response and subsequent changes in red cell volume and VO_{2max} .

If the classification of ‘responder’ and ‘non-responder’ is a fixed trait, possibly related to underlying genetics, individual athletes should respond similarly whenever undergoing altitude exposure of similar duration and altitude. Only two studies to date (Robertson et al., 2010b; Wachsmuth et al., 2013) have investigated the repeatability of responses to altitude. Robertson et al. (2010b) followed eight highly-trained runners during two blocks of hypoxic exposure (3 weeks LHTL), separated by a 5-week wash-out period and reported reproducible group mean increases for VO_{2max} (~2.1%) and haemoglobin mass [Hb_{mass} (~2.7%)], but not for mean changes in time trial performance (+0.5% and -0.7% following exposure 1 and 2, respectively). Moreover, there was a moderate but unclear negative correlation for change in Hb_{mass} from one exposure to the next, (Robertson et al., 2010b) demonstrating high variability in individual responsiveness between exposures. Similarly, Wachsmuth et al. (2013) reported a weak correlation ($r = 0.379$, $p = 0.160$) between Hb_{mass} responses following two altitude training camps conducted approximately three months apart with elite swimmers, despite very reproducible increases in serum erythropoietin ($R = 0.95$, $p < 0.001$).

Team sport athletes often complete pre-season altitude training camps in an attempt to optimise running performance and we recently reported improved running performance (~1.5% above matched sea-level control) accompanied with a 3.6% increase in Hb_{mass}

following 19 days of LHTH in elite team sport athletes (McLean et al., 2013b). Similar to previous investigations (Stray-Gundersen et al., 2001; Friedmann et al., 2005), we reported high variability in erythropoietic responses, which may be partially explained by levels of initial Hb_{mass} (Robach and Lundby, 2012), however, much of this variability remains unexplained. Possible identification of ‘responders’ and ‘non-responders’ after a single altitude training camp, or an acute hypoxic exposure, may allow prescription of altitude training to be targeted towards those athletes who ‘respond’ well, and/or alternative methods/altitudes adopted in an attempt to enhance the response of potential ‘non-responders’.

Therefore, the primary aim of this study was to examine the variability of physiological responses between two similar LHTH pre-season training camps in professional Australian-Football players. A secondary aim was to identify potential factors that may influence responsiveness to altitude exposure, such as pre-intervention Hb_{mass} , energy balance and health status. It was hypothesised that there would be a moderate-strong correlation between the magnitude of physiological changes from exposure 1 to exposure 2.

METHODS

Subjects

Twelve Australian-Football players completed a 19- (ALT1) and 18-day (ALT2) moderate altitude (~2,100m) training camp, separated by 12 months. Results from ALT1 have been previously reported (McLean et al., 2013b). An additional 20 subjects completed only one of the two training camps, (ALT1 – additional N = 9, ALT2 – additional N = 11). All training and testing took place during the Australian Football League pre-season from November to January, following a six-week, off-season break. All subjects provided written informed consent and this study was approved by the University Human Research Ethics Committee. All subjects were supplemented with oral ferrous sulphate (325 mg/day) throughout the intervention period, and athletes identified as having low serum ferritin pre-intervention (serum ferritin $\leq 30 \mu\text{g/L}$; $n = 3$) were given a single, 2 mL ferrum H injection (Aspen Pharmacare, St Leonards, Australia; equivalent to 100 mg of iron) prior to the altitude camp.

Training

During ALT1, subjects completed an eight-week training block, including 19 days living and training at moderate altitude in Flagstaff, Arizona, USA (elevation ~ 2,100 m). One year later, subjects completed a similar eight-week training block, including 18 days living and training at moderate altitude in Park City, Utah, USA (elevation ~ 2,000 m). Both training blocks included endurance training, resistance exercise and football specific training (see table 4.1). All training was prescribed by team coaches and monitored via session rating of perceived exertion (RPE) (Foster et al., 2001). This method calculates a total load (arbitrary units [AU]) by multiplying the session-RPE (Borg's category ratio 10-scale) by the session duration, which is valid for quantifying training loads in AF (Scott et al., 2013).

Table 4.1. Typical training week during intervention period.

Day	1	2	3	4	5	6
AM		Football	Technical	Football	Football	Resistance
PM	Cross-train	Resistance	Resistance	Cross-train		Hike
Day	7	8	9	10	11	12
AM	Hike	Football	Resistance		Football	
PM			Cross-train		Resistance	Cross-train
Day	13	14	15	16	17	18
AM	Football	Resistance	Football	Resistance	Resistance	Football
PM	Resistance	Technical		Technical	Cross-train	

Football = Australian-Football specific skills and running (90-120 min), resistance = strength training (40-70 min), cross train = non-specific training (e.g. swimming, cycling, boxing; 20-60 min), technical = skills based Australian-Football session (light intensity; 20-60 min), hike = outdoor recreational hiking (120-240 min).

Haematological measures

All subjects were measured for Hb_{mass} using the optimised carbon monoxide (CO) re-breathing technique (Schmidt and Prommer, 2005) five days (D₋₅) before altitude exposure ([PRE] see figure 4.1). Hb_{mass} was measured again one (D₁), thirteen (D₁₃) and seventeen days (D₁₇/POST₁) after ascent during ALT2 only, and five days post-decent (POST₁) in ALT1 only. Hb_{mass} was measured again 28 days post-decent in both groups (POST₂). Change in Hb_{mass} (Δ Hb_{mass}) was calculated from PRE in ALT1, and from the mean of D₋₅ and D₁ in ALT2. Briefly, subjects re-breathed a bolus of 99% CO equivalent to 1.0 mL/kg of body

mass through a glass spirometer (BloodTec, Bayreuth, Germany) for two min. Percent carboxyhaemoglobin (%HbCO) in fingertip capillary blood was measured using the same OSM3 hemoximeter (Radiometer, Copenhagen, Denmark) before and seven min after administration of the CO dose. CO doses administered at altitude were adjusted for changes in the partial pressure (doses at ~2,000 m equivalent to 1.3 ml/kg). Six repeat measures of %HbCO were made for improved precision in Hb_{mass} estimation (Alexander et al., 2011). All Hb_{mass} measurements were performed by the same technician. Venous blood samples were collected at PRE, three days (D₃) and sixteen days (D₁₆) after ascent and thirteen days post-descent (POST_{D13}) in ALT2 only. Samples were centrifuged, serum decanted and frozen at -80°C, and transported to Canberra, Australia for analysis of serum EPO (for ALT2 only), in one batch, using an automated solid-phase, sequential chemiluminescent Immulite assay (Diagnostics Product Corporation, Los Angeles, USA). PRE and POST_{D13} samples were analysed for reticulocyte count using a Sysmex XE-5000 Automated Haematology Analyser (Roche Diagnostics, Castle Hill, Australia) at St Vincent's Hospital Pathology (Fitzroy, Australia), while D₃ and D₁₆ samples were analysed using a Sysmex XT-4000i Automated Haematology Analyser (Sysmex, Lincolnshire, USA) at Park City Medical Centre (Park City, USA). Serum ferritin was assessed at PRE using an AU5800 immuno-turbidimetric assay (Beckman Coulter, Lane Cove, Australia) to identify iron-deficient subjects.

Body mass and illness

Body mass was monitored daily upon waking (7-8 am) in a well hydrated state [confirmed via urine specific gravity measures (URC-N_E, Atago, Tokyo, Japan)] throughout the intervention period using electronic scales (Tanita, Kewdale, Australia). Changes in body mass were calculated from the first to the last day at altitude during both camps. Athletes were classified as 'ill' if any training session was missed throughout the intervention period due to physical illness, excluding musculoskeletal injuries.

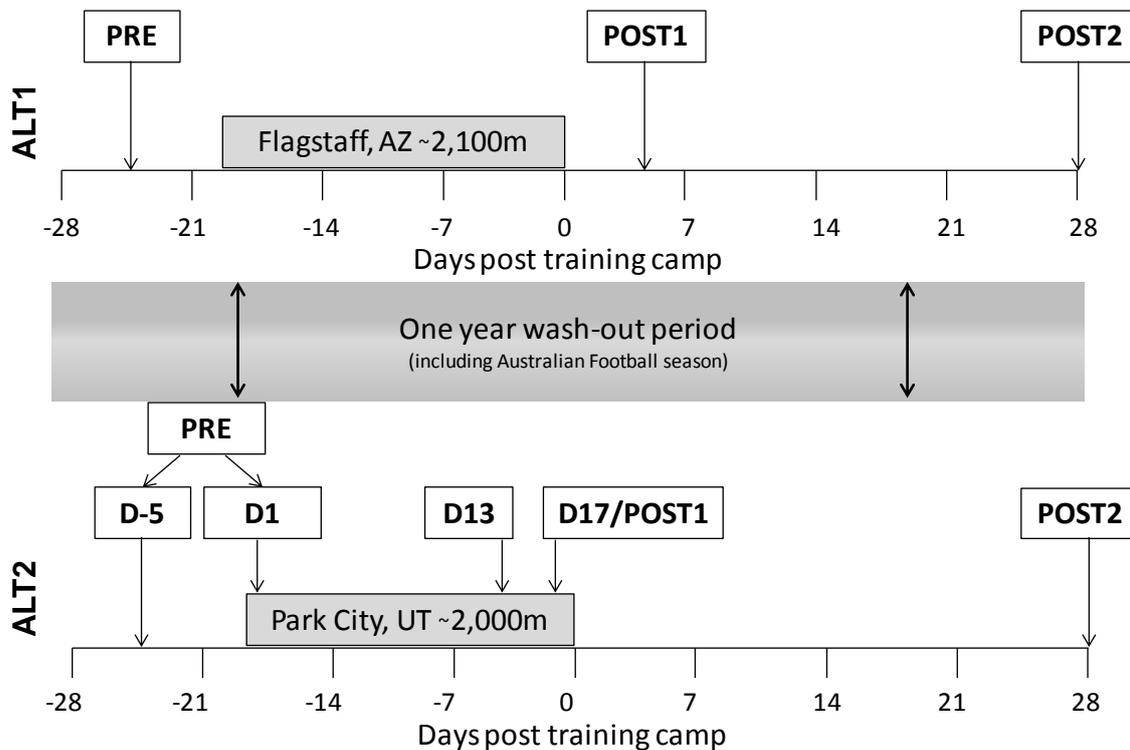


Figure 4.1. Timeline of Hb_{mass} collection over ALT1 and ALT2.

Statistical Analysis

A magnitude-based statistical approach (Hopkins et al., 2009) was used to detect small effects of practical importance. Data were log-transformed to account for non-uniformity error. Differences within and between groups were assessed with dependent and independent t-tests for unequal variance (Hopkins et al., 2009). The magnitude of changes were assessed in relation to the smallest worthwhile change (SWC), set to 2% for Hb_{mass} and a small effect size ($d = 0.2 \times$ the between-subject standard deviation for PRE) for other variables. Observed effects were reported as the mean change or difference \pm 90% confidence limits. Effects were termed positive, trivial, or negative depending on the magnitude of the change relative to the SWC and assigned a qualitative descriptor according to the likelihood of the change exceeding the SWC: 50–74% “possible”, 75–94% “likely”, 95–99% “very likely”, >99% “almost certainly” (Batterham and Hopkins, 2006). Effects where the 90% confidence interval overlapped simultaneously the substantially positive and negative thresholds were deemed “unclear”. The magnitude of individual responses were calculated from the square root of the difference in the variance of the change scores (Hopkins, 2003). A Pearson’s correlation coefficient was used to examine the relationship between percentage change in Hb_{mass} from ALT1 to ALT2 and the relationship between initial Hb_{mass} and change in Hb_{mass} .

Because one subject's results appeared to heavily influence the relationship from year-to-year, a Pearson's correlation was also performed after removing these data. Subjects who reported illness (ALT1 N=2, ALT2 N=3, total N=5) were removed from correlation analyses, as pro-inflammatory cytokines suppress EPO production (Jelkmann, 1998). Subjects were also retrospectively divided into groups classified as BodyMass_{stable} (gained mass or lost < 2.0 kg), or BodyMass_{loss} (lost > 2.0 kg).

RESULTS

Training load, duration and RPE

Weekly training load, duration and RPE for all subjects are presented in Table 4.2A. Overall training load was *likely* $5.5 \pm 4.8\%$ (mean \pm 90% confidence interval) higher and mean RPE *almost certainly* $7.2 \pm 2.6\%$ higher in ALT1 compared with ALT2. Training duration was *very likely* $12.6 \pm 3.6\%$ higher in ALT2 compared to ALT1.

Weekly training load, duration and RPE for healthy repeat subjects (N=9) are presented in Table 4.2B. There was an unclear $0.5 \pm 3.5\%$ difference in overall training load between ALT1 and ALT2. The overall mean RPE was *very likely* $5.1 \pm 2.8\%$ lower and training duration was *almost certainly* $13.5 \pm 2.4\%$ higher in ALT2 compared to ALT1.

Table 4.2A. Weekly training load, training duration and rate of perceived exertion (mean \pm SD) during ALT1 and ALT 2

	ALT1 (N=21)	ALT2 (N=23)	% Chances for ALT1 to be greater/similar/smaller than the SWC compared with ALT2
Training load (Arbitrary Units)			
Pre-intervention	2260 \pm 571	2668 \pm 488	2/14/84 (ALT2 <i>likely</i> higher)
Week 1	4765 \pm 685	4861 \pm 695	42/43/15 (<i>Unclear</i> difference)
Week 2	4962 \pm 313	4790 \pm 538	74/24/3 (ALT1 <i>possibly</i> higher)
Week 3	4536 \pm 240	3714 \pm 194	100/0/0 (ALT1 <i>almost certainly</i> higher)
Training duration (min)			
Pre-intervention	536 \pm 118	705 \pm 128	0/0/100 (ALT2 <i>almost certainly</i> higher)
Week 1	702 \pm 95	885 \pm 59	0/0/100 (ALT2 <i>almost certainly</i> higher)
Week 2	748 \pm 41	697 \pm 37	0/0/100 (ALT1 <i>almost certainly</i> higher)
Week 3	597 \pm 9	620 \pm 12	0/0/100 (ALT2 <i>almost certainly</i> higher)
Rate of Perceived Exertion			
Pre-intervention	4.84 \pm 0.58	5.37 \pm 0.51	0/0/100 (ALT2 <i>almost certainly</i> higher)
Week 1	6.97 \pm 0.26	5.48 \pm 0.58	100/0/0 (ALT1 <i>almost certainly</i> higher)
Week 2	6.35 \pm 0.13	6.12 \pm 0.49	92/7/1 (ALT1 <i>likely</i> higher)
Week 3	6.49 \pm 0.38	5.94 \pm 0.28	100/0/0 (ALT1 <i>almost certainly</i> higher)

Table 4.2B. Weekly training load, training duration and rate of perceived exertion (mean \pm SD) for the nine subjects completing both ALT1 and ALT2

	ALT1	ALT2	% Chances for ALT1 to be greater/similar/smaller than the SWC compared with ALT2
Training load (Arbitrary Units)			
Pre-intervention	2497 \pm 349	2838 \pm 201	1/2/97 (ALT2 <i>very likely</i> higher)
Week 1	5138 \pm 150	5091 \pm 323	49/31/20 (<i>Unclear</i> difference)
Week 2	5002 \pm 116	4929 \pm 337	45/47/8 (<i>Unclear</i> difference)
Week 3	3833 \pm 257	3711 \pm 185	75/18/7 (ALT2 <i>possibly</i> higher)
Training duration (min)			
Pre-intervention	550 \pm 93	765 \pm 80	0/0/100 (ALT2 <i>almost certainly</i> higher)
Week 1	730 \pm 15	902 \pm 10	0/0/100 (ALT2 <i>almost certainly</i> higher)
Week 2	763 \pm 8	696 \pm 39	0/0/100 (ALT1 <i>almost certainly</i> higher)
Week 3	625 \pm 13	597 \pm 21	0/0/100 (ALT1 <i>almost certainly</i> higher)
Rate of Perceived Exertion			
Pre-intervention	4.71 \pm 0.57	5.56 \pm 0.28	100/0/0 (ALT2 <i>almost certainly</i> higher)
Week 1	6.17 \pm 0.19	5.74 \pm 0.38	99/1/0 (ALT1 <i>very likely</i> higher)
Week 2	6.36 \pm 0.13	6.17 \pm 0.37	85/11/3 (ALT1 <i>likely</i> higher)
Week 3	6.59 \pm 0.35	5.94 \pm 0.30	100/0/0 (ALT1 <i>almost certainly</i> higher)

Haemoglobin Mass

Hb_{mass} (all subjects): The typical error of Hb_{mass} for the pooled data of all 32 subjects, for measures made approximately five days apart, was 2.6% (90% confidence interval of 2.1-3.3%). Mean (\pm SD) Hb_{mass} in ALT1 (N=21) and ALT2 (N=23) groups pre-intervention was 992 \pm 129 g and 1008 \pm 159 g, respectively. Percentage change in Hb_{mass} ($\% \Delta Hb_{mass}$) from baseline is displayed in figure 4.2. Mean $\% \Delta Hb_{mass}$ (\pm 90% CL) was *very likely* increased at POST₁ in ALT1 (3.6 \pm 1.6%, with individual responsiveness of 1.3%) and *almost certainly* increased at D₁₃ (3.9 \pm 1.0%) and D₁₇ (4.0 \pm 1.3%, with individual responsiveness of 2.2%) in ALT2. Differences in $\% \Delta Hb_{mass}$ between ALT1 and ALT2 from PRE to post-intervention (POST₁ and D₁₇ during ALT1 and ALT2, respectively) were *unclear* (-1.3 \pm 7.0%). Hb_{mass} returned to baseline at four weeks post-altitude in ALT1 and ALT2. There was a negative correlation (R=-0.51, p=0.04; see 4. 3) between initial Hb_{mass} relative to body mass (Rel Hb_{mass}) and $\% \Delta Hb_{mass}$ at POST₁ in healthy, BodyMass_{stable} subjects (n=30).

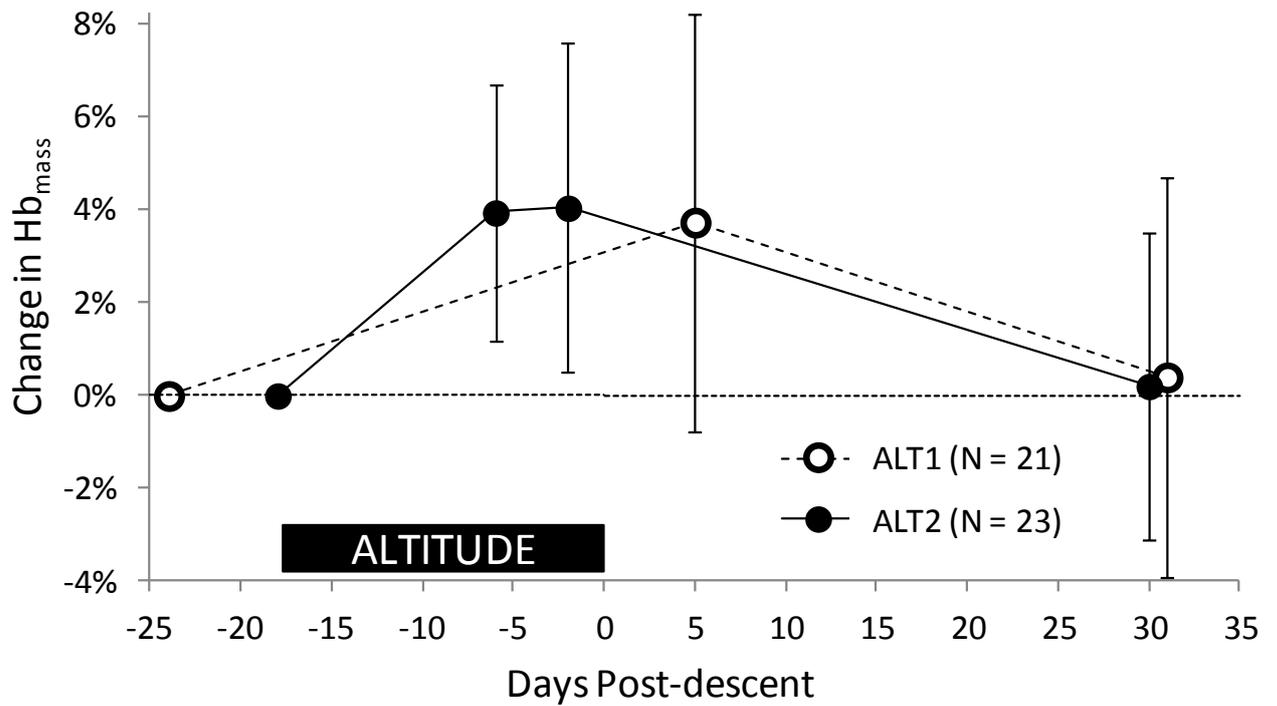


Figure 4.2. Percentage changes in Hb_{mass} (mean ± 90% confidence interval) after ALT1 and ALT2.

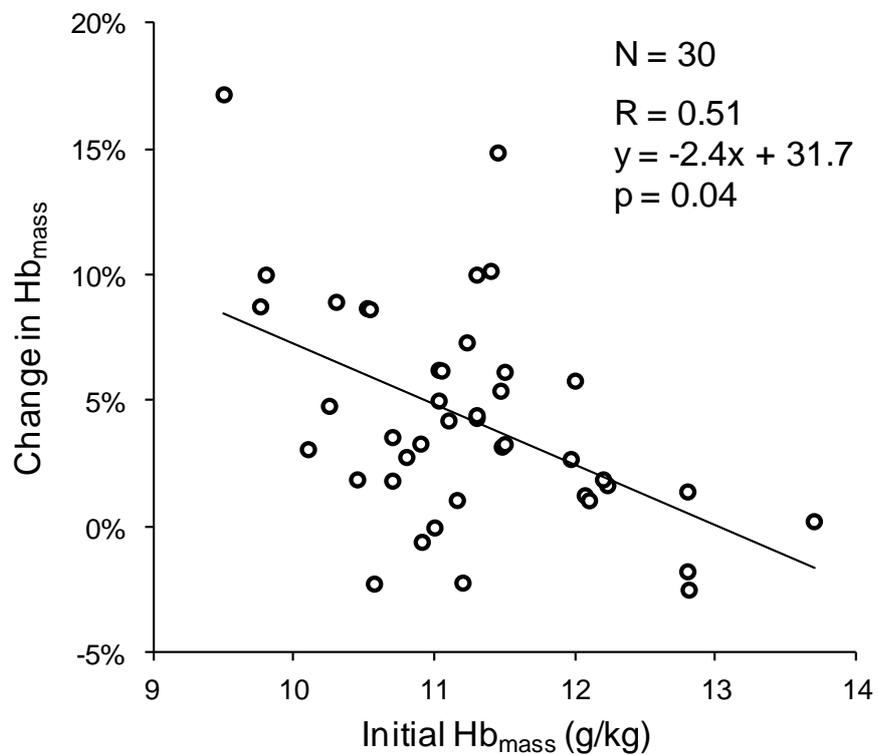


Figure 4.3. Change in Hb_{mass} vs. initial RelHb_{mass} in healthy, BodyMass_{Stable} subjects (pooled for both ALT1 and ALT2 altitude camps)

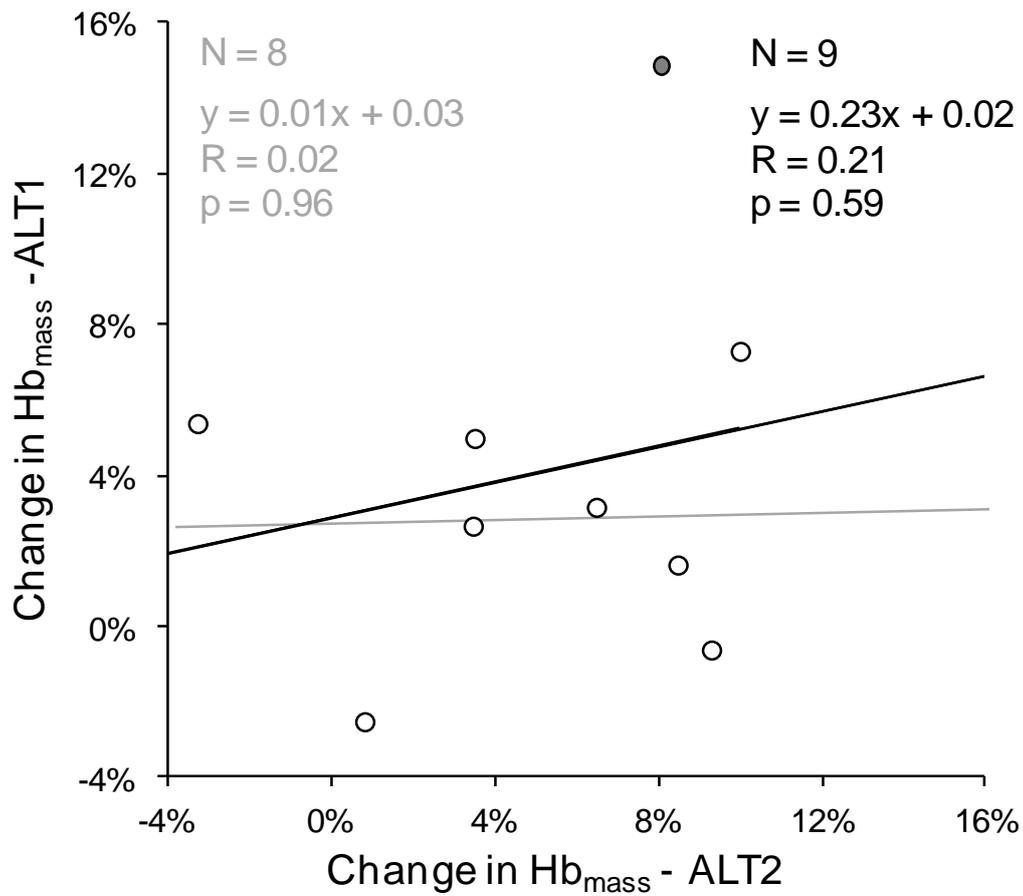


Figure 4.4. Change in Hb_{mass} during ALT1 vs. change in Hb_{mass} during ALT2. Three subjects were removed from this analysis due to illness throughout the study period (N = 9). Grey filled circle represents an outlier; regression line and associated equations when outlier is removed are shown in grey.

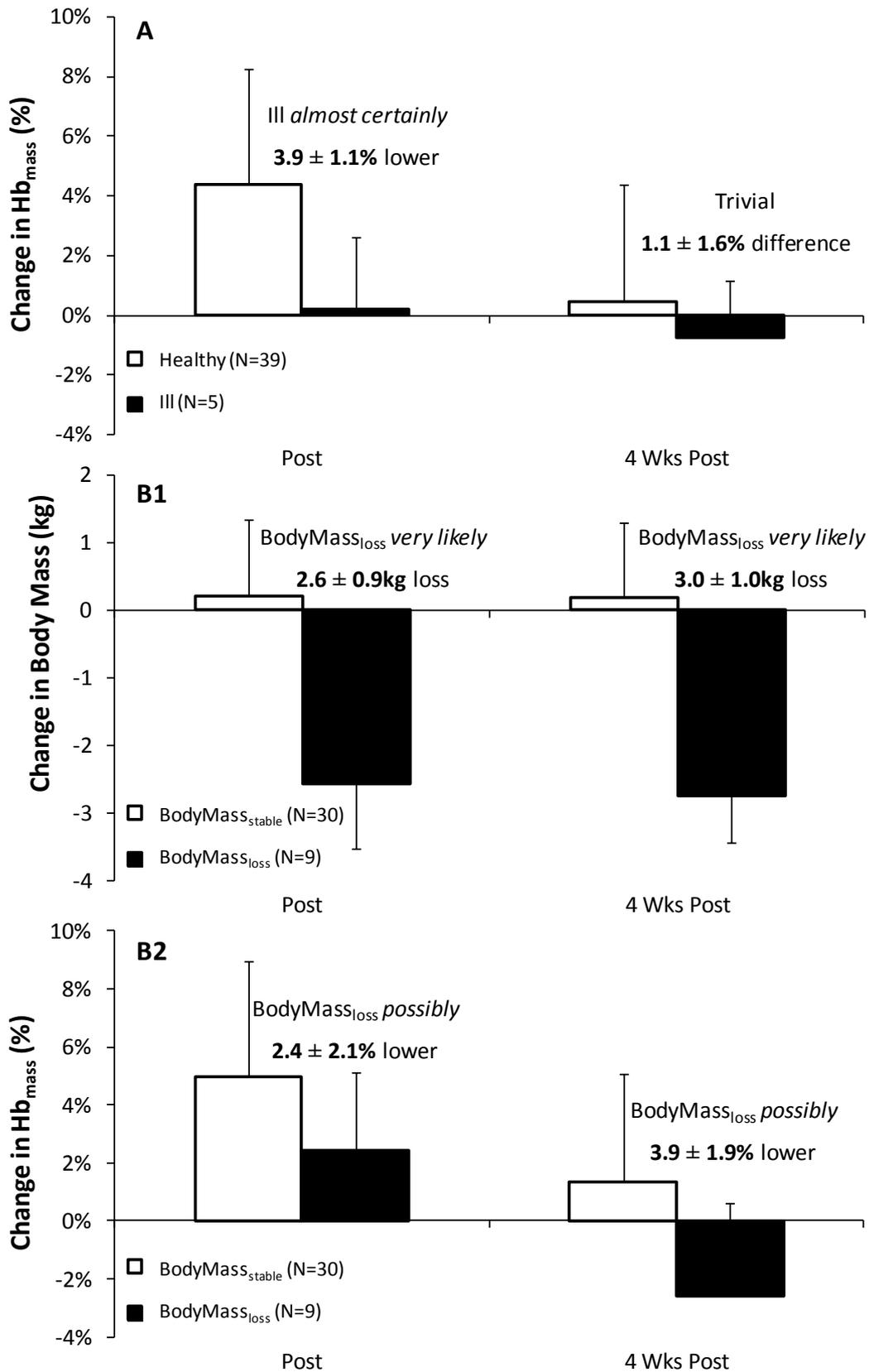


Figure 4.5. Change in Hb_{mass} in ill and healthy subjects (panel A), as well as change in mass (panel B1) and change in Hb_{mass} (panel B2) in BodyMass_{stable} and BodyMass_{loss} groups.

Hb_{mass} (subjects completing both ALT1 and ALT2): There was a *trivial* difference in Hb_{mass} at PRE for ALT1 (1,023 ±143 g) versus ALT2 (1,017 ±135 g) in subjects completing both training camps (N=12). In these subjects, Hb_{mass} *likely* increased at POST₁ in ALT1 (3.7 ± 2.5%) and at D₁₇ in ALT2 (4.2 ± 2.4%). A weak correlation between individual %ΔHb_{mass} was observed between ALT1 and ALT2 (N = 12; R = 0.12, p = 0.71), which was marginally stronger when subjects who reported illness in ALT2 were not included [(N = 9; R = 0.21, p = 0.59) see figure 4.4]. After removing one subject who was heavily influencing the results, the correlation between Hb_{mass} from year-to-year reduced to R = 0.02 (N = 8, p = 0.96, figure 4.4).

Hb_{mass} (ill v healthy subjects): Figure 4.5A shows %ΔHb_{mass} for subjects separated into healthy (N=39) and ill (N=5) groups. Ill subjects had a *trivial* 0.2 ± 2.4% change in Hb_{mass} at POST₁, which was *almost certainly* 3.9 ± 1.1% lower than changes healthy subjects. There was a *trivial* 1.1 ± 1.6% difference in Hb_{mass} between ill and healthy subjects at POST₂.

Hb_{mass} (BodyMass_{stable} v BodyMass_{loss} subjects): Changes in Hb_{mass} and body mass for BodyMass_{stable} (N=30) and BodyMass_{loss} (N=9) groups are presented in figure 4.5B1 and 4.5B2, respectively. BodyMass_{loss} subjects *very likely* lost 2.6 ± 0.9 kg from PRE to POST₁, while BodyMass_{stable} subjects had a *trivial* 0.2 ± 1.3 kg change in body mass. BodyMass_{loss} subjects *possibly* increased in Hb_{mass} by 2.6 ± 1.8% at POST₁, which was *possibly* 2.4 ± 2.1% lower than changes in the BodyMass_{stable} group. At POST₂, BodyMass_{loss} subjects had a *possible* 2.3 ± 2.7% decrease in Hb_{mass} from PRE, which was *very likely* 3.9 ± 1.9% lower than the BodyMass_{stable} group.

Reticulocytes and EPO - for ALT2 only

Figure 4.6 shows reticulocyte percentage and EPO concentrations for ALT2. Compared with PRE, reticulocytes were *almost certainly* increased by 39 ± 12% at D₃ and *very likely* increased 16 ± 10% at D₁₆. Reticulocytes were *almost certainly* reduced from PRE by 27 ± 10% at POST_{D13}. EPO *almost certainly* increased by 36 ± 10% and 22 ± 10% from PRE at D₃ and D₁₆, respectively, and *almost certainly* reduced from PRE by 16 ± 16% at POST_{D13}.

DISCUSSION

The main finding of the current investigation is that, while two pre-season moderate altitude camps yield a similar group mean increase (~4%) in Hb_{mass} of elite footballers, there is wide variability in this erythropoietic response and individual athletes do not exhibit consistency in changes in Hb_{mass} from year-to-year. Thus, a ‘responder’ or ‘non-responder’ to altitude does not appear to be a fixed trait with respect to changes in Hb_{mass} . Some of the variability in erythropoietic response may be explained by incidence of illness, reductions in body mass, and pre-camp Hb_{mass} , but large variability is still evident in healthy athletes who maintain body mass. Another important finding is that almost all of the erythropoietic response of an 18-day altitude camp (ALT2) occurred within the first 13 days of exposure.

Repeatability of changes in Hb_{mass}

We previously reported that elite team sport athletes achieved a mean increase of ~3.6% in Hb_{mass} over a 19-day altitude camp (McLean et al., 2013b). During a subsequent 18-day exposure (ALT2) our subjects achieved a similar mean increase in Hb_{mass} of 4%. However, in agreement with other work (Scoggin et al., 1978; Chapman et al., 1998; Friedmann et al., 2005). there was considerable individual variability in the erythropoietic response. Chapman et al. (1998) introduced the concept of ‘responders’ and ‘non-responders’ to altitude when they found that subjects who achieved the greatest improvements in 5000 m running performance following LHTL also demonstrated the greatest erythropoietic response. This led to suggestions that the magnitude of an individual’s response to altitude may be influenced by genetically determined traits (Chapman et al., 1998; Ge et al., 2002). This theory proposes that individuals should respond similarly on subsequent hypoxic exposures, given the same hypoxic dose (i.e., exposure time and degree of hypoxia). However, we observed a small correlation between changes in Hb_{mass} following ALT1 and ALT2 ($R=0.21$) in subjects who completed both training camps ($N=9$), which was reduced to $R=0.02$ when the sole outlier was removed. This supports previous investigations (Robertson et al., 2010b; Wachsmuth et al., 2013) that have reported weak ($R \sim 0.10-0.38$) relationships between changes in Hb_{mass} following repeated altitude exposures ~1-3 months apart, suggesting that responsiveness to a given hypoxic stimulus is not a fixed trait. The ~2% typical error of the optimised CO rebreathing technique (Schmidt and Prommer, 2005; Garvican et al., 2012b) along with natural biological variations (Garvican et al., 2010; Eastwood et al., 2012) may, in part, contribute to the variability from exposure-to-exposure.

Timeline of erythropoietic response

It is well accepted that a sufficient hypoxic dose (hr/day; number of days; degree of hypoxia) is required to induce detectable erythropoietic benefit (Wilber et al., 2007; Rasmussen et al., 2013). Wilber et al. (2007) suggested that altitude training at 2000–2500 m for at least 22 hr/day and a minimum of 4 weeks is required to optimise physiological benefits. However, these recommendations are based on data from studies that examined erythrocyte volumes pre- and post-altitude exposure, with little data addressing the time course of responses. Recently, detectable increases in Hb_{mass} have been reported with exposures as short as 11 (Garvican et al., 2012a) and 13 (Wachsmuth et al., 2013) days. Similarly, we observed ~4% increase in Hb_{mass} after 13 days at altitude, with no additional increases by day 17. This suggests that erythropoietic benefits are possible with shorter duration altitude training camps than commonly recommended, given sufficient altitude/hypoxia (Wilber et al., 2007). A two-week timecourse may have significant implications for team sport organisations who often schedule altitude camps during limited pre-season periods, and may face financial restrictions during longer duration camps due to travel/accommodation costs for athletes and support staff.

Effect of initial $RelHb_{mass}$

We found a similar relationship between initial $RelHb_{mass}$ and change in $Hb_{mass}(\%)$ as in our original investigation (McLean et al., 2013b). These data support the work of Robach and Lundby (2012) which suggests athletes starting with low $RelHb_{mass}$ have the ability to increase Hb_{mass} to a greater extent following hypoxic interventions. Others have proposed that athletes with initially high $RelHb_{mass}$ (~14.7 g/kg) may have already ‘maximised’ this component of their physiological capacity through training at sea level (Gore et al., 1998), with limited opportunity to further increase Hb_{mass} through altitude interventions. However, subsequent research from the same group showed increases in Hb_{mass} can be achieved in cyclists possessing elevated initial $RelHb_{mass}$ (~14.2 g/kg) (Garvican et al., 2012a). It is also possible that altitude training interventions may increase Hb_{mass} to a given individual’s physiological limit, and responsiveness may therefore vary depending on an individual’s baseline Hb_{mass} (Wachsmuth et al., 2013). Collectively, these data (Robach and Lundby, 2012; McLean et al., 2013b; Wachsmuth et al., 2013) suggest that team sport athletes with a higher initial $RelHb_{mass}$ may have an attenuated ability to increase Hb_{mass} with altitude training interventions.

Effect of illness on erythropoiesis

It has been suggested that athletes suffering from infection/illness/injury that results in increased inflammation may have limited erythropoietic responses to altitude exposures (McLean et al., 2013b; Wachsmuth et al., 2013). Indeed, Wachsmuth et al. (2013) reported attenuated changes in Hb_{mass} in sick/injured athletes following LHTH interventions at 2,320 m. Similarly, we observed an attenuated erythropoietic response in subjects experiencing illness during ALT1 (N=2) and ALT2 (N=3) (see figure 4.5A). Proinflammatory cytokines, such as interleukin 1(IL-1), are known to suppress the release of EPO (Jelkmann, 1998), and EPO data for ill subjects during ALT2 are highlighted in figure 4.6B. Subjects (b) and (c) (in figure 4.6B) suffered illness immediately preceding the camp, which was accompanied by low EPO levels before and throughout the camp duration. Subject (a) fell ill upon arriving at altitude and displayed suppressed EPO responses thereafter. Making statistical inferences are difficult, given instances of illness/injury are low in our investigation (N=5) and in Wachsmuth et al. (2013) (N=7). However, these data support the hypothesis that illness/injury may limit the erythropoietic benefits associated with prolonged hypoxic exposures.

Effect of body mass reductions on erythropoiesis

Subjects experiencing reductions in body mass (>2kg) achieved approximately half of the erythropoietic benefit as those maintaining body mass (~2.5% vs. ~5% increase in Hb_{mass} , respectively). Furthermore, at four weeks post-altitude $BodyMass_{loss}$ subjects displayed Hb_{mass} ~2.3% below pre-altitude levels. Our results contrast with those of Gough et al. (2013), who modelled that body mass changes over approximately 6 months did not significantly alter Hb_{mass} ; for instance, that a 10% loss of body mass would only decrease Hb_{mass} by 1.4%. In contrast, the 2.6kg reduction in body mass within the Australian-Footballer sub-group over 18-19 days suggest a mismatch between energy consumption and energy expenditure and thus an overall catabolic state, which may not support an anabolic process like erythropoiesis. Evidence in maintenance haemodialysis patients suggests that poor appetite and low protein intake is associated with increased serum concentrations of inflammatory markers (including CRP, IL-6 and TNF- α) and increased synthetic EPO dose requirements (Kalantar-Zadeh et al., 2004). While mechanisms for a poor erythropoietic response in $BodyMass_{loss}$ subjects are not clear, the combination of altitude exposure and weight loss appears to be counterproductive for Hb_{mass} maintenance.

Limitations

This research was conducted with a relatively small sample of elite Australian-Football players. Even when using a magnitude-based statistical approach (Hopkins et al., 2009), the small sample size limitations are most apparent for our attempts to quantify the effects of initial Hb_{mass} , loss of body mass and of illness on the response to altitude. A further limitation is the use of two different Sysmex haematology analysers for the reticulocytes measures, since even Sysmex analysers that are calibrated within the manufacturer tolerances can have biases in the order of 0.3-0.5% reticulocytes (Ashenden et al., 2013). The applied nature of this research also led to a number of limitations, which should be considered when interpreting our results. ALT1 and ALT2 were conducted in different locations over different durations (19 and 18 days, respectively). However, the altitude at these locations is very similar (~2,100m) and the overall hypoxic dose between camps only differed by 18 hours. The temporal measurements of Hb_{mass} differed between ALT1 and ALT2; $POST_1$ was taken five days post-altitude (ALT1) compared with the penultimate day at altitude (ALT2). Therefore, the potential confounding effects of neocytolysis upon return to sea-level (Garvican et al., 2012a; Pottgiesser et al., 2012) during ALT1 may account for some of the variability between the two exposures.

Conclusion

This investigation was conducted in an ecologically valid environment, with professional team sport athletes engaging in pre-season altitude training in an attempt to improve subsequent performance at sea level. As responsiveness to a given altitude exposure does not appear to be a fixed trait, it is not currently possible for individual athletes to be identified as ‘responders’ or ‘non-responders’ following a single altitude exposure. To optimise the erythropoietic benefit from an LHTH intervention, athletes should be well-prepared, in good health and maintain body mass throughout its duration. Healthy team sport athletes should expect a 3-4% increase in Hb_{mass} following an 18-19 day altitude training camp, with this erythropoietic response possibly achievable in as short as 13 days.

What are the new findings

- Team sport athletes produce repeatable group mean increases in Hb_{mass} (~4%) over 18-19 day moderate altitude camps, and these benefits may be achieved in as short as 13 days.
- Individual athletes do not exhibit consistency in altitude-induced changes in Hb_{mass} from year-to-year, thus a 'responder' or 'non-responder' to altitude does not appear to be a fixed trait.
- To achieve full erythropoietic benefit from altitude exposure, athletes should maintain body mass and remain free from illness immediately before and throughout the exposure.

How might it impact on clinical practice in the near future

- Athletes may gain physiological benefits from participating in shorter duration (~13 days) altitude training camps than previously recommended
- Strategies to maintain body mass and optimal health immediately before and throughout altitude training camps should be adopted

CHAPTER 4.1 – EVIDENCE OF A UNIQUE RESPONDER TO MULTIPLE ALTITUDE TRAINING CAMPS: CASE STUDY OF AN ELITE TEAM SPORT ATHLETE

PUBLICATION STATEMENT

This work was prepared as a short case study, yet to be submitted, in the International Journal of Sports Physiology and Performance, conforming to the following guidelines:

IJSP case studies may describe a single case or a small case series of physiological and/or performance aspects of a highly trained athlete, team, event, or competition; limited to 800 words, two tables or figures, and six references. A case study is appropriate when a phenomenon is interesting, novel, or unusual but logistically difficult to study with a sample. The case can exemplify identification, diagnosis, treatment, measurement, or analysis.

INTRODUCTION

The phenomenon of a *responder* and *non-responder* to altitude training was introduced by Chapman et al. (1998) and suggests that some individuals have a heightened erythropoietic response to a given altitude stimulus, which is associated with greater improvements in sea-level exercise performance. However, we recently reported a weak correlation between the change in haemoglobin mass (Hb_{mass}) from year-to-year within individual athletes participating in two altitude training camps (see Chapter 4). This finding supports previous work showing a weak correlation between responses to two altitude training camps (separated by five weeks) with respect to Hb_{mass} and performance (Robertson et al., 2010b), suggesting that individual responsiveness is not a fixed trait from exposure to exposure. In this case study, we present data of one athlete who has consistently shown an enhanced erythropoietic response to altitude exposure, and may therefore be classified as a unique *responder*.

METHODS

We followed a group of male professional Australian Footballers over two altitude training camps (~2,000 m) during December 2011 (ALT1) and December 2012 (ALT2). Group

results from these camps have been reported previously (McLean et al., 2013a; McLean et al., 2013b). One particular athlete was identified as having an enhanced Hb_{mass} response in both years and his data are reported in this case study. The subject was 21 y, 188 cm and 81.9 kg at the commencement of the study, and free from injury and illness during ALT1. One month prior to ALT2, he sustained a stress fracture of the navicular bone in his left foot and was, thus, restricted to non-weight-bearing exercise during ALT2. All training was monitored via the session RPE method and Hb_{mass} was measured via the carbon monoxide rebreathing technique (McLean et al., 2013a; McLean et al., 2013b).

RESULTS

Training load

Table 4.1.1. Training load (monitored via session RPE) during ALT1 and ALT2

Week	ALT1			ALT2		
	1	2	3	1	2	3
<i>Responder</i> total (AU)	5,281	4,940	3,960	3,245	4,020	2,040
Group total (AU)	5,138 ± 150	5,002 ± 116	3,833 ± 257	5,091 ± 323	4,929 ± 337	3,711 ± 185

ALT1 vs. ALT2 responses for Hb_{mass}

The correlation between ALT1 and ALT2 for individual changes in Hb_{mass} (ΔHb_{mass}), with the inclusion of responder data, was $R = 0.21$, $p = 0.59$ ($N=9$). This relationship weakened further ($R = 0.02$, $p = 0.96$, $N = 8$) when *responder* data were not included.

Erythropoietin (EPO) response

Baseline EPO, collected only during ALT2, was 12.7 vs. 10.7 ± 3.4 mU/mL in the *responder* and group, respectively. This increased to 14.6 vs. 14.8 ± 4.8 mU/mL after 3 days at altitude, declined to 11.6 vs. 12.9 ± 3.8 mU/mL after 17 days at altitude, and after 17 days at sea-level, was 6.2 vs. 9.1 ± 3.1 mU/mL.

Haematological responses

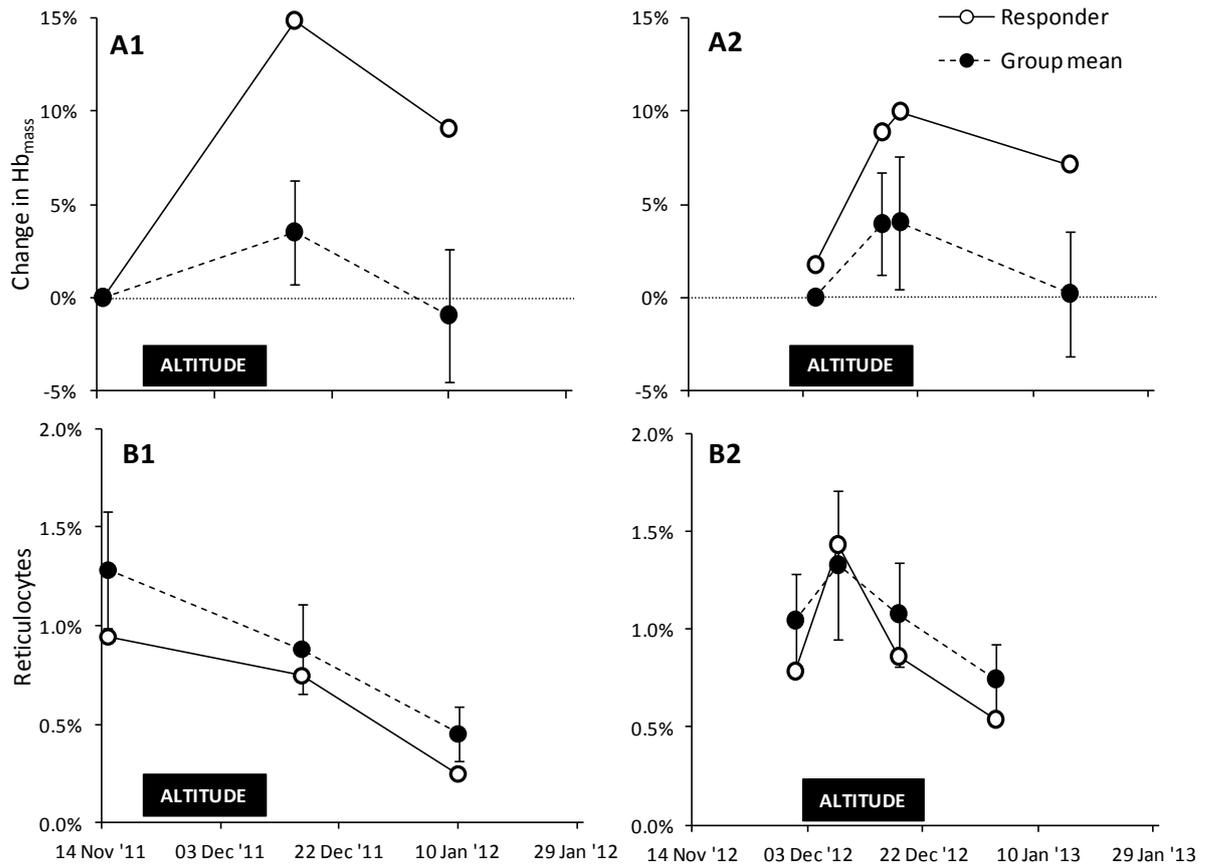


Figure 4.1.1. Change in Hb_{mass} during ALT1 (panel A1) and ALT2 (panel A2), and change in reticulocytes during ALT1 (panel B1) and ALT2 (panel B2). The *responder's* change in Hb_{mass} was 2.3 and 1.7 standard deviations above the group mean response during ALT1 and ALT2, respectively.

DISCUSSION

This case demonstrates an individual athlete who has repeatedly shown a uniquely enhanced erythropoietic response to altitude training. While we have previously shown that changes in Hb_{mass} within individual athletes (n=9) are weakly correlated from exposure to exposure (McLean et al., 2013a), it may be speculated that this athlete is pre-disposed to an enhanced response to altitude training, possibly related to inherent genetic traits (Chapman et al., 1998).

The *responder's* increase in Hb_{mass} was 2.3 and 1.7 standard deviations above the group mean increase during ALT1 and ALT2, respectively. This enhanced erythropoietic response is supported by a greater increase in reticulocytes after 3 days at altitude and a greater

suppression of reticulocytes after 16 days at altitude and upon returning to sea-level (see figure 1B). This increased erythropoiesis is evident despite EPO responses being very similar to the group mean responses during altitude exposure.

The lower reticulocyte count in this athlete upon return to sea-level may be indicative of increased neocytolysis after altitude exposure, which may be caused by increased suppression of EPO (responder 6.2 vs. group 9.1 ± 3.1 mU/mL) post-altitude. Although the *responder* had a larger suppression in EPO and reticulocytes post-altitude, he maintained his increases in Hb_{mass} for longer than is generally described following return to sea-level (McLean et al., 2013b). Indeed, in our group, mean Hb_{mass} had returned to baseline levels after 4 weeks at sea-level, whilst the *responder* was still ~7-9% above baseline. This finding was confirmed with a duplicate measure (at 4.5 weeks post-descent) in ALT2. This sustained increase in Hb_{mass} may be attributed to the greater erythropoietic response at altitude, as both the *responder* and group appeared to reduce Hb_{mass} at a similar rate. Elevated Hb_{mass} following altitude interventions may confer an enhanced physiological capacity that aids sea-level training.

Due to lower limb injury, this athlete completed a reduced training load during ALT2 compared to ALT1, and the increases in Hb_{mass} were also reduced during ALT2. This supports the prevailing theory that increases in training load augment increases in Hb_{mass} (Garvican et al., 2010). However, despite his reduced training load compared to the rest of the group, this individual still displayed an erythropoietic response well above all other participants, providing further support that he is a unique *responder*. While illness and injury appear to suppress erythropoietic responses to altitude exposure (Wachsmuth et al., 2013), thought to be caused by inflammatory factors (McLean et al., 2013a; Wachsmuth et al., 2013), the injury was not in its acute phase (during ALT2) and it is thus likely that minimal inflammation was present during altitude exposure.

The uniqueness of this *responder* is also highlighted by a further weakening of the exposure-to-exposure relationship for Hb_{mass} when his data are removed from analyses ($R = 0.21$, $p = 0.59$ vs. $R = 0.02$, $p = 0.96$, with and without *responder*, respectively).

CONCLUSION

Although previous work (Robertson et al., 2010b; McLean et al., 2013a) suggests that individual responsiveness is not a fixed trait following repeated altitude exposures, this case study suggests that the *responder* to altitude exposure does exist, but the phenomenon may be rarer than previously proposed (Chapman et al., 1998). This highlights the importance in monitoring individual erythropoietic responses to altitude training, which may help identify unique athletes who are consistent *responders* to altitude exposure, thereby facilitating more individualised prescription of altitude training interventions.

CHAPTER 5 – SUPPLEMENTARY STUDY: ALTITUDE TRAINING AND HAEMOGLOBIN MASS FROM THE OPTIMISED CARBON MONOXIDE RE-BREATHING METHOD – A META-ANALYSIS

PUBLICATION STATEMENT

Gore et al. (2013) conducted a meta-analysis using raw data from 17 LHTL and LHTH studies. This short chapter is included because the raw data from Studies 1 (Chapter 3) and 2 (Chapter 4) in this thesis formed part of this meta-analysis, thereby leading to co-authorship. The aim of this meta-analysis was to “use the raw data of only those studies that used the optimised CO rebreathing method to determine Hb_{mass}, and that were conducted since 2008” in order to “offer a more precise estimate of the effect of altitude on Hb_{mass}” (Gore et al., 2013).

This chapter will discuss the findings and practical implications of the meta-analysis conducted by Gore et al. (2013). For a detailed description of methods and further discussion, please refer to Appendix V:

Gore C, Sharpe K, Garvican-Lewis L, Saunders P, Humberstone C, Robertson EY, Wachsmuth N, Clark SA, McLean BD, Friedman-Bette B, Neyra M, Pottgiesser T, Schumacher YO and Schmidt W. (2013) Altitude training and haemoglobin mass from the optimised carbon monoxide re-breathing method – a meta-analysis. *British Journal of Sports Medicine*. 47: i31-i39.

INTRODUCTION

It is well established that a sufficient ‘hypoxic dose’ during LHTH and LHTL interventions will lead to increases in Hb_{mass} (Wilber et al., 2007). However, there is less consensus regarding the time course of changes in Hb_{mass}/red cell volume (RCV), and the minimal hypoxic dose required to stimulate detectable increases in Hb_{mass}. Clark et al. (2009) conducted serial time-course measurements of Hb_{mass} during a 21-day LHTL intervention (~14 hr/day at a simulated altitude of 3,000 m) and reported that Hb_{mass} increased by 3.3% over the duration of the camp, at a rate of approximately 1% for every 100 hours of hypoxic exposure. Conversely, a recent meta-analysis (Rasmussen et al., 2013) suggested that at altitudes of 3,000 m or below, exposure times of at least 4 weeks are needed to yield statistically significant increases in Hb_{mass} (back calculated from RCV), and that this

response may be accelerated with exposure above 4,000 m. However, this meta-analysis by Rasmussen et al. (2013) garnered data from 66 studies which used a range of methodologies to assess RCV, including carbon monoxide (CO) rebreathing (44%), radioactive labelling of albumin (19%), and various plasma dye-dilution tracer methods (37%). All of these methods have different measurement error, and Rasmussen et al. (2013) reported ‘surprisingly large’ variability in increases in RCV (49 ± 240 mL/wk). The variability in these results is likely affected by the different measurement techniques used; indeed, it has previously been shown that typical error for measurement of RCV from both CO rebreathing and Evans blue dye (a common plasma dye-dilution method) is ~7% (Gore et al., 2005). In contrast, measurement of Hb_{mass} using the CO rebreathing technique (Schmidt and Prommer, 2005) displays measurement error of ~2% (Gore et al., 2005). Thus, using the CO rebreathing method to measure changes in Hb_{mass} may be capable of detecting smaller, practically meaningful changes in red blood cells following altitude interventions.

Therefore, this meta-analysis aimed to use the raw data from 17 LHTL and LHTH studies that used the optimised CO rebreathing method to determine Hb_{mass}, all 17 studies were conducted since 2008 in order to offer a more precise estimate of the effect of altitude on Hb_{mass} (Gore et al., 2013).

FINDINGS AND DISCUSSION

The main finding of this meta-analysis was that Hb_{mass} increased by approximately 1.1% with every 100 hours of hypoxic during classic altitude camps (> 2,100 m) or normobaric hypoxia LHTL interventions (simulated altitude > 3,000 m). These results are in contrast to the recent meta-analysis conducted by Rasmussen et al. (2013), which suggests that a 1% increase in Hb_{mass} would take 13-28 days and 18–31 days of classic and LHTL altitude exposure, respectively. In the meta-analysis of Rasmussen et al. (2013), the selection of RCV as the outcome variable (needed to standardise their different data sources) and the inclusion of data from a variety of relatively ‘noisy’ techniques may have prevented this analysis from detecting small, practically important changes. Thus, these contrasting findings are likely explained by the noise/error in the data, since changes as small as 1% are below the analytical error of even the best methods. However, the large sample of raw data collected from a single, reliable technique (CO rebreathing) only in the meta-analysis of Gore et al. (2013) provides strong statistical power, allowing for detection of small changes in Hb_{mass}.

It is well established that haematological changes in response to altitude residence will eventually plateau (Brothers et al., 2010), and a fitted quadratic in the meta-analysis of Gore et al. (2013) suggests a maximum increase in Hb_{mass} of 6.6%. However, this estimation is likely specific to the data used, and the maximum exposure time among this data set was 670 hours; therefore, extrapolations beyond this time course should be interpreted with caution.

The majority of the classic and simulated LHTL interventions examined yielded increases of ~3-4% in Hb_{mass} , and modelling of post-altitude Hb_{mass} indicated that ~3% increase will likely persist for up to 20 days following the intervention. Between 20 and 32 days post-altitude (the range of the available data), the type of altitude intervention appeared to influence the decay in Hb_{mass} ; indeed, the change in Hb_{mass} was not significantly different from zero for classic altitude, but was estimated to be 1.5% higher than pre-altitude values for LHTL.

Whilst a substantial amount of individual variability is evident in response to altitude (Chapman et al., 1998) and other training (Mann et al., 2014) interventions, the meta-analysis of Gore et al. (2013) suggest that 97.5% of individuals will increase Hb_{mass} by at least 1% after 300 hours of exposure (equivalent to 12.5 days of classic altitude, or 21.4 days of LHTL with 14 h/day of hypoxia). This implies that most athletes will increase Hb_{mass} after a 2-week classic altitude camp, which is a shorter duration exposure than the minimal hypoxic ‘dose’ suggested by others as necessary to induce haematological benefits (Wilber et al., 2007; Rasmussen et al., 2013). These findings are in agreement with Study 2 (Chapter 4), where we report that athletes achieve almost all of their 4% increase in Hb_{mass} after just 13 days at altitude. Therefore, athletes who have a busy schedule of training and competition may gain small benefits from participating in short duration (~ 2 weeks) altitude training camps.

What are the new findings of this meta-analysis [as taken from Gore et al. (2013)]

- The optimised carbon monoxide rebreathing method to determine Hb_{mass} has an analytical error of ~2%, which provides a sound basis to interpret changes in Hb_{mass} of athletes exposed to moderate altitude.

- During-altitude Hb_{mass} increases by ~1.1%/100 h of adequate altitude exposure, so when living and training on a mountain (classic altitude) for just 2 weeks, a mean increase of ~3.4% is anticipated.
- Living high and training low (LHTL) at 3000 m simulated altitude is just as effective as classic altitude training at ~2320 m at increasing Hb_{mass}, when the total hours of hypoxia are matched.
- ~97.5% of adequately prepared athletes are likely to increase Hb_{mass} by at least 1% after approximately 300 h of altitude exposure, either classic or LHTL. ‘Adequately prepared’ includes being free from injury or illness, not ‘overtrained’ and with iron supplementation.

How might these findings influence clinical practice in future [as taken from Gore et al. (2013)]

- For athletes with a busy training and competition schedule, altitude training camps as short as 2 weeks of classic altitude will quite likely increase Hb_{mass} and most athletes can expect benefit.
- Athletes, coaches and sport scientists can use altitude training with high confidence of an erythropoietic benefit, even if the subsequent performance benefits are more tenuous.

CHAPTER 6 – SYSTEMATIC REVIEW: APPLICATION OF ‘LIVE LOW-TRAIN HIGH’ FOR ENHANCING NORMOXIC EXERCISE PERFORMANCE IN TEAM SPORT ATHLETES – A SYSTEMATIC REVIEW

PUBLICATION STATEMENT

This work was submitted and accepted for publication in *Sports Medicine*, please see Appendix IV:

McLean BD, Gore C and Kemp J. (2014) Application of ‘live low-train high’ for enhancing normoxic exercise performance in team sport athletes – a systematic review. *Sports Medicine*.

ABSTRACT

BACKGROUND AND OBJECTIVE. Hypoxic training techniques are increasingly used by athletes in an attempt to improve performance in normoxic environments. The ‘live low-train high (LLTH)’ model of hypoxic training may be of particular interest to athletes because LLTH protocols generally involve shorter hypoxic exposures (~2-5 sessions per week of < 3 h) than other traditional hypoxic training techniques (e.g. live high-train high or live high-train low). However, the methods employed in LLTH studies to date vary greatly with respect to exposure times, training intensities, training modalities, degrees of hypoxia and performance outcomes assessed. Whilst recent reviews provide some insight into how LLTH may be applied for performance, little attention has been given to how training intensity/modality may specifically influence subsequent performance in normoxia. Therefore, this systematic review aims to evaluate the normoxic performance outcomes of the available LLTH literature, with a particular focus on training intensity and modality.

DATA SOURCES AND STUDY SELECTION. A systematic search was conducted to capture all LLTH studies with a matched normoxic (control) training group and the assessment of performance under normoxic conditions. Studies were excluded if no training was completed during the hypoxic exposures, or if these exposures exceeded 3 h per day. Four electronic databases were searched (PubMed, SPORTDiscusTM, EMBASE and Web of Science) during August 2013, and these searches were supplemented by additional manual searches until December 2013.

RESULTS. After the electronic and manual searches, 40 papers were deemed to meet the inclusion criteria, representing 31 separate studies. Within these 31 studies, four types of LLTH were identified: (1) continuous low-intensity training in hypoxia (CHT, n=16), (2) Interval hypoxic training (IHT, n = 4) (3) Repeated sprint training in hypoxia (RSH, n=3) and (4) Resistance training in hypoxia (RTH, n=4). Four studies also used a combination of CHT and IHT. The majority of studies reported no difference in normoxic performance between the hypoxic and normoxic training groups (n=19), while 9 reported greater improvements in the hypoxic group and 3 reported poorer outcomes compared to the control group. Selection of training intensity (including matching relative or absolute intensity between normoxic and hypoxic group) was identified as a key factor in mediating the subsequent normoxic performance outcomes. Five studies included some form of normoxic training for the hypoxic group and 14 studies assessed performance outcomes not specific to the training intensity/modality completed during the training intervention.

CONCLUSIONS. Four modes of LLTH are identified in the current literature (CHT, IHT, RSH and RTH), with training mode and intensity appearing to be key factors in mediating subsequent performance responses in normoxia. Improvements in normoxic performance appear most likely following high-intensity, short term and intermittent training (e.g. IHT, RSH). LLTH programs should carefully apply the principles of training and testing specificity and include some high-intensity training in normoxia. For RTH, it is unclear whether the associated adaptations are greater than those of traditional (maximal) resistance training programs.

INTRODUCTION

Over the past decade, the use of hypoxic training techniques has become increasingly popular in team sports (Billaut et al., 2012). Most commonly, athletes will both live and train at moderate to high altitude [live high-train high (LHTH)] or live at moderate to high altitude whilst training closer to sea-level [live high-train low (LHTL)]. These two techniques require relatively long exposure times (>12 h/day for a minimum of 2 weeks) to accumulate a sufficient ‘hypoxic dose’ to attain the associated physiological benefits (Wilber et al., 2007; Gore et al., 2013), which is often achieved by team sport athletes during a pre-season training camp at altitude (McLean et al., 2013b) or by sleeping in hypoxic chambers (Buchheit et al., 2012b). Implementing such techniques in-season is more challenging, as weekly competition does not allow for two-week-long training blocks at altitude, meaning that long exposures are only possible if teams have access to hypoxic sleeping chambers near their training base.

An alternative hypoxic training technique gaining popularity with team sports (Billaut, 2011) is the ‘live low-train high (LLTH)’ model of hypoxic training. This technique involves athletes living in normoxic conditions and performing some training sessions under hypoxic conditions. Such hypoxic exposures typically last < 3 h, 2-5 times per week and, therefore, do not provide a sufficient hypoxic stimulus (Wilber et al., 2007) to induce the haematological changes associated with LHTH and LHTL protocols. Another short duration (< 3 h) hypoxic technique is intermittent hypoxic exposure (IHE) where no training is performed during exposure sessions; but a comprehensive review concluded that IHE alone does not lead to sustained physiological adaptations or improved exercise performance (Millet et al., 2010). Conversely, a meta-analysis (Bonetti and Hopkins, 2009) concluded that IHE may improve performance in sub-elite athletes, but these effects are not evident in elite athletes, possibly due to the fact that elite athletes experience more hypoxia in their muscles from higher intensities of training compared with sub-elite athletes (Bonetti and Hopkins, 2009).

Although the physiological and performance effects of IHE appear minimal for elite athletic populations, there is evidence to suggest that short term exposure that includes some form of physical training (i.e. LLTH) has the ability to enhance glycolytic enzymes, glucose transport and pH regulation (Vogt et al., 2001; Zoll et al., 2006), and may also improve anaerobic power production (Hamlin et al., 2010) and repeated sprint ability (Faiss et al., 2013b). It has also been proposed recently that hypoxic stimuli may be used to enhance

adaptations gained from resistance training (Nishimura et al., 2010; Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b). Given that LLTH techniques deliver hypoxia only during training times, athletes involved in regular competition (e.g. weekly team sport competition) may be able to utilise this additional environmental stimulus to enhance the training process throughout the in-season period.

Three recent reviews (Millet et al., 2010; Billaut et al., 2012; Faiss et al., 2013a) and one meta-analysis (Bonetti and Hopkins, 2009) provide some insight into the potential applications of hypoxic training techniques, and how these might be applied in an attempt to enhance performance in normoxic environments. However, these reviews do not discuss in detail the effect of training modality/intensity during LLTH protocols, which may be important variables that affect performance outcomes following such interventions (Millet and Faiss, 2012). Therefore, the aim of this systematic review was to examine the LLTH literature to assess the efficacy of this technique, with a particular focus on training modality/intensity, and how these findings may be applied to enhance team sport (normoxic) performance.

METHODS

Data sources and searches

A systematic review of the literature was performed from the earliest record up to August 2013. An electronic literature search was performed using four online databases – PubMed, SPORTDiscusTM, EMBASE and Web of Science. The following terms were searched for in ‘all fields’ – [(hypoxic OR hypoxic strength OR hypoxia OR altitude OR kaatsu OR IHT) AND train*] while the terms patients, pregnancy, diabetes, rats, rodents and mice were excluded (using NOT). Results were limited to ‘English language’ and the following filters were applied to each database: PubMed - *adult 19-44 years OR young adult 19-24 years*; SPORTDiscusTM - *Academic Journals*; EMBASE - *adult 18-64 years*; Web of Science - *Document type (article) AND Category (sport sciences or physiology)*. This search was performed by two authors (BM & JK) and articles were then screened, first by title and then by abstract using the eligibility criteria below. After screening titles and abstracts, full text was retrieved for all potentially relevant articles and assessed according to the selection criteria outlined below. Reference lists for all selected articles were then screened and searches were supplemented by reviewing the reference lists of other recent reviews (Bonetti

and Hopkins, 2009; Millet et al., 2010; Billaut et al., 2012) and consulting one expert in the area of hypoxic training, who reviewed the list of included studies and made suggestions on any other potentially relevant work. Following the initial search in August 2013, relevant journals within the field were monitored closely during preparation of this manuscript and any new articles meeting the inclusion criteria and published up to December 2013 were added (n = 2).

Selection criteria

To assess the influence of LLTH interventions on normoxic performance outcomes, the following inclusion criteria were used: (1) subjects were exposed to short term (≤ 3 h/day) hypoxia throughout an intervention period ≥ 7 days; (2) some form of physical training was completed during the hypoxic exposures (hypoxic exposures with no training excluded); (3) the intervention group was compared to a control group completing matched training under normoxic conditions; (4) exercise performance under normoxic conditions (definition of 'performance' below) was assessed; (5) subjects were adults aged between 19 and 44 y. Studies were excluded according to the following criteria: (1) hypoxic exposures were > 3 h per day; (2) subjects were previously acclimatised to hypoxia (e.g. high altitude natives); (3) a within-subject unilateral research design was employed (i.e. one leg trained in hypoxia, opposite leg trained in normoxia). 'Performance' was defined as any physical test leading to a non-physiological based outcome, and included (but was not limited to); graded exercise tests [assessing time to exhaustion and/or maximal power output (W_{max})], time to exhaustion tests, time trials, repeated sprint ability tests and various maximal or near maximal strength tests. All studies reporting only physiological outcomes to LLTH were not included in this review, including those only reporting VO_{2max} without any other performance variable.

Data analysis

There is wide variety in the methodologies used in LLTH studies, including differences in exercise mode, exercise intensity, degree of hypoxia, use of normobaric or hypobaric hypoxia, number of exposures per week, weeks of exposure/training, amount/modality/intensity of additional normoxic training conducted, and the performance measures/outcome variables. Therefore, a systematic review was conducted without meta-analysis.

Initial analysis of the included studies revealed four distinct training modalities performed under hypoxic conditions, three of which have been previously identified/defined (Millet et al., 2013). As a result, studies were categorised into four types based on training modality, as the training modality completed during hypoxic exposures is likely critical to the performance outcomes following the intervention period: (1) Continuous hypoxic training (CHT) - involves continuous sub-maximal training sessions under hypoxic conditions lasting greater than 20 min (adapted from suggestion of Millet et al. (2013)), usually in an attempt to improve endurance based performance (e.g. running, cycling, swimming, rowing); (2) Interval hypoxic training (IHT) – involves medium duration (~ 30 s to 5 min), high-intensity (> 70% VO₂max) intervals with similar duration recoveries, generally performed in an attempt to improve high-intensity running ability; (3) Repeated sprint training in hypoxia (RSH) - involves short duration (~5 to 30 s) efforts followed by longer recovery periods (~20 s to 3 min), generally performed in an attempt to improve repeated sprint ability; (4) Resistance training in hypoxia (RTH) - involves resistance training under hypoxic conditions, in an attempt to increase muscular strength and power production. Two researchers individually categorised papers as CHT, IHT, RSH or RTH.

RESULTS

Figure 6.1 shows a flowchart of potentially relevant articles obtained during the search procedures. Electronic searches returned 672, 745, 724 and 708 results for PubMed, SPORTDiscusTM, EMBASE and Web of Science, respectively. From these electronic searches, duplicates were removed and 66 articles were identified as potentially relevant after examination of the titles and abstracts; of these 37 papers met the inclusion criteria (Terrados et al., 1988; Levine et al., 1992; Engfred et al., 1994; Emonson et al., 1997; Bailey et al., 2000; Bailey et al., 2001b; Geiser et al., 2001; Meeuwsen et al., 2001; Messonnier et al., 2001; Vogt et al., 2001; Friedmann et al., 2003; Hendriksen and Meeuwsen, 2003; Kime et al., 2003; Truijens et al., 2003; Ventura et al., 2003; Messonnier et al., 2004; Morton and Cable, 2005; Roels et al., 2005; Dufour et al., 2006; Ponsot et al., 2006; Zoll et al., 2006; Roels et al., 2007a; Roels et al., 2007b; Haufe et al., 2008; Beidleman et al., 2009; Mounier et al., 2009; Debevec et al., 2010; Hamlin et al., 2010; Lecoultre et al., 2010; Nishimura et al., 2010; Schmutz et al., 2010; Czuba et al., 2011; Mao et al., 2011; Faiss et al., 2013b; Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b). Additional manual searches returned two results which met all inclusion criteria. (Galvin et al., 2013; Puype et al., 2013) One article was also added that was published after the initial search date (Ho et al., 2014).

From these 40 papers, 31 separate research studies were identified (i.e. 8 cases of different papers reporting results from a common research study) and these studies are outlined in Table 6.1. There was one instance of authors using a randomised control design (Meeuwssen et al., 2001) before using a subset of the same subjects to conduct a randomised crossover study one year later (Hendriksen and Meeuwssen, 2003). Of the 31 separate studies, 16 were classified as CHT, 4 as IHT, 4 as using a combination of CHT and IHT, 3 as RSH and 4 as RTH (see Table 6.1).

There was a wide range of training modes and intensities identified among the four categories of LLTH interventions which may influence the outcomes, so training frequency, duration and intensity are summarised in table 6.1. A number of methodological considerations were also identified in the literature, including: matching of absolute or relative training intensity between hypoxic and normoxic groups; method of matching relative training intensity; and the inclusion of some portion of normoxic training for the hypoxic group (Meeuwssen et al., 2001; Friedmann et al., 2003; Hendriksen and Meeuwssen, 2003; Morton and Cable, 2005; Nishimura et al., 2010; Mao et al., 2011; Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b; Puype et al., 2013). Results of studies matching absolute training intensities ($n = 8$) between hypoxic and normoxic group should be interpreted with caution, because this likely produces a higher relative training intensity in the hypoxic group. Only six (Truijens et al., 2003; Ventura et al., 2003; Dufour et al., 2006; Ponsot et al., 2006; Zoll et al., 2006; Roels et al., 2007a; Roels et al., 2007b; Mounier et al., 2009; Lecoultre et al., 2010; Czuba et al., 2011) of the 31 studies in the present review included some form of normoxic training for the hypoxic group.

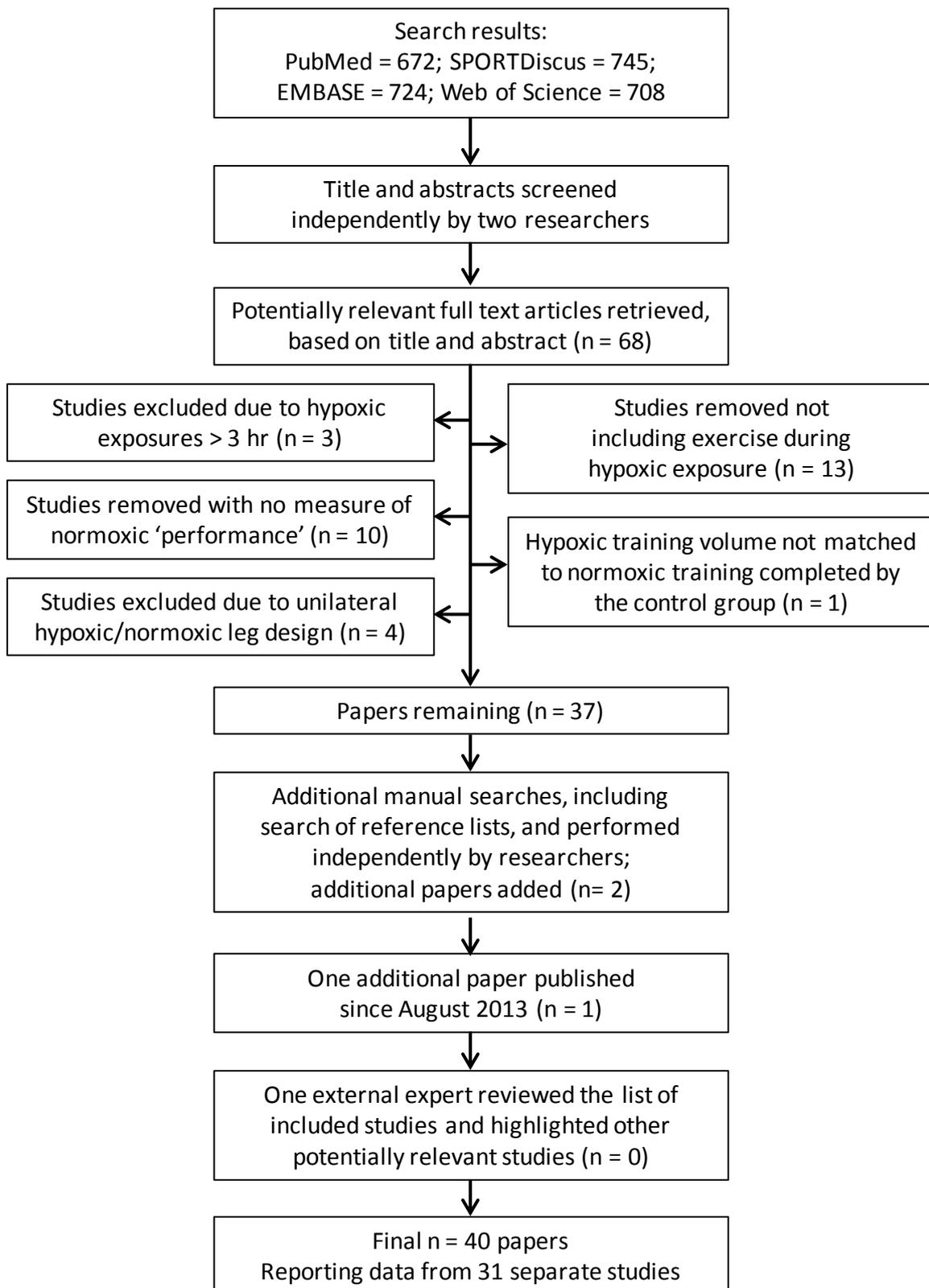


Figure 6.1. Flowchart illustrating the search and exclusion/inclusion strategy.

Table 6.1. Summary of included LLTH studies.

Author(s) (year)	Subjects <i>M or F</i> (<i>H, N</i>)	F _I O ₂ ^a H group (<i>normobaric or hypobaric</i>)	Days/weeks of training (<i>training modality</i>)	Number and duration H & control sessions (<i>additional N training</i>)	Training intensity	Absolute or relative intensity matched (H vs. N)	Performance measures	Normoxic performance (H compared to N)
Continuous hypoxic training								
Bailey et al. (2000) + Bailey et al. (2001b)	Untrained <i>M (18, 14)</i>	16.0 % (<i>Normobaric</i>)	4 weeks (<i>cycling</i>)	3 per week; 20-30 min (<i>not reported</i>)	70%–85% HRmax	Relative	Cycling GXT	No difference
Beidleman et al. (2009)	Untrained <i>M (11, 6)</i>	12.6 % (<i>Hypobaric</i>)	1 week (<i>cycling</i>)	6-7 per week; 50 min (<i>none</i>)	~80% HRmax	Relative	Cycling TT (~38 min)	No difference
Czuba et al. (2011)	Cyclists <i>M (10, 10)</i>	15.2% (<i>Normobaric</i>)	3 weeks (<i>cycling</i>)	3 per week; 60-70 min (<i>15-16 hr/week</i>)	95% LT (H) 100% LT (N)	Relative	Cycling GXT 30km TT	Improved Improved
Debevec et al. (2010)	Untrained <i>M (9, 9)</i>	12.0% (<i>Normobaric</i>)	4 weeks (<i>cycling</i>)	5 per week; 60 min (<i>none</i>)	HR at 50% Wmax	Relative	Cycling GXT TTE @ 80% VO ₂ max	No difference No difference
Emonson et al. (1997)	Untrained <i>M (9, 9)</i>	15.7 % (<i>Hypobaric</i>)	5 weeks (<i>cycling</i>)	3 per week; 45 min (<i>none</i>)	HR corresponding to 70% VO ₂ max	Relative	TTE @ 80% VO ₂ max	No difference
Engfred et al. (1994) Levine et al. (1992)	Untrained <i>M, F (14, 7)</i>	15.7 % (<i>Hypobaric</i>)	5 weeks (<i>cycling</i>)	5 per week; 45 min (<i>not reported</i>)	70% VO ₂ max	Relative + Absolute	TTE @ 85% VO ₂ max	Decreased (relative + absolute group)
Geiser et al. (2001)	Untrained <i>M (18, 15)</i>	13.4 % (<i>Normobaric</i>)	6 weeks (<i>cycling</i>)	5 per week; 30 min (<i>not reported</i>)	77%–85% HRmax	Relative + Absolute	Cycling GXT	No difference
Haufe et al. (2008)	Untrained <i>M (10, 10)</i>	15.0 % (<i>Normobaric</i>)	4 weeks (<i>running</i>)	3 per week; 60 min (<i>not reported</i>)	HR corresponding to 3 mmol·L ⁻¹ La ⁻	Relative	Running GXT	No difference
Hendriksen and Meeuwse (2003) ^b	Triathletes <i>M (12,12)</i>	15.7 % (<i>Hypobaric</i>)	10 days (<i>cycling</i>)	7 per week; 120 min (<i>none</i>)	60%–70% HRR	Absolute	WAnT Cycling GXT	Improved No difference

Author(s) (year)	Subjects <i>M or F</i> (<i>H, N</i>)	FiO ₂ ^a H group (<i>normobaric or hypobaric</i>)	Days/weeks of training (<i>training modality</i>)	Number and duration H & control sessions (<i>additional N training</i>)	Training intensity	Absolute or relative intensity matched (<i>H vs. N</i>)	Performance measures	Normoxic performance (<i>H compared to N</i>)
Continuous hypoxic training (continued)								
Kime et al. (2003)	Cyclists <i>M, F (8 – crossover)</i>	15.0 % (<i>Normobaric</i>)	3 weeks (<i>cycling</i>)	3 per week; 120 min (<i>none</i>)	LT	Relative	Cycling GXT	No difference
Mao et al. (2011)	Untrained <i>M (12, 12)</i>	15.0 % (<i>Normobaric</i>)	5 weeks (<i>cycling</i>)	5 per week; 30 min (<i>none</i>)	60% Wmax	Absolute	Cycling GXT	Improved
Meeuwse et al. (2001)	Triathletes <i>M (8, 8)</i>	15.7 % (<i>Hypobaric</i>)	10 days (<i>cycling</i>)	7 per week; 120 min (<i>none</i>)	60%–70% HRR	Absolute	WAnT Cycling GXT	Improved Improved
Messonnier et al. (2001) Messonnier et al. (2004)	Untrained <i>M, F (5, 8)</i>	11.7 % (<i>Normobaric</i>)	4 weeks (<i>cycling</i>)	6 per week; 120 min (<i>none</i>)	60-80% Wmax	Relative	Cycling GXT TTE at Wmax	No difference No difference
Schmutz et al. (2010)	Untrained <i>M (6, 6)</i>	12.0% (<i>Normobaric</i>)	6 weeks (<i>cycling</i>)	5 per week; 30 min (<i>none</i>)	65% Wmax	Relative	Cycling GXT	Decreased
Ventura et al. (2003)	Cyclists <i>M+F (7, 5)</i>	12.7 % (<i>Normobaric</i>)	6 weeks (<i>cycling</i>)	3 per week; 30 min (<i>duration not reported</i>)	73-84% Wmax	Relative	Cycling GXT	No difference
Vogt et al. (2001)	Untrained <i>M (14, 16)</i>	12.7 % (<i>Normobaric</i>)	6 weeks (<i>cycling</i>)	5 per week; 30 min (<i>none</i>)	52-67% Wmax	Relative	Cycling GXT	No difference
Continuous hypoxic training + Interval hypoxic training								
Hamlin et al. (2010)	Cyclists <i>M (9, 7)</i>	SpO ₂ ~82-88% (<i>Normobaric</i>)	10 days (<i>cycling</i>)	7 per week; 91 min (<i>none</i>)	60%–70% HRR + 2 x 30 s max.	Relative	WAnT 20km TT	Improved No difference
Lecoultre et al. (2010)	Cyclists <i>M (7, 7)</i>	14.5% (<i>Normobaric</i>)	4 weeks (<i>cycling</i>)	3 per week; 66-100 min (<i>~400 min/week</i>)	~60-120% Wmax	Relative	Cycling GXT 40km TT	No difference No difference

Author(s) (year)	Subjects <i>M or F</i> (<i>H, N</i>)	FiO ₂ ^a H group (<i>normobaric or hypobaric</i>)	Days/weeks of training (<i>training modality</i>)	Number and duration H & control sessions (<i>additional N training</i>)	Training intensity	Absolute or relative intensity matched (<i>H vs. N</i>)	Performance measures	Normoxic performance (<i>H compared to N</i>)
Continuous hypoxic training + Interval hypoxic training (continued)								
Mounier et al. (2009) Roels et al. (2007a) Roels et al. (2007b)	Cyclists & triathletes <i>M (10, 8)</i>	13.1% (<i>Normobaric</i>)	3 weeks (<i>cycling</i>)	5 per week; 60-90 min (<i>duration not reported</i>)	60-100% W _{max}	Relative	Cycling GXT Cycling TT (10 min)	No difference No difference
Terrados et al. (1988)	Cyclists <i>M (4, 4)</i>	16.1% (<i>Hypobaric</i>)	3-4 weeks (<i>cycling</i>)	4-5 per week; 105-150 min (<i>none</i>)	60-130% W _{max}	Relative	Cycling GXT	No difference
Interval hypoxic training								
Dufour et al. (2006) Ponsot et al. (2006) Zoll et al. (2006)	Distance runners <i>M (9,6)</i>	14.5 % (<i>Normobaric</i>)	6 weeks (<i>running</i>)	2 per week; 39-55 min (<i>3 per week; ~83 min</i>)	77% & 88% of N VO ₂ max for H & N, respectively	Relative	TTE @ VO ₂ max	Improved
Morton and Cable (2005)	Team sport athletes <i>M (8, 8)</i>	15.1 % (<i>Normobaric</i>)	4 weeks (<i>cycling</i>)	3 per week; 30 min (<i>none</i>)	80% W _{max}	Absolute	Cycling GXT WAnT	No difference No difference
Roels et al. (2005)	Cyclists & triathletes <i>M (20, 8)</i>	13.1% (<i>Normobaric</i>)	7 weeks (<i>cycling</i>)	2 per week; 60 min (<i>none</i>)	90-100% W _{max}	Relative	Cycling GXT Cycling TT (10 min)	No difference No difference
Truijens et al. (2003)	Swimmers <i>M+F (8,8)</i>	15.3 % (<i>Normobaric</i>)	5 weeks (<i>swimming</i>)	3 per week; ~25 min (<i>≥ 3 per week</i>)	69-94% VO ₂ max	Relative	Swimming TT (100m and 400m)	No difference
Repeated sprint in hypoxia								
Faiss et al. (2013b)	Cyclists <i>M (20, 20)</i>	14.6 % (<i>Normobaric</i>)	4 weeks (<i>cycling</i>)	2 per week; 36 min (<i>not reported</i>)	Maximal repeated 10 s sprints (<i>active recovery</i>)	Maximal	Cycling RSA WAnT 3 min W _{max}	Improved No difference No difference
Galvin et al. (2013)	Rugby players <i>M (26 total)</i>	13.0 % (<i>Normobaric</i>)	4 weeks (<i>running</i>)	3 per week; 6 min (<i>not reported</i>)	Maximal repeated 6 s sprints (<i>passive recovery</i>)	Maximal	Yo-Yo IR1 20m RSA test 20m Sprint	Improved No difference No difference
Puype et al. (2013)	Untrained <i>M (10, 9)</i>	14.4 % (<i>Normobaric</i>)	6 weeks (<i>cycling</i>)	3 per week; 30-55 min (<i>none</i>)	80% maximal sprinting power (<i>active recovery</i>)	Absolute	Cycling GXT Cycling TT (~10 min)	No difference No difference

Author(s) [year]	Subjects <i>M or F</i> (<i>H, N</i>)	FiO ₂ ^a H group (<i>normobaric or hypobaric</i>)	Days/weeks of training (<i>training modality</i>)	Number and duration H & control sessions (<i>additional N training</i>)	Training intensity	Absolute or relative intensity matched (H vs. N)	Performance measures	Normoxic performance (H compared to N)
Resistance training in hypoxia								
Friedmann et al. (2003)	Untrained <i>M (10, 9)</i>	12.0 % (<i>Normobaric</i>)	4 weeks (<i>knee flex. & exten.</i>)	3 per week; 6 sets, 25 reps (<i>none</i>)	30% 1RM	Absolute	Isokinetic torque Isokinetic work during 50 reps	No difference No difference
Ho et al. (2014)	Untrained <i>M (18 total)</i>	15.0 % (<i>Normobaric</i>)	6 weeks (<i>dynamic squat</i>)	3 per week; 3 sets, 10 RM (not reported)	10 RM (approx. 75% 1 RM)	Relative	1 RM Squat Isometric torque Isokinetic Torque	No difference No difference No difference
Manimmanakorn et al. (2013a) + (2013b)	Netball players <i>F (10, 10)</i>	SpO ₂ ~80% (<i>Normobaric</i>)	5 weeks (<i>knee flex. & exten.</i>)	3 per week; 6 sets, ~30 reps (<i>not reported</i>)	20% 1RM	Absolute	MVC ₃ MVC ₃₀ Reps _{20%1RM}	Improved Improved Improved
Nishimura et al. (2010)	Untrained <i>M (7, 7)</i>	16.0 % (<i>Normobaric</i>)	6 weeks (<i>elbow flex. & exten.</i>)	2 per week; 4 sets, 10 reps (<i>none</i>)	70% 1RM	Absolute	10RM arm curl 10RM standing French press	No difference No difference

M = Male, F = Female, H = Hypoxic, N = Normoxic, TTE = Time to exhaustion, TT = time trial, WAnT = Wingate Anaerobic Test, GXT = graded exercise test to exhaustion, HR = heart rate, HRR = heart rate reserve, RSA = repeated sprint ability, Reps = repetitions, 1RM = one repetition maximum, MVC = maximal voluntary contraction, MVC₃ = 3 s maximal voluntary contraction, MVC₃₀ = 30 s maximal voluntary contraction, Reps_{20%1RM} = Number of Reps at 20% or one repetition maximum, W = Watts, Wmax = maximal W achieved during GXT, LT = Lactate Threshold, Yo-Yo IR1 = Yo-Yo Intermittent Recovery test level 1, La⁻ = Lactate, HRmax = maximum heart rate, VO₂max = maximum oxygen consumption, RM = repetition maximum, FiO₂ = fraction of inspired oxygen, SpO₂ = saturation of peripheral oxygen, flex. = flexion, exten = extension, approx. = approximately. a Except where stated otherwise, b Hendriksen and Meeuwsen (2003) is a crossover study involving 12 subjects who completed Meeuwsen et al. (2001) (one year wash-out period)

DISCUSSION

Within the current literature, the efficacy of LLTH for improving sea-level performance is unclear. Although rarely discussed, exercise mode and intensity are likely key factors in mediating the response to the LLTH program, with higher training intensities appearing to be more beneficial than sub-maximal workloads. LLTH also appears to have a greater impact on the performance of highly anaerobic tasks, such as short-term high-intensity work and maximal intermittent exercise (i.e. repeated sprint ability), and these benefits are more likely identified when performance tests are specific to the training modality/intensity performed during LLTH sessions.

Methodological considerations

The effect of hypoxia on training intensity and inclusion of normoxic training

Training in moderate hypoxic environments effectively limits the amount of energy that can be produced oxidatively during exercise, and it is well established that this reduction in oxidative energy production leads to a reduced exercise performance in both endurance (Buchheit et al., 2012a) and team sport (Garvican et al., 2014) athletes. Although absolute exercise intensity is reduced under hypoxic conditions, the reduced oxygen availability may produce a greater peripheral physiological stimulus than training in normoxia, and this is thought to be the major contributing factor leading to possible increases in performance following LLTH interventions. Whilst this peripheral physiological strain may be beneficial for subsequent athletic performance, there is undoubtedly a reduction in cardiovascular stress during hypoxic training sessions, which is directly related to the reduced exercise intensity (Buchheit, 2012). Indeed, maximal cardiac output and stroke volume are known to be reduced under acute hypoxic conditions (Calbet et al., 2009). As one of the major cardiovascular training adaptations is an increase in stroke volume, induced by invoking large training stroke volumes (Lepretre et al., 2004; Buchheit and Laursen, 2013), training in hypoxia appears to limit cardiovascular overload. Therefore, training in hypoxia alone will apparently limit training induced cardiovascular adaptations; thus, a mixture of training in hypoxia and normoxia seems more preferable than training in hypoxic environments only. Despite this, only six (Truijens et al., 2003; Ventura et al., 2003; Dufour et al., 2006; Ponsot et al., 2006; Zoll et al., 2006; Roels et al., 2007a; Roels et al., 2007b; Mounier et al., 2009; Lecoultre et al., 2010; Czuba et al., 2011) of the 31 studies examined in this review included some normoxic training for the hypoxic group. Furthermore, only two of these studies

prescribed well-periodised training programs in normoxia, with some portion of high-intensity interval training (Dufour et al., 2006; Ponsot et al., 2006; Zoll et al., 2006; Czuba et al., 2011), whilst the hypoxic groups in the other three studies completed only sub-maximal work under normoxic conditions (Truijens et al., 2003; Roels et al., 2007a; Roels et al., 2007b; Mounier et al., 2009; Lecoultre et al., 2010).

Matching relative or absolute training intensity

When interpreting results from LLTH studies, it is important to consider how training intensity was matched between hypoxic and normoxic training groups. A number of the LLTH studies within this review have aimed to match the absolute training intensity between these groups (Meeuwsen et al., 2001; Friedmann et al., 2003; Hendriksen and Meeuwsen, 2003; Morton and Cable, 2005; Nishimura et al., 2010; Mao et al., 2011; Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b; Puype et al., 2013). However, any group training in normoxia will have an increased exercise capacity when training, compared to individuals training in hypoxic conditions. Therefore, the matching of absolute training intensity will limit the training adaptations for the normoxic group and provide a higher relative training stimulus for those training in a hypoxic environment. This theory is supported by the work of Desplanches et al. (1993) who included two normoxic training groups matched for absolute and relative training intensity with the hypoxic group. These authors reported a significant increase in mitochondrial density following three weeks of training at 70-80% VO_2peak (relative to training condition) in the normoxic and hypoxic groups, respectively, but found no changes in the group training in normoxia with absolute workload matched to the hypoxic group. Thus, groups that train in normoxia at the same absolute intensity as in hypoxia are not likely to experience sufficient overload to induce the associated training benefits. For this reason, results from studies matching absolute training intensities between normoxic and hypoxic groups (Meeuwsen et al., 2001; Friedmann et al., 2003; Hendriksen and Meeuwsen, 2003; Morton and Cable, 2005; Nishimura et al., 2010; Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b; Puype et al., 2013) should be interpreted with caution, because any greater improvements in performance in the hypoxic group may be solely attributed to a higher relative training intensity.

The method of prescribing matched relative workloads during hypoxia needs to be carefully considered. Some CHT studies use a percentage of maximum heart rate (HR_{max}) (Bailey et al., 2000; Bailey et al., 2001a; Geiser et al., 2001; Beidleman et al., 2009) or heart rate reserve

(Meeuwsen et al., 2001; Hendriksen and Meeuwsen, 2003). Although heart rate is commonly used for prescription of training intensity, there is evidence to suggest that this is not an accurate method for matching relative intensities between hypoxic and normoxic conditions. Bailey et al. (2000) attempted to match relative intensity by having subjects train between 70-80% HRmax, as determined via a pre-test in a hypoxic or normoxic environment for the LLTH and control group, respectively. During training, there was no difference in average heart rate between these groups, suggesting that relative exercise intensity was matched. As the Wmax of the LLTH group was reduced by ~10% in hypoxia (Bailey et al., 2001a), this group should therefore be training at a lower absolute intensity than the normoxic group (to match relative workloads). However, there was no difference in power data from the training intervention between the hypoxic and normoxic groups (Bailey et al., 2000), meaning that both groups were actually training at the same absolute workload. While other studies use HRmax (Geiser et al., 2001; Beidleman et al., 2009) or heart rate reserve (Meeuwsen et al., 2001; Hendriksen and Meeuwsen, 2003) methods to prescribe relative training intensities in normoxia and hypoxia, they do not provide data on training power/velocity to allow determination of actual training intensity. But the results of Bailey et al. (2000) suggest that heart rate based methods are problematic when prescribing relative training intensities during LLTH sessions.

Training mode and intensity on performance outcomes

Training modality/intensity has been largely ignored when interpreting the effectiveness of LLTH protocols within the literature. The importance of exercise intensity as a key factor in modulating the response to LLTH has recently been highlighted (Millet et al., 2010; Millet and Faiss, 2012) where Millet and Faiss (2012) suggest that greater responses occur with maximal or near-maximal training interventions (e.g. RSH) compared with sub-maximal training protocols. Millet and Faiss (2012) also suggest that sub-maximal training intensities in the majority of LLTH studies may explain why many fail to demonstrate additional performance benefits when compared with similar normoxic training. While high-intensity RSH training appears to be a more desirable method for enhancing normoxic exercise performance compared with lower intensity training, and may be beneficial for team sport athletes (Millet and Faiss, 2012; Galvin et al., 2013), little attention has focused on high-intensity IHT and its effects on intermittent exercise performance (i.e. no IHT studies examined in the current review use a measure of high-intensity intermittent exercise performance). The principle of specificity, in relation to matching the training performed

and the performance tests, is also often overlooked when interpreting LLTH literature. For example, a number of studies have used training intensities of ~50-70% W_{max} , but have assessed performance outcomes with higher intensity exercise (e.g. time trial at 80% W_{max}) (Emonson et al., 1997; Debevec et al., 2010). This importance of testing specificity is highlighted in the work of Faiss et al. (2013b) who found greater improvements in the number of sprints completed to exhaustion by a hypoxic group following four weeks of repeated sprint training compared to a normoxic control group, but found similar improvements between groups in 30 s Wingate Anaerobic Test performance and no change in 3-min maximal exercise for either group.

Placebo and nocebo effects

The placebo effects of training in a ‘beneficial’ hypoxic environment should also be considered when interpreting results from LLTH studies. Similarly, nocebo effects may negatively influence the performance of control groups, if they are informed (or deduce) that they are not receiving the ‘beneficial’ treatment. In LLTH studies, this limitation can be overcome by having both experimental and control groups under the assumption that they are training in hypoxia. For example, Czuba et al. (2011) and Faiss et al. (2013b) achieved this by having their hypoxic and normoxic groups train in a hypoxic chamber with the hypoxic generator switched on, albeit simulating very different altitudes for each group. Additionally, Faiss et al. (2013b) informed all subjects, including those in the normoxic group, that all training sessions were being completed in hypoxia. Studies that report improved performance in the hypoxic training group without describing the steps taken to control for potential placebo/nocebo effects should be interpreted with caution. For example, Dufour et al. (2006) report improved running performance after six weeks of IHT, with the hypoxic treatment delivered ‘by breathing through face masks connected to a mixing chamber’. However, the authors make no mention of whether participants in the normoxic group also trained while connected to a face mask, or if they had knowledge that they were not receiving a hypoxic treatment. Future studies should carefully consider methodology to control for placebo/nocebo effects and be sure to carefully report these methods, so that the effects of the hypoxia *per se* can be interpreted more confidently.

Performance outcomes following LLTH interventions

Continuous hypoxic training (CHT)

Of the LLTH studies that only include CHT (i.e. no portion of IHT) and match relative training intensity between the hypoxic and normoxic groups, most report no additional benefit of training in hypoxia (Terrados et al., 1988; Emonson et al., 1997; Bailey et al., 2000; Bailey et al., 2001b; Geiser et al., 2001; Messonnier et al., 2001; Vogt et al., 2001; Kime et al., 2003; Ventura et al., 2003; Messonnier et al., 2004; Haufe et al., 2008; Beidleman et al., 2009; Debevec et al., 2010; Lecoultre et al., 2010; Wang et al., 2010). The reduction in cardiovascular function during hypoxic training sessions, which is directly related to the reduced absolute exercise intensity under hypoxic conditions (Buchheit, 2012), may explain the lack of aerobic-based performance improvements in these studies. Moreover, given that LLTH interventions are thought to induce primarily peripheral adaptations related to anaerobic capacity (i.e. carbohydrate metabolism; rate of glycolysis; pH regulation) (Zoll et al., 2006), the training intensities used in the majority of CHT studies may not have been sufficient to stimulate these adaptations and produce performance outcomes greater than matched normoxic training.

The outcomes of Hamlin et al. (2010) support this, reporting no change in 20 km time trial performance but a *likely* improvement in mean power during a 30 s Wingate Anaerobic Test. A distinguishing feature of this study is the inclusion of a small portion of IHT, which was not included in most other CHT studies. Specifically, while the participants in Hamlin et al. (2010) predominantly performed CHT (90 min per day at 60%–70% of heart rate reserve); they also completed 1 min of daily anaerobic-based IHT (2 x 30 s maximal efforts, separated by 5 min recovery). The only well controlled CHT study to show improvements in prolonged endurance based performance (Czuba et al., 2011) also presents a distinguishing feature to other CHT studies, in that their hypoxic group maintained a significant amount of training in normoxia, including high-intensity interval training. Alongside the increase in maximal workload in a graded exercise test following the CHT intervention, Czuba et al. (2011) also reported improvements in 30 km time trial performance, which suggests the necessity to include training in normoxia (to accompany CHT) in order to achieve a sufficient cardiovascular overload for aerobic adaptation.

Interval hypoxic training (IHT)

Of the eight studies in this review that include IHT, the majority report no change in performance following a hypoxic intervention (Terrados et al., 1988; Truijens et al., 2003; Roels et al., 2005; Roels et al., 2007a; Roels et al., 2007b; Lecoultre et al., 2010), while two report enhanced performance (Dufour et al., 2006; Hamlin et al., 2010) compared with matched controls. These differing outcomes might again be related to study design – specifically, the intensity of training and/or the performance measures used – with high-intensity training and short duration performance tests more likely to show a beneficial effect of the hypoxic stimulus.

The outcomes of these studies, when taken together, also extend the proposition raised in the previous section: that a mixture of training in hypoxia and normoxia is not only preferable to training in hypoxic environments alone, but that the intensity of the supplementary normoxic training is important if seeking improvements in performance. For example, Dufour et al. (2006) found an improvement in run time to exhaustion at $v\text{VO}_2\text{max}$ following six weeks of IHT in trained distance runners, with the hypoxic group performing three sessions per week of high-intensity training in normoxia (in addition to two IHT sessions). In contrast, a study by Roels et al. (2005) reported no change in mean power for a 10 min cycling time trial (performance of similar intensity and duration to that of Dufour et al. (2006)) following seven weeks of IHT in endurance cyclists and triathletes. However, no normoxic training by the hypoxic group was reported. Of the other studies that did report some portion of normoxic training in addition to IHT for their hypoxic group (Truijens et al., 2003; Roels et al., 2007a; Roels et al., 2007b; Mounier et al., 2009; Lecoultre et al., 2010), none reported performance improvements. But the additional normoxic training, as described in these papers, appears to be of low-moderate intensity only. Therefore, when implementing IHT, maintaining additional training in normoxia at high-intensity might be an important factor if the recognised reduction in cardiovascular function during hypoxia is to be overcome, eliciting performance improvements greater than those normoxic training alone.

The importance of the design (e.g. training intensity/volume) of supplementary normoxic sessions is highlighted within the literature. For example, Roels et al. (2007a) reported a 5.0% improvement in VO_2max for their normoxic training group of endurance-trained cyclists and triathletes, but no change (-0.3%) for their hypoxic group, after completing only low-moderate intensity supplementary normoxic sessions. After completing similar

additional normoxic training (i.e. low-moderate intensity), Lecoultre et al. (2010) reported changes in VO_2max of +7.4% and +1.4% for their normoxic and hypoxic training groups of well-trained cyclists, respectively (although this difference was $p = 0.12$). In contrast, with the provision of high-intensity training sessions in normoxia as accompaniment to IHT, Dufour et al. (2006) reported a 5% increase in VO_2max in their hypoxic group, with no change in the normoxic group. This suggests that the high-intensity normoxic training was important in achieving cardiovascular overload. Similarly, although Czuba et al. (2011) delivered a CHT intervention, high-intensity interval training was part of the supplementary normoxic exposures for their hypoxic group, and this group showed improvements in endurance performance and VO_2max . Therefore, from the data available, it appears that when IHT is being implemented, low-moderate training in normoxia is sufficient to maintain aerobic power, but high-intensity training of sufficient volume in normoxia would be necessary to drive aerobic improvements.

In summary, while the majority of well-controlled IHT studies report no additional benefit of the hypoxic stimulus, the limited literature available suggests that greater improvements with IHT might be more likely if the following criteria are followed: 1) high-intensity intervals are completed during the hypoxic exposures; 2) anaerobic rather than aerobic performance is measured; and 3) a sufficient intensity and volume of normoxic training accompanies IHT.

Repeated sprint training in hypoxia (RSH)

Traditionally, LLTH has targeted endurance-based athletes, but more recently, the effect of repeated sprint training in hypoxia on high-intensity exercise performance (Faiss et al., 2013b; Galvin et al., 2013; Puype et al., 2013) has gained attention. Faiss et al. (2013b) hypothesised that, compared with repeated sprint training in normoxia (RSN), RSH could induce beneficial adaptations at the muscular level, along with improved blood perfusion, which may lead to greater improvements in repeated sprint ability. These authors assessed 40 trained male cyclists completing four weeks of RSH or RSN (see table 6.1), and both groups significantly improved power output during repeated sprints post-intervention. However, only the RSH group delayed the onset of fatigue post-intervention, with subjects improving from 9 to 13 sprints until exhaustion, whilst the RSN group showed no such improvement. Despite this improved repeated sprint ability following RSH, improvement in

a single 10-s sprint and 30 s Wingate Anaerobic Test performance did not differ between RSH and RSN, while 3-min maximal exercise performance was not altered.

Similarly, Galvin et al. (2013) found that four weeks of maximal RSH training induced greater improvements in Yo-Yo IR1 performance in a group of elite rugby players compared with matched controls completing the same training under normoxic conditions. In contrast, Puype et al. (2013) found no additional benefit of RSH over RSN in untrained men completing a 10 min cycling time trial following four weeks of training. However, although we have classified Puype et al. (2013) as RSH, the authors refer to their training protocol as ‘repeated sprint interval training,’ with subjects completing longer duration (30 s), sub-maximal (~80% of the mean power output measured in the first sprint) sprints during training. The sub-maximal nature of these sprints may produce a different physiological response than maximal RSH training (Faiss et al., 2013b; Galvin et al., 2013). Furthermore, the performance measures in this study were continuous in nature (cycling GXT and 10 min time trial), thereby lacking specificity to the intermittent training stimulus.

Resistance training in hypoxia (RTH)

Recently, hypoxic environments have been proposed to enhance some of the adaptations associated with resistance training. Resistance training is known to improve maximal strength, power production and reduce fatigability through a number of adaptations, including hypertrophy (Schoenfeld, 2013) and altered motor recruitment patterns (McCaulley et al., 2009). More than a decade ago, a Japanese group investigated the effects of resistance training with simultaneous vascular occlusion (Takarada et al., 2000), and reported greater strength improvements in subjects using the occlusion technique (Takarada et al., 2000; Takarada et al., 2002). One of the proposed mechanisms related to these performance gains is local hypoxia (created by vascular occlusion) within the active muscle tissue (Takarada et al., 2002), which is a key component for the anabolic effects of resistance training (Schoenfeld, 2013). Thus, a number of groups have since investigated the effects of systemic hypoxia on resistance training adaptations (Friedmann et al., 2003; Nishimura et al., 2010; Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b; Ho et al., 2014).

Of the four RTH studies in this review, only one found greater performance improvements in the hypoxic group (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b) compared with training in normoxic conditions. Three of the four studies matched the

absolute workloads between hypoxic and normoxic groups, and used protocols involving ≥ 10 repetitions with sub-maximal workloads; primarily ranging from 20-30% 1RM (Friedmann et al., 2003; Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b), with one study examining the effects of training at 70% 1RM (Nishimura et al., 2010). As discussed in the section '*Matching relative or absolute training intensity*', matching absolute workloads provides a greater relative training stimulus in the hypoxic condition. Furthermore, these studies all prescribed the number of 'repetitions' for subjects throughout the training protocol, as opposed to using a set load and asking participants to lift until failure. This design suggests that both groups were training at sub-maximal intensities, but with the hypoxic group training at a higher relative intensity and experiencing a greater training stimulus. This different relative intensity of training may explain the improved performance reported for the hypoxic group in the study by Manimmanakorn et al. (2013a); Manimmanakorn et al. (2013b) and may also explain the greater hypertrophy seen in the hypoxic group of Nishimura et al. (2010). Furthermore, the absence of training to failure in Friedmann et al. (2003) suggests that their subjects were training sub-maximally and, thus, hypoxia did not stimulate additional adaptations; a contention supported by the absence of improved maximal strength in both of their training groups following the intervention period. One recent RTH study did attempt to address the limitation of matching absolute workload between the hypoxic and normoxic groups (Ho et al., 2014). During a six-week RTH intervention, Ho et al. (2014) initially matched normoxic and hypoxic training intensities based on normoxic 1 RM (75%). However, these investigators then increased the load when subjects successfully completed all of the prescribed loads in two consecutive training sessions. This may have lead to a greater increase in training load in the normoxic group, if exercise intensity was limited by the hypoxic environment. Unfortunately, training load data were not reported in this study, and it is therefore not possible to determine how the hypoxic stimulus may have affected training progression. Despite training volumes not being available, the RTH intervention appeared to have no performance effect in these untrained males. In summary, methodological flaws in RTH studies to date preclude any definitive conclusions about the effectiveness of LLTH for enhancing performance gains from maximal intensity resistance training programs.

PRACTICAL APPLICATIONS

The 'Live low-train high' model has the potential to contribute to a number of training adaptations, and these appear to be more related to anaerobic metabolism. Thus, LLTH interventions may have the greatest benefit for high-intensity, short-term and intermittent

performance (e.g. team sports). This is supported by the current LLTH literature that suggests performance improvements are more likely following high-intensity LLTH compared with sub-maximal training intensities while, similarly, evidence is equivocal for endurance benefits subsequent to LLTH.

In an applied setting, LLTH interventions can be difficult to implement given (1) hypoxic training rooms may have limited space available for training, or; (2) if athletes are connected to a hypoxic gas supply (i.e. no hypoxic room available), movement will be limited. The recent development of portable, inflatable hypoxic tents may overcome some of these limitations and provide a versatile alternative for practitioners wishing to implement LLTH in field settings (Girard et al., 2013). Delivering appropriate training intensities in hypoxic environments is also difficult, given the prescription issues surrounding some traditional measures of intensity (e.g. heart rate) and the reduced work capacity evident in hypoxia. Current literature may guide some exercise prescription in hypoxia (Buchheit et al., 2012a) if prescription is based on some measure of aerobic capacity (e.g. $\dot{V}O_2$ max, maximal aerobic speed, etc.). Indeed, Buchheit et al. (2012a) suggest that 5 x 90 s interval speed is decreased by ~6% in hypoxia (15.4% O_2) compared with that in normoxia. An alternative method may be to have participants perform maximally for the duration of the interval, as with current RSH methodologies (Faiss et al., 2013b; Galvin et al., 2013). Furthermore, given that training in hypoxia limits exercise intensity and training-induced cardiovascular stress, practitioners and researchers should include some portion of high-intensity normoxic training when designing programs that include LLTH, to ensure that all physiological systems are being overloaded.

With respect to resistance training, the available research (albeit limited) suggests that greater strength and hypertrophy gains are possible following sub-maximal resistance training in hypoxia compared with matched sub-maximal training in normoxia (Nishimura et al., 2010; Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b). While this may have applications for populations where the mechanical stress imposed during training needs to be limited (e.g. rehabilitation, elderly), evidence of the efficacy of RTH compared with well periodised maximal strength training in normoxia is lacking. Therefore, at this time, there is no support for the prescription of RTH for healthy athletes who are able to engage in traditional strength training programs, but this is an interesting area for future research.

CONCLUSION

The majority of LLTH literature reports no additional benefits of training under hypoxic conditions. However, much of this literature has used continuous, sub-maximal intensity training during hypoxic exposures, in an attempt to improve prolonged endurance performance. The majority of benefits following LLTH interventions appear to be more related to high-intensity, anaerobic performance, which may be more beneficial in short-duration, high-intensity athletic events and intermittent team sports. LLTH programs and studies should carefully apply the principles of training specificity, whilst considering that improvements are more likely following high-intensity, short-term and intermittent training (e.g. IHT and RSH), and should always include some portion of high-intensity training in normoxia, given that some physiological systems are limited under hypoxic conditions. While hypoxia may augment metabolic and neuromuscular adaptations associated with sub-maximal resistance training, it is not clear whether RTH induces greater adaptations than traditional (maximal) strength training programs. Therefore, RTH cannot be recommended for healthy athletes who are able to undertake traditional resistance training programs.

CHAPTER 7 – STUDY 3: A SELF-PACED INTERMITTENT PROTOCOL ON A NON-MOTORISED TREADMILL; A RELIABLE ALTERNATIVE TO ASSESSING TEAM SPORT RUNNING PERFORMANCE

This chapter outlines the development of a self-paced team sport running protocol, and assessment of its reliability with team sport athletes. This protocol was developed as a part of this candidature to offer an assessment tool of team sport locomotor activity. This was developed and was used to assess the impact of the interval hypoxic training intervention presented in Chapter 8 – Study 4.

PUBLICATION STATEMENT

This work was submitted to the *International Journal of Sports Physiology and Performance* in March 2014 and is currently under review.

ABSTRACT

PURPOSE. To assess the reliability of a ‘self-paced’ team sport running simulation on a Woodway Curve 3.0 non-motorised treadmill (NMT).

METHODS. Ten male team sport athletes (20.3 ± 1.2 y, 74.4 ± 9.7 kg, $\dot{V}O_{2\text{peak}} 57.1 \pm 4.5$ ml.kg⁻¹.min⁻¹) attended five testing sessions ($\dot{V}O_{2\text{peak}}$ testing + familiarisation of team sport running simulation; four reliability trials). The 30-min team sport protocol consisted of three identical 10-min activity blocks, with visual and audible commands to direct locomotor activity; however, actual locomotor speeds were self-selected by participants. Reliability of variables was estimated using typical error \pm 90% confidence limits expressed as a percentage [coefficient of variation (CV)]. The smallest worthwhile change (SWC), defined as the smallest change of practical importance, was calculated as $0.2 \times$ between participant standard deviation.

RESULTS. Peak and mean speed and distance variables assessed across the entire 30-min protocol exhibited a CV < 5%, and for each 10-min activity block a CV < 6%. All peak and mean power variables exhibited a CV < 7.5%, except walking (CV 8.3-10.1%). The most reliable variables were maximum and mean sprint speed for the entire trial (CV 1.8% and 1.9%, respectively). All variables analysed produced a CV% greater than the SWC.

CONCLUSIONS. An entirely self-paced, team sport running simulation performed on a curved NMT produces reliable data across a range of speed and distance variables. Importantly, this protocol required just one familiarisation session to achieve acceptable levels of reliability. Given the self-paced design, this type of protocol provides an ecologically valid alternative to externally-paced team sport running simulations.

INTRODUCTION

Running performance in team sports has been shown to influence overall team success (Mooney et al., 2011; Gabbett et al., 2013; Manzi et al., 2014). The activity profile within team sports consists of periods of high-intensity running, interspersed with lower intensity activity and/or complete rest (Brewer et al., 2010). Therefore, the physiological determinants of team sport running performance differ somewhat from traditional endurance exercise. As an alternative to more traditional endurance tests, a number of high-intensity intermittent performance tests have been developed to assess running performance specific to team sports (Bangsbo et al., 2008). While these tests provide greater specificity when testing team sport athletes, most do not incorporate the wide range of locomotor activities experienced in team sport competition (i.e., walking to sprinting). Furthermore, the majority of current high-intensity, intermittent running performance tests are externally paced (e.g. shuttle speeds guided by sound, running speeds guided by visual feedback), whereas locomotor speeds during team sport competition are determined by the individual athlete, dependent on game situations. The use of non-motorised treadmills (NMT)(Lakomy, 1987) has allowed for the development of simulated team sport running protocols that mimic team sport running (i.e., rapid speed changes) in a controlled environment in which different performance variables (e.g., speed, distance, power) can be systematically measured (Highton et al., 2012). Assessing the reliability of these protocols is an important consideration for researchers and practitioners in determining the smallest practically important change that may be detected following training interventions (Pyne, 2003; Sirotic and Coutts, 2008). Original NMT models (e.g., Woodway Force, Woodway, USA) require runners to wear a tether belt around the waist and be anchored behind, allowing them to overcome the inertia of the treadmill belt to perform locomotor activities. Recently, a curved NMT has been manufactured (Woodway Curve 3.0., Woodway, USA) allowing participants to complete locomotor tasks without being anchored via a waist tether. While this technology provides a promising tool to assess team sport specific running performance, the reliability of these measures collected on a Woodway Curve 3.0 NMT has not been reported. To date, all published, treadmill-based team sport running simulation protocols (Sirotic and Coutts, 2007; Sirotic and Coutts, 2008) use externally-paced movement velocities (e.g., percentage of maximal sprinting speed), or a very small portion of self-selected velocity (2.7% of total activity)(Aldous et al., 2013), in order to assess team sport specific running performance. As the self-paced nature of team sports may have a significant impact on movement strategies adopted throughout a game (Aughey, 2010), internally paced performance tests may provide a more ecologically valid assessment tool than externally paced alternatives. Although some partial or

completely self-paced, field-based team sport running tests exist (Williams et al., 2010; Ali et al., 2014), these do not allow for the detailed measurement of variables such as power output. Therefore, the purpose of this study was to assess the reliability of a self-paced team sport running protocol on the Woodway Curve 3.0 NMT. A secondary purpose was to assess the number of familiarisation sessions needed to produce reliable data.

METHODS

Ten amateur team sport athletes (20.3 ± 1.2 y, 74.4 ± 9.7 kg, $\dot{V}O_{2\text{peak}} 57.1 \pm 4.5$ ml.kg⁻¹.min⁻¹) were recruited to participate in this study. All participants were required to have an aerobic capacity ($\dot{V}O_{2\text{peak}} \geq 50$ ml.kg⁻¹.min⁻¹) (tested during the initial laboratory visit) and be currently competing or training in team sports [e.g., soccer, Australian Football, rugby, field hockey] at least three times per week. Participants were required to attend five testing sessions, involving an initial pre-test and familiarisation session, followed by four team sport running simulations (trials 1-4), each separated by one week. Prior to each laboratory visit, participants completed a 48 h food diary and were asked to refrain from any strenuous physical activity preceding the testing day. Participants were asked to follow the same diet (as recorded in initial food diary) and exercise routine for 48 h prior to subsequent laboratory visits. Laboratory conditions were constant (21.4 ± 0.7 °C; $44.6 \pm 2.9\%$ relative humidity) and each individual was tested at the same time of day to limit diurnal fluctuations in performance.

Non-motorised treadmill model

The treadmill used in the present study was a curved, non-motorised design (Woodway Curve 3.0, Woodway, USA). Unlike previous NMTs, the curved design allows for untethered running (Sirotic and Coutts, 2008). The static incline of the treadmill surface was set at 140 mm and 90 mm (distance from floor to the frame of the NMT) for the front and rear feet, respectively, per manufacturer specifications. The Curve 3.0 contains four load cells (on the left and right side at the front and rear of the treadmill belt) that measure vertical ground reaction force at 200 Hz, while treadmill belt speed is measured via photomicrosensors (Omron EE-SX670, Omron Corporation, Osaka, Japan) mounted on the running drum shaft. All data are collected and analysed through the manufacturer's software (Pacer Performance System, Innervations, Australia). The aforementioned software package calculates horizontal force using the formula: horizontal force = acceleration * (body mass

* belt friction), and power output was calculated via the product of horizontal force and horizontal displacement. Data were then exported to Microsoft Excel for detailed analysis of specific speed zones.

Protocol Development

Previous NMT-based team sport running simulations have been developed to replicate time-motion profiles of a number of team sports (e.g., soccer, rugby league, rugby union, Australian Football) (Sirotic and Coutts, 2007). These protocols achieve the desired activity profiles by prescribing running speeds based on percentage of maximal sprinting speed, requiring participants to match these speeds via visual feedback cues (Sirotic and Coutts, 2007; Sirotic and Coutts, 2008).

In contrast, the protocol in the current study used visual and audible commands to direct participant locomotor speed (i.e., ‘Stand Still’, ‘Walk’, ‘Jog’, ‘Run’, ‘Sprint’), however actual locomotor speeds were self-selected. Before commencing the protocol, participants were asked to follow visual and audible commands (as above) and were instructed that during ‘run’ periods they should be performing a ‘hard run’, as if pushing to the next contest within a game and to ‘sprint maximally’ during ‘sprint’ periods. This initial guidance was provided to assist participants in differentiating between the discrete speed categories. During the sprint periods, standardised verbal encouragement was provided by the investigator. No other encouragement or feedback was provided. Our performance protocol was designed to achieve mean running velocities above the Australian Football game mean ($\sim 125 \text{ m}\cdot\text{min}^{-1}$) (Wisbey et al., 2011), with the goal of creating significant physiological stress. Figure 1 shows a 10-min portion of the team sport running protocol, which was repeated three times during each trial to form a 30-min performance test. Each 10-min block was made up of 8 min of simulated ‘on-field’ activity and a 2-min period of low activity, to mimic an Australian Football interchange when the player is removed from the field of play. During these low activity periods, participants were permitted to consume water ad libitum. This duration of on-field activity and interchange period is typical of current Australian Football practices (Coutts et al., 2010). The three identical 10-min blocks allow for the assessment of changes during specific time points of the activity. Furthermore, the 30-min duration of the performance test (approximately a quarter of an Australian Football match, typically 4 x 30-min quarters) was deemed appropriate to assess changes in team sport

specific running performance, and has been utilised for a previous team sport running protocol (Sirotic and Coutts, 2008).

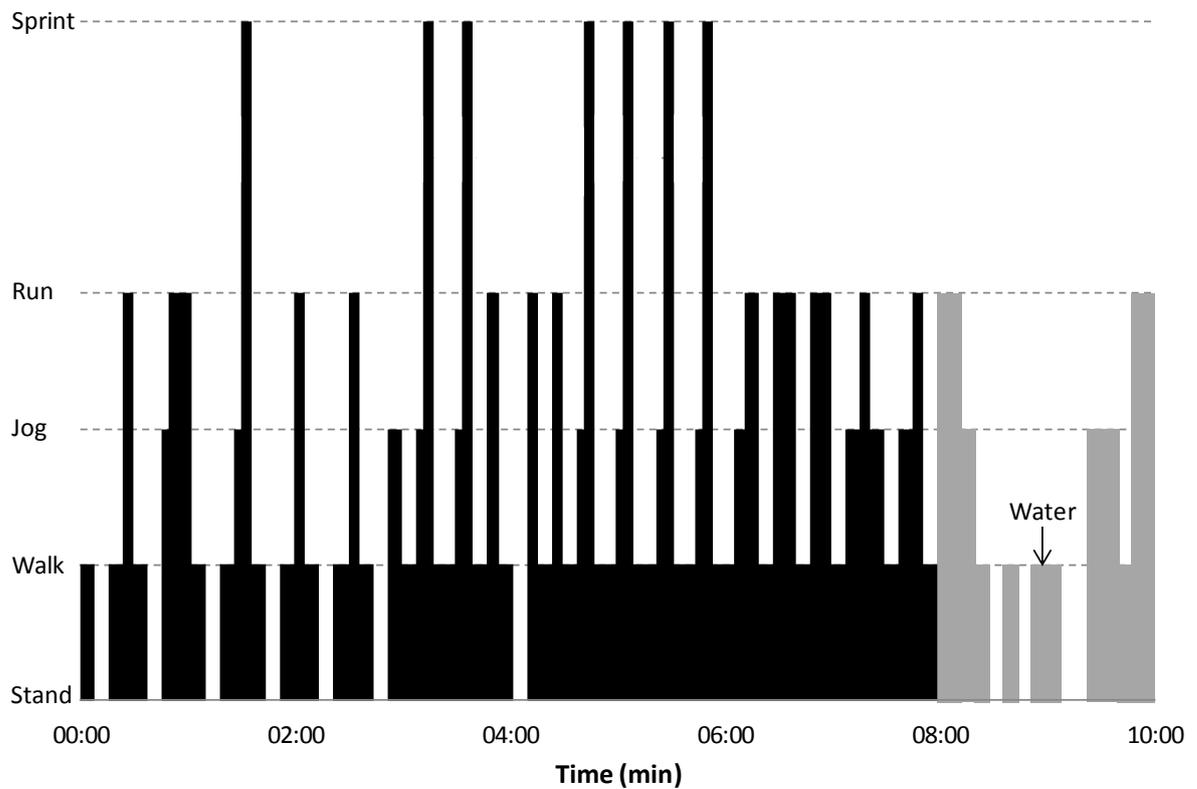


Figure 7.1. A ten minute portion of the self-paced match-simulation protocol. This 10-min period was repeated three times to make up the complete 30-min protocol. Participants self-selected their chosen running speeds. The area highlighted in grey depicts a period of ‘low’ activity, simulating a rest period (interchange) common in Australian Football. Participants were permitted to consume water during this period.

Testing Sessions

Visit 1 (pre-testing and familiarisation): Upon reporting to the laboratory, all participants underwent a standardised warm up, which involved 3 min of self-selected sub-maximal running on a NMT (Woodway Curve 3.0, Woodway, USA) before completing a sequence of dynamic stretches of the major muscle groups of the lower limbs. Participants then completed an incremental motorised-treadmill (Pulsar 3p, HP Cosmos, Nussdorf-Traunstein, Germany) run to exhaustion while being monitored via open-circuit spirometry (TrueOne 2400, Parvo Medics, Utah, USA) for assessment of $\dot{V}O_{2peak}$. The incremental test involved two 3-min stages at 8 and 12 km•h⁻¹ with a grade of 0%. Thereafter, speed was increased by 1 km•h⁻¹ every min to 18 km•h⁻¹, at which point speed remained constant and grade was increased by 2% every minute until volitional exhaustion. After completing the run to exhaustion, participants rested for ~10 min before returning to the NMT to complete an initial familiarisation of the 30-min team sport running simulation.

Visits 2-5 (reliability trials 1-4): Before completing trials 1-4, participants underwent the same standardised warm up as described above before performing a 3-min portion of the team sport simulation, which included one sub-maximal sprint. Participants then rested for 5 min, towel dried and obtained body mass (PW-200KGL, A&D Weighing, Kensington, Australia) wearing shorts only, before commencing the 30-min team sport running simulation.

Data Analysis

All variables were log transformed to reduce bias because of non-uniformity of error, and analysis was performed using a custom spreadsheet (Hopkins, 2011). Data were separated into locomotor zones for analysis of reliability, as defined by the speed commands described earlier, with designated standing periods removed from analysis. The inter-trial (e.g., Trial 1 v Trial 2) reliability of mean speed, mean/total distance and mean power output in all speed zones was estimated using the typical error \pm 90% confidence limits (CL) expressed as a percentage [coefficient of variation (CV)]. The smallest worthwhile change (SWC), defined as the smallest change of practical importance, was calculated as $0.2 \times$ the between participant standard deviation (SD). Variables were considered capable of detecting the SWC if $CV\% \leq SWC$ (Pyne, 2003). Reliability was also calculated for total, maximum, and mean distance, speed, and power output per zone, and between 10-min blocks.

RESULTS

Tables 7.1-7.4 display mean \pm SD, SWC, CV% \pm 90% CL, and percentage change in mean for distance and speed covered across each trial (Trials 1-4) and separated for 10-min blocks, respectively. All variables produced a CV% greater than the SWC.

Speed and Distance Reliability

The most reliable variables were maximum speed and mean sprint speed for the entire trial (CV 1.8% and 1.9%, respectively). The least reliable of all variables was the inter-trial jogging distance/mean speed of Block 3 (CV 5.7%). The range of CV% for all variables between trials 2-1 was 1.8 to 6.8%, similar to trials 3-2 (CV 1.8 to 4.9%) and 4-3 (CV 2.1 to 5.7%).

Power Reliability

Overall, mean power output during sprint periods was the most reliable power measure (CV 2.7%). Mean and between block power output during walking were the least reliable measures (range CV 8.3 – 10.1%). All other power output variables displayed a CV% < 7.5%.

Table 7.1. Reliability of speed and distance measures between trials.

	Trial					CV [(%) 90 CL]				Mean. % Change in Mean	Mean SWC (%)	Mean ICC [(%) 90 CL]
	1	2	3	4	Mean	2-1	3-2	4-3	Mean			
Total Distance (m)	3951 ± 365	3857 ± 402	3890 ± 379	3857 ± 357	3889 ± 380	2.5 (1.8-4.1)	3.0 (2.1-4.9)	2.6 (1.9-4.3)	2.7 (2.1-3.6)	-0.08	2.03	0.94 (0.88-0.98)
Max Speed (m.s ⁻¹)	7.6 ± 0.3	7.7 ± 0.3	7.8 ± 0.5	7.8 ± 0.5	7.7 ± 0.4	2.1 (1.5-2.4)	1.9 (1.4-3.2)	1.5 (1.1-2.4)	1.8 (1.5-2.5)	0.19	1.09	0.91 (0.81-0.97)
Mean Speed (m.min ⁻¹)	133.6 ±10.8	129.5 ± 12.9	129.4 ±12.8	128.6 ±11.9	130.3 ± 12.1	2.2 (1.6-3.6)	3.5 (2.5-5.8)	2.7 (2.0-4.5)	2.8 (2.3-3.8)	-0.14	1.96	0.93 (0.85-0.98)
Mean Sprint Speed (m.s ⁻¹)	5.9 ± 0.3	6.0 ± 0.3	6.1 ± 0.4	6.2 ± 0.4	6.0 ± 0.3	1.7 (1.3-2.9)	1.8 (1.3-3.0)	2.0 (1.4-3.3)	1.9 (1.5-2.5)	0.32	1.12	0.92 (0.82-0.97)
Mean Run speed (m.s ⁻¹)	3.3 ± 0.5	3.2 ± 0.5	3.2 ± 0.5	3.2 ± 0.5	3.2 ± 0.5	4.4 (3.2-7.3)	4.6 (3.3-7.7)	4.0 (2.9-6.6)	4.3 (3.5-5.9)	-0.08	3.34	0.94 (0.87-0.98)
Mean Jog speed (m.s ⁻¹)	2.5 ± 0.3	2.5 ± 0.4	2.6 ± 0.3	2.5 ± 0.3	2.5 ± 0.3	3.1 (2.3-5.2)	4.9 (3.6-8.2)	4.5 (3.3-7.6)	4.3 (3.4-5.8)	-0.05	2.54	0.91 (0.80-97)
Mean Walk speed (m.s ⁻¹)	1.7 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	4.3 (3.1-7.2)	3.3 (2.4-5.4)	3.0 (2.2-5.0)	3.6 (2.9-4.9)	-0.18	2.74	0.94 (0.87-0.98)

Data presented are mean ± SD for all variables, CV (with 90% CL), mean percent change in mean, mean SWC, and mean ICC (with 90% CL).

CV: coefficient of variation; CL: confidence limit; SWC: smallest worthwhile change; ICC: intraclass correlation coefficient.

Table 7.2. Reliability of distance (and speeds) between trials and activity blocks within trials.

Trial	Distance (m)					Speed (m·s ⁻¹)	CV (90 % CL)			Mean % Change in Mean	Mean SWC (%)	Mean ICC (90 % CL)	
	1	2	3	4	Mean	Mean	2-1	3-2	4-3				
Sprinting													
Block 1	249 ±14	251 ±13	258 ±17	260 ±15	254 ±15	6.1 ±0.4	3.2 (2.3-5.3)	2.5 (1.8-4.2)	2.8 (2.0-5.6)	2.8 (2.3-3.8)	0.30	1.2	0.81 (0.62-0.93)
Block 2	247 ±13	253 ±11	258 ±16	259 ±17	254 ±15	6.1 ±0.3	1.8 (1.3-2.9)	2.4 (1.8-4.0)	2.1 (1.5-3.5)	2.1 (1.7-2.9)	0.36	1.2	0.90 (0.78-0.96)
Block 3	245 ±14	249 ±16	255 ±17	258 ±15	252 ±16	6.0 ±0.4	3.8 (2.8-6.3)	2.1 (1.5-3.4)	2.4 (1.7-3.9)	2.8 (2.3-3.8)	0.31	1.2	0.83 (0.66-0.94)
Running													
Block 1	401 ±60	384 ±58	382 ±66	386 ±66	388 ±63	3.3 ±0.5	3.1 (2.2-5.1)	4.7 (3.4-7.8)	4.3 (3.1-7.1)	4.1 (3.2-5.5)	-0.10	3.3	0.95 (0.89-0.98)
Block 2	387 ±53	371 ±66	372 ±60	373 ±67	376 ±64	3.2 ±0.5	6.1 (4.4-10.3)	5.8 (4.2-9.7)	5.7 (4.1-9.6)	5.9 (4.7-8.0)	-0.09	3.5	0.91 (0.80-0.97)
Block 3	371 ±57	354 ±64	364 ±56	367 ±58	364 ±59	3.1 ±0.5	6.8 (4.9-11.5)	5.9 (4.3-9.9)	3.7 (2.7-6.2)	5.6 (4.5-7.7)	-0.03	3.5	0.91 (0.81-0.97)
Jogging													
Block 1	236 ±27	233 ±29	237 ±28	230 ±25	234 ±28	2.5 ±0.3	3.5 (2.6-5.9)	5.1 (3.7-8.5)	4.8 (3.5-8.0)	4.5 (3.6-6.1)	-0.08	2.4	0.89 (0.76-0.96)
Block 2	228 ±24	226 ±31	230 ±27	225 ±24	227 ±28	2.4 ±0.3	4.4 (3.2-7.4)	4.3 (3.1-7.2)	5.1 (3.7-8.5)	4.6 (3.7-6.3)	-0.05	2.5	0.89 (0.76-0.96)
Block 3	223 ±34	220 ±35	224 ±30	220 ±22	222 ±29	2.4 ±0.3	5.0 (3.6-8.4)	6.6 (4.8-11.1)	5.4 (3.9-9.0)	5.7 (4.6-7.8)	-0.02	2.9	0.87 (0.73-0.95)
Walking													
Block 1	462 ±49	449 ±53	443 ±53	435 ±54	448 ±54	1.7 ±0.2	5.7 (4.1-9.5)	3.5 (2.6-5.9)	2.9 (2.1-4.8)	4.2 (3.4-5.7)	-0.19	2.5	0.91 (0.80-0.97)
Block 2	444 ±56	429 ±57	427 ±54	411 ±50	428 ±54	1.6 ±0.2	4.2 (3.0-6.9)	3.2 (2.4-5.4)	3.2 (2.3-5.3)	3.6 (2.8-4.8)	-0.21	2.8	0.95 (0.88-0.98)
Block 3	422 ±53	403 ±59	409 ±60	401 ±62	409 ±60	1.6 ±0.2	5.8 (4.2-9.7)	5.2 (3.8-8.8)	4.6 (3.3-7.7)	5.2 (4.2-7.1)	-0.14	3.2	0.91 (0.80-0.97)

Data presented are mean ± SD for all variables, CV (90% CL) for both distance and speed, average percent change in mean, average SWC, and average ICC (90% CL). Also presented are mean ± SD speeds for each block. CV: coefficient of variation; CL: confidence limit; SWC: smallest worthwhile change; ICC: intraclass correlation coefficient.

Table 7.3. Reliability of power output between trials.

	Trial				Mean	CV [(%) 90 CL]				Mean % Change in Mean	Mean SWC (%)	Mean ICC (90 % CL)
	1	2	3	4		2-1	3-2	4-3	Mean			
Peak Sprint (W)	1041 ± 84	1044 ± 100	104 ± 129	1061 ± 149	1048.2 ± 118	2.1 (1.5-3.5)	5.7 (4.1-9.5)	8.9 (6.4-15.1)	6.2 (4.9-8.4)	0.05	2.27	0.55 (0.44- 0.74)
Mean Sprint (W)	291 ± 15	290 ± 16	290 ± 15	294 ± 17	291 ± 16	2.9 (2.1-4.7)	2.1 (1.5-3.5)	3.0 (2.2-5.0)	2.7 (2.2-3.6)	0.07	1.12	0.49 (0.39- 0.66)
Mean Run (W)	140 ± 21	132 ± 24	129 ± 24	128.7 ± 24	132 ± 23	5.1 (5.6-6.5)	5.6 (4.1-9.5)	6.5 (4.7-11.0)	5.8 (4.6-7.9)	-0.17	3.77	0.33 (0.26- 0.44)
Mean Jog (W)	109 ± 16	110 ± 18	113 ± 17	109 ± 15	110 ± 17	4.6 (3.4-7.7)	5.9 (4.3-9.9)	6.5 (4.7-10.9)	5.7 (4.6-7.8)	0.00	3.20	0.37 (0.30- 0.50)
Mean Walk (W)	40 ± 6	39 ± 6	38 ± 6	38 ± 6	39 ± 6	4.1 (3.0-6.8)	9.4 (6.8-15.9)	10.2 (7.3-17.3)	8.3 (6.6-11.4)	-0.12	3.55	0.49 (0.39- 0.66)

Data presented are mean ± SD for all variables, CV (90% CL), average percent change in mean, average SWC, and average ICC (90% CL).

CV: coefficient of variation; CL: confidence limit; SWC: smallest worthwhile change; ICC: intraclass correlation coefficient.

Table 7.4. Reliability of power output between trials and activity blocks within trials.

	Power (W)					CV (90 % CL)			Mean % Change in Mean	Mean SWC (%)	Mean ICC (90 % CL)	
	1	2	3	4	Mean	2-1	3-2	4-3				Mean
Sprinting												
Block 1	297 ± 18	301 ± 20	292 ± 23	303 ± 14	299 ± 19	3.6 (2.6-6.0)	5.9 (4.3-9.9)	5.4 (3.9-9.1)	5.1 (4.1-6.9)	0.15	1.33	0.76 (0.61-1.02)
Block 2	290 ± 16	290 ± 24	288 ± 17	291 ± 17	290 ± 19	4.7 (3.4-7.8)	4.6 (3.3-7.7)	4.6 (3.4-7.7)	4.6 (3.7-6.3)	0.06	1.33	0.69 (0.56-0.94)
Block 3	285 ± 17	278 ± 15	286 ± 14	288 ± 24	284 ± 18	4.3 (3.2-7.3)	4.8 (3.5-8.0)	3.2 (2.3-5.3)	4.2 (3.3-5.7)	0.22	1.29	0.64 (0.52-0.87)
Running												
Block 1	146 ± 25	137 ± 23	134 ± 24	133 ± 24	137 ± 24	4.4 (3.2-7.3)	4.9 (3.5-8.1)	9.0 (6.5-15.2)	6.4 (5.1-8.7)	-0.21	3.61	0.37 (0.30-0.50)
Block 2	139 ± 21	133 ± 26	128 ± 25	128 ± 25	132 ± 24	7.1 (5.2-12.0)	7.2 (5.2-12.1)	7.1 (5.2-12.0)	7.1 (5.7-9.8)	-0.17	4.02	0.38 (0.30-0.51)
Block 3	133 ± 24	125 ± 26	125 ± 24	126 ± 23	127 ± 24	7.5 (5.4-12.7)	7.4 (5.4-12.5)	5.1 (3.7-8.5)	6.8 (5.4-9.2)	-0.10	4.15	0.35 (0.28-0.47)
Jogging												
Block 1	112 ± 17	110.9 ± 17.0	116.0 ± 18.3	110 ± 14	112 ± 16	4.6 (3.3-7.7)	6.4 (4.6-10.8)	8.0 (5.7-13.4)	6.5 (5.2-8.8)	-0.06	3.08	0.44 (0.35-0.59)
Block 2	109 ± 14	110.6 ± 18.6	112.3 ± 16.8	110 ± 17	111 ± 17	5.7 (4.1-9.5)	5.6 (4.0-9.4)	7.2 (5.2-12.1)	6.2 (4.9-8.4)	0.02	3.23	0.40 (0.32-0.54)
Block 3	107 ± 18	108.8 ± 20.4	109.9 ± 18.0	108 ± 15	108 ± 18	6.8 (4.9-11.5)	8.3 (6.0-14.1)	6.5 (4.7-10.9)	7.3 (5.8-9.9)	0.03	3.59	0.42 (0.34-0.57)
Walking												
Block 1	41 ± 7	40 ± 7	40 ± 7	39 ± 6	40 ± 7	5.9 (4.3-9.8)	10.5 (7.5-17.8)	10.0 (7.2-17.0)	9.0 (7.2-2.3)	-0.12	3.80	0.49 (0.40-0.67)
Block 2	40 ± 7	39 ± 7	38 ± 6	37 ± 5	39 ± 6	5.4 (3.9-9.0)	10.6 (7.6-18.0)	10.9 (7.8-18.5)	9.3 (7.4-12.7)	-0.16	3.50	0.55 (0.44-0.74)
Block 3	38 ± 6	37 ± 7	36 ± 6	37 ± 6	37 ± 6	5.4 (3.9-9.1)	12.3 (8.8-21.0)	11.3 (8.1-19.3)	10.1 (8.0-13.8)	-0.08	3.83	0.55 (0.44-0.74)

Data presented are mean ± SD, CV (90% CL) for both distance and speed, average percent change in mean, average SWC, and average ICC (90% CL). CV: coefficient of variation; CL: confidence limit; SWC: smallest worthwhile change; ICC: intraclass correlation coefficient.

DISCUSSION

To our knowledge, this is the first study to assess the reliability of entirely self-paced team sport running, incorporating a spectrum of running intensities, on a Woodway Curve 3.0 NMT. Previous treadmill-based team sport running protocols utilise external pacing, by asking participants to achieve a prescribed percentage of maximal sprinting speed (Sirotic and Coutts, 2008; Aldous et al., 2013; Nedelec et al., 2013b) or a speed relating to a percentage of VO_2max (Nicholas et al., 2000). Some externally-paced team sport running simulations have been performed on motorised treadmills and, thus, are limited by the maximal speed of the treadmill (generally $25 \text{ km}\cdot\text{h}^{-1}$) and the inability for the treadmill to change speed quickly (Drust et al., 2000; Greig et al., 2006). The use of NMTs allows for more rapid speed changes and a maximal speed limited only by the athlete's ability. For this reason, research using NMTs has gained popularity to better emulate team sport running (Oliver et al., 2007; Sirotic and Coutts, 2008; Aldous et al., 2013; Nedelec et al., 2013a). Previous investigations have shown good reliability for distance covered in all speed bands (CV ~2-5%) (Sirotic and Coutts, 2008; Aldous et al., 2013) during NMT team sport running protocols. However, all speeds were externally paced; therefore, good reliability for distance covered is not unexpected. In the present work with a self-paced running protocol, we report similar reliability for the distance variables (see table 7.2, mean CV < 6%), highlighting the ability for athletes to repeatedly 'self-select' a consistent locomotor pace based on simple instruction. A recent study which incorporated periods of variable running distance (i.e., self-paced) during a soccer-specific NMT simulation (Aldous et al., 2013) reported higher reliability (CV 1.4%) in comparison to the 'running' periods of our study (mean CV 4.4%). However, the variable running distance accounted for only 2.7% of the entire protocol, while the entire team sport running simulation in the present study was self-paced.

Although previous research using team sport running simulation protocols on a NMT recommends a minimum of two familiarisation sessions (Sirotic and Coutts, 2008; Aldous et al., 2013; Nedelec et al., 2013b), our data indicate that participants were familiarised following trial 1, with CV < 5% across all speed/distance variables (Table 7.1) between trials 1 and 2. Mean CV% for maximal and mean sprint speed, potentially the most difficult movement speed to complete on the NMT, was the lowest for any variable measured (CV 1.8% and 1.9%, respectively). This compares well with other externally paced team sport running simulations performed on a NMT, which present maximal sprinting speed reliability of CV ~1.3% (Sirotic and Coutts, 2008) and CV 4.5% (Aldous et al., 2013). Furthermore, the reliability obtained in

a specific repeat sprint test ranged from CV 0.8 to 1.5% (Spencer et al., 2006), which also compares well to the present work.

All speed/distance variables assessed in this study demonstrated high reliability, exhibiting CVs < 6%. All power output variables, except walking, returned CVs < 7.5%. However, no variables were capable of detecting the SWC (i.e., CV% > SWC). Our analysis also shows high reliability for total distance (CV 2.7%). In comparison, a 60-min self-paced test on a motorised treadmill with trained runners presented similar reliability for total distance (CV 2.7%) (Schabert et al., 1998). Similarly, trained female cyclists performing a 60-min cycle-ergometer test demonstrated a CV of 2.7% for mean power output across the whole test (Bishop, 1997). As speed is not generally measured during ergometer cycling, power output in this instance provides a surrogate for speed, as the two are very closely related in a controlled environment (Pugh, 1974). Importantly, these two comparative studies did not require changes in speed as demanded in the present study. This indicates that, even with changes in speed during a self-paced team sport running simulation protocol, athletes are able to consistently repeat their performance across testing sessions.

The CV for mean power output (2.7%) across the 6-s sprints within the team sport running protocol was the most reliable power measure, while peak power output, and mean running/jogging and peak sprint power were all similar (CV ~6%). Previous research assessing peak power reliability on an NMT has reported CVs of 7.9% (Oliver et al., 2007) and 9.0% (Sirotic and Coutts, 2008). However, the latter study analysed sprinting reliability via a separate peak sprint test, while the former, as in the present study, assessed sprinting reliability throughout the entire protocol. The CV%, coupled with the SWC, can be used to estimate sample sizes required for prospective studies using the equation proposed by Hopkins (2000): $N \approx 8 \times CV^2/d^2$ where $d = SWC$. For example, to detect a SWC of 2% in total sprint distance requires a sample size of 23, while peak power output (SWC = 2.27%) would require 60 participants. Previous research using an externally paced protocol on an NMT (Oliver et al., 2007) calculated required sample sizes of 13 and 56 for the above variables, respectively, using the same methods.

This curved NMT belt differs from the flat belt, tethered version in previous team sport running simulations (Woodway Force, Woodway, USA) (Oliver et al., 2007; Sirotic and Coutts, 2008; Aldous et al., 2013; Nedelec et al., 2013a) and may alter running ergonomics when compared to overground running. However, to date, no research has assessed potential changes in running

ergonomics on the Woodway Curve 3.0 NMT. A further limitation to the current protocol is the lack of team sport specific actions (i.e., jumping, changing direction, kicking, etc.) (Magalhães et al., 2010; Nedelec et al., 2013a). This limitation may be overcome in future NMT protocols by performing some of these activities off the treadmill; for example, subjects could dismount the treadmill to perform a series of jumps, kicks, tackles, etc.

CONCLUSION

This work shows that a team sport running simulation protocol that is entirely self-paced presents reliability similar to that of externally-paced team sport running simulations. Moreover, with as little as one familiarisation session on the Woodway Curve NMT, team sport athletes can reliably reproduce self-selected distances/speeds across a range of locomotor commands. Given the self-paced nature of the protocol in the present study, this and similar self-paced curved NMT protocols may provide a more ecologically valid, laboratory-based performance test than externally-paced alternatives.

PRACTICAL APPLICATIONS

Self-paced team sport running simulations on a curved NMT that closely match the locomotor demands of competition deliver reliable test-retest measures of speed, distance and power. Thus, self-paced protocols may provide greater ecological validity than externally-paced treadmill protocols. Therefore, this protocol can be used to assess match-like activity in a controlled environment whilst also allowing for collection of other physiological variables, which may assist in the development of ecologically valid intervention studies. Moreover, such protocols may be sensitive to changes in pacing following an intervention that cannot be detected during externally-paced tests.

CHAPTER 8 – STUDY 4: CHANGES IN RUNNING PERFORMANCE FOLLOWING FOUR WEEKS OF INTERVAL HYPOXIC TRAINING IN AUSTRALIAN FOOTBALLERS: A SINGLE BLIND, PLACEBO CONTROLLED STUDY

PUBLICATION STATEMENT

This work is ready for submission to *PLoSone* journal. Submission to this journal is planned immediately following acceptance of Study 3 (NMT reliability) for publication.

ABSTRACT

There is a paucity of data examining the impact of high-intensity interval training in hypoxia (IHT) on intermittent running performance. This study assessed the effects of IHT on 17 amateur Australian Footballers, who completed eight interval running sessions [IHT ($F_{I}O_2 = 15.1\%$) or placebo (PLA)] over four weeks, in addition to normoxic football (2/wk) and resistance (2/wk) training sessions. To match relative training intensity, IHT sessions were reduced by 6% vVO_2 peak compared with PLA. Before and after the intervention, participants' performance was assessed by a Yo-Yo intermittent recovery test level 2 (Yo-Yo IR2) and a self-paced team sport specific running protocol. Compared with PLA, IHT participants experienced: (a) smaller improvements in Yo-Yo IR2 performance [D = -0.42 (-0.82;-0.02; 90% confidence interval); (b) similar increases in high-intensity running distance during the team sport protocol [D = 0.17 (-0.50;0.84)]; and (c) greater improvements in total distance [D = 0.72 (0.33;1.10)] and distance covered during low-intensity activity [D = 0.59 (-0.07;1.11)] during the team sport protocol. The lower absolute training intensity of IHT may explain the smaller improvements in Yo-Yo IR2 performance in the hypoxic group. Conversely, the data from the self-paced protocol suggest that IHT may influence pacing strategies in team sport athletes. In conclusion, IHT may offer some performance benefits to team sport athletes, however, the inclusion of structured high-intensity normoxic running sessions (for IHT subjects) may be necessary to achieve optimal performance outcomes.

INTRODUCTION

In recent years, the development of techniques that create a normobaric hypoxic environment has led to interest in performance benefits that may be induced by intermittent training bouts in hypoxic conditions, while living in a normoxic environment [Live Low, Train High (LLTH)]. Much of the current literature examining the performance effects following LLTH interventions relates to endurance performance (McLean et al., 2014). However, many of the physiological adaptations proposed occur following LLTH protocols are related to anaerobic metabolism (Faiss et al., 2013b), suggesting that this type of training may be beneficial for performance with a high anaerobic demand.

A number of studies have found improvements in high-intensity intermittent exercise following LLTH interventions in both endurance (Faiss et al., 2013b) and team sport athletes (Galvin et al., 2013). However, improvements in high-intensity exercise performance are not universal (Messonnier et al., 2004; Roels et al., 2005; Roels et al., 2007a; Puype et al., 2013), and it appears that factors relating to the specificity and intensity of hypoxic training, as well as the volume and intensity of associated normoxic exercise (during LLTH interventions), are important in mediating performance outcomes (McLean et al., 2014). Indeed, a wide range of training intensities exist within the LLTH literature (Millet et al., 2013; McLean et al., 2014) and the differences in these training intensities are proposed as key factors in determining the impact of the LLTH intervention (Millet and Faiss, 2012).

The importance of training intensity during LLTH protocols may explain why the majority of continuous hypoxic training and interval hypoxic training (IHT) studies report no additional performance benefits for hypoxic training groups (McLean et al., 2014). Recently, a number of authors have reported beneficial effects of repeated sprint training in hypoxia (RSH) on high-intensity intermittent exercise performance (Faiss et al., 2013b; Galvin et al., 2013; Brocherie et al., 2015). For example, Galvin et al. (2013) reported improvements in Yo-Yo IR1 (Bangsbo et al., 2008) performance following four weeks of RSH in well-trained rugby players, and Faiss et al. (2013b) found improvements in repeated sprint (cycling) performance following a RSH intervention with moderately trained cyclists. Completing a combination of in-season high-intensity intermittent training, repeated sprint/agility and explosive strength/change of direction training in hypoxia has also recently been reported to

enhance improvements in repeated agility, maximal sprinting speed (10-40 m) and countermovement jump performance in youth soccer players (Brocherie et al., 2015). Although RSH interventions appear promising for improvements in team sport specific performance (Faiss et al., 2013b), the implementation of running-based RSH are often not practically feasible. This is because, ideally, RSH requires hypoxic training rooms that are equipped with non-motorised treadmills or the use of rooms or tents (Girard et al., 2013) that have sufficient space to complete overground repeated sprint or agility training (Brocherie et al., 2015). In contrast, IHT programs that can be implemented on motorised treadmills may be more accessible for running-based team sport athletes. To our knowledge, there are no studies examining the effect of IHT on high-intensity intermittent exercise performance, with IHT studies to date only assessing normoxic exercise performance using continuous exercise tests (McLean et al., 2014).

Therefore, the aims of this study were to assess the effects of four weeks of IHT on: (i) externally-paced high-intensity intermittent running performance, and (ii) self-paced performance during a team sport specific running protocol. We hypothesised that IHT would lead to greater improvements in high-intensity running performance and in distance covered during the self-paced a team sport protocol.

METHODS

Participants

Twenty-one amateur Australian Footballers (23 ± 3 y, 184 ± 8 cm, 81 ± 9 kg) were recruited to participate in this randomised controlled trial. Participants were required to be currently training for Australian Football at least three times per week. Participants were randomly assigned to an interval hypoxic training (IHT; $n=11$) or placebo (PLA; $n = 10$) training group. Four participants (IHT $n = 2$, PLA $n = 2$) from this initial group withdrew from the study before its completion due to injury. All training and testing took place during the Australian Football pre-season period in February and March, following a four-week period of preparatory training. This preparatory training involved participants completing two football/running and 1-4 resistance (depending on individual history) training sessions per week; this training was unsupervised and compliance was self-reported by the participants.

All participants provided written informed consent to participate in this study, which was approved by the Human Research Ethics Committee at Australian Catholic University.

Study outline

During the four-week intervention, participants completed two IHT (or PLA), two resistance training, and two football training sessions (see Table 8.1 for details). The combination of football, resistance and running sessions was chosen in an attempt to replicate the typical pre-season training routine of Australian Football players. All IHT, PLA and resistance training sessions were prescribed and supervised by the investigators, while football training sessions were prescribed by team coaches. All training was monitored via the session rating of perceived exertion (RPE) method (Foster, 1998), which calculates a total load (arbitrary units [AU]) by multiplying the session-RPE [Borg's category ratio 10-scale (Borg, 1998)] by the session duration, which is a valid method to quantify training loads in Australian Footballers (Scott et al., 2013). Additional measurements of training load and intensity were used during running sessions [i.e. running volumes (GPS or treadmill data); mean session heart rate (HR); see below for details].

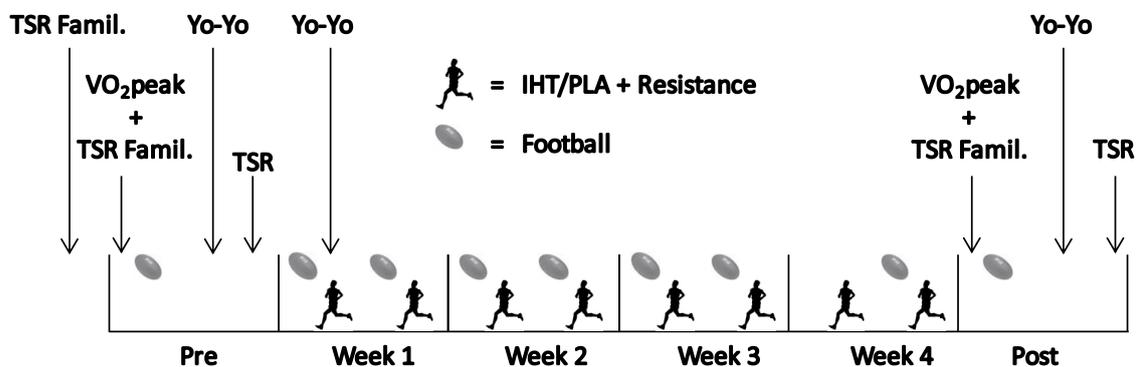


Figure 8.1. Data collection and training intervention timeline.

TSR = team sport running protocol; Famil. = familiarisation; VO₂peak = treadmill run to exhaustion for assessment of VO₂peak; Yo-Yo = Yo-Yo intermittent recovery test, level 2; IHT/PLA + resistance = supervised training session including running (under hypoxic or placebo conditions) and resistance training; Football = football training session, as prescribed by team coaches.

Blinding procedure: To minimise placebo and nocebo effects, all participants were informed that they were training at a simulated altitude of 3,000 m at the beginning of the study. During IHT and PLA training sessions, ducted air conditioning was continuously running

for both groups, to control temperature and create airflow through the training room. During IHT sessions only, target oxygen concentration was set to 14.8%, and was continuously monitored via two wall-mounted oxygen sensors (SmarTox-I; The Canary Company, Lane Cove, NSW, Australia). At the conclusion of the post-testing, participants were informed that one group actually trained ‘near sea-level’ and at this time were asked if they believed they were training ‘near sea-level’ or at ‘altitude; 44% IHT and 50% PLA participants believed they were training at ‘altitude.’ All investigators were blinded to the allocation of groups, except for the principal investigator, who set the oxygen concentration of the training room and calculated individual training speeds based on percentage of the peak velocity achieved during a $\dot{V}O_{2\text{peak}}$ test ($v\dot{V}O_{2\text{peak}}$; see Table 8.1).

Hypoxic or placebo training: During the four-week training block, participants completed eight IHT or PLA training sessions. All training sessions were ~30 min duration and consisted of 30 s to 3 min intervals of running on a motorised treadmill (Cybex 750T, Cybex, USA). All participants trained in a room (dimensions: 11.8 x 12.4 m) connected to two hypoxic generators (Altitude Training Systems, Lidcombe, NSW, Australia). Training intensity was set between 90% and 110% of $v\dot{V}O_{2\text{peak}}$ (see below for calculation) for normoxic participants. Based on previous investigations (Buchheit et al., 2012a) and pilot testing with the current training protocols, training intensity in the IHT group was reduced by 6% compared with the PLA group; that is, training intensities between 84% and 104% $v\dot{V}O_{2\text{peak}}$ for IHT, in order to match relative training intensity between groups. In addition to session-RPE measurements, training intensity during the final two weeks of the intervention was monitored via a team-based HR monitoring system (Polar Team, Pursuit Performance, Adelaide, Australia). Mean session HR was then calculated using commercially available software (Polar Precision Performance 4.03, Pursuit Performance, Adelaide, Australia) and a percentage of heart rate reserve [HRR (Karvonen et al., 1956)] was calculated using the following formula: $(HR_{\text{train}} - HR_{\text{rest}}) / (HR_{\text{max}} - HR_{\text{rest}})$, where HR_{train} = average HR during training, HR_{rest} = resting HR and HR_{max} = maximal HR. Maximal HR was recorded as the peak value achieved during pre- and post- incremental treadmill test runs to exhaustion or Yo-Yo IR2 tests. Due to logistical challenges (i.e. investigators available to prepare and fit HR monitors and lost data files during collection), full data sets for training HR were only available for 12 participants (IHT n = 8, PLA n = 4). If in a training session, participants voluntarily dismounted the treadmill during an interval (due to fatigue), they were provided a 10 s rest before re-commencing the interval to completion.

Resistance training: During the intervention period, all participants participated in two resistance training sessions per week supervised by the researchers. Resistance training sessions were ~60 min duration and comprised of 4-5 compound exercises of the upper limbs (e.g. barbell bench press, barbell bench pull, dumbbell press, dumbbell row) and 2-3 compound exercises of the lower limbs (e.g. leg press, dumbbell step-ups). These programs followed a linear progression over the four weeks from moderate volume/moderate intensity (e.g. 4 sets, 10 repetitions each exercise) to low-moderate volume/moderate-high-intensity (e.g. 4 sets, 6 repetitions each exercise).

Football training: Two football training sessions per week (~90 min duration) were prescribed by team coaches. All 17 participants completed 7 football sessions throughout the four-week intervention, totalling 119 football sessions, of which 92 were monitored via global positioning system (GPS) technology [Catapult MinimaxX (n = 11) or Catapult MinimaxX S4 (n = 6) units; Catapult Innovations, Melbourne, Australia]. Total distance, as well as distance below (“low-intensity running”) and above (“high-intensity running”) 84% vVO_{2peak} , was calculated. The threshold of 84% vVO_{2peak} for low- and high-intensity running was chosen because all running completed during IHT or PLA sessions was performed \geq 84% vVO_{2peak} . Therefore, this threshold allowed for direct comparisons between the volume of high-intensity running during football training and during IHT (or PLA) sessions.

Performance testing

Peak oxygen consumption (VO_{2peak}): During the second visit to the laboratory, participants completed an incremental run to exhaustion on a treadmill (Pulsar 3p, HP Cosmos, Nussdorf-Traunstein, Germany) while monitored via open-circuit spirometry (TrueOne 2400, Parvo Medics, Utah, USA) for assessment of VO_{2peak} . A run to exhaustion test was used as described previously (Buchheit et al., 2012a), which involved initial speed being set to $10 \text{ km}\cdot\text{h}^{-1}$ with a grade of 0%. Thereafter, speed was increased by $1 \text{ km}\cdot\text{h}^{-1}$ every min until volitional exhaustion. If the last stage was not fully completed, the peak treadmill speed was calculated using the following formula: peak treadmill speed = $S_f + (t/60 \times 0.5)$, where S_f was the last completed speed in $\text{km}\cdot\text{h}^{-1}$ and t is the time in seconds of the uncompleted stage (James et al., 2002; Buchheit et al., 2012a). After completing the run to exhaustion, participants rested for ~10 min before completing an initial familiarisation of the 30-min team sport running protocol on a non-motorised treadmill.

Team sport running protocol: A previously reported team sport running protocol was used to assess self-paced, sport-specific running performance (see Chapter 7). Briefly, this protocol involves participants completing 30 min of self-paced, intermittent running on a non-motorised treadmill (Woodway Curve 3.0., Woodway, USA). The protocol uses visual and audible commands to direct a participant's movement category (i.e. 'Stand Still', 'Walk', 'Jog', 'Run', 'Sprint'). Before commencing the protocol, participants were asked to follow visual and audible commands (as above) and instructed that during 'run' periods they should be completing a 'hard run, as if pushing to the next contest within a match', and during 'sprint' periods to 'sprint maximally'. Standardised verbal encouragement was provided by the investigator during the sprint periods. No other encouragement, verbal or visual feedback was provided (i.e. participants had no knowledge of speeds/distances or time elapsed). The team sport specific running protocol was designed to achieve mean running velocities above the Australian Football game mean [$\sim 125 \text{ m}\cdot\text{min}^{-1}$ (Wisbey et al., 2011)] with the goal of creating significant physiological stress representing a similar running intensity that may be experienced during a 30 min 'worst case scenario' period of an Australian Football game (Brewer et al., 2010; Wisbey et al., 2011). In the two weeks prior to the study, participants underwent two familiarisation sessions on the non-motorised treadmill, where 20 min and 15 min of this protocol were completed, respectively. This protocol has previously been shown to be reliable after one familiarisation session (see Chapter 7).

Before completing the team sport running protocol, participants underwent a standardised warm up which involved 3-min of sub-maximal treadmill running at a self-selected speed, followed by a sequence of dynamic stretches of the major muscle groups of the lower limbs, and a 3-min portion of the team sport protocol, which included one sub-maximal sprint. Participants then rested for ~ 5 min and their body mass was obtained (PW-200KGL, A&D Weighing, Kensington, Australia) wearing shorts only, before commencing the 30-min team sport running protocol.

Statistical Analysis

A contemporary analytical approach involving magnitude-based inferences (Hopkins et al., 2009) was used to detect small effects of practical importance. All data were log-transformed for analysis to account for non-uniformity of error. The differences within and between groups for the changes from Pre to Post intervention were assessed with dependent and

independent t tests for unequal variance (Hopkins et al., 2009). The magnitudes of change (Δ) were assessed in relation to the smallest worthwhile change (SWC), where a small effect size ($0.2 \times$ between participants standard deviation at Pre for both groups pooled) was used for all variables. For all performance variables, baseline measurements were used as a covariate in the analysis of changes, to account for different responses that may result from initial exercise capacity. Changes in the IHT group (Δ IHT) were termed greater, similar, or smaller than changes in the PLA group (Δ PLA). These differences were assigned a qualitative descriptor according to the likelihood of the difference exceeding the SWC as follows: 0% to 49%, *trivial*; 50% to 74%, *possible*; 75% to 94%, *likely*; 95% to 99%, *very likely*; and $>99\%$, *almost certainly* (Hopkins et al., 2009). Effects where the 90% confidence interval overlapped the positive and the negative thresholds simultaneously were deemed *unclear*. Standardised effect size statistics were calculated using Cohen's D, to allow for comparisons across different running performance measures which exhibit different levels of normal variability. Effects of 0.2, 0.5 and > 0.8 were considered small, moderate and large, respectively.

RESULTS

Training load

Total training distances are displayed in Figure 8.2A. There were *unclear* differences in overall or high-intensity ($\geq 84\%$ $v\text{VO}_2\text{peak}$) weekly distance covered between the two groups during football and during IHT or PLA training sessions. Likewise, there were *unclear* differences in weekly IHT/PLA, resistance, football or overall session-RPE training load between groups (see Figure 8.2B).

Training intensity

The session RPE was similar between groups for each of the eight treadmill based running sessions; that is, all differences between groups were *unclear*. Mean training HRR was *likely* higher in the PLA group during sessions 3.1 (Cohen's D \pm 95% Confidence Interval = 0.69 ± 0.91), 3.2 (1.93 ± 1.16) and 4.1 (0.67 ± 1.01), as well as *possibly* higher in the PLA group during session 4.2 (D = 0.46 ± 0.62).

Running performance

VO₂peak: There were *unclear* differences between IHT and PLA in VO₂peak at baseline. Improvements in VO₂peak and running performance following the training intervention are summarised in Table 8.2. All participants experienced a *possible* increase in VO₂peak ($D = 0.26 \pm 0.17$), with *unclear* differences between groups. Time to exhaustion was *possibly* greater in IHT (617 ± 91 s) versus PLA (581 ± 64 s) at baseline. Time to exhaustion during the VO₂peak test *almost certainly* increased ($D = 0.56 \pm 0.14$), with *trivial* differences between groups.

Yo-Yo IR2: There were *unclear* differences between IHT and PLA in Yo-Yo IR2 performance at baseline. All participants *almost certainly* experienced increases in Yo-Yo IR2 performance ($D = 0.88 \pm 0.23$), but these increases were *likely* smaller ($D = -0.42 \pm 0.40$) in the IHT group than in the PLA group.

Team sport running protocol: At baseline, walking, jogging, running, sprinting and total distance covered during the team sport running protocol were all *likely* greater in IHT (total = $4,565 \pm 496$ m) versus PLA (total = $4,258 \pm 254$ m). Pooled data for both groups showed a *likely* increase in total distance covered during the team sport protocol ($D = 0.47 \pm 0.28$), with these increases *very likely* greater in the IHT group than the PLA group ($D = 0.72 \pm 0.39$). For both groups combined, there was a *very likely* increase in sprint ($D = 0.57 \pm 0.31$) and run ($D = 0.63 \pm 0.30$) distance and a *likely* increase in jog ($D = 0.42 \pm 0.29$) distance. IHT participants experienced a *likely* increase in walking distance ($D = 0.72 \pm 0.35$), while the corresponding changes were *unclear* in the PLA group ($D = -0.22 \pm 0.41$).

Table 8.1. Training distance, session rating of perceived exertion (sRPE) and training heart rate reserve (HRR).

Week. session number	Group	Oxygen concentration (%)	Intensity (% vVO ₂ peak)	Interval duration (recovery)	Heart rate reserve (%)	sRPE	Number 10 s rests during training (from n participants) [^]
1.1	IHT	15.3 ± 0.2	84 %	3 min	-	8.2 ± 1.8	7 (4)
	PLA	21.0 ± 0.3	90 %	(1 min)	-	8.7 ± 1.0	9 (4)
1.2	IHT	15.3 ± 0.0	92 %	2 min	-	8.5 ± 1.1	8 (4)
	PLA	20.7 ± 0.1	98 %	(1 min)	-	8.2 ± 1.3	4 (2)
2.1	IHT	15.1 ± 0.1	98 %	1 min	-	5.1 ± 1.3	0 (0)
	PLA	21.0 ± 0.2	104 %	(1 min)	-	6.3 ± 1.4	0 (0)
2.2	IHT	14.9 ± 0.2	104 %	30 sec	-	5.7 ± 1.8	0 (0)
	PLA	20.3 ± 0.2	110 %	(30 sec)	-	6.3 ± 1.2	0 (0)
3.1	IHT	15.2 ± 0.2	84 %	3 min	80 ± 4*	5.1 ± 1.1	0 (0)
	PLA	20.8 ± 0.1	90 %	(1 min)	76 ± 5	6.7 ± 1.7	0 (0)
3.2	IHT	14.9 ± 0.2	92 %	2 min	85 ± 2*	8.5 ± 1.2	10 (4)
	PLA	20.0 ± 0.2	98 %	(1 min)	80 ± 4	8.8 ± 1.3	18 (5)
4.1	IHT	15.1 ± 0.1	98 %	1 min	76 ± 4*	6.0 ± 1.6	0 (0)
	PLA	20.7 ± 0.1	104 %	(1 min)	73 ± 6	6.9 ± 1.2	0 (0)
4.2	IHT	15.2 ± 0.3	104 %	30 sec	79 ± 3 [#]	5.9 ± 1.3	0 (0)
	PLA	20.3 ± 0.1	110 %	(30 sec)	77 ± 5	6.2 ± 1.4	0 (0)

Values are mean ± 90 % confidence interval. * and [#] denote HRR *likely* and *possibly* higher in PLA, respectively. IHT n = 9, PLA = 8 (except for HRR, where IHT n = 8, PLA n = 4). [^] If participants voluntarily dismounted the treadmill during an interval (due to fatigue), they were provided a 10 s rest before re-commencing the interval to completion.

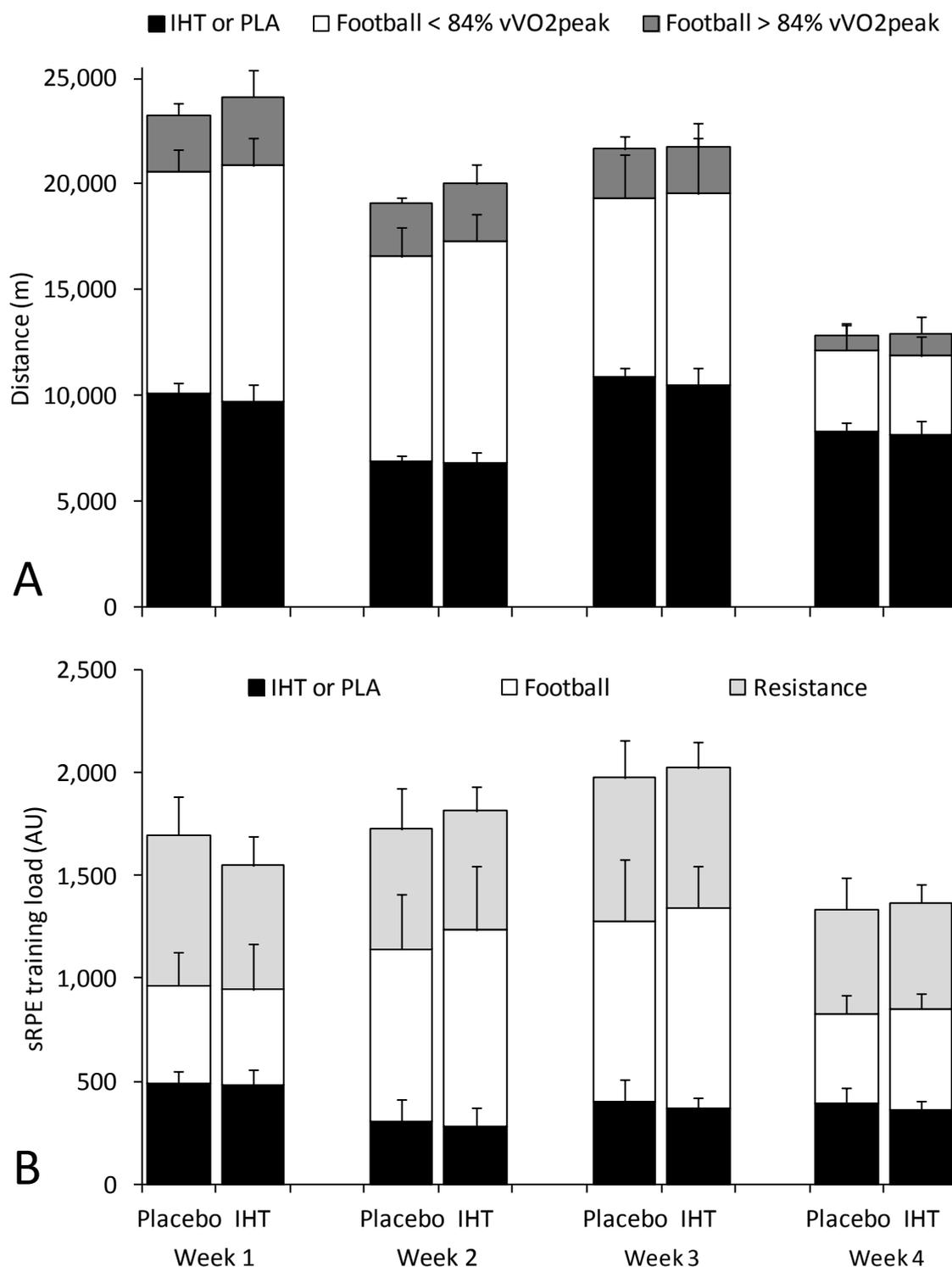


Figure 8.2. A) Weekly running volumes, and B) session rating of perceived exertion (sRPE) training load, during the four-week intervention period. IHT = interval hypoxic training group, PLA = placebo training group, vVO₂peak = peak velocity achieved during a VO₂peak test, AU = arbitrary units

Table 8.2. Summary of running performance outcomes.

Running measure	Group	Pre-intervention	Post-intervention	Standardised difference of changes between groups (Δ IHT- Δ PLA; D and 90% CI)	Chances (in %) for greater/similar/smaller changes for IHT compared with PLA
Yo-Yo IR2 distance (m)	PLA	973 \pm 185	1373 \pm 182	-0.42 (-0.82;-0.02)	1/16/83 – <i>likely</i> smaller
	IHT	1040 \pm 312	1262 \pm 351		
VO ₂ peak (L/min)	PLA	4.22 \pm 0.30	4.28 \pm 0.35	0.26 (-0.09;0.60)	61/37/2 – <i>unclear</i>
	IHT	4.46 \pm 0.55	4.64 \pm 0.52		
TTE (s)	PLA	581 \pm 64	634 \pm 47	0.03 (-0.16;0.22)	7/90/3 – <i>likely</i> trivial
	IHT	617 \pm 91	661 \pm 76		
TSR total distance (m)	PLA	4259 \pm 254	4365 \pm 253	0.72 (0.33;1.10)	98/2/0 – <i>very likely</i> greater
	IHT	4564 \pm 496*	4849 \pm 236		
TSR walking distance (m)	PLA	1371 \pm 124	1342 \pm 117	0.64 (0.15;1.14)	93/6/1 – <i>likely</i> greater
	IHT	1493 \pm 197*	1571 \pm 127		
TSR jogging distance (m)	PLA	726 \pm 70	754 \pm 75	0.37 (-0.24;0.98)	68/26/6 – <i>unclear</i>
	IHT	805 \pm 129*	873 \pm 82		
Low-intensity activity [jog + walk distance (m)]	PLA	2098 \pm 162	2096 \pm 141	0.59 (-0.07;1.11)	89/10/1 – <i>likely</i> greater
	IHT	2298 \pm 296*	2444 \pm 170		
TSR running distance (m)	PLA	1369 \pm 95	1457 \pm 100	0.21 (-0.46;0.89)	51/34/15 – <i>unclear</i>
	IHT	1442 \pm 190*	1558 \pm 121		
TSR sprinting distance (m)	PLA	743 \pm 30	768 \pm 23	0.00 (-0.73;0.73)	32/36/32 – <i>unclear</i>
	IHT	772 \pm 49*	798 \pm 31		
High-intensity activity [run + sprint distance (m)]	PLA	2112 \pm 120	2225 \pm 109	0.17 (-0.50;0.84)	47/36/17 – <i>unclear</i>
	IHT	2215 \pm 226*	2357 \pm 139		

IHT = interval hypoxic training group (n = 9), PLA = placebo group (n = 8); Δ IHT = post minus pre change within the IHT group; Δ PLA = post minus pre change within the PLA group; TSR = team sport running protocol; TTE = time to exhaustion during the incremental treadmill test. * IHT *likely* greater than PLA at pre-intervention.

DISCUSSION

To our knowledge, this single-blinded, randomised controlled trial is the first to assess the effects of IHT on high-intensity intermittent, and self-paced, exercise performance. The main findings are that four weeks of IHT in Australian Footballers resulted in: (i) smaller improvements in externally-paced high-intensity running (Yo-Yo IR2) performance compared with training in normoxia; (ii) IHT and PLA participants exhibiting similar increases in high-intensity running distance during a self-paced team sport protocol; and (iii) IHT participants exhibiting greater improvements in the self-paced protocol for total distance and distance covered during low-intensity activity.

The findings with respect to Yo-Yo IR2 performance in this study are in contrast to the results of Galvin et al. (2013) who found greater Yo-Yo IR1 improvements in their hypoxic group following four weeks of RSH training with well-trained rugby players. Several differences in study design may explain these opposing findings, perhaps most importantly the training intensity used in each of the studies [repeated 6 s sprints in Galvin et al. (2013) vs. 30 s to 3 min intervals in this study]; thus, the protocol in the study of Galvin et al. (2013) may have a greater impact on high intensity repeated efforts, compared to the current protocol which has a greater focus on prolonged intervals. Differences in the initial running capacity of the groups in each study may also play a part, with the hypoxic group in the study of Galvin et al. (2013) starting with a poorer high-intensity running capacity than the normoxic group (Yo-Yo IR1 distance: 1237 ± 265 and 1374 ± 361 m, respectively). This suggests that the hypoxic group may have had greater potential to improve performance following their training intervention, an idea supported by the similar Yo-Yo IR1 results at completion of the intervention (Yo-Yo IR1 distance 1621 ± 364 m and 1594 ± 379 m for the hypoxic and normoxic groups, respectively). However, in the current study, there were *unclear* differences in baseline Yo-Yo IR2 performance, and we attempted to control for any small differences by using these baseline data as a covariate in statistical analysis. The hypoxic participants in the current study ended with substantially lower Yo-Yo IR2 results than those in the placebo group (1262 ± 351 m and 1373 ± 182 m, respectively). These differences may be related to the reduced absolute training intensity ($\approx 6\%$ $v\dot{V}O_{2peak}$) of the hypoxic training sessions, which made up the majority of the high-intensity running volume (9-10 km per week) during the intervention. Given that IHT participants completed only 2-3 km per week of high-intensity running in normoxia (during football sessions), this may have limited some of the training adaptations stimulated by high-intensity (absolute) interval training. A greater portion of high-intensity training in normoxia may overcome this

limitation in future interventions, and is an important consideration for practitioners when implementing IHT protocols (McLean et al., 2014).

The novel combination of externally- and self-paced performance tests in the current study highlights the importance of testing specificity when assessing the influence of LLTH, and other training interventions. Yo-Yo IR2 is often used as a measure of team sport specific running performance (Bangsbo et al., 2008); however, this measure is externally-paced and, therefore, does not detect changes in pacing strategies that may be adopted, which is important given the self-paced nature of team sport competition (Duffield et al., 2009; Aughey, 2010). Within the current investigation, Yo-Yo IR2 results would suggest that the hypoxic intervention blunted some of the training-induced improvements in team sport high-intensity intermittent running performance. In direct contrast, the IHT group had greater increases in total distance covered in the team sport running protocol, with no differences between groups in the amount of high-intensity activity (i.e. running and sprinting) performed. However, IHT participants covered extra distance during low-intensity periods which may support the theory that team sport athletes regulate low-intensity activity throughout a match to maintain high-intensity activity (Aughey et al., 2014). While the reasons for the change in pacing strategies between the IHT and PLA groups are unclear, these changes may be related to central regulation. Indeed, cortical voluntary activation has been shown to parallel changes in cerebral oxygen delivery (Goodall et al., 2012), and hypoxic training interventions can lead to enhanced cerebral oxygen delivery during high-intensity intermittent exercise (Galvin et al., 2013). Therefore, training in hypoxia presents an intervention that may influence central fatigue during exercise, which in turn could impact on self-pacing strategies. It should be acknowledged that cerebral oxygenation was not measured in this study and, therefore, our contention that our hypoxic intervention has altered oxygen delivery to the brain and influenced self-pacing is speculative. However, this is the first study to report changes in self-pacing during high-intensity intermittent exercise following a LLTH intervention.

Pooled data from both groups in this study show a small increase in VO_{2peak} following the training intervention. However, there were *trivial* differences between ΔIHT and ΔPLA , which suggests that the hypoxic stimulus had little additional effect on changes in VO_{2peak} . Training in hypoxia is known to limit absolute exercise intensity (Buchheit et al., 2012a), which invariably leads to a reduction in cardiovascular load (training HR and VO_2). This was evident in our study, where hypoxic participants were prescribed velocities at 6% less vVO_{2peak} than normoxic participants, which resulted in *likely* lower training heart rates (see

Table 8.1). Despite this apparent reduction in cardiovascular load, both groups experienced similar gains in VO_2peak . Therefore, it may be that a four-week training intervention was too short to elicit differences between IHT and PLA participants in cardiovascular adaptations. Alternatively, reductions in central overload may have been offset by an increase in peripheral adaptation in hypoxic participants; indeed, training in hypoxia has been shown to augment a range of peripheral mRNA transcripts related to glycolytic potential and mitochondrial biogenesis and metabolism (Zoll et al., 2006). However, it should be noted that these mechanisms were not investigated in this study, so any proposed peripheral adaptations are purely speculative. Regardless of the underlying mechanism, both groups experienced similar increases in VO_2peak during our four-week intervention, which suggests that any differences in performance are not related to changes in aerobic capacity.

The placebo and nocebo effects are always an important consideration in intervention studies, and logistical challenges often make these difficult to control in hypoxic training studies (Bonetti and Hopkins, 2009; McLean et al., 2014). In the present study, all participants were informed that they were training in hypoxia, and training sessions were set-up to promote this belief. Indeed, all participants trained in a hypoxic training room with air conditioning running (thereby creating similar air flow in both the hypoxic and normoxic conditions), and the alterations in absolute training intensity (of which the participants were not informed) led to similar session ratings of perceived exertion between groups. These data indicated that we adequately controlled for placebo/nocebo effects given that, when asked, ~ 50% of participants from both groups believed that their training was performed in hypoxia.

LIMITATIONS

A number of limitations must be considered when interpreting results from the current study. This study was conducted with a group of sub-elite amateur Australian Footballers and, therefore, their responses may differ from responses of highly trained professional football athletes. However, comparison with published data for professional Australian Footballers (Buchheit et al., 2013) shows that the athletes in the current study displayed similar Yo-Yo IR2 running performance at baseline as professional athletes who had completed a 3-week training camp in the heat (current study $\approx 1,013$ m, professional $\approx 1,024$ m). Despite this comparatively elite level of team sport running performance before the intervention, both

our groups experienced large ($D = 0.88$) increases in Yo-Yo IR2 performance following the training intervention, suggesting that elite players may also benefit from similar protocols.

Matching prescription of training intensity between hypoxic and normoxic groups is difficult when implementing sub-maximal training protocols (i.e. any training intervention that is not a maximal sprint protocol). Although it is well established that training in hypoxia will limit absolute exercise intensity (Buchheit et al., 2012a), there is currently no consensus on the magnitude of such limitations (McLean et al., 2014). In this study, we used the recommendations of Buchheit et al. (2012a) whereby interval training intensity is reduced by 6% vVO_{2max} in hypoxia, in an attempt to match relative training intensity between groups. This method resulted in similar perceived exertion between groups but substantially higher training HR in the normoxic group, reflecting the greater absolute training intensity compared with the IHT group.

The prescription of training intensity based on vVO_{2peak} also has a number of limitations which are apparent in the current study. During training sessions 1.1, 1.2 and 3.2, approximately half of the participants required \geq one 10 s rest period in order to complete the exercise protocol. Similar numbers of participants required 10 s rest periods in both the IHT and PLA groups, which suggest this prescription issue was not caused by the hypoxic stimulus. While these results suggest that the training velocities prescribed were too high, \sim 50% of participants were still able to complete the stipulated protocol in these three sessions (that is, sessions 1.1, 1.2 and 3.2). Therefore, we propose that the sole use of % vVO_{2peak} is insufficient for accurate prescription of high-intensity interval training in team sport athletes. Some combination of aerobic power measurement and anaerobic speed reserve/maximal speed may be more efficacious for training prescription in these athletes [e.g. integration of maximal speed data or use of high intensity intermittent field protocols such as the 30-15 intermittent field test (Buchheit, 2008)].

We recently highlighted the importance of sufficient volume and intensity of normoxic training during IHT interventions (McLean et al., 2014) and, in this study, we attempted to provide this training stimulus through ‘football’ training sessions, as prescribed by team coaches. However, in retrospect, this resulted in only small amounts of high-intensity running (see figure 8.2A) in normoxia. Thus, the normoxic training for the IHT group may not have been of sufficient volume and intensity to maximise adaptations related to high-intensity (absolute) interval training. Future IHT interventions should consider the inclusion

of structured high-intensity normoxic training sessions to optimally overload all physiological systems that are important in team sport running performance.

This study was completed in a highly ecologically valid environment, during the pre-season phase with a group of AF players. However, conducting this study in such an applied -setting brought with it some inherent limitations which must be acknowledged. There is a relatively small sample size in the current investigation, due to limited availability of athletes and a number of withdrawals throughout the study. Future studies should aim for larger sample sizes for greater confidence in the observed results. The possible mechanisms contributing to different performance responses between PLA and IHT groups are not addressed in this study. The inclusion of such information would enhance the understanding of how the hypoxic stimulus contributes to changes in performance and how these techniques may be best applied with team sport athletes.

CONCLUSIONS AND PRACTICAL APPLICATIONS

The main findings of this study are that four weeks of IHT lead to greater improvements in self-paced performance during a team sport running protocol, but smaller improvements in externally-paced intermittent running performance (that is, Yo-Yo IR2), when compared to matched training in normoxia, where an attempt was made to match relative training intensity. Thus, we propose that hypoxic training positively influences pacing strategies in team sport athletes and, therefore, improves movement strategies adopted in team sport competition. Differences in the volume of high-intensity training in normoxia may explain the smaller improvements in externally-paced running performance in the hypoxic group, and we recommend that practitioners and researchers include greater volumes of targeted normoxic training in all groups undertaking hypoxic training.

CHAPTER 9 – DISCUSSION

Before this work, there was a paucity of information on the responses of team sport athletes to hypoxic training techniques (e.g. LHTH, LHTL and LLTH). This work begins to explore how team sport athletes respond to these interventions, complementing other recent work in this area (Buchheit et al., 2013; Galvin et al., 2013; Brocherie et al., 2014). Our work suggests that altitude training camps (LHTH) may offer both physiological (McLean et al., 2013a; McLean et al., 2013b) and running performance (McLean et al., 2013b) benefits for Australian Footballers, which may be related to the hypoxic stimulus itself and/or increases in training dose during training camps. In a systematic review of the literature (McLean et al., 2014), some important methodological considerations when implementing LLTH interventions are highlighted, including the importance of well-prescribed normoxic training for athletes involved in LLTH programs. In Study 3 of this work, a reliable alternative for assessing team sport running performance is presented. This self-paced protocol may provide a more ecologically-valid, laboratory-based performance test for team sport athletes than existing externally-paced tests. Study 4 suggests that IHT may lead to smaller improvements in externally-paced intermittent running performance compared with normoxic high-intensity training, but greater improvements in self-paced exercise performance. This final chapter addresses these findings from an applied perspective, discussing the practical implications of these research outcomes.

EFFICACY OF LHTH

Although altitude training camps (primarily LHTH) have been popular with Australian Football teams in recent years, this is the first published work to examine the physiological and performance responses of team sport athletes to this type of training intervention. Our results suggest that Australian Footballers may enhance improvements in running performance during the pre-season period by participating in a LHTH intervention.

These improvements in running performance may be partially explained by ~ 4% increase in Hb_{mass} following the LHTH intervention. However, subjects who participated in the altitude training camp reported in Chapter 3 also completed a greater volume (duration) and intensity (session RPE) of training than those players who remained at their home, sea-level training base during this period. Whilst this difference is a limitation when determining the efficacy of hypoxia *per se*, this may be an important practical finding for coaches and athletes

regarding the implementation of pre-season training camps. At the beginning of this study, the aim of the conditioning and coaching staff was to closely replicate training for those players training at altitude and for the group training at their sea-level home base. This objective was not achieved, as shown by the differences in overall training load between these groups. It may be that the environment of an intensified training camp, be it at altitude or sea-level, led coaches to prescribe and players to complete a greater volume and higher intensity of training. Despite this, an attempt was made to account for these training load differences through covariate analysis. This still showed a benefit for LHTH over sea-level training for running performance.

The LHTH studies in this work demonstrated a repeatable ~4% increase in Hb_{mass} over two pre-season altitude training camps in professional Australian Footballers. However, these data show wide variability in the individual responsiveness for changes in Hb_{mass} . Moreover, individual athletes do not exhibit consistency in changes from year to year. Thus, a 'responder' or 'non-responder' to altitude does not appear to be a fixed trait, with respect to changes in Hb_{mass} . Blunted erythropoietic responses are also demonstrated for athletes who fall ill or lose >2 kg of body mass during an 18-19 day altitude training camp. Therefore, to maximise the benefits of altitude training camps, athletes should adopt strategies to maintain body mass and optimal health immediately before and throughout altitude training camps.

Even though the LHTL intervention was not investigated in this work, it should be noted that LHTL interventions warrant consideration for Australian Football teams wishing to implement prolonged hypoxic exposures. Some authors suggest LHTL is a superior methodology compared with LHTH for improving endurance performance, as absolute exercise intensity can be maintained by training in normoxic conditions (Levine and Stray-Gundersen, 1997). Recently, 12 nights of LHTL in normobaric hypoxia, in combination with training in the heat, led to small increases in Hb_{mass} (2.6%) in Australian Football players (Buchheit et al., 2013). Despite the increases in Hb_{mass} , these subjects experienced no immediate performance benefits over a matched normoxic control group. However, in the four weeks following the 'LHTL in the heat' intervention, hypoxic subjects experienced additional increases in Hb_{mass} , which paralleled a better maintenance of running performance compared to the control group (Buchheit et al., 2013).

PRACTICAL CONSIDERATIONS WHEN IMPLEMENTING LHTH CAMPS WITH TEAM SPORT ATHLETES

As discussed in Chapters 3 and 4, pre-season LHTH training camps can be used to increase Hb_{mass} and improve running performance in team sport athletes. However, there are a number of important practical aspects that must be considered when planning and implementing these types of interventions, and these are discussed here.

Timing of pre-season altitude training camps

The timing of an altitude training camp to achieve peak performance at sea-level is an important consideration for endurance athletes aiming to optimise the benefits of any prolonged hypoxic exposure (Chapman et al., 2014). The optimal time to achieve peak sea-level performance following altitude exposures is thought to be primarily influenced by three factors; time course of decay of increases in Hb_{mass} , ventilatory re-acclimatization, and alterations in neuromuscular function following weeks of training at altitude (Chapman et al., 2014). Thus, coaches and scientists both acknowledge that the timing of altitude camps is an important factor to consider when planning for a one-off event, such as a world championships, Olympic Games or multistage road race (Chapman et al., 2014). However, team sport athletes are faced with very different challenges, being required to compete in a prolonged season, ending with the most important competitive matches. Indeed, in professional Australian Football, the season runs from March to September, with the longest break from competition during this period lasting ~ 14 days. As there are only short breaks between games during the season, it is difficult to schedule extended (2-3 week) training camps which may precede the most important competition periods.

An additional challenge in scheduling LHTH camps for Australian Football is that venues which are at sufficient altitude to provide the recommended 'hypoxic dose' (Wilber et al., 2007) are not readily available in Australia. Therefore, the pre-season period presents the only opportunity for a team to travel to altitude and complete a 2-3 week training camp. As a result, LHTH interventions are often completed 6-8 months before the most important competition period (as occurred in Studies 1 and 2) and, therefore, any physiological adaptations occurring during the altitude exposure will have re-acclimated to normal sea-level values by this time. Thus, the impact of a LHTH stimulus on performance throughout the season, and in important late season matches, must be questioned. It may be, that if a LHTH intervention increases physiological and running capacity during the pre-season,

athletes are then able to train harder during the post-intervention period, perhaps stimulating greater training adaptations that are maintained in subsequent weeks. However, whilst we have shown improvements in running capacity following a LHTH intervention (Study 1), the impact of this on training intensity and the maintenance of these improvements throughout the season is not known. Additionally, the transfer of improvements in running performance (Study 1) to football performance is not yet established.

A further complication when timing pre-season camps for Australian Football is the influence of two rest periods where structured training cannot be mandated for players (as per AFL rules). This includes a 5-7 week block of rest immediately preceding the commencement of pre-season training, and a two-week Christmas training break in the middle of the pre-season period (Buchheit et al., 2014). LHTH interventions are often completed by AFL clubs during this pre-Christmas training block (as in Studies 1 and 2 of this work) and, therefore, players often face limited preparation leading into the training camp (usually only 0-3 weeks of structured training prior to the camp). This timing may compromise the benefits of the LHTH intervention in two ways; (1) athletes may be under-prepared to cope with the stressful demands of training in hypoxia and the typically large training loads that are completed during concentrated camps, and (2) by resting during the Christmas break, athletes may under-utilise any enhanced physiological capacity resulting from LHTH, which will be progressively returning to sea-level baseline values.

Impact of LHTH camps on overall preparation for Australian Football

While LHTH interventions may offer small (~2%) improvements in running performance (McLean et al., 2013b), the impact of these camps on other physical and technical/tactical areas must also be considered. As a multi-disciplinary collision sport, it is important for Australian Footballers to be strong and powerful, technically skilful, have a strong tactical awareness, and possess excellent decision making skills. Therefore, the preparatory phases of training should also address these components. As previously mentioned, many of the altitude venues appropriate for LHTH intervention lie outside of Australia, prompting many Australian Football teams to visit North American venues during the pre-season period (McLean et al., 2013a; McLean et al., 2013b). As these camps usually take place during November and December (i.e., North American winter), many venues at altitude are beginning to experience snowfall, making outdoor Australian Football training difficult and non-specific to playing conditions. Indoor training facilities at these venues can overcome

this challenge, but the field size in these venues is usually limited to the size of an American Football field, which has a total area less than 50% of that of a full-sized Australian Football playing arena. It has previously been shown that pitch area per player can significantly influence activity demands (Hill-Haas et al., 2011), Therefore, team practice often lacks both physical and tactical specificity whilst at altitude, with match simulation during training not possible.

Cost/benefit of LHTH interventions

The cost of training interventions is always an important consideration for professional sporting clubs which operate on limited budgets. Recently, this has become even more evident for AFL teams, as the league has now introduced a cap on non-player football expenditure (AFL, 2014). Therefore, the cost/benefit relationship must be considered when implementing all areas of the football program. Altitude training camps can be expensive for AFL teams, with the costs of travel and accommodation for 40-45 players and 20 or more support staff estimated at over \$500,000 for a 2-3 week international camp (Fjeldstad, 2013).

While this work has shown that LHTH may improve physiological capacity and running ability in Australian Footballers, it must be acknowledged that the magnitude of these improvements are relatively small, and the physiological improvements do not last for the duration of the season. Therefore, practitioners and administrators should consider the large costs to limited football department expenditure when making decisions regarding LHTH interventions for Australian Football players.

Together, the current results and other recent work in team sport athletes (Buchheit et al., 2013) suggest that prolonged hypoxic exposure (i.e. LHTH or LHTL) may offer some physiological and performance benefits. However, prolonged international training camps are becoming increasingly difficult to implement for Australian Footballers given scheduling and budget related issues, and the benefit/transfer to 'football performance' is yet to be established. The use of prolonged normobaric hypoxic exposures, within Australia, to implement LHTL interventions is an alternative option that may be used at various times throughout a season to provide additional performance benefits for Australian Footballers.

LLTH FOR TEAM SPORTS

Through a systematic review of the literature (Chapter 6), this work highlights some important methodological considerations when implementing LLTH interventions. Within this review, it is concluded that the LLTH model has the potential to contribute to a number of training adaptations, and these appear to be more related to anaerobic metabolism. Thus, LLTH interventions may have the greatest benefit for high-intensity, short-term and intermittent performance (e.g. team sports). To maximise the benefits of LLTH interventions, practitioners should be sure to include sufficient volume and intensity of normoxic training, as reductions in absolute training intensity may blunt some of the training adaptations if only training in hypoxic conditions.

A number of recent investigations have shown that completing high-intensity, intermittent training in hypoxia may provide additional performance benefits for team sport athletes (Galvin et al., 2013; Brocherie et al., 2014). In this work, Study 4 suggests that training in hypoxia may lead to smaller improvements (compared with matched training in normoxia) in externally-paced intermittent running performance (i.e. Yo-Yo IR2 performance), but greater improvements in self-pacing during a team sport running protocol. These blunted improvements in externally-paced performance may be related to reductions in absolute training intensity for IHT subjects, given that in retrospect, these subjects may not have received a sufficient volume of structured, high-intensity normoxic training. As highlighted in Chapter 6 (systematic review), training in hypoxia may limit some of the training adaptations associated with maximal absolute training intensities. Therefore, well-structured, high-intensity training in normoxia needs to accompany hypoxic training to optimise performance outcomes when implementing LLTH programs.

Practical application for LLTH for team sport athletes

As previously discussed, the implementation of LLTH programs may have additional physical performance benefits for team sport athletes. However, a number of practical considerations need to be taken into account when implementing LLTH programs in a team sport environment. Positive physical performance outcomes following LLTH are most likely if the training mode is specific to the competition exercise modality (e.g. running sessions for a predominantly running based team sport athlete). Furthermore, positive outcomes appear most likely if these sessions are completed at very high intensities, given low-to-moderate intensity LLTH programs do not appear to provide additional performance

benefits. While there may be scope to include specific, high-intensity sessions during the pre-season period, the additional load of these sessions may have a negative impact on team sport athletes who often face a demanding congested playing schedule. Therefore, such interventions may have the greatest impact during the pre-competition phase of training. The nature of hypoxic training facilities also provides a logistical challenge when attempting to implement high-intensity repeated efforts with running based athletes. Traditional treadmills (which are often found in hypoxic training rooms) may not change speed fast enough between intervals, or have a high enough maximal speed to complete repeated sprint sessions. In contrast, portable, inflatable hypoxic rooms (that can be placed over practice fields) and non-motorised treadmills may overcome these issues when implementing hypoxic training sessions with team sport athletes. Team coaches and fitness staff should therefore consider if these techniques are practically viable in their team's training environment.

The implementation of high intensity training in normoxia (alongside LLTH) is also important to consider for team sport athletes. As previously discussed, absolute training intensity is limited during LLTH sessions and, therefore, some physiological capacities are not stressed to the same extent as in high intensity normoxic training sessions. Coaches and fitness staff need to carefully consider the periodization and volume of LLTH interventions, whilst also integrating some high intensity training in normoxia, to optimally overload all physiological systems.

LIMITATIONS

The current work was conducted in a highly ecologically-valid environment, with professional (Studies 1 and 2) and amateur (Study 4) Australian Football athletes. Whilst this gives the findings strong practical relevance, the applied setting also creates many research challenges which have led to a number of limitations.

One of the major limitations in Study 1 is the difference in training load between the altitude and sea-level (control) group. This difference in training load may account for some of the differences in performance between the two groups, which should be considered when interpreting the results. However, the greater training loads completed during concentrated pre-season camps may be an important practical consideration. Additionally, placebo and nocebo effects may have influenced the running performance in Study 1; while this may

complicate scientific interpretation, placebo benefits (and conversely, detrimental nocebo effects) may influence practically important changes in performance. The specificity of the performance tests used in Study 1 should also be considered with respect to AF running performance. In this study, performance was measured using a 2 km TT, which is a continuous exercise test lasting approximately 6-7 minutes (with elite AF players). This type of running is not particularly specific to the intermittent demands of AF and, therefore, caution needs to be used when interpreting the results for Australian Footballers.

Study 2 also has a number of limitations associated with the applied nature of the research. One limitation is that ALT1 and ALT2 were conducted in different locations over different durations (19 and 18 days, respectively). However, as discussed in Study 2, the altitude at these locations is very similar (~2100 m) and the overall hypoxic dose between camps differed by only 18 hours. The timing of Hb_{mass} measurement also differed between ALT1 and ALT2; POST1 was taken 5 days post-altitude (ALT1) compared with the penultimate day at altitude (ALT2). Despite these limitations, the data from Study 1 and 2 show consistency, with ~4% increase in Hb_{mass} reported in ALT1 and ALT2.

Study 4 was conducted with a group of sub-elite amateur Australian Footballers and, therefore, their responses may differ from responses of highly-trained professional athletes. However, athletes in this study displayed similar Yo-Yo IR2 running performance at baseline as professional Australian Footballers (Buchheit et al., 2013). In Study 4, we used the recommendations of Buchheit et al. (2012a) for matching prescription of training intensity between hypoxic and normoxic groups, whereby interval training intensity is reduced by 6% vVO_{2peak} in the hypoxic group, in an attempt to match relative training intensity between groups. The prescription of training intensity based on vVO_{2peak} also has a number of limitations, some of which are apparent in the current study. During three training sessions in this study, approximately half of the subjects were unable to complete all of the prescribed exercise protocol. The number of participants who could not complete the protocol were similar in both the hypoxic and placebo groups, which suggests that this prescription issue was not caused by the hypoxic stimulus. While these results suggest that the training velocities prescribed were too high, ~50% of participants were still able to complete the same stipulated protocol. Therefore, we propose that the sole use of %vVO_{2peak} is insufficient for accurate prescription of high-intensity interval training in team sport athletes. Some combination of aerobic power measurement and anaerobic speed

reserve/maximal speed may be more efficacious for training prescription in these athletes [e.g. training prescription based on 30-15 intermittent field test (Buchheit, 2008) results].

In Study 4, we attempted to provide a normoxic training stimulus to all subjects through ‘football’ training sessions, as prescribed by team coaches. These sessions, in retrospect, included only small amounts of high-intensity running and, thus, the normoxic training for the IHT group in this study may not have been of sufficient volume and intensity to maximise adaptations related to high-intensity (absolute) interval training. Future IHT interventions should consider the inclusion of structured high-intensity normoxic training sessions in order to optimally overload all physiological systems (e.g. cardiovascular) that are important in team sport running performance.

FUTURE RESEARCH DIRECTIONS

As traditional LLTH interventions are becoming increasingly difficult to implement in Australian Football, future work into the application of LLTL interventions using normobaric hypoxic rooms may have important practical implications for Australian Footballers. The timing of these interventions and the impact on running performance in-season, particularly in the important final weeks of the season, also remains unknown.

LLTH techniques appear to have some additional benefits for team sport athletes, if implemented correctly and with the inclusion of well periodised training in normoxia. There is more work needed to understand what volumes of normoxic and hypoxic training may be optimal for performance. There is also much discussion around the optimal training intensity (Millet et al., 2013) and exercise-to-rest ratios (Millet and Faiss, 2012) during LLTH interventions, as it is likely that low-intensity continuous exercise in hypoxia provides no additional benefits over training in normoxia (see Chapter 6 – Systematic Review). More work is needed to determine the most beneficial training intensities and exercise-to-rest ratios that optimise high-intensity intermittent performance. Furthermore, the optimal degree of hypoxia to use during LLTH techniques is currently unknown – indeed, this may vary depending on the LLTH method (CHT, IHT, RSH or RTH) being used. Further understanding of the mechanisms related to changes in performance, including peripheral and central factors, is also needed.

CONCLUSIONS AND PRACTICAL APPLICATIONS

A variety of hypoxic techniques can be used to enhance physiological capacities and running performance in team sport athletes. These techniques should be applied as part of a holistic training program, ensuring athletes are well prepared to adapt to additional hypoxic stimuli. The influence of hypoxic environments on training intensity, during both LHTH and LLTH interventions, should also be carefully considered when planning training.

Athletes may gain a physiological benefit from altitude training camps with as little as 2 weeks of exposure. However, this type of intervention is becoming increasingly difficult to implement within professional Australian Football, due to limited availability of training venues within Australia and only short periods available to implement expensive international training camps. Therefore, LHTL techniques using hypoxic sleeping houses (e.g. within Australia) may offer an alternative to obtain some of the physiological benefits associated with altitude training camps. An advantage of this technique is that it may also be utilised close to, or within, the competition period if facilities are available at or near the athletes' home training base.

LLTH techniques may also be implemented throughout an Australian Football season, and using these techniques may enhance pacing strategies in team sport athletes. Practitioners implementing LLTH interventions should be careful to include a sufficient volume of well-periodised training in normoxia, to maximise high-intensity running performance.

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APPENDIX I – RESEARCH PORTFOLIO

PUBLICATIONS

1. **McLean, B.D.**, D. Buttifant, C.J. Gore, K. White, C. Liess and J. Kemp (2013). Physiological and performance responses to a pre-season altitude training camp in elite team sport athletes. *International Journal of Sports Physiology Performance*. 8(4): pages 391-399.

Contribution statement: BM was primarily responsible for the design, implementation and overall management of this project. BM and DB were involved in the concept and design of the study, conducted the data collection and analysis and prepared the manuscript. CG and JK were involved in the concept and design of the study, data analysis and interpretation and preparation of the manuscript. KW and CL assisted with data collection and analysis and manuscript preparation.

Approximate percentage contributions – B. D. McLean 75%; D. Buttifant 5%; C.J. Gore 5%; K. White 5%; C. Liess 5%; J. Kemp 5%.

I acknowledge that my contribution to the above paper is 75%

B.D. McLean:  Date: 24/07/2014

As principal supervisor of this project, I certify that the above contributions are true and correct:

J. Kemp:  Date: 24/07/2014

2. **McLean, B.D.**, D. Buttifant, C.J. Gore, K. White and J. Kemp (2013). Year-to-year variability in haemoglobin mass response to two altitude training camps. *British Journal of Sports Medicine*. 47(Supplement 1): pages i51-i58.

Contribution statement: BM was primarily responsible for the design, implementation and overall management of this project. BM and DB were involved in the concept and design of the study, conducted the data collection and analysis and prepared the manuscript. CG and JK were involved in the concept and design of the

study, data analysis and interpretation and preparation of the manuscript. KW assisted with data collection and analysis and manuscript preparation.

Approximate percentage contributions – B. D. McLean 80%; D. Buttifant 5%; C.J. Gore 5%; K. White 5%; J. Kemp 5%.

I acknowledge that my contribution to the above paper is 80%

B.D. McLean:  Date: 24/07/2014

As principal supervisor of this project, I certify that the above contributions are true and correct:

J. Kemp:  Date: 24/07/2014

3. **McLean, B.D.,** C. Gore and J. Kemp (2014). Application of ‘live low-train high’ for enhancing normoxic exercise performance in team sport athletes – a systematic review. *Sports Medicine*. Online first 22 May.

Contribution statement: BM was primarily responsible for the concept, design and project management of this systematic review. BM then led the systematic review process which was also carried out by JK. All authors were involved in interpreting the results and preparation of the manuscript.

I acknowledge that my contribution to the above paper is 80%

B.D. McLean:  Date: 24/07/2014

As principal supervisor of this project, I certify that the above contributions are true and correct:

J. Kemp:  Date: 24/07/2014

4. Tofari, P.,* **B.D. McLean**,* J. Kemp & S. Cormack (Under review). A self-paced intermittent protocol on a non-motorised treadmill: A reliable alternative to assessing team sport running performance. *International Journal of Sports Physiology and Performance*.

* Authors made an equal contribution to the manuscript

Contribution statement: BM and PT were jointly responsible for the design, implementation and overall management of this project. All authors were involved in the concept and design of the study, data analysis and preparation of the manuscript. PT and BM were responsible for the data collection. All authors agree that BM and PT made an equal contribution to the manuscript as lead authors (which was noted on the publication submission).

Approximate percentage contributions – B. D. McLean 45%; P. Tofari 45%; J. Kemp 5%; S. Cormack 5%.

I acknowledge that my contribution to the above paper is 45%

B.D. McLean:  Date: 24/07/2014

As principal supervisor of this project, I certify that the above contributions are true and correct:

J. Kemp:  Date: 24/07/2014

5. **McLean, B.D.**, P. Tofari, C. Gore and J. Kemp. Changes in running performance following four weeks of interval hypoxic training in Australian Footballers: a single blind, placebo controlled study.

Manuscript to be submitted once paper 4 (above) is accepted.

Contribution statement: BM was primarily responsible for the design, implementation and overall management of this project. All authors were involved in the concept and design of the study, data analysis and preparation of the manuscript. BM was responsible for the implementation of the training intervention. BM and PT were responsible for performance data collection.

Approximate percentage contributions – B. D. McLean 70%; P. Tofari 10%; C.J. Gore 10%; J. Kemp 10%.

I acknowledge that my contribution to the above paper is 70%

B.D. McLean:  Date: 24/07/2014

As principal supervisor of this project, I certify that the above contributions are true and correct:

J. Kemp:  Date: 24/07/2014

CONFERENCE PRESENTATIONS

1. **McLean, B.D.**, D. Buttifant, C.J. Gore, K. White, C. Liess and J. Kemp. Changes in haemoglobin mass and intramuscular carnosine during a 19 day altitude training camp in elite Australian Rules Footballers. *World Conference on Science and Soccer*. Ghent, Belgium; 16th May 2012.

Contribution statement: This conference presentation was based on the work from paper 1 (see above for author contributions). The presentation was primarily compiled by BM and subsequently reviewed by DB, CG, KW and JK. The oral presentation was delivered by BM.

2. **McLean, B.D.**, D. Buttifant, C.J. Gore, K. White, and J. Kemp. Year-to-year variability in response to a pre-season altitude training camp in elite team sport athletes. *Altitude Training and Team Sports Conference*. Doha, Qatar; 25th March 2013.

Contribution statement: This conference presentation was based on the work from paper 2 (see above for author contributions). The presentation was primarily compiled by BM and subsequently reviewed by DB, CG, KW and JK. The oral presentation was delivered by BM.

OTHER PRESENTATIONS

1. **McLean, B.D.**. Changes in haemoglobin mass and intramuscular carnosine during a 19 day altitude training camp in elite Australian Rules Footballers. *ACU 3 Minute Thesis*. Melbourne, Australia; 27th August 2013.

Contribution statement: This conference presentation was based on the work from papers 1 and 2 (see above for author contributions). The presentation was primarily compiled by BM and subsequently reviewed by DB, CG, KW and JK. The oral presentation was delivered by BM.

OTHER PUBLICATIONS

1. Gore, C., K. Sharpe, L. Garvican-Lewis, P. Saunders, C. Humberstone, E.Y. Robertson, N. Wachsmuth, S.A. Clark, **B.D. McLean**, B. Friedman-Bette, M. Neya, T. Pottgiesser, Y.O. Schumacher W. Schmidt, W (2013). Altitude training and haemoglobin mass from the optimised carbon monoxide re-breathing method – a meta-analysis. *British Journal of Sports Medicine*. 47(Supplement 1): pages i31-i39.

Contribution statement: BM collected data during publications 1 and 2 (listed above) which directly contributed to this meta-analysis. BM's specific contribution to this publication was to compile the data from his work and provide it to CG for analysis. BM was then involved in revision of the final version of the manuscript in conjunction with all co-authors.

APPENDIX II – STUDY 1 PUBLICATION

REFERENCE

McLean BD, Buttifant D, Gore CJ, White K, Liess C and Kemp J. (2013) Physiological and performance responses to a pre-season altitude training camp in elite team sport athletes. *International Journal of Sports Physiology and Performance*. 8: 391-399.

Physiological and Performance Responses to a Preseason Altitude-Training Camp in Elite Team-Sport Athletes

Blake D. McLean, David Buttifant, Christopher J. Gore, Kevin White, Carsten Liess, and Justin Kemp

Purpose: Little research has been done on the physiological and performance effects of altitude training on team-sport athletes. Therefore, this study examined changes in 2000-m time-trial running performance (TT), hemoglobin mass (Hb_{mass}), and intramuscular carnosine content of elite Australian Football (AF) players after a preseason altitude camp. **Methods:** Thirty elite AF players completed 19 days of living and training at either moderate altitude (~2130 m; ALT, $n = 21$) or sea level (CON, $n = 9$). TT performance and Hb_{mass} were assessed preintervention (PRE) and postintervention (POST1) in both groups and at 4 wk after returning to sea level (POST2) in ALT only. **Results:** Improvement in TT performance after altitude was likely 1.5% (± 4.8 –90%CL) greater in ALT than in CON, with an individual responsiveness of 0.8%. Improvements in TT were maintained at POST2 in ALT. Hb_{mass} after altitude was very likely increased in ALT compared with CON ($2.8\% \pm 3.5\%$), with an individual responsiveness of 1.3%. Hb_{mass} returned to baseline at POST2. Intramuscular carnosine did not change in either gastrocnemius or soleus from PRE to POST1. **Conclusions:** A preseason altitude camp improved TT performance and Hb_{mass} in elite AF players to a magnitude similar to that demonstrated by elite endurance athletes undertaking altitude training. The individual responsiveness of both TT and Hb_{mass} was approximately half the group mean effect, indicating that most players gained benefit. The maintenance of running performance for 4 wk, despite Hb_{mass} returning to baseline, suggests that altitude training is a valuable preparation for AF players leading into the competitive season.

Keywords: football, hypoxia, hemoglobin mass, carnosine

High-intensity-running performance is vital to success in team sports,¹ requiring athletes to have a highly developed aerobic system together with a large anaerobic capacity.² Therefore, training modalities that increase an athlete's ability to perform high-intensity work should benefit overall team performance. A recent modality adopted by professional teams in an attempt to improve performance is altitude training, where athletes are exposed to a hypobaric, hypoxic environment. Altitude training has been reported to induce a range of physiological adaptations,^{3,4} and 2 of these responses—increases in oxygen-carrying capacity of the blood⁴ and increases in muscle buffering capacity^{5,6}—would be expected to improve high-intensity-running ability. For improved

oxygen transport, the primary physiological adaptation after altitude exposure is an increase in total hemoglobin mass (Hb_{mass}),⁴ and this can lead to increases in VO_{2max} .⁷ With respect to increases in muscle buffering capacity after altitude exposure, the underpinning mechanism is unknown, but several authors propose that an increase in an intramuscular buffer, carnosine, is likely responsible.^{6,8}

The live-high, train-high (LHTH) model, involving living and training at moderate altitudes in the range of 2000–3000 m,⁹ is a common practice for hypobaric, hypoxic exposure. Endurance athletes have used this model for more than half a century, attempting to improve performance at sea level. Similarly, for professional team sports, the ultimate goal of a preseason altitude camp is to elicit performance improvements and increase training capacity on return to sea level. However, there are no published data describing the physiological or performance responses after altitude exposure in elite team-sport athletes. Given the unique physical attributes and performance demands of these athletes, it is important to determine how they respond to an altitude camp and whether there are performance gains on return to sea level.

Therefore, the current study examined the physiological and performance changes in professional

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Australian Football players after a 19-day altitude training camp. These data were compared with those of a control group of elite Australian Football players undertaking similar training at sea level. We hypothesized that increases in Hb_{mass} and intramuscular carnosine content would occur after altitude exposure, and after an LHTH camp athletes would show greater improvements in running performance than their matched controls completing similar training at sea level.

Methods

Subjects

Thirty elite Australian Football players (mean \pm SD; age 23 ± 3 y, height 188.2 ± 8.0 cm, body mass 88.0 ± 9.0 kg, sum of 7 skinfolds = 44.9 ± 4.7 mm) were examined throughout an 8-week training block (see Figure 1). Subjects were split into altitude (ALT, $n = 21$) and control (CON, $n = 9$) training groups. All training and testing took place during the Australian Football League preseason from November to January, after a 6-week off-season break. All subjects provided written informed consent, and this study was approved by the human research ethics committee at Australian Catholic University.

Nutritional Supplementation

Nutritional supplements were prescribed throughout the study by club dietitians. All subjects were supplemented with oral ferrous sulfate (325 mg/d) throughout the intervention period, and athletes identified as having low serum ferritin preintervention (serum ferritin ≤ 30 $\mu\text{g/L}$; $n = 2$) were given a single, 2-mL ferrum H injection (equivalent to 100 mg of iron) before the altitude camp. No supplements containing β -alanine were prescribed, and no subjects had used β -alanine within the preceding 6 months. As β -alanine is known to increase intramuscular carnosine concentrations,¹⁰ players were asked to

refrain from consuming any supplements containing β -alanine throughout the study, and they reported 100% compliance.

Training

During an 8-week training block, the ALT training group completed 19 days living and training at altitude, involving endurance- and resistance-exercise training, in Flagstaff, AZ (elevation ≈ 2130 m). The CON group training in Melbourne, Australia (elevation ≈ 30 m), completed training similar to that of the ALT group for the first 4 weeks of the intervention; thereafter training differed between groups. As such, data for the CON group are only reported through the first 4 weeks of the intervention period. All training was prescribed by team coaches and monitored via session rating of perceived exertion (RPE).¹¹ This method calculates a total load (arbitrary units [AU]) by multiplying the session RPE (Borg's CR 10-scale) by the session duration.

Running Performance

To assess high-intensity-running performance, subjects completed a 2000-m running time trial (TT) at the commencement (PRE) and 4 weeks into the training block (POST1). In addition, TT performance was assessed again in ALT subjects 8 weeks into the training block (POST2). TT performance was assessed on the same outdoor running track at sea level in Melbourne, Australia, between 8 and 9 AM (temperature 18 – 26°C , humidity 50 – 80%) and measured via handheld stop watches. All players were familiar with the TT from previous years—most had completed 6 to 10 (minimum 3) similar time trials in the preceding 1 to 3 years.

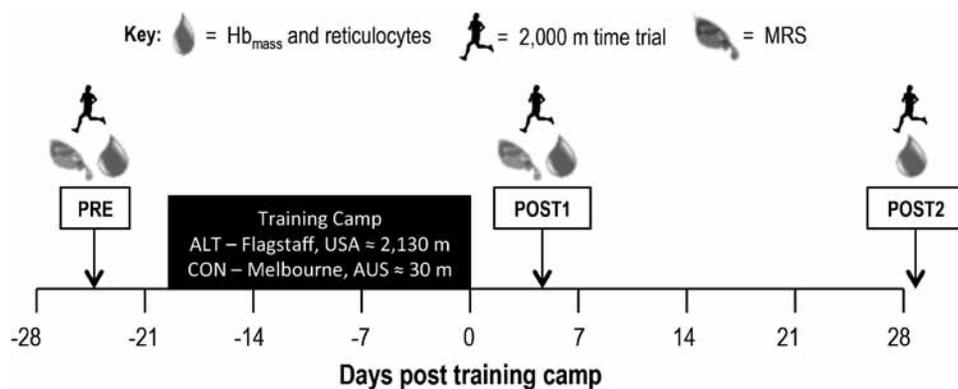


Figure 1 — Schematic timeline of study design. The altitude-training group (ALT) was measured preintervention (PRE), postintervention (POST1), and 4 weeks after returning to sea level (POST2). The sea-level control group (CON) was measured at PRE and POST1. Abbreviations: MRS, magnetic resonance spectroscopy.

Hematological Measures

All subjects were measured for Hb_{mass} using the optimized carbon monoxide rebreathing technique¹² at PRE and POST1 (see Figure 1). In addition, Hb_{mass} was measured again in ALT subjects at POST2. Briefly, subjects rebreathed a bolus of 99% CO equivalent to 1.0 mL/kg of body mass through a glass spirometer (BloodTec, Bayreuth, Germany) for 2 minutes. Percent carboxy-hemoglobin (%HbCO) in fingertip capillary blood was measured using an OSM3 hemoximeter (Radiometer, Copenhagen, Denmark) before and 7 minutes after administration of the CO dose. Six repeat measures of %HbCO were made for improved precision in Hb_{mass} estimation.¹³ Venous blood samples were sent to a local hospital laboratory (St Vincent's Pathology, Fitzroy, VIC, Australia) for assessment of reticulocyte count in both groups at PRE and POST1 and in the ALT group only at POST2. Preceding venous blood-sample collection, subjects' ambulation was limited for 15 minutes, with the majority of this time spent sitting. Concentration of serum ferritin was also assessed at PRE to identify subjects who might be iron deficient.

Magnetic Resonance Spectroscopy

Magnetic resonance spectra were acquired in the soleus and gastrocnemius of ALT only at a field strength of 3T on a Philips Achieva system (Philips Medical Systems, Best, The Netherlands) using the point-resolved spectroscopy technique^{14,15} with a repetition time of 2000 milliseconds and an echo time of 33 milliseconds. Voxel sizes varied depending on the size of the muscle but were generally in the range of 10 mm × 20 mm × 40 mm. Care was taken to position the voxel in such a way as to avoid contributions from fasciae across the chemical shift range of the voxel. Voxels were shimmed to between 12 and 19 Hz; 256 water-suppressed and 8 non-water-suppressed averages were acquired at the same receiver gain. Spectral data analysis was carried out using jMRUI version 4.0¹⁶ after zero filling and line broadening by 5Hz; metabolite peaks were fitted and expressed relative to the non-water-suppressed signal. Peaks were assigned as follows: carnosine C2-H (8 ppm) and C4-H (7 ppm), residual water (around 4.7 ppm), phosphocreatine and creatine (3.05 and 3.95 ppm), containing metabolites (3.20 ppm). All analyses presented in this article refer to the carnosine C2-H peak at 8 ppm and the carnitine and choline peak at 3.20 ppm. No relaxation-time corrections were made.

Statistical Analysis

A contemporary analytical approach involving magnitude-based inferences¹⁷ was used to detect small effects of practical importance. All data were log-transformed to account for nonuniformity error. The percentage changes in the mean TT and Hb_{mass} from prealtitude to each time point after altitude were calculated. The

differences within and between groups were assessed with dependent and independent *t* tests for unequal variance.¹⁷ The magnitudes of changes were assessed in relation to the smallest worthwhile change (SWC), which was set to 2% for TT and Hb_{mass} , and a small effect size ($d = 0.2$) was used for all other variables. Analysis of overall training load revealed differences between the CON (3229 ± 447 AU per week; mean \pm SD) and ALT (4249 ± 351) groups. As a result, training load was used as a covariate in the analysis of changes in TT and Hb_{mass} . The observed effects were reported as the mean change or difference \pm 90% confidence limits (CL). Effects were termed positive, trivial, or negative depending on the magnitude of the change relative to the SWC and were assigned a qualitative descriptor according to the likelihood of the change exceeding the SWC as follows: 50% to 74%, *possible*; 75% to 94%, *likely*; 95% to 99%, *very likely*; and >99%, *almost certainly*.¹⁸ Effects where the 90% confidence interval overlapped simultaneously the substantially positive and the negative thresholds were deemed unclear. The individual response was also quantified for TT performance and Hb_{mass} . For each, the magnitude of individual responses was calculated from the square root of the difference in the variance of the change scores of the CON and ALT groups.¹⁹ A Pearson correlation coefficient was used to examine the relationship between initial Hb_{mass} and change in Hb_{mass} at POST1—however, 2 subjects reported illness during the study and were subsequently removed from the correlation analysis, as proinflammatory cytokines that are increased with infection are known to suppress EPO production.²⁰

Results

Training Load

Training load, training duration, and RPE are presented in Table 1. Throughout the first 4 weeks of the intervention period, overall training load was almost certainly 24.5% \pm 10% higher in ALT than in CON. Training duration was very likely 11.5% \pm 7.2% higher and mean RPE was almost certainly 13.3% \pm 5.4% higher in ALT than in CON.

2000-m Time-Trial Performance

Time-trial performance was possibly faster in ALT (413 ± 14 s) than in CON (422 ± 23 s) before the altitude-training camp (Figure 2). The mean improvement in TT performance (\pm 90% CL) at POST1 was likely 2.1% \pm 2.1% greater in ALT than in CON. When training load was used as a covariate, change in TT was possibly 1.5% \pm 4.8% greater in ALT than in CON at POST1. The individual variation in TT performance at POST1 was 0.9% without training load used as a covariate and 0.8% when load was used as a covariate. Thirty days postdescent (POST2), improvement in TT performance in ALT had been maintained, with the change from POST1 to POST2 trivial ($-0.8\% \pm 0.9\%$).

Table 1 Training Load, Training Duration, and Rating of Perceived Exertion (Mean ± SD) in ALT and CON During the First 4 Weeks of the Intervention

	ALT (n = 21)	CON (n = 9)	% Chances for ALT to be greater/similar/smaller than the SWC compared with CON
Training load (arbitrary units)			
preintervention week	2260 ± 571	2553 ± 952	25/8/67 (CON possibly higher)
week 1	4765 ± 685	2961 ± 382	100/0/0 (ALT almost certainly higher)
week 2	4962 ± 313	3771 ± 848	99/0/0 (ALT very likely higher)
week 3	4536 ± 240	3631 ± 477	100/0/0 (ALT almost certainly higher)
Training duration (min)			
preintervention week	536 ± 118	446 ± 157	87/4/9 (ALT likely higher)
week 1	702 ± 95	562 ± 59	100/0/0 (ALT almost certainly higher)
week 2	748 ± 41	663 ± 115	92/4/4 (ALT likely higher)
week 3	597 ± 9	623 ± 29	0/11/88 (CON likely higher)
Rating of perceived exertion			
preintervention week	4.84 ± 0.58	5.38 ± 0.68	1/4/95 (CON likely higher)
week 1	6.97 ± 0.26	5.01 ± 0.75	100/0/0 (ALT almost certainly higher)
week 2	6.35 ± 0.13	5.38 ± 0.48	100/0/0 (ALT almost certainly higher)
week 3	6.49 ± 0.38	5.64 ± 0.65	99/1/0 (ALT very likely higher)

Abbreviations: ALT, altitude-training group; CON, sea-level control group; SWC, smallest worthwhile change.

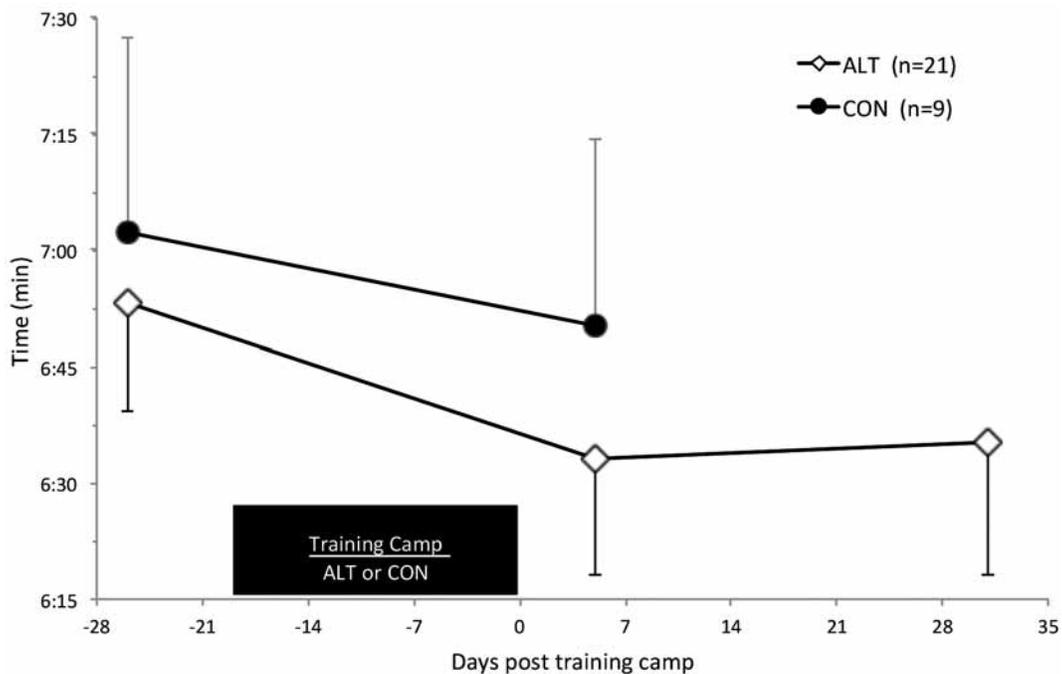


Figure 2 — Performance in 2000-m time-trial running at sea level (mean ± SD). Abbreviations: ALT, altitude-training group; CON, sea-level control group.

Hb_{mass} and Reticulocytes

The mean (\pm SD) Hb_{mass} in the ALT and CON groups before the intervention were 992 ± 129 g and 980 ± 151 g, respectively. Percentage change in Hb_{mass} ($\% \Delta$ Hb_{mass}) from baseline is displayed in Figure 3. At POST1, mean $\% \Delta$ Hb_{mass} (\pm 90% CL) was very likely increased in ALT ($3.6\% \pm 1.6\%$), while changes in CON were trivial ($0.5\% \pm 2.4\%$). The changes in Hb_{mass} at POST1 were likely $2.8\% \pm 3.5\%$ greater in ALT than in CON. When training load was used as a covariate, the mean $\% \Delta$ Hb_{mass} was possibly $2.2\% \pm 7.7\%$ higher in ALT than in CON. The individual variation in Hb_{mass} at POST1 was 1.7% without training load used as a covariate and 1.3% (90% CL -4.2 to 4.8) when load was used as a covariate. Thirty days after the camp, Hb_{mass} was not likely different from PRE in ALT ($0.3\% \pm 1.6\%$). A Pearson correlation revealed a significant negative correlation ($R = -.484$, $P = .036$) between initial Hb_{mass} relative to body mass (RelHb_{mass}) and $\% \Delta$ Hb_{mass} postaltitude exposure in ALT (2 subjects who reported illness throughout the study removed from this analyses). Reticulocytes were almost certainly lower in ALT than in CON at POST1 ($-26.2\% \pm 8.6\%$) and were almost certainly lower than PRE at POST2 in ALT ($-64.5\% \pm 12.6\%$).

Intramuscular Metabolites

Intramuscular carnosine of ALT was almost certainly $35.5\% \pm 15.0\%$ higher in the gastrocnemius (34.9 ± 8.6 AU) than in the soleus (22.4 ± 5.2 AU) at PRE. Changes in carnosine were trivial from PRE to POST1 in gastrocnemius ($3.9\% \pm 9.0\%$) and unclear in soleus (-1.6 ± 13.1). The pooled carnitine and choline was likely $26.3\% \pm 15.5\%$ lower in gastrocnemius (228 ± 60 AU) than in soleus (283 ± 50 AU) at PRE. Pooled carnitine and choline was likely increased in soleus ($8.8\% \pm 6.1\%$) but trivial in gastrocnemius from PRE to POST1 ($0.6\% \pm 14.1\%$).

Discussion

The main finding of the current study is that professional team-sport athletes undertaking an LHTH preseason training camp at moderate altitude had $\sim 1.5\%$ greater improvements in running performance than matched controls living and training at sea level. These performance improvements were accompanied by $\sim 3\%$ increase in Hb_{mass} not observed in control subjects. Changes in Hb_{mass} returned to baseline 4 weeks postaltitude, but improvements in running performance were maintained. A novel finding of the current study is that there was no clear change in intramuscular carnosine concentration after altitude exposure, suggesting that changes in this intramuscular protein may not be responsible for previously reported changes in muscle buffering capacity⁶ or that the changes were too small for magnetic resonance spectroscopy (MRS) measurements to identify.

Performance

The $\sim 1.5\%$ greater improvement in running performance in ALT over that observed in CON subjects in the current investigation is of similar magnitude to improvements seen in endurance athletes after altitude-training interventions.^{4,21} However, the individual variability in this study was approximately half of the mean change in TT performance, which is less than previously reported in endurance athletes.²¹ This suggests that team-sport athletes may experience more consistent improvements in performance than endurance athletes after an altitude intervention, which may be related to some endurance athletes' approaching their physiological limits, with only small opportunities for improvements. It has previously been suggested that, after altitude exposure, athletes are able to train at higher intensities, thereby increasing the training stimuli and consequent improvements in performance.²² As improvements in running performance were maintained for at least 4 weeks postdescent in our subjects, we propose that an altitude-training camp may positively influence subsequent preseason training in team-sport athletes, thus improving preparation leading into the competitive season.

Hb_{mass}

A change in the oxygen-carrying capacity of the blood is often proposed as the major physiological adaptation leading to improved performance after altitude exposure.⁴ In the current study, athletes achieved a mean increase in Hb_{mass} of 3.6%, which is similar to changes observed in endurance cyclists after 19 days residing at 2760 m.²³ Hb_{mass} returned to baseline 30 days postdescent, which is also similar to the results of Garvican et al,²³ suggesting that team-sport athletes can achieve improvements in Hb_{mass} after an altitude intervention comparable to those observed in endurance athletes and that such changes follow a similar time course with ascent to altitude and descent to sea level. The depression of reticulocytes on return to sea level in the ALT group provides additional evidence of accelerated erythropoiesis after altitude exposure. Pottgiesser et al²⁴ observed an $\sim 20\%$ depression of reticulocytes 9 days after 26 nights spent at 3000 m, which is similar to the 26% depression we observed 7 days postaltitude.

Although it is commonly accepted that prolonged exposure to hypoxic conditions can elicit increases in Hb_{mass}, it is also acknowledged that there is high variability in this response between individuals.^{21,25} Similar to previous findings,²¹ our data demonstrate high inter-individual variability, with individual responsiveness in Hb_{mass} approximately half the magnitude of the mean change in ALT. The causes of such variability between individuals are not well understood and may be related to a number of factors. The proinflammatory cytokine interleukin 1 (IL-1) suppresses the release of erythropoietin,²⁰ so athletes experiencing infections that lead to increases in IL-1 before or during an altitude camp may

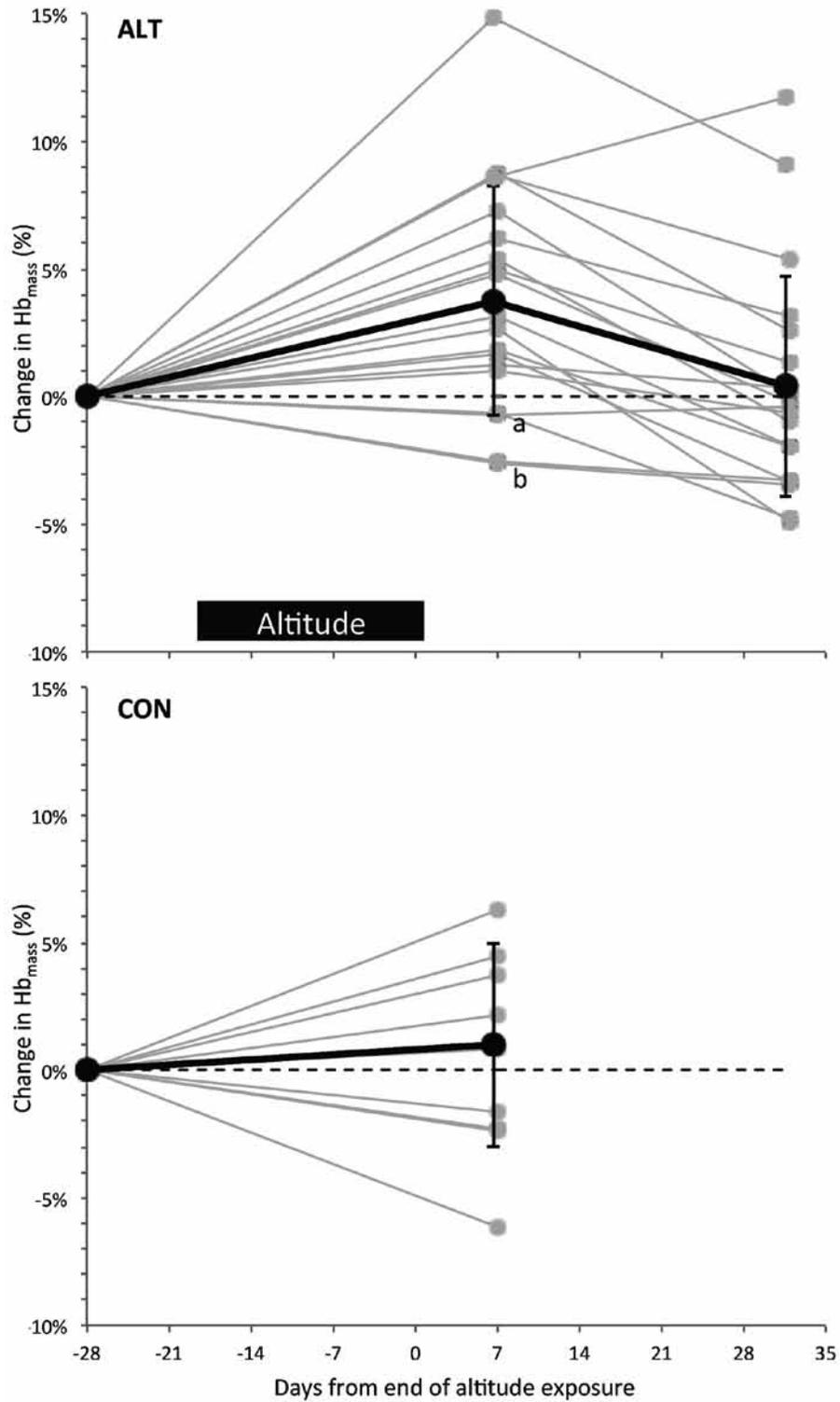


Figure 3 — Changes in hemoglobin mass (Hb_{mass}) after altitude exposure (ALT, $n = 21$) or control conditions (CON, $n = 9$). Black circles and error bars show group change (%) as mean \pm SD, and gray circles show individual responses. The letters *a* and *b* depict responses of 2 subjects who reported illness before (*b*) and during (*a*) altitude exposure.

have a blunted erythropoietic response. This is supported by data from the current study in which 2 athletes reported illness, either before or during altitude exposure, and neither athlete demonstrated an increase in Hb_{mass} after the altitude-training camp ($\Delta Hb_{mass} = -0.8\%$ and -2.7% , see Figure 3).

However, there is still large variability in the responses of our other subjects. Some have proposed that athletes starting with high initial Hb_{mass} may have a limited ability to stimulate further increases.²⁶ Robach and Lundby²⁶ combined data from 9 altitude-training studies involving elite endurance athletes and found an inverse correlation between initial $RelHb_{mass}$ and change in Hb_{mass} ($R = .86$, $P < .01$). Our results also show a significant inverse correlation between initial $RelHb_{mass}$ and percentage change in Hb_{mass} after altitude exposure, but the magnitude was only about half that of Robach and Lundby.

Intramuscular Metabolites

While increased erythropoiesis appears to be an important adaptation with altitude exposure, it cannot completely account for increases in performance after such exposures, even in situations where the strongest relationships are observed.⁴ Nonhematological adaptations may also play a role in improved performance,³ including increases in muscle buffering capacity.^{5,6,8} However, such findings are not universal,²⁷ and none of the aforementioned studies have been able to elucidate the mechanism responsible for this adaptation. Despite this, Saltin et al⁶ proposed that changes in muscle buffering capacity after altitude training may be due to changes in intramuscular carnosine, a hypothesis further supported by others.⁸ The current study is the first to assess intramuscular carnosine levels before and after an altitude-training intervention. However, we found no clear changes in carnosine content in the gastrocnemius or soleus after the altitude intervention. This finding may be due to the availability of β -alanine, which limits carnosine synthesis.¹⁰ If altitude exposure were to alter carnosine concentrations within skeletal muscle, such exposure would need to alter the availability of β -alanine, but there is no apparent mechanism for this to occur during altitude exposure. The current results, combined with the current understanding of factors limiting carnosine synthesis, would suggest that changes in carnosine are not responsible for previously observed changes in muscle buffering capacity, although muscle buffering capacity was not directly measured in the current study.

An interesting finding in our data was an increase in the carnitine/choline peak observed in soleus after the intervention period. The mechanism for such an increase is unclear, but as pooled carnitine and choline increased only in soleus, and not in gastrocnemius, we propose that these increases are related to changes in the mitochondria, which are found in higher concentrations in soleus due to the greater proportion of type I muscle fibers.²⁸ Carnitine acts as an acceptor for the acetyl groups by

forming acetylcarnitine when the rate of acetyl coenzyme A formation from glycolysis is high during high-intensity exercise.²⁹ Therefore, we propose that possible increases in carnitine may be related to improved capacity for high-intensity exercise. Further research is needed to fully understand this finding, including whether this change was due to a training effect or altitude exposure, as the control group was not included in the MRS analysis in this study.

Limitations

One limitation in this study was the difference in training load observed between groups. However, although ALT subjects completed higher training loads than controls during the intervention period, covariate analyses suggest that improvements in performance and changes in Hb_{mass} were still greater in ALT subjects when controlling for training load. Furthermore, using RPE-based methods to assess training load during LHTH interventions may overestimate the mechanical and physiological training load. When training in hypoxic conditions, RPE is higher than in similar training completed in normoxic conditions, even when training velocities and markers of physiological load (eg, oxygen consumption and heart rate) are lower.³⁰ RPE was higher in the ALT subjects throughout the intervention period, and it is not possible to determine if this is merely due to perception of effort or if the ALT group actually completed higher-intensity training throughout this period. Although differences in RPE may account for some of the observed differences in training load, training duration was also $\sim 12\%$ higher in the ALT group.

Possible placebo, nocebo, and training-camp effects also cannot be eliminated from having an influence on the current results. However, while placebo and training-camp effects may confound scientific results, these changes are nevertheless of interest to practitioners looking to achieve the best possible performance outcomes during the preseason period.

Practical Applications

The current investigation was conducted in an ecologically valid environment with professional team-sport athletes; therefore, the findings have strong practical relevance. Our results show improved running performance in team-sport athletes completing a preseason LHTH intervention. Altitude exposure also increased Hb_{mass} , and this benefit may be greatest in athletes starting with lower initial $RelHb_{mass}$. Such physiological changes may lead to improved quality of training on returning to sea level, and although we found performance benefit for a month, practitioners should not expect physiological changes to be maintained throughout the duration of a team-sport season. Athletes experiencing illness leading into or during an altitude-training camp are also unlikely to see erythropoietic benefit from the altitude exposure.

Conclusions

Team-sport athletes completing a 19-day altitude-training camp experience greater improvements in running performance than athletes completing similar training at sea level. Improvements in running performance are maintained for at least 4 weeks postdescent and may therefore lead to improved quality of training throughout this period, thereby improving preparation for competition. Therefore, we conclude that a preseason altitude-training camp is a worthwhile intervention for improving running performance in elite team-sport athletes.

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APPENDIX III – STUDY 2 PUBLICATION

REFERENCE

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OPEN ACCESS

Year-to-year variability in haemoglobin mass response to two altitude training camps

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ABSTRACT

Aim To quantify the year-to-year variability of altitude-induced changes in haemoglobin mass (Hb_{mass}) in elite team-sport athletes.

Methods 12 Australian-Footballers completed a 19-day (ALT1) and 18-day (ALT2) moderate altitude (~2100 m), training camp separated by 12 months. An additional 20 participants completed only one of the two training camps (ALT1 additional n=9, ALT2 additional n=11). Total Hb_{mass} was assessed using carbon monoxide rebreathing before (PRE), after (POST₁) and 4 weeks after each camp. The typical error of Hb_{mass} for the pooled data of all 32 participants was 2.6%.

A contemporary statistics analysis was used with the smallest worthwhile change set to 2% for Hb_{mass} .

Results POST₁ Hb_{mass} was very likely increased in ALT1 ($3.6\pm 1.6\%$, n=19; mean \pm ~90 CL) as well as ALT2 ($4.4\pm 1.3\%$, n=23) with an individual responsiveness of 1.3% and 2.2%, respectively. There was a small correlation between ALT1 and ALT2 ($R=0.21$, $p=0.59$) for a change in Hb_{mass} , but a moderately inverse relationship between the change in Hb_{mass} and initial relative Hb_{mass} (g/kg ($R=-0.51$, $p=0.04$)).

Conclusions Two preseason moderate altitude camps 1 year apart yielded a similar (4%) mean increase in Hb_{mass} of elite footballers, with an individual responsiveness of approximately half the group mean effect, indicating that most players gained benefit. Nevertheless, the same individuals generally did not change their Hb_{mass} consistently from year to year. Thus, a 'responder' or 'non-responder' to altitude for Hb_{mass} does not appear to be a fixed trait.

INTRODUCTION

High variability in physiological and performance responses exists between individuals following live high, train high (LHTH) and live high, train low (LHTL) altitude training. It has been proposed that some individuals respond better than others, possibly due to inherent genetic traits, and that 'responders' and 'non-responders' might explain the high-variability in adaptations to altitude training.¹⁻⁵ Indeed, Chapman *et al*⁴ retrospectively classified a group of distance runners as 'responders' or 'non-responders', based on their change in 5000 m time trial performance, following 28 days of LHTL. These authors⁴ reported that athletes who improved their time trial also exhibited the greatest erythropoietin (EPO) response and subsequent changes in red cell volume and VO_{2max} .

If the classification of 'responder' and 'non-responder' is a fixed trait, possibly related to underlying genetics, individual athletes should respond similarly whenever undergoing altitude exposure of

similar duration and altitude. Only two studies until now^{3,5} have investigated the repeatability of responses to altitude. Robertson *et al*³ followed eight highly-trained runners during two blocks of hypoxic exposure (3 weeks LHTL), separated by a 5-week washout period, and reported reproducible group mean increases for VO_{2max} (~2.1%) and haemoglobin mass (Hb_{mass} (~2.7%)), but not for mean changes in time trial performance (+0.5% and -0.7% following exposures 1 and 2, respectively). Moreover, there was a moderate but unclear negative correlation for change in Hb_{mass} from one exposure to the next,³ demonstrating high variability in individual responsiveness between exposures. Similarly, Wachsmuth *et al*⁵ reported a weak correlation ($r=0.379$, $p=0.160$) between Hb_{mass} responses following two altitude training camps conducted approximately 3 months apart with elite swimmers, despite very reproducible increases in serum EPO ($R=0.95$, $p<0.001$).

Team-sport athletes often complete preseason altitude training camps in an attempt to optimise running performance and we recently reported improved running performance (~1.5% above matched sea level control) accompanied by a 3.6% increase in Hb_{mass} following 19 days of LHTH in elite team-sport athletes.⁶ Similar to previous investigations,^{1,2} we reported high variability in erythropoietic responses, which may be partially explained by levels of initial Hb_{mass} ⁷; however, much of this variability remains unexplained. The possible identification of 'responders' and 'non-responders' after a single altitude training camp, or after an acute hypoxic exposure, may allow prescription of altitude training to be targeted towards those athletes who 'respond' well, and/or alternative methods/altitudes adopted in an attempt to enhance the response of potential 'non-responders'.

Therefore, the primary aim of this study was to examine the variability of physiological responses between two similar LHTH preseason training camps in professional Australian-Football players. A secondary aim was to identify potential factors that may influence responsiveness to altitude exposure, such as preintervention Hb_{mass} , energy balance and health status. It was hypothesised that there would be a moderately strong correlation between the magnitude of physiological changes from exposure 1 to exposure 2.

METHODS

Subjects

Twelve Australian-Football players completed a 19-day (ALT1) and 18-day (ALT2) moderate altitude (~2100 m) training camp, separated by



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12 months. Results from ALT1 have been previously reported.⁶ An additional 20 participants completed only one of the two training camps (ALT1—additional n=9, ALT2—additional n=11). All training and testing took place during the Australian Football League preseason from November to January, following a 6-week, off-season break. All participants provided written informed consent. All participants were supplemented with oral ferrous sulfate (325 mg/day) throughout the intervention period, and athletes identified as having low serum ferritin pre-intervention (serum ferritin ≤ 30 $\mu\text{g/L}$; n=3) were given a single, 2 mL ferrum H injection (Aspen Pharmacare, St Leonards, Australia; equivalent to 100 mg of iron) prior to the altitude camp.

Training

During ALT1, participants completed an 8-week training block, including 19 days living and training at moderate altitude in Flagstaff, Arizona, USA (elevation ~ 2100 m). One year later, participants completed a similar 8-week training block, including 18 days living and training at moderate altitude in Park City, Utah, USA (elevation ~ 2000 m). Both training blocks included endurance training, resistance exercise and football specific training (see table 1). All training was prescribed by team coaches and monitored through session rating of perceived exertion (RPE).⁸ This method calculates a total load (arbitrary units (AU)) by multiplying the session-RPE (Borg's Category Ratio 10-Scale) by the session duration, which is a valid method to quantify training loads in high-intensity, intermittent team sports.⁹

Haematological measures

All participants' Hb_{mass} was measured using the optimised carbon monoxide (CO) rebreathing technique¹⁰ 5 days (D₋₅) before altitude exposure (PRE see figure 1). Hb_{mass} was measured again one (D₁), 13 (D₁₃) and 17 days (D₁₇/POST₁) after ascent during ALT2 only, and 5 days postdescent (POST₁) in ALT1 only. Hb_{mass} was measured again 28 days postdescent in both groups (POST₂). Change in Hb_{mass} ($\Delta\text{Hb}_{\text{mass}}$) was calculated from PRE in ALT1, and from the mean of D₋₅ and D₁ in ALT2. Briefly, participants rebreathed a bolus of 99% CO equivalent to 1.0 mL/kg of body mass through a glass spirometer (BloodTec, Bayreuth, Germany) for 2 min. Per cent carboxyhaemoglobin (%HbCO) in fingertip capillary blood was measured using the same OSM3 hemoximeter (Radiometer, Copenhagen, Denmark) before and 7 min after administration of the CO dose. CO doses administered at altitude were

adjusted for changes in the partial pressure (doses at ~ 2000 m equivalent to 1.3 mL/kg). Six repeat measures of %HbCO were made for improved precision in Hb_{mass} estimation.¹¹ All Hb_{mass} measurements were performed by the same technician. Venous blood samples were collected at PRE, 3 days (D₃) and 16 days (D₁₆) after ascent and 13 days postdescent (POST_{D13}) in ALT2 only. Samples were centrifuged, serum decanted and frozen at -80°C , and transported to Canberra, Australia for analysis of serum EPO (for ALT2 only), in one batch, using an automated solid-phase, sequential chemiluminescent Immulite assay (Diagnostics Product Corporation, Los Angeles, USA). PRE and POST_{D13} samples were analysed for reticulocyte count using a Sysmex XE-5000 Automated Haematology Analyser (Roche Diagnostics, Castle Hill, Australia) at St Vincent's Hospital Pathology (Fitzroy, Australia), while D₃ and D₁₆ samples were analysed using a Sysmex XT-4000i Automated Haematology Analyser (Sysmex, Lincolnshire, USA) at Park City Medical Centre (Park City, USA). Serum ferritin was assessed at PRE using an AU5800 immuno-turbidimetric assay (Beckman Coulter, Lane Cove, Australia) to identify iron-deficient participants.

Body mass and illness

Body mass was monitored daily on waking (07:00–08:00) in a well hydrated state (confirmed through urine specific gravity measures (URC-N_E, Atago, Tokyo, Japan)) throughout the intervention period using electronic scales (Tanita, Kewdale, Australia). Changes in body mass were calculated from the first to the last day at altitude during both camps. Athletes were classified as 'ill' if any training session was missed throughout the intervention period due to physical illness, excluding musculo-skeletal injuries.

Statistical analysis

A magnitude-based statistical approach¹² was used to detect small effects of practical importance. Data were log-transformed to account for non-uniformity error. Differences within and between groups were assessed with dependent and independent t tests for unequal variance.¹² The magnitude of changes were assessed in relation to the smallest worthwhile change (SWC), set to 2% for Hb_{mass} and a small effect size ($d=0.2 \times$ the between-participant SD for PRE) for other variables. Observed effects were reported as the mean change or difference $\pm 90\%$ confidence limits. Effects were termed positive, trivial or negative depending on the magnitude of the change relative to the SWC and assigned a qualitative descriptor according to the likelihood of the change exceeding the SWC: 50–74% 'possible', 75–94% 'likely', 95–99% 'very likely', $>99\%$ 'almost certainly'.¹³ Effects where the 90% CI overlapped simultaneously the substantially positive and negative thresholds were deemed 'unclear'. The magnitude of individual responses were calculated from the square root of the difference in the variance of the change scores.¹⁴ A Pearson's correlation coefficient was used to examine the relationship between percentage change in Hb_{mass} ($\%\Delta\text{Hb}_{\text{mass}}$) from ALT1 to ALT2 and the relationship between initial Hb_{mass} and change in Hb_{mass}. Because one participant's results appeared to heavily influence the relationship from year to year, a Pearson's correlation was also performed after removing these data. Participants who reported illness (ALT1 n=2, ALT2 n=3, total n=5) were removed from correlation analyses, as proinflammatory cytokines suppress EPO production.¹⁵ Participants were also retrospectively divided into groups classified as BodyMass_{stable} (gained mass or lost <2.0 kg), or BodyMass_{loss} (lost >2.0 kg).

Table 1 Typical training week during intervention period

Day	1	2	3	4	5	6
AM		Football	Technical	Football	Football	Resistance
PM	Cross train	Resistance	Resistance	Cross train		Hike
Day 7	8	9	10	11	12	
AM	Hike	Football	Resistance		Football	
PM			Cross Train		Resistance	Cross train
Day 13	14	15	16	17	18	
AM	Football	Resistance	Football	Resistance	Resistance	Football
PM	Resistance	Technical		Technical	Cross train	

Football=Australian-Football specific skills and running (90–120 min), resistance=strength training (40–70 min), cross train=non-specific training (eg, swimming, cycling and boxing; 20–60 min), technical=skills based Australian-Football session (light intensity; 20–60 min), hike=outdoor recreational hiking (120–240 min).

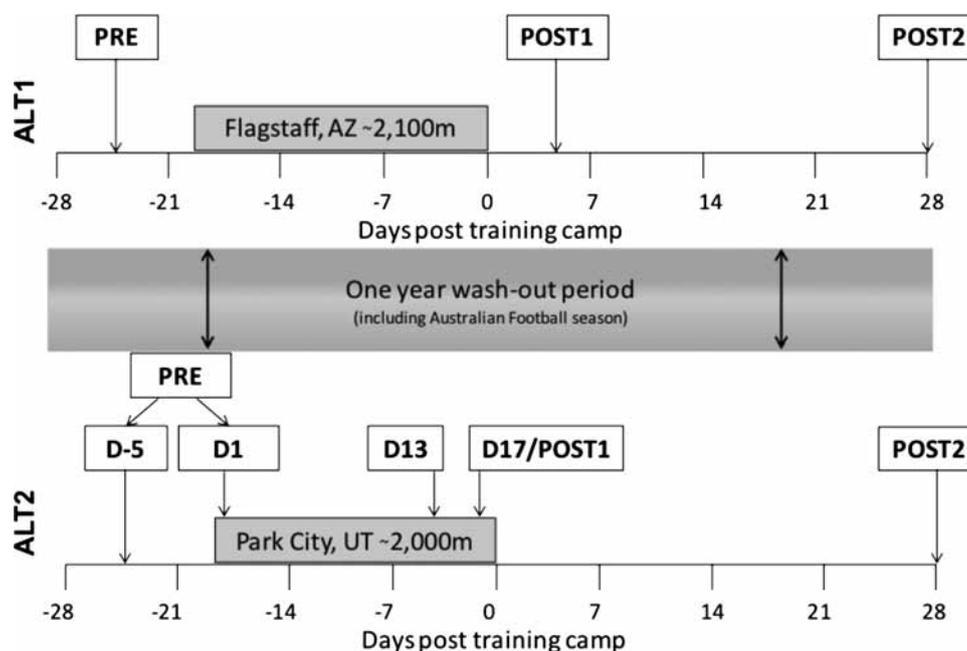


Figure 1 Timeline of haemoglobin mass (Hb_{mass}) collection over ALT1 and ALT2.

RESULTS

Training load, duration and RPE

Weekly training load, duration and RPE for all participants are presented in table 2A. The overall training load was *very likely* $5.5 \pm 4.8\%$ (mean $\pm 90\%$ CI) higher and mean RPE *almost certainly* $7.2 \pm 2.6\%$ higher in ALT1 compared to ALT2. Training duration was *very likely* $12.6 \pm 3.6\%$ higher in ALT2 compared to ALT1.

The weekly training load, duration and RPE for healthy repeat participants ($n=9$) are presented in table 3. There was an unclear $0.5 \pm 3.5\%$ difference in the overall training load between ALT1 and ALT2. The overall mean RPE was *very likely* $5.1 \pm 2.8\%$ lower and training duration was *almost certainly* $13.5 \pm 2.4\%$ higher in ALT2 compared to ALT1.

Haemoglobin mass

Hb_{mass} (all participants): The typical error of Hb_{mass} for the pooled data of all 32 participants, for measures made

approximately 5 days apart, was 2.6% (90% CI of 2.1% to 3.3%). Mean (\pm SD) Hb_{mass} in ALT1 ($n=21$) and ALT2 ($n=23$) groups preintervention was 992 ± 129 and 1008 ± 159 g, respectively. $\% \Delta Hb_{mass}$ from baseline is displayed in figure 2. Mean $\% \Delta Hb_{mass}$ ($\pm 90\%$ CL) was *very likely* increased at $POST_1$ in ALT1 ($3.6 \pm 1.6\%$, with individual responsiveness of 1.3%) and *almost certainly* increased at D_{13} ($3.9 \pm 1.0\%$) and D_{17} ($4.0 \pm 1.3\%$, with individual responsiveness of 2.2%) in ALT2. Differences in $\% \Delta Hb_{mass}$ between ALT1 and ALT2 from PRE to postintervention ($POST_1$ and D_{17} during ALT1 and ALT2, respectively) were *unclear* ($-1.3 \pm 7.0\%$). Hb_{mass} returned to baseline at 4 weeks postaltitude in ALT1 and ALT2. There was a negative correlation ($R=-0.51$, $p=0.04$; see figure 3) between initial Hb_{mass} relative to body mass ($RelHb_{mass}$) and $\% \Delta Hb_{mass}$ at $POST_1$ in healthy $BodyMass_{stable}$ participants ($n=30$).

Hb_{mass} (participants completing ALT1 and ALT2): There was a *trivial* difference in Hb_{mass} at PRE for ALT1 (1023 ± 143 g)

Table 2 Training load, training duration and rate of perceived exertion (mean \pm SD) during ALT1 and ALT2

	ALT1 (n=21)	ALT2 (n=23)	Per cent chances for ALT1 to be greater/similar/smaller than the SWC compared with ALT2
Training load (arbitrary units)			
Preintervention week	2260 \pm 571	2668 \pm 488	2/14/84 (ALT2 <i>very likely</i> higher)
Week 1	4765 \pm 685	4861 \pm 695	42/43/15 (<i>Unclear</i> difference)
Week 2	4962 \pm 313	4790 \pm 538	74/24/3 (ALT1 <i>possibly</i> higher)
Week 3	4536 \pm 240	3714 \pm 194	100/0/0 (ALT1 <i>almost certainly</i> higher)
Training duration (min)			
Preintervention week	536 \pm 118	705 \pm 128	0/0/100 (ALT2 <i>almost certainly</i> higher)
Week 1	702 \pm 95	885 \pm 59	0/0/100 (ALT2 <i>almost certainly</i> higher)
Week 2	748 \pm 41	697 \pm 37	0/0/100 (ALT1 <i>almost certainly</i> higher)
Week 3	597 \pm 9	620 \pm 12	0/0/100 (ALT2 <i>almost certainly</i> higher)
Rate of perceived exertion			
Preintervention week	4.84 \pm 0.58	5.37 \pm 0.51	0/0/100 (ALT2 <i>almost certainly</i> higher)
Week 1	6.97 \pm 0.26	5.48 \pm 0.58	100/0/0 (ALT1 <i>almost certainly</i> higher)
Week 2	6.35 \pm 0.13	6.12 \pm 0.49	92/7/1 (ALT1 <i>very likely</i> higher)
Week 3	6.49 \pm 0.38	5.94 \pm 0.28	100/0/0 (ALT1 <i>almost certainly</i> higher)

Table 3 Training load, training duration and rate of perceived exertion (mean±SD) for the nine participants completing both ALT1 and ALT2

	ALT1	ALT2	Per cent chances for ALT1 to be greater/similar/smaller than the SWC compared with ALT2
Training load (arbitrary units)			
Preintervention week	2497±349	2838±201	1/2/97 (ALT2 <i>very likely</i> higher)
Week 1	5138±150	5091±323	49/31/20 (<i>Unclear</i> difference)
Week 2	5002±116	4929±337	45/47/8 (<i>Unclear</i> difference)
Week 3	3833±257	3711±185	75/18/7 (ALT2 <i>possibly</i> higher)
Training duration (min)			
Preintervention week	550±93	765±80	0/0/100 (ALT2 <i>almost certainly</i> higher)
Week 1	730±15	902±10	0/0/100 (ALT2 <i>almost certainly</i> higher)
Week 2	763±8	696±39	0/0/100 (ALT1 <i>almost certainly</i> higher)
Week 3	625±13	597±21	0/0/100 (ALT1 <i>almost certainly</i> higher)
Rate of perceived exertion			
Preintervention week	4.71±0.57	5.56±0.28	100/0/0 (ALT2 <i>almost certainly</i> higher)
Week 1	6.17±0.19	5.74±0.38	99/1/0 (ALT1 <i>very likely</i> higher)
Week 2	6.36±0.13	6.17±0.37	85/11/3 (ALT1 <i>very likely</i> higher)
Week 3	6.59±0.35	5.94±0.30	100/0/0 (ALT1 <i>almost certainly</i> higher)

versus ALT2 (1017±135 g) in participants completing both training camps (n=12). In these participants, Hb_{mass} *very likely* increased at POST₁ in ALT1 (3.7±2.5%) and at D₁₇ in ALT2 (4.2±2.4%). A weak correlation between individual %ΔHb_{mass} was observed between ALT1 and ALT2 (n=12; R=0.12, p=0.71), which was marginally stronger when participants who reported illness in ALT2 were not included (n=9; R=0.21, p=0.59) see figure 4). After removing one participant who was influencing the results heavily, the correlation between Hb_{mass} from year to year reduced to R=0.02 (n=8, p=0.96, figure 4).

Hb_{mass} (ill vs healthy participants): figure 5A shows % ΔHb_{mass} for participants separated into healthy (n=39) and ill (n=5) groups. Ill participants had a *trivial* 0.2±2.4% change in Hb_{mass} at POST₁, which was *almost certainly* 3.9±1.1% lower than changes in healthy participants. There was a *trivial* 1.1±1.6% difference in Hb_{mass} between ill and healthy participants at POST₂.

Hb_{mass} (BodyMass_{stable} vs BodyMass_{loss} participants): Changes in body mass and Hb_{mass} and body mass for the BodyMass_{stable} (n=30) and BodyMass_{loss} (n=9) groups are presented in figure 5B, C, respectively. BodyMass_{loss} participants *very likely* lost 2.6±0.9 kg from PRE to POST₁, while BodyMass_{stable} participants had a *trivial* 0.2±1.3 kg change in body mass. BodyMass_{loss}

participants *possibly* increased in Hb_{mass} by 2.6±1.8% at POST₁, which was *possibly* 2.4±2.1% lower than the changes in the BodyMass_{stable} group. At POST₂, BodyMass_{loss} participants had a *possible* 2.3±2.7% decrease in Hb_{mass} from PRE, which was *very likely* 3.9±1.9% lower than the BodyMass_{stable} group.

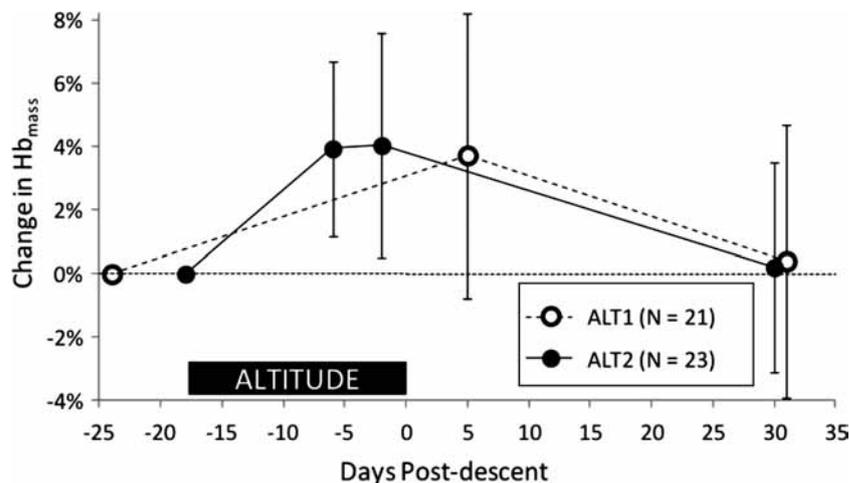
Reticulocytes and EPO—for ALT2 only

Figure 6 shows reticulocyte percentage and EPO concentrations for ALT2. Compared with PRE, reticulocytes *almost certainly* increased by 39±12% at D₃ and *very likely* increased 16±10% at D₁₆. Reticulocytes *almost certainly* reduced from PRE by 27±10% at POST_{D13}. EPO *almost certainly* increased by 36±10% and 22±10% from PRE at D₃ and D₁₆, respectively, and *almost certainly* reduced from PRE by 16±16% at POST_{D13}.

DISCUSSION

The main finding of the current investigation is that, while two preseason moderate altitude camps yield a similar group mean increase (~4%) in Hb_{mass} of elite footballers, there is wide variability in this erythropoietic response and individual athletes do not exhibit consistency in changes in Hb_{mass} from year to year.

Figure 2 Percentage changes in haemoglobin mass (Hb_{mass}) (mean ±90% CI) after altitude ALT1 and ALT2.



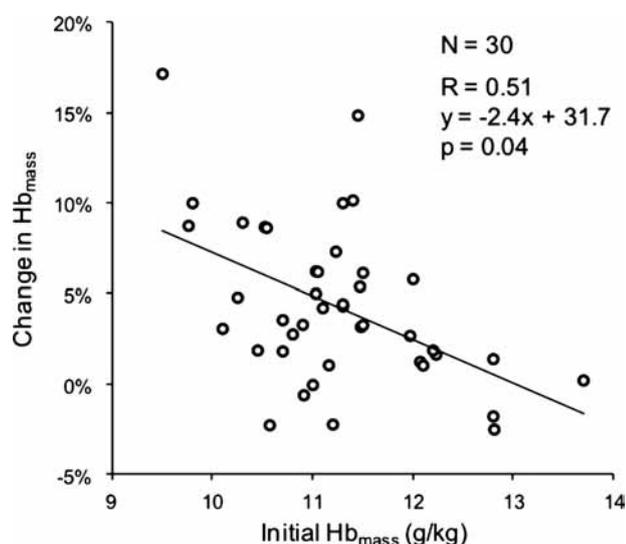


Figure 3 Change in haemoglobin mass (Hb_{mass}) versus initial RelHb_{mass} in healthy and BodyMass_{stable} participants pooled for ALT1 and ALT2 altitude camps.

Thus, a ‘responder’ or ‘non-responder’ to altitude does not appear to be a fixed trait with respect to changes in Hb_{mass}. Some of the variability in erythropoietic response may be explained by the incidence of illness, reductions in body mass and precamp Hb_{mass}, but large variability is still evident in healthy athletes who maintain body mass. Another important finding is that almost all of the erythropoietic response of an 18-day altitude camp (ALT2) occurred within the first 13 days of exposure.

Repeatability of changes in Hb_{mass}

We previously reported that elite team-sport athletes achieved a mean increase of ~3.6% in Hb_{mass} over a 19-day altitude camp.⁶ During a subsequent 18-day exposure (ALT2), our

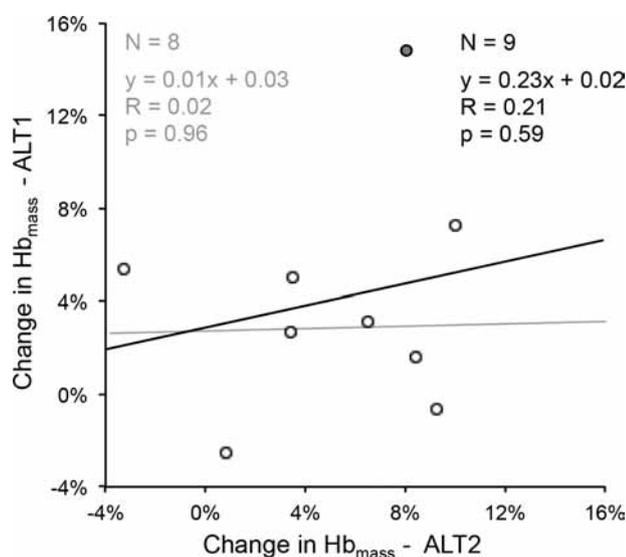


Figure 4 Change in haemoglobin mass (Hb_{mass}) during ALT1 versus change in Hb_{mass} during ALT2. Three participants were removed from this analysis due to illness throughout the study period (n=9). Grey filled circle represents an outlier; regression line and associated equations when the outlier is removed are shown in grey.

participants achieved a similar mean increase in Hb_{mass} of 4%. However, in agreement with other work,^{1 4 16} there was considerable individual variability in the erythropoietic response. Chapman *et al*⁴ introduced the concept of ‘responders’ and ‘non-responders’ to altitude when they found that participants who achieved the greatest improvements in 5000 m running performance following LHTL also demonstrated the greatest erythropoietic response. This led to suggestions that the magnitude of an individual’s response to altitude may be influenced by genetically determined traits.^{4 17} This theory proposes that individuals should respond similarly on subsequent hypoxic exposures, given the same hypoxic dose (ie, exposure time and degree of hypoxia). However, we observed a small correlation between changes in Hb_{mass} following ALT1 and ALT2 (R=0.21) in participants who completed both training camps (n=9), which was reduced to R=0.02 when the sole outlier was removed. This supports previous investigations^{3 5} that have reported weak (R~0.10–0.38) relationships between changes in Hb_{mass} following repeated altitude exposures ~1–3 months apart, suggesting that responsiveness to a given hypoxic stimulus is not a fixed trait. The ~2% typical error of the optimised CO rebreathing technique^{10 18} along with natural biological variations^{19 20} may, in part, contribute to the variability from exposure to exposure.

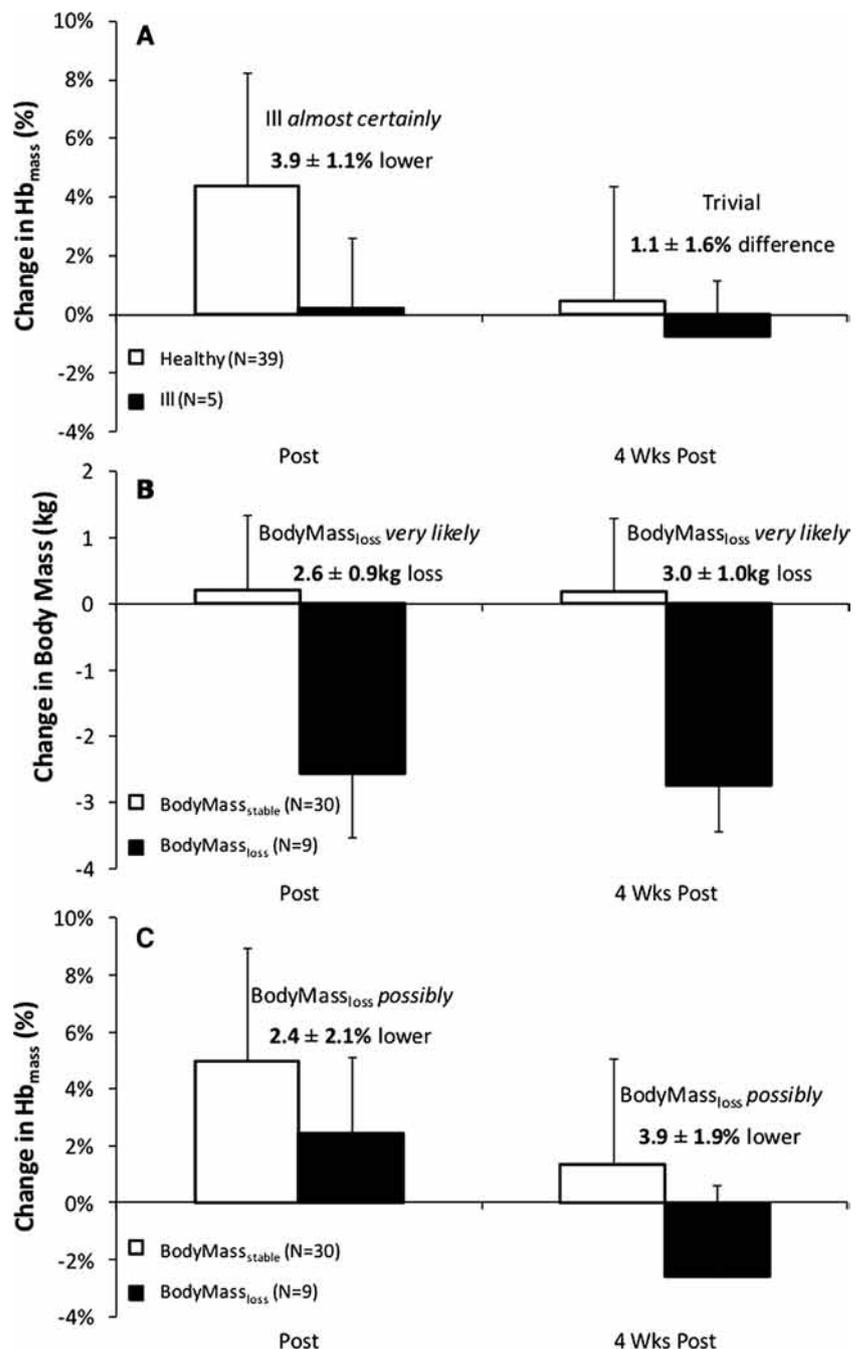
Timeline of erythropoietic response

It is well accepted that a sufficient hypoxic dose (h/day; number of days; degree of hypoxia) is required to induce detectable erythropoietic benefit.^{21 22} Wilber *et al*²¹ suggested that altitude training at 2000–2500 m for at least 22 h/day and a minimum of 4 weeks is required to optimise physiological benefits. However, these recommendations are based on data from studies that examined erythrocyte volumes pre and postaltitude exposure, with little data addressing the time course of responses. Recently, detectable increases in Hb_{mass} have been reported with exposures as short as 11²³ and 13 days⁵. Similarly, we observed an ~4% increase in Hb_{mass} after 13 days at altitude, with no additional increases by day 17. This suggests that erythropoietic benefits are possible with shorter duration altitude training camps than commonly recommended, given sufficient altitude/hypoxia.²¹ A 2-week time course may have significant implications for team-sport organisations, which often schedule altitude camps during limited preseason periods and may face financial restrictions during longer duration camps due to travel/accommodation costs for athletes and support staff.

Effect of initial RelHb_{mass}

We found a similar relationship between initial RelHb_{mass} and %ΔHb_{mass} as in our original investigation.⁶ These data support the work of Robach and Lundby,⁷ which suggests that athletes starting with low RelHb_{mass} have the ability to increase Hb_{mass} to a greater extent following hypoxic interventions. Others have proposed that athletes with initially high RelHb_{mass} (~14.7 g/kg) may have already ‘maximised’ this component of their physiological capacity through training at sea level,²⁴ with limited opportunity to further increase Hb_{mass} through altitude interventions. However, subsequent research from the same group showed that increases in Hb_{mass} can be achieved in cyclists possessing elevated initial RelHb_{mass} (~14.2 g/kg).²³ It is also possible that altitude training interventions may increase Hb_{mass} to a given individual’s physiological limit, and responsiveness may therefore vary depending on an individual’s baseline Hb_{mass}.⁵ Collectively, these data^{5–7} suggest that team-sport athletes with a

Figure 5 Change in haemoglobin mass (Hb_{mass}) in ill and healthy participants (A), as well as change in mass (B) and change in Hb_{mass} (C) in $BodyMass_{stable}$ and $BodyMass_{loss}$ groups.



higher initial $RelHb_{mass}$ may have an attenuated ability to increase Hb_{mass} with altitude training interventions.

Effect of illness on erythropoiesis

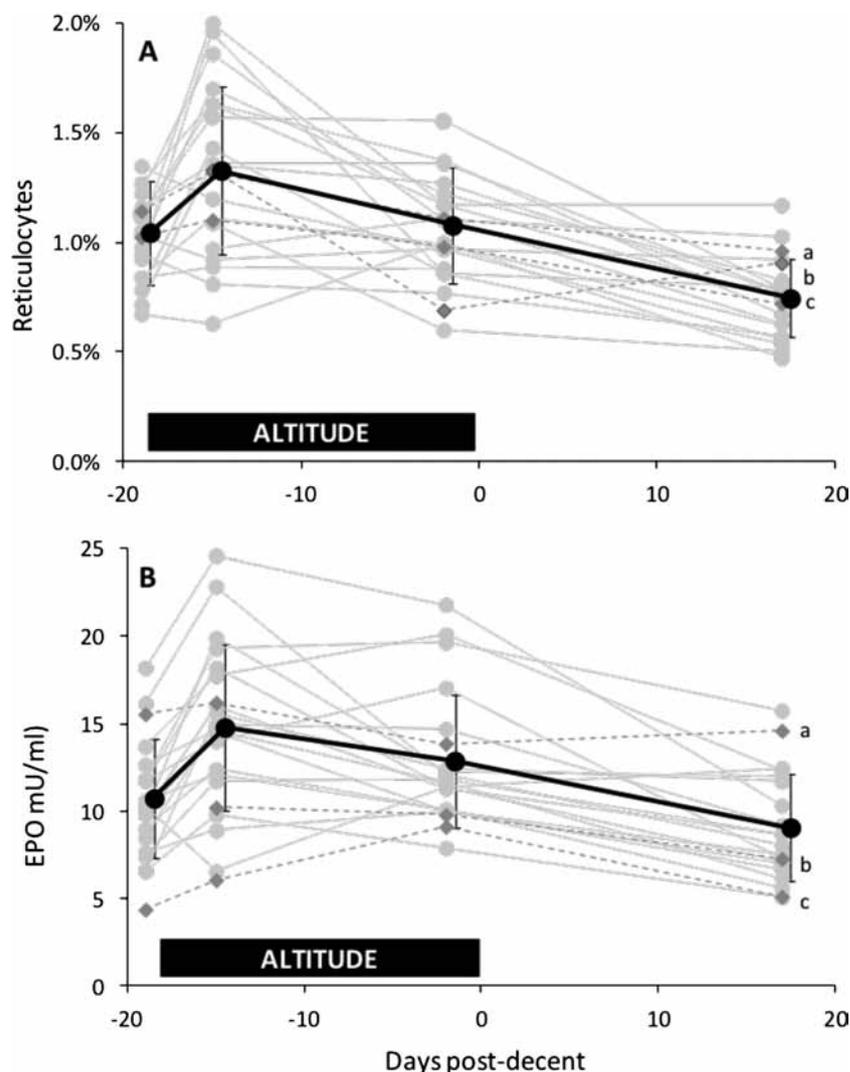
It has been suggested that athletes suffering from infection/illness/injury that results in increased inflammation may have limited erythropoietic responses to altitude exposures.^{5, 6} Indeed, Wachsmuth *et al*⁵ reported attenuated changes in Hb_{mass} in sick/injured athletes following LHTH interventions at 2320 m. Similarly, we observed an attenuated erythropoietic response in participants experiencing illness during ALT1 (n=2) and ALT2 (n=3) (see figure 5A). Proinflammatory cytokines, such as interleukin 1 (IL-1), are known to suppress the release of EPO,¹⁵ and EPO data for ill participants during ALT2 are highlighted in figure 6B. Participants (b) and (c) (in figure 6B) suffered illness immediately preceding the camp, which was accompanied by low EPO levels before and throughout the

camp duration. Participant (a) fell ill on arriving at a high altitude and displayed suppressed EPO responses thereafter. Making statistical inferences are difficult, given that instances of illness/injury are low in our investigation (n=5) and in Wachsmuth *et al*⁵ (n=7). However, these data support the hypothesis that illness/injury may limit the erythropoietic benefits associated with prolonged hypoxic exposures.

Effect of body mass reductions on erythropoiesis

Participants experiencing reductions in body mass (>2 kg) achieved approximately half of the erythropoietic benefit as those maintaining body mass (~2.5% vs ~5% increase in Hb_{mass} , respectively). Furthermore, at 4 weeks postaltitude, $BodyMass_{loss}$ participants displayed Hb_{mass} ~2.3% below prealtitude levels. Our results are in contrast with those of Gough *et al*,²⁵ who modelled that body mass changes over approximately 6 months did not significantly alter Hb_{mass} ; for instance, that a 10% loss of

Figure 6 Reticulocyte (A) and erythropoietin (EPO) (B) response during ALT2. Black circles and error bars show group change as mean \pm SD and grey circles show individual responses. Dark grey broken lines depict responses of three participants who reported illness before (b+c) and during (a) altitude exposure. NB. EPO data at PRE missing for participant (b) due to being absent from testing as a result of illness.



body mass would only decrease Hb_{mass} by 1.4%. In contrast, the 2.6 kg reduction in body mass within the Australian-Footballer subgroup over 18–19 days suggests a mismatch between energy consumption and energy expenditure and thus an overall catabolic state, which may not support an anabolic process like erythropoiesis. Evidence in maintenance haemodialysis patients with haemodialysis suggests that poor appetite and low protein intake are associated with increased serum concentrations of inflammatory markers (including C reactive protein, IL-6 and tumour necrosis factor- α) and increased synthetic EPO dose requirements.²⁶ While mechanisms for a poor erythropoietic response in $BodyMass_{loss}$ participants are not clear, the combination of altitude exposure and weight loss appears to be counter-productive for Hb_{mass} maintenance.

Limitations

This research was conducted with a relatively small sample of elite Australian-Football players. Even when using a magnitude-based statistical approach,¹² the small sample size limitations are most apparent in our attempts to quantify the effects of initial Hb_{mass} , as well as loss of body mass and of illness on the response to altitude. A further limitation is the use of two different Sysmex haematology analysers for the reticulocyte measures, since even Sysmex analysers that are calibrated within the manufacturer's tolerances can have biases of the order of 0.3–0.5% reticulocytes.²⁷ The applied nature of this research also led to a

number of limitations, which should be considered when interpreting our results. ALT1 and ALT2 were conducted in different locations over different durations (19 and 18 days, respectively). However, the altitude at these locations is very similar (~2100 m) and the overall hypoxic dose between camps differed by only 18 h. The temporal measurements of Hb_{mass} differed between ALT1 and ALT2; $POST_1$ was taken 5 days postaltitude (ALT1) compared with the penultimate day at altitude (ALT2). Therefore, the potential confounding effects of neocytolysis upon return to sea level^{23–28} during ALT1 may account for some of the variability between the two exposures.

CONCLUSION

This investigation was conducted in an ecologically valid environment, with professional team-sport athletes engaging in pre-season altitude training in an attempt to improve subsequent performance at sea level. As responsiveness to a given altitude exposure does not appear to be a fixed trait, it is not currently possible for individual athletes to be identified as 'responders' or 'non-responders' following a single altitude exposure. To optimise the erythropoietic benefit from an LHTH intervention, athletes should be well prepared, in good health and maintain body mass throughout its duration. Healthy team-sport athletes should expect a 3–4% increase in Hb_{mass} following an 18–19 day altitude training camp, with this erythropoietic response possibly achievable in as short as 13 days.

What are the new findings

- ▶ Team sport athletes produce repeatable group mean increases in Hb_{mass} (~4%) over 18–19 day moderate altitude camps, and these benefits may be achieved in as short as 13 days.
- ▶ Individual athletes do not exhibit consistency in altitude-induced changes in Hb_{mass} from year to year, and thus a 'responder' or 'non-responder' to altitude does not appear to be a fixed trait.
- ▶ To achieve full erythropoietic benefit from altitude exposure, athletes should maintain body mass and remain free from illness immediately before and throughout the exposure.

How might it impact on clinical practice in the near future?

- ▶ Athletes may gain physiological benefits from participating in shorter duration (~13 days) altitude training camps than previously recommended.
- ▶ Strategies to maintain body mass and optimal health immediately before and throughout altitude training camps should be adopted.

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Contributors BM and DB were involved in the concept and design of the study, conducted the data collection and analysis and prepared the manuscript. CG and JK were involved in the concept and design of the study, data analysis and interpretation and preparation of the manuscript. KW assisted with data collection and analysis and manuscript preparation.

Competing interests None.

Ethics approval Australian Catholic University Human Research Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

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APPENDIX IV – SYSTEMATIC REVIEW PUBLICATION

REFERENCE

McLean BD, Gore C and Kemp J. (2014) Application of ‘live low-train high’ for enhancing normoxic exercise performance in team sport athletes – a systematic review. *Sports Medicine*.

Application of ‘Live Low-Train High’ for Enhancing Normoxic Exercise Performance in Team Sport Athletes

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Justin Kemp

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Abstract

Background and Objective Hypoxic training techniques are increasingly used by athletes in an attempt to improve performance in normoxic environments. The ‘live low-train high (LLTH)’ model of hypoxic training may be of particular interest to athletes because LLTH protocols generally involve shorter hypoxic exposures (approximately two to five sessions per week of <3 h) than other traditional hypoxic training techniques (e.g. live high-train high or live high-train low). However, the methods employed in LLTH studies to date vary greatly with respect to exposure times, training intensities, training modalities, degrees of hypoxia and performance outcomes assessed. Whilst recent reviews provide some insight into how LLTH may be applied to enhance performance, little attention has been given to how training intensity/modality may specifically influence subsequent performance in

normoxia. Therefore, this systematic review aims to evaluate the normoxic performance outcomes of the available LLTH literature, with a particular focus on training intensity and modality.

Data Sources and Study Selection A systematic search was conducted to capture all LLTH studies with a matched normoxic (control) training group and the assessment of performance under normoxic conditions. Studies were excluded if no training was completed during the hypoxic exposures, or if these exposures exceeded 3 h per day. Four electronic databases were searched (PubMed, SPORTDiscus™, EMBASE and Web of Science) during August 2013, and these searches were supplemented by additional manual searches until December 2013.

Results After the electronic and manual searches, 40 papers were deemed to meet the inclusion criteria, representing 31 separate studies. Within these 31 studies, four types of LLTH were identified: (1) continuous low-intensity training in hypoxia (CHT, $n = 16$), (2) interval hypoxic training (IHT, $n = 4$), (3) repeated sprint training in hypoxia (RSH, $n = 3$) and (4) resistance training in hypoxia (RTH, $n = 4$). Four studies also used a combination of CHT and IHT. The majority of studies reported no difference in normoxic performance between the hypoxic and normoxic training groups ($n = 19$), while nine reported greater improvements in the hypoxic group and three reported poorer outcomes compared with the control group. Selection of training intensity (including matching relative or absolute intensity between normoxic and hypoxic groups) was identified as a key factor in mediating the subsequent normoxic performance outcomes. Five studies included some form of normoxic training for the hypoxic group and 14 studies assessed performance outcomes not specific to the training intensity/modality completed during the training intervention.

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Conclusion Four modes of LLTH are identified in the current literature (CHT, IHT, RSH and RTH), with training mode and intensity appearing to be key factors in mediating subsequent performance responses in normoxia. Improvements in normoxic performance appear most likely following high-intensity, short-term and intermittent training (e.g. IHT, RSH). LLTH programmes should carefully apply the principles of training and testing specificity and include some high-intensity training in normoxia. For RTH, it is unclear whether the associated adaptations are greater than those of traditional (maximal) resistance training programmes.

1 Introduction

Over the past decade, the use of hypoxic training techniques has become increasingly popular in team sports [1]. Most commonly, athletes will both live and train at moderate to high altitude [live high-train high (LHTH)] or live at moderate to high altitude whilst training closer to sea-level [live high-train low (LHTL)]. These two techniques require relatively long exposure times (>12 h/day for a minimum of 2 weeks) to accumulate a sufficient ‘hypoxic dose’ to attain the associated physiological benefits [2, 3], which is often achieved by team sport athletes during a pre-season training camp at altitude [4] or by sleeping in hypoxic chambers [5]. Implementing such techniques in-season is more challenging, as weekly competition does not allow for 2-week-long training blocks at altitude, meaning that long exposures are only possible if teams have access to hypoxic sleeping chambers near their training base.

An alternative hypoxic training technique gaining popularity with team sports [6] is the ‘live low-train high (LLTH)’ model of hypoxic training. This technique involves athletes living in normoxic conditions and performing some training sessions under hypoxic conditions. Such hypoxic exposures typically last <3 h, two to five times per week and, therefore, do not provide a sufficient hypoxic stimulus [2] to induce the haematological changes associated with LHTH and LHTL protocols. Another short-duration (<3 h) hypoxic technique is intermittent hypoxic exposure (IHE) where no training is performed during exposure sessions; but a comprehensive review concluded that IHE alone does not lead to sustained physiological adaptations or improved exercise performance [7]. Conversely, a meta-analysis [8] concluded that IHE may improve performance in sub-elite athletes, but these effects are not evident in elite athletes, possibly owing to the fact that elite athletes experience more hypoxia in their muscles from higher intensities of training compared with sub-elite athletes [8].

Although the physiological and performance effects of IHE appear minimal for elite athletic populations, there is evidence to suggest that short-term exposure that includes some form of physical training (i.e. LLTH) has the ability to enhance glycolytic enzymes, glucose transport and pH regulation [9, 10], and may also improve anaerobic power production [11] and repeated sprint ability [12]. It has also been proposed recently that hypoxic stimuli may be used to enhance adaptations gained from resistance training [13–15]. Given that LLTH techniques deliver hypoxia only during training times, athletes involved in regular competition (e.g. weekly team sport competition) may be able to use this additional environmental stimulus to enhance the training process throughout the in-season period.

Three recent reviews [1, 7, 16] and one meta-analysis [8] provide some insight into the potential applications of hypoxic training techniques, and how these might be applied in an attempt to enhance performance in normoxic environments. However, these reviews do not discuss in detail the effect of training modality/intensity during LLTH protocols, which may be important variables that affect performance outcomes following such interventions [17]. Therefore, the aim of this systematic review was to examine the LLTH literature to assess the efficacy of this technique, with a particular focus on training modality/intensity, and how these findings may be applied to enhance team sport (normoxic) performance.

2 Methods

2.1 Data Sources and Searches

A systematic review of the literature was performed from the earliest record up to August 2013. An electronic literature search was performed using four online databases—PubMed, SPORTDiscusTM, EMBASE and Web of Science. The following terms were searched for in ‘all fields’—[(hypoxic OR hypoxic strength OR hypoxia OR altitude OR kaatsu OR IHT) AND train*] while the terms patients, pregnancy, diabetes, rats, rodents and mice were excluded (using NOT). Results were limited to ‘English language’ and the following filters were applied to each database: PubMed—*adult 19–44 years OR young adult 19–24 years*; SPORTDiscusTM—*Academic Journals*; EMBASE—*adult 18–64 years*; Web of Science—*Document type (article) AND Category (sport sciences or physiology)*. This search was performed by two authors (BM and JK) and articles were then screened, first by title and then by abstract using the eligibility criteria below. After screening titles and abstracts, full text was retrieved for all potentially relevant articles and assessed according to the selection criteria outlined below. Reference lists for all selected articles were

then screened and searches were supplemented by reviewing the reference lists of other recent reviews [1, 7, 8] and consulting one expert in the area of hypoxic training, who reviewed the list of included studies and made suggestions on any other potentially relevant work. Following the initial search in August 2013, relevant journals within the field were monitored closely during preparation of this manuscript and any new articles meeting the inclusion criteria and published up to December 2013 were added ($n = 2$).

2.2 Selection Criteria

To assess the influence of LLTH interventions on normoxic performance outcomes, the following inclusion criteria were used: (1) subjects were exposed to short-term (≤ 3 h/day) hypoxia throughout an intervention period ≥ 7 days; (2) some form of physical training was completed during the hypoxic exposures (hypoxic exposures with no training excluded); (3) the intervention group was compared with a control group completing matched training under normoxic conditions; (4) exercise performance under normoxic conditions (definition of 'performance' below) was assessed; and (5) subjects were adults aged between 19 and 44 years. Studies were excluded according to the following criteria: (1) hypoxic exposures were > 3 h per day; (2) subjects were previously acclimatised to hypoxia (e.g. high-altitude natives); and (3) a within-subject unilateral research design was employed (i.e. one leg trained in hypoxia, opposite leg trained in normoxia). 'Performance' was defined as any physical test leading to a non-physiological-based outcome, and included (but was not limited to); graded exercise tests [assessing time to exhaustion and/or maximal power output (W_{\max})], time to exhaustion tests, time trials, repeated sprint ability tests and various maximal or near maximal strength tests. All studies reporting only physiological outcomes to LLTH were not included in this review, including those only reporting $VO_{2\max}$ without any other performance variable.

2.3 Data Analysis

There is wide variety in the methodologies used in LLTH studies, including differences in exercise mode, exercise intensity, degree of hypoxia, use of normobaric or hypobaric hypoxia, number of exposures per week, weeks of exposure/training, amount/modality/intensity of additional normoxic training conducted, and the performance measures/outcome variables. Therefore, a systematic review was conducted without a meta-analysis.

Initial analysis of the included studies revealed four distinct training modalities performed under hypoxic

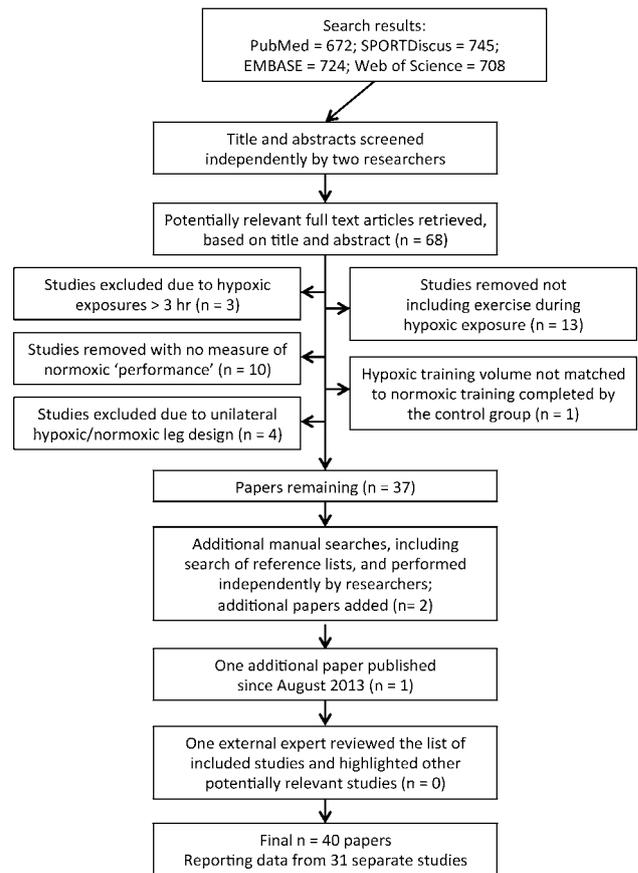


Fig. 1 Flowchart illustrating the search and exclusion/inclusion strategy

conditions, three of which have been previously identified/defined [18]. As a result, studies were categorised into four types based on training modality, as the training modality completed during hypoxic exposures is likely critical to the performance outcomes following the intervention period: (1) continuous hypoxic training (CHT)—involves continuous sub-maximal training sessions under hypoxic conditions lasting greater than 20 min (adapted from suggestion of Millet et al. [18]), usually in an attempt to improve endurance-based performance (e.g. running, cycling, swimming, rowing); (2) interval hypoxic training (IHT)—involves medium duration (~ 30 s to 5 min), high-intensity ($> 70\%$ $VO_{2\max}$) intervals with similar duration recoveries, generally performed in an attempt to improve high-intensity exercise capacity; (3) repeated sprint training in hypoxia (RSH)—involves short duration (~ 5 to 30 s) efforts followed by longer recovery periods (~ 20 s to 3 min), generally performed in an attempt to improve repeated sprint ability; (4) resistance training in hypoxia (RTH)—involves resistance training under hypoxic conditions, in an attempt to increase muscular strength and power production. Two researchers individually categorised papers as CHT, IHT, RSH or RTH.

Table 1 Summary of included intermittent hypoxic training studies

References	Subjects M or F (H, N)	F _I O ₂ H group (normobaric or hypobaric)	Days/weeks of training (training modality)	Number and duration H and control sessions (additional N training)	Training intensity	Absolute or relative intensity matched (H vs. N)	Performance measures	Normoxic performance (H compared to N)
Continuous hypoxic training								
Bailey et al. [30, 35]	Untrained M (18, 14)	16.0 % (normobaric)	4 weeks (cycling)	3 per week; 20–30 min (not reported)	70–85 % HR _{max}	Relative	Cycling GXT	No difference
Beidleman et al. [39]	Untrained M (11, 6)	12.6 % (hypobaric)	1 week (cycling)	6–7 per week; 50 min (none)	~80 % HR _{max}	Relative	Cycling TT (~38 min)	No difference
Czuba et al. [37]	Cyclists M (10, 10)	15.2 % (normobaric)	3 weeks (cycling)	3 per week; 60–70 min (15–16 h/ week)	95 % LT (H) 100 % LT (N)	Relative	Cycling GXT 30 km TT	Improved Improved
Debevec et al. [41]	Untrained M (9, 9)	12.0 % (normobaric)	4 weeks (cycling)	5 per week; 60 min (none)	HR at 50 % W _{max}	Relative	Cycling GXT TTE @ 80 % VO _{2max}	No difference No difference
Emonson et al. [40]	Untrained M (9, 9)	15.7 % (hypobaric)	5 weeks (cycling)	3 per week; 45 min (none)	HR corresponding to 70 % VO _{2max}	Relative	TTE @ 80 % VO _{2max}	No difference
Engfred et al. [46]	Untrained M, F (14, 7)	15.7 % (hypobaric)	5 weeks (cycling)	5 per week; 45 min (not reported)	70 % VO _{2max}	Relative + absolute	TTE @ 85 % VO _{2max}	Decreased (relative + absolute group)
Levine et al. [33]								
Geiser et al. [29]	Untrained M (18, 15)	13.4 % (normobaric)	6 weeks (cycling)	5 per week; 30 min (not reported)	77–85 % HR _{max}	Relative + absolute	Cycling GXT	No difference
Haufe et al. [44]	Untrained M (10, 10)	15.0 % (normobaric)	4 weeks (running)	3 per week; 60 min (not reported)	HR corresponding to 3 mmol·L ⁻¹ La ⁻	Relative	Running GXT	No difference
Hendriksen and Meeuwssen [38] ^b	Triathletes M (12, 12)	15.7 % (hypobaric)	10 days (cycling)	7 per week; 120 min (none)	60–70 % HRR	Absolute	WAnT Cycling GXT	Improved No difference
Kime et al. [43]	Cyclists M, F (8– crossover)	15.0 % (normobaric)	3 weeks (cycling)	3 per week; 120 min (none)	LT	Relative	Cycling GXT	No difference
Mao et al. [36]	Untrained M (12, 12)	15.0 % (normobaric)	5 weeks (cycling)	5 per week; 30 min (none)	60 % W _{max}	Absolute	Cycling GXT	Improved
Meeuwssen et al. [32]	Triathletes M (8, 8)	15.7 % (hypobaric)	10 days (cycling)	7 per week; 120 min (none)	60–70 % HRR	Absolute	WAnT Cycling GXT	Improved Improved
Messonnier et al. [26, 31]	Untrained M, F (5, 8)	11.7 % (normobaric)	4 weeks (cycling)	6 per week; 120 min (none)	60–80 % W _{max}	Relative	Cycling GXT TTE at W _{max}	No difference No difference
Schmutz et al. [19]	Untrained M (6, 6)	12.0 % (normobaric)	6 weeks (cycling)	5 per week; 30 min (none)	65 % W _{max}	Relative	Cycling GXT	Decreased
Ventura et al. [28]	Cyclists M + F (7, 5)	12.7 % (normobaric)	6 weeks (cycling)	3 per week; 30 min (duration not reported)	73–84 % W _{max}	Relative	Cycling GXT	No difference
Vogt et al. [10]	Untrained M (14, 16)	12.7 % (normobaric)	6 weeks (cycling)	5 per week; 30 min (none)	52–67 % W _{max}	Relative	Cycling GXT	No difference

Table 1 continued

References	Subjects M or F (H, N)	F _I O ₂ H group (normobaric or hypobaric)	Days/weeks of training (training modality)	Number and duration H and control sessions (additional N training)	Training intensity	Absolute or relative intensity matched (H vs. N)	Performance measures	Normoxic performance (H compared to N)
Continuous hypoxic training + interval hypoxic training								
Hamlin et al. [11]	Cyclists M (9, 7)	SpO ₂ ~82–88 % (normobaric)	10 days (cycling)	7 per week; 91 min (none)	60–70 % HRR + 2 × 30 s max	Relative	WAnT 20 km TT	Improved No difference
Lecoultre et al. [20]	Cyclists M (7, 7)	14.5 % (normobaric)	4 weeks (cycling)	3 per week; 66–100 min (~400 min/week)	~60–120 % W _{max}	Relative	Cycling GXT 40 km TT	No difference No difference
Mounier et al. [47]	Cyclists and triathletes M (10, 8)	13.1 % (normobaric)	3 weeks (cycling)	5 per week; 60–90 min (duration not reported)	60–100 % W _{max}	Relative	Cycling GXT Cycling TT (10 min)	No difference No difference
Roels et al. [22, 23]	Cyclists M (4, 4)	16.1 % (hypobaric)	3–4 weeks (cycling)	4–5 per week; 105–150 min (none)	60–130 % W _{max}	Relative	Cycling GXT	No difference
Interval hypoxic training								
Dufour et al. [45]	Distance runners M (9,6)	14.5 % (normobaric)	6 weeks (running)	2 per week; 39–55 min (3 per week; ~83 min)	77 and 88 % of N VO _{2max} for H and N, respectively	Relative	TTE @ VO _{2max}	Improved
Morton and Cable [34]	Team sport athletes M (8, 8)	15.1 % (normobaric)	4 weeks (cycling)	3 per week; 30 min (none)	80 % W _{max}	Absolute	Cycling GXT WAnT	No difference No difference
Roels et al. [25]	Cyclists and triathletes M (20, 8)	13.1 % (normobaric)	7 weeks (cycling)	2 per week; 60 min (none)	90–100 % W _{max}	Relative	Cycling GXT Cycling TT (10 min)	No difference No difference
Truijens et al. [21]	Swimmers M + F (8;8)	15.3 % (normobaric)	5 weeks (swimming)	3 per week; ~25 min (≥3 per week)	69–94 % VO _{2max}	Relative	Swimming TT (100 and 400 m)	No difference
Repeated sprint in hypoxia								
Faiss et al. [12]	Cyclists M (20, 20)	14.6 % (normobaric)	4 weeks (cycling)	2 per week; 36 min (not reported)	Maximal repeated 10-s sprints (active recovery)	Maximal	Cycling RSA WAnT	Improved No difference
Galvin et al. [48]	Rugby players M (26 total)	13.0 % (normobaric)	4 weeks (running)	3 per week; 6 min (not reported)	Maximal repeated 6-s sprints (passive recovery)	Maximal	3-min W _{max} Yo-Yo IR1 20-m RSA test	No difference Improved No difference
Puype et al. [49]	Untrained M (10, 9)	14.4 % (normobaric)	6 weeks (cycling)	3 per week; 30–55 min (none)	80 % maximal sprinting power (active recovery)	Absolute	20-m Sprint Cycling GXT Cycling TT (~10 min)	No difference No difference No difference
Resistance training in hypoxia								
Friedmann et al. [27]	Untrained M (10, 9)	12.0 % (normobaric)	4 weeks (knee flex. and exten.)	3 per week; 6 sets, 25 reps (none)	30 % 1RM	Absolute	Isokinetic torque Isokinetic work during 50 reps	No difference No difference

Table 1 continued

References	Subjects M or F (H, N)	F _I O ₂ H group (normobaric or hypobaric)	Days/weeks of training (training modality)	Number and duration H and control sessions (additional N training)	Training intensity	Absolute or relative intensity matched (H vs. N)	Performance measures	Normoxic performance (H compared to N)
Ho et al. [50]	Untrained M (18 total)	15.0 % (normobaric)	6 weeks (dynamic squat)	3 per week; 3 sets, 10RM (not reported)	10RM (approx. 75 % IRM)	Relative	IRM squat Isometric torque Isokinetic torque	No difference No difference No difference
Manimmanakorn et al. [14, 15]	Netball players F (10, 10)	SpO ₂ ~80 % (normobaric)	5 weeks (knee flex. and exten.)	3 per week; 6 sets, ~30 reps (not reported)	20 % IRM	Absolute	MVC ₃ MVC ₃₀	Improved Improved
Nishimura et al. [13]	Untrained M (7, 7)	16.0 % (normobaric)	6 weeks (elbow flex. and exten.)	2 per week; 4 sets, 10 reps (none)	70 % IRM	Absolute	Reps _{20%IRM} 10RM arm curl 10RM standing French press	No difference No difference

M male, F female, H hypoxic, N normoxic, TTE time to exhaustion, TT time trial, WAnT Wingate Anaerobic Test, GXT graded exercise test to exhaustion, HR heart rate, HRR heart rate reserve, RSA repeated sprint ability, Reps repetitions, IRM one repetition maximum, MVC maximal voluntary contraction, MVC₃₀ 30-s maximal voluntary contraction, MVC₃₀-s 30-s maximal voluntary contraction, Reps_{20%/IRM} number of reps at 20 % or one repetition maximum, W Watts, W_{max} maximal W achieved during GXT, LT lactate threshold, Yo-Yo IR1 Yo-Yo Intermittent Recovery test level 1, La lactate, HR_{max} maximum heart rate, VO_{2max} maximum oxygen consumption, RM repetition maximum, F_IO₂ fraction of inspired oxygen, SpO₂ saturation of peripheral oxygen, flex. flexion, exten. extension, approx. approximately

^a Except where stated otherwise

^b Hendrikssen and Meeuwse [38] is a crossover study involving 12 subjects who completed Hendrikssen et al. [32] (1-year wash-out period)

3 Results

Figure 1 shows a flowchart of potentially relevant articles obtained during the search procedures. Electronic searches returned 672, 745, 724 and 708 results for PubMed, SPORTDiscusTM, EMBASE and Web of Science, respectively. From these electronic searches, duplicates were removed and 68 articles were identified as potentially relevant after examination of the titles and abstracts; of these 37 papers [9–15, 19–47] met the inclusion criteria. Additional manual searches returned two results that met all inclusion criteria [48, 49]. One article was also added that was published after the initial search date [50]. From these 40 papers, 31 separate research studies were identified (i.e. eight cases of different papers reporting results from a common research study) and these studies are outlined in Table 1. There was one instance of authors using a randomised control design [32] before using a subset of the same subjects to conduct a randomised crossover study 1 year later [38]. Of the 31 separate studies, 16 were classified as CHT, 4 as IHT, 4 as using a combination of CHT and IHT, 3 as RSH and 4 as RTH (see Table 1).

There was a wide range of training modes and intensities identified among the four categories of LLTH interventions that may influence the outcomes, so training frequency, duration and intensity are summarised in Table 1. A number of methodological considerations were also identified in the literature, including: matching of absolute or relative training intensity between hypoxic and normoxic groups; method of matching relative training intensity; and the inclusion of some portion of normoxic training for the hypoxic group [13–15, 27, 32, 34, 36, 38, 49]. Results of studies matching absolute training intensities ($n = 8$) between hypoxic and normoxic group should be interpreted with caution, because this likely produces a higher relative training intensity in the hypoxic group. Only six [9, 20–24, 28, 37, 45, 47] of the 31 studies in the present review included some form of normoxic training for the hypoxic group.

4 Discussion

Within the current literature, the efficacy of LLTH for improving sea-level performance is unclear. Although rarely discussed, exercise mode and intensity are likely key factors in mediating the response to the LLTH programme, with higher training intensities appearing to be more beneficial than sub-maximal workloads. LLTH also appears to have a greater impact on the performance of highly anaerobic tasks, such as short-term high-intensity work and maximal intermittent exercise (i.e. repeated sprint ability), and these benefits are more likely identified when

performance tests are specific to the training modality/intensity performed during LLTH sessions.

4.1 Methodological Considerations

4.1.1 *The Effect of Hypoxia on Training Intensity and Inclusion of Normoxic Training*

Training in moderate hypoxic environments effectively limits the amount of energy that can be produced oxidatively during exercise, and it is well established that this reduction in oxidative energy production leads to a reduced exercise performance in both endurance [51] and team sport [52] athletes. Although absolute exercise intensity is reduced under hypoxic conditions, the reduced oxygen availability may produce a greater peripheral physiological stimulus than training in normoxia, and this is thought to be the major contributing factor leading to possible increases in performance following LLTH interventions. Whilst this peripheral physiological strain may be beneficial for subsequent athletic performance, there is undoubtedly a reduction in cardiovascular stress during hypoxic training sessions, which is directly related to the reduced exercise intensity [53]. Indeed, maximal cardiac output and stroke volume are known to be reduced under acute hypoxic conditions [54]. As one of the major cardiovascular training adaptations is an increase in stroke volume, induced by invoking large training stroke volumes [55, 56], training in hypoxia appears to limit cardiovascular overload. Therefore, training in hypoxia alone will apparently limit training-induced cardiovascular adaptations; thus, a mixture of training in hypoxia and normoxia seems more preferable than training in hypoxic environments only. Despite this, only six [9, 20–24, 28, 37, 45, 47] of the 31 studies examined in this review included some normoxic training for the hypoxic group. Furthermore, only two of these studies prescribed well-periodised training programmes in normoxia, with some portion of high-intensity interval training [9, 24, 37, 45], whilst the hypoxic groups in the other three studies completed only sub-maximal work under normoxic conditions [20–23, 47].

4.1.2 *Matching Relative or Absolute Training Intensity*

When interpreting results from LLTH studies, it is important to consider how training intensity was matched between hypoxic and normoxic training groups. A number of the LLTH studies within this review have aimed to match the absolute training intensity between these groups [13–15, 27, 32, 34, 36, 38, 49]. However, any group training in normoxia will have an increased exercise

capacity when training, compared with individuals training in hypoxic conditions. Therefore, the matching of absolute training intensity will limit the training adaptations for the normoxic group and provide a higher relative training stimulus for those training in a hypoxic environment. This theory is supported by the work of Desplanches et al. [57] who included two normoxic training groups matched for absolute and relative training intensity with the hypoxic group. These authors reported a significant increase in mitochondrial density following 3 weeks of training at 70–80 % $\dot{V}O_{2peak}$ (relative to training condition) in the normoxic and hypoxic groups, respectively, but found no changes in the group training in normoxia with absolute workload matched to the hypoxic group. Thus, groups that train in normoxia at the same absolute intensity as in hypoxia are not likely to experience sufficient overload to induce the associated training benefits. For this reason, results from studies matching absolute training intensities between normoxic and hypoxic groups [13–15, 27, 32, 34, 38, 49] should be interpreted with caution, because any greater improvements in performance in the hypoxic group may be solely attributed to a higher relative training intensity.

The method of prescribing matched relative workloads during hypoxia needs to be carefully considered. Some CHT studies use a percentage of maximum heart rate (HR_{max}) [29, 35, 39, 58] or heart rate reserve [32, 38]. Although heart rate is commonly used for prescription of training intensity, there is evidence to suggest that this is not an accurate method for matching relative intensities between hypoxic and normoxic conditions. Bailey et al. [35] attempted to match relative intensity by having subjects train between 70 and 80 % HR_{max} , as determined via a pre-test in a hypoxic or normoxic environment for the LLTH and control group, respectively. During training, there was no difference in average heart rate between these groups, suggesting that relative exercise intensity was matched. As the W_{max} of the LLTH group was reduced by ~10 % in hypoxia [58], this group should therefore be training at a lower absolute intensity than the normoxic group (to match relative workloads). However, there was no difference in power data from the training intervention between the hypoxic and normoxic groups [35], meaning that both groups were actually training at the same absolute workload. While other studies use HR_{max} [29, 39] or heart rate reserve [32, 38] methods to prescribe relative training intensities in normoxia and hypoxia, they do not provide data on training power/velocity to allow determination of actual training intensity. However, the results of Bailey et al. [35] suggest that heart rate-based methods are problematic when prescribing relative training intensities during LLTH sessions.

4.1.3 Training Mode and Intensity on Performance Outcomes

Training modality/intensity has been largely ignored when interpreting the effectiveness of LLTH protocols within the literature. The importance of exercise intensity as a key factor in modulating the response to LLTH has recently been highlighted by Millet et al. [7, 17], where they suggest that greater responses occur with maximal or near-maximal training interventions (e.g. RSH) compared with sub-maximal training protocols. Millet et al. [17] also suggest that sub-maximal training intensities in the majority of LLTH studies may explain why many fail to demonstrate additional performance benefits when compared with similar normoxic training. While high-intensity RSH training appears to be a more desirable method for enhancing normoxic exercise performance compared with lower intensity training, and may be beneficial for team sport athletes [17, 48], little attention has focused on high-intensity IHT and its effects on intermittent exercise performance (i.e. no IHT studies examined in the current review use a measure of high-intensity intermittent exercise performance). The principle of specificity, in relation to matching the training performed and the performance tests, is also often overlooked when interpreting LLTH literature. For example, a number of studies have used training intensities of $\sim 50\text{--}70\%$ W_{\max} , but have assessed performance outcomes with higher intensity exercise (e.g. time trial at 80% W_{\max}) [40, 41]. This importance of testing specificity is highlighted in the work of Faiss et al. [12], who found greater improvements in the number of sprints completed to exhaustion by a hypoxic group following 4 weeks of repeated sprint training compared with a normoxic control group, but found similar improvements between groups in 30-s Wingate Anaerobic Test performance and no change in 3-min maximal exercise for either group.

4.1.4 Placebo and Nocebo Effects

The placebo effects of training in a ‘beneficial’ hypoxic environment should also be considered when interpreting results from LLTH studies. Similarly, nocebo effects may negatively influence the performance of control groups, if they are informed (or deduce) that they are not receiving the ‘beneficial’ treatment. In LLTH studies, this limitation can be overcome by having both experimental and control groups under the assumption that they are training in hypoxia. For example, Czuba et al. [37] and Faiss et al. [12] achieved this by having their hypoxic and normoxic groups train in a hypoxic chamber with the hypoxic generator switched on, albeit simulating very different altitudes for each group. Additionally, Faiss et al. [12]

informed all subjects, including those in the normoxic group, that all training sessions were being completed in hypoxia. Studies that report improved performance in the hypoxic training group without describing the steps taken to control for potential placebo/nocebo effects should be interpreted with caution. For example, Dufour et al. [45] report improved running performance after 6 weeks of IHT, with the hypoxic treatment delivered ‘by breathing through face masks connected to a mixing chamber’. However, the authors make no mention of whether participants in the normoxic group also trained while connected to a face mask, or if they had knowledge that they were not receiving a hypoxic treatment. Future studies should carefully consider methodology to control for placebo/nocebo effects and be sure to carefully report these methods, so that the effects of the hypoxia per se can be interpreted more confidently.

4.2 Performance Outcomes Following Live Low-Train High Interventions

4.2.1 Continuous Hypoxic Training

Of the LLTH studies that only include CHT (i.e. no portion of IHT) and match relative training intensity between the hypoxic and normoxic groups, most report no additional benefit of training in hypoxia [10, 20, 26, 28–31, 35, 39–44, 59]. The reduction in cardiovascular function during hypoxic training sessions, which is directly related to the reduced absolute exercise intensity under hypoxic conditions [53], may explain the lack of aerobic-based performance improvements in these studies. Moreover, given that LLTH interventions are thought to induce primarily peripheral adaptations related to anaerobic capacity (i.e. carbohydrate metabolism; rate of glycolysis; pH regulation) [9], the training intensities used in the majority of CHT studies may not have been sufficient to stimulate these adaptations and produce performance outcomes greater than matched normoxic training.

The outcomes of Hamlin et al. [11] support this, reporting no change in 20-km time trial performance but a *likely* improvement in mean power during a 30-s Wingate Anaerobic Test. A distinguishing feature of this study is the inclusion of a small portion of IHT, which was not included in most other CHT studies. Specifically, while the participants in Hamlin et al. [11] predominantly performed CHT (90 min per day at $60\text{--}70\%$ of heart rate reserve); they also completed 1 min of daily anaerobic-based IHT (2×30 s maximal efforts, separated by 5-min recovery). The only well controlled CHT study to show improvements in prolonged endurance-based performance [37] also presents a distinguishing feature to other CHT studies, in that their hypoxic group maintained a significant amount of

training in normoxia, including high-intensity interval training. Alongside the increase in maximal workload in a graded exercise test following the CHT intervention, Czuba et al. [37] also reported improvements in 30-km time trial performance, which suggests the necessity to include training in normoxia (to accompany CHT) to achieve a sufficient cardiovascular overload for aerobic adaptation.

4.2.2 Interval Hypoxic Training

Of the eight studies in this review that include IHT, the majority report no change in performance following a hypoxic intervention [20–23, 25, 42], while two report enhanced performance [11, 45] compared with matched controls. These differing outcomes might again be related to study design—specifically, the intensity of training and/or the performance measures used—with high-intensity training and short-duration performance tests more likely to show a beneficial effect of the hypoxic stimulus.

The outcomes of these studies, when taken together, also extend the proposition raised in the previous section: that a mixture of training in hypoxia and normoxia is not only preferable to training in hypoxic environments alone, but that the intensity of the supplementary normoxic training is important if seeking improvements in performance. For example, Dufour et al. [45] found an improvement in run time to exhaustion at $v\dot{V}O_{2\max}$ following 6 weeks of IHT in trained distance runners, with the hypoxic group performing three sessions per week of high-intensity training in normoxia (in addition to two IHT sessions). In contrast, a study by Roels et al. [25] reported no change in mean power for a 10-min cycling time trial (performance of similar intensity and duration to that of Dufour et al. [45]) following 7 weeks of IHT in endurance cyclists and triathletes. However, no normoxic training by the hypoxic group was reported. Of the other studies that did report some portion of normoxic training in addition to IHT for their hypoxic group [20–23, 47], none reported performance improvements. However, the additional normoxic training, as described in these papers, appears to be of low-moderate intensity only. Therefore, when implementing IHT, maintaining additional training in normoxia at high intensity might be an important factor if the recognised reduction in cardiovascular function during hypoxia is to be overcome, eliciting performance improvements greater than those following normoxic training alone.

The importance of the design (e.g. training intensity/volume) of supplementary normoxic sessions is highlighted within the literature. For example, Roels et al. [22] reported a 5.0 % improvement in $\dot{V}O_{2\max}$ for their normoxic training group of endurance-trained cyclists and triathletes, but no change (−0.3 %) for their hypoxic group, after completing only low-moderate intensity supplementary normoxic

sessions. After completing similar additional normoxic training (i.e. low-moderate intensity), Lecoultré et al. [20] reported changes in $\dot{V}O_{2\max}$ of +7.4 and +1.4 % for their normoxic and hypoxic training groups of well-trained cyclists, respectively (although this difference was $p = 0.12$). In contrast, with the provision of high-intensity training sessions in normoxia as accompaniment to IHT, Dufour et al. [45] reported a 5 % increase in $\dot{V}O_{2\max}$ in their hypoxic group, with no change in the normoxic group. This suggests that the high-intensity normoxic training was important in achieving cardiovascular overload. Similarly, although Czuba et al. [37] delivered a CHT intervention, high-intensity interval training was part of the supplementary normoxic exposures for their hypoxic group, and this group showed improvements in endurance performance and $\dot{V}O_{2\max}$. Therefore, from the data available, it appears that when IHT is being implemented, low-moderate training in normoxia is sufficient to maintain aerobic power, but high-intensity training of sufficient volume in normoxia would be necessary to drive aerobic improvements.

In summary, while the majority of well-controlled IHT studies report no additional benefit of the hypoxic stimulus, the limited literature available suggests that greater improvements with IHT might be more likely if the following criteria are followed: (1) high-intensity intervals are completed during the hypoxic exposures; (2) anaerobic rather than aerobic performance is measured; and (3) a sufficient intensity and volume of normoxic training accompanies IHT.

4.2.3 Repeated Sprint Training in Hypoxia

Traditionally, LLTH has targeted endurance-based athletes, but more recently, the effect of repeated sprint training in hypoxia on high-intensity exercise performance [12, 48, 49] has gained attention. Faiss et al. [12] hypothesised that, compared with repeated sprint training in normoxia (RSN), RSH could induce beneficial adaptations at the muscular level, along with improved blood perfusion, which may lead to greater improvements in repeated sprint ability. These authors assessed 40 trained male cyclists completing 4 weeks of RSH or RSN (see Table 1), and both groups significantly improved power output during repeated sprints post-intervention. However, only the RSH group delayed the onset of fatigue post-intervention, with subjects improving from 9 to 13 sprints until exhaustion, whilst the RSN group showed no such improvement. Despite this improved repeated sprint ability following RSH, improvement in a single 10-s sprint and 30-s Wingate Anaerobic Test performance did not differ between RSH and RSN, while 3-min maximal exercise performance was not altered.

Similarly, Galvin et al. [48] found that 4 weeks of maximal RSH training induced greater improvements in Yo-Yo IR1 performance in a group of elite rugby players compared with matched controls completing the same training under normoxic conditions. In contrast, Puype et al. [49] found no additional benefit of RSH over RSN in untrained men completing a 10-min cycling time trial following 4 weeks of training. However, although we have classified Puype et al. [49] as RSH, the authors refer to their training protocol as ‘repeated sprint interval training,’ with subjects completing longer duration (30-s), sub-maximal (~80 % of the mean power output measured in the first sprint) sprints during training. The sub-maximal nature of these sprints may produce a different physiological response than maximal RSH training [12, 48]. Furthermore, the performance measures in this study were continuous in nature (cycling GXT and 10-min time trial), thereby lacking specificity to the intermittent training stimulus.

4.2.4 Resistance Training in Hypoxia

Recently, hypoxic environments have been proposed to enhance some of the adaptations associated with resistance training. Resistance training is known to improve maximal strength, power production and reduce fatigability through a number of adaptations, including hypertrophy [60] and altered motor recruitment patterns [61]. More than a decade ago, a Japanese group investigated the effects of resistance training with simultaneous vascular occlusion [62], and reported greater strength improvements in subjects using the occlusion technique [62, 63]. One of the proposed mechanisms related to these performance gains is local hypoxia (created by vascular occlusion) within the active muscle tissue [63], which is a key component for the anabolic effects of resistance training [60]. Thus, a number of groups have since investigated the effects of systemic hypoxia on resistance training adaptations [13–15, 27, 50].

Of the four RTH studies in this review, only one found greater performance improvements in the hypoxic group [14, 15] compared with training in normoxic conditions. Three of the four studies matched the absolute workloads between hypoxic and normoxic groups, and used protocols involving ≥ 10 repetitions with sub-maximal workloads; primarily from 20 to 30 % 1RM [14, 15, 27], with one study examining the effects of training at 70 % 1RM [13]. As discussed in Sect. 4.1.2, matching absolute workloads provides a greater relative training stimulus in the hypoxic condition. Furthermore, these studies all prescribed the number of ‘repetitions’ for subjects throughout the training protocol, as opposed to using a set load and asking participants to lift until failure. This design suggests that both groups were training at sub-maximal intensities, but with

the hypoxic group training at a higher relative intensity and experiencing a greater training stimulus. This different relative intensity of training may explain the improved performance reported for the hypoxic group in the study by Manimmanakorn et al. [14, 15] and may also explain the greater hypertrophy seen in the hypoxic group of Nishimura et al. [13]. Furthermore, the absence of training to failure in Friedmann et al. [27] suggests that their subjects were training sub-maximally and, thus, hypoxia did not stimulate additional adaptations; a contention supported by the absence of improved maximal strength in both of their training groups following the intervention period. One recent RTH study did attempt to address the limitation of matching absolute workload between the hypoxic and normoxic groups [50]. During a 6-week RTH intervention, Ho et al. [50] initially matched normoxic and hypoxic training intensities based on normoxic 1RM (75 %). However, these investigators then increased the load when subjects successfully completed all of the prescribed loads in two consecutive training sessions. This may have led to a greater increase in training load in the normoxic group, if exercise intensity was limited by the hypoxic environment. Unfortunately, training load data were not reported in this study, and it is therefore not possible to determine how the hypoxic stimulus may have affected training progression. Despite training volumes not being available, the RTH intervention appeared to have no performance effect in these untrained male athletes. In summary, methodological flaws in RTH studies to date preclude any definitive conclusions about the effectiveness of LLTH for enhancing performance gains from maximal intensity, resistance training programmes.

5 Practical Applications

The LLTH model has the potential to contribute to a number of training adaptations, and these appear to be more related to anaerobic metabolism. Thus, LLTH interventions may have the greatest benefit for high-intensity, short-term and intermittent performance (e.g. team sports). This is supported by the current LLTH literature that suggests performance improvements are more likely following high-intensity LLTH compared with sub-maximal training intensities while, similarly, evidence is equivocal for endurance benefits subsequent to LLTH.

In an applied setting, LLTH interventions can be difficult to implement given (1) hypoxic training rooms may have limited space available for training, or; (2) if athletes are connected to a hypoxic gas supply (i.e. no hypoxic room available), movement will be limited. The recent development of portable, inflatable, hypoxic tents [64] may overcome some of these limitations and provide a versatile

alternative for practitioners wishing to implement LLTH in field settings. Delivering appropriate training intensities in hypoxic environments is also difficult, given the prescription issues surrounding some traditional measures of intensity (e.g. heart rate) and the reduced work capacity evident in hypoxia. Current literature may guide some exercise prescription in hypoxia [51] if prescription is based on some measure of aerobic capacity (e.g. VO_{2max} , maximal aerobic speed). Indeed, Buchheit et al. [51] suggest that 5×90 s interval speed is decreased by $\sim 6\%$ in hypoxia ($15.4\% O_2$) compared with that in normoxia. An alternative method may be to have participants perform maximally for the duration of the interval, as with current RSH methodologies [12, 48]. Furthermore, given that training in hypoxia limits exercise intensity and training-induced cardiovascular stress, practitioners and researchers should include some portion of high-intensity normoxic training when designing programmes that include LLTH, to ensure that all physiological systems are being overloaded.

With respect to resistance training, the available research (albeit limited) suggests that greater strength and hypertrophy gains are possible following sub-maximal resistance training in hypoxia compared with matched sub-maximal training in normoxia [13–15]. While this may have applications for populations where the mechanical stress imposed during training needs to be limited (e.g. rehabilitation, elderly individuals), evidence of the efficacy of RTH compared with well periodised maximal strength training in normoxia is lacking. Therefore, at this time, there is no support for the prescription of RTH for healthy athletes who are able to engage in traditional strength training programmes, but this is an interesting area for future research.

6 Conclusion

The majority of LLTH literature reports no additional benefits of training under hypoxic conditions. However, much of this literature has used continuous, sub-maximal, intensity training during hypoxic exposures, in an attempt to improve prolonged endurance performance. The majority of benefits following LLTH interventions appear to be more related to high-intensity, anaerobic performance, which may be more beneficial in short-duration, high-intensity athletic events and intermittent team sports. LLTH programmes and studies should carefully apply the principles of training specificity, whilst considering that improvements are more likely following high-intensity, short-term and intermittent training (e.g. IHT and RSH), and should always include some portion of high-intensity training in normoxia, given that some physiological

systems are limited under hypoxic conditions. While hypoxia may augment metabolic and neuromuscular adaptations associated with sub-maximal resistance training, it is not clear whether RTH induces greater adaptations than traditional (maximal) strength training programmes. Therefore, RTH cannot be recommended for healthy athletes who are able to undertake traditional resistance training programmes.

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APPENDIX V – SUPPLEMENTARY MET-ANALYSIS
BY GORE ET AL. (2013)

REFERENCE

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Altitude training and haemoglobin mass from the optimised carbon monoxide rebreathing method determined by a meta-analysis

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ABSTRACT

Objective To characterise the time course of changes in haemoglobin mass (Hbmass) in response to altitude exposure.

Methods This meta-analysis uses raw data from 17 studies that used carbon monoxide rebreathing to determine Hbmass prealtitude, during altitude and postaltitude. Seven studies were classic altitude training, eight were live high train low (LHTL) and two mixed classic and LHTL. Separate linear-mixed models were fitted to the data from the 17 studies and the resultant estimates of the effects of altitude used in a random effects meta-analysis to obtain an overall estimate of the effect of altitude, with separate analyses during altitude and postaltitude. In addition, within-subject differences from the prealtitude phase for altitude participant and all the data on control participants were used to estimate the analytical SD. The 'true' between-subject response to altitude was estimated from the within-subject differences on altitude participants, between the prealtitude and during-altitude phases, together with the estimated analytical SD.

Results During-altitude Hbmass was estimated to increase by $\sim 1.1\%/100$ h for LHTL and classic altitude. Postaltitude Hbmass was estimated to be 3.3% higher than prealtitude values for up to 20 days. The within-subject SD was constant at $\sim 2\%$ for up to 7 days between observations, indicative of analytical error. A 95% prediction interval for the 'true' response of an athlete exposed to 300 h of altitude was estimated to be 1.1–6%.

Conclusions Camps as short as 2 weeks of classic and LHTL altitude will quite likely increase Hbmass and most athletes can expect benefit.

INTRODUCTION

An increase in erythropoiesis resulting from altitude exposure has been described for over 100 years,¹ and is quite apparent among lifelong residents of high altitude (>3000 m).^{2–4} However, for short-term sojourns, a comprehensive new meta-analysis and Monte Carlo simulation of data spanning the last 100 years concluded that there was no statistically significant increase in red cell volume (RCV) unless the exposure exceeded 4 weeks at an altitude of at least 3000 m.⁵ This altitude is substantially higher than that recommended for athletes,⁶ where a lower altitude (2000–2500 m) is advised to minimise the loss of training intensity evident at higher elevations.

Rusko *et al*⁷ have suggested that an exposure of 3 weeks is sufficient at altitudes $>\sim 2000$ m, provided the exposure exceeds 12 h/day. Furthermore, Clark *et al*⁸ have concluded, based on the serial measurements of haemoglobin mass (Hbmass) and on other recent altitude studies, that Hbmass increases at a mean rate of 1%/100 h of exposure to adequate altitude.

Rasmussen *et al*,⁵ who were careful in the selection criteria for studies to include in their meta-analysis, noted that a variety of methodologies to measure RCV were adopted in the different studies, ranging from carbon monoxide (CO) rebreathing to radioactive labelling of albumin to various plasma dye-dilution tracer methods. As with any meta-analysis, the veracity of the conclusions is only as good as the quality of the data included, and Rasmussen *et al* formally tested that there was no significant effect of the method of measurement on their conclusions. However, all methods of estimating RCV or Hbmass are subject to error, and a meta-analysis of raw data demonstrated that the measurement error of CO rebreathing (2.2%) was, if anything, slightly less than that (2.8%) for the gold standard method of ⁵¹chromium-labelled red blood cells.⁹ In contrast, the measurement error for the common plasma dilution method of Evans Blue dye was estimated to be 6.7%. If the effects of moderate altitude (2000–3000 m)¹⁰ are relatively small, then it is quite likely that inclusion of studies with greater error (eg, refs. ¹¹ and ¹²) may obfuscate the effects of studies using more reliable methods (eg, refs. ¹³ and ¹⁴).

In 2005, Schmidt and Prommer¹⁵ validated a variation on the CO rebreathing method with a low error of measurement of $\sim 1.7\%$, which has been successively refined.^{16–18} Thus, rather than using many data sources, some of which include relatively noisy data, the aim of this meta-analysis was to use the raw data of only those studies that used the optimised CO rebreathing method to determine Hbmass, and that were conducted since 2008. This approach should offer a more precise estimate of the effect of altitude on Hbmass.

METHODS

Data sources

The data used in the meta-analysis were obtained from authors who had, since 2008, published the



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Review

results of studies that used the 'optimised CO rebreathing' method to evaluate the effects of altitude training on Hbmass. Briefly, this rebreathing method involves a known CO dose of ~1.2 ml/kg body mass being administered and rebreathed for 2 min. Capillary fingertip blood samples are taken before the start of the test and 7 min after administration of the CO dose. Both blood samples are measured a minimum of five times for determination of percentage of carboxyhaemoglobin (%HbCO) using an OSM3 Hemoximeter (Radiometer, Copenhagen). Hbmass is calculated from the mean change in %HbCO before and after rebreathing CO.

Individual, deidentified raw data were provided from 17 studies^{8 13 14 19–31}; seven were classic altitude training (ie, living and training on a mountain) studies,^{13 22 23 28 29 31 32} eight were live high train low (LHTL) studies^{8 14 19–21 25 27 30} and two included a mixture of classic and LHTL modalities.^{24 26} Regardless of the form of altitude or simulated altitude, hereafter, all such studies will be referred to as 'altitude' studies for simplicity.

Coding of predictor variables

Altitude treatments

In addition to the recorded altitude of each study, the total hours spent in hypoxia was calculated from the hours per day and numbers of days of exposure. This approach allowed for comparison of classic and LHTL modes, since the former affords continuous altitude exposure, while the latter is intermittent. All but one of the LHTL studies used ≥ 14 h/day in hypoxia, while the other used 10 h/day.²⁴ Several of the classic and LHTL studies made serial measurements of Hbmass during altitude exposure (table 1), which enabled multiple estimates of

the change in Hbmass over time. All studies included prealtitude and postaltitude measurements of Hbmass.

Where included in the study design, control participants were coded as controls and those exposed to altitude were coded as altitude participants. The one exception was the LHTL study of Neya *et al*²⁴ where the control group resided at 1300 m 24 h/day; these participants were coded as classic altitude, albeit at the lowest altitude of any group and very much lower than that conventionally associated with an increase in RCV.³³

Statistical analysis

The approach taken was first to fit linear mixed models separately to the data from each of the 17 studies to estimate the effects of altitude. The resultant estimates were then used in a random effects meta-analysis to obtain an overall estimate of the effect of altitude, with separate analyses for the during-altitude and postaltitude phases. In addition, all possible within-subject differences for control participants and those during the prealtitude and during-altitude phases for altitude participants were used to evaluate within-subject variation. All analyses were conducted using the statistical package R,³⁴ with the mixed model analyses conducted using the mle procedure available in R's nlme library.³⁵

In the initial analyses of the 17 studies, Hbmass values were log transformed (natural logarithms (ln)) and linear mixed models fitted with treatment (control or altitude), days during altitude, days postaltitude and sex as fixed effects and participant as a random effect. In addition, where appropriate, the models allowed for different within-subject SDs for men and women (some of the studies used all male or all female participants) and within-subject autocorrelation. Some of the studies

Table 1 Data sources

Reference	Altitude mode	Altitude (m)	Duration (h)	Sport	Calibre of athletes	N at altitude	N in control	Number of measures per participant
Clark <i>et al</i> ⁸	LHTL	3000	294	Cycling	International	12 m	–	7
Frese and Friedmann-Bette ²⁸	Classic	1300–1650	480–528	Running	Junior	7 f, 4 m	2 f, 6 m	2–6
Garvican <i>et al</i> ¹⁴	LHTL	3000	416	Cycling	International	12 f	–	8–12
Garvican <i>et al</i> ²⁹	Classic	2760	456	Cycling	International	8 m	7 m	5–9
Garvican-Lewis <i>et al</i> ²⁵	LHTL	3000	154–266	Water polo	International	9 f	–	4
Gough <i>et al</i> ²⁶	Classic LHTL	2100–2320	204–504	Swimming	International	3 f, 14 m	–	2–4
		3000	294			9 f, 1 m	–	
Humberstone <i>et al</i> ³⁰	LHTL	3000	238	Triathlon	International	2 f, 5 m	6 f, 11 m	4
McLean <i>et al</i> ^{22 23}	Classic	2130	456	Football	National	21 m	9 m	3–9
	Classic	2100	432	Football	National	23 m	–	4–6
Neya <i>et al</i> ²⁴	Classic	1300	504	Running	Collegiate	7 m	–	3
	LHTL	3000	210			7 m	–	
Pottgiesser <i>et al</i> ²⁷	Classic	1816	504	Cycling	International	7 m	–	3
Robertson <i>et al</i> ²⁰	LHTL	3000	294	Running	National	4 f, 6 m	3 f, 5 m	6–12
Robertson <i>et al</i> ¹⁹	LHTL	3000	294	Running	National	2 f, 6 m	2 f, 7 m	6
Saunders <i>et al</i> ²¹	LHTL	3000	294	Race walking	International	3 f, 3 m	6 f, 5 m	7
Wachsmuth <i>et al</i> ¹³ (overall	Classic	2320	672	Swimming	International	6 f, 13 m	–	2–5
35 participants (17 f, 18 m)	Classic	2320	672	Swimming	International	4 f, 6 m	6 f, 5 m	(within each
with 2–11 measures)	Classic	2320	504	Swimming	International	2 f, 5 m	–	subsection)
	Classic	1360	552	Swimming	International	7 f, 4 m	–	
Wachsmuth <i>et al</i> ³¹	Classic	3600	288	Football	Junior	17 m	16 m	3–5
Wachsmuth <i>et al</i> ³²	Classic	2300	504	Swimming	National	3 f, 6 m	3 f, 4 m	3–7
Total						73 f, 175 m	28 f, 75 m	

f, females; LHTL, live high train low; m, males.

had too few observations on each participant to warrant inclusion of the assumed autocorrelation structure but, for consistency, the same form of model was fitted to all 17 data sets.

The next stage fitted linear mixed models with response variable estimates of the mean (over participants within each study) differences between baseline and subsequent values of $\ln(\text{Hbmass})$ on altitude participants, with separate analyses for the during-altitude and postaltitude phases.

For the during-altitude phase, the time at altitude, both in terms of the number of days and the number of hours, the altitude and the type of altitude (classic or LH TL) were treated as fixed effects; whereas for the postaltitude phase, altitude, the number of days and hours at altitude, the type of altitude and number of days postaltitude were treated as fixed effects. For both analyses, the study was treated as a random effect and the SEs associated with the estimates of mean differences, as provided by the initial analyses, were used to determine suitable weightings.

Estimates obtained from these analyses were back transformed (via the exponential function) to express results as percentage changes on the Hbmass scale.

Variability of measurements

Without some form of intervention, Hbmass is considered to be constant, especially over a period of a few days, so that differences in measurements taken under stable conditions can be attributed almost exclusively to measurement, or analytical, error.³⁶ Using the within-subject variability of the recorded Hbmass values among the control participants and the within-subject variability during (just) the prealtitude phase among the altitude participant, it is possible to estimate the magnitude of the analytical error. In addition, following Hopkins,³⁷ it is also possible to estimate the overall magnitude of between-subject differences in response to altitude training using the within-subject differences in Hbmass measurements between the prealtitude and during-altitude phases, and between pairs of during-altitude measurements, made on the altitude participants.

Within-subject measurements

Using the control participant data and just the prealtitude values from altitude participants, an estimate of the analytical SD was obtained as follows. Separately for each study, estimates of within-subject SDs associated with each difference in days were obtained as the square root of half the average of the squared differences in $\ln(\text{Hbmass})$. A linear mixed model was then fitted to the \ln -transformed (estimated) SDs with the number of days between estimates as a fixed effect, study as a random effect and weights determined by the numbers of differences used to estimate the SDs. The results of this analysis were back transformed (twice, using exponentials) to obtain coefficients of variation (CVs) on the Hbmass scale, with the analytical CV estimated as the value associated with readings zero days apart.

Between-subject 'true' responses

Using data from (just) the altitude participants, estimates of the between-subject variation in the 'true' response to altitude were obtained as follows. First, estimates of the within-subject SDs of $\ln(\text{Hbmass})$ associated with differences between prealtitude and altitude values, and between values obtained while at altitude, for different values of the difference in the number of hours at altitude, were obtained as the SD of the relevant differences divided by $\sqrt{2}$. Linear mixed models were then fitted to the \ln -transformed SDs with the difference in the number of hours at altitude as a fixed effect, study as a random effect and

weights determined by the degrees of freedom of the estimated SDs. The models considered were constrained so that the estimated within-subject SD after zero (additional) hours at altitude agreed with the estimate of the analytical SD. This was achieved by subtracting the natural log of the estimated analytical SD from each of the \ln -transformed SDs and then fitting models without an intercept term. Estimates of the SDs of the between-subject 'true' responses were then obtained as

$$\sqrt{((\text{fitted within-subject SD})^2 - (\text{estimated analytical SD})^2)}$$

for a range of values of (differences in) the hours at altitude; these SDs were then used to estimate the likely range of 'true' responses to different exposures to altitude.

RESULTS

Raw data

A total of 1624 measures of Hbmass were made on 328 participants, 18 of whom participated in more than one study (14 in 2 studies, 3 in 3 studies and 1 in 4 studies); 225 participants participated as altitude-only participants, 96 as control-only participants and 7 as altitude and control participants, but in different studies (table 1). The mean (\pm SD) number of measures per participant was 4.8 (\pm 2.7). As the serial measures were made virtually during all studies, there were 76 estimates of the change in $\ln(\text{Hbmass})$ from the prealtitude values, 40 estimates during altitude and 36 estimates after altitude exposure.

The median classic altitude was 2320 m (range 1360–3600 m); while all but one LH TL study used 3000 m, the other used 2500 m.

During altitude

Of the 40 estimates of the change in $\ln(\text{Hbmass})$ from prealtitude to during altitude available for analysis, one appeared to be an obvious outlier, see figure 1. This estimate was associated with an altitude of 1360 m, but it did not show up as having an especially large standardised residual (-2.06). Omission of this observation has a negligible effect on the results, and for consistency with the treatment of the outliers identified during the postaltitude phase, it has been omitted from the results reported here; all other estimates were associated with an altitude of at least 2100 m.

After allowing for the time at altitude, none of the other altitude-related fixed effects (altitude, days at altitude and type of altitude) made a significant additional contribution ($p=0.642$ for the combined additional contribution). Various ways of modelling the effect of time at altitude were considered, including

1. A simple linear relationship passing through the origin (ie, forcing the change in $\ln(\text{Hbmass})$ (or just Hbmass) to be zero after zero hours at altitude);
2. A quadratic relationship, also passing through the origin;
3. Grouping the different times at altitude to form a factor with seven levels.

As a first approximation, for those studies ≥ 2100 m, there was a 1.08% (95% CI 0.94% to 1.21%) increase in Hbmass per 100 h of LH TL and classic altitude exposure (figure 1; table 2). However, the quadratic model was significantly better than the linear model ($p=0.015$), while treating time at altitude as a factor with seven levels (table 2) was not significantly better than a quadratic based on the seven levels ($p=0.334$). There was also no evidence of different quadratics being appropriate for classic and LH TL altitude ($p=0.271$), while for both the linear and the quadratic models, formal tests of departure from

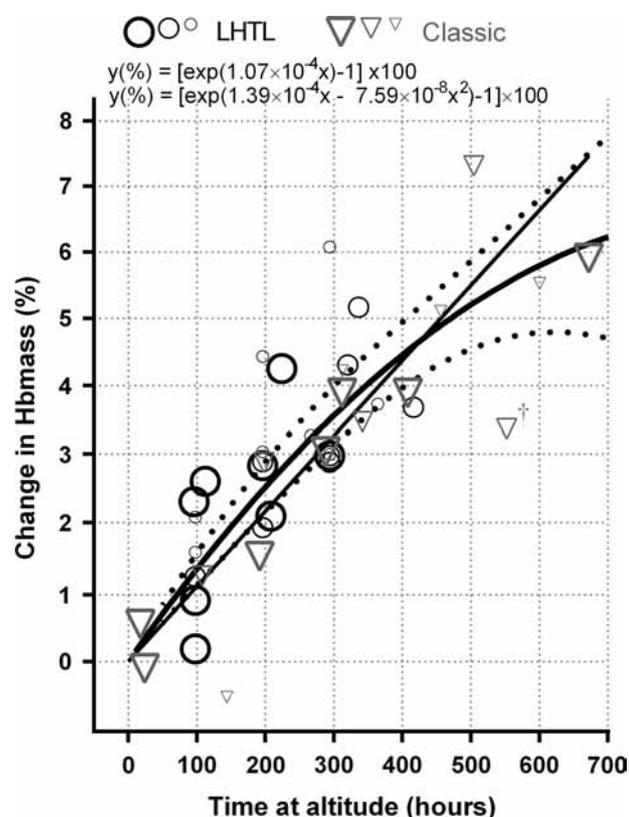


Figure 1 Estimates of the change in haemoglobin mass (Hbmass) during live high train low (LHTL, n=24) and classic (n=16) altitude exposure. Fitted lines are for the linear and quadratic models. Dashed lines are the upper and lower 95% confidence limits of the quadratic model. The relative weightings of estimates are indicated by symbol size and border thickness—the largest symbols are for the highest weighted estimates, the estimates with the smallest SEs. †The study at 1360 m,¹³ and has been omitted from the reported analyses.

the line or quadratic passing through the origin were not significant with p values of 0.186 and 0.759, respectively. For altitude as a factor, the estimated mean change in Hbmass for all levels apart from the first (duration up to 24 h) was significantly greater than zero ($p < 0.001$). See table 2 for parameter estimates, SEs and CIs.

Postaltitude

Two of the 36 estimates of the change in $\ln(\text{Hbmass})$ from pre-altitude to postaltitude available for analysis were obvious outliers (standardised residuals of -3.49 and -3.36). Both these estimates were associated with an altitude less than 1800 m, and rather than just omitting the two outliers, it was decided to omit all five estimates associated with an altitude of < 1800 m from the results reported here (figure 2); omission of the extra three estimates had a negligible effect on any of the fitted models.

The most significant effect was the number of days postaltitude, though only in terms of whether or not the number was greater than 20 (days). There was also evidence of an effect of type of altitude, but only after 20 days postaltitude, with LHTL resulting in significantly higher values than classical altitude ($p = 0.039$). After allowing for the number of days postaltitude and the type of altitude, none of the other fixed effects (altitude, days or hours at altitude) added significantly to the model ($p = 0.666$ for the combined additional contribution). Up to 20 days postaltitude Hbmass was estimated to be, on average, 3.4% higher than prealtitude values, while for between 20 and 32 days postaltitude (the range of the available data), the change in Hbmass was not significantly different from zero for classic altitude, but was estimated to be 1.5% higher than prealtitude values for LHTL (table 3, figure 2).

Variability of within-subject measurements

For the results from the control participants' data and just the prealtitude data from the altitude participants, a simple step

Table 2 Parameter estimates for changes in $\ln(\text{Hbmass})$ from baseline (prealtitude) values during altitude exposure, derived via linear mixed modelling, and their interpretation in terms of percentage changes (increases) in Hbmass

Model/parameter	Change in $\ln(\text{Hbmass})$ from prevalues			Percentage of increase in Hbmass		
	Estimate	95% CI	p Value	Time at altitude (h)	Estimate	95% CI
Linear*						
slope	1.07×10^{-4}	$(0.94 \times 10^{-4} \text{ to } 1.20 \times 10^{-4})$	< 0.001	100	1.08	$(0.94 \text{ to } 1.21)$
Quadratic*						
Linear	1.39×10^{-4}	$(1.10 \times 10^{-4} \text{ to } 1.69 \times 10^{-4})$	< 0.001	100	1.33	$(1.10 \text{ to } 1.56)$
Quadratic	-7.59×10^{-8}	$(-13.95 \times 10^{-8} \text{ to } -1.23 \times 10^{-8})$	0.021	200	2.52	$(2.14 \text{ to } 2.89)$
				300	3.56	$(3.13 \text{ to } 4.00)$
Time as a factor (h)						
18–24	0.22×10^{-4}	$(-0.80 \times 10^{-4} \text{ to } 1.23 \times 10^{-4})$	0.664	18–24	0.22	$(-0.80 \text{ to } 1.24)$
96–112	1.29×10^{-4}	$(0.66 \times 10^{-4} \text{ to } 1.93 \times 10^{-4})$	< 0.001	96–112	1.30	$(0.66 \text{ to } 1.95)$
144–224	2.41×10^{-4}	$(1.82 \times 10^{-4} \text{ to } 3.01 \times 10^{-4})$	< 0.001	144–224	2.44	$(1.84 \text{ to } 3.06)$
266–294	3.25×10^{-4}	$(2.50 \times 10^{-4} \text{ to } 4.00 \times 10^{-4})$	< 0.001	266–294	3.30	$(2.53 \text{ to } 4.08)$
312–364	3.89×10^{-4}	$(3.09 \times 10^{-4} \text{ to } 4.70 \times 10^{-4})$	< 0.001	312–364	3.97	$(3.14 \text{ to } 4.81)$
408–456	4.00×10^{-4}	$(2.91 \times 10^{-4} \text{ to } 5.09 \times 10^{-4})$	< 0.001	408–456	4.08	$(2.95 \text{ to } 5.22)$
504–672	6.28×10^{-4}	$(4.96 \times 10^{-4} \text{ to } 7.59 \times 10^{-4})$	< 0.001	504–672	6.48	$(5.09 \text{ to } 7.89)$

*For the linear and quadratic models, the time at altitude is measured in hours so that, for example, the linear model implies an increase in $\ln(\text{Hbmass})$ of 0.0107/100 h, which translates to an increase of 1.08% in Hbmass.

p Values refer to testing whether the associated parameter is equal to zero. Hbmass, haemoglobin mass; $\ln(\text{Hbmass})$, natural log of Hbmass.

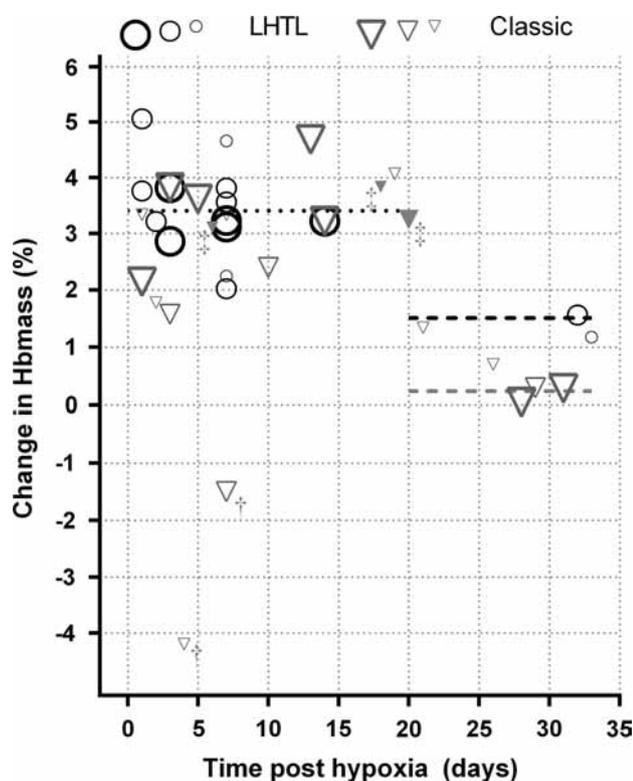


Figure 2 Estimates of the change in haemoglobin mass (Hbmass) after live high train low (LHTL, n=15) and classic (n=21) altitude exposure. The relative weightings of estimates are indicated by symbol size and border thickness—the largest symbols are for the highest weighted studies which have the smallest SEs. †Outliers, the estimate at day 4 from Frese and Friedmann-Bette,²⁸ and the estimate at day 7 from Neya *et al.*²⁴ ‡The other three estimates from Frese and Friedmann-Bette²⁸ (classic altitude and filled triangles) omitted from the reported analysis. Dotted (≤ 20 days) and dashed (> 20 days) lines are the modelled estimates indicated in table 3.

function between days 7 and 8 was found to be the best predictor of the within-subject SD of $\ln(\text{Hbmass})$, although there was some evidence ($p=0.061$) of an additional increase in the SD as the number of days between measurements increased. Using the model with a jump between days 7 and 8, and the additional increase with days, the CV for Hbmass was estimated to be reasonably constant at $\sim 2\%$ (with $\sim 95\%$ CI 1.80% to 2.35%) for measurements taken up to 7 days apart, after which it increased to $\sim 2.5\%$ (2.48% to 2.58% with $\sim 95\%$ CI 2.16% to 3.00% for measurements taken between 8 and 40 days apart; figure 3). An extended model, with six additional levels of a factor for days (roughly weeks), did not produce a significant improvement ($p=0.901$).

Table 3 Estimates of changes in Hbmass from baseline (prealtitude) to postaltitude values derived via linear mixed modelling

Condition	Percentage of increase in Hbmass		
	Estimate	95% CI	p Value
≤ 20 days (after LHTL or classic)	3.41	(2.89 to 3.92)	<0.001
> 20 days after LHTL	1.51	(0.43 to 2.59)	0.009
> 20 days after classic	0.24	(-0.55 to 1.04)	0.523

p Values refer to testing whether the associated parameter is equal to zero. Hbmass, haemoglobin mass; LHTL, live high train low.

The best estimate of the analytical CV for Hbmass was 2.04% for zero days between measurements with $\sim 95\%$ CI 1.80% to 2.33%.

Between-subject 'true' responses

For the within-subject SDs obtained from differences in $\ln(\text{Hbmass})$ between measurements taken prealtitude and while at altitude, a simple linear model in hours of exposure, with the SD equal to the estimated analytical SD for zero hours of exposure (the intercept), fitted the data reasonably well. Formal tests were carried out for departure from the forced intercept, for different responses to prealtitude to during altitude versus during altitude to during altitude, and for adding a quadratic term in altitude exposure, none of which were significant with p values of 0.331, 0.721 and 0.353, respectively. The results of this modelling are presented in figure 4, which gives estimated 95% prediction intervals for the 'true' increase in $\ln(\text{Hbmass})$, for individuals, for different durations of altitude exposure. For example, while it is estimated that after 300 h of exposure the estimated median increase in Hbmass will be 3.52%, it is also estimated that 95% of individuals will have a 'true' increase in Hbmass of between 1.14% and 5.95%.

DISCUSSION

The main findings of this meta-analysis are that Hbmass increases at approximately 1.1%/100 h of altitude exposure regardless of whether the exposure is classic altitude (> 2100 m) or LHTL (~ 3000 m), and that after a typical exposure of 300–400 h the increase above prealtitude values persists for ~ 3 weeks. In addition, modelling suggests that 97.5% of individuals will have a 'true' increase in Hbmass after 100 h of altitude exposure.

During altitude

In 2004, Rusko *et al.*³⁸ combined the results of eight studies using simple linear regression and concluded that LHTL could increase Hbmass by approximately 0.3%/day of altitude. In 2009, Clark *et al.*⁸ applied the same methodology as Rusko *et al.* to more recent studies and estimated that Hbmass increases at a mean rate of 1%/100 h of exposure to an adequate LHTL altitude, but with large uncertainty of this estimate (SE of estimate $\pm 3.5\%$). In a review of their own data, Levine and Stray-Gundersen³⁹ concluded that 3 weeks of classic altitude exposure or LHTL for 12 h/day each generated an increase in RCV of $\sim 4\%$. The current meta-analysis confirms the estimate of $\sim 1\%/100$ h for the pooled data of LHTL and classic altitude; this finding contrasts with Rasmussen *et al.*⁵ who concluded that below 3000 m of classic altitude there is no statistically significant increase in RCV within 4 weeks. The Monte Carlo simulation of Rasmussen *et al.*⁵ (their table 3) indicates that a 1% increase in RCV at a 95% level of probability would take between 13–28 days and 18–31 days of classic and LHTL altitude exposure, respectively. Their simulation results contrast with the current estimate of ~ 100 h, which equates to approximately 4 and 7 days for classic and LHTL, respectively, when the latter uses ~ 14 h/day. What are the possible reasons for these contrasting time estimates?

Two explanations are tenable and both relate to noise/error in the data, since changes as small as 1% are below the analytical error of even the best methods. The first consideration is that Rasmussen *et al.*⁵ selected RCV instead of Hbmass as their outcome variable. They needed to do so in order to standardise their data sources that were 44% from CO rebreathing, 37% from plasma dye dilution methods and 19% from radiolabelled albumin methods. Gore *et al.*⁹ demonstrated that the typical error

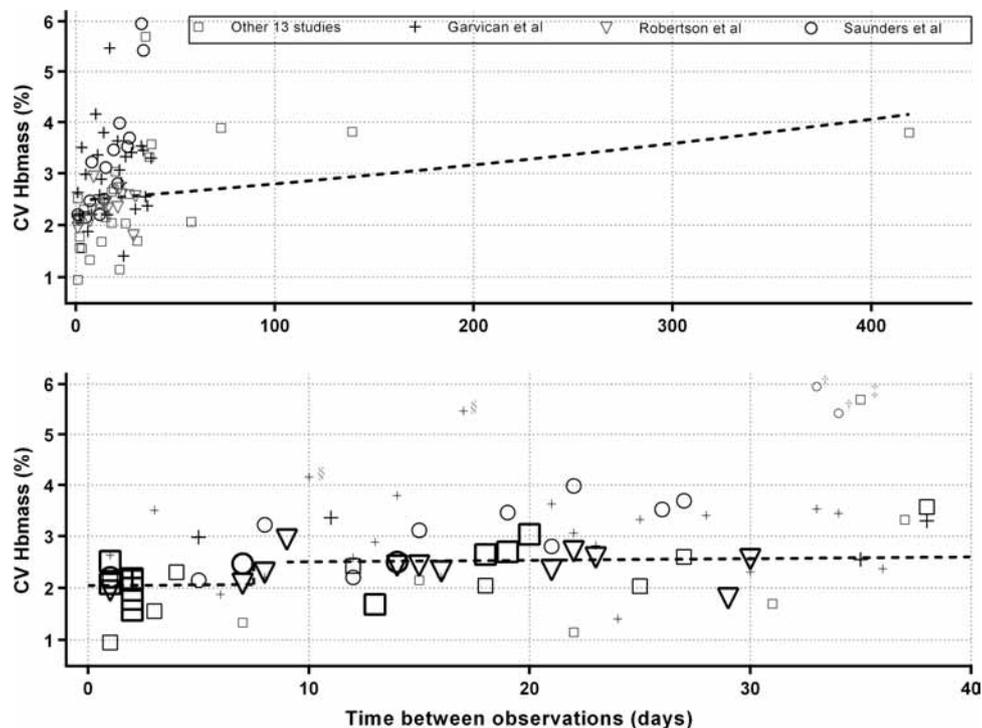


Figure 3 Estimates of the within-subject coefficient of variation (CV (%)) of haemoglobin mass (Hbmass) obtained using all of the pairwise differences in natural log of Hbmass ($\ln(\text{Hbmass})$) over time from the 17 studies using either repeated measures on control participants or the prealtitude replicates on the altitude participants. A total of 80 estimates were obtained from 1003 paired differences. Three studies provided 51 estimates as a consequence of frequent serial measures on their control participants and duplicate measures at baseline on their altitude participants: Garvican *et al*²⁹ (n=25), Robertson *et al*²⁰ (n=12) and Saunders *et al*²¹ (n=14). The lower panel is a repeat of the upper panel expanding the first 40 days, with the symbol sizes indicating how many pairwise observations (on individuals) were used to generate the estimate; a small symbol indicates ≤ 7 observations, a medium symbol 8–14 observations and a large symbol ≥ 15 observations. A dashed line on both panels shows the fitted models; for x (days) ≤ 7

$$y = \sqrt{\exp(e^{-7.8004+0.0024x}) - 1} \times 100$$

whereas for $x > 7$

$$y = \sqrt{\exp(e^{-7.4342+0.0024x}) - 1} \times 100,$$

Superscripted symbols indicate studies with the five largest estimates, each of which was $>4\%$; [†]Frese and Friedmann-Bette,²⁸ [§]Garvican *et al*²⁹ and [†]Saunders *et al*.²¹

of RCV from CO rebreathing and Evans blue dye (a common plasma dye-dilution method) is $\sim 7\%$, with 90% confidence limits of $\sim 3\text{--}14\%$ and $5\text{--}9\%$, respectively). In contrast, the corresponding typical error for Hbmass from CO rebreathing was estimated at 2.2% (90% confidence limits of 1.4% to 3.5%) for measures taken 1 day apart.⁹ Our best estimate of the analytical error for Hbmass from the current meta-analysis is 2.04% (95% confidence limits of 1.80% to 2.33%) for observations taken zero days apart. The approximate tripling of error using RCV instead of Hbmass relates partially to error propagation due to the extra steps of measuring the haemoglobin concentration and haematocrit required to estimate RCV and partially to the greater imprecision of dye-dilution methods.⁹ The second consideration is that relatively noisy data for RCV from a variety of sources will cloud small effects even with a statistically powerful meta-analytic approach as discussed by Rasmussen *et al*.⁵ They state that “the inclusion of results obtained with different methods may have increased the variance and reduced the power of the analysis.” Indeed, they also comment that the variability in their modelled increase in RCV across the pooled data set was ‘surprisingly large’, being an average of 49 ± 240 mL/week. The median RCV of Rasmussen *et al*’s participants was 2518 mL, so the uncertainty of ± 240 mL equates to a variation of $\pm 9.5\%$

about the median RCV. This is consistent with the conclusion of the 2005 meta-analysis⁹ that RCV has $\sim 7\%$ error for measures 1 day apart and closer to $\sim 8\%$ for measures taken a month apart, as would be more typical with an altitude intervention. With 20% of Rasmussen *et al*’s⁵ data from radiolabelled methods, one would have expected that their overall error may have been attenuated, but substantial noise is apparent in their data for the average estimates of the change in RCV for time spent at altitude.

Notwithstanding the large uncertainty, the average increase of $49 (\pm 240)$ mL/week reported by Rasmussen *et al*⁵ corresponds to 1.95%/week of the median RCV, or an increase of 1.16%/100 h of altitude. This is similar to our estimate of 1.08%/100 h for Hbmass (table 2). Therefore, despite examining mostly different data sets, there is good agreement about the general magnitude of increase from altitude exposure between the current meta-analysis and that of Rasmussen *et al*⁵ albeit that the latter included substantially higher altitudes than the former.

Finally, data from lifelong altitude residents show that Hbmass will not increase indefinitely when athletes train at altitude; for instance, Schmidt *et al*⁴⁰ found that the Hbmass relative to the body mass of cyclists from 2600 m was $\sim 10\%$ higher than that of cyclists from sea level. So after some period of months, the increase that we report (figure 1) will plateau.^{41 42}

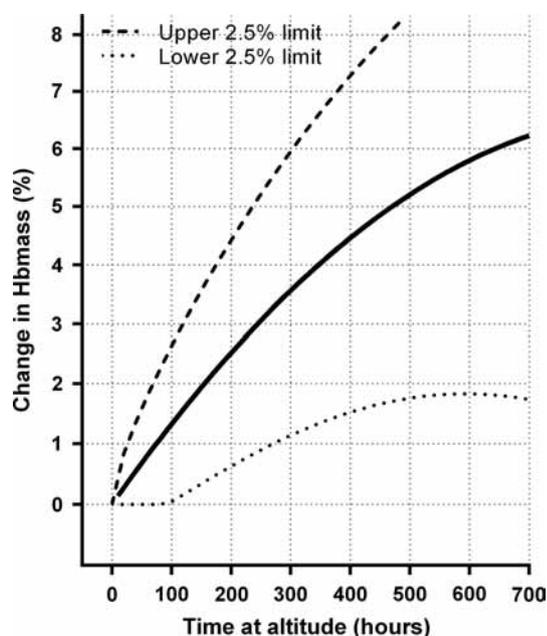


Figure 4 Estimated median and estimated between-subject 'true' change in haemoglobin mass (Hbmass) in response to altitude exposure. The solid line refers to the same quadratic model as in figure 1 with dashed lines being the upper and lower 95% individual response limits. Where the lower limit of the individual responses was estimated to be negative, it has been truncated at zero.

Various models with such a plateau were tried but did not fit our data as well as the reported quadratic model, which should not be extrapolated beyond the range of our data; the maximum exposure to altitude among our data was 670 h. The fitted quadratic implies a maximum increase in Hbmass of 6.6% after 920 h, albeit that this result is quite likely specific to the data set that was examined and 920 h is well beyond the range of our data. Indeed, theoretically one would have expected that a model that would have best fitted our data would comprise a delay constant and two exponential functions, one for rapid changes and one for slow changes, which are typical of multi-component acclimatisations. There should be a delay because of the time needed for increased red cell production⁴³ and a steeper curve of increasing Hbmass during the first phase,²⁹ before improved arterial oxygen content following a decrease in plasma volume⁴⁴ and an increase in ventilation caused by water and bicarbonate excretion.⁴⁵ Thereafter, a second slower phase of increasing Hbmass would be expected.³ However, multi-component models of this sophistication did not fit our data better than the models we derived. More elaborate models would require more extensive data than were available, but our parsimonious linear model (a linear increase of $\sim 1\%/100$ h) is simple for practitioners to apply.

Postaltitude

The veracity of the estimated increase in Hbmass during altitude exposure is supported by the results posthypoxia from our current meta-analysis, with a significant increase of $\sim 3\text{--}4\%$ evident following typical exposures to classic and LHTL altitude exposure. In addition, this is the first meta-analytic attempt to characterise the time course of Hbmass after altitude exposure.

Our modelling indicated a $\sim 3\%$ increase in Hbmass for up to 20 days post classic and LHTL altitude exposure. Prommer *et al*⁴⁶ have most carefully characterised the time course of Hbmass in Kenyans living temporarily near sea level; they observed no discernible change in Hbmass for the first 14 days and then a significant ($\sim 2.5\%$) decrease after 21 days, which was $\sim 3.3\%$ in magnitude after 28 days and $\sim 6\%$ after 40 days. However, one might expect that the time course for lifelong altitude residents might differ from that of athletes who had only sojourned to altitude for a few weeks.

Neocytolysis, the preferential destruction of young circulating RBCs (neocytes) by reticuloendothelial phagocytes,⁴⁷ is considered the likely cause of the response after descent from altitude.⁴⁸ Pottgiesser *et al*⁴⁹ explored serum erythropoietin, serum ferritin, percentage of reticulocytes and Hbmass as indirect markers of neocytolysis in athletes subsequent to LHTL and concluded that there was evidence of rapid red blood cell destruction, at least in some athletes. The data of Pottgiesser *et al* are included in the current meta-analysis as part of Garvican *et al*¹⁴ but when pooled with the other available studies, the evidence for a rapid decrease in Hbmass is not present. A recent study of Bolivians transitioning from La Paz (3600 m) to near sea level for 6 days also reached the same conclusion; specifically, that Hbmass did not decrease rapidly for altitude natives during a few days near sea level.³¹ However, it needs to be acknowledged that for our meta-analysis there is a paucity of data in excess of 7 days posthypoxia; specifically, 19 of the 31 values (61%) used in this meta-analysis were for data collected within the first week postaltitude. In addition, the quality of the data was noticeably better (smaller SEs) for the data collected ≤ 14 days postaltitude, which adds supports to the confidence in our conclusion that the posthypoxia increase is significant. To better understand the time course of Hbmass postaltitude exposure, future research should focus particularly on generating data up to 4 weeks after exposure.

Variability of within-subject measurement

Measures less than or equal to a week apart were associated with a within-subject CV of $\sim 2\%$, which is very similar to both the 2.2% estimated in a previous meta-analysis for measures taken 1 day apart⁹ and the estimates of 1.9 and 2% for men and women, respectively, for measures taken on the same day.³⁶ The most recent estimate of the CV of Hbmass of 1.6% for measures taken ~ 12 days apart is even lower,¹⁸ which is much less than in the present study and may reflect successive refinements to the CO rebreathing method over time, such as higher doses of CO and more replicate measurements of carboxyhaemoglobin,¹⁶ as well as the use of custom quality control materials.¹⁸ In the current meta-analysis, the SD of paired observations increased over time for measures conducted more than 1 week apart, such that for measures 40 days apart the within-subject CV is $\sim 2.68\%$. It should be appreciated that the estimate of within-subject SD taken more than a few days apart includes biological and analytical components that are independent and additive.³⁶ In the current meta-analysis, there was little evidence of biological variation for the first week, but thereafter the additional progressive increase in SD is evidence of biological variation. Sources of biological variation in Hbmass of athletes include illness, injury,⁵⁰ training⁵¹ and energy intake.²²

Of the 80 estimates of within-subject CV, there were 5 which exceeded 4%, Frese and Friedmann-Bette,²⁸ Garvican *et al*,²⁹ and Saunders *et al*,²¹ and the largest of these was nearly 6% (figure 3). The latter two of these studies provided 39 estimates of the within-subject CV and when using small samples ($n < 7$), a

range of estimates is expected by chance alone. For the >7-day estimate of within-subject CV of 2.5% of this meta-analysis, the 95% limits for a change (calculated as $\pm 1.96 \times \sqrt{2 \times 2.5}$) between successive measures on an individual is from -6.9% to 6.9%, since both the first and second measures will be subject to error. So changes as large as ~7% are likely between two measures of Hbmass just over a week apart, and will occur 5% of the time (ie, one in every 20 measures). Furthermore, one in every 100 measures for a control participants will quite likely show a random change as much as 9.1% ($2.57 \times \sqrt{2 \times 2.5}$), even though the Hbmass is stable. Such data should not be discarded as outliers; they are the inevitable consequence of measurement imprecision.

Given that the 7-day within-subject CV is ~2% (the vast majority of which is likely to be due to analytical errors), how can this meta-analysis conclude that the 1% increase in Hbmass after ~100 h of altitude exposure is statistically significant? The estimated change after ~100 h at altitude (1.33%, table 2) is about half the uncertainty of the measure. But with adequate sample sizes, and hence statistical power, even small changes can be detected from a conventional statistical approach. The current meta-analysis of multiple estimates of the change in Hbmass, derived from time series measurements using CO rebreathing, has provided the means to do so. Radiolabelled methods such as ^{51}Cr to estimate RCV are the criterion, but it is not practical to make multiple measurements on healthy athletes before, during and after altitude exposure due to radiation concerns.⁹ Indeed, given the findings of this meta-analysis, it would be potentially unethical to use radiolabelled methods, which have similar measurement error to CO rebreathing.⁹

Between-subject 'true' response

Statistically removing the analytical component of error from the measured change in Hbmass from prealtitude to during altitude and while at altitude allows a method to approximate the 'true' between-individual responsiveness to altitude exposure—albeit that it is inexact (figure 4).³⁷ Our analysis reveals that 97.5% of individuals will increase Hbmass by at least 1% after 300 h of exposure (equivalent to 12.5 days of classic altitude or 21.4 days of LHTL with 14 h/day of hypoxia); the corresponding upper limit is 6%. Although these estimates are relatively crude approximations, they imply that a 2-week camp at classic altitude will increase the Hbmass of most athletes. Therefore, with adequate preparation,⁵² coaches and athletes can undertake such short camps with confidence such that they will quite likely be worthwhile for most individuals. However, if measured changes in Hbmass after a 2-week altitude camp are greater than ~5.5% (eg, 10%), this would be indicative of measurement imprecision, as described in the previous subsection, rather than a 'true' increase of this magnitude.

Limitations

The limitations of this study are as follows: (1) all but one of the LHTL studies used the same simulated altitude of 3000 m, (2) only one of the LHTL studies was conducted at terrestrial altitude,²⁴ (3) there are relatively few data in excess of 7 days postaltitude exposure, (4) none of the studies used double-blind placebo interventions and (5) training data are not included. With respect to the latter point, Garvican *et al*⁵¹ have modelled that a 10% change in a 42-day training load is associated with a 1% change in Hbmass. Thus, for a typical 3-week altitude camp, a 20% increase in training load, which would be large for an elite athlete, might be associated with a 1% increase in Hbmass. However, for a classic altitude camp of 3 weeks, the mean estimated increase in Hbmass is 5.2%, so the majority of the increase is quite likely

attributable to altitude per se. If, as is common practice, the training load was actually reduced during the altitude camp, then any increase in Hbmass could reasonably be attributed to the altitude stimulus. Finally, while laboratory or competition performance postaltitude is susceptible to placebo effects,⁵³ it is not tenable that Hbmass can be influenced by belief.

Application

In a busy schedule of training and competition, athletes may not be able to afford the time for the recommended 3–4 week blocks of altitude training,⁶ instead opting for camps as short as 2 weeks.³⁹ The results of this meta-analysis support the notion that a 2-week classic camp (336 h) may be sufficient to increase Hbmass by a mean of ~3% and by at least 1% for 97.5% of athletes. Athletes have been using altitude training of various forms for many years⁵⁴ and, given that a worthwhile increase in performance for an elite athlete is of the order of 0.3–0.4%,^{55 56} it is possible that they are attuned to small but important changes in their physiology that might improve race performance. As indicated by Jacobs,⁵⁷ the possibility of type II errors (falsely accepting null findings due to modest statistical power) may cloud our interpretation of studies of altitude training, particularly when it comes to performance. Atkinson *et al*⁵⁸ support this idea and state specifically that “statistical significance and non-significance can no longer be taken as sole evidence for the presence or absence of a practically meaningful effect.”

What are the new findings

- ▶ The optimised carbon monoxide rebreathing method to determine haemoglobin mass (Hbmass) has an analytical error of ~2%, which provides a sound basis to interpret changes in Hbmass of athletes exposed to moderate altitude.
- ▶ During-altitude Hbmass increases by ~1.1%/100 h of adequate altitude exposure, so when living and training on a mountain (classic altitude) for just 2 weeks, a mean increase of ~3.4% is anticipated.
- ▶ Living high and training low (LHTL) at 3000 m simulated altitude is just as effective as classic altitude training at ~2320 m at increasing Hbmass, when the total hours of hypoxia are matched.
- ▶ ~97.5% of adequately prepared athletes are likely to increase Hbmass by at least 1% after approximately 300 h of altitude exposure, either classic or LHTL. 'Adequately prepared' includes being free from injury or illness, not 'overtrained' and with iron supplementation.

How might it impact on clinical practice in the near future

- ▶ For athletes with a busy training and competition schedule, altitude training camps as short as 2 weeks of classic altitude will quite likely increase Hbmass and most athletes can expect benefit.
- ▶ Athletes, coaches and sport scientists can use altitude training with high confidence of an erythropoietic benefit, even if the subsequent performance benefits are more tenuous.

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Competing interests None.

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**APPENDIX VI – ETHICS APPROVALS, LETTERS TO PARTICIPANTS
AND CONSENT FORMS**

STUDY 1 AND 2 ETHICS APPROVAL,
LETTER TO PARTICIPANTS AND CONSENT FORMS
ACU HUMAN ETHICS COMMITTEE APPROVAL NUMBER V2011 108

INFORMATION LETTER TO PARTICIPANTS

TITLE OF PROJECT: Physiological response to a three-week altitude training camp'

INVESTIGATOR 1: Associate Professor Justin Kemp

INVESTIGATOR 2: Associate Professor David Buttifant

INVESTIGATOR 3: Dr Doug Whyte

STUDENT RESEARCHER: Mr Blake McLean

PROGRAMME IN WHICH ENROLLED: PhD – Exercise Science

Dear Participant,

You are invited to participate in the research project 'Physiological response to a three-week altitude training camp' being conducted by Associate Professor Justin Kemp, Associate Professor David Buttifant, Dr Doug Whyte and Mr Blake McLean. The project aims to determine the physiological responses to a three-week altitude training camp and also identify how individual athletes respond to altitude training. This will be achieved through a series of performance tests (e.g. running and strength tests completed as part of your normal training program), body composition scans and blood analyses. For this, the study will involve the collection of a total of three venous blood samples and nine finger-prick blood samples before, during and after a 3 week training camp in Flagstaff, Arizona. These blood samples will help us identify possible markers of adaptation to altitude such as blood, hormone and DNA related characteristics that might help us understand why some players respond more than others to altitude training.

Should you choose to participate in the study, you will be required, to provide blood samples and complete muscle and a body composition skinfold tests immediately before, immediately after and two weeks after the training camp in Arizona.

All procedures used in this study are used in your current training/monitoring programs. However, there are a number of small risks associated with these practices, which include: risk of injury during maximal exercise testing (however, this is minimal as you are used to high exercise intensities), and a small risk of infection with blood sampling (no more than a usual blood collection); however every precaution will be taken to ensure safe sampling. As always, with blood sampling, there may be some small amount of bruising associated with drawing the sample.

The study also involves a Magnetic Resonance Spectroscopy (MRS) scan, similar to scans you may have had for some injuries. Whilst this scan involves no radiation, it does use a magnet, so you must inform us if you have any metal plates or irremovable piercings in your body. If this is the case, you will not be able to perform this scan, but you will be able to participate in the rest of the study.

From your blood sample we will also analyse one part of your DNA which is thought to be related to training responses. Only this portion of your DNA (androgen receptor gene) will be analysed and DNA samples will be destroyed once this has taken place. You may request the manner in which your DNA is destroyed to abide by any cultural or religious beliefs. Should you choose to, you may opt not to have your DNA analysed and still participate in the rest of the research study.

Your participation in this study will help us understand what physiological changes occur at altitude and how quickly these changes occur. The findings from the study will also help us understand how we might best prescribe altitude training on an individual basis by identifying factors that might make some people respond to this type of training more effectively than others. This project will hopefully

provide insight into this issue and help us to better prescribe training for you and other players in the future.

Participation in the study is voluntary and your standing with coaching staff and Collingwood Football Club will not be affected by your decision to participate or not in the research study. If you do choose to participate but later change your mind for any reason, you may withdraw without any consequences.

Your personal information and any data collected during this study will be kept completely confidential throughout and after the study period. The only people that will have access to this information are the researchers (Blake McLean, David Buttifant, Doug Whyte and Justin Kemp) and Collingwood Football Club medical and sports science staff. After all data have been collected and analysed, data will be available to Collingwood Football Club medical and sports science staff in order to optimise your future training programs. Average scores for the group as a whole will also be used in sports science presentations and publications; however, no individually identifiable data will be apparent in such presentations/publications.

Any questions regarding this project should be directed to investigators and the student researcher:

Associate Professor Justin Kemp
Ph: 03 9953 3031

Associate Professor David Buttifant
Ph: 0429 481 184

Associate Professor Doug Whyte
Ph: 03 9953 3557

Mr Blake McLean
Ph: 0468 646 765

School of Exercise Science
Australian Catholic University
115 Victoria Pde
Fitzroy, Victoria, 3065

Upon the completion of the project all of your results will be available to you and also given to your in a printed summary.

This study has been approved by the Human Research Ethics Committee at Australian Catholic University. In the event that you have any complaint or concern, or if you have any query that the Supervisor or Student Researcher have not been able to satisfy, you may write to the Chair of the Human Research Ethics Committee care of the nearest branch of the Research Services Office:

Chair, HREC
C/- Research Services
Australian Catholic University
Melbourne Campus
Locked Bag 4115
FITZROY VIC 3065
Tel: 03 9953 3158

Any complaint or concern will be treated in confidence and fully investigated. The participant will be informed of the outcome.

If you agree to participate in this project, you should sign both copies of the Consent Form, retain one copy for your records and return the other copy to the Supervisor or Student Researcher.

.....
Investigator 1

.....
Student Researcher

CONSENT FORM

TITLE OF PROJECT: Physiological and genetic responses to altitude training

INVESTIGATOR 1: Dr Justin Kemp

INVESTIGATOR 2: Dr David Buttifant

INVESTIGATOR 3: Dr Doug Whyte

STUDENT RESEARCHER : Mr Blake McLean

I..... have read and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in this study involving blood collections, body composition scans and exercise testing over a two-month period, realising that I can withdraw my consent at any time without any adverse consequences. I understand that as part of this study DNA analysis of the androgen receptor gene will be performed on my blood samples. I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.

Do you have any metal plates in your body and/or irremovable piercings?

Yes / No

Do you agree to have your DNA analysed for the purposes of this study (androgen receptor gene only)?

Yes / No

NAME OF PARTICIPANT:

SIGNATURE DATE

SIGNATURE OF INVESTIGATOR 1 :..... DATE:.....

SIGNATURE OF INVESTIGATOR 2 :..... DATE:.....

SIGNATURE OF INVESTIGATOR 3 :..... DATE:.....

SIGNATURE OF STUDENT RESEARCHER:.....DATE:.....

STUDY 3 ETHICS APPROVAL,

LETTER TO PARTICIPANTS AND CONSENT FORMS

ACU HUMAN ETHICS COMMITTEE APPROVAL NUMBER V2012 257V

INFORMATION LETTER TO PARTICIPANTS

TITLE OF PROJECT: The reliability of running performance during simulated team-sport running on a non-motorised treadmill

INVESTIGATOR 1: Dr Stuart Cormack

INVESTIGATOR 2: Professor Justin Kemp

STUDENT RESEARCHER: Mr Blake McLean

PROGRAMME IN WHICH ENROLLED: PhD – Exercise Science

Dear Participant,

You are invited to participate in the research project ‘The reliability of running performance during simulated team-sport running on a non-motorised treadmill’ being conducted by Dr Stuart Cormack, Associate Professor Justin Kemp, and Mr Blake McLean. The project aims to determine the reliability of a simulated team sport protocol on the newly developed Curve 3 Non-motorised treadmill (NMT). This will be achieved with a running protocol on the NMT designed to simulate a team sport match. For this, the study will involve five visits to the laboratory, with the first visit involving physiological testing and familiarisation with running on the NMT. Visits 2-5 will involve a 30min simulated team sport protocol on the NMT. During the first visit, oxygen consumption will be measured via analyses of expired air (i.e. you will have a mask attached to you, measuring what you breathe out) and eight to ten finger-prick blood samples will be collected during the exercise protocols for immediate analysis of blood lactate.

Should you choose to participate in the study, you will be required to complete treadmill exercise protocols on five separate visits to the laboratory and provide fingerprick blood samples during these exercise tests.

There are a number of small risks associated with these practices, which include: risk of injury during maximal exercise testing (however, this is minimal as you are used to high exercise intensities), and a very small risk of infection with blood sampling (this is unlikely due to strict aseptic techniques being used); however every precaution will be taken to ensure safe sampling.

Your participation in this study will help with understanding the reliability of testing team sport athletes on a NMT. These findings will help future projects determine what effect different training protocols, supplement interventions etc. have on team sport running performance, and ultimately lead to optimal training strategies for team sport athletes.

Participation in the study is voluntary and if you do choose to participate but later change your mind for any reason, you may withdraw without any consequences.

Your personal information and any data collected during this study will be kept completely confidential. The only people who will have access to this information are the researchers (Stuart Cormack, Blake McLean, and Justin Kemp). After all data have been collected and analysed, average scores for the group as a whole will also be used in sports science presentations and publications; however, no individually identifiable data will be apparent in such presentations/publications.

Any questions regarding this project should be directed to investigators and the student researcher:

Dr Stuart Cormack
Ph: 03 9953 3133

Professor Justin Kemp
Ph: 03 9953 3031

Mr Blake McLean
Ph: 0468 646 765

School of Exercise Science
Australian Catholic University
115 Victoria Pde
Fitzroy, Victoria, 3065

Upon the completion of the project all of your results will be available to you and also given to your in a printed summary.

This study has been approved by the Human Research Ethics Committee at Australian Catholic University. In the event that you have any complaint or concern, or if you have any query that the Supervisor or Student Researcher have not been able to satisfy, you may write to the Chair of the Human Research Ethics Committee care of the nearest branch of the Research Services Office:

Chair, HREC
C/- Research Services
Australian Catholic University
Melbourne Campus
Locked Bag 4115
FITZROY VIC 3065
Tel: 03 9953 3158
Fax: 03 9953 3315

Any complaint or concern will be treated in confidence and fully investigated. The participant will be informed of the outcome.

If you agree to participate in this project, you should sign both copies of the Consent Form, retain one copy for your records and return the other copy to the Investigator or Student Researcher.

.....
Investigator 1

.....
Student Researcher

CONSENT FORM

TITLE OF PROJECT: The reliability of running performance during simulated team-sport running on a non-motorised treadmill

INVESTIGATOR 1: Dr Stuart Cormack

INVESTIGATOR 2: Prof Justin Kemp

STUDENT RESEARCHER: Mr Blake McLean

I have read and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in this study involving blood collections and treadmill performance test over five laboratory visits, realising that I can withdraw my consent at any time without any adverse consequences. I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.

NAME OF PARTICIPANT:

SIGNATUREDATE

SIGNATURE OF INVESTIGATOR 1 :.....DATE:.....

SIGNATURE OF INVESTIGATOR 2 :.....DATE:.....

SIGNATURE OF STUDENT RESEARCHER:.....DATE:.....

STUDY 4 ETHICS APPROVAL,
LETTER TO PARTICIPANTS AND CONSENT FORMS
ACU HUMAN ETHICS COMMITTEE APPROVAL NUMBER V2013 79V

PARTICIPANT INFORMATION LETTER

PROJECT TITLE: Performance benefits of five weeks of intermittent hypoxic training in team sport athletes

PRINCIPAL INVESTIGATOR: Professor Justin Kemp

STUDENT RESEARCHER: Mr Blake McLean

STUDENT'S DEGREE: Doctorate of Philosophy

Dear Participant,

You are invited to participate in the research project described below.

What is the project about?

The research project investigates the physiological responses to five weeks of intermittent hypoxic training, and how this type of training affects running performance specific to team sports.

Who is undertaking the project?

This project is being conducted by Mr Blake McLean and will form the basis for the degree of Doctorate of Philosophy at Australian Catholic University under the supervision of Professor Justin Kemp.

Are there any risks associated with participating in this project?

There are a number of small risks associated with these practices, which include: risk of injury during maximal exercise testing and exercise training (however, this is minimal as you are used to high exercise intensities), and a small risk of infection with blood sampling (no more than a usual blood collection); however every precaution will be taken to ensure safe sampling. As always, with blood sampling, there may be some small amount of bruising associated with drawing the sample.

What will I be asked to do?

Should you choose to participate in this study, you will be asked to be involved in the following testing:

- Four laboratory visits to ACU, over the space of 3 weeks which will include:
 - Four 30 min team sport running simulations on a non-motorised treadmill
 - One VO₂max test on a motorized treadmill (final laboratory visit)
- Complete five weeks of intermittent hypoxic training, including running and resistance training, at the Westpac Centre (Collingwood Football Club training facility) – this will involve 3 training sessions per week, all including some running and resistance training
- Upon completion of the 5 weeks of training, you will be asked to return to the laboratory at ACU and again complete a 30 min team sport simulation and another VO₂max test

How much time will the project take?

Each laboratory visit at ACU will take around 1 hour to complete, and each training session throughout the 5 weeks will last around 2 hours. Testing session at ACU will most likely be held on Saturdays and training session will most likely be Monday, Wednesday and Friday evenings – however, this training and testing days may be altered to suit your schedule, where possible.

What are the benefits of the research project?

By participating in this project, you will have access to new training facilities at the Collingwood Football Club (throughout training days in the study) and be participating in training programs designed to optimise team sport performance. You will also be participating in some intermittent hypoxic training, a new technique which has the possibility to further enhance running performance. By participating in this training, you should

improve your fitness and be well prepared for any upcoming team sport competition that you are involved in.

Your participation in this study will also help us understand what performance and physiological changes occur with intermittent hypoxic training and understand how to best use this type of training with team sport athletes in the future, in order to optimise running performance in matches.

Can I withdraw from the study?

Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate, you can withdraw from the study at any time without adverse consequences.

Will anyone else know the results of the project?

Your personal information and any data collected during this study will be kept completely confidential throughout and after the study period. The only people that will have access to this information are the researchers (Blake McLean and Justin Kemp). Average scores for the group as a whole will also be used in sports science presentations and publications; however, no individually identifiable data will be apparent in such presentations/publications.

Will I be able to find out the results of the project?

After all data have been collected and analysed, you will be provided with a summary of your results as well as an overview of the group's average results.

Who do I contact if I have questions about the project?

Any questions regarding this project should be directed to investigators and the student researcher:

Professor Justin Kemp

Ph: 03 9953 3031

Mr Blake McLean

Ph: 0468 646 765

School of Exercise Science

Australian Catholic University

115 Victoria Pde

Fitzroy, Victoria, 3065

What if I have a complaint or any concerns?

The study has been approved by the Human Research Ethics Committee at Australian Catholic University (approval number 2012 0000017257). If you have any complaints or concerns about the conduct of the project, you may write to the Chair of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).

Chair, HREC

c/o Office of the Deputy Vice Chancellor (Research)

Australian Catholic University

Locked Bag 4115

FITZROY, VIC, 3065

Ph: 03 9953 3150

Email: res.ethics@acu.edu.au

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

I want to participate! How do I sign up?

If you would like to participate in this study, please contact Blake McLean (E-mail: Blake.Mclean@acu.edu.au, Phone: 0468 646 765) to arrange a time to meet and return your informed consent forms and arrange a time for your first laboratory testing session.

Yours sincerely,

Mr Blake McLean

Prof. Justin Kemp

CONSENT FORM

TITLE OF PROJECT: Performance benefits of five weeks of intermittent hypoxic training in team sport athletes

PRINCIPAL INVESTIGATOR 1: Dr Justin Kemp

STUDENT RESEARCHER: Mr Blake McLean

I..... have read and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in this study involving 5 weeks of intermittent hypoxic training, blood collections, Hb_{mass} testing and treadmill based performance tests, realising that I can withdraw my consent at any time without any adverse consequences. I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.

NAME OF PARTICIPANT:

SIGNATURE DATE

SIGNATURE OF PRINCIPAL INVESTIGATOR:

DATE:.....

SIGNATURE OF STUDENT RESEARCHER:

DATE:.....