University of Brighton

Hypoxic Exposure to Optimise Altitude Training Adaptations in Elite Endurance Athletes

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ABSTRACT

The purpose of this thesis was to examine the physiological and haematological responses to altitude training and hypoxic exposures. Furthermore to investigate if additional hypoxic exposure around a "live high-train high" altitude training camp could maximise adaptations.

Study one provided a detailed insight into the current practices and perceptions of elite British endurance athletes and coaches to altitude training. A survey found that the athletes and support staff's concerns included maintaining training load at altitude, reducing the acclimatisation period, maximising haematological adaptations and when to compete on return to sea level. These challenges were prioritised and investigated further in the thesis.

Confidence in the optimised carbon monoxide (CO) rebreathing method (oCOR-method) is paramount when assessing haematological adaptations. Study two found that Radiometer ABL80 hemoximeter provided a more valid and reliable determination of percent carboxyhaemoglobin (%HbCO) with a minimum of three replicate blood samples to obtain an error of ≤1%. Study three found that administering different boluses of CO produced significantly different haemoglobin mass (tHbmass) results (0.6 mL·kg^{-1} = 791 ± 149 g; 1.0 mL·kg^{-1} = 788 ± 149 g; and 1.4 mL·kg^{-1} = 776 ± 148 g). A bolus of 0.6 to 1.0 mL·kg^{-1} provided sufficient precision and safety to determine %HbCO with the ABL80 hemoximeter.

Additional hypoxic exposures have been identified as a strategy to maintain altitude haematological adaptations gained from altitude training camps. Study four investigated the time course of erythropoietin (EPO) and inflammatory markers after acute (2 h passive rest) hypoxic exposures (FiO_2: 0.135, 0.125, 0.115, and 0.209). [EPO] increased in all hypoxic conditions 2 h post-exposure, being maintained until 4 h post-exposure, however, the largest increase came from the FiO_2: 0.115 condition increasing by ~50% ($P < 0.001$). There were no differences found between hypoxic exposures in IL-6 or TNFα.

Study five investigated the effect of acute hypoxia as a priming tool, by measuring the effect of increased circulating EPO on endurance performance. A 10 min pre-loaded treadmill running time trial (TT_{10}) was preceded by 2 h normobaric hypoxia (HYPO; FiO_2: 0.115), hyperoxia (HYPER; FiO_2: 0.395) or normoxia (CON; FiO_2: 0.209) 3.5 h prior to the TT_{10}. No differences ($P = 0.082$) were found in distance covered during TT_{10} (HYPO: 2726 ± 277 vs. CON: 2724 ± 279 vs. HYPER: 2742 ± 281 m).

Study six monitored physiological and haematological variables of elite endurance runners completing four weeks of live high-training high (LHTH; ~2,300 m) altitude training (ALT) compared to a control group (CON). A hypoxic sensitivity test (HST) was completed pre (PRE) and post-altitude (POST-2), alongside a treadmill test and oCOR-method. From PRE to POST-2 a difference in average lactate threshold (LT) (6.1 ± 4.6% vs. 1.8 ± 4.5%) and lactate turnpoint (LTP) (5.4 ± 3.8% vs. 1.1 ± 3.2%) was found within ALT, but not CON. Mean $\dot{V}O_{2max}$ increased by 2.7 ± 3.5% in ALT, and decreased by 3.3 ± 6.3% in the CON group ($P = 0.042$). Total Hbmass increased by 1.9 ± 2.9% and 0.1 ± 3.3% ($P > 0.05$) from PRE to POST-2 in the ALT and CON group, respectively. No changes were found in mean tHbmass post-LHTH; however, EPO was lower at POST-1. The HST revealed desaturation at rest and hypoxic ventilatory response at exercise predicted individual changes in tHbmass and hypoxic cardiac response at rest predicted changes in $\dot{V}O_{2max}$.

The evidence reported supports the notion that additional hypoxic exposures around an altitude training camp can maximise physiological and haematological adaptation via a prior understanding of an athlete's response to hypoxia and therefore the optimisation the athlete's altitude training needs.

TABLE OF CONTENTS

PUBLICATIONS AND PRESENTATIONS

The work in this thesis has been presented at scientific meetings and/or published in peer reviewed journals as listed below:

1.1 Publications

Turner, G, Richardson, AJ, Maxwell, NS and Pringle, JSM (2014). Comparison of total haemoglobin mass measured with the optimized carbon monoxide rebreathing method using different Radiometer™ hemoximeters. *Phys Measure*, 35(12): N41-N49

Turner, G, Pringle, JSM, Ingham, SA, Fudge BW, Richardson, AJ and Maxwell, NS (2014). The influence of carbon monoxide bolus on the measurement of total haemoglobin mass using the optimized CO-rebreathing method. *Phys Measure*, 35(2): N11-N19.

Turner, G, Gibson, OR, Watt, PW, Pringle, JSM, Richardson, AJ and Maxwell, NS (2016). The time course of endogenous erythropoietin, IL-6, and TNFα in response to acute hypoxic exposures. *Scand J Med Sci Sport*, [Epub ahead of print]

1.2 Presentations

Turner, G, Pringle, JSM, Ingham, SA, Fudge BW and Maxwell, NS (2013). The stability and accuracy of the measurement of tHbmass using the optimised CO-rebreathing method. *ECSS* Barcelona 2013.

Turner, G, Ross, EZ, Richardson, AJ, Maxwell, NS and Pringle, JSM (2014). Dose-response relationship of endogenous erythropoietin in response to an acute hypoxic exposure. *ECSS* Amsterdam 2014.

1.3 Other

Fudge, BW, Pringle, JSM, Maxwell, NS, **Turner, G,** Ingham, SA & Jones, AM. (2012). Altitude training for elite endurance performance: a 2012 update. *Curr Sports Med Rev*, 11(3): 148-154.

Turner, G, Gibson, OR and Maxwell, NS (2014) Simulated moderate hypoxia reduces intermittent sprint performance in games players. *J Sports Med Phys Fitness*, 54(5): 566-74.

Gibson, OR, **Turner, G,** Tuttle, JA, Taylor, L, Watt, PW and Maxwell, NS (2015) Heat acclimation attenuates physiological strain and the cellular stress response to acute normobaric hypoxia. *J Appl Physiol*, 119(45) 889-899.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

The following abbreviations are commonly used in the field of exercise physiology and altitude training research and will be abbreviated once at the start of the thesis. The abbreviations will also be used in all tables and figures, including titles.

Erythropoietin	EPO
Live high-train high	LHTH
Live high-train low	LHTL
Live low-train high	LLTH
Intermittent hypoxic exposure	IHE
Intermittent hypoxic training	IHT
Maximal oxygen consumption	$\dot{V}O_{2max}$
Total haemoglobin mass	tHbmass
Haemoglobin mass	Hbmass
Optimised CO-rebreathing method	oCOR-method
Red blood cell	RBC
Haemoglobin concentration	[Hb]
Haematocrit	Hct
Fraction of inspired oxygen	FiO_2
Arterial oxygen saturation	SaO_2
Arterial oxygen saturation (measured with pulse oximetry)	SpO_2
Analysis of variance	ANOVA
Adenosine triphosphate	ATP
Standard Deviation	SD
English Institute of Sport	EIS
University of Brighton	UoB

Other abbreviations that are less common, specific to each study or a key physiological measurement will be abbreviated in each chapter to ensure that the reader has continued clarity. These abbreviations form a key component of the study in question; therefore will help with the flow of the topic.

Heart Rate	HR
Cardiac Output	Q
Ratings of perceived exertion	RPE
Carbon monoxide	CO
Carboxyhaemoglobin	HbCO
Percent carboxyhaemoglobin	%HbCO
Hypoxic-inducible factor-1α	HIF-1α
Hypoxic Ventilatory Response	HVR
Blood volume	BV
Plasma volume	PV
Red cell volume	RCV
Red cell mass	RCM
Intermittent hypoxic exposure with interval training	IHIT
Live high-train low and high	LHTL+H
Erythropoietin concentration	[EPO]
Lactate threshold	LT
Lactate turnpoint	LTP
Running Economy	RE
Partial pressure of oxygen	PO_2
Ventilation	$\dot{V}_E$
Oxygen consumption	$\dot{V}O_2$
Carbon dioxide production	$\dot{V}CO_2$
Lake Louise Questionnaire	LLQ
Acute Mountain Sickness	AMS
Hypoxic Sensitivity Test	HST
Blood-brain barrier	BBB

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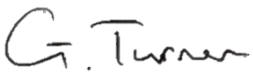
DECLARATION

I declare that the research contained in this thesis, unless otherwise formally indicated with the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed: 

Date: 30/09/2016

CHAPTER 1

1 INTRODUCTION

1.1 Endurance Performance

The goal in competitive distance running is to run a given distance in the least time, or at least faster than the next best competitor (Barnes and Kilding 2014). Performance in endurance events is heavily dependent upon the aerobic resynthesis of adenosine triphosphate (ATP), which requires an adequate delivery of oxygen from the atmosphere to the mitochondria, as well as the supply of fuel in the form of carbohydrate and lipid, in order to maintain a given velocity (Jones and Carter 2000). Endurance training aims to enhance the pathway for oxygen from the atmosphere to the mitochondria in four key areas: 1) the pulmonary diffusing capacity, 2) maximal cardiac output, 3) oxygen carrying capacity of the blood, and 4) skeletal muscle characteristics (Bassett and Howley 2000). The first three of these factors can be classified at *central*, with the fourth being *peripheral*.

For endurance athletes a vital goal is to increase the amount of oxygen that can be transported, extracted and consumed during exercise, defined as maximal aerobic power ($\dot{V}O_{2max}$). In whole body exercise, it is widely accepted that $\dot{V}O_{2max}$ is limited by the aforementioned *central* factors, i.e. the rate at which oxygen can be supplied to the muscles, and not by the (*peripheral*) muscle's ability to extract oxygen from the blood it receives (Jones and Carter 2000). Therefore, if an athlete is able to enhance the oxygen transport system, such as an increase in oxygen carrying capacity, then $\dot{V}O_{2max}$ may increase (Schmidt and Prommer 2010). Although an enhancement in endurance exercise performance is also reflected by improvements in exercise economy, the lactate/ventilatory threshold and the critical power which will influence the oxygen uptake kinetics (Jones and Carter 2000).

One such factor that influences oxygen carrying capacity is the amount of haemoglobin in the body. Haemoglobin is an iron-containing globular protein pigment molecule carried within red blood cells (RBCs) and carries almost all of the oxygen in the blood (Otto et al. 2013). Total haemoglobin mass (tHbmass) is the absolute mass of circulating haemoglobin in the body (Otto et al. 2013) and can be quickly, safely, cheaply and reliably measured using the optimised carbon monoxide (CO) re-breathing method (oCOR-method) (Schmidt and Prommer 2005; Prommer and Schmidt 2007). A change in tHbmass by 1 g causes a change in $\dot{V}O_{2max}$ by approximately 4 $mL \cdot min^{-1}$ (Schmidt and Prommer 2010) and change of 20 g is meaningful to endurance performance. Therefore increasing tHbmass has become a focus of an endurance athlete's training programme.

1.2 Performance at Altitude

The 1968 Mexico City Olympic Games was the first of several Olympic Games in which the middle- and long-distance events in men's athletics were dominated by countries from East Africa – in

particular Kenya and Ethiopia (Wilber and Pitsiladis 2012). The East African nations won 50% of the medals available in the 800 m to marathon distances, having won just two medals in the previous two Olympic Games. This Mexico City Olympic Games was held at 2,420 m and many of the East African athletes were born, raised and trained at 2,000-2,500 m near the Great Rift Valley. Prior to 1968, coaches arguably underestimated the effects of altitude and the ensuing hypoxia on maximal performance and fatigue (Fiskerstrand and Seiler 2004), with many lowlanders reportedly collapsing during competition (including the Australian 5,000 and 10,000 m world record holder, Ron Clarke, who nearly died in a race, despite training in the Alps to get acclimatised to the high altitudes of Mexico City). This, along with the East African dominance, led researchers to believe that living and training at altitude had a role to play in enhancing sea level endurance performance. Early positive reports of altitude training improving $\dot{V}O_{2max}$ and competition performance (Daniels and Oldridge 1970; Dill and Adams 1971) thus started an era of altitude training camps for elite endurance athletes.

Many modern-day elite endurance athletes incorporate altitude and hypoxic training within their year-round training plan, believing that it will provide the "competitive edge" to succeed at the Olympic level (Wilber 2007a). The primary model of adaptation to hypoxia is that the lower partial pressure of oxygen (PO_2) associated with ascending to higher altitudes [or reduced fraction of inspired oxygen (FiO_2) with simulated altitude], induces erythropoietin (EPO) production, which in turn stimulates the production of RBCs, increasing tHbmass and; in turn allowing an increased oxygen delivery and $\dot{V}O_{2max}$, and potentially improving endurance performance (Saunders et al. 2013).

Within the coaching and athletic community there is widespread belief that altitude training and hypoxic exposures can also enhance sea level endurance performance (Saunders et al. 2004b). However, from a scientific research perspective the effectiveness of altitude and hypoxic training relative to sea level performance remains inconclusive. In some cases this is believed to be as a result of poorly designed studies or a limited understanding of the mechanistic basis of altitude training and adaptation (Fudge et al. 2012). Achieving improvements in sea level performance after field based altitude training studies are also difficult due to notably large individual variation in physiological responses to the altitude stressor, such as ventilation, heart rate and arterial oxygen saturation (SaO_2) (Chapman 2013). It is surprising, therefore that current research has not attempted to characterise this individual variation by profiling elite athletes before an altitude training camp. Prior screening of the individual physiological responses could provide the opportunity to optimise the specific altitude training strategy and ultimately improve the resultant adaptations.

Despite there appearing to be a strong rationale behind altitude training for endurance athletes, there is still an ongoing debate surrounding the effectiveness of methods, such as, live high-train low (LHTL), at both natural and simulated altitudes (Levine and Stray-Gundersen 2005; Jacobs 2013; Wilber 2013). Initially, altitude training research was guided by the experiences of elite coaches (Owen 1972; Dick 1992) and physiological testing was built around altitude training camps that were already taking place (Bailey et al. 1998). However, more recently specific scientific questions have driven altitude training investigations (e.g. Garvican et al. 2011b; Siebenmann et al. 2012). Bailey and Davies (1997) produced an early review of altitude training for endurance performance at sea level. The

authors concluded that future research should focus on the optimum height and duration of altitude training strategy, as well as potential mediators after the return to sea level, and the role of the immune response to training at altitude. Eighteen years later and researchers are still attempting to define the optimal 'dose' of altitude training (Chapman et al. 2014a), reporting suppression of the immune system (Wachsmuth et al. 2013), investigating the timing of return for optimal sea level performance (Chapman et al. 2014b) and discussing the mechanisms involved (Gore et al. 2013).

1.3 Research Overview

The present thesis aims to add to the growing body of research surrounding altitude training by identifying and answering the questions of elite coaches within the British High Performance System, by understanding the individual athlete's haematological response to both acute and chronic exposure to hypoxia and by providing strategies around an altitude training camp that can be utilised by endurance athletes as part of their preparation for major global competitions. The combination of current scientific literature and applied experience could help to bridge the gap in this area of altitude and hypoxic training research (Fudge et al. 2012) – the context and concept explored in Chapter 2. The proposed research questions and hypotheses are presented in section 2.10 and the aims of the thesis are presented in the following chapters:

- Chapter 2 reviews the literature surrounding the use of altitude training in elite endurance sport, the physiological adaptations that occur and the effect they have on athletic performance. Particular interest is paid to the haematological responses and adaptations that arise as a result of acute and chronic altitude and hypoxic exposures.
- Chapter 3 describes the general methods used throughout all experimental chapters.
- Chapter 4 (Study 1) outlines the current altitude and hypoxic training practices and perceptions as well as challenges faced by of elite endurance athletes and coaches.
- Chapter 5 (Study 2) assesses the reliability of two different hemoximeter analysers (Radiometer™ ABL80 CO-OX Flex and OSM3 hemoximeters) used to measure %HbCO – a key dependent variable of the thesis work and integral to accurate measurement of tHbmass
- In continuation from the previous chapter, Chapter 6 (Study 3) evaluates the reliability of the tHbmass measurement to small methodological changes in the oCOR-method. The oCOR-method is routinely used to assess the success of an altitude training camp or series of hypoxic exposures. The findings from both chapters 5 and 6 are utilised in subsequent chapters.
- Chapter 7 (Study 4) investigates the individual variations in endogenous EPO and inflammatory markers in response to acute hypoxia. This chapter describes the time-course of EPO and inflammatory markers after different severities of hypoxic and examines the dose-response relationship.
- Chapter 8 (Study 5) follows on from Chapter 7. The experiment describes the effect of an acute hypoxic exposure on subsequent time trial performance in endurance runners.

- Chapter 9 (Study 6) describes a field based study that investigates the physiological and haematological adaptations after a four week altitude training camp at 2,300 m. The study also examines the efficacy of a hypoxic sensitivity test used as a pre-screening tool to identify individual differences in response and adaptation.
- Chapter 10 discusses the general findings from all the experimental studies, with particular detail surrounding an athlete's individual response to hypoxia and altitude training and the sensitivity of the various markers tested in this thesis to quantify that response. This chapter also examines the role of additional hypoxic exposures to predict an athlete's response to altitude training, as well as methods to optimise training at altitude itself, and to maximise physiological and haematological adaptations to altitude training camps.

CHAPTER 2

2 REVIEW OF LITERATURE

2.1 Introduction

This review will outline the physiological determinants and limitations of endurance performance, specifically in elite middle- and long-distance endurance runners. Areas such as $\dot{V}O_{2max}$ and the pathway of oxygen from mouth to muscle will be presented. Previous research suggests that altitude and hypoxic training can enhance oxygen carrying capacity and therefore improve a runner's performance. The different methods of altitude and hypoxic training, the physiological adaptations that occur as a result, and ultimately how additional hypoxic strategies can help to improve the performance of an elite endurance runner will be discussed.

The review also outlines the role of tHbmass and EPO on physiological capacity and resulting performance. Attention will be paid to the quantification of tHbmass and the physiological determinants of endurance performance, and how these can be measured accurately and reliably. The application of these markers, with regard to quantifying the success of an altitude training camp or series of hypoxic exposures will also be evaluated. Finally, the review will introduces the concept of a hypoxic sensitivity test, to be implemented prior to an altitude training camp, and the basis by which this may offer insight into subsequent adaptation. The typical responses of elite endurance runners will be evaluated and the topic of individual variability in response to hypoxia will be introduced in an attempt to aid prescription of training at altitude in elite runners.

2.2 Defining and Researching the Elite Athlete

The present thesis aims to provide practical applications to elite athletes. Elite athletes have long been tested to discover what makes them different from recreationally active populations and sedentary individuals. There is a large amount of research that claims to use elite athletes, and describing an athlete of any standard is difficult with the different terminology cited (De Pauw et al. 2013). For example, a review of sport psychology research found a wide range of definitions of an elite athlete, from Olympic gold medallists and world-record holders, to regional and university level athletes (Swann et al. 2015). The study concluded that definitions should be based on the athletes' highest standard of performance, their success at that level, and the amount of experience that they have gained at that level (Swann et al. 2015). Physiological variables have also been used to describe 'eliteness' of an athlete. De Pauw et al. (2013) stated $\dot{V}O_{2max}$ to be one the most important performance predictor in cyclists. Based on previous research, Jones (2006a) stated that international standard male distance runners typically have $\dot{V}O_{2max}$ values of 70–85 $mL \cdot kg^{-1} \cdot min^{-1}$ while their female

counterparts have $\dot{V}O_{2max}$ values of 60–75 $mL \cdot kg^{-1} \cdot min^{-1}$. The issue being that the variables, nor the standard of that variable to classify as 'elite', have never been agreed or defined.

For the remainder of the literature review and when attempting to identify if an athlete is elite or not, the categories suggested by Swann et al. (2015) will be utilised (Table 2.1). For example, if an athlete is described as 'elite' within the study description then they must fulfil a minimum of two of the criteria referenced by Swann et al. (2015).

Table 2.1: Athlete description and criteria to identify the level of the individual

Proposed description	**Highest standard of performance**	**Success at that level**	**Experience at that level**	**Measured $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)**
Elite	International Honours	Olympic, World or Continental Championships	8+ years	>71
Highly Trained	National Honours	National Championships	5-8 years	>65
Well Trained	Collegiate/ University	Regional/University Championships	2-5 years	>55
Trained	Local club runner	Local events	<2 years	>45

Adapted from Swann et al. (2015)

Conducting research with elite athletes presents additional challenges. A major limitation of some research into altitude training with controlled designs is the inclusion of lesser trained subjects (rather than elite athletes). Elite athletes may not be as responsive to a given stimulus as less trained volunteers (Lundby et al. 2012). For example, Robach and Lundby (2012) concluded that athletes with an already high tHbmass may not increase their tHbmass any further with altitude training, whereas an increase may be possible if tHbmass is low to begin with. This concept has been termed 'symmorphosis' and suggests that for each step in the oxygen transport chain, animals maintain sufficient structure capacity to support flux rates at $\dot{V}O_{2max}$, but not higher (Taylor and Weibel 1981; Hoppeler and Weibel 1998). This concept further proposes that for athletic species, including human elite athletes, each step would have evolved toward optimal function, allowing little room for further adaptive improvements (Robach and Lundby 2012).

The present thesis will therefore be cautious when interpreting other research to ensure that if participants are described as 'elite' that they have fulfilled enough of the criteria to be truly defined as such. Irrespective, whilst there is clear and pressing need for controlled studies with elite athletes, it is acknowledged this is difficult to achieve when such athletes likely have tightly controlled and busy competition schedules and are not often able to deviate from their prescribed training programmes.

2.3 Endurance Performance

Endurance running can be defined as the capacity to sustain a given velocity for the longest possible time, therefore, performance in endurance events is heavily dependent upon the aerobic re-synthesis of ATP; which requires an adequate delivery of oxygen from the atmosphere to the mitochondria (Jones and Carter 2000). This pathway can be improved with endurance training via adaptations in the pulmonary, haematological, cardiovascular and neuromuscular systems (Jones and Carter 2000). Different types of endurance training practices will have different effects upon the key determinants of aerobic fitness and the parameters that are targeted through training, which will ultimately depend upon an individual's personal, physiological 'strengths' and 'weaknesses' and the duration of the event they are training for.

Success in elite level middle- and long-distance running involves both aerobic and anaerobic metabolism (Brandon and Boileau 1992). Consequently, middle- and long-distance runners use a variety of training methods that lead to different adaptations (Rabadán et al. 2011). Whilst training for long-distance events, an athlete is primarily attempting to optimise endurance performance by sustaining a high fraction of $\dot{V}O_{2max}$ (Costill et al. 1973), whereas during middle-distance running the intensity is higher, therefore they train to compete at an even higher percentage of $\dot{V}O_{2max}$ or indeed, above $\dot{V}O_{2max}$ (Daniels 1985). The physiological adaptations of endurance training are reflected in improvements in the key parameters of aerobic fitness, namely $\dot{V}O_{2max}$, running economy, the lactate/ventilatory threshold and the critical power, and the oxygen uptake kinetics (Jones and Carter 2000). Improvement in one or more of these parameters may result in an enhancement in endurance exercise performance. Although the aerobic parameters are important determinants of endurance exercise performance, it should be noted that competitive performance also depends upon psychological factors, aspect of central and peripheral fatigue, race tactics and the prevailing environmental conditions.

2.3.1 Physiological determinants of endurance performance

Elite athletic performance involves integration of muscular, cardiovascular and neurological factors that function together to transfer efficiently the energy from aerobic and anaerobic ATP turnover into velocity and power (Joyner and Coyle 2008). Performance in all Olympic endurance running events (excluding race walking) are decided at intensities above 85% of $\dot{V}O_{2max}$ (Joyner and Coyle 2008). In order for an athlete to exercise, energy (in the form of ATP) must be supplied to the muscle. As running velocity increases over a longer distance there is a greater reliance on ATP production via oxidative phosphorylation (Bassett and Howley 2000). As a result, the consumption, delivery and utilisation of oxygen during prolonged submaximal exercise become a marker of the rate at which ATP is generated, and therefore energy provided to the muscle. Hence, it is evident that the delivery of oxygen plays an important role in endurance performance and this has been termed an 'endurance performance limitation'.

Distance running performance is determined by; the maximal amount of oxygen you can take up and utilise (more commonly known as $\dot{V}O_{2max}$), how much of this $\dot{V}O_{2max}$ you can maintain at a given pace (also known as fractional utilisation or lactate threshold) and finally how you transfer the oxygen uptake into running speed (known as running economy) (see Figure 2.1). In the diagram performance oxygen uptake ($\dot{V}O_2$) represents the highest mean $\dot{V}O_2$ that can be sustained during the race and running economy refers to how efficient the runner is at converting available energy into running speed (Midgley et al. 2007). The interplay of these physiological variables determine the performance capabilities of an individual in endurance events, and if enhanced can result in improved performance.

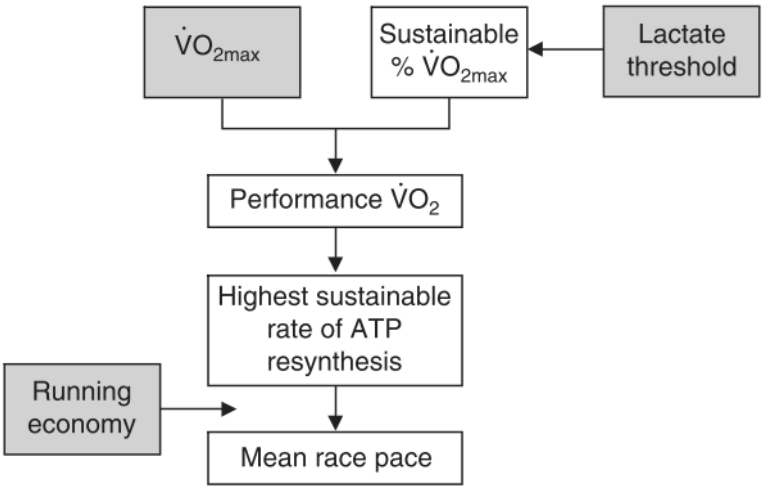


Figure 2.1: The determinants of long-distance running performance (Midgley et al. 2007).

2.3.1.1 Maximal oxygen uptake ($\dot{V}O_{2max}$)

Maximal oxygen uptake ($\dot{V}O_{2max}$) reflects an individual's maximal rate of aerobic energy expenditure and has long been associated with success in endurance sports (Saltin and Astrand 1967). The determination of $\dot{V}O_{2max}$ is not only a measure of aerobic turnover, but also offers a precise measure of the capacity to transport and utilise oxygen, i.e., the functional capacities of lungs, cardiovascular system, and muscle mitochondria combined (Saltin and Strange 1992). Although $\dot{V}O_{2max}$ is not performance, it clearly is one of the major characteristics that determine performance in endurance sport (di Prampero 2003). With respect to performance over a given distance there are other factors that will affect the result. $\dot{V}O_{2max}$ alone does not adequately predict a winning performance among runners with similar aerobic capacities (Costill et al. 1973). Bassett & Howley (1997) believed that $\dot{V}O_{2max}$ sets the upper limit for performance in endurance events; yet, it is not always the best predictor of athletic ability.

2.3.1.2 Fractional utilisation of $\dot{V}O_{2max}$ and blood lactate

The ability to exercise for long periods at high fractions of the $\dot{V}O_{2max}$ is a characteristic of elite endurance athletes and is an important determinant of performance (Costill et al. 1973; Sjödin and Svedenhag 1985). Strong correlations have been reported between fractional utilisation of $\dot{V}O_{2max}$ and running performances. Over a 10 mile road race those men who displayed low B[La] during exercise at 85-90% of $\dot{V}O_{2max}$ are able to utilise a large fraction of their aerobic power during prolonged endurance activity, and subsequently performed better (Costill et al. 1973). The fractional utilisation of the $\dot{V}O_{2max}$ during endurance competition also appears to be closely linked to markers of blood lactate (B[La]) accumulation during exercise, such as the lactate or ventilatory threshold, or more specifically, the higher intensity of lactate turnpoint (LTP), maximal lactate steady state (MLSS) or 'onset of blood lactate accumulation' (Jones 2006).

Elevations in B[La] are seen to arise when NADH and hydrogen ions (H+) from cytosolic reactions are produced at rates in excess of mitochondrial capacity (Robergs et al. 2004). Although not directly associated in effects of muscle fatigue itself, B[La] is known to be an indicator for increased H+ production and a subsequent lowering of cellular and blood pH (Robergs et al. 2004). During low intensity exercise B[La] initially remains close to the resting value (i.e. ~ 1.0 mM). However, at a particular running speed (or, more precisely, a particular metabolic rate), B[La] begins to increase above the resting value (Jones et al. 1999), which is also known at the lactate threshold (LT). The LT typically occurs at 50–70% $\dot{V}O_{2max}$, although it can be as high as 80–85% $\dot{V}O_{2max}$ in highly-trained marathon runners and exercise near the LT can be sustained for >2 hours with B[La] being perhaps slightly elevated but not accumulating over time (Sjödin and Svedenhag 1985).

As exercise intensity increases to running speeds exceeding the LT a second 'sudden and sustained' increase in B[La] (at around 2–4 mM) can often be discerned and this second threshold has become known as the LTP in incremental exercise (Jones 2006), and its corollary in constant pace exercise, the maximal lactate steady state. Continuous running speeds between the LT and the LTP can be sustained for 20-60 minutes – the typical duration of the half marathon event – and whilst B[La] will be elevated above baseline values it will remain relatively stable over time. Conversely, during continuous exercise at running speeds above the LTP, B[La] will continue to increase with time until the exercise is terminated (Jones 2006).

Endurance training confers the benefit of a rightward shift of the B[La] curve to higher running speeds with the adaptation allowing a higher absolute (running speed) and relative (% $\dot{V}O_{2max}$) exercise intensity to be sustained without the accumulation of blood lactate (Jones and Carter 2000). The resulting LT and LTP also occur at a higher fraction of $\dot{V}O_{2max}$.

2.3.1.3 Running economy

Running economy (RE), defined as the metabolic cost to cover a given distance, is a primary physiological determinant of endurance running performance (Shaw et al. 2014). Irrespective of how

RE is expressed, the lower the metabolic cost assessed at a given exercise intensity, the better the RE of an individual; the better the running economy, the greater work and hence running speed therefore can be achieved for the same energy cost. There is considerable inter-individual variability in RE, even in elite distance runners and when expressed in units of $mL \cdot kg^{-1} \cdot km^{-1}$, a value of around 200 is considered average, with values above and below this value representing poor and good economy, respectively (Jones 2006). Running economy reflects the interaction of numerous factors including muscle morphology, elastic elements and joint mechanics in the efficient transfer of ATP to running speed (Joyner and Coyle 2008). Good RE, i.e. a low $\dot{V}O_2$ for a given running speed (typically ~180 $mL \cdot kg^{-1} \cdot km^{-1}$), results in the utilisation of a lower percentage of the athlete's $\dot{V}O_{2max}$ while running at that speed (and consequently a reduction in muscle glycogen utilisation, and potentially less reliance on oxygen-independent metabolism resulting in a reduction in metabolic acidosis) (Jones 2006). Physiological (ventilation, core temperature, muscle metabolism), anthropometric (muscle fibre type, body composition) and biomechanical (running technique, muscle stiffness, storage/return of elastic energy) factors are thought to influence RE (Saunders et al. 2004a)

Running economy represents the translation of energy turnover into running velocity and is cited as a stronger indicator of endurance performance than $\dot{V}O_{2max}$ alone within athletically homogenous populations (Daniels 1985). Cavanagh (1989) suggested that modest enhancements in running economy could result in substantial performance gains for elite distance runners. Accordingly, improvements in RE are highly desirable to maximise athletic performance (Shaw et al. 2013). Figure 2.2 illustrates the interaction of $\dot{V}O_{2max}$ and RE in two athletes who have the same $\dot{V}O_{2max}$ of 70 $mL \cdot kg^{-1} \cdot min^{-1}$ but differing running economy characteristics. Athlete B has better running economy and therefore in this example will run faster for their metabolic capacity, provided $\dot{V}O_{2max}$ is retained.

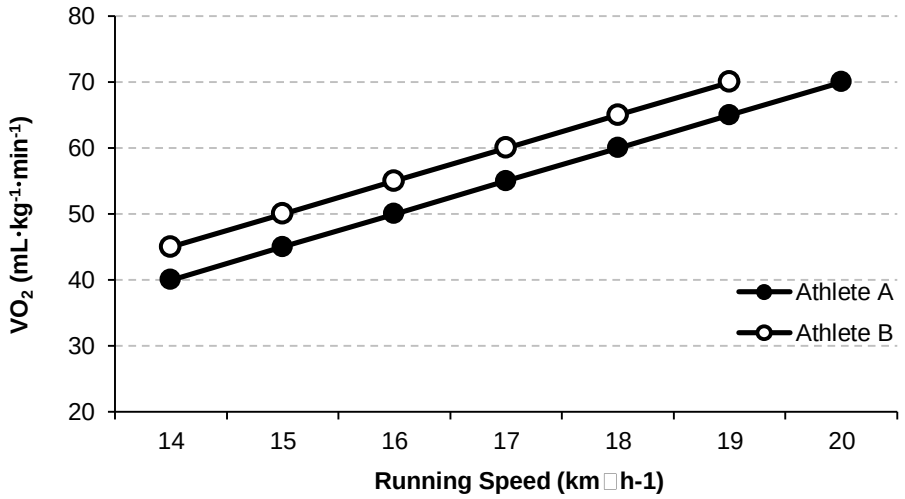


Figure 2.2: $\dot{V}O_{2max}$ and Running Economy (figure taken from Jones (2006))

2.3.2 Physiological limitations of $\dot{V}O_{2max}$

The physiological limitations of $\dot{V}O_{2max}$ are illustrated in Figure 2.3. Similar to Bassett and Howley (2000), Rowell (1986) divided the potential physiological factors limiting $\dot{V}O_{2max}$ in 1) respiration, 2) central circulation, 3) peripheral circulation and 4) muscle metabolism. The limitations reported are directly influenced by hypoxic exposure and interestingly it has been suggested that there are a number of mechanistic similarities in human physiology between adaptations for endurance performance and for hypoxia tolerance (Hochachka et al. 1998), an area which will be discussed later.

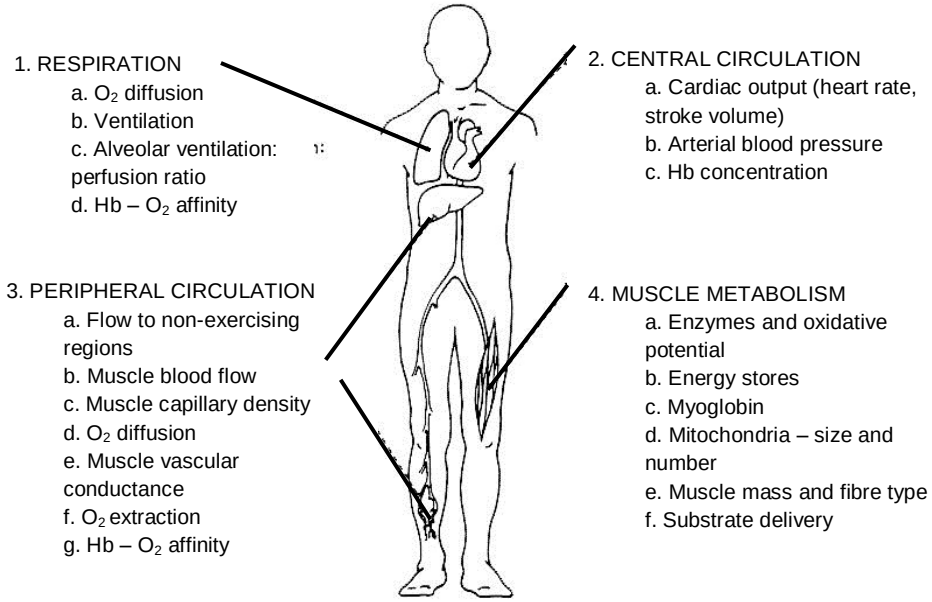


Figure 2.3: Potential physiological factors limiting $\dot{V}O_{2max}$ (Rowell 1986).

Figure 2.4 (Page 30) combines Figure 2.1 and Figure 2.3 into a conceptual framework presented by Joyner and Coyle (2008) that focused on 'performance velocity' and how it is determined by maximum rates of aerobic energy production, anaerobic capacity and how efficiently the energy being used is converted to movement. The model suggests that $\dot{V}O_{2max}$ and LT interact to determine how long a given rate of aerobic and anaerobic metabolism can be sustained (i.e. performance $\dot{V}O_2$) and efficiency then determines how much speed or power (i.e. performance velocity) can be achieved at a given amount of energy consumption (Joyner and Coyle 2008). The schematic also introduces the physiological adaptions that will affect the determinants of endurance performance.

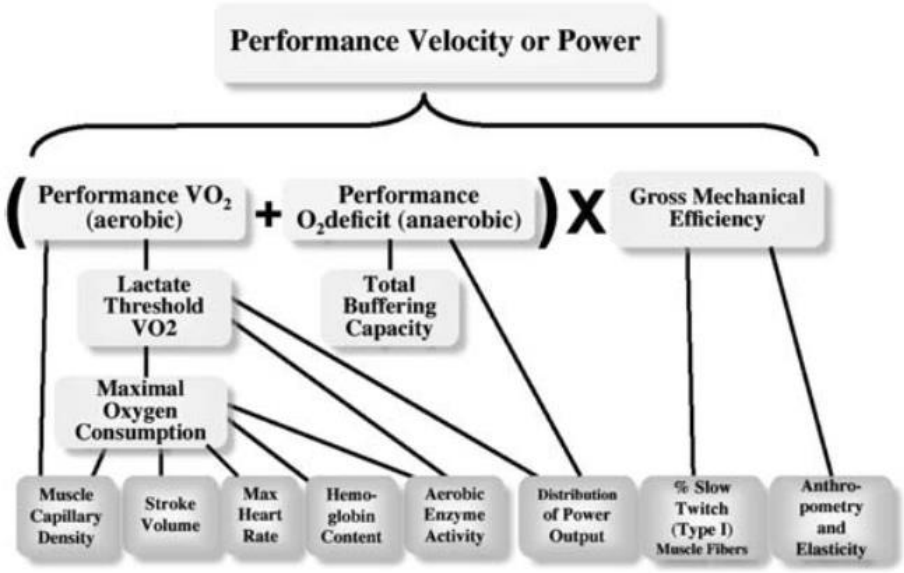


Figure 2.4: The multiple physiological factors that interact as determinants of performance velocity or power output (Joyner and Coyle 2008).

2.4 Altitude Training

The following section will review the various altitude training methods, analyse the research that both supports and refutes performance improvements and establish what can be done to optimise altitude and hypoxic training in elite endurance athletes.

The idea that athletes might benefit from a period of several weeks training at a high altitude camp first gained belief in the four years preceding the Mexico Olympics (Shephard 1974). The 1968 Olympic Games was held in Mexico City at an altitude of 2,240 m. Previous studies had shown that mountain climbers underwent a progressive physiological adaptation to the stresses of high altitude (Pugh 1958; Pugh et al. 1964), albeit at higher altitudes. Therefore, it was reasoned that a period of altitude training or acclimatisation might minimise the potentially disastrous early effects that the altitude of Mexico City was alleged to cause, in coaches and athletes alike (Shephard 1974).

Further to this the success of Kenyan and Ethiopian athletes in middle- and long-distance events was believed to be associated with a physiological advantage gained by residing at moderate altitude (Wilber and Pitsiladis 2012). The altitude in Mexico City proved to be detrimental to aerobic capacity, as middle- to long-distance events were run slower than previous world records and the sprint and jump events all broke world records (Hamlin et al. 2015). Table 2.2 highlights the dominance of male East African runners in Mexico City. As a result, coaches thought it would be beneficial for sea level athletes to live and train at altitude in a bid to gain the favourable adaptations shown by the East African athletes (Lundby et al. 2012).

Table 2.2: Men's results from the 1968 Mexico City Olympic Games held at 2,240 m altitude

Event	Winner	Country	Result	Record
100 m	Hines	USA	9.95 s	World
200 m	Smith	USA	19.83 s	World
400 m	Evans	USA	43.86 s	World
400 m hurdles	Hemery	GB	48.12 s	World
800 m	Doubell	USA	1 min 44.3 s	World
1500 m	**Keino**	**Kenya**	**3 min 43.91 s**	**Olympic**
3000 m steeple chase	**Biwott**	**Kenya**	**8 min 51.0 s**	**4% slower vs. WR**
10,000 m	**Temu**	**Kenya**	**29 min 27.4 s**	**7% slower vs. WR**
Marathon	**Wolde**	**Ethiopia**	**2 h 20 min 26 s**	**8.5% slower vs. WR**
Long Jump	Beamon	USA	8.90 m	World
Pole Vault	Seagren	USA	5.40 m	World

Results obtained from http://www.olympic.org/

An observational study by Tucker et al. (2015) concluded that Kenya's unique dominance in distance running is a complex and intriguing multifactorial mix of the interaction between genotype, phenotype, environment, and socioeconomic factors. It should be noted that Kenyan coaches do not believe that the success of their athletes is solely attributed to residing at moderate altitude.

> "*If running success is based on altitude residence, then why doesn't Colombia and Nepal produce great runners like Kenya? Our success is based on hard work and attitude, not altitude*." Kenyan coach Mike Kosgei (Tanser 1997)

The basic premise of altitude training is that when an athlete ascends from sea level to moderate altitude, the decrease in partial pressure of oxygen (PO_2) initially impairs endurance training and performance. However, after a few weeks at altitude, training and performance recover to some extent as the athlete adapts (Bonetti and Hopkins 2009). The efficacy of altitude training relative to sea level performance remains controversial from a research perspective; however, elite athletes have utilised altitude training for several years in preparation for elite level competition (Wilber 2007a). Briefly, it is thought that chronic adaptions in response to moderate altitude, such as an increase in tHbmass and therefore oxygen carry capacity (Gore et al. 2013) and enhanced muscular efficiency (Gore et al. 2007), can facilitate an increase in $\dot{V}O_{2max}$ and potentially improve endurance performance.

2.4.1 Altitude and hypoxic training methods

To date several forms of altitude/hypoxic training exist: the traditional or classic 'live high-train high' (LHTH), the contemporary 'live high-train low' (LHTL) and 'live low-train high' (LLTH) approaches (Wilber 2007a). More recently interest has focused on using 'intermittent hypoxic exposure' (IHE),

'intermittent hypoxic training' (IHT), 'intermittent hypoxic exposure with interval training' (IHIT) and 'live high-train low and high' (LHTL+H) (Millet et al. 2010). Figure 2.5 outlines the different methods of altitude/hypoxic training currently used by elite athletes. These altitude and hypoxic training methods have been previously reviewed in greater detail (Wilber 2007a; Bonetti and Hopkins 2009; Saunders et al. 2009a; Millet et al. 2010; Fudge et al. 2012; Lundby et al. 2012).

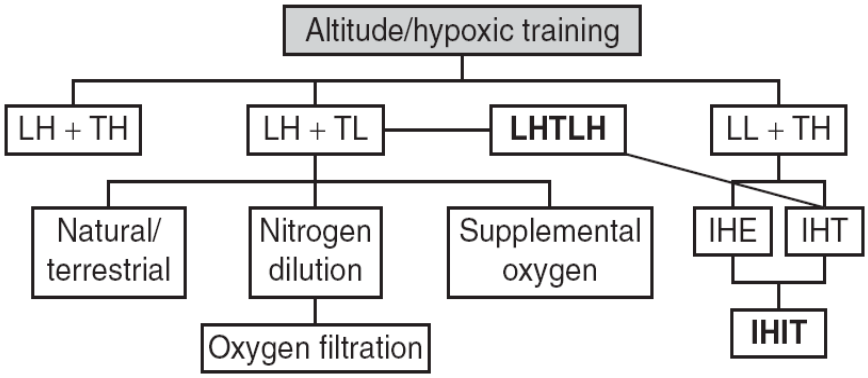


Figure 2.5: Different altitude/hypoxic training methods (Millet et al. 2010).

LH + TH (live high + train high), LH + TL (live high + train low), LL + TH (live low + train high), LHTLH (live high-train low and high), IHE (intermittent hypoxic exposure), IHT (intermittent hypoxic training), IHIT (intermittent hypoxic exposure during interval training)

For the purpose of this thesis when describing a specific altitude for either living or training the definitions from Table 2.3 will be used in general commentary. The descriptions of altitude training methods such as LHTH, LHTL and LLTH only define the method being used and do not include the specific altitude of the living and training. Where appropriate the specific altitude will be described separately.

Table 2.3: Characteristics of altitude ranges (Bärtsch et al. 2008)

Altitude	**Definition**
0–600 m *	Near sea level
Above 600–2000 m	Low altitude: minor impairment of aerobic performance becomes detectable
Above 2000–3000 m	Moderate altitude: mountain sickness starts to occur and acclimatisation is increasingly important for performance
Above 3000–5500 m	High altitude: mountain sickness and acclimatisation become clinically relevant; performance is considerably impaired
Above 5500 m	Extreme altitude: prolonged exposure leads to progressive clinical deterioration

* Amended from 500 m to 600 m to take into consideration the Australian Institute of Sport residence in Canberra where 'sea level' training takes place at ~600 m

The traditional LHTH was the first method of altitude training that was tested by coaches and athletes. An experiment by Daniels & Oldridge (1970) produced equivocal results, with some athletes improving their sea level performance, some declining and the remainder finding no change in performance. This was thought to be due to a poor control of training volume and intensity when switching between altitude and poor reliability of testing procedures. The traditional LHTH method has been subsequently reviewed by Friedmann-Bette (2008) who concluded that improvements in sea level aerobic capacity or performance were more likely in well-trained athletes but not elite athletes. There were however, some uncontrolled studies that found significant performance increases in elite athletes after living and training at > 2,000 m. It is possible that the measurement tools within the studies, e.g. $\dot{V}O_{2max}$, were not ideal to evaluate performance in elite athletes.

As a result of the mixed findings from LHTH, the method of LHTL was introduced by Levine & Stray-Gundersen (1997) where athletes would live at higher altitude to gain the advantages of breathing a lower partial pressure of oxygen but then descend to lower altitudes to train at an intensity that would not result in detraining. Again, the method produced conflicting results, but was largely a success and has been replicated in many different ways. The LHTL method initially involved ascending and descending to different altitudes during a camp, which presents additional challenges. According to Millet et al. (2010) if the correct 'dose' of hypoxia is administered then both physiological and haematological enhancements can be achieved. Natural LHTL may not always be a viable option for many athletes because athletes may not have the financial means to access the geographic locations, laboratory facilities, or the equipment to conduct effective natural camp (Wilber 2004).

Simulated, or artificial, LHTL has gained more attention as an alternative. In recent years, there has been a remarkable increase in the number of techniques designed to "bring the mountain to the athlete" (Levine 2002). Breathing hypoxic air through a mask or in a tent can simulate altitude and if used in the correct way, may improve performance through physiological enhancements. Hahn et al. (2001) summarised several LHTL normobaric hypoxia studies and stated that sleeping in moderate normobaric hypoxia (2650–3000m) for longer than 3 weeks could induce practical advantages for elite athletes, but that most of these potential benefits were not likely to result from haematological (i.e. increased tHbmass or increased $\dot{V}O_{2max}$) but rather from peripheral adaptations (i.e. muscle buffer capacity or mechanical efficiency)

In the search for marginal gains in endurance performance some more novel methods of altitude and hypoxic training have been tested. IHE has been suggested as a way of artificially increasing EPO production and therefore tHbmass. IHE consists of repeatedly switching between breathing hypoxic and normoxic air during a session, usually lasting 60 – 90 min, with the intermittent application of severe hypoxia between FiO_2 = 0.12% (equivalent to a PIO_2 of 4,500m) to 0.10% (6,000 m) (Bartsch et al. 2008). A review found that IHE alone cannot be recommended for improvements in aerobic or anaerobic performance, however, more prolonged exposures prior to or after LHTH or LHTL may be effective (Bartsch et al. 2008).

Another alternative approach is for athletes to breathe a hypoxic inspirate during some of their usual exercise training sessions while living in normoxia, termed IHT (Holliss et al. 2014). IHT is also referred to as LLTH and unlike other methods of altitude training the aim is not to increase tHbmass and therefore oxygen carrying capacity. It is thought that IHT might play a role on adaptations at the molecular level in skeletal muscle tissue, such as increases in mitochondrial and capillary density, capillary-to-fibre ratio, fibre cross-section area, myoglobin content and oxidative enzyme activity (Hoppeler et al. 2003). The limitation with IHT is the duration of time spent in hypoxia and assuming the right 'dose' is obtained then endurance sports could take advantage (Millet et al. 2010).

2.4.2 Physiological response to altitude and hypoxia

The next section of literature review will first describe the initial physiological responses (hours to days) to altitude, followed by the acclimatisation process that occurs as a result of prolonged periods of time (3-4 weeks) at altitude and finally the role these adaptions have on endurance performance. The physiological response and acclimatisation will be divided into four main areas as summarised by Rowell (1986) and Bartsch and Saltin (2008); the respiratory (or pulmonary) system, the cardiovascular system, the circulatory system (haematology and oxygen delivery) and, the skeletal muscle. The literature review will focus on the typical altitudes where altitude and hypoxic training would take place (1,500 – 3,000 m) and reference will be made to resting and exercising at altitude. Finally, an evaluation of the research studies completed on elite endurance runners will take place.

Altitude exposure challenges the cardiovascular system to maintain oxygen delivery to the mitochondria under conditions of hypoxic stress (Stembridge et al. 2015). There are a series of physiological responses that occur when exposed to increasing altitude that ultimately results in adaptations to the oxygen transport chain or 'oxygen cascade'. These physiological and haematological responses and subsequent adaptations are illustrated in Figure 2.6 and will be examined in the literature review.

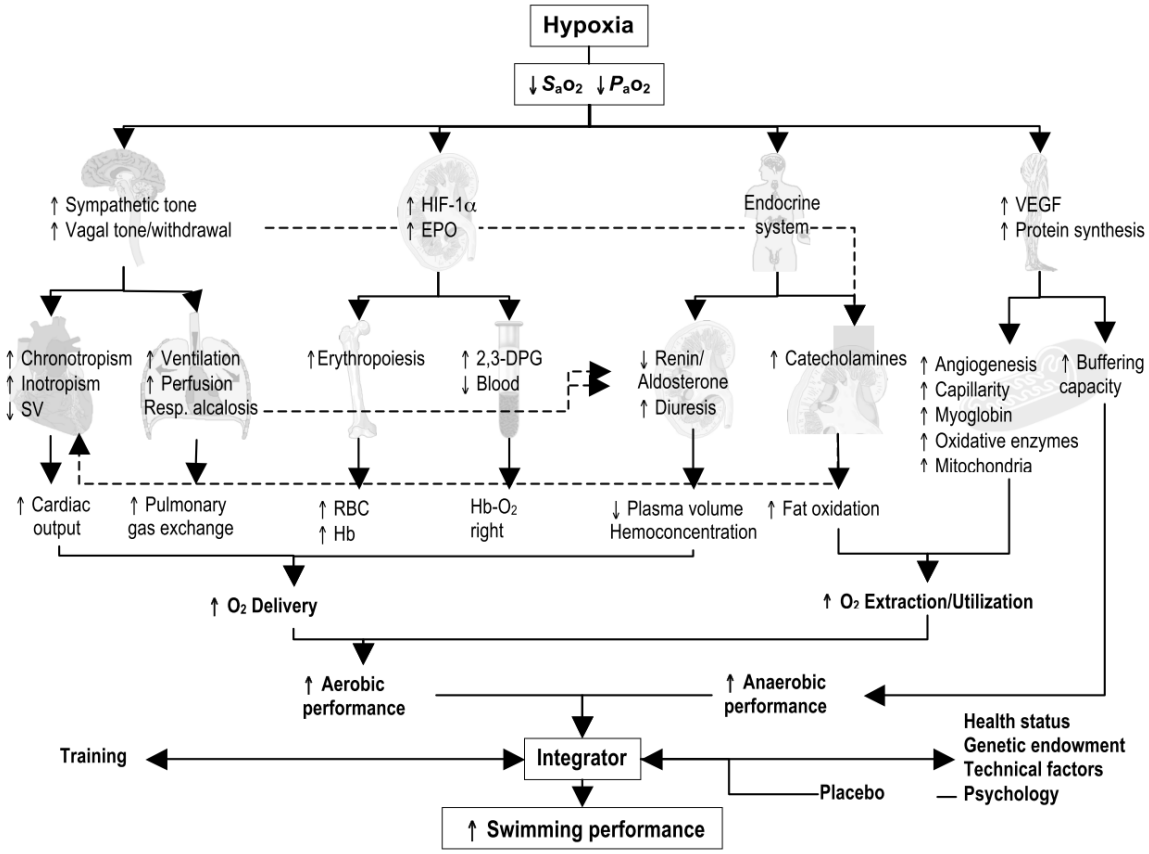


Figure 2.6: Summary of the purported physiological mechanisms involved in the use of hypoxia for performance enhancement (Truijens and Rodríguez 2011). Modified from Rodríguez et al. (2007). Although the model is from swimming performance the mechanisms can be applied to endurance performance.

When exercising at altitude, there are two different independent stresses to which the body must respond and adapt; hypoxia and exercise (Mazzeo 2008). The adaptations that are associated with acclimatisation to altitude and aim to reduce the physiological stress imposed during exercise at altitude to ensure that the body has an adequate amount of oxygen delivered to the exercise muscles (Mazzeo 2008). This is achieved through adaptions to the various links in the 'oxygen cascade' (See Figure 2.7).

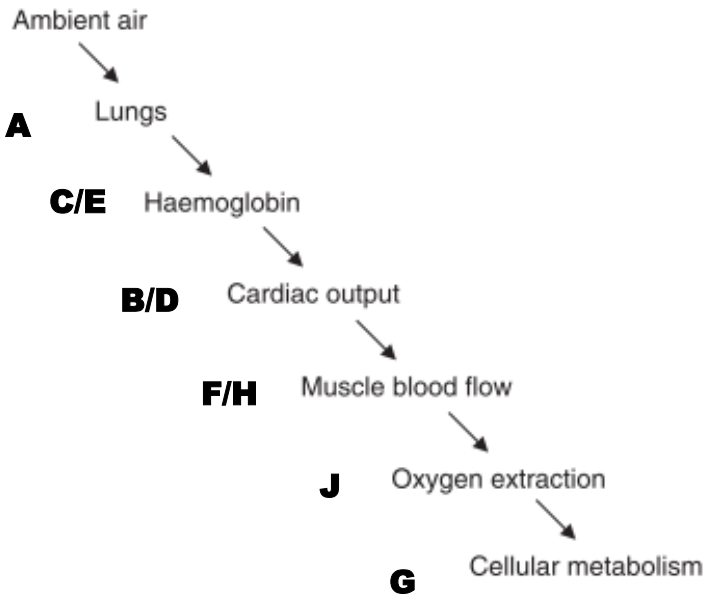


Figure 2.7: The 'oxygen cascade'. The letters in the boxes refer to additional graphs in Figure 2.8, which illustrates the time course of each physiological response

Figure 2.8 illustrates how different altitudes (1,500 and 3,000 m at rest and during exercise) affect the physiological links in the 'oxygen cascade' and shows the immediate adjustments and acclimatisation to altitude. The threshold altitude and the magnitude of the responses vary considerably between the different systems and also show a large inter-individual variability (Bartsch and Saltin 2008). The figure is split into ten separate panels each depicting a physiological variable. The x-axis denotes the time scale and the y-axis denotes relative changes, using sea level as baseline.

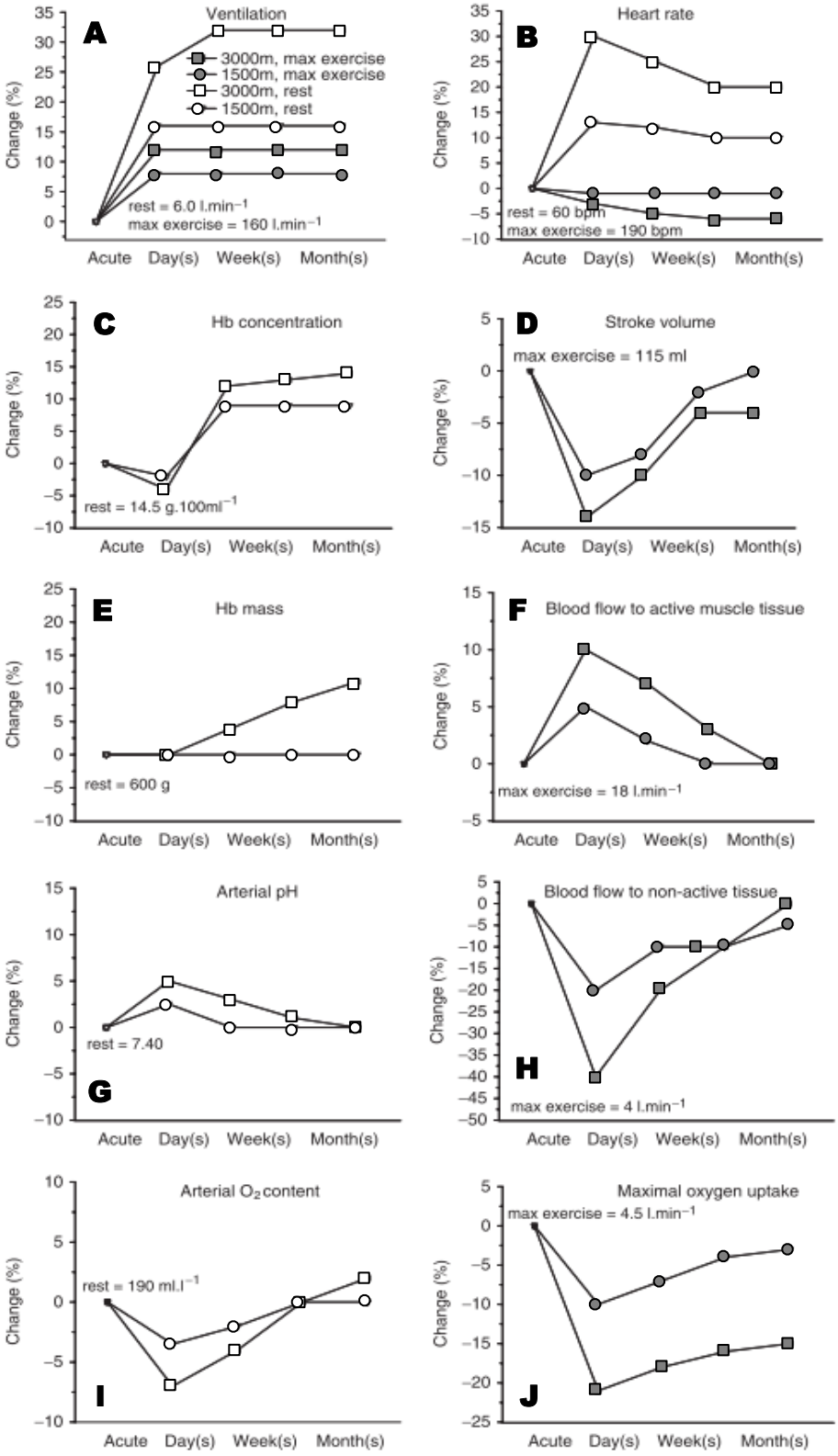


Figure 2.8: Schematic summary of key physiological variables and how they are altered at two altitudes (1,500m and 3,000m) (Bartsch and Saltin 2008)

2.4.2.1 The pulmonary system

Intense exercise is difficult to perform within the first 2–3 days at altitudes of around 2,000 m. One of the acute alterations when exposed to both hypobaric and normobaric hypoxia is an increase in minute ventilation, which arises because the reduction in arterial oxygen partial pressure (PaO_2) stimulates peripheral chemoreceptors (Lomax 2010). Given the influence of PaO_2 on haemoglobin oxygen saturation, it is unsurprising that SaO_2 declines during ascent from sea level to altitude (Mazzeo 2008). The increase in ventilation in hypoxia is critical to minimising the reduction in SaO_2 (Dempsey et al. 2013). Increased ventilation at altitude also causes respiratory alkalosis that is fully counter-regulated within one day at 2,200 m (Dempsey et al. 1972). Compensation of respiratory alkalosis is accomplished by increased renal bicarbonate excretion, resulting in a reduced buffer capacity of plasma (Böning et al. 2008). At moderate altitudes acid–base balance is restored rapidly in blood but it may take longer at the cellular level (Bartsch and Saltin 2008).

After an initial rise, ventilation continues to increase over 10–14 days (see Figure 2.8A) due to increasing sensitivity of the peripheral chemoreceptors to hypoxia, which results in a substantial increase in SaO_2 during the first 2 weeks at a given altitude (Bartsch and Saltin 2008). Ventilatory acclimatisation to hypoxia is driven by an increase in the hypoxic ventilatory response (HVR) (Powell and Fu 2008). As the duration of exposure continues, environmental hypoxia appears to be unique in causing a sequence of pulmonary changes that progressively enhance functioning of the individual in the environment, especially under conditions of increased energy demand, such as muscular exercise (Dempsey and Forster 1982). The major response to short-term hypoxia is an efficient increase in atmospheric-to-alveolar gas exchange that buffers against tissue anoxia and in the long-term hyperventilation is alleviated by pulmonary, hematologic, vascular and tissue adaptations (Dempsey and Forster 1982). Further evidence of this can be seen in highlanders who show only minimal hyperventilation during exercise (i.e., beyond that at rest), in their hypoxic environment. However, they preserve their PaO_2 and SaO_2 during exercise at about the same level as in the markedly hyperventilating sojourner (Dempsey et al. 1972). In lowlanders exercise causes PaO_2 and consequently SaO_2 to decrease at altitude because of a diffusion limitation predominantly due to lower alveolar PO_2 and increased pulmonary blood flow with exercise, a decrease that is larger in endurance trained athletes because of their higher maximal cardiac output (Bartsch and Saltin 2008).

2.4.2.2 The cardiovascular system

On acute exposure to a hypoxic environment it is generally accepted that cardiac output (Q) increases, as a result heart rate also increases due to increased sympathetic drive (Hooper and Mellor 2011). At higher altitudes a greater increase in heart rate occurs. The increase is an attempt to compensate for the reduced oxygen content of blood, which ensures adequate amounts of oxygen are transported to tissues, including exercising muscles (Mazzeo 2008). This is further evidenced by the increase in Q exactly matching the decrease in arterial oxygen content, so that the oxygen delivery to the tissues, remains unchanged (Naeije 2010). Stroke volume is the other factor contributing to Q and appears to be only marginally affected (lower compared to sea level) during submaximal exercise, however, at

higher exercise intensities it is reduced as oxygen delivery cannot keep pace with muscle demand, thereby creating a mismatch (Mazzeo 2008).

Submaximal heart rate and Q remains elevated after long-term exposure to altitude to ensure adequate amounts of oxygen are transported to exercising muscles; as a result VO_2 remains unchanged to ensure that submaximal exercise intensity can be maintained (Mazzeo 2008). As an individual acclimatises, the resting heart rate generally returns to that of sea level values (Hooper and Mellor 2011). The sympathetic nervous system is activated during both short- and long-term exposure to altitude and may play a role in the regulation of heart rate during acclimatisation to altitude (Hopkins et al. 2003). With a more prolonged exposure to altitude, stroke volume declines over time, stabilising after 1–2 weeks, which may be as a result of a decline in plasma volume (PV) and therefore a reduction in venous return and left ventricular filling (Mazzeo 2008). At a given exercise intensity, acclimatisation to altitude (1–3 weeks) causes a further reduction in Q as SV, HR and muscle blood flow decrease, however, oxygen delivery during submaximal exercise remains unchanged primarily due to an increase in arterial oxygen content coupled with enhanced oxygen extraction by muscle (Mazzeo 2008).

When exercising, heart rate for a given work load is greater than at sea level, except at maximal exercise where maximal heart rate is reduced compared to sea level values (Hooper and Mellor 2011). It has been widely debated as to why maximal heart rate is lower at altitude compared to sea level, including hypoxia down regulates β-adrenergic receptors (in animal models) (Richalet et al. 1990), partial parasympathetic withdrawal (Hopkins et al. 2003), an increased vagal tone (Boushel et al. 2001) or lower pulmonary blood flow to allow an increased mean transit time for the red cells passing through the lungs (Bartsch and Saltin 2008). During maximal exercise both maximal stroke volume and heart rate are decreased, whilst there is a higher Q (Naeije 2010). Initially, Lundby et al. (2001) found that the drop in maximal heart rate occurs after 4 h, and after 8 h it is noticeable, with maximal heart rate continuing to decrease in the days thereafter and a reduced maximal heart rate has been reported between 2,100-3,000 m (Saltin 1996; Friedmann et al. 2005b). For a summary see Table 2.4.

Table 2.4: Acute response and chronic acclimatisation responses of heart rate, cardiac output and stroke volume at rest, during submaximal and maximal exercise

	Acute Response		
	Rest	**Submaximal Exercise**	**Maximal Exercise**
Heart Rate	Increased	Increased	Decreased
Cardiac Output	Increased	Increased	Decreased
Stroke Volume	Unchanged	Decreased	Decreased

	Chronic Acclimatisation		
	Rest	**Submaximal Exercise**	**Maximal Exercise**
Heart Rate	Increased	Increased	Decreased
Cardiac Output	Unchanged	Unchanged	Decreased
Stroke Volume	Decreased	Decreased	Decreased

2.4.2.3 The circulatory system

At altitude there is generally a reduction in PV as a result of diuresis and dehydration, possibly due to drinking insufficient fluid to offset urinary losses (Sawka et al. 2000), or evaporative losses from high respiratory rates (Hooper and Mellor 2011) or hypoxic stimulation of the carotid bodies reducing sodium reabsorption in the kidneys (Honig 1979). The reduction in PV, although haematocrit (Hct) has increased, causes a reduction in blood volume (BV) (until red cell production increases) (Hooper and Mellor 2011). Additionally, haemoglobin concentration ([Hb]) increases in lowlanders sojourning at altitude (Svedenhag et al. 1997), however, the extent to which this represents erythrocyte volume expansion as opposed to haemoconcentration remains contentious (Sawka et al. 2000). Upon rapid ascent to altitude results suggest that the magnitude of haemoconcentration relates to the magnitude of hypoxic stress, i.e., elevation, (Sawka et al. 2000).

As previously discussed, the reduction in partial pressure of inspired oxygen (P_iO_2) causes fewer oxygen molecules to bind to haemoglobin, which subsequently results in a decrease in SaO_2. Hypoxia-induced factor 1α (HIF-1α) detects a low oxygen pressure and mediates erythropoiesis. Under conditions of decreased oxygen availability, such as hypoxia, EPO is produced in the kidneys and the liver (Gunga et al. 2007). The glycoprotein EPO, stimulates new red cell production from bone marrow. EPO is the major humoral factor responsible for maintaining a normal blood erythrocyte count under conditions of constant oxygen (Gunga et al. 2007). The increase in production of EPO causes erythrocytosis, an increased production of new RBCs (reticulocytes). The resultant increase in RBCs also causes an increase in 2,3-diphosphoglycerate (2,3-DPG), which controls how much oxygen is released once the blood gets out into the tissues. An increased amount of 2,3-DPG in the cell, ensures more oxygen is delivered to body tissues.

As lowlanders acclimatise to altitude, BV adjustments and the elevation of [Hb] and Hct (from plasma loss) are considered central contributors to the performance improvement at altitude by facilitating oxygen transport and delivery to metabolically active tissues (Sawka et al. 2000). Within the following weeks to months at altitude, PV remains low, whereas tHbmass increases until the initial BV is achieved (Heinicke et al. 2003). An example of the desired adaptation can be observed in natives of the Andes, who display high tHbmass, low PV, and elevated [Hb] (Heinicke et al. 2003). Erythropoietin increases transiently up to two days after ascent to altitude and remains elevated after

several days at altitude, EPO then decreases and reaches a constant level slightly above the initial baseline (Ge et al. 2002; Garvican et al. 2012) (for more detail see Section 2.8.1).

2.4.2.4 The skeletal muscle

Hypoxia has been proposed as a key inducer of morphology adaptation in skeletal muscle not only at altitude but also with physical training (Baar 2006). The vast majority of research into the physiological responses of altitude on skeletal muscle morphology and metabolism focuses on the effect of mountaineers exercising at high altitude (<3,000 m). Although hypoxia might be an important stimulus related to exercise in muscle tissue, the stimulus of hypoxia could be negated under chronic altitude conditions such that hypoxia may negatively interfere with recovery processes including signalling events, transcription, translation and protein synthesis (Hoppeler et al. 2003).

Geiser et al. (2001) and Vogt et al. (2001) studied the specific response of skeletal muscle to hypoxia training, and the involvement of HIF-1α. The transcription factor HIF-1α targets genes coding for proteins involved in oxygen transport (EPO and vascular endothelial growth factor, VEGF) as well as coding for glycolytic enzymes and glucose transporters (Hoppeler and Vogt 2001). Muscle biopsies revealed that levels of the regulatory subunit of HIF-1α increased after training under hypoxic conditions but not in normoxia. Additionally high intensity training in hypoxia increased mRNAs coding for myoglobin, VEGF and phosphofructokinase (Hoppeler and Vogt 2001). Following the LLTH method of high-intensity training at around 3,850 m subsarcolemmal mitochondria and capillary length density were significantly increased. This evidence supports the application of altitude training in athletes as training in hypoxia leads to adaptations that compensate for a reduced availability of oxygen during training.

Conversely, Lundby et al. (2004) found when studying VEGF expression in human skeletal muscle, VEGF mRNA is not enhanced at 4,100 m. Further studies were in agreement showing that muscle capillarisation is not increased when expressed as number of capillaries per fibre (Mizuno et al. 1990). Mechanisms responsible for this observed improvement in performance after exposure to hypoxia appear to be a HIF-1 driven response at a molecular level and are likely to include improved exercise efficiency related to tighter coupling of muscular intracellular bioenergetics and mitochondrial function leading to improved mitochondrial efficiency, and/or improved muscle pH regulation and muscle buffering (Gore et al. 2007).

2.5 The application of altitude training

The next section of the review will examine the altitude and hypoxic training research studies. Data for this section has been derived from literature searches of databases (PubMed, Sport Discus, and Google Scholar) and existing reviews on altitude training. Further longitudinal research studies and those with advanced study designs will also be analysed.

2.5.1 The 'hypoxic dose'

The optimal 'dose' to train at altitude has been discussed as early as Shephard in 1974. When defining the optimal 'hypoxic dose', many researchers have addressed three key questions: 1) What is the optimal altitude at which to live? 2) How many days are required at altitude? and 3) How many hours per day are required? (Wilber et al. 2007; Wilber 2013).

Millet et al. (2010) completed an extensive review and revealed the following:

1) The optimal height of the altitude stimulus (2,000-2,500 m) will determine if a large increase in EPO is achieved and therefore an optimal erythropoietic effect. Increasing the altitude may also be important for additional non-haematological parameters.
2) The duration of the camp may also determine the magnitude of erythropoietic enhancements with four weeks being optimal. Three weeks are long enough for beneficial changes in economy, muscle buffering capacity, and the hypoxic ventilatory response.
3) The daily 'dose' of altitude is the 'longer is better' with regards to haematological changes since additional benefits have been shown as hypoxic exposure increases beyond 16 $h \cdot day^{-1}$, with a minimum of 12 $h \cdot day^{-1}$. However, for non-haematological changes, the implementation of a much shorter duration of exposure is possible

Table 2.5 provides a summary of the optimal 'dose' of altitude training as recommended by researchers in Finland, Australia, Switzerland and the USA. These recommendations primarily aim to optimise the erythropoietic response to hypoxia using the natural LHTH methods and simulated LHTL method. Based on these findings there appears to be an optimal height of 2,000 – 2,500 m for 3 – 4 weeks for LHTH and a sleeping height of 2,500 – 3,000 m for 3 – 4 weeks whilst accumulating greater than 12 $h \cdot day^{-1}$ at altitude for LHTL. The recommendations are established upon the current published research where either tHbmass, red cell mass (RCM) or red cell volume (RCV) has been measured. A common theme throughout all of the studies is that groups of athlete have been sent to live and train at the same altitude, and conclusions have been made from the mean response of the group. This is despite individual variation in response to altitude training being reported (Chapman et al. 1998), yet there are very few studies that have attempted to tailor the altitude training strategy to the individual athletes physiological characteristics. The concept of an individual's 'hypoxic tolerance' will be discussed in the subsequent sections of the literature review (see Section 2.7).

Table 2.5: Optimal 'hypoxic dose' for haematological gains as a result of altitude training

Study	Method	Height (m)	Duration (weeks)	Minimum daily 'dose'
Rusko et al. (2004)	LHTH	< 2,000 – 2,200	< 3 – 4	n/a
	LHTL	2,100 – 2,500	3	> 12 h·day^{-1}
Levine and Stray-Gundersen (2006)	LHTL		3	12 h·day^{-1}
Wilber (2007)	LHTH	1,800 – 2,500	3 – 4	> 22 h·day^{-1}
	LHTL	2,500 – 3,000		12-16 h·day^{-1}
Millet et al. (2010)		2,200 – 2,500	4	12-16 h·day^{-1}
Rasmussen et al. (2013)		> 3,000	> 4	n/a
Gore et al. (2013)	LHTH	>2,100	~3	14-19 h·day^{-1}
	LHTL	~3,000		
Chapman et al. (2014a)	LHTL	2,085 – 2,454	4	n/a

Further to these recommendations, Bonetti & Hopkins (2009) conducted a meta-analysis for elite athletes, and found that enhancements in sea level endurance performance were only possible with the natural LHTL method and unclear with LHTH or LLTH. The study provided some important considerations (see Table 2.6) for optimising the current methods used. For example, in elite athletes, by modifying the altitude and hypoxic training method with appropriate manipulation (degree of altitude, number of days of exposure, and the post-altitude test day) there may be clear positive effects with both natural LHTH/LHTL and simulated LHTL. This analysis supports the need for an individualise approach to altitude training, where modifications should be made to an altitude training strategy based on previously collected empirical evidence or potentially by pre-screening athletes to assess their hypoxic tolerance.

Table 2.6: Meta-analysis of effects on sea level mean power output following adaptation to hypoxia (Bonetti and Hopkins 2009), with suggested modifications and subsequent performance improvements

	Natural altitude methods		**Simulated altitude methods**
	LHTH	**LHTL**	**LH (8-18 h·day^{-1} continuous) TL**
Effect of mean protocol (%); ± 90% CL	1.6 ± 2.7	4.0 ± 3.7	0.6 ± 2.0
Modification to protocol	+ altitude - days + test day	- days - test day	+altitude + hours hypoxia - days exposure
Effect of enhanced protocol (%); ± 90% CL	5.2 ± 4.1	4.3 ± 4.1	4.0 ± 5.5

'Effect of mean protocol' = the performance improvement from reviewed altitude/hypoxic training studies; '+' = increase; '-'= decrease; 'altitude' = the height of the training camp; 'days' = the duration of the training camp; 'test day' = when post-altitude testing took place; 'hours hypoxia' = number of hours of simulated altitude exposure; 'days exposure' = the number of days the simulated altitude exposure took place for and 'Effect of enhanced protocol' = potential performance improvement with modifications.

2.5.2 Longitudinal research studies

Much of the research in altitude and hypoxic training has focussed on the effects of a single 3-4 week training camp, at no particular time of the season, with pre- and post-measures to assess the changes that have occurred (Gore et al. 1998; Stray-Gundersen et al. 2001; Rodríguez et al. 2007; Truijens et al. 2008; Frese and Friedmann-Bette 2010). Wachsmuth et al. (2013) stated that this type of one camp study only focused on a few aspects of training and did not embed the altitude training measurements into the long-term training process. Therefore, it is not known if the statistically significant increases in tHbmass after a single altitude training camp disappear within the normal biological variability that occurs during long-term training periods on measured tHbmass exclusively. The author subsequently monitored the German swimming federation in preparation for the 2008 Olympic Games in Beijing following six natural altitude training camps over a two year period. The key findings included:

- low oscillations in tHbmass when hypoxic influences are excluded. i.e. elite athletes do not substantially increase tHbmass by sea level training
- participation at natural altitude training camps at 2,300 m for 3–4 weeks elevates the mean tHbmass above the normal oscillation at sea level
- the erythropoietic activity at altitude seems to depend on baseline fitness conditions and tHbmass increases up to an individual limit
- illness and injury blunt the erythropoietic response, poor health status may be one important reason for athletes failing to respond to altitude
- competition performance tends to be slightly reduced (-0.4 %) immediately after return from altitude and is best (+0.8 %) 3–5 weeks thereafter

- over the whole season, tHbmass is positively related to performance but its role after return from altitude remains unclear.

More recently there is an understanding that the height of the altitude training camps and type of training completed should be periodised into an athletes training programme. One of the most difficult tasks of the coach is to lead their athletes at their peak fitness at the appropriate time i.e. the main competition and the periodization of hypoxic training is very challenging (Millet et al. 2010). For endurance sports LHTH during the winter should be appropriate as the decline in intensity is not detrimental during this phase of the season (Millet et al. 2010). Ideally this will be repeated on several occasions to help speed up the acclimatisation from one camp to the next and at an altitude of 2,000 – 2,500 m, for 3 - 4 weeks as the threshold altitude for a sustained increase in blood EPO concentration is about 2200m (Millet et al. 2010).

Gough et al. (2012) observed international swimming competitions over a season long period. The swimmers were split into LHTH, LHTL and a control group with the race times collected as a record of swimming performance. The findings of the study found that performance was slower for up to one week after altitude and no period of peak form was identified. A unique aspect of this study was that it was a season long analysis, which compared two tapered performances to minimise the influence of fatigue and despite positive haematological adaptation in the altitude group there was no clear advantage when competing. The findings questioned the utility of altitude training for competition preparation (Gough et al. 2012b).

2.5.3 Increased tHbmass

Although there are physiological mechanisms that would suggest altitude training and hypoxic exposures result in an increase in tHbmass, and therefore enhanced endurance performance, there is contradictory evidence (Lundby et al. 2012). Various research groups have suggested the reasons for this includes: individual variation in responses (Chapman et al. 1998), insufficient time spent at altitude (Rasmussen et al. 2013), insufficient altitude stimulus (Chapman et al. 2014a), existing supreme aerobic capacities (Robach et al. 2012), illness or injury (Wachsmuth et al. 2013) and decreased training intensity (Bailey et al. 1998). There is however evidence to suggest altitude training (both LHTH and LHTL) does increase tHbmass (Gore et al. 2013).

Rasmussen et al. (2013) produced a meta-analysis and Monte Carlo simulation of RCV expansion at altitude and concluded that an increase in tHbmass occurs relatively slowly with only minor chances for an increase within two weeks; therefore an exposure time longer than four weeks is generally required. The authors also believed it should be accepted that altitudes <3000 m are insufficient to trigger red cell expansion, particularly if the athletes initial RCV is high. As a result, athletes may need to spend more time at altitude to see an effect on RCV than commonly recommended. Gore et al. (2013) stated Rasmussen et al. (2013) were careful in the selection criteria for studies to include in their meta-analysis, which included a variety of methodologies to measure

RCV from different studies, ranging from CO-rebreathing (2.2% measurement error) to radioactive labelling of albumin (2.8% measurement error) to various plasma dye-dilution tracer methods (6.7% measurement error). Therefore, if the effects of moderate altitude (2000–3000 m) are relatively small, then it is quite likely that inclusion of studies with greater error may confuse the effects of studies using more reliable methods (Gore et al. 2013).

The findings of an original study by Chapman et al. (2013) also disagreed with Rasmussen's conclusions. The study randomly assigned 48 collegiate track and cross country runners into four separate groups of LHTL+H. The live high altitudes were 1,780 m, 2,085 m, 2,454 m and 2,800 m, whilst standardised training sessions look place between 1,250 m and 3,000 m. The study found that the groups living at 2,085 m and 2,454 m achieved a significantly improved sea level race performance (2-3% improvement in 3,000 m time trial) and $\dot{V}O_{2max}$, whereas the athletes living at elevations lower (1,780 m) and higher (2,800 m) than those altitudes demonstrated no changes in sea level performance after the altitude camp. These performance changes occurred despite equivalent increases in RCM (~6% increase) within all four altitude groups, suggesting that altitude-induced erythropoiesis may be necessary and there is an optimal living altitude for producing improvements in sea level performance.

2.5.4 Altitude training in endurance runners

Altitude training is frequently used to enhance endurance performance at sea level, from 800 m up to marathon distance (Fudge et al. 2011). Different altitude and hypoxic training methods (i.e. LHTH, LHTL, IHT etc.) are used to achieve improvements in competition performance, but from a research perspective the effectiveness of a protocol is usually judged by its ability to present classic physiological responses. There are currently differing opinions as to whether the physiological markers measured in altitude training studies transfer into improved performance (Jacobs 2013; Wilber 2013) and in some cases if altitude training is just a 'training camp effect' or placebo effect (Lundby et al. 2012). Traditional physiological and haematological markers in response altitude training include: increase in tHbmass, increase in $\dot{V}O_{2max}$, decrease in submaximal and maximal heart rate, and muscular adaptations (Fudge et al. 2012).

Much of the initial research studies on altitude training took place with groups of well-trained endurance runners using the traditional LHTH method. As a result the findings are difficult to interpret and transfer into an elite athlete population. The studies presented in Table 2.7, Table 2.8 and Table 2.9 are limited to trained endurance runners with a minimum of five participants and evidence of at least three markers of altitude training adaptations. The criteria were selected based on the most common and valuable measures associated with altitude and hypoxic training. Criteria 1-4 are physiological measures that are taken during the altitude exposure and criteria 5-10 are measures taken post-altitude exposure.

Table 2.7: Natural (hypobaric) altitude training studies in runners

			Altitude Training criteria (see footer for detail)										
Reference	**Participants**	**Protocol**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**P**
Levine and Stray-Gundersen (1997)	27M, 12F; HT	EXP_1: 4-wk LHTH (LH = 2,500 m, TH = 2,500-2,700 m)	-	-	-	-	✓	✓	✗	-	-	-	✗
		EXP_2: 4-wk LHTL (LH = 2,500 m, TL = 1,200-1,400 m)	-	-	-	-	✓	✓	✗	-	-	-	✓
		CON: 4-wk LLTL (LL = 150 m, TL = 150 m)	-	-	-	-	✗	✗	✗	-	-	-	✗
Bailey et al. (1998)	16M, 7F; EL	EXP_1: 4-wk LHTH; 1,500-2,000 m	-	-	-	-	-	-	-	✓	-	-	-
	22M, 7F; EL	EXP_2: 4-wk LHTH; 1,640 m	-	-	-	-	-	✗	-	-	✗	-	-
Chapman et al. (1998)	9M, 6F; HT;	EXP_1: 4-wk LHTH, LHTL or LHTL+H (3,000-1,200 m) "Responders"	✓	✓	-	-	✓	✓	✓	-	-	-	✓
	13M, 4F; HT;	EXP_2: 4-wk LHTH, LHTL or LHTL+H (3,000-1,200 m) "Non-Responders"	✓	✗	-	-	✗	✗	✗	-	-	-	✓
Stray-Gundersen et al. (2001)	14M, 8F; EL	EXP: LHTL+H (LH = 2,500 m, TL+H= 1,250-2,500 m)	-	✓	-	-	-	✓	-	-	✗	-	✓
Frese and Friedmann-Bette (2010)	4M, 8F; EL	EXP: LMTM (20 days @ 1,300 m and 22 days @ 1,650 with 19 day break)	-	✓	-	-	✓	-	-	-	-	-	-
		CON: LLTL (similar training at SL)	-	-	-	-	✗	-	-	-	-	-	-
Chapman et al. (2014a)	32M, 16F; WT	EXP_1: LHTL+H (LH = 1,780 m, TL+H = 1,250-3,000 m)	✓	✓	-	-	✓	✗	-	-	✓	-	✗
		EXP_2: LHTL+H (LH = 2,085 m, TL+H = 1,250-3,000 m)	✓	✓	-	-	✓	✓	-	-	✓	-	✓
		EXP_3: LHTL+H (LH = 2,454 m, TL+H = 1,250-3,000 m)	✓	✓	-	-	✓	✓	-	-	✓	-	✓
		EXP_4: LHTL+H (LH = 2,800 m, TL+H = 1,250-3,000 m)	✓	✓	-	-	✓	✓	-	-	✗	-	✗
Wilhite et al. (2013)	6M, 1F; EL	EXP: LHTL+H (LH = 2,150 m, TL+H = 1,150-2,850 m)	-	-	-	-	-	✓	✗	-	✗	-	-
Garvican-Lewis et al. (2015)	13M, 3F; HT	EXP: LHTH (LH = 1,800 m, TL = 1,700 – 2,200 m)	-	-	-	✓	✓	-	-	-	-	-	-
		CON: LLTL (similar training at ~ 600 m)	-	-	-	✗	✗	-	-	-	-	-	-

Table 2.8: Simulated (normobaric) LHTL altitude training in runners

			Altitude Training criteria (see footer for detail)										
Reference	**Participants**	**Protocol**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**P**
Ashenden et al. (2000)	11M; WT	EXP: LHTL 24 nights (5 nights @ 2,650 m/3 nights off) x 3; 8-11h·day^{-1}	✓	✓	-	✗	-	-	-	-	-	-	-
		CON: LLTL 24 nights @ ~600 m	✗	✗	-	✗	-	-	-	-	-	-	-
Saunders et al. (2004b)	22M; EL	EXP$_1$: LHTL 4-wk (5 nights @ 3,100 m/2 nights off) x 4; 9-12h·day^{-1}	-	-	-	-	✗	-	✓	✗	✗	-	-
		EXP$_2$: LMTM 4-wk (Natural LM = 1,570 m, TM = 1,500-2,000 m)	-	-	-	-	✗	-	✗	✗	✗	-	-
		CON: LLTL @ ~600 m for 20 days	-	-	-	-	✗	-	✗	✗	✗	-	-
Brugniaux et al. (2006)	12M; EL	EXP: LHTL (LH = 18 nights @ 2,500-3,000 m; 14 h·day^{-1}, TL = 1,200 m)	✓	✗	-	✗	✓	✓	✓	-	✓	-	-
		CON: "LLTL" @ 1,200 m	✗	✗	-	✗	✗	✗	✗	-	✗	-	-
Neya et al. (2007)	25M; WT	EXP$_1$: LHTL (LH = 29 nights @ 3,000 m for 10-12 h·day^{-1}, TL = SL)	-	-	-	-	✗	✗	✓	✗	✗	-	-
		EXP$_2$: LLTH (LL = SL, TH = 30 min training at 3,000 m for12/31 days)	-	-	-	-	✗	✗	✗	✗	✗	-	-
		CON: LLTL @ SL	-	-	-	-	✗	✗	✗	✗	✗	-	-
Saunders et al. (2009b)	18M; EL	EXP: LHTL 12 wks [LH = 5 nights on/2 nights off @ 2860 m (consisting of 46 ± 8 nights or 415±75 h), TL = ~600 m]	-	-	-	-	✓	✗	✓	✗	✗	-	-
		CON: LLTL @ ~600 m for 12 wks	-	-	-	-	✗	✗	✗	✗	✗	-	-
Robertson et al. (2010b)	11M, 5F; HT	EXP: LHTL (LH = 2 x 3-wk (14 h·day^{-1}, ~ 300 h) @ 3,000 m with 5 wk washout; TL = ~ 600 m)	-	✓	-	-	✓	✓	✗	✓	-	-	✗
		CON: LLTL @ ~600 m for same period	-	✗	-	-	✗	✗	✗	✗	-	-	✗
Robertson et al. (2010c)	13M, 4F; HT	EXP$_1$: LTLH+H (TH = 3-wk (14 h·day^{-1}, ~ 300 h) @ 3,000 m, TL = ~ 600 m, +H = 12 training sessions at 2,200 m)	-	✓	-	✓	✓	✓	✗	✗	-	-	✓
		EXP$_2$: LLTL+TH (LLTL = 3-wk @ ~600 m, = +TH = 12 training sessions at 2,200 m)	-	✗	-	✗	✗	✓	✗	✗	-	-	✗
Humberstone-Gough et al. (2013)	17M, 7F; EL	EXP$_1$: LHTL (LH = 14h·day^{-1} for 17 days @ ~3,000 m, TL = ~ 600 m	✓	-	-	-	✓	✗	✓	✓	✓	-	-
		EXP$_2$: IHE for 17 days (6 min on, 4 min off for 60 min every day) Target stimulus 3,500-6,000 m. 10.2 h hypoxia in total	✓	-	-	-	✗	✗	✗	✓	✓	-	-
		CON: Placebo IHE for 17 days	✗	-	-	-	✗	✗	✗	✗	✗	-	-

Table 2.9: Simulated (normobaric) LLTH altitude training in runners

			Altitude Training criteria (see footer for detail)										
Reference	**Participants**	**Protocol**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**P**
Katayama et al. (2003)	12M; MD	EXP: 3-wk LLTL+IHE (IHE = 90 min @ 4,500 m 3 times a week)	✓	✓	-	✗	-	✗	✓	-	✗	-	✓
		CON: 3-wk LLTL	✗	✗	-	✗	-	✗	✗	-	✗	-	✗
Julian et al. (2004)	14M, 3F; HT	EXP: 4-wk IHE [70 min of 5 min on (FiO2 0.12-0.10), 5 min off (FIO2 0.29) 5 times a week]	✓	✗	-	✓	-	✗	✗	✗	✗	-	✗
		CON: 4 –wk PLA [70 min of 5 min on (FiO2 0.29), 5 min off (FIO2 0.29) 5 times a week]	✗	✗	-	✓	-	✗	✗	✗	✗	-	✗
Dufour et al. (2005)	18M; WT	EXP: 6-wk IHT (2 sessions of 12-20 min per week at 3,000 m)	✓	-	-	-	-	✓	✗	✗	✗	-	-
		CON: 6-wk IHT (2 sessions of 12-20 min per week at 0 m)	✗	-	-	-	-	✓	✗	✗	✗	-	-
Ponsot et al. (2006)	15M; WT	EXP: 6-wk IHT (2 sessions of 12-20 min per week at 3,000 m)	-	-	-	-	-	✓	✓	-	-	✓	-
		CON: 6-wk IHT (2 sessions of 12-20 min per week at 0 m)	-	-	-	-	-	✗	✗	-	-	✗	-
Burtscher et al. (2010)	11M, 1F; WT	EXP: 5 wks IHE (2 h·day^{-1}, 3 day·wk^{-1} @ 3,200-5,500 m) x 2	-	-	-	-	-	✗	✓	-	✗	-	-
		CON: 5 wks no intervention	-	-	-	-	-	✗	✓	-	✗	-	-
Holliss et al. (2014)	12M; EL	EXP: IHT [2 sessions of 40 min per week @ ~2,150 m (FiO_2 = 0.16)]	✗	-	-	-	-	✗	✓	✗	✓	-	-
		CON: IHT [2 sessions of 40 min per week @ 0 m (FiO_2 = 0.29)]	✗	-	-	-	-	✗	✗	✗	✓	-	-

1 = resting SaO_2 decreased; 2 = increased EPO; 3 = increased HIF1α; 4 = increased reticulocytes; 5 = increased tHbmass; 6 = increased $\dot{V}O_{2max}$; 7 = improved submaximal economy; 8 = decreased blood lactate production/increased buffering capacity; 9 = decreased HR during exercise; 10 = increased capillarisation/mitochondria; P = improved performance; ✓= criteria achieved; ✗ = criteria not achieved; - = not measured; M = male; F = female; EL = elite, HT = highly trained; WT = well trained; MT = moderately trained; EXP = experimental; CON = control; PLA = placebo; LH = live high; TL = train low; LL = live low; TH = train high, LM = live moderate, TM = training moderate; TL+H = low/moderate intensity training at moderate altitude and high intensity training at low to moderate altitude; IHT = intermittent hypoxic training; IHE = intermittent hypoxic exposure; SL = sea level.

In the studies using natural altitude training, i.e. going up the mountain, there are a variety of responses depending on the participants; methodology, training completed and 'hypoxic dose' (see Table 2.7). Levine and Stray-Gundersen (1997), Frese and Friedmann-Bette (2010), Chapman et al. (2014a) and Garvican-Lewis et al. (2015) were all able to elicit increases in tHbmass as a result of LHTH, LHTL and LHTL+H at varying living altitudes (LH = 1,300-2,800 m). A living altitude of ~2,200 m was thought to be a pre-requisite of an erythropoietic response to altitude training (Millet et al. 2010), however Garvican-Lewis et al. (2015) subsequently found an increase in tHbmass after 3-weeks at 1,800 m.

Studies using simulated (normobaric) LHTL altitude training (see Table 2.8) have been increasingly implemented since the introduction of hypoxic generators. The method of LHTL using generators and altitude houses has enable more controlled studies, which are also able to measure more variables and standardise training programmes. Using the oCOR-method studies have found an increase in tHbmass (Brugniaux et al. 2006; Saunders et al. 2009b; Robertson et al. 2010b; Robertson et al. 2010c; Humberstone-Gough et al. 2013), however others have not (Saunders et al. 2004b; Neya et al. 2007). It would appear that 12 h·day^{-1} of hypoxia is not sufficient to increase tHbmass regardless of the severity of the hypoxic stimulus. As a result, the recommendations of Rusko et al. (2004) and Levine and Stray-Gundersen (2006), stating 12 h·day^{-1} would be insufficient with 14 h·day^{-1} more realistic. It is worth noting that in all the studies the sleeping altitude is fixed throughout the entire study duration, this does not account for the acclimatisation process or the hypoxic tolerance of the individual. If sleeping height is adjusted based on changes in physiological measures of hypoxic tolerance, such as SaO_2, HVR or [EPO], then the physiological response could be further enhanced.

There is little evidence to suggest that IHE (Bartsch et al. 2008) or IHT/LLTH (Lundby and Robach 2016) induce greater increases in tHbmass and therefore endurance performance. There is however some evidence which supports the use of LLTH altitude training methods in improving some physiological determinants of endurance performance (see Table 2.9). Dufour et al. (2005) and Ponsot et al. (2006) found 6 weeks of IHT (2 sessions of 12-20 min per week at 3,000 m) improved $\dot{V}O_{2max}$, however, Burtscher et al. (2010) and Holliss et al. (2014) did not find increases in $\dot{V}O_{2max}$ with similar protocols. Interestingly the later studies did report and improved submaximal economy, which may be reflective of the type of training completed by the athletes.

2.6 Measuring the Success of Altitude and Hypoxic Training

The challenge of assessing athlete performance after altitude training has been discussed previously by Gore (2014) in an invited editorial. The author suggested that there were four key areas for interrogation: red cell volume (RCV), $\dot{V}O_{2max}$, performance, and measurement precision. The next section of the review will examine those areas.

2.6.1 Total haemoglobin mass (tHbmass)

Although many different mechanisms have been proposed to explain the improved performance at sea level with altitude training or hypoxic exposure, the majority of data (from adequately controlled studies) leads us to conclude that the erythropoietic effect of altitude and hypoxia acclimatisation is of primary importance (Levine and Stray-Gundersen 2006). Changes to systemic oxygen transport via alterations to BV and [Hb] can have significant implications for $\dot{V}O_{2max}$ and potentially endurance performance (Gledhill et al. 1999). Previously, measures such as Hct and [Hb] were used to evaluate haematological enhancements as a result of altitude and hypoxic training (Ingjer and Myhre 1992). Unfortunately, Hct and [Hb] alone are inadequate to explain changes in tHbmass, as they are concentrations in the total BV, meaning they are subject to acute changes as a result of alterations in BV itself seen in ascent and decent from altitude (Sawka et al. 2000), and changes in training status (Mørkeberg et al. 2009) or hydration status (Jimenez et al. 1999).

Kjellberg et al. (1949) were among the first group of investigators to identify the relationship between BV expansion and exercise training. Brotherhood et al. (1975) went on to examine the primary components of blood and found there to be no significant differences in the Hct and [Hb] between trained and untrained subjects, despite trained subjects having a larger BV in comparison to untrained. These data clearly demonstrate that both the plasma and red cell components of BV contributed to the hypervolemia and total circulating haemoglobin in endurance trained athletes (Convertino 1991). However, this also highlighted the importance of being able to measure the absolute tHbmass of an athlete, as [Hb] is not a good enough indicator of the bloods ability to transport more oxygen to the exercising muscles. For example, one athlete could have a [Hb] of 15.0 $g \cdot dL^{-1}$ and a BV of 6 L, with a second athlete having the same [Hb] but a BV of 7 L. The measurement of [Hb] alone would be misleading, as the second athlete is likely to have a higher absolute tHbmass but it would be undetectable.

Consequently, a more accurate measurement of oxygen carrying capacity was established to quantify tHbmass pre and post- altitude training using CO-rebreathing methods (Burge and Skinner 1995; Schmidt and Prommer 2005). Worldwide physiologists and practitioners now utilise the oCOR-method to quantify changes in tHbmass, however these changes vary in magnitude depending on the altitude training method used with small gains in tHbmass of ~3-4% arising as a result of LHTH and LHTL (Gore et al. 2013). However, confidence in the validity and reliability of the oCOR-method is therefore vital when evaluating these small changes and judging the success of an altitude training camp.

According to Fick's equation, $\dot{V}O_{2max}$ is determined by the oxygen supply of the blood and by the oxygen consumption of the muscles. As haemoglobin is the sole transporter of oxygen from the lung to the muscle, tHbmass is important. Schmidt & Prommer (2010) believed that tHbmass linearly depends on BV over a broad range in a sex-related manner and the scattering of tHbmass related to a certain value of BV reflects different [Hb]. Consequently, [Hb] and tHbmass are considered to be different physiological parameters, which may exert different effects on endurance performance (see

Figure 2.9). Total Hbmass is thought to be an excellent predictor of $\dot{V}O_{2max}$ and therefore, endurance performance (Schmidt and Prommer 2010).

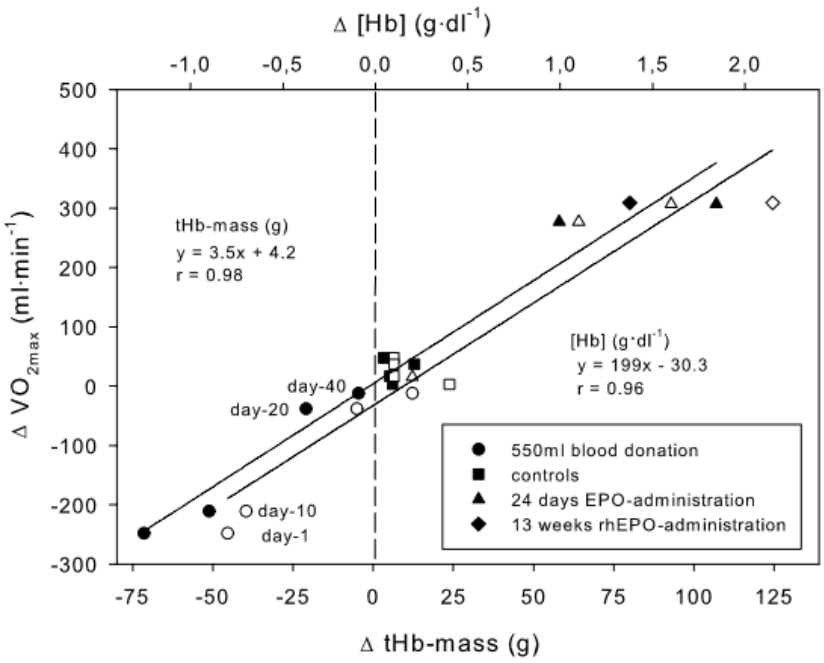


Figure 2.9: Relationship between changes in maximal oxygen uptake (V˙O2max) and total haemoglobin mass (tHbmass) (closed symbols) as well as haemoglobin concentration (open symbols).Taken from Schmidt and Prommer (2010)

2.6.1.1 Determining tHbmass with the oCOR-method

Total Hbmass has been measured frequently in altitude training studies with the Evans blue dye (Gibson and Evans 1937) and also an extended CO-rebreathing method (Burge and Skinner 1995). However, these methods are too expensive, time consuming and invasive. As a result of the optimisation of CO-rebreathing (the oCOR-method), Schmidt and Prommer (2005) has made measurement of tHbmass more accessible. Since the method became readily accessible, many research groups and sporting associations have sought reduce the measurement error of the method. Measurement error of the oCOR-method has been estimated from studies that include interventions, where it will include the analytic error, the day-to-day biological variation, and the inter-individual variation in response to the intervention (Gore et al. 2005). The critical analysis of the oCOR-method has been tested and validated in many different scenarios (see Table 2.11).

Further to the technical error of measurement, Garvican et al. (2010b) studied the seasonal variation of tHbmass in internationally competitive female endurance cyclists. The study revealed that tHbmass varied by ~3 % during a competitive season. This may be partly related to changes in training load, as a 10% change in training load over a 6-week period was associated with a 1% change in tHbmass. Eastwood et al. (2011) investigated within subject variation in tHbmass in elite athletes over the period of one year and also the effect of altitude training on tHbmass. The investigation found a variation in males and females of ~4% and that altitude training can potentially increase or decrease

tHbmass by 3%. Ulrich et al. (2011) also monitored tHbmass over an extended period in endurance-trained (canoeing, swimming and distance running) and non-endurance-trained (basketball, tennis and weightlifting), male and female elite adolescent athletes. The study found there were no differences in relative tHbmass, beyond the changes due to growth and maturation, between endurance-trained and non-endurance-trained elite adolescent athletes over an 18-month period. Therefore, regular endurance training at the ages of 15-17 years does not significantly increase tHbmass as was hypothesised. Studies that find a low variability in tHbmass (Eastwood et al. 2008; Garvican et al. 2010b; Eastwood et al. 2012c; Gough, Clare Elizabeth, Sharpe, K., Garvican, L. A., Anson, J.M., Saunders, P.U., Gore 2013; Wachsmuth et al. 2013) demonstrate that the method can be used in multiple populations and any gains in tHbmass can be attributed to altitude training as opposed to training load, sex or age.

Schmidt & Prommer (2010) reviewed the impact of tHbmass on $\dot{V}O_{2max}$ to distinguish between the effects of tHbmass and [Hb] on $\dot{V}O_{2max}$. Figure 2.10 illustrated how variations of these parameters affect $\dot{V}O_{2max}$. The authors stated that a change in tHbmass of 1 g is related to a change in $\dot{V}O_{2max}$ of approximately 4 $mL \cdot min^{-1}$ and this was via erythropoietic stimulation and a change in oxygen carrying capacity. Using the equation from Schmidt & Prommer (2010), Table 2.10 illustrates the potential effect of a 2% increase in tHbmass on 10 km performance. Total Hbmass determines $\dot{V}O_{2max}$ by two mechanisms: (i) endurance training and (ii) adaptation to altitude training, although care should be taken when interpreting altitude training results as observed haematological changes could simply reflect the greater training stimulus that accompanies a training camp (Siebenmann 2016). Total Hbmass can increase $\dot{V}O_{2max}$ via the following mechanisms: (i) a balanced increase of tHbmass and PV augmenting cardiac out and/or (ii) by increasing [Hb] due to an increase of tHbmass accompanied by a reduced or unchanged PV augmenting arteriovenous oxygen difference (Schmidt and Prommer 2010).

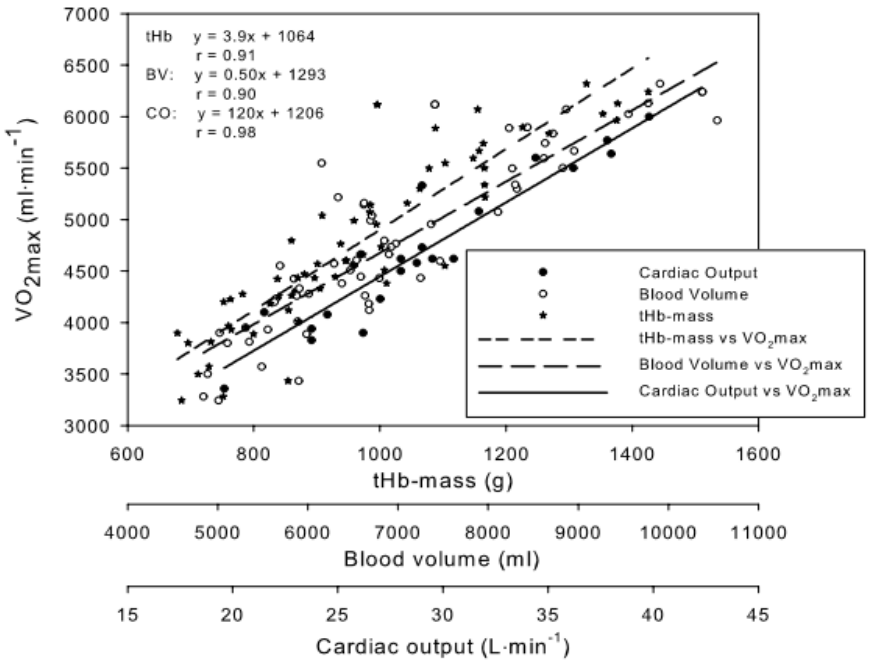


Figure 2.10: Relationship between $\dot{V}O_{2max}$ and tHbmass, blood volume, and cardiac output. Taken from Schmidt and Prommer (2010)

Table 2.10: Example of how a 2% increase in tHbmass may affect performance (Schmidt and Prommer 2010)

	PRE	**POST**
$\dot{V}O_2$ (L·min^{-1})	3.9	3.9
tHbmass (g)	1000	1020
	1064	1064
$\dot{V}O_{2max}$ = (3.9 * tHbmass) + 1064 (Schmidt and Prommer 2010)		
$\dot{V}O_2$ (mL·min^{-1})	4964	5042
$\dot{V}O_2$ (L·min^{-1})	4.96	5.04
Body mass (kg) *	70.0	70.0
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	70.9	72.0
Economy (mL·kg^{-1}·min^{-1}) *	200	200
v$\dot{V}O_{2max}$ (km·h^{-1})	21.3	21.6
90% of v$\dot{V}O_{2max}$	19.1	19.4
10km time (min:s) **	31:20.2	30:51.1

* The outcome of the equation assumes that body mass and economy remain constant
** 10 km time is estimated from speed maintained at 90% of v$\dot{V}O_{2max}$

In athletes the greatest legal increase in $\dot{V}O_{2max}$ is as a consequence of training and living at altitude. Prommer et al. (2010) attempted to characterise the tHbmass of elite male Kenyan runners to find out whether it contributed to their performance superiority. The study found there was no difference in tHbmass compared to German runners during the competitive season; therefore superior performance cannot be solely explained by the oxygen transport of the blood.

Table 2.11: Studies measuring the validity and reliability of the oCOR-method

Author	Title	Findings
Gore et al. (2006)	Time and Sample Site Dependency of the oCOR-method	• The oCOR-method using capillary blood had typical error for tHbmass of 1.1%. • Capillary blood should be sampled at 8 and 10 min (typical error of 1.2%) after CO-rebreathing.
Prommer & Schmidt (2007)	Loss of CO from the intravascular bed and its impact on the optimised CO-rebreathing method	• The oCOR-method found a CO flux out of the vascular bed resulting in a 2% increase in tHbmass. • Only a small volume of the administered CO (0.32% min^{-1}) leaves the vascular space. • The best agreement between venous and capillary %HbCO is found at min 8 & 10, with minor at 6 min. • Using 6–8 min for sampling is recommended.
Garvican et al. (2010)	Carbon monoxide uptake kinetics of arterial, venous and capillary blood during CO rebreathing	• The CO mixing time at rest varies between subjects, but is largely complete 10 min post-CO rebreathe. • A total of~1.8%, of the CO bolus, was lost to myoglobin during the oCOR-method. • Based on the CO flux loss to myoglobin tHbmass will be overestimated by~2.2%.
Alexander et al. (2011)	Standardising analysis of carbon monoxide rebreathing for application in anti-doping.	• Measuring tHbmass via oCOR-method may be a suitable for inclusion into the biological passport • Analyser error of the method can be reduced to ≤1% • Running five replicates, to obtain %HbCO, reduces the analyser error of the OSM3 hemoximeter.
Gough et al. (2011)	Quality control technique to reduce the variability of longitudinal measurement of haemoglobin mass.	• The analytical variation associated with measuring tHbmass was increased when tests were conducted in ''different laboratories,'' • The adjustment of tHbmass values using quality control solutions can eliminate additional variation. • A larger bolus of CO was used (1.4 $mL \cdot kg^{-1}$ body mass) and this is associated with increased precision.
Steiner & Wehrlin (2011)	Comparability of haemoglobin mass measured with different carbon monoxide-based rebreathing procedures and calculations	• Differences for tHbmass measured with various CO-rebreathing approaches of up to 9.7%. • This is relevant as estimating tHbmass always depends on the procedure and the subsequent calculations, as these two factors can influence the resultant tHbmass. • Measuring %HbCO at 8 and 10 min not 6 and 8 min post-CO inhalation would reduce Δ%HbCO by about 0.1%, and therefore increase the estimated tHbmass by about 1 – 2%.

2.6.2 Determinants of endurance performance

It is a commonly held view that $\dot{V}O_{2max}$ can be used to predict endurance performance. Saltin and Astrand (1967) reported that successful distance running performance is highly correlated with a high $\dot{V}O_{2max}$, which is influenced by muscle capillary density, tHbmass, stroke volume, aerobic enzyme activity and muscle fibre type composition (Coyle 1999). Although $\dot{V}O_{2max}$ is important, other physiological factors including sustaining a high percentage of $\dot{V}O_{2max}$ (fractional utilisation or LT) and running with relatively low energy expenditure (a good running economy) are also crucial (Jones 2006). Saunders et al. (2010) found that $\dot{V}O_{2max}$, running economy and LT (as a three-predictor) were able to predict the within-subject changes in performance (measured by peak running speed). Consequently, the use of longitudinal monitoring of performance and physiological variables in distance runners over a training period using standard treadmill testing is supported (Saunders et al. 2010).

It is worth noting that race performance isn't purely a consequence of physiology but a multifaceted approach, taking into account several biological, environmental and technological issues (Lippi et al. 2008). However, assessing running performance in the laboratory in controlled environmental conditions is essential when trying to understand if an intervention has worked. A combination of one or multiple physiological determinants of endurance performance has been measured in research studies related to altitude and hypoxic training. A meta-analysis of altitude and hypoxic training methods by Bonetti and Hopkins (2009) reported that 37 studies measured $\dot{V}O_{2max}$, 12 measured tHbmass and 14 measured running economy. Figure 2.11 illustrates the relationship between the 'estimated effect of performance' and (a) $\dot{V}O_{2max}$, (b) tHbmass and (c) exercise economy utilising different altitude and hypoxic training methods.

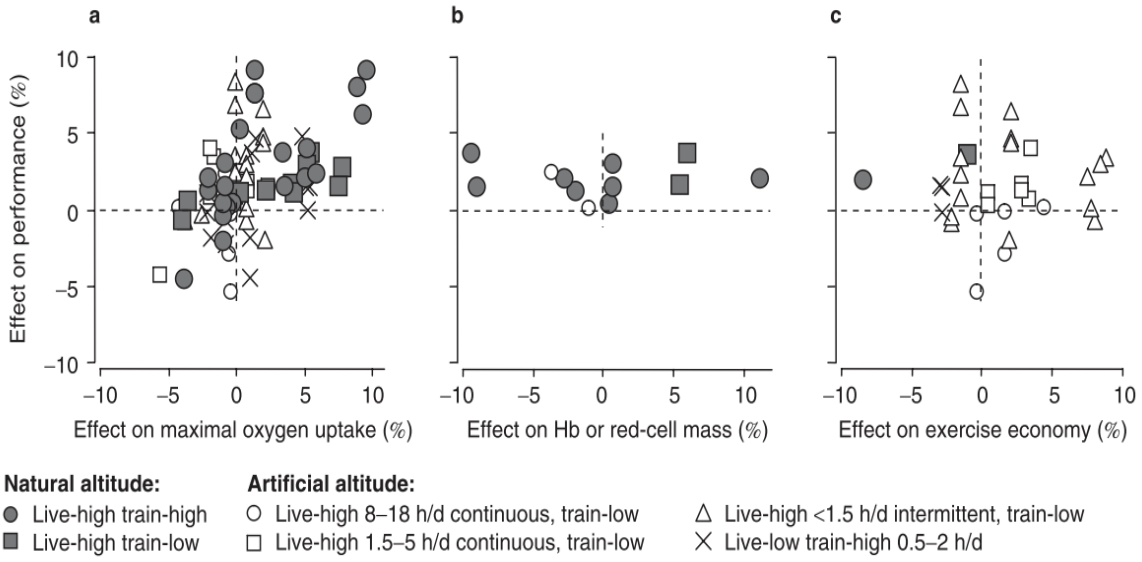


Figure 2.11: Individual study-estimates of effects on performance plotted against $\dot{V}O_{2max}$, haemoglobin (Hb) or red cell mass, and exercise economy with natural and artificial altitude training methods (Bonetti and Hopkins 2009).

2.6.3 Performance

Relatively few studies have monitored elite athletes during a competitive racing season with altitude exposure incorporated into their training (Saunders et al. 2009b). Stray-Gundersen et al. (2001) recruited 26 elite level distance runners and completed a 27 days altitude training camp at 2,500 m 3-weeks after the NCAA Championships and the USA Track and Field Championships. Additional 3,000 m performance trials were completed immediately before and after the training camp. 3,000 m performance at sea level significantly improved after LHTL, reducing the time trial time by 5.8 s (95% confidence limits 1.8–9.8 s) or by 1.1% (95% confidence limits 0.3–1.9%). Although the altitude training camp was placed within the competition season, a competitive race did not take place after the altitude training camp, only controlled 3,000 m time trials.

Fudge et al. (2011) investigated the effect of four weeks of living at 1,850 m and training between 1,537 – 2,000 m in 14 elite British 800-10,000 m athletes. The altitude training camps took place from 2008-2010 during the track and field competition phase of the season (typically May to September). A retrospective analysis found that the number of personal bests increased significantly from 15 in 2008 and 16 in 2009 to 24 in 2010. The overall improvement in performance was 0.8%, 0.9% and 1.4% in 2008, 2009 and 2010 respectively (Fudge et al. 2011). The findings suggested a progressive development of the athletes over the three seasons of altitude inclusion, and greater than seen in the preceding years. Although it should be noted there was no control group included in this study. Saunders et al. (2009b) also analysed the race performance during a competitive season in 7 elite Australian middle-distance runners. The altitude programme included an extended simulated LHTL exposures (44 ± 7 nights at 2,846 ± 32 m over 7-weeks) combined with a series of short LHTL+H training camps to natural moderate altitude (4 x 7-10 days at 1,700 – 2,200 m). The programme improved season's best race performance by 1.9% (90% confidence interval 1.3–2.5%) in competitive 800, 1500, and 3000 m races compared with their personal best times before the intervention. Unfortunately both studies did not report any physiological or haematological markers alongside race performance.

2.6.4 Other haematological markers

The measurement of EPO has received a great deal of attention with regards to altitude and hypoxic training research. Specifically in endurance athletes, the magnitude of the individual EPO response may ultimately determine the level of haematological adaptation and performance enhancement following altitude training (Chapman et al. 1998). It has also been shown that the rate of EPO production appears to be directly related to the severity of the hypoxic stress (Eckardt et al. 1989) but there is a substantial inter-individual variability (Friedmann et al. 2005a). Measuring EPO before, during or after altitude and hypoxic training may help to determine which athletes are more sensitive to the hypoxic stimulus and the adaptations associated with it. Ultimately, it might come down to genetic differences in hypoxia sensing and hypoxic response pathways to understand the wide variation in EPO response to a fixed level of hypoxia (Chapman et al. 2010). Although this theory was

disputed by Jedlickova et al. (2003) who found no convincing association between genetic markers tightly linked to EPO and eight genes involved in EPO regulation or EPO differential responses to hypoxia.

Other biochemical markers, such as iron and ferritin, have also been measured in relation to altitude training. According to Mazzeo (2005) the lack of adaptations from altitude training are often related to illness or depleted iron stores prior to altitude exposure. Iron deficiency is common in endurance runners possibly due to prolonged training and repeated ground impacts (Burden et al. 2015) and iron supplementation has been extensively researched in relation to endurance performance (Garvican et al. 2014). It is well known that iron plays a key role in number of cellular processes, and is an essential element of haemoglobin, the blood's oxygen carrier, as a result iron depletion can affect physical performance (Schumacher et al. 2002). Availability of sufficient amounts of iron is critically important for normal and stress-induced erythropoiesis and circulating iron levels are affected by intestinal absorption from the diet, iron transport capacity of the blood, iron losses via bleeding and cellular breakdown, and the release of iron from cells such as macrophages and hepatocytes (Goetze et al. 2013). Additional stress to endurance athletes' altitude training causes hypoxia-induced erythropoiesis, which increases the iron demand in the erythropoietic compartment and induces adaptive changes in the human body, such as increased intestinal iron uptake, augmentation of serum iron-binding capacity and enhanced mobilisation of iron from cellular stores (Haase 2010). Provided the potential side effects associated with oral iron supplementation are considered, such as nausea, flatulence and gastrointestinal discomfort, the development of altitude-specific, iron supplementation guidelines could help to optimise athletes' haematological adaptations to prolonged altitude exposure (Wachsmuth et al. 2014; Govus et al. 2015).

2.7 Hypoxic Tolerance

In 1992 the well-respected coach Frank Dick published 'Training at altitude in practice' (Dick, 1992). He stressed that the individual response to altitude training is very varied, even amongst athletes who work with the same coach and who perform very similar training programmes. The author did not offer an explanation for this statement; despite this he did provide a series of practical recommendations for all athletes. Research groups since have investigated the individual variation in physiological, haematological and performance markers after altitude training (Chapman et al. 1998; Friedmann et al. 2005a; Chapman 2013). Despite this research, the individual's characteristics or experiences of the sensitivity to, or tolerances of hypoxia, have received relatively little attention.

2.7.1 Individual variation

Altitude and hypoxic training does not appear to work for everyone. For example, Dill and Adams (1971) were the first to report that, at altitude, highly trained athletes were unexpectedly impaired during exercise and to an unusually greater extent compared to lesser trained individuals. Subsequent

retrospective analysis by Chapman et al. (1998) identified notable individual variation in 5,000 m run time after 4 weeks of altitude training at 2,500 m. As a result, the athletes were divided into two groups; the 'responders' who improved their performance and the 'non-responders' whose performance declined. Their analysis revealed that the individual variability in the response to altitude training may be accounted for by two mechanistic pathways: 1) an altitude-acclimatization effect, i.e., an increase in EPO concentrations and total RCV, therefore oxygen carrying capacity and 2) $\dot{V}O_{2max}$, and a training effect, i.e., maintenance of interval training velocity and oxygen flux near sea level values, facilitating improvements in $\dot{V}O_{2max}$ and race performance (Chapman et al. 1998). The 'responders' group demonstrated a greater acute and sustained increase in EPO, and therefore increase in total RCV, increase in $\dot{V}O_{2max}$ and finally significant improvement in 5,000 m run time (Chapman et al. 1998). Individual variation in EPO has since been reported in response to acute hypobaric hypoxia (Ge et al. 2002), to acute normobaric hypoxia (MacKenzie et al. 2008) and also to chronic exposure to natural altitude (Friedmann et al. 2005a).

Friedmann et al. (2005) tested the hypothesis that EPO response after 4 h acute exposure to simulated altitude, i.e. normobaric hypoxia (at an ambient PO_2 similar to the training altitude) could be indicative to the extent of the acute EPO increase during a training camp at real moderate altitude. Furthermore, that a large EPO increase after acute hypoxic exposure was associated with a large increase tHbmass after training at moderate altitude. In this work, a group of 20 elite junior swimmers (with 5 years of training history lived) and trained at an altitude of 2,100 – 2,300 m for 3-weeks. The study reported a significant correlation ($r = 0.74$) between the change in EPO after 4 h exposure to normobaric hypoxia (FiO_2 0.15), and the subsequent change in EPO after 1-2 days at real altitude. However, the increase in tHbmass measured after the altitude training period was not correlated with the acute EPO increase or with the EPO values measured in the middle and at the end of the 3-week altitude training camp.

A more recent review by (Chapman 2013) found that the variability of responses to altitude is associated with the extent to which performance, in the form of $\dot{V}O_{2max}$, is impaired at altitude. The explanation for this phenomenon resides at the level of the lung, as there is also a significant negative correlation between SaO_2, measured either in normoxia or hypoxia, and the decline in $\dot{V}O_{2max}$. Therefore, individuals who are least able to maintain SaO_2 are likely end up being the ones with the largest drop in $\dot{V}O_{2max}$ (Chapman 2013). This would suggest that SaO_2 during heavy or maximal exercise could be used to predict who may or may not be more negatively affected at altitude and potentially who is likely to gain the greatest adaptation. The use of a pre-screening tool has gained more credibility (Chapman et al. 2010; Billaut et al. 2012) to identify where athletes sit on the 'high to low responder' continuum. Optimising an altitude training strategy for an individual athlete's response to a hypoxic stimulus introduces a challenge in relation to taking training groups altitude training. Elite athletes rarely train alone and a training group is considered key to success, therefore the group would have to travel to the same altitude location.

2.7.2 Hypoxic tolerance

The theory that there are mechanistic similarities in human physiology between adaptations for endurance performance and for hypoxic tolerance was first introduced by Hochachka et al. (1998). The Hypoxic tolerance has arisen from analysis of human responses to hypobaric hypoxia in different lineages (lowlanders, Andean natives, Himalayan natives, and East Africans), which indicate 'conservative' and 'adaptable' physiological characteristics (Hochachka et al. 1999). Hochachka et al. (1999) stated that the usual timeline for response to hypoxia is divided into three categories: acute, acclamatory, and phylogenetic (or genetic adaptation). Hochachka et al. (1998) stated that the key components involved in the response are:

1) Sensing mechanisms that tell the organism when an oxygen limitation problem arises and how serious it is, therefore initiating the cascade of events
2) The information is then transduced at various levels of organisation into the appropriate functional responses
3) A specific set of signal transduction pathways are involved in acute responses to the stress
4) The same or different sets of signal transduction pathways may be used to orchestrate more complex acclamatory responses
5) All of the above (the sensing step, the signal transduction pathways, the acute response, and the acclamatory responses) may be changed gradually through evolutionary time.

In lowlanders, hypoxia defences are initiated by several oxygen sensing, signal transduction pathways, which can be described as five general hypoxia response systems, each initiated by a different hypoxia-sensing mechanism (see Figure 2.12): (i) carotid body; (ii) pulmonary vasculature; (iii) oxygen sensors in the vasculature of other tissues; (iv) oxygen sensors in kidney and liver; and (v) tissue-specific oxygen sensing and signal transduction pathways lead to metabolic (Hochachka et al. 1998).

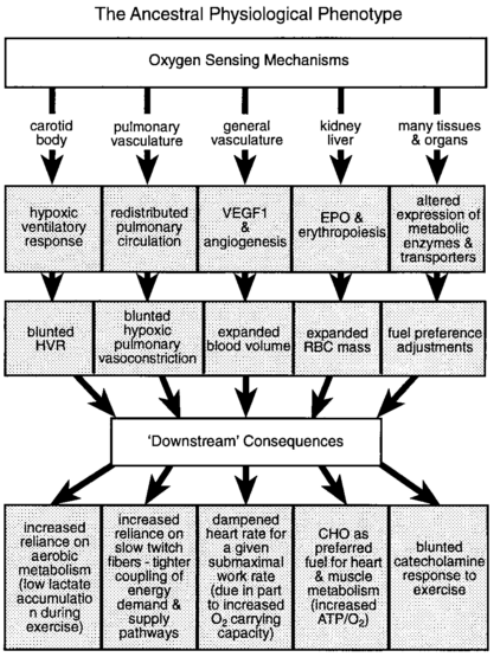


Figure 2.12: Summary of the proposed ancestral physiological phenotype as a phylogenetic adaptation to hypobaric hypoxia (Hochachka et al. 1998).

To assess how these traits change through generational time, Hochachka et al. (1998) compared the acute and acclamatory patterns of lowlanders to those found in indigenous highlanders. The conservative physiological characters are dominant and make up most of our physiology and therefore conserved through phylogenetic time by negative selection (see Table 2.12). The evidence for adaptable characters was found in Quechuas and Sherpas, where adjustments in the five previously mentioned response systems seem to be a key to the complex physiology of hypoxia tolerance (Hochachka et al. 1998). The hypoxic tolerance of an athlete may provide a valuable insight into their individual physiological make-up. The outcome of this would not be to class athletes as 'conservative' or 'adaptable' but to identify sensitive areas that can be targeted with strategies to optimise acclimatisation during the initial days at altitude or maximise the desired adaptations on return to sea level.

Table 2.12: Examples of conservative and adaptable characters in response to hypoxia.

Conservative characters	**Adaptable characters**
Haemoglobin oxygen affinity and regulation	Blunted HVR
Muscle organization into different fibre types	Blunted hypoxic pulmonary vasoconstrictor response
Region-specific organization of brain metabolism with the brain's almost exclusive preference for glucose as a fuel	Up-regulated expression of vascular endothelial growth factor 1
	Up-regulated EPO expression
	Regulatory adjustments of metabolic pathways

There are also parallels between the individual responses of endurance athletes to altitude training and the susceptibility of altitude illnesses for high altitude mountaineers. Burtscher et al. (2004) stated that since hypoxia is primarily responsible for the development of acute mountain sickness (AMS), individual ventilatory response to hypoxia and thus differences in tissue oxygenation during acute altitude exposure might partly explain this variability. The extent to which exercise is impaired at altitude also shows substantial individual variability across athletic populations (Chapman 2013). The cause of this variability could result from (a) a wide range of individual sensitivities to a fixed level of hypoxia by the EPO producing cells of the kidney, (b) differences between oxygen transport and oxygen consumption in the kidney across individuals (Bauer and Kurtz 2003), and/or (c) ventilatory mediated differences in arterial oxygen content, altering the degree of arterial hypoxemia sensed by the kidney (Chapman et al. 2010). Jedlickova et al. (2003) postulated that individual variability is a function of genetic differences in hypoxia-sensing and hypoxia-responsive pathways as oxygen-sensing pathway regulates a number of genes that determine the response to hypoxia, including, genes that control erythropoiesis, angiogenesis, energy metabolism, carcinogenesis, and apoptosis. However, this theory was not proven.

Many of these pathways reside at a molecular level, which are too invasive to measure in elite athlete populations. In high altitude medicine the physiological risk for AMS has been linked to the severity of the hypoxic exposure, the efficiency of the physiological adaptive responses, the ventilatory response to hypoxia and the decrease in SaO_2, at rest or exercise. Subsequently, Richalet et al. (1988) implemented a hypoxic sensitivity test (HST) designed to predict those mountain climbers who would be more susceptible to AMS. The HST has also been used to determine if there is a the relationship between severe hypoxia and the impairment of cycling time trial performance (Bourdillon et al. 2014). The metrics derived from the HST, including arterial oxygen desaturation, hypoxic ventilatory response and cardiac response to hypoxia could provide a useful pre-screening tool for athletes before they ascend to altitude to predict how they will respond in the initial days. Coaches may be able to optimise the training that they complete to maximise the desired physiological and haematological adaptations.

2.8 Challenges of Altitude Training

2.8.1 Returning to sea level and preparing for competition

Traditionally, altitude training camps were designed to fit around the athlete's competition schedule. A camp would usually last between 4-6 weeks and the return to sea level would be determined by the coach's previous experiences and anecdotal evidence. An early review of the practical implications of altitude training, which was based upon (albeit anecdotal) experiences of elite coaches, revealed that on return from altitude, that time was needed to reach a stage were performance shows a clear sign of benefit (Dick, 1992). The author stated that athletes began to feel fatigued during the later stages of the altitude camp and on descent, that initial performance may be depressed. Three to four days after returning to sea level there can be a brief return to 'normal' performance but then performance returns to a relatively depressed state until days 8-11 post-return (Dick 1992). Related to this, Dick reported that coaches believed that for those athletes who used altitude training regularly over a period of years, the peak performance period occurred around 14 days following descent (Dick 1992).

The optimum time to compete or resume normal training post-altitude training has been reviewed recently (Chapman et al. 2014b). This was first discussed in 1998 by Gore and colleagues who stated that there is considerable debate as to when, if at all, performance is enhanced at sea level after return from altitude. His findings along with that of others were unable to identify an optimal group mean time for sea level performance of endurance athletes during the first three weeks after altitude training. Robertson et al. (2010b) were only able to suggest that the timing of competition after altitude exposure and the management of training are particularly important factors in realising performance gains after altitude exposure. There is limited empirical data charting the time course response of performance changes after altitude training, which is a key issue frequently debated by coaches and warrants systematic investigation (Gough et al. 2012).

The time course of red cell production during and after LHTL and natural altitude training strategies has been previously studied (Heinicke et al. 2005; Brugniaux et al. 2006; Clark et al. 2009; Robertson et al. 2010b; Pottgiesser et al. 2012). Table 2.13 summaries the altitude training method implemented and study design.

Figure 2.13 illustrates that the hypoxia-induced increases in tHbmass may be lost within 1-2 weeks after an altitude training camp. Only recently has the variable 'performance on return from altitude' been associated with any physiological markers. The rapid decrease in tHbmass and other haematological markers such as EPO on return to sea level from altitude has been linked to the observations of Prommer et al. (2009), who found that when natural altitude dwellers reside at sea level for sustained durations a steady reduction in tHbmass occurs. Garvican et al. (2012) suggested that removal of the altitude stimulus results in a 're-acclimatisation' to the normoxic environment. Rice et al. (2001) also showed there is a relationship between descending from high altitude and decreases in tHbmass, which has been termed neocytolysis. Neocytolysis is the selective destruction of youngest erythrocytes (neocytes), which may be stimulated by a sudden drop in EPO concentration upon descent to sea level (Rice et al., 2001). A decrease in EPO has also been associated with a decline in performance (Schuler et al. 2012); therefore, preventing this decrease in EPO may attenuate the impairment in endurance performance that has been reported.

Neocytolysis has been proposed as a physiological process which could shorten RBCs lifespan in response to a changed external environment and lead to a reduction in tHbmass, however the factors determining the lifespan of cells (including RBCs) that circulate in blood are not fully understood (Risso et al. 2014). The study by Rice et al. (2001) argued that young RBC destruction was as a result of elevation of ferritin levels, which was interpreted as the transfer of iron to stores. However, ferritin saturation was not measured in the study, and this, combined with the observation that the levels of circulating transferrin receptors did not change, raises the suspicion that ferritin might have been elevated for other reasons (Risso et al. 2014). Further to this, Rice et al. (2001) concluded in favour of the neocytolysis process being triggered by descent to sea level as reticulocyte counts did not change with respect to baseline values during the first 6 days at sea level. Yet, when neocytolysis should be at its peak, because RBCs are at a maximum and EPO levels are at their lowest, the reticulocytes are spared by the process, as if they were different from the neocytes (Risso et al. 2014). Evidently, different features in the reticulocyte and neocyte must be invoked to accept this explanation, but a discussion about this aspect has never been conducted (Risso et al. 2014).

In order to confirm the link between the descent from high altitude and neocytolysis causing a rapid decline in tHbmass further research is needed. Risso et al. (2014) suggested the systematic and accurate measurements of the RBC age parameter 4.1a/4.1b, which is an absolute marker of RBC age independent on cell density, metabolic activity or imponderable side effects of radiolabelling should be conducted. Further studies are needed to establish whether the reduction tHbmass, which is an established physiological adaptive responses, is due to the removal of a selected population of RBCs (e.g. 'neocytes') and what are the features of the targeted cells (Risso et al. 2014).

Table 2.13: Studies that have measured the time course response of haematological adaptions during and after natural and artificial altitude training

Study	**Participants**	**Camp Duration and Altitude**			**Measures**			
		Method	Duration	Altitude		Pre	During	Post
Heinicke et al. (2005)	10 world class biathletes	Terrestrial LHTH+H	3 weeks	Living: 2,050 m Training: ~4-6 hours at 1,850-2,600 m	tHbmass: EPO:	- Pre-camp (SL)	Day1, 20 Day 1, 2, 4, 10, 20	Day 16 Day 16
Brugniaux et al. (2006)	12 Elite Runners	Artificial LHLT	18 days	Living: 14 h·day^{-1} (6 days at 2,500 m and 12 days at 3,000 m) Training: 1,200 m	tHbmass: EPO: $\dot{V}O_{2max}$:	Pre-camp Pre-camp Pre-camp	- Day 6 -	Day2, 15 Day 2, 15 Day 3, 15
Clark et al. (2009)	12 well-trained endurance cyclists	Artificial LHTL	4 weeks	Living: ~14 h·day^{-1} at 3,000 m Training: ~ 600 m	tHbmass: EPO: $\dot{V}O_{2max}$:	Pre-camp x 2 Pre-camp x 2 Pre- camp	Day 7, 14, 21 Day 1, 2, 6, 13, 20	Day 2, 6 Day 2, 6 Post
Robertson et al. (2010b)	16 highly trained middle- and long-distance runners	Artificial LHTL	2 x 3 weeks separated by 5 week wash out	Living: h·day^{-1}, ~300 h at 3,000 m Training: ~ 600 m	tHbmass: EPO: $\dot{V}O_{2max}$:	Pre-camp x 2 Pre-camp x 2 Pre-camp	Day 2, 6, 20 Day 2, 6, 20 -	Day 6 Day 6 Post
Garvican et al. (2012)	13 international competitive cyclists	Terrestrial LHTL+H	3 weeks	Living: 2,760 m Training: 1,000–3,000 m (2–6 h·day^{-1})	tHbmass: EPO:	Pre-camp x 2 Pre-camp x 2	Day 2, 6, 11, 20 Day 2, 6, 11, 20	Day 2, 10 Day 2, 10
Pottgiesser et al. (2012)	5 well trained	Artificial LHTL	28 days	Living: h·day^{-1} at 3,000 m Training: ~ 600 m	tHbmass: EPO:	Pre-camp Pre-camp	- -	Day 1, 9 Day 2, 5, 9

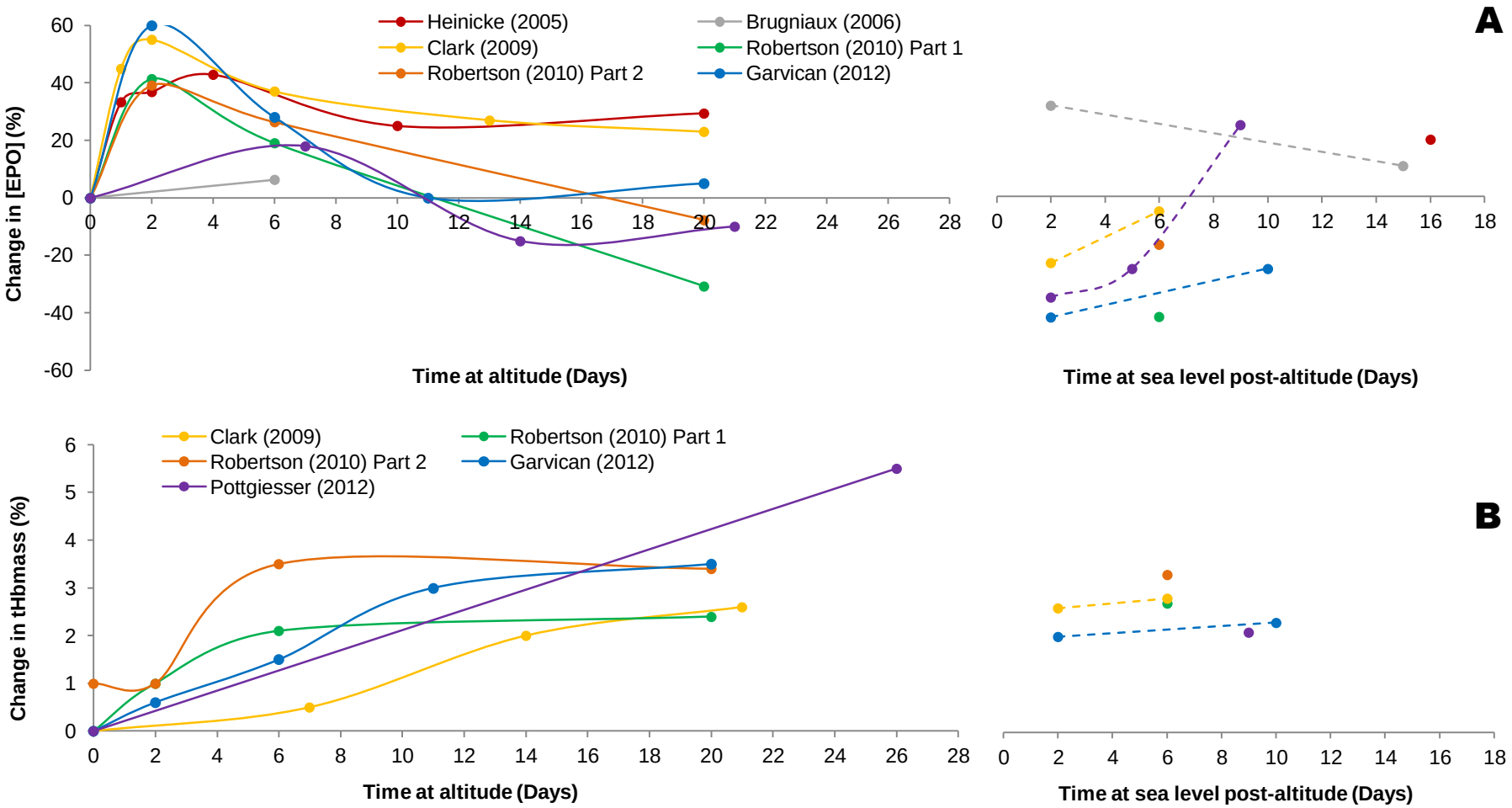


*Figure 2.13: The time course response for percentage change in EPO (**A**) and tHbmass (**B**) following altitude training methods as detailed in Table 2.13.*

2.8.2 Illness and inflammation

The additive stress of the extensive training loads employed by athletes in combination with a reduction in the inspiratory PO_2 at altitude may explain the less favourable modulation of immune function during acute and chronic exposure to hypobaric altitude (Bailey and Davies 1997). Data from elite British endurance runners found a marked increase in the frequency of upper respiratory and gastrointestinal tract infections during altitude sojourns lasting 4 weeks at 1,500 – 2,000 m (Bailey et al. 1998). These infections may have been as a result of depression of normal immunoreactivity rather than poor hygiene or the ingestion of foreign foods. Hypobaric hypoxia causes some adverse changes in immune function, possible due to the immunomodulatory roles of endogenous glucocorticoids and neuropeptides, which increase at altitude (Meehan 1987). Wachsmuth et al. (2013) also reported illness whilst altitude training, which compared to healthy athletes, resulted in a smaller increased in tHbmass.

Environmental stress of altitude and hypoxia exposure alone is sufficient to cause an elevation in circulating IL-6 even under resting conditions (independent of exercise stress) (Mazzeo 2005). It is possible that IL-6 levels were elevated during exercise with acute altitude exposure, due to the exaggerated epinephrine response associated with initial exposure to hypoxia (Mazzeo et al. 2001a). Aside from incidences of illness the increased production of pro-inflammatory cytokines, such as IL-6 and TNFα, have been shown to impair the production of EPO and therefore potentially reducing RBC production (Faquin et al. 1992). Impaired erythropoiesis is most likely due to apoptosis induction, cell growth inhibition and EPOr down regulation as a result of a local increased production of the cytokines, but also iron metabolism damage (Morceau et al. 2009). Staying healthy and illness free therefore appears to be crucial to a successful altitude training camp that has the aim of increasing tHbmass and oxygen carrying capacity.

2.8.3 Endurance training at altitude

At low altitudes, resting SaO_2 is generally well maintained and thus results in only a marginal disruption in homeostasis (Mazzeo 2008). During ascent to moderate altitude a slight, but significant, decrease in resting SaO_2 to 95 - 92% is observed, decreasing further to ~80% and lower at higher altitudes (Mazzeo 2008). Physiological responses to this challenge include increased heart rate, increased ventilation rate and haematological alterations, which are intended to avoid serious medical conditions such as AMS (Hochachka and Rupert 2003). Secondary to the disruption of homeostasis, elite athletes have to adjust (reduce) the intensity of the exercise bout at whilst training altitude. As observed at sea level, the relative stress/intensity of an exercise bout plays a major role in the subsequent physiological and metabolic adjustments needed by the body to maintain performance (Mazzeo 2008). The decline in $\dot{V}O_{2max}$ with increasing elevation (Wehrlin and Hallén 2006) requires a greater adjustment to exercise intensity when performing similar exercise tasks at altitude compared with sea level (Mazzeo 2008), otherwise the consequent greater relative exercise intensity and hence additional stress elicits a greater disruption to homeostasis (Mazzeo 2008).

Related to this and fundamental to the success of an altitude training camp, is the quality of the training itself completed at altitude. Saunders et al. (2009a) believed that effective altitude training requires a foundation of several years of training at a high level. Further, he does not recommend altitude training for developmental athletes – who usually lack fundamental experience, and who can readily gain a more than a 1% performance benefit from conventional training at sea level. The impaired $\dot{V}O_{2max}$ at progressively higher altitudes (Wehrlin and Hallén 2006) will reduce absolute training intensity in endurance exercise measured by velocity for a given distance (Saunders et al. 2009a). However, the relative training intensity is higher at any given speed due to the hypoxia-induced reduction in $\dot{V}O_{2max}$ and, therefore altitude training can be used as an added stimulus to train at higher intensities than possible at sea level (Friedmann-Bette 2008). Recommendations from Saunders et al. (2009a) when training athletes at altitude include:

- taking the first few days to a week to acclimatize to the altitude;
- that lower-intensity, higher-volume training is accompanied by shorter-interval work to maintain competition velocities;
- that as acclimatization occurs, the intensity of longer training intervals can be increased.

2.9 Aims of the Thesis

The haematological adaptations that occur as a result of altitude training camps or sleeping in a hypoxic tent have been well documented (Gore et al. 2013; Rasmussen et al. 2013), however improvements in endurance performance after altitude training are not always attributed to hypoxia-induced adaptations (Lundby et al. 2012; Jacobs 2013). To maximise the benefits of traditional LHTH altitude training, such as an increased tHbmass, $\dot{V}O_{2max}$ and subsequent endurance performance, a prior understanding of an individual athlete's physiological and haematological response to hypoxia is essential. This thesis will therefore initially consist of a series of investigations which outline the altitude training strategies utilised by British endurance athletes and ensure the measurement tools which assess the success of an altitude training camp are reliable and valid. Subsequent experiments will investigate the individual responses to acute hypoxia and the role of additional hypoxic exposures in optimising and predicting a broad spectrum of physiological and haematological adaptations to LHTH altitude training.

2.9.1 Research questions arising from the literature review

The thesis literature review has outlined some areas for investigation still outstanding in altitude and hypoxic training. It has been reported that altitude training can improve endurance performance in a variety of different endurance events (Saunders et al. 2009c; Fudge et al. 2011). Observational and experimental studies have identified an optimal 'hypoxic dose' that has resulted in enhanced physiological and haematological markers allied to endurance performance, yet the findings across a range of studies are inconsistent. Whilst the mechanisms associated with successful altitude and hypoxic training are becoming well understood, these do not always occur with every athlete that trains at altitude. Developments in the altitude training method and optimising the 'hypoxic dose' have been extensive; however individualising the 'hypoxic dose' and training at altitude based on the athlete's hypoxic tolerance are scarce. Altitude training adaptions are dependent on endogenous physiological strain – with an emphasis placed on the level of arterial oxygen desaturation and subsequent increase in EPO production. Identifying an athlete's hypoxic tolerance and individual response to hypoxia may augment potential adaptions, providing a more effective altitude training method, thus further investigation is warranted.

2.10 Proposed Research Studies and Hypotheses

The following research questions and associated hypotheses are proposed for this thesis.

2.10.1 Methods and perceptions of altitude training in elite British endurance athletes

- What are the current altitude and hypoxic training methods used by elite British endurance runners and the methods prescribed by coaches?
- What are the athlete's and coaches' perceptions of altitude training and the challenges associated with its use?

It is primarily hypothesised that altitude and hypoxic training methods undertaken by athletes and prescribed by coaches will be the same. Secondly, athletes and coaches' perceptions of altitude training will be the same. And finally, open-ended questions will provide insight into the challenges and opportunities of altitude training.

2.10.2 Differences in tHbmass measured with five Radiometer™ hemoximeters

- What is the effect of different Radiometer™ hemoximeters (two *old* OSM3 and three *new* ABL80 CO-OX Flex) on the measurement of tHbmass, using the oCOR-method?

It is primarily hypothesised that the *new* ABL80 hemoximeter would require fewer samples than its *old* OSM3 counterpart to yield an analyser error of ≤1%. Secondly, it is hypothesised that there will be no difference between tHbmass measured across the five different Radiometer™ hemoximeters.

2.10.3 Differences in tHbmass measured with different administration of CO bolus

- What is the effect of different boluses of CO (0.6 $mL \cdot kg^{-1}$, 1.0 $mL \cdot kg^{-1}$ and 1.4 $mL \cdot kg^{-1}$), administered during the rebreathing phase of the oCOR-method, on the measurement of tHbmass?

It is hypothesised that there will be no difference in tHbmass measured using different bolus of CO.

2.10.4 Differences in haematological response to acute hypoxic exposures

- What is the effect of different severities of normobaric hypoxia on the production of endogenous [EPO], IL-6 and TNFα?
- Do basal levels of IL-6 and TNFα inhibit the subsequent production of [EPO]?

It is primarily hypothesised that the magnitude of increased [EPO], following two hour normobaric hypoxic exposure, would occur in accordance with the severity of hypoxia. Secondly, it is hypothesised that basal levels of pro-inflammatory cytokines would inhibit the production of [EPO].

2.10.5 Differences in endurance performance after acute hypoxic exposures

- Is time trial performance in endurance runners affected by endogenous [EPO] production, in response to different fractions of inspired oxygen?

It is primarily hypothesised that there will be a difference in endogenous [EPO] production in response to normobaric hypoxia, hyperoxia and normoxia. Secondly, it is hypothesised that distance covered during the time trial would be increased after hypoxia.

2.10.6 Predicting an athlete's physiological and haematological response to altitude training

- What is the effect of a four week LHTH altitude training camp on the determinants of endurance performance and tHbmass?
- What is the time course of haematological markers post-LHTH?
- What are the predictive capabilities of a hypoxic sensitivity test on changes in tHbmass and $\dot{V}O_{2max}$ post-LHTH?

It is primarily hypothesised that LHTH will improve physiological capacity and tHbmass in elite endurance runners. Secondly, it is hypothesised that the time course of haematological markers post-altitude will effect concomitant changes in physiological capacity. Thirdly, it is hypothesised that there will be a relationship between Richalet HST indices and the haematological response of endurance runners after a LHTH altitude training camp.

CHAPTER 3

3 GENERAL METHODS

This chapter describes the materials and methods used frequently in each experimental chapter. Additional, specific or modified methods are described within the methods section of the relevant study chapters.

3.1 Health and Safety

The Internal Review Board of the English Institute of Sport (EIS) or the University of Brighton (UoB) Research Ethics and Governance Committee approved all experimental procedures before testing was carried out (See Appendix 11). Studies 2 (Chapters 5), 5 (Chapter 8) and 6 (Chapter 9) were carried out within the EIS Performance Physiology Laboratory, Loughborough. Study 3 (Chapters 6) and 4 (Chapter 7) were carried out at the UoB Welkin Laboratories, Eastbourne.

In studies 4 (Chapter 7), 5 (Chapter 8) and 6 (Chapter 9) a hypoxic chamber or hypoxic generator was used. On each occasion, at least two experimenters; one within the hypoxic chamber attending to the participant and one experimenter outside the chamber, ensured all individuals were safe. There was also a qualified first aider present within the laboratory building when the experiment took place.

Exercise was stopped if any of the following criteria were met:

- the participant asked to stop the test. Participants were not required to give any reason for this;
- the experimenter felt it appropriate to stop the test whether it be for equipment problems or the participant displaying signs of illness such as chest pain, dyspnoea, nausea, vomiting, generic pain/discomfort, faintness or dizziness;
- the participants pulse oxygen saturation fell below 70% for consecutive measurements;
- the Lake Louise Questionnaire (LLQ) for the diagnosis was Acute Mountain Sickness (AMS) was 3-5 for mild symptoms and 6 for severe.

3.1.1 Hazardous substances

In studies 2, 3, 5 and 6 (Chapter 5, 6, 8 and 9) the oCOR-method was used; therefore, a small amount of CO was present in the laboratory. A Control of Substances Hazardous to Health (COSHH) form was completed for each potentially hazardous gas used during a study, including CO, Albumin Bovine Serum, Phosphate Buffered Saline tablets, sulphuric acid and Tetramethylbenzidine used for

biochemistry analysis. Additional risk assessments were also completed for use in all laboratories, hypoxic facilities, exercise and invasive techniques such as cannulation and venepuncture.

3.1.2 Hygiene

All apparatus and surfaces were cleaned before and after use. Equipment associated with pulmonary gas analysis and the oCOR-method was soaked in Virkon disinfectant (1% Antec Int. Suffolk, UK) or Descogen®-1 (Antiseptica; Pulheim/Brauweiler, Germany) for at least 10 minutes, followed by a thorough rinse in cold water and drying prior to use. Electrical equipment contacting the body such as heart rate monitors and saturation devices were cleaned using alcohol wipes, after each use. Sharps, such as needles and cannulae from the measurement of blood were disposed of in a designated sharps bin.

3.2 Participants and Recruitment

Throughout the programme of study a variety of different participants were recruited to take part. The descriptive details of each of these cohorts are explained in the study chapters, however a brief summary is provided in Table 3.1. As a result of the varied participants recruited, any specific exclusion criteria are found is the study chapters and were dependent upon the constraints of the study.

Experimental trials were started after the participants provided written informed consent as approved by the UoB Research Ethics and Governance Committee in accordance with the declaration of Helsinki 1975 (revised 2013). For each individual study, participants were provided with a participant information sheet detailing the study design and the participant requirements. Participants were also invited to ask questions regarding the study, before they consented to undertaking the research. Participants were informed they could withdraw from a study at any time without providing justification or explanation.

Table 3.1: Participants recruited throughout the duration of the study programme

Study	Participants	Recruitment Approach
1 (Chapter 4)	Elite, highly training athletes, coaches and physiology practitioners	Through the EIS network and British Athletics athlete/coach database
2 (Chapter 5)	Healthy, non-smoking and physically active individuals	Through posters around university campus
3 (Chapter 6)	Healthy, non-smoking and physically active individuals	Through posters around university campus

4 (Chapter 7)	Trained, physically active participants	Emails sent to local sports and fitness clubs
5 (Chapter 8)	Well-trained runners	Emails sent to local running clubs
6 (Chapter 9)	Elite, highly trained middle and long-distance runners	Selected by British Athletics 'Head of Endurance'

For each experiment (excluding Chapter 4 and 9) participants were recruited via voluntary response to advertising posters and emails. All participants were explained verbally the protocol, dangers and discomforts possible, while also given a participant information pack to read. Participants could then decide if they still wanted to take part. Participation in each experiment was conditional upon the completion of a medical questionnaire with no contraindications. Participants were excluded:

- if they had been verified, or documented as having any blood carried infections (Hepatitis, HIV), were diabetic, or presented with a known history of haematological, cardiac, respiratory, or renal disease;
- if they a history of symptoms of nausea or light-headedness resulting from needles, probes or other medical-type equipment were not recruited; and
- if they were smokers

In studies 2-6 (Chapters 5-9) participants had not performed exhaustive exercise or consumed alcohol or caffeine in the 24 hours (Richardson et al. 2008) prior to starting the relevant trial. Throughout each studies' testing period, the participants were encouraged not to change physical activity patterns or dietary habits, but this was not controlled (Lundby et al. 2011). During all exercise trials participants were required to wear clothing that they would usually train or compete in and replicated this for all subsequent exercise trials. Similarly, participants were required to wear the same footwear for each exercise trial. In studies 4 and 5 (Chapters 7 and 8) a 1 week wash out period was allowed between acute hypoxic trials (Richardson et al. 2009a). In chapter 9 participants had not spent any time above 1,600 m in the preceding 8 weeks (MacKenzie et al. 2008). Participants performed acute hypoxic exposures between 09:00 and 11:00, to control for diurnal variations of serum [EPO] in trained and untrained participants (Klausen et al. 1993) and level of altitude (Klausen et al. 1996).

3.3 Anthropometry

3.3.1 Height

Participants were required to stand vertically in the anatomical position facing away from the stadiometer (SECA) scale, which was fixed to the wall. The stadiometer arm was lowered until it rested horizontally on the most superior aspect of the head and stature was recorded to the nearest 0.1 cm.

3.3.2 Body mass

Nude body mass was recorded using electronic scales (Adams Equipment, Model GFK 150; Milton Keynes, UK) at the UoB's Welkin Laboratories and different electronic scales (SECA, Model 876; Birmingham, UK) at the EIS Performance Centre, Loughborough. The Adams scales had a maximum capacity of 150 kg and readability of 0.01 g. The SECA scales had a maximum capacity of 250 kg, and a readability of 0.1 g. Both scales were calibrated weekly using a known weight.

3.3.3 Body composition

Assessment of body composition was measured using Harpenden callipers (Harpenden, Idass, England). Skinfold thickness was determined from the right side of each participant whilst stood in the anatomical position. All measurements were taken twice (see Table 3.2 for TEM) in accordance with International Society for the Advancement of Kinanthropometry (ISAK) and the experimenter held a level 1 ISAK qualification. The sum of eight site skinfolds: biceps, triceps, subscapular, supraspinale, iliac crest, abdominal, mid-thigh and calf was recorded. Body density was calculated in accordance with (Durnin and Womersley 1974) using the iliac crest, subscapular, bicep and triceps sites and the following equation.

Equation 3.1: Calculation of body density for adult males (Durnin and Womersley 1974)

$$\text{Body density} = 1.1610 - 0.0632 \text{ Log } \Sigma \text{ iliac crest, subscapular, bicep and triceps}$$

Following the determination of body density, % body fat was calculated according to the method described by (Siri 1956) below.

Equation 3.2: Calculation of percentage body fat for human populations (Siri 1956)

$$\text{Body Fat \%} = [(4.95/\text{density}) - 4.50] \times 100$$

Table 3.2: Intra-sample reliability data for skinfold thickness (mm) measured with skinfold callipers for the calculation of body fat percentage.

n = 87	**Skinfold-1**	**Skinfold-2**
Mean (mm)	7.1	7.0
Standard Deviation (mm)	3.8	3.8
Absolute TEM (mm)	0.36	
Relative TEM (%)	1.28	
Correlation	r = 0.998 *P* < 0.001	

3.4 Exercise Testing

3.4.1 Treadmill ergometry

Two treadmills were used during the experimental testing. A Woodway treadmill (PPS Med, Woodway USA Inc., Waukesha, WI, USA) was used during study 5 (Chapter 8) and an HP Cosmos Saturn treadmill (Traunstein, Germany) was used during study 6 (Chapter 9). Both treadmills were running at a known treadmill speed and were maintained according to the manufacturer's recommendations (see Table 3.3). Treadmill incline was set at 1% throughout submaximal assessments in order to reflect the energetic cost of outdoor running (Jones and Doust 1996). The treadmill incline was checked weekly during the testing period with an inclinometer at 5% and 10% using trigonometric procedures. If the measurement was not correct then the treadmill was re-calibrated.

Table 3.3: Treadmill service and maintenance history.

	Woodway	**HP Cosmos Saturn**
Service History	Every 6 months	Yearly
Cleaning	Monthly	Monthly
Maintenance	n/a	Belt alignment and oil check every 90 days

All checks were completed by the EIS laboratory technician

3.4.2 Submaximal running assessment

Treadmill start speed was decided based upon either previous step testing results, or criteria set by Jones (2007). The treadmill starting speed aimed to provide ~4 speeds prior to lactate turnpoint (LTP) (Shaw et al. 2015). Following a warm-up (~10 min at 10–12 km·h^{-1}), participants completed a discontinuous, submaximal incremental test consisting of six-to-nine stages of 3 minutes continuous running, with increments of 1 km·h^{-1} interspersed by 30 s rest periods for blood sampling. Capillary blood was sampled from the ear lobe and is detailed in section 3.5.3. The test was stopped when B[La] increased by greater than 1 mmol·L^{-1} or increased above 4 mmol·L^{-1}. The participant then placed their hands on the safety bars as the treadmill slowed. Heart rate was recorded in the final 30 s of each stage (see section 3.5.2) and oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and RER values were quantified for the final 30 s of each stage of the submaximal protocol (see section 3.5.1).

During Chapter 8 and 9 lactate threshold (LT) and lactate turnpoint (LTP) were determined using the submaximal running assessment. Lactate threshold was identified as the running speed at which B[La] begins to increase above the resting values (i.e. ~1.0 mmol·L^{-1}), typically a rise of approximately 0.4 mmol·L^{-1} (Thoden 1991). Lactate turnpoint was defined as the running speed above

which there was a second 'sudden and sustained' increase in B[La] of >1.0 mmol·L^{-1} from the previous stage, typically between 2–4 mmol·L^{-1} (Jones 2006).

3.4.3 Maximal running assessment

A continuous incremental, treadmill running ramp test to volitional exhaustion was used to determine $\dot{V}O_{2max}$. After a warm-up, participants initially ran at a speed 2 km·h^{-1} below the final speed of the submaximal test (see previous) and at a 1% gradient. Each minute, the incline was increased by a further 1% until volitional exhaustion. The test duration was typically 6–8 minutes (Jones 2006).

Criteria for determining whether $\dot{V}O_{2max}$ had been achieved was guided by the criteria set by the British Association of Sport and Exercise Sciences (Winter 2007), which was as follows:

- A plateau (less than 2 mL·kg^{-1}·min^{-1} or 150 mL·min^{-1}) in $\dot{V}O_{2max}$ despite an increase in exercise intensity to next stage
- Heart rate within 10 b·min^{-1}of age predicted maximum,
- Blood lactate above 8 mmol·L^{-1}
- A respiratory exchange ratio (RER) of 1.15 or above

If these criteria were not met then the participant would have been instructed to complete the test at a later date, although this did not occur during any of the experimental chapters.

3.5 Physiological Measurement

3.5.1 Pulmonary gas analysis

Breath-by-breath gas exchange data were measured with an automated open circuit metabolic cart (Oxycon Pro; Carefusion, San Diego, CA). This system has previously been shown to be a valid apparatus for the determination of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) at both low and maximal exercise intensities (Rietjens et al. 2001). Prior to each test, a two-point calibration of the gas sensors was completed (calibration range set by manufacturer), using a known gas mixture (FiO_2 = 0.16 and $FiCO_2$ = 0.05) (BOC Gases; Guildford, UK) and ambient air (FiO_2 = 0.2093 and $FiCO_2$ = 0.0005). Ventilatory volume was calibrated with simulated inspiration and expiration via a manual 3 L (±0.4%) syringe (Hans Rudolph, Germany) for 10 acceptable cycles eliciting a flow rate of 2-4 L·s^{-1}. Participants breathed through a low–dead space mask, with air sampled at 60 mL·kg^{-1}·min^{-1}.

3.5.2 Heart rate

Heart rate was measured by telemetry (Polar Electro Oyo, Temple, Finland) in all chapters involving exercise or hypoxia. The HR sensor detected R-R time interval of the electrocardiogram signal, and transmitted this to a single heart beat signal in real time, via telemetry. The signal was received by a

wristwatch, which was held within a ~3 m transmission range of the sensor. According to the manufacturer the accuracy of HR measurement was ± 1 b·min^{-1} with a measureable range of 15-240 b·min^{-1}.

3.5.3 Blood lactate

Blood lactate concentration (B[La]) was taken from the ear lobe during studies 5 and 6 (Chapters 9 and 10). A 20-µL capillary blood sample was required for analysis with a Biosen C-line (EKF Diagnostics, Germany). A single capillary blood sample was analysed immediately to determine lactate threshold (LT), LTP and peak B[La] during submaximal and maximal testing. The Biosen analyser was calibrated upon starting the analyser and every 60 min with a multi standard solution of 12.0 mmol·L^{-1} (EKF Diagnostics, Germany). Linearity tests (all error ranges were set by the manufacturer) were also undertaken every 90 days with control solutions of 2.0 (range = 1.8-2.2 mmol·L^{-1}), 7.0 (range = 6.75-7.25 mmol·L-1) and 18.0 (range = 17.5-18.5 mmol·L^{-1}) (EKF Diagnostics, Germany) and control solutions of 3.0 and 15.0 mmol·L^{-1} (EKF Diagnostics, Germany) are run every week. The Technical Error of Measurement (TEM) (see Section 3.13.1 for details) is outlined in Table 3.4, which shows the reliability of the measurement.

Table 3.4: Intra-sample reliability data for blood lactate concentration measured at rest and during exercise.

n = 20	**B[La]-1 (Rest)**	**B[La]-2 (Rest)**	**B[La]-1 (Exercise)**	**B[La]-2 (Exercise)**
Mean (mmol·L^{-1})	0.99	0.98	5.76	5.71
Standard Deviation (mmol·L^{-1})	0.17	0.14	1.84	1.81
Absolute TEM (mmol·L^{-1})	0.06		0.13	
Relative TEM (%)	1.56		0.55	
Correlation	r = 0.977 *P* < 0.001		r = 0.999 *P* < 0.001	

3.5.4 Arterial oxygen saturation (SaO_2)

Arterial oxygen saturation was estimated using fingertip pulse oximetry (SpO_2). A pulse oximeters determine oxygen saturation by measuring light absorption of arterial blood at two specific wavelengths, 660 nm (red) and 940 nm (infrared). The pulse oximeter used in each experiment is detailed in the relevant chapters. The pulse oximeter was placed on the right index finger while the participant was seated, and the SpO_2 remained stable for 1 minute before recording (Wagner et al. 2012). The following pulse oximetry limitations (Jubran 2004) were taken into consideration:

- falsely low SpO_2 readings have been reported with fluorescent and xenon arc surgical lamps;

- low cardiac output, vasoconstriction, or hypothermia can make it difficult for a sensor to distinguish the true signal from background noise.
- elevated carboxyhaemoglobin (HbCO) and methaemoglobin levels can cause inaccurate oximetry readings.
- nail polish, if blue, green, or black, causes inaccurate SpO_2 readings, however, acrylic nails do not interfere with readings; and
- inaccurate oximetry readings have been observed in pigmented participants.

If there were any problems with the measurement of SpO_2, e.g. no reading due to nail polish, then the participant was asked to remove the nail polish from their index finger during the experimental testing period.

3.6 Environmental Controls

3.6.1 Ambient testing conditions

During preliminary testing and experimental testing, participants performed exercise or rested in ambient conditions maintained at 18-22°C using industrial air conditioning. Temperature, humidity (although this was not controlled) and barometric pressure were measured and recorded in studies 2, 5 and 6 (Chapters 5, 8 and 9) with a Vaisala Transmitter (PTU301; Birmingham, UK) and in Studies 3 and 4 (Chapters 6 and 7) with a Weather Station (Oregon Scientific, Oregon, USA).

3.6.2 Altitude and hypoxia

Simulated altitude and natural altitude were utilised throughout the experimental chapters. Due to the multiple locations of the studies different equipment was used, which is detailed in the relevant study chapters. General methodological details are given here.

3.6.3 University of Brighton, Eastbourne

A large (3.2 x 4.0 x 2.8 m) custom-built normobaric hypoxic chamber (The Altitude Centre™, London, UK) was installed into the architecture of Welkin Laboratories (see Figure 3.1). The chamber used nitrogen-enriched gas facilitated by computer-controlled generators to produce and control the level of hypoxia. FiO_2 was constantly monitored throughout each trial with three sensors (Air check O_2 Oxygen Deficiency Monitor, PureAire Monitoring Systems Inc., Illinois, USA) affixed around the internal and external walls of the chamber within the laboratory. A computer programme (The Altitude Centre™, London, UK) controlled the level of FiO_2. Recording of the FiO_2 was performed every 5 minutes to describe accurately the environment experienced by participants. A large internal air conditioning unit set to 20°C and facilitated thermal control of the hypoxic chamber.

Figure 3.1: Hypoxic chamber used during study 4 at the UoB Welkin Laboratory

3.6.4 EIS Performance Laboratory, Loughborough

Portable simulated altitude generators (McKinley Altitude Simulator, Higher Peak Performance) were used in studies 5 and 6 (Chapter 8 and 9) to create a hypoxic environment (see Figure 3.2). The altitude generators used an air compressor to trap N molecules and allow oxygen molecules to pass through into a waste exhaust. The air compressor fed into two cylinders one of which was pressurised and to release N enriched air from an outlet pipe, this formed the hypoxic inspirate. The process was repeated within the generator to allow a continuous flow of hypoxic air. A computer programme enabled a user defined FiO_2 to be maintained within a range of ~0.175 to 0.115. A steady flow of air (30-90 litres per min) was created and connected to either breathing mask system or Douglas bag.

Figure 3.2: Hypoxic generator (left) and mask set up (right) used during study 5 at the EIS Laboratory

The accuracy of the FiO_2 production was checked before use using a high accuracy paramagnetic oxygen analyser (Servomex 5200, Servomex, Crowborough, UK) and recorded (see Table 3.5).

Table 3.5: Calibration record for McKinley Altitude Simulator used in experimental studies 5 and 6.

Control Panel Setting	Air Flow (%)	FiO_2 (%)	Simulated Altitude (m)
4.0	90	17.5	1,600
	70	16.7	2,000
	50	14.0	3,477
5.0	90	16.8	1,949
	70	15.8	2,469
	50	12.8	4,210
6.0	90	16.2	2,258
	70	14.8	3,017
	50	11.9	4,796
7.0	90	14.5	3,187
	70	13.9	3,536
	50	11.5	5,067

Note: Equivalent altitude conversion from McKinley Altitude Simulator® User Manual

3.6.5 Haemoglobin concentration [Hb]

Fingertip capillary blood was collected using Hemocue slides (Hb 201 Microcuvettes, Hemocue; Angelhom, Sweden) and then placed into a portable Hemocue haemoglobin device (HemoCue® Hb 201 System Photometer; Angelhom, Sweden). This device quantified haemoglobin to 1 $g \cdot dL^{-1}$. Blood was collected in triplicate to obtain an average sample value. The HemoCue Hb 201 Analyser has an internal electronic 'self-test', therefore every time the device is turned on, it will automatically verify the measurement performance. The device was also checked on a daily basis by measuring the control cuvette and a standard of known concentration (Hb 201 Calibration Slide, Hemocue; Angelhom, Sweden) of a known value (13.1 $g \cdot L^{-1}$). On each occasion the HemoCue was used it passed the quality control checks. Two devices were used to calculate [Hb], the Hemocue and Pentra ES 60 (Horiba Medical; Kyoto, Japan), which are detailed in Section 3.11.4.1. For the TEM of [Hb] using both devices refer to Table 3.6.

Table 3.6: Intra-sample reliability data for haemoglobin concentration.

	HemoCue		Pentra	
n = 20	**[Hb]-1**	**[Hb]-2**	**[Hb]-1**	**[Hb]-2**
Mean ($g \cdot L^{-1}$)	15.2	15.3	14.4	14.4
Standard Deviation ($g \cdot L^{-1}$)	0.8	0.9	0.7	0.8
Absolute TEM ($g \cdot L^{-1}$)	0.39		0.11	
Relative TEM (%)	0.65		0.19	
Correlation	r = 0.891 *P* < 0.001		r = 0.991 *P* < 0.001	

3.6.6 Haematocrit (Hct)

Blood for the measurement of Hct was collected in heparinised capillary tubes (Hawksley & Sons Ltd.; Lancing, UK) from either finger prick or ear lobe blood sampling. Blood was collected in triplicate to allow for breakages of the delicate capillary tubes and to obtain an average sample value. Capillary tubes were spun in a centrifuge (Hematospin 1300, Hawksley & Sons Ltd.; Lancing, UK) at 2000 rpm for 5 minutes. Hct was then measured using a Hct capillary tube slide measure (Hawksley Reader 01502, Hawksley & Sons Ltd.; Lancing, UK) by measuring the RBCs from the separated blood plasma. Two methods were used to calculate Hct, the Hawksley reader and Pentra ES 60 (Horiba Medical; Kyoto, Japan), which are detailed in Section 3.11.4.1. For the TEM of Hct using both devices, refer to Table 3.7.

Table 3.7: Intra-individual reliability data for Hct (%)

	Hawksley Reader		Pentra	
n = 20	**Hct-1**	**Hct -2**	**Hct -1**	**Hct -2**
Mean (%)	46.5	45.3	43.5	43.4
Standard Deviation (%)	1.8	1.9	2.5	2.5
Absolute TEM (%)	0.57		0.43	
Relative TEM (%)	0.31		0.25	
Correlation	r= 0.959 *P* < 0.001		r = 0.985 *P* < 0.001	

3.7 Optimised Carbon Monoxide Re-breathing Method

Participants completed the oCOR-method (Schmidt and Prommer 2005; Prommer and Schmidt 2007) in studies 2, 3, 5 and 6 (Chapters, 5, 6, 8 and 9). Participants were required to maintain their normal training regimen (Eastwood et al. 2008). The repeat tests were completed a maximum of one week apart; according to Eastwood *et al* (2012a) tHbmass remains stable following 40 days of moderate physical activity.

The method required participants to remain seated for 20 min to allow plasma volume (PV) to stabilise (Harrison 1985). Participants then completely exhaled to residual volume into a CO gas meter (Pac 7000, Dräger; Pittsburg, PA, USA). Baseline %HbCO was measured from fingertip or earlobe capillary blood samples (350 µl) using a lancet (Accu-chek, Safe-T-Pro Plus, Roche Diagnostics GnbH; Mannheim, Germany). Samples were collected in triplicate into glass heparinised capillary tubes (Hawksley; Lancing, West Sussex, UK) and were analysed immediately using a hemoximeter (ABL80 and OSM3 Radiometer™, Copenhagen, Denmark). Calibration and measurement information can be found in Table 3.8.

Table 3.8: Equipment specifications and measurement guidelines

Equipment	Measurement Range	Resolution	Calibration
Dräger CO gas meter	0 - 1999 ppm	1 ppm	The unit is equipped with a fresh air calibration function, which was completed on testing days
ABL80 CO-oximeter	0 – 100 %	0.1%	- An automatic two point calibration was completed every 8 hours - A tHb calibration was complete manually every 90 days

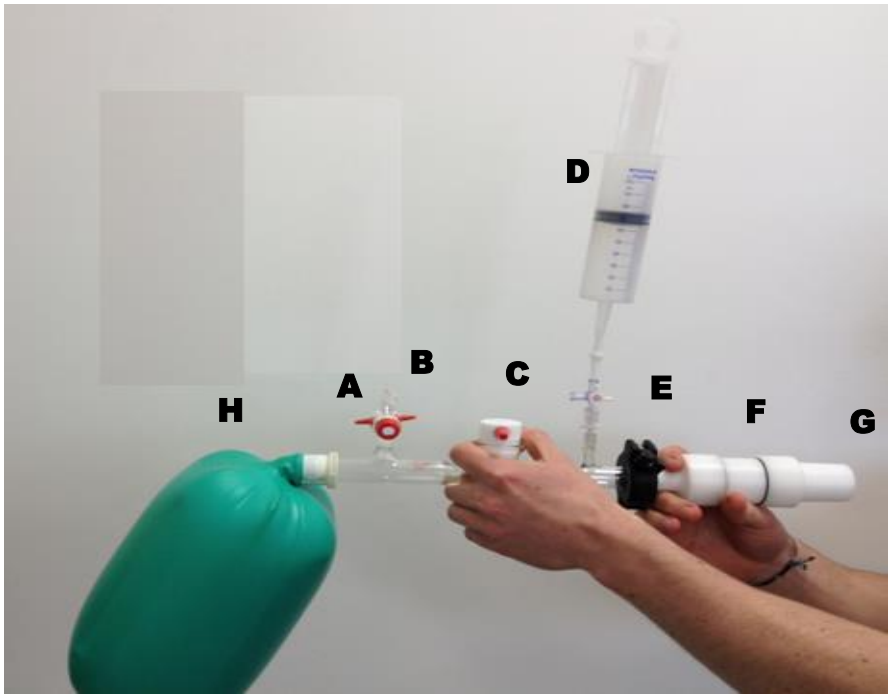


Figure 3.3: Spirometer developed by Schmidt & Prommer (2005).

A = Connection for oxygen tube, **B** = oxygen valve (closed during test), **C** = valve of the oxygen reservoirs (open during test), **D** = CO syringe, **E** = adapter to connect mouthpiece, **F** = compartment for soda lime, **G** = mouthpiece, **H** = anaesthetic bag filled with oxygen.

A custom-made glass spirometer (Blood Tec spirometer, SpiCo; Bayreuth, Germany) was used for the 2 min of rebreathing (see Figure 3.3). The spirometer was designed to permit the inhalation of the whole bolus of CO with the participant's first breath. Participants exhaled to their residual volume and then whilst connected to the mouthpiece (**G**, Figure 3.3), performed a maximal inhalation of the CO bolus (volume was study dependant: 0.6-1.4 $mL \cdot kg^{-1}$), which was administered via a pre-filled syringe (Kendall Monoject™ Syringe, Tyco Healthcare Group; Mansfield MA) (**D**). At the same time, the valve (**C**) between the participant and the oxygen reservoir (**H**) was opened. The oCOR-method ensured the entire CO bolus was inhaled in the first part of the breath and distributed within the alveoli. Given the high affinity of CO for haemoglobin (Douglas et al. 1912), it was expected that a large part of CO diffused into the blood during the first seconds (Schmidt and Prommer 2005). To further support the diffusion process the subject held their breath for 10 s after the first inspiration, after which they continued to breathe at a comfortable rate from the spirometer for a further 1 min 50 s. Soda lime (10 g) was added (**F**) to absorb carbon dioxide (CO_2). The participant was then disconnected after exhaling to residual volume to attempt to fill the anaesthetic bag (**H**), which was closed thereafter by valve **C**. The full exhalation was necessary to quantify the volume of CO, which was not taken up by the body. A post–measure of CO in the body was taken at 4 min and post measures of %HbCO were taken at 6 and 8 min. The volume of the anaesthetic bag (**H**) and CO concentration in the bag was also recorded.

3.7.1.1 Reliability of the oCOR-method

To determine the reliability of the oCOR-method for the measurement of tHbmass, the method was completed twice, separated by a minimum of 7 days. The participants included in the reliability analysis were from Chapters 5 and 9 and undertook the two tests at similar times of the day. Participants were given the same instructions on each occasion on how to complete the oCOR-method. The absolute and relative technical error of measurement (TEM) was calculated from the two repeated measures (see Table 3.9).

Table 3.9: Intra-sample reliability data for tHbmass, BV and PV.

n = 23	**tHbmass-1 (g)**	**tHbmass-2 (g)**	**BV-1 (mL)**	**BV-2 (mL)**	**PV-1 (mL)**	**PV-2 (mL)**
Mean	793	796	5611	5609	3309	3356
Standard Deviation	145	140	988	949	568	554
Absolute TEM	39.3		329.0		303.6	
Relative TEM (%)	1.24		1.47		2.28	
Correlation	r = 0.981 *P* < 0.001		r = 0.971 *P* < 0.001		r = 0.927 *P* < 0.001	

3.7.1.2 Syringe calibration

The 140 mL syringe used during the investigation was calibrated to determine if the measurement was reading what it specified. Table 3.10 outlines the specified gas volume and the mass of the syringe measured during the calibration. A difference was found between what was specified and what was measured. A correlation was plotted (see Figure 3.4) between the gas volume and water mass and a line of best fit produced an equation of $y = 0.8814x^{1.0222}$, where, y = corrected syringe volume and x = specified syringe volume. The correct volume equation was used for the calculation of tHbmass.

Table 3.10: Syringe calibration details

Specified Gas Volume (mL)	Measured Mass (g)	Difference
10.0	9.2	0.8
20.0	18.9	1.1
30.0	28.6	1.4
40.0	38.4	1.6
50.0	48.2	1.8
60.0	57.7	2.3
70.0	67.9	2.1
80.0	77.6	2.4
90.0	87.8	2.2
100.0	97.4	2.6
110.0	107.4	2.6
120.0	117.8	2.2

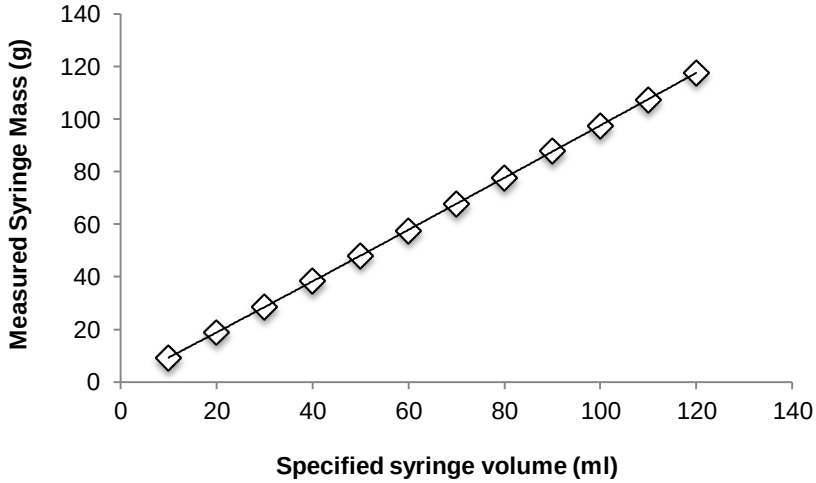


Figure 3.4: Syringe calibration correlation ($y = 0.8814x^{1.0222}$; $R^2 = 0.999$)

3.7.1.3 The reliability and stability of total haemoglobin mass measured using the oCOR-method

The following abstract describes pilot testing of the effect of hydration status on tHbmass:

Purpose: The oCOR-method is routinely used to measure tHbmass. The tHbmass measure is subject to a test-retest typical error of ~2%, error is attributed to the precision of the %HbCO measurement. We hypothesized that tHbmass would be robust to changes in the hydration status during the oCOR-method. **Methods:** Seven participants (6 males and 1 females; age 26 ± 4 yr, height 178 ± 7 cm, weight 75.8 ± 12.7 kg and body fat 11 ± 3 %) completed the oCOR-method on four occasions. Participants completed the oCOR-method, with 1.0 mL/kg of CO administered, under different states of hydration (HYPO: hypohydrated to a 2% decrease in body mass; EU1: euhydrated and HYPER: hyperhydrated with sodium loading); to determine the reliability of EU1, a second trial was conducted (EU2). **Results:** There were no significant differences in tHbmass when performed in HYPO (757 ± 135 g), EU1 (769 ± 138 g) or HYPO (768 ± 149 g) trials. EU2 was 771 ± 145 g. **Conclusions:** The measurement of tHbmass is reliable to within 0.8%. Total Hbmass also remained stable under different states of hydration, which is important as athletes are often tested when hypohydration or hyperhydration. The present study has reaffirmed the stability of tHbmass measured with the oCOR-method.

For further information on the pilot testing please see Appendix 1.

3.8 Hydration Measurement

3.8.1 Urine Specific Gravity

Urine specific gravity (USG) was assessed using a refractometer (Specific Gravity Refractometer Model 32, Atago; USA). A small volume (~2 mL) of urine was placed into the glass window and the window flap was closed. The refractometer was then held up to the light, while the experimenter looked through the eyepiece and recorded the values from the scale within. The refractometer was quality assured daily through the administration of a known value using distilled water (Uspg = 1.000). For the TEM of USG using both devices refer to Table 3.11. Euhydration was accepted as USG < 1.020 (Sawka et al. 2007).

Table 3.11: Intra-sample reliability data for urine specific gravity

n = 20	**USG-1**	**USG-2**
Mean	1.011	1.011
Standard Deviation	0.005	0.005
Absolute TEM	0.001	
Relative TEM (%)	0.02	
Correlation	r = 0.991	
	P < 0.001	

3.8.2 Urine osmolality

Urine osmolality (UOSM) was measured using a handheld osmometer (Osmocheck™ Pocket, Vitech Scientific Ltd.; UK.). A small volume of urine (~2 mL) was placed on the aperture and the start button was pressed. The result was displayed to within ±10 mOsmols·kg^{-1}·H_2O^{-1} immediately. The measuring surface was then safely cleaned with water. The osmometer was quality assured daily through the administration of a known value using distilled water (Uosm = 0). For the TEM of UOSM using both devices refer to Table 3.12. Euhydration was accepted as UOSM = < 700 mOsm·kg^{-1}·H_2O^{-1} (Sawka et al. 2007).

Table 3.12: Intra-sample reliability data for urine osmolality

n = 20	**UOSM-1**	**UOSM-2**
Mean (mOsm·kg^{-1}·H_2O^{-1})	515	517
Standard Deviation (mOsm·kg^{-1}·H_2O^{-1})	236	234
Absolute TEM (mOsm·kg^{-1}·H_2O^{-1})	11.0	
Relative TEM (%)	0.53	
Correlation	r = 1.000 *P* < 0.001	

3.10 Subjective Scales

3.10.1 Ratings of Perceived Exertion

The Ratings of Perceived Exertion (RPE) scale was used to quantify perceived exertion during exercise using a Borg scale (Borg et al. 1985) (6, very very light – 20, maximal, see Figure 3.5). The RPE was based on a clear understanding of "anchoring" the top and bottom ratings to previously experienced sensations of no exertion at all (RPE = 6) and extremely hard or maximal exertion (RPE = 20) (Mauger et al. 2013). The RPE-scale is constructed so that the rating increases linearly with increasing speed and HR during an exercise test on the cycle ergometer (or running treadmill) (Borg et al. 1985). During Study 5 (Chapter 8) RPE was recorded as part of the time trial assessment as has been previously recorded (Pedlar et al. 2008; Crowcroft et al. 2015).

rating	description
6	NO EXERTION AT ALL
7	
8	EXTREMELY LIGHT
9	VERY LIGHT
10	
11	LIGHT
12	
13	SOMEWHAT HARD
14	
15	HARD (HEAVY)
16	
17	VERY HARD
18	
19	EXTREMELY HARD
20	MAXIMAL EXERTION

Figure 3.5: Rating of Perceived Exertion (RPE)

3.10.2 Lake Louise Questionnaire

The Lake Louise Questionnaire (LLQ) (Roach et al. 1993a) was used with the sleep questions extracted (see Figure 3.6), as this is irrelevant during acute exposures (Bartsch et al. 2004). The LLQ quantified symptoms of acute mountain sickness (AMS) using the sum of four questions scored 0-3, including; headache, gastrointestinal upset, fatigue or weakness, dizziness or light-headedness. A total score of 3-5 indicates mild AMS and a score of >6 was thought to represent severe AMS, and this reference value was used in the remainder of this thesis.

Lake Louise Score (LLS) for the diagnosis of Acute Mountain Sickness (AMS)

A diagnosis of AMS is based on:
1. A rise in altitude within the last 4 days
2. Presence of a headache

PLUS
3. Presence of at least one other symptom
4. A total score of 3 or more from the questions below

Total score of:
3 to 5 = mild AMS
6 or more = severe AMS

Note:
Do not ascend with symptoms of AMS
Descend if symptoms are not improving or getting worse
Descend if symptoms of HACE or HAPE develop

SELF-REPORT QUESTIONNAIRE
Add together the individual scores for each symptom to get the total score.

Name: _____ Age: _____ Sex: _____ Date: _____ Trial: _____

			Time	0 min	30 min	1 h	1 h 30	2 h	3 h	4 h
Headache:	No headache	0								
	Mild headache	1								
	Moderate headache	2								
	Severe headache, incapacitating	3								
Gastrointestinal symptoms:	None	0								
	Poor appetite or nausea	1								
	Moderate nausea &/or vomiting	2								
	Severe nausea &/or vomiting	3								
Fatigue and/or weakness:	Not tired or weak	0								
	Mild fatigue/ weakness	1								
	Moderate fatigue/ weakness	2								
	Severe fatigue/ weakness	3								
Dizziness/lightheadedness:	Not dizzy	0								
	Mild dizziness	1								
	Moderate dizziness	2								
	Severe dizziness, incapacitating	3								
Difficulty sleeping: (removed from analysis)	Slept as well as usual	0								
	Did not sleep as well as usuall	1								
	Woke many times, poor sleep	2								
	Could not sleep at all	3								
	Symptom Score									

Figure 3.6: Lake Louise Questionnaire (LLQ) for Acute Mountain Sickness (AMS)

3.11 Haematology, Phlebotomy and Biochemistry

3.11.1 Capillary blood sampling

Capillary blood samples were collected from the participant's non-dominant hand at rest or ear lobe during exercise. The finger/ear was cleaned using alcohol wipes and pricked using a sterile single-use lancing device (Accu-chek® Safe-T-Pro Plus, Roche; Mannheim, Germany). The first appearance of blood was wiped away for the sample to then be collected. Reliability sampling and analysis was carried out prior to experimental studies. Capillary blood was collected for lactate, [Hb], Hct and HbCO analysis.

3.11.2 Venepuncture blood sampling

All venepuncture samples were taken from the ante-cubital fossa using a sterile needle (21G x 1.25 BD Eclipse Safety Needle, BD; New Jersey, USA) and vacutainer (2 mL BD Vacutainer® Plus Plastic K_2 EDTA Tubes, BD; New Jersey, USA). Prior to venepuncture the participant was placed in a sitting or lying position, an appropriate arm was chosen and the area cleaned using alcohol wipes. A tourniquet was used to select a vein and allow insertion of the needle. The necessary volume of blood (typically 10 mL) was collected. For the measurement of HbCO blood samples were analysed immediately.

3.11.3 Cannulation

Participants were placed into a lying position and an appropriate arm was chosen and cleaned with alcohol wipes. A tourniquet was used to select a vein and a cannula (18G BD Venflon I.V. Cannula with Port, BD; New Jersey, USA) was then inserted into the ante-cubital fossa. A 3-way stopcock (BD Connecta 3 Way Stopcock, BD; New Jersey, USA) was connected to the cannula. A solution administration kit (Pump/gravity solution administration set, Baxter Health Care Ltd; Norfolk, UK) was attached to the stopcock and a 500 mL bag of saline solution (0.9% Sodium Chloride I.V. Infusion, Baxter Health Care Ltd.; Norfolk, UK). A drip rate of one drip every second was set up to ensure the line was flushed.

A 10 mL syringe (BD Plastipak Sterile Syringes; New Jersey, USA) was used to draw blood via the cannula. Blood was immediately transfused into 5 mL EDTA (32.332, Sarsedt, Akteingesellscaft & Co; Numbrecht, Germany) so the blood could be spun in a centrifuge (5702R Centrifuge, Eppendorf®; Hamburg, Germany) for 15 min at 2100 rpm at a temperature of 5°C. The plasma was then immediately separated from the blood and placed in 2 mL microtubes (Eppendorf®; Hamburg, Germany). Two aliquots per sample were stored at -84°C (VIP Series, Sanyo Biomedical; Loughborough. UK) for no longer than 3 months before biochemical analysis.

3.11.4 Haematology analysers

A further two biochemistry methods were used throughout the experimentation.

3.11.4.1 Pentra ES 60

The Pentra ES 60 was used to quantify [Hb] and Hct during all experimentation at the EIS Performance Laboratory. The analyser was calibrated quarterly using the ABX Minocal calibrator. If technical interventions (installation, maintenance, service intervention) were administered then the further calibration took place. Three control levels were also tested (Low, Normal, High) monthly for linearity assessment. The reliability of the Pentra ES 60 was evaluated automatically through the consecutive analyses of the same blood sample. A coefficient of variation (in %) was set from the results obtained and if results fell outside of these limits, then the sample was re-analysed.

3.11.4.2 Standard ELISA analysis

Enzyme-linked immunosorbent assay (ELISA) plates are a commercially available tool used to measure the concentration of an analyte in a solution. Table 3.13 outlines the different plates used for each experimental chapter and the manufacturer intra/inter-assay variability. Further details are provided in the specific sections for the analytes measured.

Table 3.13: ELISA details, sensitivity and coefficient of variation (CV) for each manufacturer used

Measure	Study	Details	Sensitivity	CV%
Plasma [EPO]	4 (Chapter 7)	Roche Diagnostics – Roche Applied Science Human Erythropoietin ELISA	Range = 3 – 200 mIU·mL^{-1}	Intra-assay = 7.1% (low), 2.7% (medium), 3.9% (high). Inter-assay = 8.3% (low), 1.9% (medium), 2.8% (high)
Plasma IL-6	4 (Chapter 7)	R&D Systems UK Human IL-6 DuoSet ELISA	Range = 9.38 – 600 pg·mL^{-1}	n/a *
Plasma TNFα	4 (Chapter 7)	R&D Systems UK Human TNF-α DuoSet ELISA	Range = 15.60 – 1,000 pg·mL^{-1}	n/a *
Plasma [EPO]	5 (Chapter 8)	BioVendor Human Erythropoietin ELISA	Mean = 8.5 mIU·m^{-1} Range = 1.6 – 57.2 mIU·m^{-1}	Intra-assay = 3.4% Inter-assay = 6.2%
Plasma [EPO]	6 (Chapter 9)	BioVendor Human Erythropoietin ELISA	Mean = 8.5 mIU·m^{-1} Range = 1.6 – 57.2 mIU·m^{-1}	Intra-assay = 3.4% Inter-assay = 6.2%

* The DuoSet ELISA does not have any manufacturer CV% data available as DuoSet ELISA Development kit contains the basic components required for the development of sandwich ELISAs, which the experimenter builds.

3.11.4.3 Erythropoietin Analysis

Erythropoietin concentrations were measured in plasma, drawn from whole blood samples and concentrations were determined using an ELISA kit (see Table 3.13). EPO quantitative determination was performed according to manufacturer's guidelines. Incubation of the 96 well kit, including the required quality control standards was performed on an orbital shaker (Heidolph Titramax 1000) at 450rpm, and read via a plate reader with optical density set at 450nm (El_x800 Universal Microplate reader, Bio-Tek Instruments Inc., Vermont, USA). The Microplate reader was calibrated and maintained according to the manufacturers' recommendations.

Plotting a graph for linearity between known sample concentrations and optical density confirmed the accuracy of the sample data. A linear trend line and equation was used to translate raw plate reader results into EPO units ($mlU \cdot mL^{-1}$). For the TEM of [EPO] measured in each experimental chapter refer to Table 3.14, Table 3.15 and Table 3.16.

3.11.4.4 Interleukin 6 and TNFα Analysis

Interleukin 6 (IL-6) and Tumor-necrosis factor alpha (TNFα) concentrations were measured in plasma, drawn from whole blood samples and concentrations were determined using a sandwich ELISA kit (see Table 3.13). IL-6 and TNFα quantitative determination was performed according to manufacturer's guidelines of a DuoSet ELISA Development Kit. The kit is made up of its component parts (Albumin Bovine Serum, Phosphate Buffered Saline tablets, sulphuric acid and Tetramethylbenzidine) and the DuoSet kit, each of which had a COSHH form completed. The 96-well microplate was prepared with the capture antibody overnight then the assay procedures were followed. Incubation of the 96-well kit, including the required quality control standards was performed on an orbital shaker (Heidolph Titramax 1000) at 450rpm, and read via a plate reader with optical density set at 450nm (El_x800 Universal Microplate reader, Bio-Tek Instruments Inc., Vermont, USA). To view the TEM of IL-6 and TNFα measured in chapter 7 refer to Table 3.17and Table 3.18.

Table 3.14: Intra-sample reliability data for [EPO] plate for study 5 (Chapter 8)

	EPO Plate 1		**EPO Plate 2**		**EPO All Plates**	
	n = 36		**n = 36**		**n = 72**	
Mean ($mlU \cdot mL^{-1}$)	4.61	4.99	6.42	6.47	**5.51**	**5.73**
Standard Deviation ($mlU \cdot mL^{-1}$)	1.59	1.85	0.97	1.03	**1.59**	**1.66**
Absolute TEM ($mlU \cdot mL^{-1}$)	1.00		0.75		**0.89**	
Relative TEM (%)	5.23		2.93		**3.95**	
Correlation	r = 0.948		r = 0.858		**r = 0.934**	
	P < 0.001		*P* < 0.001		***P* < 0.001**	

Table 3.15: Intra-sample reliability data for [EPO] plate for study 6 (Chapter 9)

	EPO Plate 1		**EPO Plate 2**		**EPO All Plates**	
	n = 40		**n = 39**		**n = 79**	
Mean (mlU·mL^{-1})	2.50	2.49	3.15	3.30	**2.82**	**2.89**
Standard Deviation (mlU·mL^{-1})	0.89	0.97	1.92	2.12	**1.52**	**1.69**
Absolute TEM (mlU·mL^{-1})	0.77		0.98		**0.88**	
Relative TEM (%)	7.69		7.62		**7.71**	
Correlation	r = 0.829		r = 0.947		**r = 0.930**	
	P < 0.001		*P* < 0.001		***P* < 0.001**	

Table 3.16: Intra-sample reliability data for each ELISA [EPO] plate for study 4 (Chapter 7)

	EPO Plate 1		**EPO Plate 2**		**EPO Plate 3**		**EPO Plate 4**		**EPO Plate 5**		**EPO All Plates**	
	n = 40		**n = 39**		**n = 40**		**n = 40**		**n = 39**		**n = 198**	
Mean (mlU·mL^{-1})	4.81	4.79	4.63	4.86	5.36	5.78	3.46	3.27	5.24	4.97	**4.70**	**4.74**
Standard Deviation (mlU·mL^{-1})	2.18	2.01	1.39	1.44	1.97	1.85	1.21	1.26	0.93	0.90	**1.72**	**1.74**
Absolute TEM (mlU·mL^{-1})	1.59		1.18		1.40		1.73		1.12		**1.42**	
Relative TEM (%)	8.28		6.19		6.30		12.87		5.49		**7.52**	
Correlation	r = 0.855		r = 0.837		r = 0.888		r = 0.507		r = 0.660		**r = 0.831**	
	P < 0.001		*P* < 0.001		*P* < 0.001		*P* < 0.001		*P* < 0.001		***P* < 0.001**	

Table 3.17: Intra-sample reliability data for each ELISA IL-6 plate for study 4 (Chapter 7)

	IL-6 Plate 1 n = 40		**IL-6 Plate 2 n = 34**		**IL-6 Plate 3 n = 39**		**IL-6 Plate 4 n = 35**		**IL-6 All Plates n = 151**	
Mean (pg·mL^{-1})	5.62	5.06	12.68	10.53	3.38	3.05	17.18	15.02	**9.46**	**8.32**
Standard Deviation (pg·mL^{-1})	3.76	3.34	7.59	3.91	2.16	2.08	10.00	8.68	**8.43**	**7.08**
Absolute TEM (pg·mL^{-1})	1.86		8.60		1.50		5.86		**5.55**	
Relative TEM (%)	8.72		18.53		11.66		9.10		**15.60**	
Correlation	r = 0.949		r = 0.666		r = 0.885		r = 0.936		**r = 0.897**	
	P < 0.001		*P* < 0.001		*P* < 0.001		*P* < 0.001		***P* < 0.001**	

Table 3.18: Intra-sample reliability data for each ELISA TNFα plate for study 4 (Chapter 7)

	TNFα Plate 1 n = 40		**TNFα Plate 2 n = 39**		**TNFα Plate 3 n = 40**		**TNFα Plate 5 n = 39**		**TNFα All Plates n = 154**	
Mean (pg·mL^{-1})	2914	2709	6251	6419	233	270	3329	3377	**3277**	**3288**
Standard Deviation (pg·mL^{-1})	1719	1449	5884	6043	176	168	3284	3338	**4086**	**4161**
Absolute TEM (pg·mL^{-1})	1099		1466		281		872		**1043**	
Relative TEM (%)	9.77		5.78		27.89		6.50		**7.94**	
Correlation	r = 0.899		r = 0.985		r = 0.333		r = 0.982		**r = 0.899**	
	P < 0.001		*P* < 0.001		*P* < 0.051		*P* < 0.001		***P* < 0.001**	

3.12 Calculations

3.12.1 Total haemoglobin mass

Equation 3.3:Calculation for tHbmass (Schmidt and Prommer 2005)

$tHbmass\ (g) = K \times MCO \times 100 \times (\Delta\%HbCO \times 1.39)^{-1}$, where:

- K = current barometric pressure $\times 760^{-1} \times [1 + (0.003661 \times \text{current temperature})]$
- $MCO = CO_{adm} - (CO_{system + lung\ (after\ disconnection)} + CO_{exhaled\ (after\ disconnection)})$
- CO_{adm} (mL) = CO volume administered into the system
- $CO_{system + lung\ (after\ disconnection)}$ = CO concentration in spirometer × (spirometer volume + lung residual volume)
- $CO_{exhaled\ (after\ disconnection)}$ = end-tidal CO concentration × alveolar ventilation × time
- $\Delta\%HbCO$ (%) = difference between basal HbCO and HbCO in the blood samples after CO administration
- 1.39 (mL $O_2 \cdot g^{-1}$ of Hb) = Hufner's number (ml CO × g Hb^{-1})

3.12.2 Circulating blood

Equation 3.4: Calculation for blood volume (BV) (Wachsmuth et al. 2014)

$BV\ (mL) = tHbmass \times 100 \times [Hb]^{-1} \times 0.91^{-1}$

Equation 3.5: Calculation for red cell volume (RCV) (Wachsmuth et al. 2014)

$RCV\ (mL) = tHbmass\ (MCHC \times 100)^{-1}$

Equation 3.6: Calculation for plasma volume (PV) (Wachsmuth et al. 2014)

$PV\ (mL) = BV - RCV$

where:

- tHbmass (g) = total haemoglobin mass (previously calculated, see Equation 3.3)
- [Hb] ($g \cdot dL^{-1}$) = haemoglobin concentration
- 0.91 = the cell factor (Chaplin et al. 1953)
- MCHC = mean corpuscular haemoglobin concentration

3.13 Statistical Analyses

Data were checked for normality and sphericity and were adjusted using the Huynh-Feldt method. One way analysis of variance with repeated measures and Tukey's Honestly Significantly Different post hoc analysis was used to compare between test conditions. All data were analysed using a standard statistical package (SPSS Statistics 22; International Business Machines Corp., Armonk, New York). Data were reported as mean ± SD, with the significance level set at $P < 0.05$. Please refer to *Statistical Analyses* sections within individual experimental chapters for more details.

3.13.1 Technical Error of Measurement

The most common way to express the error margin in a measurement is by means of the technical error of measurement (TEM), which is an accuracy index and represents the measurement quality and control dimension (Perini et al. 2005). TEM is the variability encountered between dimensions when the same specimens are measured at multiple sessions and within the experimental chapters of this thesis the aim is to minimise TEM, as this can affect results and inferences made (Harris and Smith 2009). Statistically, TEM is part of the residual ('unexplained') variance in a statistical test, so accounting for TEM, which requires repeated measurements, enhances the chances of finding a statistically significant difference if one exists (Harris and Smith 2009).

Equation 3.7: Calculation of absolute TEM (Norton et al. 1996)

$$\text{Absolute TEM} = \sqrt{\Sigma d^2 / 2n}$$

Equation 3.8: Calculation of relative TEM (Norton et al. 1996)

$$\text{Relative TEM (\%)} = (\text{TEM}/[\text{Mean1}+\text{Mean2}]/2) \times 100$$

CHAPTER 4

4 ALTITUDE AND HYPOXIC TRAINING IN ENDURANCE RUNNING: PERCEPTIONS OF ELITE ATHLETES AND SUPPORT STAFF

4.1 Abstract

Purpose: Recent research has challenged the worth of altitude and hypoxic training in elite endurance athletes. Elite British endurance runners were surveyed to establish the altitude and hypoxic training methods utilised, the reasons for use and any situational, cultural and behaviour factors influencing their use. **Methods:** Initially, five practitioners were interviewed to provide insight into the altitude and hypoxic training methods used by and to help guide the survey questions. Prior to and during the 2012 Olympics Games thirty-nine athletes and 20 coaches/practitioners (support staff) from 'British Athletics' completed an internet-based survey. **Results:** The semi-structured interviews revealed practitioners had questions surrounding topics, such as; '*training too hard at altitude*', *'competing on return to sea level'* and *'the use of additional hypoxic exposure post-altitude training camp'*. A very high proportion of the athletes (98%) and support staff (95%) surveyed had utilised altitude and hypoxic training, or advised it to athletes. 75% of athletes believed altitude and hypoxia to be a very important part of their training regime, with 50% of support staff believing the same. Athletes and support staff were in agreement of the methods of altitude training (i.e. 'hypoxic dose' and strategy) utilised with camps lasting 3-4 weeks at 1,500 to 2,500 m being the most popular. Open-ended questions frequently raised challenges of '*over training at altitude'*, '*loss of training intensity at altitude*', '*the timing of racing on return to sea level*' and '*being away from home'*. **Conclusions:** The survey is the first to analyse elite British track, road and cross country athletes on their current practices and perceptions of altitude and hypoxic training. The internet-based survey provided a detailed insight into the current practices of elite British endurance athletes and support staff. Generally, British athletes and support staff are applying the appropriate altitude and hypoxic training methods; however perceptions suggest there are still concerns. Many of the challenges raised in this study could provide priority and scope to optimise altitude training for endurance performance. To date there is no survey that has evaluated altitude training strategies alongside the insights and observations of elite athletes and coaches. The original findings may enhance and develop the current practices of British Athletics and also other endurance sports utilising altitude and hypoxic training.

4.2 Introduction

The effects of training at moderate altitude on subsequent performance at near sea level became important at the 1968 Mexico City Olympic Games (~2,240 m), and has subsequently been researched extensively (Saunders et al. 2013). Altitude training has since become an accepted mode of training and is widely endorsed by elite athletes, coaches and sports organisations as a crucial component of serious training regimes (Lundby et al. 2012). The theoretical concept is the independent and combined effects of the physiological processes of acclimatisation to chronic hypoxia and those derived from training under the additional stress imposed by exercising in a hypoxic environment (Rodríguez et al. 2015).

The physiological enhancements and performance improvements as a result of altitude and/or hypoxic training have been well documented (Bonetti and Hopkins 2009; Millet et al. 2010; Fudge et al. 2012), with mechanisms such as accelerated erythropoiesis (Levine and Stray-Gundersen 2005), improved running economy (Saunders et al. 2004b) and enhanced skeletal muscle buffering capacity (Gore et al. 2001) being reported. The elite British sporting system has empirical evidence that supports the use of altitude training for race performance improvements in middle-to-long-distance runners (Fudge et al. 2011). However earlier research on middle- and long-distance runners by Bailey et al. (1998) does not support these findings. It has been suggested that altitude training may depress immune function (Bailey et al. 1998), reduce training intensity/volume (Levine and Stray-Gundersen 1997) and does not induce erythropoiesis (Gore et al. 1998), factors which may contribute to the equivocal positions. As a result, 'Pro' (Wilber 2013) and 'Con' (Jacobs 2013) statements have been produced on altitude training.

The optimal 'hypoxic dose' for sea level performance adaptation is critical; too long or too extreme an exposure and training/performance are compromised and too short or too low an exposure may be insufficient to stimulate worthwhile physiological adaptations (Garvican-Lewis et al. 2016b). The adequacy of the 'hypoxic dose' could be the difference between the success and failure of an altitude training camp. Additionally, personal factors, such as homesickness, boredom, and well-being, may affect the mood of an athlete on a training camp or during a period of heavy training. It has been previously shown that mood state may affect recovery, and ultimately performance, during intensive training blocks (Kellmann and Günther 2000; Steinacker et al. 2000). With altitude training camps lasting 3-4 weeks, further knowledge of athletes' perceptions of altitude training would provide coaches with the tools to ensure a camp is successful.

Training at altitude provides a variety of different challenges, such as location (altitude, venue, and facilities), training prescription (volume, intensity, and recovery), individual responses (haematological, physiological and performance) and competition on return to sea level (timing, competition schedule and re-acclimatisation) (Dick 1992). Additionally, measuring the success of altitude training has recently been discussed (Gore 2014), with four key areas outlined as crucial (red cell volume, $\dot{V}O_{2max}$, performance and measurement precision). It is possible that these areas will be rated in varying degrees of importance for athlete, coaches and practitioners. Recognising the risks

associated with training in a different environment would help to scope research questions (Bergeron et al. 2012) and ensure athletes optimise their training at altitude, therefore maximising sea level performance. Further to this, empirical evidence of the use of altitude training by athletes is limited and there appears to be a disconnect between what is believed to work in research and what is actually practiced by coaches and used by athletes (Álvarez-Herms et al. 2015).

The application of an altitude training camp or series of hypoxic exposures not only depends upon physiological outcomes, but also the perceptions of coaches/athletes and the practical viability of implementing altitude training with elite athletes. Recent meta-analysis that examined the effects of various methods of altitude training on sea level performance reported 1.6-1.9% (Bonetti and Hopkins 2009) and 1.8-2.5% (Saunders et al. 2009a) improvements in endurance performance. Despite this evidence, the altitude training methods used across the EIS network have not undergone any systematic scrutiny to evaluate their efficacy. The altitude training camps organised by British Athletics operate on a system where athletes are invited to attend (Barden 2012) and once the invite is accepted the athletes are supervised by a senior coach, not their own coach. The individual athlete's coach writes their athlete's training programme, however the senior altitude training camp coaches may alter the programme during the camp. This may lead to conflicting opinion between the coaches, which may also affect an athlete's understanding of the correct altitude training strategy. To continue the development of the altitude and hypoxic training methods used within the EIS network, the practices and perceptions of elite athletes, coaches and practitioners, need to be evaluated to clarify some of the real challenges facing elite athletes and coaches; therefore enabling future research questions and investigation to be focussed and appropriate.

Various types of surveys have been used in sport, which vary in sample size, length, rigour, reliability and validity and application. Methods such as semi-structured interviews (Dunn et al. 2010), close-response formats (Likert scale ratings) and open-ended questions (Gould et al. 2002) and online questionnaires (Towlson et al. 2013), have been used by the Australian Institute of Sport, US Olympic Committee and on English football Premier League players, respectively. Despite there being limitations to the use of survey research in athletic training, survey research is a very worthwhile tool and an important method of gathering information about issues involving athletic training (Turocy 2002). The design and administration of the survey is vital as questionnaires tend to fail because participants don't understand them, can't complete them, get bored or offended by them, or dislike how they look (Boynton 2004). Recently, Álvarez-Herms et al. (2015) utilised an online survey and gathered responses "in situ" from endurance athletes (runners, cyclists, triathletes and duathletes) from Europe, Russia, USA, South Africa, Australia and New Zealand. The survey was administered by New Zealand and Spanish universities to gain an insight into the popularity of altitude training in professional and amateur endurance athletes.

The primary purpose of the study was to gain an up-to-date insight into the altitude and hypoxic training practices of elite endurance runners and the support staff, compared to current, peer-reviewed recommendations. It is hypothesised that there will be no differences between the altitude training practices of athletes, support staff and current literature A secondary aim was to understand

the challenges that athletes and support staff face when undertaking and prescribing altitude and hypoxic training methods.. This study investigated the following research questions:

- How well do the current altitude training practices (altitude 'dose', timing and management of strategy) of elite British endurance runners and support staff compare to each other and to the accepted best practice within the research literature?
- What are the athlete and support staff perceptions (positive and negative) of altitude training in relation to physiology, performance, lifestyle, training and understanding?
- What are the main challenges that athletes and support staff encounter when planning, performing and evaluating altitude training strategies?

4.3 Methods

4.3.1 Participants

'British Athletics' invited athletes, coaches and practitioners to take part in a survey. The British Athletics Head of Endurance sent the invitations via email. Thirty-nine elite British endurance runners and 19 endurance coaches/practitioners (hereafter referred to as 'support staff') completed the survey. Inclusion criteria included representing or coaching Great Britain at junior, U23 or senior level in track (outdoor and indoor) and/or cross-country running. This group included participants who had and had not utilised altitude and hypoxic training. Two athletes and two support staff started the survey but did not complete it and were excluded from the analysis, providing a 91% completion rate. It was not possible to determine the exact response rate of the survey as invitations were sent out via the National Governing Bodies (NGB) and the data protection act prevented the accessibility of that information. All methods were approved by the EIS with participants providing informed consent though reading the opening statements at the beginning of the survey, following the principles outlined by the Declaration of Helsinki, as revised in 2013.

4.3.2 Experimental design

Prior to the survey, five semi-structured interviews took place with physiology practitioners from British Athletics, British Rowing, British Triathlon, British Cycling and British Swimming. The findings of the semi-structured interviews shaped the direction of the questions and ensured the content of the survey was appropriate. Subsequent alterations were made and the final survey was distributed to the main participants electronically with an email of invitation and guidelines for the online survey (surveymonkey.com, California, Palo Alto, USA) (see Appendix 10). The survey was piloted with athletes, coaches and academics that had also either completed research in altitude and hypoxic training, or implemented it regularly. Appendix 2 outlines the semi-structured interviews that took place. The survey invitations were circulated prior to and after the 2012 London Olympic Games from May to October 2012. The survey comprised of 20 to 40 multiple-choice questions and two open-ended questions (depending upon answers to initial questions).

4.3.3 Experimental procedures

4.3.3.1 The Survey

The survey went through three stages of piloting. After the semi-structured interviews a draft survey was created. For two weeks three EIS physiology practitioners were asked to complete the survey and provide feedback. As a result of the feedback a second draft was distributed to three academic researchers and the third draft to two athletes and two coaches, within the same time scale. The

groups were sampled due to their expertise in the area of altitude training and survey writing. Appendix 3 provides examples of the feedback given. During each stage of the piloting, the survey was altered to enable the survey to be easily understood, have the potential to provide valuable answers and also ensure that there was a high response rate.

Following the pilot testing the final survey was created. Estimated survey completion time was ≤ 20 min. Approximately 90% of the survey questions used closed-response formats including Likert scale ratings (1: strongly agree, 2: agree, 3: neither agree nor disagree, 4: disagree and 5: strongly disagree) and yes/no frequency responses, with the remaining 10% using opened-ended formats. After section (a) the participants only completed questions relevant to who they were (i.e. athlete, coach or practitioner) and after section (b) the participants only completed questions related to their altitude and hypoxia experiences.

The final survey was divided into five sections: (a) *participant characteristics*, (b) *altitude and hypoxic training – uses and values*, (c) *altitude and hypoxic training – current practices*, (d) *athlete and support staff perceptions of altitude training*, and (e) *benefits and drawbacks*. In section (d) *individual perceptions of altitude/hypoxic training*, athletes and support staff were required to respond to the five-point Likert scale. The athletes were required to answer 45 statements, which were placed within 15 sub-topics (3 statements per sub-topic) and these were grouped into five higher-order topics (3 sub-topics per topic) to provide an overarching description. The support staff was required to complete 24 statements, which were modified so they were applicable to the population. The statements were placed into 8 sub-topics (3 statements per sub-topic) and these were grouped into four higher-order topics (2 sub-topics per topic). For a full list of the statements answered by athletes and coaches, and the topics and sub-topics see Appendix 4.

4.3.4 Data analyses

The present study was a descriptive cross-sectional survey design and, therefore, the analyses and presentation of data were shown in a way that expresses this. A Chi–Squared analysis compared the responses of the athletes to coaches and practitioners, who were grouped to form a larger cohort of 'support staff'. If the assumptions of the Chi-squared test were violated, then the Fisher's exact test was used. Within the sub-topics of section (d), one of the statements was phrased in a negative way to prevent responder bias (Baumgartner and Steenkamp 2001). The negative score was reversed into a positive one before the scores were averaged. A conventional content analysis (Hsieh and Shannon 2005) was used to analyse the open ended questions, with the aim of finding themes within the answers. Although there was a question related to participants who had not used altitude and hypoxic training, this section was not included in the analysis as there were not enough respondents (n = 4) to draw any meaningful conclusions.

4.4 Results

4.4.1 Semi-structured interviews

Table 4.1 is a summary of the findings from the semi-structured interviews of practitioners at the EIS. The statements and questions represent the views of practitioners that regularly advise and support altitude training programmes. The statements were used to create the questions used in the remainder of the survey.

Table 4.1: Findings of semi-structured interviews sent out to practitioners at the EIS

Theme	Statement/Question
Successes	Buy in from the coaches and athletes to the benefits of altitude/hypoxic training
	Actually getting the athletes to real altitude training camps regularly
	Athletes winning major championship medals off the back of altitude training camps
	Supporting altitude training camps with tent exposure in between
Current Practices	Structured camps throughout the season based around competitions
	Taking athletes to different locations, therefore different heights to suit the current training phase
	Using haemoglobin mass as a means of measuring the success of a camp
	Using altitude as part of a rehab process for athletes who are unable to fully train
Perceptions and Experiences	Would like to send more athletes to camps but logistically it is not possible
	There are non-haematological adaptation that take place at altitude as well
	Most endurance athletes are now using altitude/hypoxia as part of a year round programme
	The aim of a camp is not always performance based, but also to prepare for the next training block
Concerns and Pressures	Supplying every athlete with an altitude generator
	Athletes going 'too hard' at altitude and then 'wrecking' the remainder of their year
	Poor performance in competitions being ascribed to altitude and athletes not wanting to return
	Pre-competition altitude camps and what happens when the athletes return to sea level
Questions	What happens when you take the altitude stimulus away and you have to go and compete?
	Why does an acute hypoxic exposure in a tent alleviate the perceived feeling of lethargy after altitude?
	What else can be done to force an adaptation when an athlete is not responding very well?
	What is the optimum time to come back from altitude pre-competition?
	Why do 'first timers' have a different experience compared to 'regular' users'?
	Can athletes use the chamber or a tent to acclimatise themselves before a camp?
The Future	Is it possible to build an altitude house at an EIS site?
	Using haemoglobin mass measurements with a larger proportion of the squad
	How to inform the best practices to the sport
	Designing a new technique for measuring haemoglobin mass
	Altitude/hypoxia for strength training and rehab

4.4.1.1 (a) Participant characteristics

Thirty-nine athletes completed the survey, of which 75% were senior, 22% were U23/Junior and 3% were masters' athletes. Nineteen of the support staff completed the survey, of which 25% were currently working with senior athletes, 25% with U23/Junior athletes and 50% were working with a mixture of senior, U23/Junior and masters athletes. Figure 4.1 illustrates the highest level of competition that the participants surveyed have competed at or were working with.

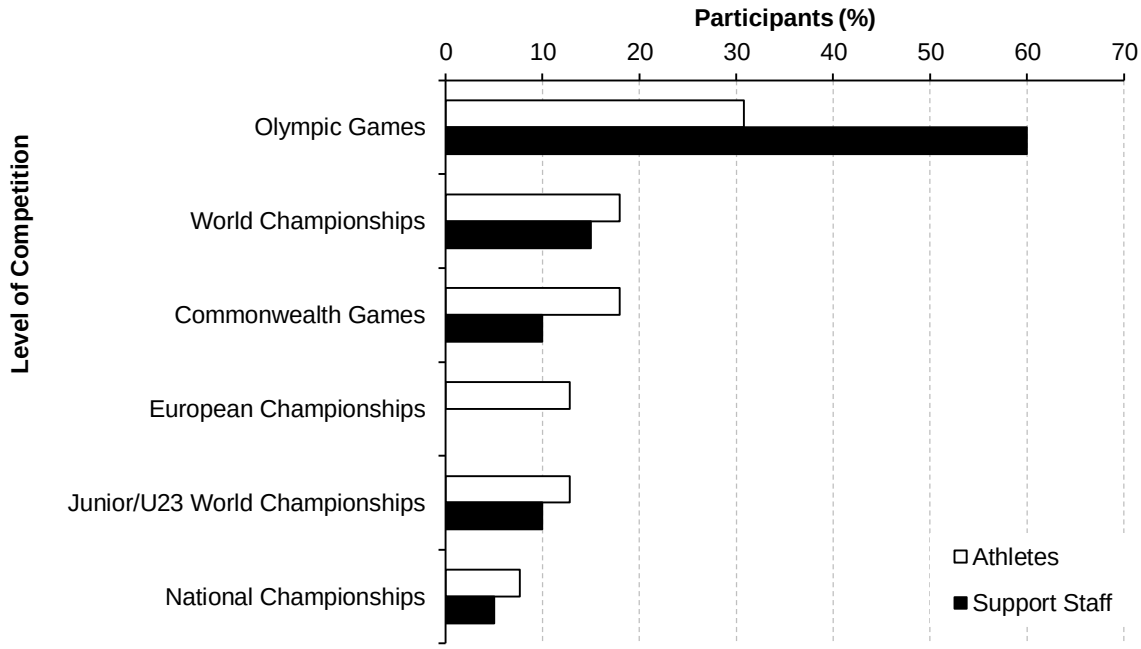


Figure 4.1: Please indicate the highest level of competition you have competed/worked at.

4.4.1.2 (b) Altitude and Hypoxic Training Methods – Value and Usage

Initially, to ascertain how the participants' valued altitude and hypoxic training they were asked, "How important would you rate altitude/hypoxia use (living or training at it) amongst your training regime or coaching practise?" The majority of athletes (74%) and half of the support staff (50%) believed that altitude and hypoxic training was very important or above (see Table 4.2), although there was no difference between the two groups (χ^2 = 3.75 (2); P = 0.154). There was also no difference (χ^2 = 1.87 (4); P = 0.759) between the competitive level of the athletes and support staff and how they valued altitude and hypoxic training: 65% Olympic/World Championship participants rated altitude and hypoxic training as very important, with 57% of the European/Commonwealth and 82% of the U23/Junior and National participants completing the same rating.

Table 4.2: How important would you rate altitude/hypoxia use (living or training at it) amongst your training regime or coaching practise?

	Athletes N = 39	Support Staff N = 19
Essential	9 (23%)	2 (10%)
Very Important	20 (51%)	8 (40%)
Important	6 (15%)	7 (35%)
Minor Importance	4 (10%)	2 (10%)
Not important at all	0 (0%)	1 (5%)

4.4.1.3 (c) Altitude and Hypoxic Training Methods – Current Practices

Participants went on to describe their uses of the different altitude and hypoxic training equipment and methods. Figure 4.2 illustrates the athlete responses and Figure 4.3 illustrates the coach responses. The figures illustrate that there is no difference between what athletes are currently doing and what support staff are prescribing with regard to living at real altitude (χ^2 = 4.04 (3); P = 0.257), sleeping in an altitude tent (χ^2 = 0.86 (3); P = 0.836), using a mask/generator at rest (χ^2 = 6.47 (3); P = 0.091), using intermittent hypoxia at rest (χ^2 = 4.36 (3); P = 0.225), exercising at real altitude (χ^2 = 2.12 (3); P = 0.549), exercising in an altitude chamber (χ^2 = 6.78 (3); P = 0.079), exercising using a mask/generator (χ^2 = 0.93 (3); P = 0.818), or using hypoxia for rehabilitation (χ^2 = 1.46 (3); P = 0.691).

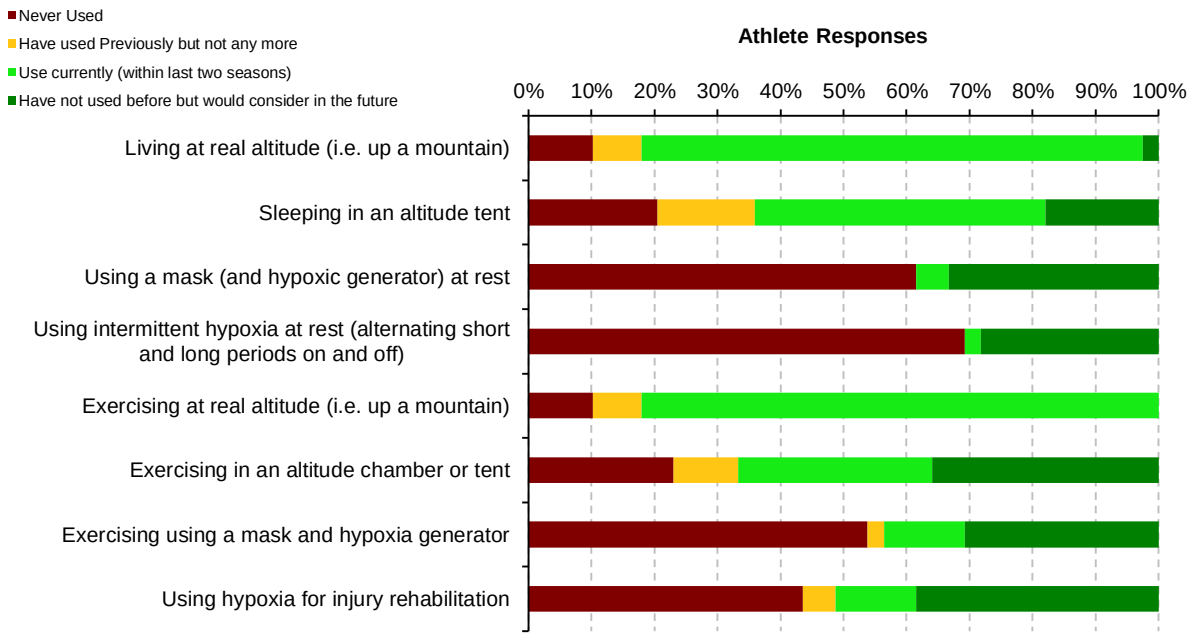


Figure 4.2: Question: When considering the various equipment and methods of altitude training/hypoxic use, please indicate your opinion on the statements.

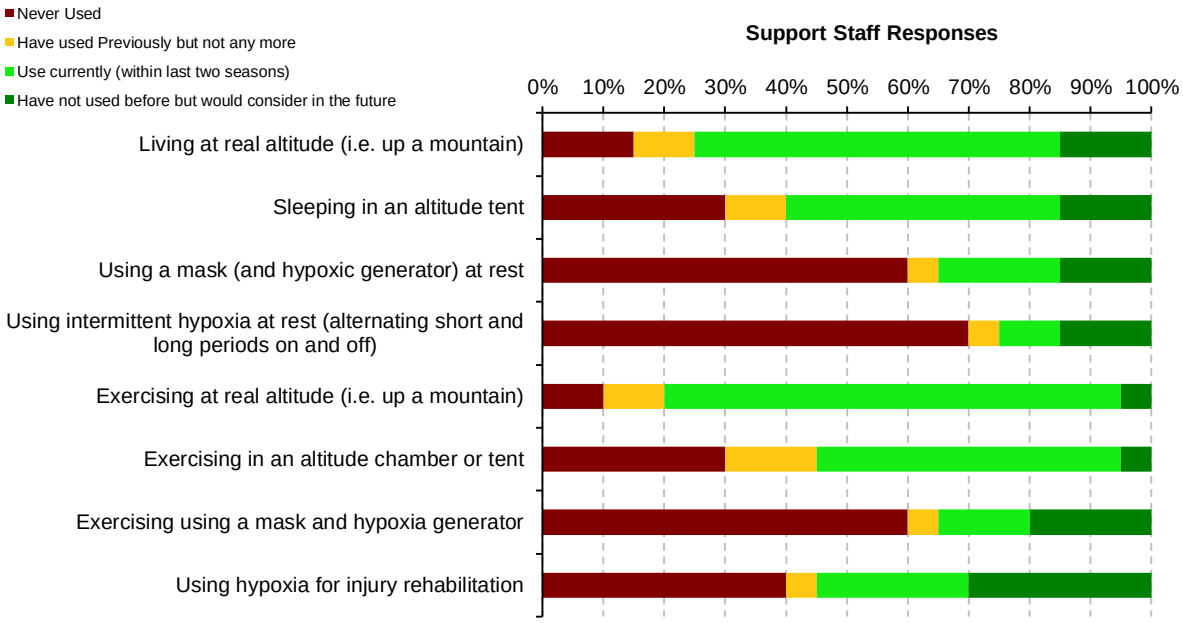


Figure 4.3: Question: When considering the various equipment and methods of altitude training/hypoxic use, please indicate your opinion on the statements.

Participants reported the altitude and hypoxic training methods that they had previously utilised (see Table 4.3). As a result of the answer to this question, participants were directed to a series of more specific questions on altitude and hypoxic training methodology.

Table 4.3: Question: Please indicate which of the following you have experienced

	Athletes (n = 39)	**Support Staff (n = 19)**
Altitude and Hypoxic Training Methods		
Natural Altitude Training Camp	14 (35%)	9 (45%)
Simulated Normobaric Hypoxia	0 (0%)	1 (5%)
Both	25 (63%)	9 (45%)

4.4.1.3.1 Natural Altitude Training

Table 4.4 summarises the athlete and support staff responses when asked about the natural altitude training methods that they used. Approximately half of the athletes surveyed reported that they went on an altitude training camp twice a year (44%), for 4 weeks (54%) at an altitude of 1,500 – 2,500 m (49%), favouring the LHTH method (95%). There were no differences between athletes and support staff in; altitude training cycles per year (χ^2 = 0.753 (1); P = 0.386), duration of altitude training camp (χ^2 = 0.08 (1); P = 0.780), height of altitude training camp (χ^2 = 4.04 (3); P = 0.257), altitude training camp living method (χ^2 = 4.91 (2); P = 0.086), however, there was a significant difference in the training method whilst altitude training (χ^2 = 7.41 (2); P = 0.025). There was a discrepancy between

the altitude that athletes and coaches believed they should be training at, with 92% of athletes preferring LHTH compared to 63% of support staff.

Table 4.4: Athlete and support staff experiences of altitude training

	Athletes (n = 39)	**Support Staff (n = 19)**
Altitude training cycles per year		
Once	14 (36%)	6 (32%)
Twice	17 (44%)	11 (58%)
Three times	6 (15%)	1 (5%)
Four or more	2 (5%)	1 (5%)
Duration of altitude training camp		
2 weeks or less	1 (3%)	0 (0%)
3 weeks	9 (23%)	4 (26%)
4 weeks	21 (54%)	13 (68%)
5 weeks or more	8 (21%)	1 (5%)
Height of altitude training camp (multiple responses permitted)		
1,000 - 1,500 m	0 (0%)	2 (6%)
1,500 - 2,000 m	32 (49%)	14 (45%)
2,000 - 2,500 m	31 (48%)	13 (42%)
2,500 - 3,000 m	2 (3%)	2 (6%)
Method of altitude training camp - living		
Below height of altitude venue (LL)	0 (0%)	0 (0%)
Similar height to altitude venue (LH)	38 (97%)	15 (84%)
Higher than altitude venue (LH)	0 (0%)	2 (11%)
Mixture of all methods (LL+H)	1 (3%)	1 (5%)
Method of altitude training camp - training		
Below height of altitude venue (TL)	0 (0%)	2 (11%)
Similar height to altitude venue (TH)	36 (92%)	12 (63%)
Higher than altitude venue (TH)	0 (0%)	0 (0%)
Mixture of all methods (TL+H)	3 (8%)	4 (26%)

Data are numbers of participants who answered each question and the proportion as a percentage in brackets.

4.4.1.3.2 Simulated Hypoxic Exposures

Table 4.5 summarises the athlete and support staff responses when asked about the simulated hypoxic exposures that were utilised. The Chi-Squared analyses revealed that there were no differences between athletes and support staff when asked how many simulated hypoxic exposures were

completed per year (χ^2 = 0.86 (2); *P* = 0.652). There was no difference between athletes and support staff in the ideal duration of simulated hypoxic exposure (χ^2 = 2.13 (2); *P* = 0.344), ideal height of simulated hypoxic exposure (χ^2 = 1.39 (2); *P* = 0.500), ideal hours per day (χ^2 = 0.37 (1); *P* = 0.539), however, differences were found in the method of simulated hypoxic exposure (χ^2 = 5.23 (1); *P* = 0.022).

Table 4.5: Details of the athlete and support staff simulated hypoxic exposure experiences

	Athletes (n = 24)	**Support Staff (n = 8)**
Simulated hypoxic training exposure(s) per year		
Once	12 (50%)	1 (13%)
Twice	3 (13%)	3 (38%)
Three times	4 (17%)	2 (25%)
Four or more	4 (17%)	1 (13%)
Continuous throughout the year	1 (4%)	1 (13%)
Duration of simulated hypoxic exposure(s)		
2 weeks or less	3 (13%)	0 (0%)
3 weeks	6 (25%)	1 (13%)
4 weeks	7 (29%)	3 (38%)
5 weeks or more	7 (29%)	3 (38%)
Continuous throughout the year	1 (4%)	1 (13%)
Height (and FiO_2) of simulate hypoxic exposure(s)		
1,500 - 2,000 m (17.4 – 16.4%)	5 (21%)	1 (13%)
2,000 - 2,500 m (16.4 – 15.4%)	9 (38%)	2 (25%)
2,500 - 3,000 m (15.4 – 14.4%)	7 (29%)	2 (25%)
3,000 – 3,500 m (14.4 – 13.6%)	2 (8%)	2 (25%)
Don't know	1 (4%)	1 (13%)
Hours per day of simulate hypoxic exposure(s)		
7 hours or less	0 (0%)	0 (0%)
8-9 hours	12 (50%)	3 (38%)
10-11 hours	8 (33%)	2 (25%)
12-13 hours	4 (17%)	3 (38%)
14 hours or more	0 (0%)	0 (0%)
Method of simulate hypoxic exposure(s)		
Continuous – all in one period	17 (71%)	2 (25%)
Mainly continuous – with shorter periods	7 (29%)	5 (63%)
Mainly shorter period through day and night	0 (0%)	1 (13%)

Data are numbers of participants who answered each question and the proportion as a percentage in brackets.

4.4.1.3.3 Sea level competition and training

The experiences of athlete and support staff returning to sea level for competition and training is reported in Table 4.6. There was a difference between athlete and support staff responses (χ^2 = 8.32 (3); *P* = 0.040) in their perspectives on when they should race on return to sea level. 46% of athletes stated they would race within four days post-altitude, while 32% of coaches believed they should wait until after 17 days at sea level. Similarly, when asked when they should commence training on return from altitude, 82% of athletes said one day or less and 58% of support staff said to wait one week or more (χ^2 = 38.27 (2); *P* = 0.001).

Table 4.6: Details of post-altitude and hypoxic training questions

	Athletes N = 39	**Support Staff N = 19**
Cease exposure to altitude pre-race		
Continuous to night before race	0 (%)	1 (5%)
2-4 days pre-race	18 (46%)	2 (11%)
5-10 days pre-race	7 (18%)	5 (26%)
11-16 days pre-race	9 (23%)	2 (11%)
17 days or more	4 (10%)	6 (32%)
Other	1 (3%)	3 (16%)
Re-start training post altitude training camp or simulated hypoxia		
On the same day as arriving	16 (41%)	0 (0%)
1 days after	16 (41%)	0 (0%)
2 -3 days after	3 (8%)	6 (32%)
4-5 days after	3 (8%)	2 (11%)
1 week after	1 (3%)	1 (5%)
More than 2 weeks after	0 (0%)	10 (53%)

Data are numbers of participants who answered each question and the proportion as a percentage in brackets.

4.4.1.4 (d) Perceptions of altitude/hypoxic training

Figure 4.4 illustrates the athlete response and Figure 4.5 illustrates the support staff response to a series of statements to determine their perceptions of altitude and hypoxic training from a Likert Scale.

4.4.1.5 (e) Benefits and drawbacks

The full conventional content analysis can be found for the athlete 'benefits' and 'drawbacks' in Appendix 6 and Appendix 7, respectively, and for the support staff 'benefits' and 'drawbacks' in

Appendix 8 and Appendix 9, respectively. The analyses is organised into different themes to provide a better understanding of the athlete and support staff opinions. The content of these themes will be examined further in the discussion section of this chapter.

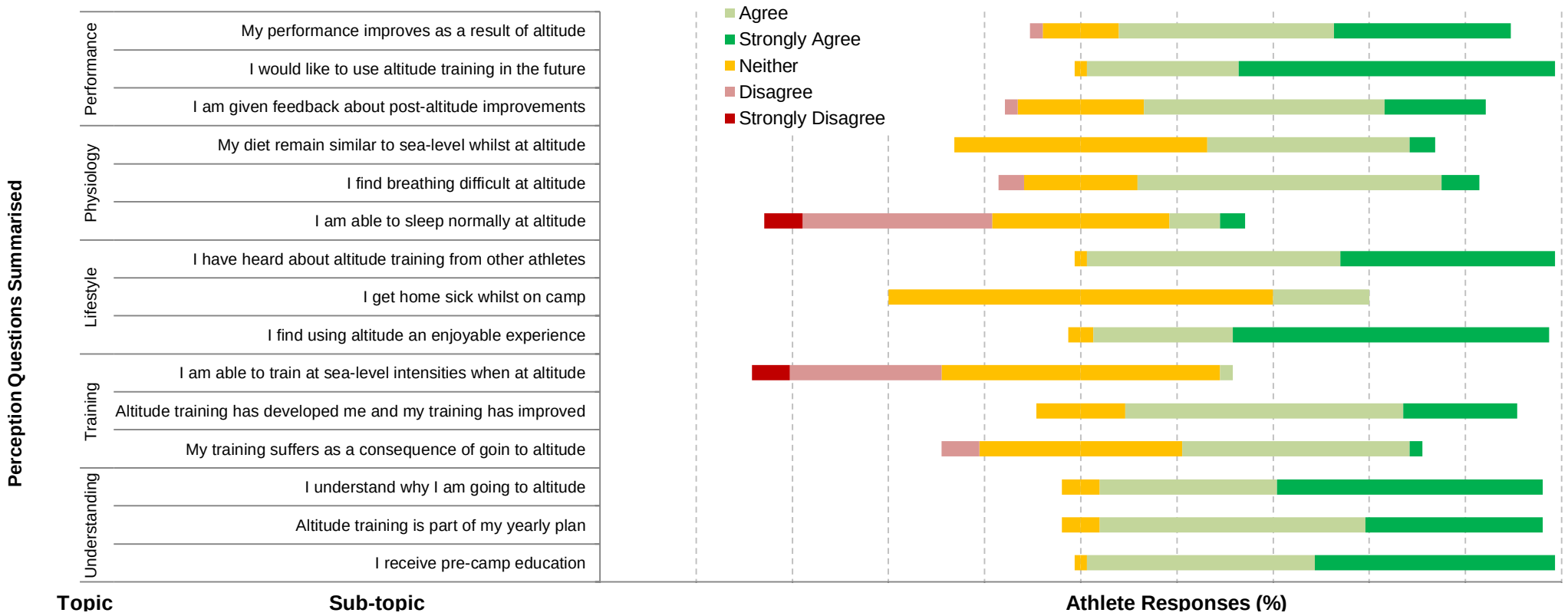


Figure 4.4: Athlete's perceptions of altitude training and hypoxia

Each coloured bar represents a percentage of athletes and how they perceived a series of statements. Each sub-topic is placed within a topic (e.g. performance) and the sub-topics above are a summary of three statements of which the mean response is illustrated. Each statement is based upon the responses from three questions, which have been averaged. The figure illustrates the trends of the athlete responses with bars sitting further to the left with more red/pink disagreeing with the statements and bars sitting further to the right with more dark/light green agreeing with the statements.

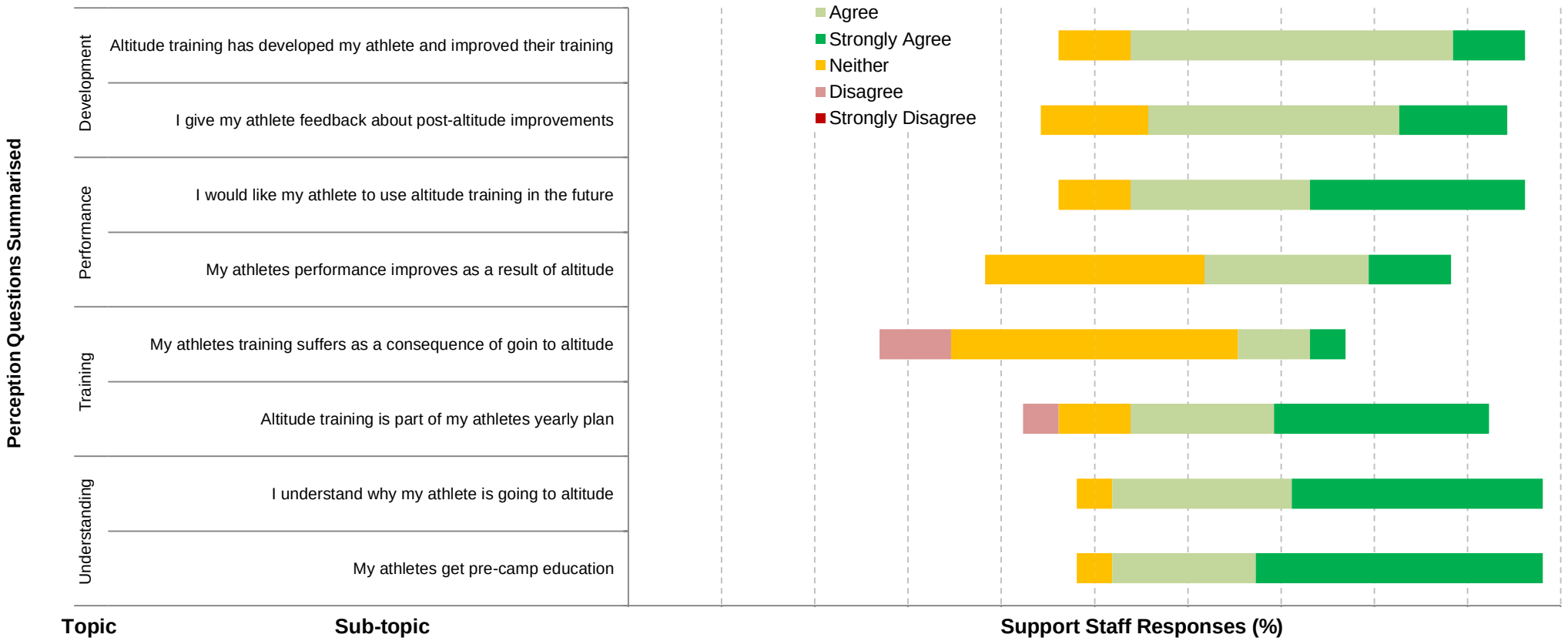


Figure 4.5: Support staff perceptions of altitude training and hypoxia

Each coloured bar represents a percentage of athletes and how they perceived a series of statements. Each sub-topic is placed within a topic (e.g. performance) and the sub-topics above are a summary of three statements of which the mean response is illustrated. Each statement is based upon the responses from three questions, which have been averaged. The figure illustrates the trends of the support staff responses with bars sitting further to the left with more red/pink disagreeing with the statements and bars sitting further to the right with more dark/light green agreeing with the statements.

4.5 Discussion

The aim of the present study was to identify data on the usage, correctness of usage, and difference in opinions of athletes and support staff regarding altitude and hypoxic training methods in elite British endurance runners. Furthermore, the study aimed to clarify some of the real challenges facing elite athletes and support staff, and enable future research questions to be more focussed and appropriate. The first finding of the survey was that both British endurance athletes and support staff believe altitude and hypoxic training to be an important part of their training programmes. Across a series of statements relating to altitude and hypoxic training methodology for athletes and support staff there was generally no difference between the methods used and this closely mirrored the recommendations of the most recent peer-reviewed literature. Finally, the perceptions and open-ended questions helped to gain insight into challenges, such as maintaining training load, returning to sea level and individual responses, that athletes and support staff face and how the success of an altitude training camp is multi-faceted.

4.5.1 (a) Participant characteristics

Participant characteristics revealed that the majority of the athletes were senior (74%), and that 25% of the support staff have worked with senior athletes. The calibre of athletes and support staff was high with 49% and 75%, respectively, having competed at or worked with athletes who have been to the Olympic Games or World Championships. A key finding of the survey was that athletes (76%) and support staff (50%) both agreed that altitude and hypoxic training was a 'very important' or 'essential' part of their training regime. Interestingly, across the different competition levels of the athlete and support staff surveyed (from Olympic down to national) there were no differences in how they valued altitude and hypoxic training, with the vast majority of them believing it to be 'very important'. It is possible that this is not only due to the reported successes of altitude training on competition performance (Fudge et al. 2011), but also due to the historical evidence of British coaches using altitude training (Dick 1992) and the British Athletics Federation's (now British Athletics) investment into researching altitude training (Bailey and Davies 1997; Bailey et al. 1998).

4.5.2 (b) Altitude and hypoxic training methods – Value and usage

The findings of the survey suggested that athletes and support staff are in agreement that living and training up a real mountain (i.e. LHTH) and sleeping in an altitude tent (i.e. LHTL) are currently taking place, using simulated hypoxia at rest or whilst training (i.e. IHE or IHT), however is not used very often and would be considered. The use of additional hypoxia during rehabilitation has also rarely been used and would be considered in the future if it were accessible. The survey did not however, ascertain at what time of the season these methods of altitude training have been taking place. Therefore, it is difficult to evaluate if British endurance runners are completing the correct method at the correct time of the season.

Millet et al. (2010) suggested that combining of different methods of hypoxic training might increase the chances of improvements in sea level performance and proposed a combination LHTH, LHTL and IHT across an entire season. Although there is currently no study that has directly investigated how to incorporate hypoxic training into the athlete's general training programme, the authors suggested LHTH (3–4 weeks each between 2,200 and 2,500m) during the preparation phase of the season, with LHTH (18–21 days at 1,800–2,000m) during the pre-competition phase and simulated IHT alongside living high (3,000 m for 5 days – sleeping at sea level for 2 days with two IHT per week) during the competition period (Millet et al. 2010). This is a model that UK Athletics (now British Athletics) has followed since the 2012 season (Barden 2012), with varying degrees of success. It should be noted that the survey was administered to British endurance athletes within the governing body's database therefore, not all levels of athletes would have received it. Therefore, it is possible that those who have not had the opportunity to go to altitude might value it differently.

Less of the athletes and support staff surveyed had previously used a hypoxic generator at rest (62% and 60%), intermittent hypoxia at rest (69% and 70%), or exercised using a mask with a hypoxic generator (54% and 60%). These methods of altitude and hypoxic training require specific equipment that might not be accessible to all athletes and they have also been researched less. The research that has taken place has found conflicting outcomes with both IHT and IHE (Levine 2002; Bartsch et al. 2008), which may have contributed to less athletes and coaches using the method. This area of altitude and hypoxic training could also provide opportunities of use during the competition phase of the season when it is not logistically possible to live 'up the mountain', or to provide an additional stimulus on return to sea level from an altitude training camp (Chapman et al. 2014b). These findings are further investigated in Chapter 7 (Study 4). Athletes and support staff do not commonly practice using hypoxia for rehabilitation, however, it would be considered in the future. This is another area which requires further research as to its appropriate application with elite endurance athletes.

4.5.3 (c) Altitude and hypoxic training methods – Current practices

The survey revealed that a very high percentage of the elite British endurance runners (97%) have previously been on an altitude training camp, or used a hypoxic generator for sleeping. Further, support staff (90%) had also recommended the use of these methods. Survey analysis by Álvarez-Herms et al. (2015) also found that altitude and hypoxic training was common in professional endurance athletes (from Europe, Russia, USA, South Africa, Australia and New Zealand) with 80 out of 95 (84%) stating they had used the methods previously.

4.5.3.1 Natural Altitude Training

For a full review of the optimal 'hypoxic dose' see 2.5.1. The present survey found that British endurance runners typically went on overseas altitude training camps 1-2 times per year (80%), which lasted 3-4 weeks (77%). They mostly attended camps situated at 1,500 to 2,500 m (97%) and reported

living at or near to the height of the altitude camp. Both athletes and support staff was in agreement with the use of these methods, however support staff appeared to favour LHTL method of altitude training.

By following the research recommendations, British endurance runners are increasing their chances of achieving the desired haematological adaptations, although there are other factors that have been previously discussed that might prevent these adaptions. Rasmussen et al. (2013) completed a meta-analysis and Monte Carlo simulation to determine the optimal altitude 'dose' that was required to increase total RCV in healthy low-landers. The analysis found that to exert a significant effect on RCV, altitude exposure must exceed 4 weeks at an altitude of at least 3,000 m. It is unlikely that altitude training camps will be situated any higher than 2,500 m due to the quality of training needed, therefore, it is possible that altitude training camps need to last longer than 4 weeks to ensure a significant increase in tHbmass. The meta-analysis by Rasmussen et al. (2013) used all methods of estimating RCV or tHbmass some of which are subject to more error than others.

Gore et al. (2013) produced a meta-analysis only using the oCOR-method, which has a lower error of measurement (Schmidt and Prommer 2005; Gore et al. 2005), and found that tHbmass increases at approximately 1.1%·100 h^{-1} of altitude exposure regardless of whether the exposure is LHTH (>2,100 m) or LHTL (~3,000 m), and that after a typical exposure of 300–400 h the increase above pre-altitude values persists for ~3 weeks. British endurance runners are currently fulfilling the recommended height and duration of exposure required to improve their chances of increasing tHbmass. However, due to inconclusive findings on the optimal 'hypoxic dose', this is studied further in Chapter 9 (Study 6).

The suitability of each of these methods has received a great deal of attention in elite athletes (Bonetti and Hopkins 2009; Lundby et al. 2012), with section 4.5.4 revealing the maintenance of sea level training to be a major concern for both athletes and support staff. This challenge regarding the 'hypoxic dose' has recently been discussed by Garvican-Lewis et al. (2016) who stated the modality, height and duration of altitude exposure is a trade-off between conflicting needs of athletes including safety, time efficiency, training quality, competition schedules and ability to travel.

4.5.3.2 Simulated Hypoxic Exposures

As previously mentioned, LHTL using altitude tents has found benefits for haematological changes providing the daily exposure is for a minimum of 12 h/day, with 'longer is better' with regards to further enhancements (Millet et al. 2010). Despite this fewer athletes had experienced sleeping in an altitude tent with simulated hypoxia compared to going to an altitude training camp. Athletes typically used simulated altitude 1-2 times a year (63%), lasting 3-4 weeks (54%), at a FiO_2 of 16.4-14.4% (67%) for 8-11 h per day (83%). The findings also suggest that the majority of athletes and support staff are not currently achieving this minimum daily requirement, however the 'hypoxic dose' is sufficient (~2,100-3,200 m). Richalet & Gore (2008) reviewed "live/sleep high – train low" in normobaric hypoxia (simulated altitude) and concluded in order to stimulate erythropoiesis and

moderately increase blood oxygen carrying capacity, simulated altitude needs to exceed ~2,500 m (FiO_2 ~15.8-15.7%), for 18 days and 12 h·day^{-1}.

Lundby et al. (2012) believed that at the moderate altitudes relevant for altitude training, $\dot{V}O_{2max}$ gradually increases over time with acclimation; therefore the relevance of 'training low' should decrease with acclimatisation. A double-blinded and placebo-controlled study by Siebenmann et al. (2012) did not demonstrate any effects on any haematological or performance parameters in elite cyclists following LHTL 16 h·day^{-1} at 3,000 m normobaric hypoxia for four full weeks. The authors speculated that the already high RCV values of the athletes might have caused the absence of a positive response, and although the general recommendations for LHTL may be sufficient for lower-end athletes; this is not necessarily the case for elite level athletes. Consequently, it is possible that the British endurance athletes should be sleeping at a lower FiO_2 provided there is no harmful effects on sleep quality and hence recovery, which is believed to occur above 3,000 m (Lundby et al. 2012).

4.5.3.3 Sea level competition and training

The best time to return from altitude training prior to a major competition for peak performance remains undocumented from a physiological standpoint (Chapman et al. 2014b). See Section 2.8.1 for a full review of the previous research, mechanisms and practical applications. Amongst the elite British endurance runners and support staff there were opposing responses to questions that surrounded when to complete and train on return to sea level. Forty-six percent of athletes typically ceased using altitude less than 4 days before competition, whereas, 60% of the support staff advised waiting more than 17 days to compete. Similarly 82% of athletes typically trained on the same day or one day after an altitude training camp, whereas 58% of support staff advised waiting one week or more to resume full training. The differing responses of athletes and support staff correspond with the current gaps in the literature and reliance on anecdotal evidence in deciding when to compete after altitude training and hypoxic exposure.

A long-term observational study by Wachsmuth et al. (2013) with swimmers found an immediate decrease in performance after return from altitude, a small trend toward lower performance until 14 days, unchanged performance at 14–24 days and a delayed performance peak 3-5 weeks after return. The performance markers did not show a clear relationship to tHbmass measurements which showed a small drop immediately after the return from altitude, an elevated plateau for the following 10 days, a sustained gain of 50 % after ~3 weeks, and values that return to baseline after ~5 weeks (Wachsmuth et al. 2013). The time course of tHbmass is subsequently investigated in Chapter 9 (Study 6).

Based on the responses of athletes and support staff it would appear both are unsure about when to train and complete when the altitude/hypoxic stimulus is removed. This period of time is crucial as the success of an altitude camp hinges on the performance of the athletes when they return to sea level. Future research should simultaneously explore in detail the time course of the changes in RBC mass and performance in elite endurance athletes after return to sea level (Chapman et al. 2014b).

4.5.4 (d) Perceptions of altitude and hypoxic training

There are many different factors, outside of physiology and performance, which will impact an athlete's decision to train at altitude or a coach's decision to send their athlete to altitude. These might include fitting the camp around their competition schedule, having the funding so that they can go, family and personal considerations and are the proposed benefits of altitude training currently limiting their performance. Figure 4.4 and Figure 4.5 illustrate that athletes and support staff, respectively, generally understood why they were going to altitude and believed it to be beneficial to their sea level performance.

The responses to the perceptions questions showed that, overall, both athlete and support staff had positive view about altitude and hypoxia. Athletes and support staff admitted that altitude was more physiologically strenuous than sea level training and therefore training intensity was reduced. Is it well documented that training at altitude causes a reduction in training intensity (Levine and Stray-Gundersen 1997; Wilber 2001; Wehrlin and Hallén 2006). Conversely, Pugliese et al. (2014) believed that elite athletes with extensive altitude training experience and several years of training at high level can maintain the same absolute intensity during LHTH compared to sea level. Clearly altitude venues with multiple training altitudes appear to be crucial to the success of an altitude training camp as lower altitude for high-quality anaerobic running is also necessary to maximize the quality of exercise in the pre-competition phase of the season (Saunders et al. 2009c).

Athletes and support staff also stated that sleep was disturbed compared to sea level. This has previously been reported by (Flaherty et al. 2016), however Girard et al. (2013) suggested that sleeping at moderate altitude does not cause major disruption to an athletes sleep, which may alternatively be as a result of sleeping in a different location rather than the altitude. Generally, there was a belief that the positives of altitude training (i.e. improved performance, physiological enhancements) outweigh the negatives (i.e. homesickness and disrupted sleep and diet) and both athletes and support staff felt it would develop them in the long run.

4.5.5 (e) Benefits and drawbacks

Common themes that arose from the benefits of altitude training according to athletes were '*Lifestyle'*, '*Physiological'*, and '*Returning to sea level*' and '*other influences'*. Interestingly, the '*Lifestyle*' benefit of altitude training falls outside of the traditional physiological and training benefits, citing the following: the training camp effect ("*simply being in a training camp environment*"), location ("*usually great training venues, perfect weather*"), having less distractions ("*can focus easier on training and recovering*") and increased time for rest and recovery ("*you get the recovery time in that is well needed*") as being beneficial. The notion of a 'training camp effect' or placebo effect causing performance improvements as a result of an altitude training camp has been previously suggested (Levine and Stray-Gundersen 1997; Bonetti and Hopkins 2009; Lundby et al. 2012). Although '*Lifestyle*'

choices may be the case many of the athletes still cited '*Physiological*' enhancements as a benefit of altitude training.

Physiological benefits accounted for most of the benefits, such as haematological adaptations ("*increasing haemoglobin mass*"), building an aerobic base ("*enhance my aerobic base*") and increasing training load ("*I can get a really good base, and good volume of training, without as much stress on the legs*"). There also appeared to be a feeling of a psychological benefit ("*mentally to know that I have gone some way to matching my African rivals*") to altitude training. Finally athletes quoted that race performance was improved on return to sea level ("*my best performances have come after a spell of high altitude training*") and they are able to increase their training intensity ("*I feel that I am often fitter… and my performances are better*"). Appendix 6 details all of the comments from the conventional analysis.

Support staff comments on the benefits of altitude also comprised of similar themes, with most emphasis being placed upon '*Physiological*', specifically aerobic physiology themes, for example, "*an increase in aerobic qualities*" and "*as a means to develop aerobic conditioning*". Coaches also stated that the location ("*can also include challenging terrain*") and environment ("*training environment, group ethos and relaxing atmosphere*") created at a training camp was beneficial for their athletes. The opinions of international endurance coaches have previously been represented by Dick (1992), who based on the practical experiences produced a series of altitude training recommendations to athletes.

For the development of an altitude training programmes the drawbacks must be considered. High proportions were themed around '*Training*' and '*Return to sea level'*. Within the training theme "*avoiding overtraining*" and "*difficulties controlling pacing*" were frequently reported. There was also a high incidence of personal issues, such as, "*some altitude training camps are too basic*" or "*there are more health risks*". A suppression of the immune system has been reported when training at altitude, which can be prevented with adequate personal hygiene and nutrition (Flaherty et al. 2016). These types of negatives directly affect what happens during the camp and can be alleviated with physiological monitoring.

Interestingly, the drawbacks from support staff did not mention return to sea level for competition but were centred around a variety of different issues, which appeared to be very specific to the athletes they were coaching. The concerned ranged from the trivial ("*travel boredom*") to fundamental ("*weight loss during camps*") and obstacles that were hard to control ("*limited by finance and time away*"). Support staffs were also concerned about disruption of training ("*disrupted training when travelling overseas*") and sleep loss ("*disrupted sleep when using tents, hypoxia*"). Again many of these issues have been previously reported (Bailey and Davies 1997) and with sufficient pre-screening of individual athlete responses to training at altitude some of these concerns can be alleviated. Pre-screening may provide support staff with a tool to reduce the incidence of overtraining, illness, reduced training intensity and poor performance at sea level. The role of the hypoxic ventilatory response, degree of oxygen saturation and EPO have been previously studied (Friedmann et al. 2005a; Chapman et al. 2010), but require further investigation due to inconclusive findings. This area will be investigated further in Chapter 9 (Study 6).

Since the optimisation of the CO-rebreathing method (Schmidt and Prommer 2005; Prommer and Schmidt 2007), the measurement of tHbmass has been become fundamental to altitude training research (see meta-analysis by Gore et al. (2013)). Álvarez-Herms et al. (2015) reported that the majority (81%) of professional athletes believed improvements with hypoxic training came via haematological changes. Similarly, within the benefits sections of the open-ended question both athletes and support staff stated that increased RBCs, and therefore oxygen carrying capacity is a priority with statements such as, "*extra red blood cells is the primary benefit*", "*increase in red blood cells on return*" and "*greater oxygen transport*". Too fully understand the adaptions associated with altitude training tHbmass should be measured, however, meaningful interpretation of serial changes in blood parameters in experimentally induced erythropoiesis requires quantification of the errors of the measurement techniques (Gore et al. 2005). Secondary to this, the athletes and support staff must believe that the changes in haematological markers, such as tHbmass, are accurate as they are likely to amend altitude training strategies off the back of results obtained. Chapter 5 and 6 (Study 2 and 3) will investigate the oCOR-method in greater detail.

4.5.6 Limitations

The present study has chosen to utilise mixed survey methods to investigate altitude training in elite British endurance runners. Measures were put in place to ensure the data collected was both valid and reliable, such as seeking advice from experts in the area, pilot testing the survey and developing a research question. There are, however, various different methods within survey collection and also ways of analysing the data, which may have been over looked. One area, which should be addressed, is the population that completed the survey. Due to the very specific nature of altitude training only endurance runners were asked to complete the survey, subsequently the findings are from a homogenous population. In order to understand the altitude training methods used by elite runners and the challenges they face fully, this population had to be targeted. Similarly to the present study, Álvarez-Herms et al. (2015) also found that a very high percentage of elite endurance athletes utilised altitude training compared to those who had not. As previously stated participants who had not been to or advised an altitude training camp were not included in the analyses due to a small sample size. Although this population might provide further insight as to why they have not chosen to use altitude and hypoxic training it was not feasible to use the data. Further data collected from other endurance sports might increase the power of this group and provide additional information as to why research should be directed to altitude training in the future.

4.6 Conclusion

The original findings of the study are that the altitude and hypoxic training practices of elite British athletes are similar to that of previously surveyed international endurance athletes (Álvarez-Herms et al. 2015). The present survey also suggests that the current practices of elite British runners are

generally in agreement with the altitude training reviews and meta-analysis (Bonetti and Hopkins 2009; Millet et al. 2010; Gore et al. 2013). Further to this, open-ended questions disclosed that there were many different individual reasons relating to the benefits (e.g., training camp effect, increased RBCs, and additional physiological stimulus) and drawbacks (e.g. costs, travel, homesickness, and decreased training intensities) of altitude and hypoxic training. Many of the physiological reasons stated by the participants could be associated with an individual's response to the hypoxic stimulus; therefore, enhancing the understanding of this area of altitude training and hypoxic exposures could increase the chances of success. The study is the first to provide a detailed insight into the current practices of elite British endurance runners and the challenges they face when undertaking an altitude training camp.

CHAPTER 5

5 COMPARISON OF TOTAL HAEMOGLOBIN MASS MEASURED BY THE OPTIMISED CARBON MONOXIDE REBREATHING METHOD USING DIFFERENT RADIOMETER™ HEMOXIMETERS

5.1 Abstract

Purpose: The oCOR-method is used to measure tHbmass. A *new* Radiometer™ hemoximeter has recently become available and will be used at three different EIS regional laboratories, therefore, the precision, agreement and bias of tHbmass determination using the old OSM3 and *new* ABL80 was determined. **Methods:** Six male and one female (age 30 ± 6 yr, stature 180 ± 7 cm, body mass 78.1 ± 10.6 kg) undertook the oCOR-method. Venous blood (~ 2 mL) was sampled immediately before and at 7 min during the oCOR-method; with seven replicates from each time point analysed on five different Radiometer hemoximeters (OSM_1, OSM_2, ABL_1, ABL_2 and ABL_3). **Results:** There were no differences ($p > 0.05$) between mean tHbmass analysed with five different hemoximeters (OSM_1: 886 ± 169 g; OSM_2: 896 ± 160 g: ABL_1: 904 ± 157 g; ABL_2: 906 ± 164 g: ABL_3: 906 ± 162 g). However, the Bland-Altman plot revealed that there was closer agreement between tHbmass determined using the *new* ABL80, e.g. OSM_1 vs. OSM_2: $r = 0.96$, mean bias -10 g with 95% limits of agreement -76 g to +56 g; ABL_2 vs. ABL_3: $r = 0.99$, mean bias -0.2 g with 95% limits of agreement -10 g to 10 g. The variance (i.e. % error) across the replicate samples decreased as the number of samples increased, with the error derived from the 'worse-case' scenario being 1.2 to 1.6 fold greater in the OSM3 than the ABL80. **Conclusions:** Although there were no differences in the tHbmass measured across five different hemoximeters, ABL80 hemoximeters were in better agreement compared to the OSM3. The ABL80 also showed less error, therefore fewer replicates are required to produce a TEM of ≤1%.

5.2 Introduction

Measuring tHbmass and blood volume (BV) is important in clinical, sports medicine, and athletic contexts (Gore et al. 2006a; Prommer et al. 2008). The findings from Chapter 4 (Study 1) also revealed that elite British athlete's and support staff valued the measurement of tHbmass. An athlete's endurance capacity can be significantly improved due to the blood's capacity to deliver more oxygen to the exercising muscles (Hoppeler and Vogt 2001), therefore, athletes have attempted to increase tHbmass through endurance training and adaptation to altitude (Fudge et al. 2012). Schmidt & Prommer (2010) proposed a change of tHbmass by 1 g would cause a change in $\dot{V}O_{2max}$ of approximately 4 $mL \cdot min^{-1}$. In order for this marginal gain to be detected the method of measuring tHbmass needs to be reliable and accurate. Methods such as radioactive labelling by ^{51}Cr, the Evans blue, hydroxyethyl-starch and indocyanine green dilution techniques have been previously used to measure tHbmass, however, these are too invasive to use on an athletic population.

A break though for sport science and medicine came with the availability of diode-array spectrophotometers (hemoximeters), which measure blood percent carboxyhaemoglobin (%HbCO), allowing practical determination of tHbmass via CO-rebreathing. Thomsen et al. (1991) was the first to use CO-rebreathing to determine tHbmass, with Burge & Skinner (1995) further refining the method to identify sources of error. Schmidt & Prommer (2005) further optimised the method (oCOR-method) to allow its practical use with athletes. The method has been studied (Gore et al. 2005; Prommer and Schmidt 2007; Garvican et al. 2010a) to identify sources of error and although there are potentially a number of methodological procedures that could contribute to the technical error of tHbmass, the analyser error (as a sub-component of technical error) can be most readily quantified and improved (Alexander et al. 2011). Therefore, the reliability and precision of multi-wavelength spectrophotometer, such as those made by Radiometer (Copenhagen, Denmark), is a fundamental requirement (Gore et al. 2005).

The manufacturer of the Radiometer OSM3 hemoximeter (OSM3) claims that the imprecision of a single-sample analysis is ±0.l% and that this decreases to ±0.045% if five analyses are performed on the one sample (Christensen et al. 1993). Two recent studies have attempted to determine the technical error of an OSM3. Gough et al. (2011) examined the variation in tHbmass when consecutive measurements were made over a short period of time in three different OSM3 analysers. The mean ± standard deviation (SD) of tHbmass was 674 ± 181, 701 ± 184 and 699 ± 185 g. A within-subject variation in tHbmass profiles was found, which is likely to be partly due to the variation in %HbCO measured. This will pose problems if an athlete was to get measured at a different laboratory.

Figure 5.1: The Radiometer OSM3 (left) and ABL80 FLEX CO-OX (right)

The reliability of the OSM3 is the critical determinant of analyser error because this hemoximeter reports %HbCO to only 0.1% resolution (Alexander et al. 2011). However, multiple measures on each blood sample will attenuate error as a function of √n replicates (Hopkins 2000). The number of replicates used during oCOR-method had varied in different studies; one replicate (Pottgiesser *et al* 2007, Schumacher *et al* 2008), duplicates (Schmidt and Prommer 2005; Prommer and Schmidt 2007; Prommer et al. 2008), triplicates (Robach et al. 2006b), quadruplicates (Burge and Skinner 1995; Wehrlin et al. 2006), quintuplicates (Gore et al. 2006a; Eastwood et al. 2008), sextuplicates (Gore et al. 2006b), octuplicates (Neya et al. 2007) and decuplicates (Gough et al. 2011). As a result Alexander et al. (2011) attempted to determine empirically the number of replicates required to yield a theoretical analyser error of ≤1% on three OSM3 hemoximeters. The study found to reduce the analyser error of measuring %HbCO with an OSM3 to ≤1%, that five replicates are necessary for a typical 5.5% ΔHbCO.

The previous two studies have demonstrated that different OSM3 analysers produce variable measurements of %HbCO, and require quintuplicate samples to yield a low analyser error, however, the OSM3 model has now been discontinued, and therefore studies will need to be repeated with the newer model. The purpose of the investigation was firstly to determine the agreement across three *new* ABL80 FLEX CO-OX hemoximeters in comparison to the *old* OSM3 and secondly to determine how many replicate samples were required to yield an analyser error of ≤1%. It is hypothesised that the *new* ABL80 device would require less samples than its *old* OSM3 counterpart.

5.3 Methods

5.3.1 Participants

Seven physically active healthy, non-smoking and physically active volunteers, six males and one female, participated in the study. The participants were age 30 ± 6 yr, height 180 ± 7 cm and body mass 78.1 ± 10.6 kg. The protocol and procedures described were approved by the institute Research

Ethics and Governance Committee and conducted in accordance to the Declaration of Helsinki. All participants gave their written informed consent to participate. For full participant inclusion and exclusion criteria see General Methods section 3.2.

5.3.2 Experimental design

Each participant completed one oCOR-method (Schmidt and Prommer 2005; Prommer and Schmidt 2007). Blood samples were analysed immediately and in septuplicate on the five Radiometer hemoximeters (OSM_1, OSM_2, ABL_1, ABL_2 and ABL_3).

5.3.3 Experimental procedures

Total Hbmass was determined using the oCOR-method (Schmidt and Prommer 2005; Prommer and Schmidt 2007). The protocol and equipment used was previously described (see section 3.7).

Venous blood samples were taken into one 2 mL BD Vacutainer® Plus Plastic K_2 EDTA Tubes (BD Vacutainer®; New Jersey, USA) from the antecubital fossa, for determination of %HbCO. Samples were taken before the start of the rebreathing procedure (pre-%HbCO) and at 7 min after (post-HbCO) the CO bolus was administered. Blood samples were mixed and drawn in two 1 mL syringes (Terumo; Leuven, Belgium) and measured immediately in seven replicates on each of the three *new* ABL80 CO-OX Flex hemoximeters and two *old* OSM3 hemoximeters (Radiometer™; Copenhagen, Denmark). A Pentra ES 60 (Horiba Medical; Kyoto, Japan) was used to determine Hct and [Hb]. Total Hbmass was calculated (Prommer and Schmidt 2007) from the mean change in %HbCO before and after rebreathing CO.

5.3.4 Statistical analyses

The mean, SD and coefficient of variation (CV) of %HbCO were calculated for each of the five hemoximeters. To establish if differences existed between machines, a repeated measure ANOVA was calculated for the mean pre-%HbCO, post-%HbCO and derived tHbmass. To establish accuracy a 'best case' scenario analysis was designed: the seven samples each of pre and post %HbCO were arranged in numerical order, allowing the largest pre-post delta change (and hence largest derived tHbmass) to be identified between any pre-post combination of single samples across the data set (e.g. lowest pre-%HbCO combined with highest post-%HbCO), and the smallest delta change (e.g. highest pre-%HbCO and lowest post-%HbCO) – these being the 'worst-case scenarios' attainable from using single samples in the divergent data set. Accordingly, with the data set organised into ascending (pre) and descending order (post), this delta change was calculated (and tHbmass derived) for differing multiple repeats (duplicate, triplicates etc.) converging on the 'best case' scenario of all seven pre and seven post samples being used to establish delta HbCO% and derived tHbmass. This analysis allows quantification of the propagation and size of error in tHbmass (away from that derived from what we deem to be the

'best' practicable method available to practitioners; i.e. seven replicates) for any number of sample replicates. Although the data were artificially arranged the order of samples within this scenario, (the likelihood of the various permutations occurring in the specific order within the data set is reported elsewhere), this analysis allows a comparative illustration of the accuracy of each hemoximeter for differing scenarios. This process was completed separately for each of the five hemoximeters. To visualise the difference between the Radiometer™ hemoximeters, as well as to compare tHbmass calculated from Δ%HbCO, thereby assessing the agreement, the Bland-Altman plot (Bland and Altman 1986) was used. All values are reported at mean ± SD.

5.4 Results

Table 5.1 provides a summary of the mean, SD and CV of the %HbCO, Δ%HbCO and tHbmass for each of the five hemoximeters. Coefficient of variation (CV) is smaller in the ABL80 pre o-COR-method (low %HbCO: 0-2%) and 7 min of the o-COR-method (high %HbCO: 4-8%) compared to the OSM3.

Before and at 7 min of the oCOR-method there were no differences ($P > 0.05$) in %HbCO between ABL_1 vs. ABL_2, ABL_2 vs. ABL_3 and ABL_1 vs. ABL_3, however, differences ($P < 0.05$) were found between all other hemoximeters. Despite some significant differences in %HbCO from the OSM3 and ABL80 there were no differences ($P = 0.13$) in Δ%HbCO and as a result there were no differences in the tHbmass calculated from the Δ%HbCO ($P = 0.86$). There was, however, a 2.1% difference between OSM_1 and ABL_3, and only a 0.3% difference between all of the ABL hemoximeters.

Figure 5.2 illustrates the best and worst case scenario analysis. The bottom row of graphs illustrate that to achieve a 1% error in tHbmass ~5 replicate samples are required when using the OSM3 and ~3 replicate samples are required when using the ABL80. Figure 5.3 illustrates the Bland-Altman plots comparing the tHbmass result from each of the 5 hemoximeters. It is clear that there is closer agreement between the ABL80 than between the OSM3. For example, OSM_1 vs. OSM_2: r = 0.96, mean bias -10 g with 95% limits of agreement -76 g to +56 g; ABL_2 vs. ABL_3: r = 0.99, mean bias -0.2 g with 95% limits of agreement -10 g to 10 g.

Table 5.1: Mean, SD and CV of %HbCO from each hemoximeter and subsequent Δ%HbCO used to calculate tHbmass.

	%HbCO									
	OSM_1		**OSM_2**		**ABL_1**		**ABL_2**		**ABL_3**	
	Pre	*7 min*	*Pre*	*7 min*	*Pre*	*7 min*	*Pre*	*7 min*	*Pre*	*7 min*
Mean	-0.1 *	5.5 *	0.8 *	6.4 *	1.3	6.9	1.4	6.9	1.2	6.7
SD	0.3	0.8	0.3	0.8	0.2	0.7	0.3	0.8	0.3	0.8
CV	5.8	1.3	8.7	0.6	3.4	0.6	3.7	0.7	4.1	0.7
	Δ%HbCO									
	OSM_1		**OSM_2**		**ABL_1**		**ABL_2**		**ABL_3**	
Mean	5.6		5.6		5.5		5.5		5.5	
SD	0.6		0.6		0.6		0.6		0.6	
CV	10.3		11.3		10.7		10.8		10.7	
	tHbmass (g)									
	OSM_1		**OSM_2**		**ABL_1**		**ABL_2**		**ABL_3**	
Mean	886		896		904		906		906	
SD	169		160		157		164		162	
CV	18.9		17.9		17.4		18.0		17.9	

* denotes significant difference between machine type and comparative time point

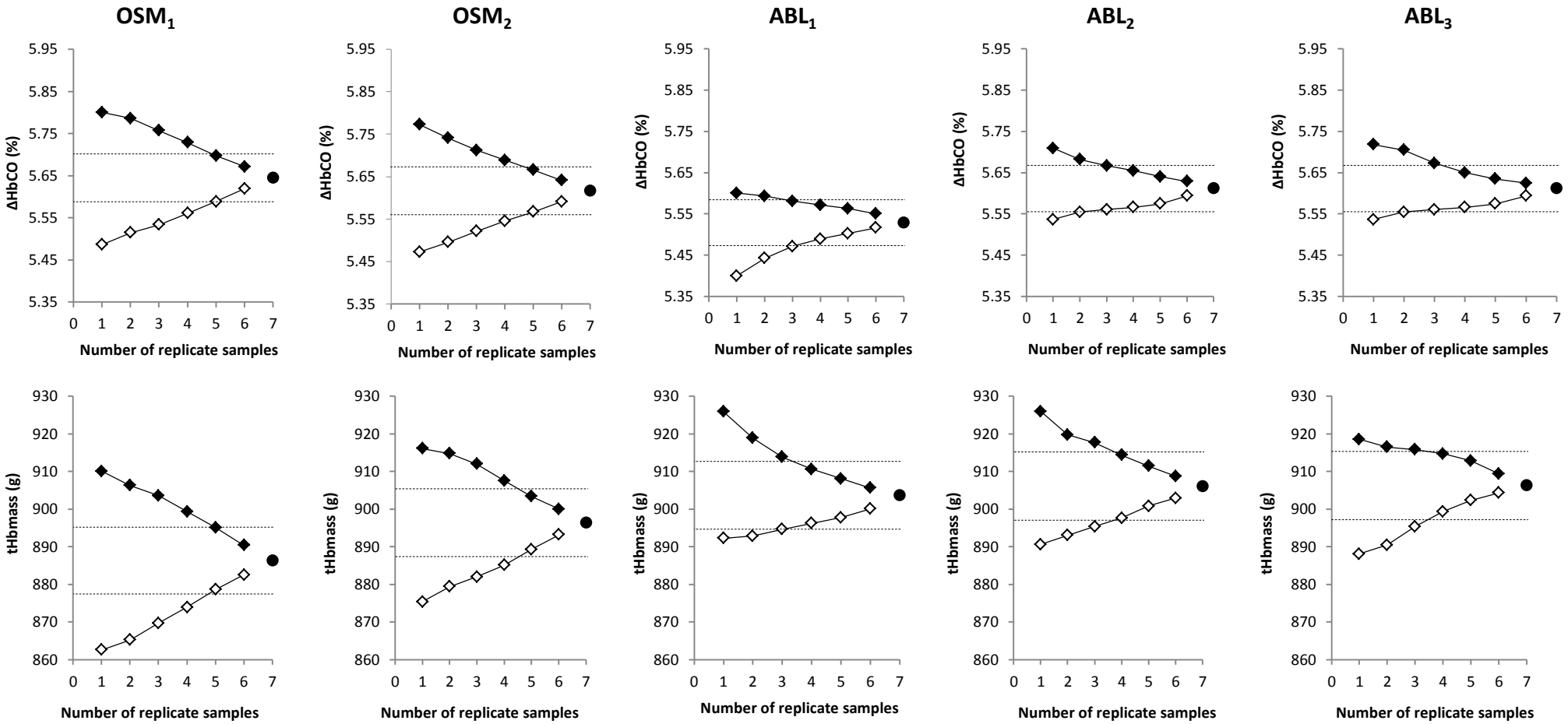


Figure 5.2: The best and worst case scenario analysis. The black diamond represents 'approaching from maximum change'; the white diamond represents 'approaching from minimum change'; and the black circle represents the 'entire data' set of seven replicate samples.

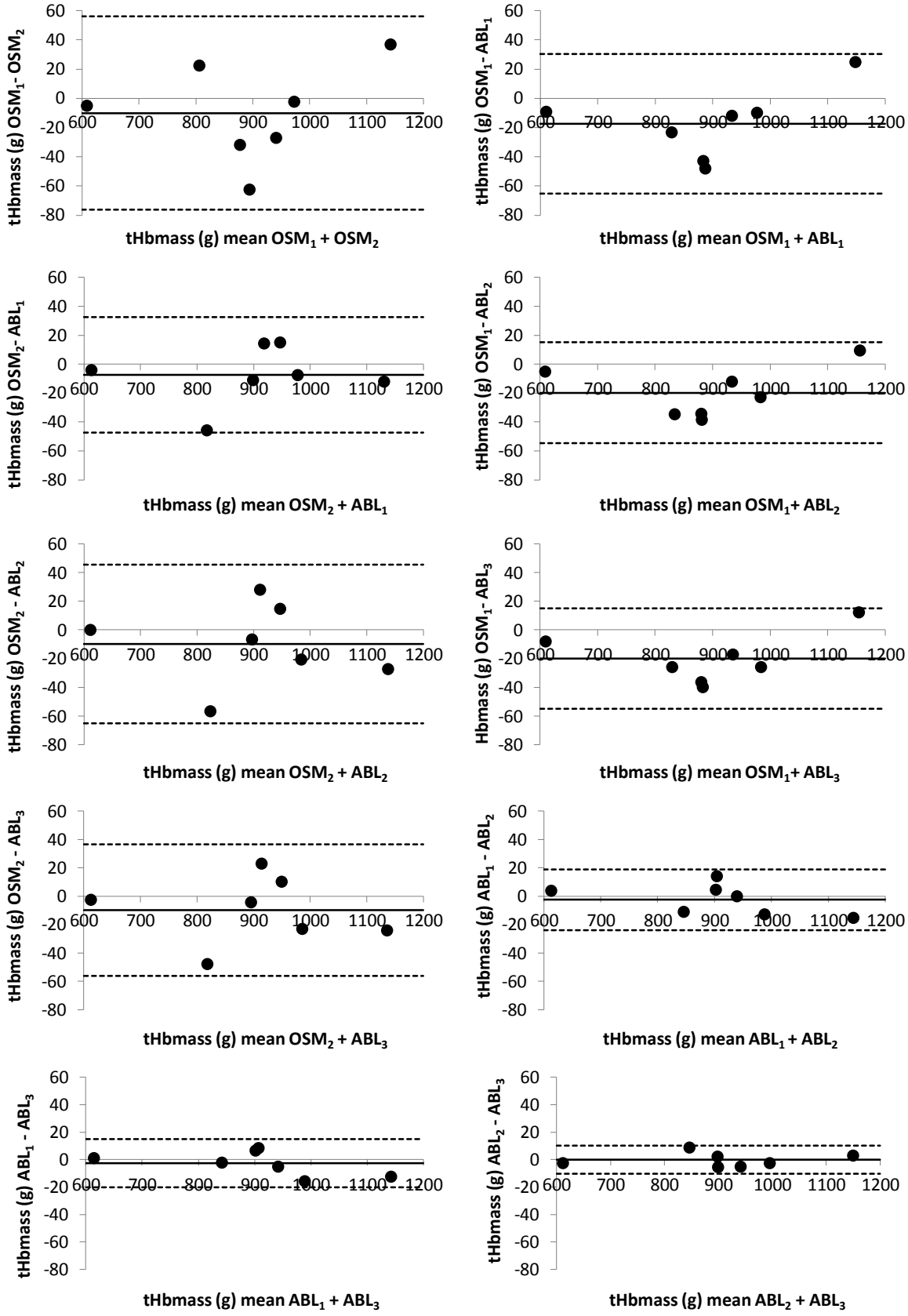


Figure 5.3: The difference between tHbmass calculated from the oCOR-method using different analysers

5.5 Discussion

The determination of tHbmass depends heavily on the reliability and precision of measuring blood %HbCO (Gore et al. 2005). This study determined the variability of seven replicates of %HbCO during the oCOR-method using five different Radiometer hemoximeters and the subsequent derived tHbmass values. Whilst there were differences ($P < 0.05$) found between the %HbCO measured on OSM3 and that on the ABL80, there were no differences ($P > 0.05$) in tHbmass calculated from Δ%HbCO between all of the hemoximeters. The oCOR-method uses this difference between pre- and 7 min %HbCO to calculate tHbmass. The ABL80 were less variable (mean cv pre sample = 3.5% and mean cv 7-min sample = 0.6%) compared to the OSM3 (mean CV pre sample = 6.7% and mean cv 7-min sample = 1.2%), meaning that fewer replicates were required for the ABL device to achieve a desirable level of precision compared to the OSM device (three replicates and five repicates, respectively).

Consequently, tHbmass results from the new ABL_1, ABL_2 and ABL_3 are reliable and accurate, which in the context of their usage across the EIS network is critical as they are located at three different laboratories. Experimenters can therefore be confident that an athlete can be tested at any of these laboratories and will elicit comparable results, providing the experimenter error is also low. Gough *et al* (2011) found that when using the OSM3, within-subject tHbmass profiles were more variable when tests were conducted in three different laboratories than in a single laboratory, due to differences between operators and equipment. In a separate study Alexander et al. (2011) also stated that the accuracy and reliability of the OSM3 influences the precision of tHbmass estimation. The present data found the influence was less in the ABL80 than the OSM3 hemoximeter. The machine-to-machine agreement between the ABL80 was better compared to the more variable OSM3.

Gough *et al* (2011) believed that the variability of tHbmass calculated in their study was most likely represented by the analytical component of variation associated with the technique, however, the error of measurement is a combination of biological and analytical variability (Gore et al. 2005). The present investigation conducted one oCOR-method per participant therefore the biological variation and reliability could not be assessed in the present experiment. The ABL_2 analyser has been previously shown to have a technical error of 0.8 ± 2.3% where the maximum period between tests was 16 days, therefore biological variation is likely to be minimal (see Chapter 6).

The results from the present study are comparable with that of Alexander *et al* (2011), who found that in order to reduce the theoretical Analyser Error of measuring %HbCO to ≤1% (using an *old* OSM3 hemoximeter), five replicates were necessary and that the change in %HbCO between before and after rebreathing a dose of CO should be ≥5.5% for the optimised oCOR-method. The present study found that the ABL80 hemoximeter required three replicates to reduce analyser error to ≤1%. The data demonstrated similar reliabilty for each of the three ABL80 hemoximeters, which indicates the performance characteristics of any well-calibrated ABL80 could be expected to be similar.

The number of replicates and differences in dose of CO administered may partially explain the wide variation in measurement error for tHbmass that was observed in the Gore *et al* (2005) meta-analysis. It should be noted that this study also used the OSM3 hemoximeter. Alexander *et al* (2011)

stated that researchers using only one or two measures of %HbCO could reach spurious conclusions about the imprecision of the oCOR-method, whereas those using larger doses of CO (to attain Δ%HbCO of ~5.5%), and using quintuplicate (or more) replicates, are more likely to be confident of their findings. Using the ABL80 the present data suggests that three replicates are sufficient to allow confidence in tHbmass. The present investigation administered a CO bolus of 1.0 $mL \cdot kg^{-1}$ during the oCOR-method and the blood samples were then analysed on OSM3 and ABL80 hemoximeters. It has been previously recommended (Burge and Skinner 1995; Gore et al. 2005) that an increased CO dose is associated with an increased precision of tHbmass estimation using a OSM3. The influence of CO dose using an ABL80 is investigated in Chapter 6 (Study 3).

The relative contribution of analytical/biological variation to tHbmass has been debated (Gough et al. 2011). However, Prommer *et al* (2008) concluded that the majority of variation over a period of 1 year in athletes was analytical in origin, rather than biological. Eastwood et al. (2008) also found little evidence of biological variation in their estimates of tHbmass in six men over a period of ~100 days. The use of a different analyser to calculate the same athletes tHbmass is likely to increase the analyitical error of the measurement rather than the biological variation. Radiometer has now discontinued the manufacture of the OSM3 analyser and with the availability of replacement parts and consumables likely to diminish, this inter-machine comparison process should be completed to establish the agreement to a contemporary hemoximeter or across multiple devices, as in the context of this original work.

5.6 Conclusion

The magnitude of biological error of tHbmass is uncertain (Eastwood et al. 2008). Alexander et al. (2011) reported the total error of measurement for tHbmass is almost completely technical in origin, with one component of technical error being analyser error and the remainder being residual technical error. The data from the present study is the first to show the agreement across multiple *new* Radiometer™ ABL80 hemoximeters is sufficient, both for the %HbCO and the derived tHbmass. Further, when compared to the *old* Radiometer OSM3 hemoximeters, the ABL80 hemoximeters are more reliable and consequently, less replicate samples are required (3 compared to 5) to achieve an acceptable level of precision. Total Hbmass can therefore be estimated with confidence in any EIS laboratories, so long as these recommendations are followed and experimenter error is minimised.

CHAPTER 6

6 THE INFLUENCE OF CARBON MONOXIDE BOLUS ON THE MEASUREMENT OF TOTAL HAEMOGLOBIN MASS USING THE OPTIMIZED CO-REBREATHING METHOD

6.1 Abstract

Purpose: The oCOR-method is routinely used to measure tHbmass. The tHbmass measure is subject to a test-retest typical error of ~2%, mostly from the precision of carboxyhaemoglobin (HbCO) measurement. It is hypothesised that tHbmass would be robust to differences in the bolus of CO administered during the oCOR-method. **Methods:** Twelve participants (10 males and 2 females; age 27 ± 6 yr, height 177 ± 11 cm and mass 73.9 ± 12.1 kg) completed the oCOR-method on four occasions. Different boluses of CO were administered (LOW: 0.6 mL·kg^{-1}; MED_1: 1.0 mL·kg^{-1} and HIGH: 1.4 mL·kg^{-1}); to determine the reliability of MED_1, a second trial was conducted (MED_2). **Results:** tHbmass was found to be significantly less from the HIGH CO bolus (776 ± 148 g) when compared to the LOW CO (791 ± 149 g) or MED_1 CO (787 ± 149 g) trials. MED_2 CO was 785 ± 150 g. **Conclusions:** The measurement of tHbmass is reliable to within 0.8%, but a small and notable difference was seen when using a HIGH CO bolus (1.4 to 1.9% less), potentially due to differences in CO uptake kinetics. Previously, an improved precision of the oCOR-method was thought to require a higher bolus of CO (i.e. larger Δ%HbCO), as commercial hemoximeters only estimate %HbCO levels to a single decimal place (usually ± 0.1%). With the new hemoximeter used in the present study, a bolus of 1.0 mL·kg^{-1} is reliable and falls within acceptable %HbCO safety limits.

6.2 Introduction

The oCOR-method (Schmidt and Prommer 2005) is minimally invasive and easy to perform (for both experimenter and participant), and is primarily used to quantify the tHbmass in blood. Survey analysis (Chapter 4, Study 1) found elite British athletes and support staff regularly utilise the measurement of tHbmass. In the field of sports medicine the routine determination of tHbmass and blood volume (BV) is valuable (Barker 1998) and small increases in tHbmass between 3-8% have been found when using altitude training (Fudge et al. 2012). As a result, small changes in tHbmass need to be quantified accurately, therefore the error of its measurement must only be 1-2% (Gore et al. 2005). The initial oCOR-method (Schmidt and Prommer 2005) was found to have a test-retest error for tHbmass of 1.7% and that was recently confirmed by Gore *et al* (2006) who attained a typical error of 1.2%. It should be noted that these studies used an *old* OSM3 hemoximeter.

A major contributor to test-retest analytic error of CO rebreathing is an inadequate dose of CO (Gore et al. 2005). In clinical settings, a CO dose of 1.0 mL·kg^{-1} (aiming for a ~6% increase in %HbCO) to a maximum dose of 100 mL, is believed to be adequate (Gore et al. 2005). However, in both a clinical and athletic settings, the precision of the analyser is important to detect small, but functionally meaningful changes in tHbmass and in this instance a dose of 1.0 mL·kg^{-1} may not be sufficient, and therefore a higher dose of CO may be required to obtain increased precision. The original study described by Schmidt and Prommer (2005) stated that the CO bolus should be 1.0 mL·kg^{-1} for trained males, 0.8 mL·kg^{-1} for untrained males, 0.7 mL·kg^{-1} for trained females and 0.6 mL·kg^{-1} for untrained females. Despite this numerous studies clearly defining their participants are still administering different bolus of CO (see Table 6.1). This variation could be problematic in relation to the Bruce and Bruce (2003) model of CO uptake kinetics throughout the vascular system whereby CO exhalation, loss to myoglobin (Mb) and ΔHbCO have substantial implications for the calculation of tHbmass (Garvican et al. 2010a). The bolus of CO administered during the oCOR-method will affect each of these mechanisms.

Table 6.1: The bolus of CO administered during the oCOR-method (as reported in the study).

Study	Participants	Bolus of CO (mL·kg^{-1})
Alexander *et al* (2011)	Recreationally active female volunteers	0.6
Clark *et al* (2009)	Well-trained males endurance cyclists	0.8
Robertson *et al* (2010a)	Elite male and female swimmers	1.0
Steiner and Wehrlin (2011)	Healthy & recreationally active male volunteers	1.2
Gough *et al* (2011)	Recreationally active participants	1.4
Siebenmann *et al* (2012)	Highly trained male and female participants	1.5

Sawka *et al* (2000) has criticized the CO-rebreathing method for not properly accounting for CO loss Mb, and thereby overestimating tHbmass. During the oCOR-method, CO enters the circulatory system via diffusion at the alveoli and binds to haemoglobin in competition with oxygen forming HbCO,

however, CO may diffuse to extravascular compartments, binding with other molecules, e.g. Mb (Garvican et al. 2010a), although according to Stewart (1975) to a lesser extent than Hb. The oCOR-method was most recently validated by Prommer and Schmidt (2007), using a CO bolus of 1.0 mL·kg^{-1}, and only a small volume of the administered CO (0.32% min^{-1}) was found to leave the vascular space, resulting in about a 2% increase in tHbmass values. The investigation has recommended using a correction factor of 0.3% of administered CO per min to exclude this source of error. Small errors relating to ΔHbCO have significant implications for the calculation of tHbmass; an error in ΔHbCO of only 0.1 unit value (equating to -1.7% at a ΔHbCO of 6.0%. i.e. 5.9% or 6.1%), results in a +1.7% error in derived tHbmass (e.g. 17 g if tHbmass = 1000 g) (Garvican et al. 2010a). It is probable that a smaller or larger bolus of CO will affect the sensitivity of the measurement of tHbmass, due to lesser or greater delta changes in HbCO and hence differences across the precision and linearity of the hemoximeters operation. It is conceivable that a smaller CO bolus may be useful in reducing the health risk in inhaling CO, whilst alternatively using a larger CO bolus may increase the change in HbCO and therefore, the precision of the method.

The purpose of this investigation was to examine the reliability of the oCOR-method and precision of the oCOR-method in response to different bolus of CO with a new hemoximeter compared to those employed previously. Chapter 5 (Study 2) found three replicates of HbCO necessary to achieve an analyser error of >1% using an ABL80 hemoximeter. A CO bolus of 1.0 mL·kg^{-1} is reflective of the 'gold standard' as a component of the testing procedure, as this was used by Prommer and Schmidt (2007). It is hypothesised that the tHbmass would be independent of the CO bolus used.

6.3 Methods

6.3.1 Participants

Twelve healthy and physically active participants (10 males and 2 females) age 27 ± 6 yr, height 177 ± 11 cm, body mass 73.9 ± 12.1 kg and body fat 12 ± 4 % volunteered for the study. The protocol and procedures described were approved by the institute Research Ethics and Governance Committee and conducted in accordance to the Declaration of Helsinki. All participants gave their written informed consent to participate. All trials were completed between the hours of 08:00 and 12:00 to ensure that any fluctuations in body mass were limited. The trials were completed in 12 ± 5 days Eastwood *et al* (2012a). For full participant inclusion and exclusion criteria see General Methods section 3.2.

6.3.2 Experimental design

On arrival participants provided a urine sample, which was analysed for urine specific gravity [(Uosm) Refractometer, Atago U.S.A. Inc.; WA, USA] and urine osmolality [(Uosm) Pocket PAL-OSMO Osmocheck™, Vitech Scientific Ltd; West Sussex, UK] to ensure they were euhydrated (Uosm < 400 mOsm·kg-^{1}) prior to testing. Participants then weighed themselves nude (Adams Equipment, Model GFK 150; Milton Keynes, UK). The experimental protocol consisted of each subject visiting the laboratory on four separate occasions completing the oCOR-method each time with the following bolus of CO administered in a randomized order, determined by a Latin squares design: 0.6 mL·kg^{-1} (LOW), 1.0 mL·kg^{-1} (MED_1) and 1.4 mL·kg^{-1} (HIGH). To determine the between day reliability of the oCOR-method, a second trial was conducted also with 1.0 mL·kg^{-1} (MED_2).

6.3.2.1 CO-rebreathing procedure

Total Hbmass, BV and PV were determined using the o-COR-method (Schmidt and Prommer 2005; Prommer and Schmidt 2007). The protocol and equipment used were previously described (see section 3.7). Fingertip capillary blood samples for the analysis [Hb] and Hct have been previously described in section 3.6.5.

6.3.3 Statistical analyses

Data were assessed for normality and sphericity and adjusted where necessary using the Huynh-Feldt method. The reliability of the oCOR-method (using 1.0 mL·kg^{-1} of CO) was evaluated using typical error of measurement (TEM) (Norton et al. 1996). Differences in tHbmass, BV, PV and ΔHbCO were calculated with one-way repeated measures ANOVA. Tukey's HSD post hoc tests were used to analyse significant pairwise differences according to the intervention utilized (CO bolus). Differences between test and retest, and the bias and limits of agreement of tHbmass from the different CO bolus administered, were also assessed (Bland and Altman 1986). All statistical tests were completed using SPSS Statistics 20 (International Business Machines Corp., Armonk, New York). Significance was accepted at $P < 0.05$. Values are reported as mean ± SD unless otherwise indicated.

6.4 Results

Using 1.0 mL·kg^{-1} of CO the TEM (± 95% confidence intervals) for tHbmass, BV and PV, were 0.8% (± 2.3%), 2.2% (± 6.0%) and 3.8% (± 10.5%), respectively. There was good agreement between tHbmass determined using a MED_1 vs. MED_2 bolus of CO during the oCOR-method [787 ± 149 g vs. 785 ± 150 g, r = 0.99, mean bias 2.4 g with 95% limits of agreement -24.7 to 29.4 g (Figure 6.1**A**)]. Table 6.2 outlines the tHbmass, BV and PV according to the different bolus of CO administered. As expected, whilst baseline %HbCO was the same, the Δ%HbCO was significantly different [F (2, 22) = 906.7, P < 0.001)] according to the different bolus of CO administered. The HIGH bolus resulted in a lower tHbmass [F (2, 22) = 4.865, P = 0.018)], BV [F (2, 22) = 5.237, P = 0.014)] and PV [F (2, 22) = 5.126, P = 0.015)] compared to LOW and MED_1. Total Hbmass determined using a MED_1 vs. LOW bolus of CO found similar bias but poorer agreement [788 ± 149 g vs. 791 ± 149 g, r = 0.98, mean bias 4.0 g with 95% limits of agreement -38.3 to 46.3 g (Figure 6.1**B**)]. tHbmass determined using a MED_1 vs. HIGH bolus of CO measured lower with better agreement [788 ± 149 g vs. 776 ± 148 g, r = 0.99, mean bias -11.0 g with 95% limits of agreement -39.9 to 17.8 g (Figure 6.1**C**)]. Finally, tHbmass determined using a LOW vs. HIGH bolus of CO again had a good agreement but was measuring higher [791 ± 149 g vs. 776 ± 148 g, r = 0.99, mean bias 15.1 g with 95% limits of agreement -12.6 to 42.7 g (Figure 6.1**D**)].

Table 6.2: oCOR-method measures from different bolus of CO.

	CO Bolus		
	LOW	**MED_1**	**HIGH**
ΔHbCO (%)	3.4 ± 0.4 *	5.6 ± 0.5 *	8.1 ± 0.8 †
tHbmass (g)	791 ± 149 *	787 ± 149 *	776 ± 148
BV (mL)	5389 ± 936 *	5234 ± 898	5110 ± 995
PV (mL)	3264 ± 601 *	3066 ± 490	3054 ± 636

* Indicates significant difference (P < 0.05) from HIGH; † Indicates significant difference (P < 0.05) from LOW

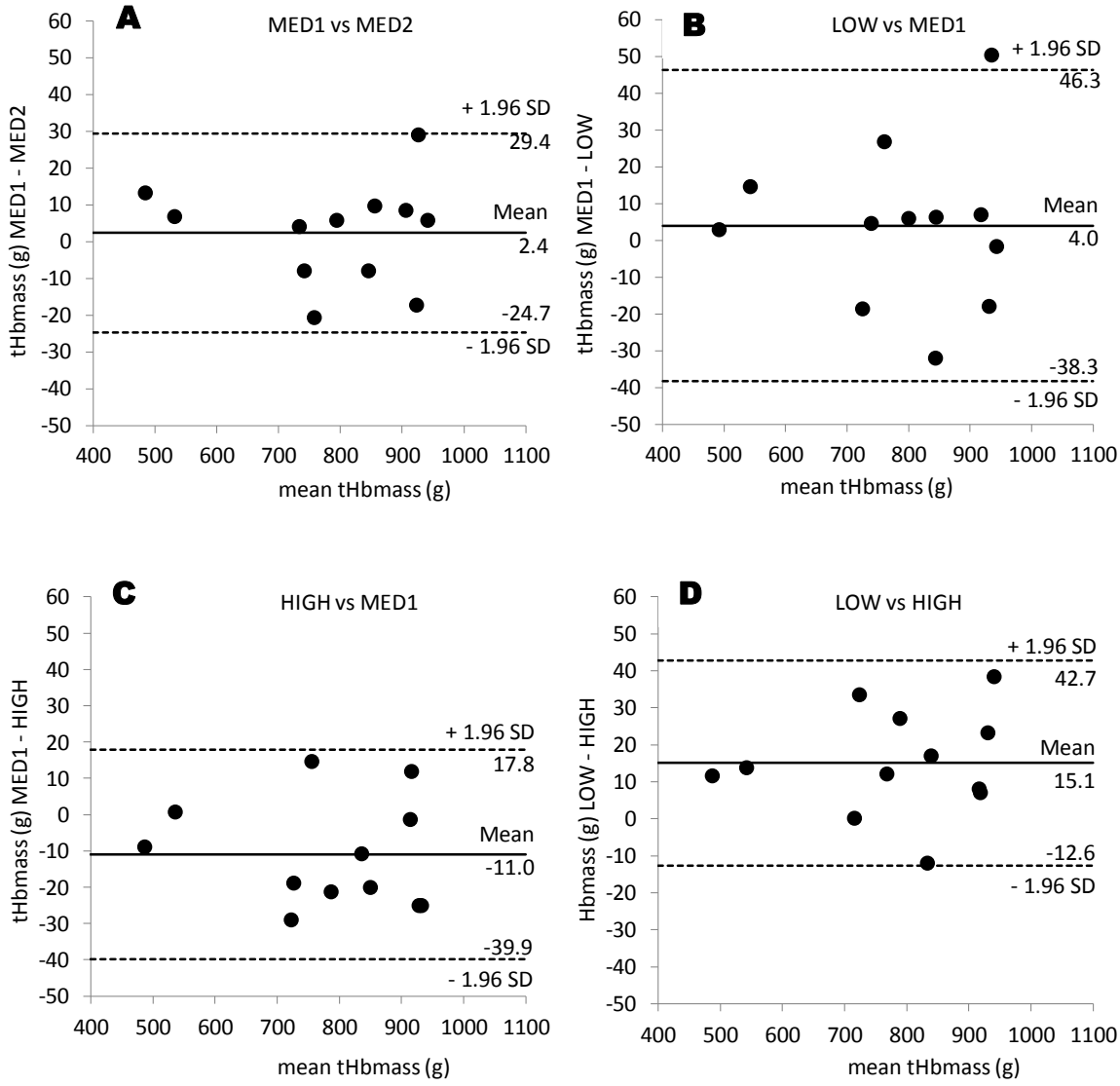


*Figure 6.1: tHbmass calculated from the different CO bolus. The individual data (n = 12) show the difference between tHbmass determined from MED_1 vs. MED_2 (**A**), from MED_1 vs. LOW (**B**), from MED_1 vs. HIGH (**C**) and from LOW vs. HIGH (**D**) on two separate occasions.*

6.5 Discussion

The present study found test-retest error for tHbmass to be 0.8% (replicating the oCOR-breathing method of Prommer and Schmidt (2007), using capillary blood and administering 1.0 mL·kg^{-1} CO). Taking into consideration the confidence limits of the error estimate, it is superior to that previously reported: 1.7 to 2.3% (Garvican et al. 2012), 1.3 to 1.5% (Wachsmuth et al. 2013) and 2.0% (Robertson et al. 2010b). The low typical error alongside the Bland-Altman plots indicates that good reliability was obtained for tHbmass using 1.0 mL·kg^{-1}. The present study also established the precision of the oCOR-method when administering different boluses of CO. The application of a CO bolus between 0.6 and 1.0 mL·kg^{-1} produced similar results, however, using a 1.4 mL·kg^{-1} bolus tHbmass was significantly less than when using 1.0 mL·kg^{-1} (-1.4%) and 0.6 mL·kg^{-1} (-1.9%).

The method of calculating tHbmass with the oCOR-method is relatively straightforward; however, the method is still subject to error. An adequate CO dose is particularly important if estimating %HbCO levels with commercial hemoximeters that display readings only to a single decimal place (usually ±0.1%) (Burge and Skinner 1995). Smaller doses of CO can be administered with well-trained technical staff (Gore et al. 2005), but with progressively lower doses of CO, a 0.1% difference in the %HbCO is associated with a substantial propagation of error in the calculation of tHbmass (Burge and Skinner 1995). A dose reduction is often made for patients with significant anaemia and/or morbid obesity (Gore et al. 2005). This suggests that if an individual with fewer RBCs needs a lower CO dose (to achieve a similar, Δ%HbCO) then an individual with a higher RBC count would need a higher CO dose to achieve the same precision of %HbCO measurement. Endurance athletes are likely to fall into that category, therefore it could be argued that a higher bolus of CO would be more appropriate for that population. The data suggested that in comparison to the reflective 'gold standard' of 1.0 mL·kg^{-1}, a higher bolus of CO would under predict tHbmass.

As previously mentioned, Bruce and Bruce (2003) model provided evidence that a 'significant fraction of inspired CO can be bound to muscle Mb'. Clark and Coburn (1975) also reported that at 1-2% HbCO, CO can move into muscular tissue and to some extent potentially bind to Mb. Richardson et al (2002) offered alternative evidence to suggest that a level of CO that induces a ~20% increase in HbCO exhibits no additional net movement out of the vascular space and no movement from blood to skeletal muscle or Mb binding. The oCOR-method aims to elevate HbCO levels by 5-8%, and this appears to be appropriate, however Richardson et al (2002) argues for the use of the higher HbCO levels, as this appears to minimize CO loss into the tissue. The uptake kinetics of CO has been investigated previously. Garvican *et al* (2010), using 1.5 mL·kg^{-1} of CO, found a 1.8% loss of CO to Mb and Prommer and Schmidt (2007), using 1.0 mL·kg^{-1} of CO, found a 2.3% loss of CO to Mb. It's difficult to compare these finding when different boluses of CO were used, however, it would appear that the findings contradict that of Richardson *et al* (2002), whereby when using less CO, more is lost to Mb. In the present study with a higher CO bolus it is unclear what percentage of CO was lost to Mb, and the effect that would have on the Δ%HbCO and the subsequent calculated tHbmass.

Whilst a difference of 0.4 mL·kg^{-1} in CO bolus makes no substantial difference to the safety of the method (Burge and Skinner 1995), we show here that 1.4 mL·kg^{-1} of CO yielded a significantly different tHbmass, BV and PV, compared to using 1.0 mL·kg^{-1} or 0.6 mL·kg^{-1}. Much of the initial

development, and more recent quality control research (Alexander et al. 2011; Gough et al. 2011), using the oCOR-method was completed using the Radiometer OSM3 hemoximeter (OSM3). This investigation used the new ABL80 FLEX CO-OX hemoximeter (ABL80), which according to Chapter 5 has a lower within-subject coefficient of variation (OSM3: pre-oCOR-method = 7.3% and at 7 min = 1.0%; ABL80: pre-oCOR-method = 3.7% and at 7 min = 0.7%). This improved response of the ABL80 may mean better detection of smaller doses of CO and certainly means less replicate blood samples are required at each time point to achieve the same acceptable sample-to-sample variance. This may explain the LOW bolus of CO being similar to MED_1 but not to the HIGH bolus, which was previously thought more valid.

CO-rebreathing has previously used a dose of 50 mL CO irrespective of body weight (Thomsen et al. 1991). Consequently, for those with a larger tHbmass, %HbCO represents exponentially larger errors in the estimation of tHbmass (Burge and Skinner 1995). Gore et al (2005) believed that modifications of the CO-rebreathing technique may affect the magnitude of measurement error for CO-rebreathing and its related volumes. The authors recommended that, to minimise likely sources of error, researchers should use relatively large doses of CO, a small rebreathing volume, and at least four replicate measures of %HbCO. At this end of the curve, increased doses of CO are associated with exponential rises in HbCO saturation. Burge and Skinner (1995) believed that a dose of CO to induce a ~6.5% ΔHbCO provides a satisfactory margin of safety and a good degree of sensitivity and precision for the estimation of tHbmass and BV. During the present study the CO bolus administered elicited Δ%HbCO ranging from 3.5% (LOW) to 5.7% (MED) and 8.2% (HIGH). Stewart (1975) stated that CO toxicity can be raised to 15% without untoward effects in normal individuals; therefore a Δ%HbCO of up to 10% is safe. It was previously assumed that an increased CO dose was required when using hemoximeter to obtain adequate precision (Burge and Skinner 1995), however, the variable doses of CO in the present study did not result in any measurement error as the coefficient of variation during all four trials was the same at 0.01%. Consequently, the ABL80 was able to measure lower doses of CO in the body with the same precision as higher doses.

The use of CO-rebreathing to measure tHbmass has been previously compared to 'gold standard' methods (Burge and Skinner 1995; Schmidt and Prommer 2005; Gore et al. 2005) and was found to have a lower coefficient of variation (Burge and Skinner 1995) and lower error of measurement (Gore et al. 2005). In the most recent validation of the oCOR-method Prommer and Schmidt (2007) used 1.0 $mL \cdot kg^{-1}$ when calculating tHbmass, therefore this is believed to be reflective of the 'gold standard'. However, despite there being a clear effect of CO bolus on the resultant tHbmass values, it is difficult to state which bolus of CO would provide the true reading for tHbmass without comparisons to the original Burge and Skinner (1995) method. As a result of our findings it is difficult to compare tHbmass values in previously published literature where different boluses of CO are administered. Therefore, caution must be taken if tHbmass values are used to assess the effectiveness of the altitude training strategies in different studies. Furthermore, when testing athletes and patients over an extended period of time, the consistent use of the same volume of CO becomes essential. The present investigation used a cohort of participants that were homogenous in their activity levels so the tHbmass results were similar and comparable to a wider population. To provide a clearer picture of

the effect of different boluses of CO on tHbmass the investigation should be extended to a clinical population with low tHbmass and to elite athletes where tHbmass is high.

6.6 Conclusion

Administering a different bolus of CO between 0.6 and 1.0 mL·kg^{-1} (LOW and MED respectively) produced similar tHbmass values, however, using a HIGH bolus (1.4 mL·kg^{-1}) tHbmass values were significantly less than MED_1 (-1.4%) and LOW (-1.9%). It is therefore important that EIS laboratories are consistent with the volume of CO used. It was previously believed that to improve the precision of the oCOR-method a higher bolus of CO was needed, to elicit a greater change in %HbCO, as commercial hemoximeters only estimate %HbCO levels to a single decimal place (usually ± 0.1%). However, with newer and less variable hemoximeters such as the ABL80 used in this study (see Chapter 5, Study 2), the present data suggests that a bolus of 0.6 to 1.0 mL·kg^{-1} provides sufficient precision and is within safety margins. The present study has reaffirmed the reliability of tHbmass measured with the oCOR-method and revealed a CO bolus of 0.6 to 1.0 mL·kg^{-1} to be appropriate, especially when taking into consideration the importance of how much CO is lost to Mb for the calculation of tHbmass. The CO bolus selected may be dependent upon the population (clinical or athletic) being tested, and always ensuring a minimum increase in HbCO of ≥ 5% is achieved.

CHAPTER 7

7 THE TIME-COURSE OF ENDOGENOUS ERYTHROPOIETIN, IL-6 AND TNFα IN RESPONSE TO ACUTE HYPOXIC EXPOSURES

7.1 Abstract

Purpose: Erythropoietin rapidly decreases on return to sea level after chronic altitude exposure. Acute hypoxia may provide an additional stimulus to prevent the decline in EPO. Pro-inflammatory cytokines, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) have been shown to inhibit EPO production. The optimal normobaric hypoxic exposure to elicit the greatest increase in EPO, whilst limiting inflammation, has not been established. **Methods:** Eight males (age 27 ± 4 yr, body mass 77.5 ± 9.0 kg, height 179 ± 6 cm) performed four passive exposures to different normobaric hypoxic severities [FiO_2: 0.209 (SL), FiO_2: ~0.135 (3,600 m), FiO_2: ~0.125 (4,200 m) and FiO_2: ~0.115 (4,800 m)] in a hypoxic chamber for 2 h. Venous blood was drawn pre-exposure and then at 1, 2, 4, 6 and 8 h to determine erythropoietin concentration ([EPO]), IL-6 and TNFα. **Results:** During 4,200 m and 4,800 m [EPO] increased from 5.9 ± 1.5 to 8.1 ± 1.5 mU·mL^{-1} (P = 0.009) and 6.0 ± 1.4 to 8.9 ± 2.0 mU·mL^{-1} (P = 0.037), respectively, with [EPO] increase peaking at 4 h (2 h post-exposure). There were no differences in IL-6 or TNFα during, or post-exposure. **Conclusions:** The present study was the first to show an increase in [EPO], peaking 2 h post-hypoxic exposure, after 2 h of normobaric hypoxia (≥4,200 m). Additionally, there was no dose-response relationship in [EPO] between simulated hypoxia severities. A novel aspect of the study was that pro-inflammatory cytokines (IL-6 and TNFα) did not inhibit the production of [EPO] or increases as a result of the 2 h of normobaric hypoxia.

7.2 Introduction

Exercise performance and haematological responses have been shown to be variable, both in response to exercise at altitude, and on return to sea level after altitude training (Chapman et al. 1998; Chapman 2013). Chapter 4 (Study 1) found that elite British athletes and support staff disagreed on the best time to compete after an altitude training camp, therefore individualising an athlete's post-altitude training strategy to optimise the hypoxia induced adaptation of the camp should be considered. Elite coach, Dick (1992), discussed training at altitude in practice and suggested that on return from altitude, time was needed to reach a stage were performance shows a clear sign of benefit. Although these assumptions were based upon the training status of the athlete, there is haematological evidence to suggest there is a 're-acclimatisation' that occurs when returning to sea level from altitude. Garvican et al. (2012) observed a 1.5% decrease in tHbmass within 3 days of decent from a 3 week natural altitude training camp, which persisted when measured 10 days after decent. Pottgiesser et al. (2012) also found that after a 26 night simulated altitude training camp there was a 3.0% reduction in tHbmass within 9 days at sea level. Likewise, Brugniaux et al. (2006) and Heinicke et al. (2005) both found an initial increase in tHbmass as a result of simulated and natural LHTL, respectively, but tHbmass returned to baseline levels within 15-16 days at sea level.

Prommer et al. (2009) found that when natural altitude dwellers reside at sea level for sustained durations, a reduction in tHbmass occurs. The study found tHbmass remained stable within the first 2 weeks at sea level followed by a reduction of ~2% per week before levelling off around 5-6 weeks post-altitude. The reduction in tHbmass was attributed to transiently suppressed EPO as a result of returning to a normoxic environment (Prommer et al. 2010). The removal of the altitude stimulus appears to result in a 're-acclimatization' to sea level (Garvican et al. 2012). Chapman et al. (2014c) stated that, if an athlete completes a 4 week altitude training camp, followed by a short time (~7–14 days) at sea level to compete, returning to altitude even for a short time may mitigate or delay these effects by re-establishing EPO levels again.

Brief periods of severe hypoxia have been suggested as a potential method to prevent the sudden decrease in EPO (Pottgiesser et al. 2012). This in turn would preserve the haematological acclimatisation response for a longer time, thereby expanding the window for endurance training and optimal competition racing (Chapman et al. 2014b). Figure 7.1 illustrates the previous investigations that have demonstrated an increase in EPO as a result of hypoxic exposures lasting 5 minutes to 5.5 hours (Eckardt et al. 1989; Knaupp et al. 1992; Rodríguez et al. 2000; Ge et al. 2002; Niess et al. 2004; Friedmann et al. 2005a; MacKenzie et al. 2008; Wahl et al. 2013). The chart bubble size represents the magnitude of EPO production in response to exposure time and simulated altitude (i.e. the larger the bubble, the greater the production of EPO). Collectively these findings suggest that a minimum time period of two hours at an altitude of >2,500 m elicit a significant increase in EPO. Although, it should be noted that these studies have used differing methods (hypobaric vs. normobaric), physical status (rest vs. exercise) and varied timing of blood sampling, that may not be accessible or possible in different scenarios.

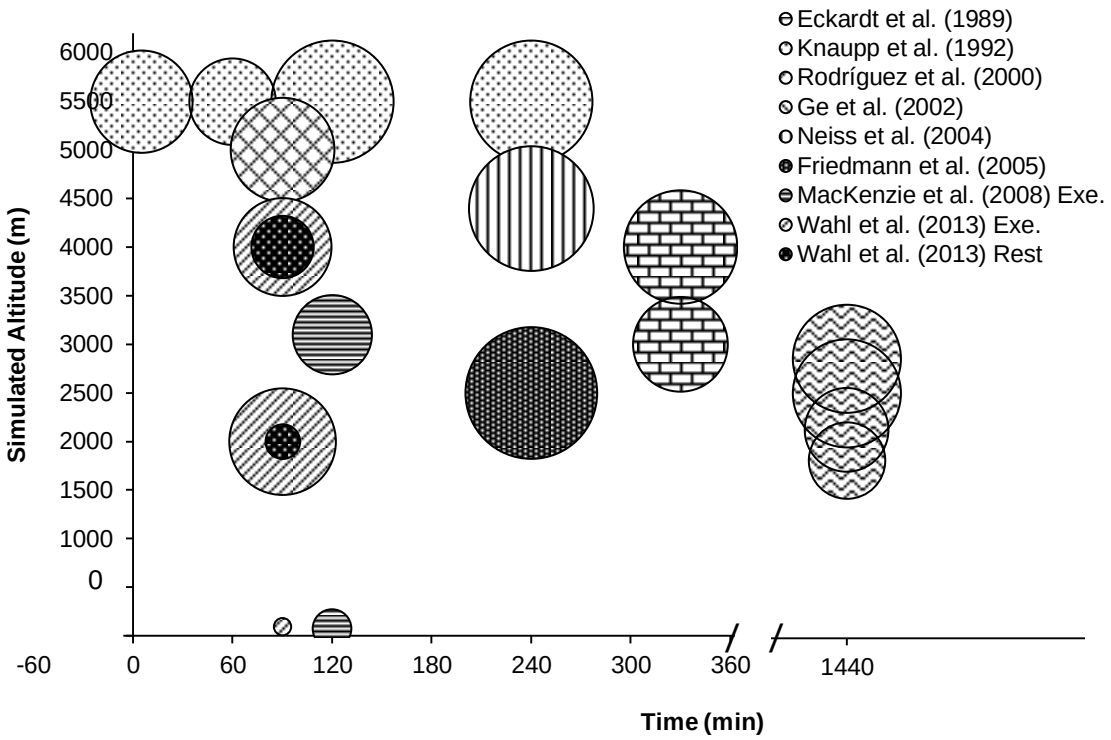


Figure 7.1: EPO response to hypoxic exposure duration and simulated altitude. The different sizes of bubbles represent the magnitude of increase in EPO as a result of hypoxic exposures. Different patterns within bubble indicate different investigations

Stray-Gundersen et al. (2001) demonstrated that when athletes attend altitude training camps it is possible that the presence of injury or infection, and therefore pro-inflammatory cytokines (such as IL-6 and TNFα), could impair the erythropoietic response to altitude (Faquin et al. 1992; Jelkmann et al. 1992). Hypoxia is a stressor that alters homeostasis (subsequent to a reduction in SaO_2) and may result in an inflammatory response (Mazzeo et al. 2001b). Previous research has found that after 3 nights at elevations higher than 3,400 m, levels of IL-6 and C-reactive protein (CRP) were increased in persons with acute mountain sickness (Hartmann et al. 2000) and similarly, after 12 nights at 4,300 m IL-6 was elevated (Mazzeo et al. 2001b). Conversely, Schobersberger et al. (2004) found that after a 3-week sojourn (living and hiking) at moderate altitude (1,700 – 2,500 m) there were no differences in IL-6 or TNFα in clinical trials of individuals with metabolic syndrome. As a result, if additional hypoxic exposures are to be considered post-altitude training then the role of pro-inflammatory cytokines and EPO production should be investigated.

Altitude training is a common practice undertaken by endurance athletes in pursuit of an enhancement of subsequent sea level performance (Fudge et al. 2012), however, identifying the best time to return to sea level prior to a major competition to optimise the haematological gains remains a question (Chapman et al. 2014b). A minimum hypoxic exposure of two hours is required to elicit an increase in EPO; however, less is understood about the effect of a two hour exposure of normobaric hypoxia on EPO and the time course of this response. Furthermore, the response of inflammatory cytokines to differing levels of hypoxia in healthy individuals is unclear. The aim of this study was to establish the dose-response relationship of EPO, alongside markers of inflammation IL-6 and TNFα,

during and following two hour exposures of normobaric hypoxia with the intention of mitigating decrements in EPO post-altitude training. It is hypothesised that [EPO] would increase following two hour normobaric hypoxic exposure, and the increase would be in accordance with the severity of hypoxia. Secondly, basal levels of pro-inflammatory cytokines would inhibit the production of [EPO] in participants leading to lesser change.

7.3 Methods

7.3.1 Participants

Eight physically active, Caucasian males participated in the study (see Table 7.1). Participants were trained, completing 8 ± 3 hours training per week. Institutional ethical approval was issued in accordance with the Declaration of Helsinki 1975 (revised 2013) and participants provided written informed consent. For full participant inclusion and exclusion criteria see General Methods section 3.2.

Table 7.1: Participant anthropometric characteristics and baseline biochemical values

Characteristics	
Age (yr)	27 ± 4
Stature (cm)	179 ± 6
Body Mass (kg)	77.5 ± 9.0
Body Fat (%)	13.1 ± 2.5
$\dot{V}O_{2max}$ (ml·kg·$^{-1}$min^{-1})	50.9 ± 8.2
[EPO] (mU·mL^{-1})	5.95 ± 1.51
IL-6 (pg·ml^{-1})	8.86 ± 7.17
TNFα (pg·ml^{-1})	3096 ± 3935

Values are mean ± SD.

7.3.2 Experimental design

The outline of this single blind, randomised and controlled study is presented in Figure 7.2. Participants attended the laboratory in Eastbourne, UK (10 m altitude) on an individual basis on five separate occasions; once for preliminary measurements; three involved resting in a hypoxic environment at three different levels of hypoxia and once resting in normoxia. Participants were required to attend the laboratory for eight and a half hours for each experimental trial. They were instructed to eat the same breakfast before each visit, were supplied with a standardised isocaloric lunch and drank water ad libitum throughout the testing procedure. The order of the trials was randomised, determined by a Latin squares design. Each trial was separated by a seven day wash out period (MacKenzie et al. 2008). All trials commenced between 07:30 and 09:30, to control for diurnal variations in EPO (Klausen et al. 1993; Klausen et al. 1996).

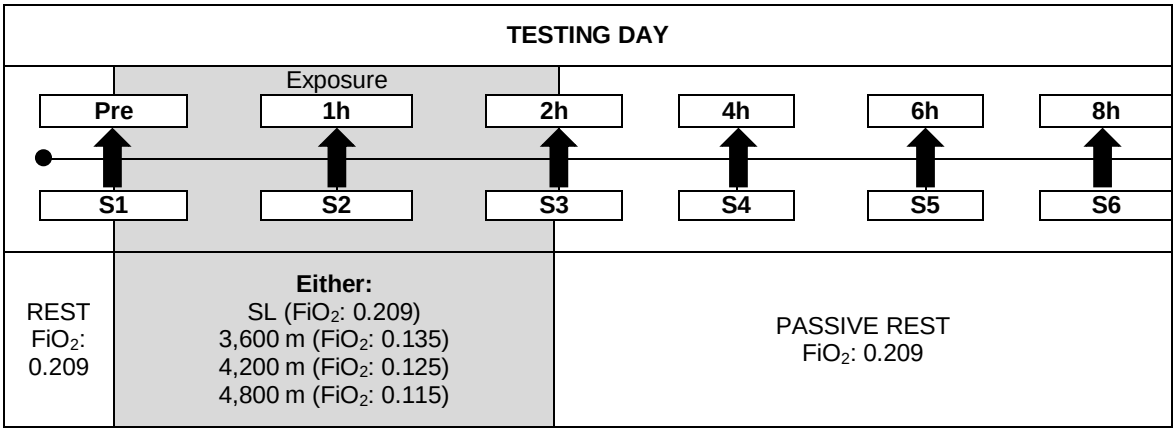


Figure 7.2: Schematic representation of the study design. Haematological data (S = blood sample, ↑ = timing of blood sample) was collected pre-exposure (Pre), during exposure (1h and 2h) and post-exposure (4h, 6h and 8h). The participants were exposed to four different conditions of; FiO_2: 0.209 (SL; sea level), FiO_2: 0.135 (3,600 m), FiO_2: 0.125 (4,200 m) and FiO_2: 0.115 (4,800 m).

7.3.3 Experimental procedures

7.3.3.1 Preliminary measures

Anthropometric data were collected with body mass measured using digital scales (GFK 150, Adam Equipment Inc., Danbury, CT, USA) and body fat assessed from four sites (biceps, triceps, subscapular and supra-iliac) as described by Durnin and Womersley (1974) using skinfold calipers (Harpenden, Burgess Hill, UK). For the assessment of aerobic capacity, participants then performed a standardised stepwise incremental test on a cycle ergometer. Cycling started at 80 W, increasing by 24 W each minute, as previously described by Gibson et al. (2015).

7.3.3.2 Hypoxic exposures

Upon arrival participants weighed themselves and provided a urine sample (see section 3.8 for description). Participants then sat and rested for 15 min for a resting heart rate (HR) and oxygen saturation (SpO_2) to be measured prior to entering the chamber.

Following this period participants spent two hours resting in a normobaric hypoxic chamber achieved using a purpose-built, nitrogen-enriched chamber (Altitude Centre; London, UK), at four different hypoxic severities [FiO_2: 0.209, (SL), FiO_2: 0.135, (3,600 m), FiO_2: 0.125, (4,200 m) and FiO_2: 0.115, (4,800 m)]. Oxygen fraction was monitored and adjusted continually by automated computer feedback. Participants remained seated during the two hours exposure. The laboratory environmental conditions [temperature = 22.8 ± 0.7°C, relative humidity = 38.4 ± 1.8%, pressure = 760 ± 2mmHg, $FiCO_2$ (range) = 0.05 – 0.1%] were stable throughout. For the remaining six hours, participants rested in normoxic conditions in temperate laboratory conditions.

FiO_2 was measured in the chamber every 15 min. SpO_2 was measured every 15 min, should participants stay below 70% they were removed from the chamber (zero incidences). The Lake Louise Questionnaire was also completed every 30 min to assess for symptoms off acute mountain sickness (AMS). Should a participant remain at 6 or above, they were removed from the chamber (zero incidences).

7.3.3.3 Physiological measures

Heart rate (Polar 810i heart rate monitor; Kempele, Finland) and SpO_2 (Nonin 2500, Nonin Medical Inc., Plymouth, MN, USA) were measured for 30 s every 15 min whilst in the hypoxic chamber and every 30 min outside of the chamber.

7.3.3.4 Haematological measures

For venous blood sampling a cannula (18G x 1.5" BD Venflon I.V. Cannula; BD Infusion Therapy AB, Helingborg, Sweden) was positioned in the antecubital fossa. Blood samples were taken before entering the chamber, after one and two hours whilst in the chamber and at four, six and eight hours outside the chamber. After discarding the first 1 ml, venous blood was collected (~8 ml) with a plastic syringe (10 ml BD Plastipak; Becton & Dickinson UK, Oxford, UK) and dispensed into two 5ml K-EDTA collection tubes (Sarstedt Ltd., Leicester, UK) prior to centrifugation at 2,200 rpm (Eppendorf Refrigerated Centrifuge Model 5702R; Eppendorf UK Ltd., Stevenage, UK) for 15 min to separate plasma. Plasma was pipetted (Eppendorf Research/Research Pro) into 1.5 ml microtubes (Western Laboratory Service, Hampshire, UK) and stored at a -85°C (Sanyo Ultra Low, VIP Series, Watford, UK) until the samples were analysed.

[EPO], IL-6 and TNFα concentrations were measured in plasma for all four trials (SL, 3,600 m, 4,200 m, and 4.800 m). Enzyme-linked immunosorbent assays were used in accordance with manufacturer instructions to determine concentrations for [EPO] (Roche Diagnostics Ltd., Lewes, UK) and for IL-6 and TNFα (DuoSet ELISA Development System; R&D Systems Inc., Abingdon, UK). The technical error of measurement (TEM) between duplicate samples for [EPO] was 3.8%, with a unit error value of 0.7 $mU \cdot mL^{-1}$, for IL-6 it was 7.1%, with a unit error value of 2.76 $pg \cdot mL^{-1}$ and for TNFα it was 4.1%, with a unit error value of 518.7 $pg \cdot mL^{-1}$.

7.3.4 Statistical analyses

Data were assessed for normality and sphericity and adjusted where necessary using the Huynh-Feldt method. Differences in [EPO], IL-6 and TNFα at each time point (e.g. Pre, 1h, 2h, 4h, 6h and 8h.), HR and SpO_2 were analysed with a two-way repeated measures ANOVA (hypoxia x time), with Bonferroni correction used to determine differences between conditions. For statistical analysis SpO_2 and HR measurements were averaged for each hour in the chamber and every two hours outside of the chamber. A Pearson product moment correlation was used to characterise the association between

peak Δ[EPO] and desaturation during hypoxic exposure, baseline IL-6 and baseline TNFα. TEM calculations were carried out on duplicate [EPO], IL-6 and TNFα samples using a method previously described by (Norton et al. 1996). All statistical tests were completed using SPSS Statistics 22 (International Business Machines Corp., Armonk, New York). Significance was accepted at $P < 0.05$. Values are reported as mean ± SD unless otherwise indicated. Effect sizes for main effects and interactions are presented as partial eta squared (η_p^2) in accordance with Lakens (2013).

7.4 Results

7.4.1 Physiological measures

There was an effect on SpO_2 over time ($F = 927.298$, $P = 0.001$, $\eta_p^2 = 0.99$), in hypoxia ($F = 258.717$, $P = 0.001$, $\eta_p^2 = 0.97$) and an interaction effect between time*hypoxia ($F = 156.411$, $P = 0.001$, $\eta_p^2 = 0.96$). Bonferroni comparison identified differences between pre-exposure (Pre) and during exposure (1 h/2 h), respectively, at 3,600 m ($P = 0.001$), 4,200 m ($P = 0.001$) and 4,800 m ($P = 0.001$). Between trials significant differences in SpO_2 were found at 1h ($P = 0.001$) and 2h ($P = 0.001$) between SL (98 ± 0 and 98 ± 1%), 3,600 m (87 ± 2 and 87 ± 3%), 4,200 m (83 ± 1 and 83 ± 1%) and 4,800 m (76 ± 2 and 75 ± 3%). No differences were found within the SL trial. Post-hoc data are presented in Figure 7.3A.

There was an effect on HR over time ($F = 21.294$, $P = 0.001$, $\eta_p^2 = 0.75$), in hypoxia ($F = 11.739$, $P = 0.001$, $\eta_p^2 = 0.63$) and an interaction effect between time*hypoxia ($F = 9.837$, $P = 0.001$, $\eta_p^2 = 0.59$). Bonferroni comparison identified significant differences ($P = 0.022$) between mean HR at SL (57 ± 7 beats·min^{-1}) and 4,800 (65 ± 10 beats·min^{-1}). At SL no differences were observed over time. At 4,200 m and 4,800 m HR at was significantly higher during the exposure period ($P < 0.05$) at 1h (respectively 66 ± 5 and 75 ± 8 beats·min^{-1}) and 2h (63 ± 6 and 72 ± 10 beats·min^{-1}), compared to the post exposure period at 4h (respectively 58 ± 4 and 61 ± 9 beats·min^{-1}), 6h (58 ± 6 and 60 ± 7 beats·min^{-1}) and 8h (55 ± 8 and 58 ± 9 beats·min^{-1}). Post-hoc data are presented in Figure 7.3B.

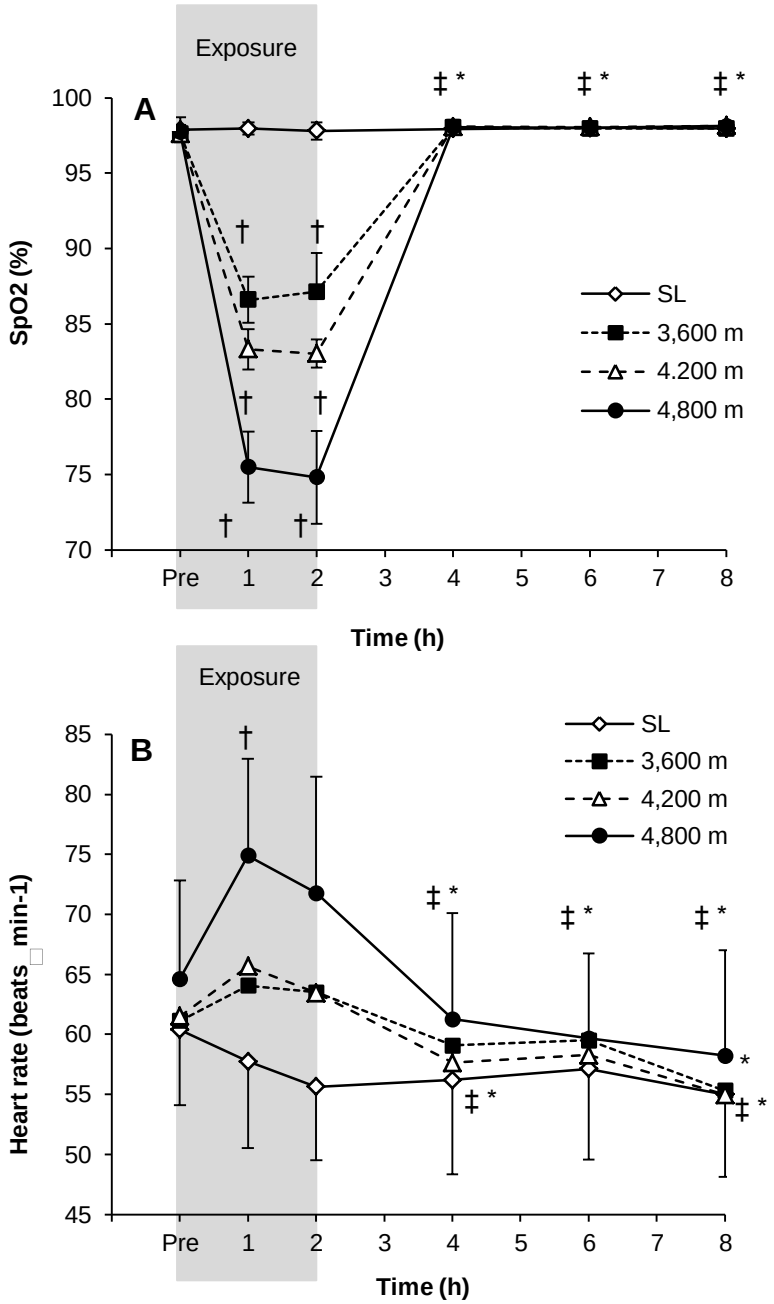


*Figure 7.3: Difference in SpO_2 (A) and HR (B) after two hour exposure to SL, 3,600 m, 4,200 m and 4,800 m. Values are means ± SD. († P ≤ 0.05 denotes differences from Pre, ‡ P ≤ 0.05 denotes differences from 1h and * P ≤ 0.05 denotes differences from 2 h). The grey box illustrates the exposure time. Note 3,600 m and 4,200 m error bars removed for clarity.*

7.4.2 Haematological Measures

An effect on [EPO] was observed over time (F = 9.959, P = 0.001, η_p^2 = 0.59). Mean [EPO] peaked at 4h in 3,600 m, in 4,200 m and in 4,800 m. No differences were observed in [EPO] from pre-exposure (5.36 ± 1.61 mU·mL^{-1}) to 8h (6.4 ± 1.45 mU·mL^{-1}) during the SL trial. Bonferroni comparison identified a difference in [EPO] between Pre and 4h at 4,200 m (P = 0.009) and at 4,800m (P = 0.037), but not at 3,600 m. There was no main effect for hypoxia (F = 0.359, P = 0.704, η_p^2 = 0.05), nor an interaction effect between time*hypoxia (F = 1.296, P = 0.250, η_p^2 = 0.16). Figure 7.4 illustrates the response of plasma [EPO] during each trial.

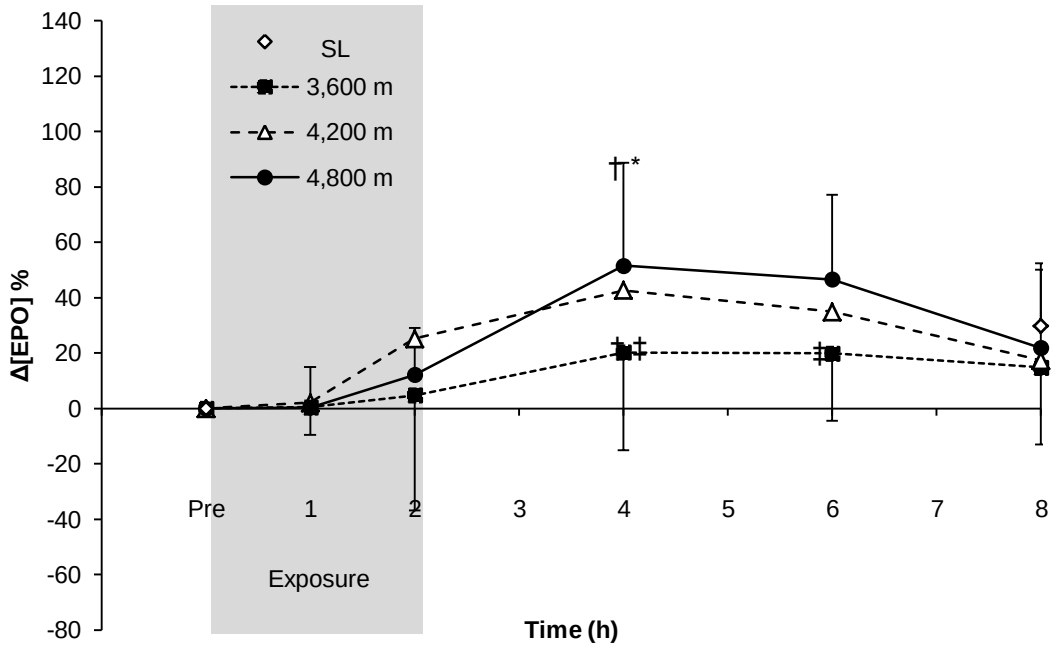


*Figure 7.4: Percentage differences from baseline in erythropoietin concentration (Δ[EPO]) of blood plasma after 2 h exposure to SL, 3,600 m, 4,200 m and 4,800 m. Values are means ± SD. († P ≤ 0.05 denotes differences from Pre, ‡ P ≤ 0.05 denotes differences from 1h and * P ≤ 0.05 denotes differences from 2 h). The grey box illustrates the exposure time. Note 4,200 m error bars removed for clarity.*

No effect on IL-6 was found over time (F = 0.683 P = 0.547, η_p^2 = 0.09), hypoxia (F = 0.242, P = 0.789, η_p^2 = 0.03), or an interaction effect between time*hypoxia (F = 0.465, P = 0.907, η_p^2 = 0.06). Figure 7.5 illustrates the response of plasma IL-6 during each trial.

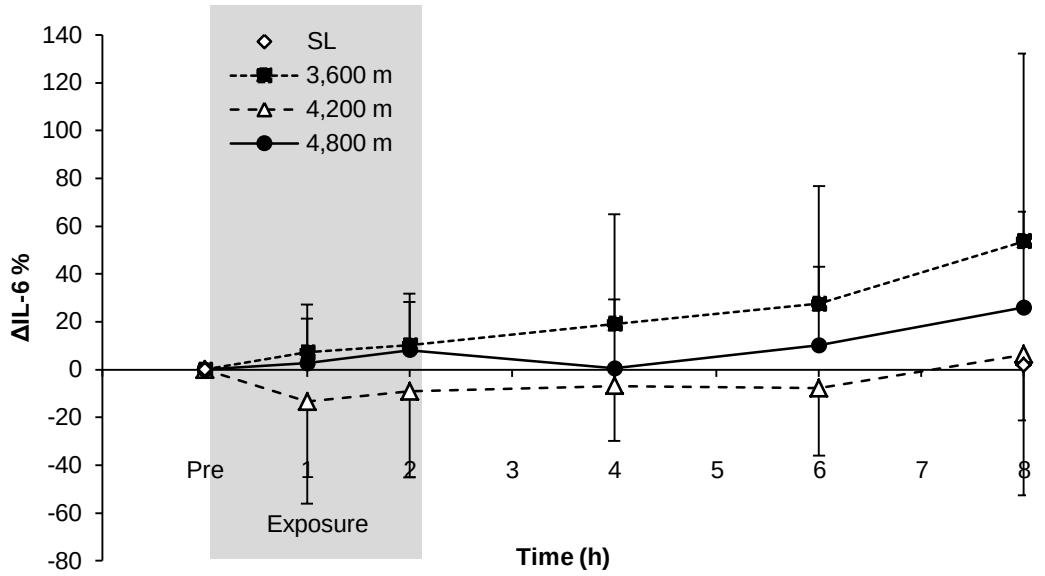


Figure 7.5: Percentage differences from baseline in interleukin-6 (Δ IL-6) of blood plasma after 2 h exposure to SL, 3,600 m, 4,200 m and 4,800 m. Values are means ± SD. The grey box illustrates the exposure time.

There was also no effect on TNFα found over time (F = 1.748, P = 0.182, η_p^2 = 0.20), hypoxia (F = 0.945, P = 0.412, η_p^2 = 0.12), or an interaction effect between time*hypoxia (F = 1.545, P = 0.142, η_p^2 = 0.18). Figure 7.6 illustrates the response of plasma TNFα during each trial.

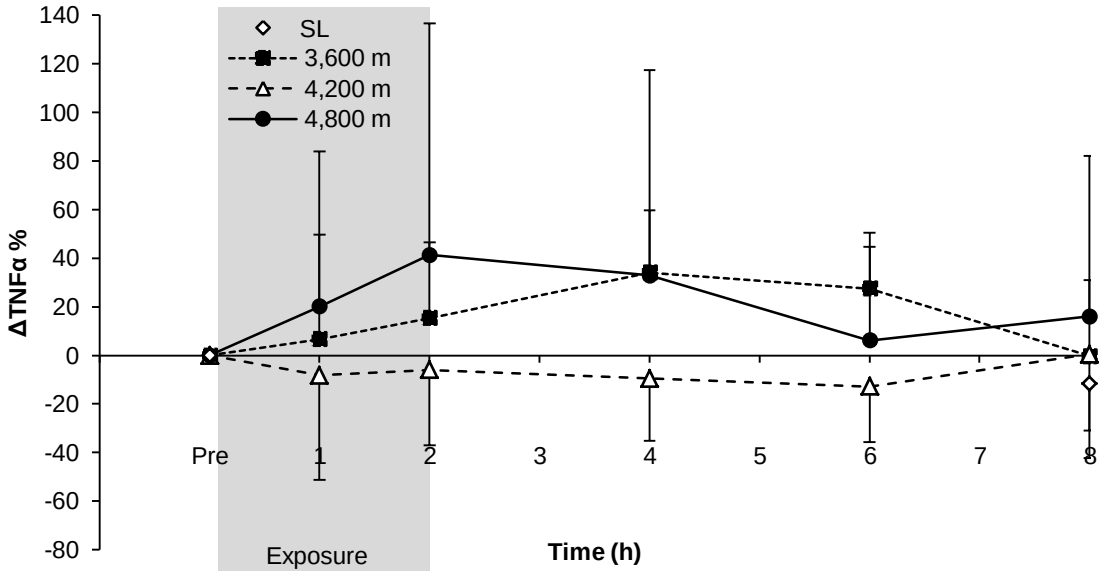


Figure 7.6: Percentage differences from baseline in tumor necrosis factor alpha (ΔTNFα) of blood plasma after 2 h exposure to SL, 3,600 m, 4,200 m and 4,800 m. Values are means ± SD. The grey box illustrates the exposure time.

Table 7.2: Haematological data measured pre-hypoxia (Pre), during hypoxia (1h and 2h) and post-hypoxia (4h, 6h and 8h).

		Pre	1h	2h	4h	6h	8h
[EPO] (mU·mL^{-1})	3,600 m	6.54 ± 3.54	6.48 ± 1.33	6.34 ± 1.17	7.61 ± 1.45	7.52 ± 1.63	7.31 ± 1.96
	4,200 m	5.86 ± 1.48	5.93 ± 1.48	7.29 ± 1.84	8.06 ± 1.47	7.96 ± 3.06	6.81 ± 2.78
	4,800 m	6.04 ± 1.40	6.07 ± 1.57	6.69 ± 1.40	8.94 ± 2.01	8.85 ± 2.75	7.50 ± 3.11
Il-6 (pg·ml^{-1})	3,600 m	8.91 ± 8.12	9.38 ± 7.84	9.52 ± 9.16	9.27 ± 8.86	9.64 ± 7.36	10.44 ± 8.41
	4,200 m	9.82 ± 8.14	9.11 ± 9.07	10.42 ± 11.36	9.20 ± 8.56	8.68 ± 7.55	9.03 ± 7.63
	4,800 m	8.99 ± 7.37	8.50 ± 6.11	9.34 ± 7.89	7.85 ± 5.68	8.70 ± 6.59	9.79 ± 7.57
TNFα (pg·ml^{-1})	3,600 m	2766 ± 3626	3113 ± 4277	3740 ± 5146	3398 ± 4539	3105 ± 4228	3190 ± 4198
	4,200 m	3372 ± 4702	3211 ± 4529	3088 ± 4380	2905 ± 3839	2993 ± 4090	3317 ± 4443
	4,800 m	3133 ± 4144	3214 ± 4111	3165 ± 3917	3399 ± 4647	3279 ± 4651	3155 ± 4553

Values are mean ± SD.

7.4.3 Relationship between peak [EPO] and other measures

No correlation (P >0.05) was found between the level of oxygen desaturation reached during the hypoxic exposure and the peak Δ[EPO] attained (r = -0.106) across pooled data from all three hypoxic trials (see Fig 7A). Further to this, no correlation (P >0.05) was found between peak Δ[EPO] and baseline IL-6 (r = 0.140) (see Fig 7B), and also between peak Δ[EPO] and baseline TNFα (r = 0.159) (see Fig 7C).

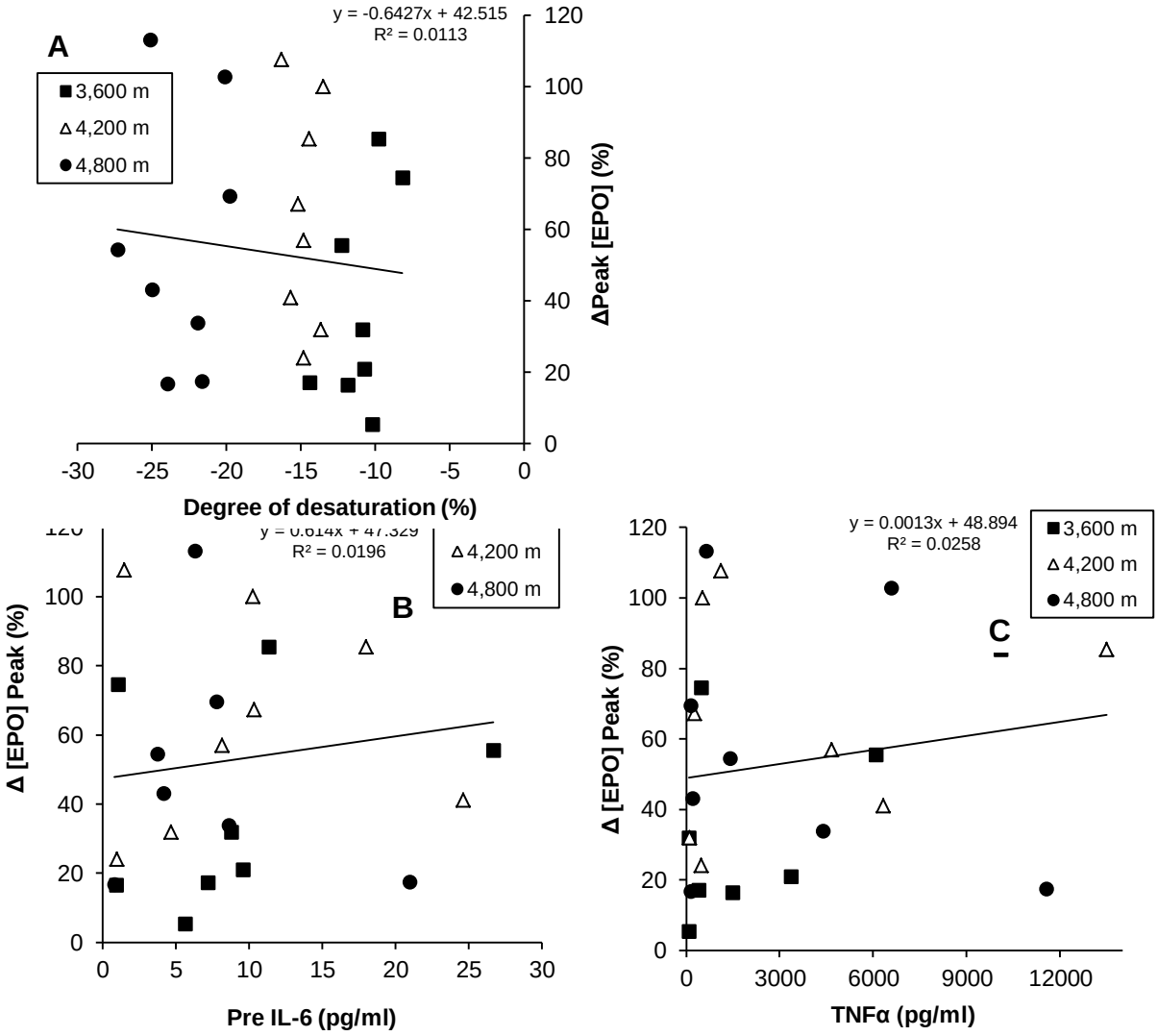


*Figure 7.7: Relationship between the degree of desaturation averaged over the 2 hours of simulated hypoxia (**A**); baseline IL-6 (**B**); baseline TNFα (**C**) and the percentage difference of the peak in [EPO] during each hypoxic condition. As a group, there was no correlation between any of the variables and Peak Δ [EPO].*

7.5 Discussion

The novel findings of this study were that a normobaric hypoxic exposure of two hours at an FiO_2 of <0.125 (>4,200 m) was sufficient to increase EPO production, with a peak [EPO] attained 2 h post-exposure and maintained up to 4 h post-exposure. Despite observing a greater increase in [EPO] as a result of increased severity of hypoxia, large individual variability (see Table 7.2) between participants resulted in no main effect from hypoxia itself. [EPO] returned to baseline levels by 6 h post-exposure. Contrary to the hypothesis, there were no differences in IL-6 and TNFα production as a result of three different hypoxic severities, and also no relationship between baseline IL-6 and TNFα and the peak Δ[EPO] attained.

7.5.1 EPO response to normobaric hypoxia

Figure 7.1 illustrates the results of the previous investigations into acute hypoxic exposures and EPO. For the application of a hypoxic exposure on return to sea level post-altitude training camp, normobaric hypoxia is the most accesible option, at a moderate to high-altitude (>4,000 m), and at a short enough duration that it would fit into an athletes daily training schedule (<2 h). The present invesigation found that two hours of normobaric hypoxia at FiO_2: ~0.135 (3,600 m), FiO_2: ~0.125 (4,200 m) and FiO_2: ~0.115 (4,800 m) caused an increase in [EPO] of 22% (range: -16—53%), 43% (range: 14—100%) and 52% (range: 16—113%), respectively, in all cases peaking two hours post-exposure, staying elevated until four hours post-exposure and returning to baseline by six hours post-exposure. Previously, Knaupp et al. (1992) demonstrated that two hours of normobaric hypoxia at ~5,500 m elicited a ~50% increase in [EPO] and Ge et al. (2002) also found an increase of ~50% in [EPO] after 24 hours of hypobaric hypoxia at ~2,500-2,800 m.

Chronically increased EPO synthesis leads to a progressive increase in tHbmass (Lundby et al. 2007), however, hypoxia-induced changes in EPO release seem to be subject to a marked inter-individual variability (Chapman et al. 1998). This may explain why there is a varied athlete response in tHbmass to altitude training camps (McLean et al. 2013). In this study despite a greater reduction in SpO_2 from an increased severity of hypoxia causing a trend for greater production in [EPO], individuals who were more oxygen desaturated did not always produce a greater [EPO] (see Figure 7.7). The findings are in agreement with previous literature (Ge et al. 2002; Friedmann et al. 2005a; MacKenzie et al. 2008) who also found a marked individual variability in EPO release at different altitudes. Although the participants in the present study were trained, similar values and variations of [EPO] have been reported in elite athletes (Clark et al. 2009; Garvican et al. 2012; Pottgiesser et al. 2012).

The exact mechanisms for individual variability in EPO response to altitude are not well determined (Fudge et al. 2012). Chapman et al. (2010) found that there was no correlation between changes in EPO at altitude and hypoxic ventilatory response measured at sea level; suggesting that peripheral chemoresponsiveness may not be responsible for the variability in EPO response, and the likely mechanisms may be downstream from the lung. Ge et al. (2002), however, stated EPO production at altitude is governed by "upstream" factors related to renal parenchymal PO_2, as well as

other undetermined mechanisms, possibly related to transcriptional regulation of EPO by renal tissue hypoxia.

MacKenzie et al. (2008) suggested EPO production is noticeably augmented by the depression of arterial oxygen content (CaO_2), as a result of decreases in SpO_2. A greater reduction in SpO_2 combined with an inability to increase HVR could facilitate a greater secretion of EPO (Jelkmann 1992). Ge et al. (2002) also suggested that the mechanism of an individual response to altitude is likely to include the greater oxyhemoglobin desaturation. This occurs as the PO_2 falls to the steep portion of the oxyhemoglobin dissociation curve and, therefore changes in SpO_2 are mirrored by EPO levels at all altitudes. The investigations by Ge et al. (2002) and MacKenzie et al. (2008) only found a moderate relationship between $\Delta CaO_2/\Delta SpO_2$ and changes in EPO, and this present study we found no relationship between ΔSpO_2 and peak Δ[EPO] percentage.

7.5.2 Inflammatory response to normobaric hypoxia

Pro-inflammatory cytokines have been shown to trigger the suppression of renal EPO production and therefore erythropoiesis (Morceau et al. 2009), with the inhibition of EPO production shown in vitro and in vivo to potentially involve IL-1, IL-6, and TNFα (Morceau et al. 2009). However, the present study found that baseline IL-6 and TNFα did not correlate with the peak Δ[EPO] and there were no differences in IL-6 and TNFα production as a result of three different levels of hypoxia.

Hypoxia and inflammation are interrelated at molecular, cellular, and clinical levels (Eltzschig and Carmeliet 2011). Oxidative stress and the release of pro-inflammatory cytokines (e.g., IL-1, IL-6, TNFα), which are systemic inflammatory markers, are closely associated with acute hypoxia and are proportional to the severity of hypoxia (He et al. 2014). Previously, Klausen et al. (1997) found that after 1 day at 4,350 m, there was a non-significant change in serum IL-6 by 56% and by day 4 had significantly increased by 86%, however, there were no changes in IL-1 or TNFα. The increase in IL-6 was significantly correlated ($r = -0.45$) with hypoxemia (mean SpO_2: 79-83%), but not HR or symptoms of AMS. The authors suggested that the increase of serum IL-6 was not secondary to increased sympathetic nervous activity or general distress during altitude acclimatisation due to the lack of relation between serum IL-6 and heart rates or AMS scores (Klausen et al. 1997).

Jelkmann et al. (1992) found that the addition of IL-1 and TNFα inhibited the production of EPO in hypoxic human hepatoma cell cultures, however, inhibition did not occur with introduction of IL-6, thus stating that IL-1 and TNFα have been shown to affect gene expression in human hepatoma cultures at the transcriptional level (Jelkmann et al. 1994). The authors reported that IL-6 does not affect EPO production *in vitro*; moreover, IL-6 appears to inhibit renal EPO formation (Jelkmann et al. 1992). Inflammatory responses to hypoxia are complex with various cytokines both inhibiting and preserving the production of EPO. The present investigation found that baseline levels of IL-6 and TNFα were not associated with the production of EPO (See Fig. 7B/C). Furthermore, there were no differences in the increases in IL-6 and TNFα between the three levels of normobaric hypoxia, despite increases in HR and decreases in SpO_2. At present it is not clear what pro-inflammatory cytokines

regulate the production of EPO, but in this investigation inflammation did compromise endogenous [EPO] increases.

7.5.3 Future directions

Additional normobaric hypoxic exposures of two hours, with the aim of increasing the production of EPO, have not been implemented on return to sea-level after an altitude training camp. Rodríguez et al. (2000) and Casas et al. (2000) exposed trained volunteers to hypobaric hypoxia at simulated altitudes of ~4,000-5,000 m for 90 minutes, three times a week for three weeks. This stimulus led to an effective stimulation of erythropoietic adaptations, such as, significant increases in RBC count, [Hb] and reticulocytes. Katayama et al. (2003), however, utilised a similar protocol (4,500 m for two hours, three times a week for three weeks) with endurance runners and found no changes in haematological parameters, including [Hb], Hct, RBC count, reticulocytes or EPO. The large individual variation and differing populations used could account for these contradictory findings; nevertheless, the protocol should be tested post-altitude training camp alongside measurement of EPO and tHbmass. Additional haematological measurements prior to the experimental period would provide a more accurate baseline interpretation.

Further to this, tighter controls surrounding exhaustive exercise pre-hypoxic exposure should be considered, as different types of exercise (concentric, eccentric, submaximal, maximal) are known to cause increases in pro-inflammatory cytokines (Pedersen et al. 1998; Nieman et al. 2001; Jürimäe et al. 2011) and intense exercise provides a physiological stimulus itself to increase EPO production (Roberts and Smith 1999). Additional modifications to the protocol could include blood samples 12, 14 and 48 hours post-hypoxic exposure to determine if there is a delayed increased in EPO or pro-inflammatory cytokines as has previously been suggested (Pedersen et al. 1998; Ge et al. 2002). The present investigation controlled for diurnal variations in haematological markers by ensuring that all trials were started between 07:30 and 09:30 as previous research has shown that EPO is subject to distinct diurnal variation in trained and untrained individuals (Klausen et al. 1993), as well as in both normoxia and hypoxia (Klausen et al. 1996). Keramidas et al. (2011) observed diurnal variation of a nadir in values of EPO in the morning hours, and zenith levels during the evening and night hours.

7.5.4 Practical applications

A decrease in EPO and tHbmass on return to sea level has been observed in athletes after altitude training camps lasting 3-4 weeks (Heinicke et al. 2005; Clark et al. 2009; Garvican et al. 2012; Pottgiesser et al. 2012). When tHbmass exceeds the physiological requirement at the altitude resided in, EPO secretion is suppressed (Rice and Alfrey 2005) and a destruction in red cells, or neocytolysis, occurs. Athletes who are acclimated after an altitude training camp, who then descend to sea level to compete may have tHbmass that is higher than necessary for homeostasis to their new environment. The rapid destruction of reticulocytes and the decline in production of new ones may depend on a drop in EPO levels (Alfrey et al. 1997). Additionally, EPO not only regulates tHbmass but also prolongs its

survival (Rice et al. 2001a). For an athlete with a faster than normal decline in tHbmass upon return to sea level, competing as soon as possible may be the most beneficial strategy but this is not always logistically achievable.

An athlete's busy travel schedule, external commitments and competition programme, make it difficult to time competing at sea level after an altitude training camp. Chapman et al. (2014c) suggested that if an athlete completes a 4 week altitude training camp, followed by a short time (~7-14 days) at sea level to compete, returning to altitude even for a short time may mitigate or delay the effects of neocytolysis by re-establishing EPO levels, although this has not been proven. This is not for added erythropoiesis, as is typically done with altitude residence, but more to preserve EPO concentrations, as a result of the athlete residing at sea level. The findings of the present study have shown that acute hypoxic exposures are sufficient enough to increase [EPO], which may provide a strategy to prevent the sudden drop off in EPO. Furthermore, improved exercise performance has also been attributed to elevated [EPO] (Schuler et al. 2012; Durussel et al. 2013a). This topic is further investigated in Chapter 8 (Study 5).

7.6 Conclusion

In Caucasian populations erythropoiesis is a key acclimatization response that increases the oxygen carrying capacity of the blood, i.e. tHbmass, as a result of chronic exposure to altitude (Chapman et al. 2014a). A change in tHbmass by 1 g causes a change in $\dot{V}O_{2max}$ by approximately 4 ml·min^{-1} (Schmidt and Prommer 2010), for an athlete increased blood gas storage capacity is very important, therefore, the maintenance of tHbmass should be considered. Chapman et al. (2014c) stated that brief, short-term periods of normobaric hypoxia may provide a sufficient stimulus to increase EPO significantly, which the findings of the present study have shown. These exposures could take place during the day, around the athlete's training schedule, to preserve the hematologic acclimatisation response for a longer time, thereby expanding the window for optimal competition. The current investigation is the first to show that a normobaric 'hypoxic dose' (i.e., FiO_2 ~0.125-0.115, equivalent to 4,200 m and above, for two hours) increases in [EPO], without an increase in IL-6 or TNFα. This method may be sufficient enough to prevent the sudden drop in EPO that has been shown post-altitude and, therefore, maintain any enhancements in tHbmass. The release of EPO is subject to a marked inter-individual variation that can only be partially explained by reductions in oxyhaemoglobin saturation, but is not effected by systemic markers of inflammation.

CHAPTER 8

8 INFLUENCE OF ENDOGENOUS ERYTHROPOIETIN ON TIME TRIAL PERFORMANCE IN ENDURANCE RUNNERS

8.1 Abstract

Purpose: Previous work has shown a strong relationship between tHbmass and $\dot{V}O_{2max}$; therefore, an increase in tHbmass may have a positive effect on endurance performance. Total Hbmass increases through an increase in production of EPO. However, less is understood about endurance performance when EPO is acutely elevated and independent of changes in tHbmass. The aim of the study was to test the effect of acutely increasing endogenous EPO, via changing the FiO_2, on running time trial performance. **Methods:** In a randomised order, seven well-trained male runners (age 29 ± 5 yr, height 181.2 ± 6.4 cm, body mass 72.9 ± 7.9 kg, $\dot{V}O_{2max}$ 64.4 ± 5.7 $mL \cdot kg^{-1} \cdot min^{-1}$; mean ± SD) performed three exercise trials consisting of a standardised warm up and a 10 min pre-loaded running time trial (TT_{10}), to replicate a competitive middle-distance running event. Trials were preceded by 2 h of a hypoxic (HYPO; FiO_2: 0.118 ± 0.001), normoxic (CON; FiO_2: ~0.208 ± 0.002) or hyperoxic (HYPER; FiO_2: 0.398 ± 0.022) exposure, 3.5 h prior to TT_{10}. Participants were blind to each exposure. Heart rate (HR), SaO_2, oxygen uptake and RPE were measured throughout. Venous blood samples were taken pre-exposure and pre-TT_{10} and analysed for EPO concentration ([EPO]). **Results:** No differences (P = 0.082) were found in distance covered during TT_{10} (HYPO: 2726 ± 277 vs. CON: 2724 ± 279 vs. HYPER: 2742 ± 281 m). [EPO] increased by 11 ± 9% in HYPO, but decreased by 20 ± 20% and 29 ± 9% in CON and HYPER, respectively. An interaction effect was found between exposure*time on [EPO] (P = 0.001), however, no main effect was found for exposure (P = 0.065) nor time (P = 0.723). **Conclusions:** Although manipulation of FiO_2 caused a variation in [EPO], this had no effect on running time trial performance in well-trained endurance runners.

8.2 Introduction

The prevailing paradigm of adaptation to hypoxia is that the lower partial pressure of oxygen, associated with ascending to higher altitudes, acutely induces EPO production, which if sufficient in duration, stimulates the production of RBCs (Levine and Stray-Gundersen 1997; Stray-Gundersen et al. 2001; Levine and Stray-Gundersen 2005), thus increasing tHbmass. Schmidt and Prommer (2010) found a change in tHbmass by 1 g causes a change in $\dot{V}O_{2max}$ by approximately 4 mL·min^{-1}, which may lead to an improvement in endurance performance. Saunders et al. (2010) revealed a 1% improvement in $\dot{V}O_{2max}$ resulted in a 0.5% change in peak running speed in well-trained male distance runners.

Over a two year period, Wachsmuth et al. (2013) observed a variable response in tHbmass after altitude training in elite German swimmers and over a whole season tHbmass was positively related to swimming performance (50 and 400 m or 22 s to 4 min 32 s) in major competitions. Conversely, Gough et al. (2012) found swimmers' race performance (100 or 200 m freestyle) was slower for up to 1 week after altitude and no period of peak form was identified, despite a positive haematological adaptation. There is further evidence to suggest that there is a rapid decrease in tHbmass and EPO on return to sea level from altitude (Clark et al. 2009; Garvican et al. 2012; Pottgiesser et al. 2012). This suggests that removal of the altitude stimulus results in a 're-acclimatisation' to the normoxic environment (Garvican et al. 2012). It has been proposed a sudden drop in [EPO] upon descent to sea level causes the selective destruction of young erythrocytes, known as neocytolysis (Rice et al. 2001a).

Aside from the well-known effects of EPO on blood parameters, EPO may also exert an effect on brain function (Rasmussen et al. 2010), as a result of EPO crossing the blood brain barrier (BBB). Prolonged doses (181–232 U.kg^{-1}.wk^{-1} three times a week for 30 d) of recombinant EPO (rhEPO) in well-trained cyclists have previously been shown to improve $\dot{V}O_{2max}$ by 7% and time to exhaustion by 9% (Birkeland et al. 2000). Schuler et al. (2012) administered an acute high dose of rhEPO to mice, which enhanced $\dot{V}O_{2max}$ by ~8% and exercise time to exhaustion by ~ 26%. This marked elevation in exercise capacity occurred independently of changes in haematological or cardiovascular parameters. Subsequent research found EPO administration could positively affect self-perception and motivation while reducing physical pain, and mechanisms of central fatigue for a given workload (Ninot et al. 2006; Miskowiak et al. 2007a; El-Kordi et al. 2009), which could therefore affect an athlete's performance and pacing strategy during a race. Rasmussen et al. (2010) suggested the feeling of improved physical condition could influence exercise capacity by modulating central fatigue, however, they were unable to enhance exercise capacity or reduce perceived exertion after three consecutive days of high-dose EPO administration, although the final EPO dose was administered ~24 h before the performance trial.

The aforementioned studies have tested high doses of rhEPO in both mice and healthy humans (Miskowiak et al. 2008). With injections of rhEPO banned by WADA the model has not been tested in athletes, or on increases in EPO as a result of acute hypoxic exposures. Acute normobaric hypoxic exposures (2 h >~4,200 m) are sufficient to increase [EPO] by up to 50%, which peaks 2-4 h post-exposure (see Chapter 7, Study 4). To understand fully the role of EPO on endurance performance, EPO must be supressed below baseline levels, which has been previously achieved with exposure to 2 h of

hyperoxia (Balestra et al. 2004; Balestra et al. 2006; Keramidas et al. 2011; Debevec et al. 2012). With these studies finding a nadir in EPO occurring 3 h post-hyperoxia and acute hypoxia finding a zenith 2-4 h post-hypoxia, this provides an opportunity to test an athlete's performance within that time frame.

Altitude and hypoxic training with endurance runners has utilised a variety of different performance tests in both a lab and field setting. The typical distances have been between 3-5 km, after acute (Katayama et al. 2003; Julian et al. 2004) and chronic strategies (Levine and Stray-Gundersen 1997; Stray-Gundersen et al. 2001; Robertson et al. 2010b; Chapman et al. 2014a). Robertson et al. (2010c) completed individual 3,000 m time trials with a pacer for lap 1 and 2 (first 800 m) to reduce the chance of a poor pacing strategy and had previously reported a typical error of measurement (TEM) of 1.3% (1.0–2.1%, 90% confidence limits) (Robertson et al. 2010b). Traditionally, time to exhaustion (TTE) exercise trials have been used to quantify the response of biological systems to various interventions, however the inherent variability of open loop trials, such as TTE, is so large that it can often mask meaningful performance changes (Walshe et al. 2010). Currell and Jeukendrup (2008) proposed time trials are more ecologically valid. Middle-distance running events that require two to 10 minutes (800 – 3,000 m) are competed at a higher percentage of $\dot{V}O_{2max}$ (Brandon 1995) and are also sufficient enough to incur arterial oxyhaemoglobin desaturation (Chapman et al. 2011). Therefore, from both an applied and mechanistic perspective, a 10 minute time trial is considered an appropriate duration to evaluate the efficacy of acute hypoxic interventions.

The aim of this study was to determine the potential performance enhancing effect of 2 h exposures to normobaric hypoxia ($FiO_{2:}$ ~0.12; to elevate [EPO]), to hyperoxia ($FiO_{2:}$ ~0.40; to supress [EPO]) and to normoxia ($FiO_{2:}$ ~0.21; as a control) on a 10 minute time trial (TT_{10}) in well-trained runners. For better comparative purposes across these trials, we chose to fix the speed of the first 3 minutes of the trial (i.e. 'pre-load') in order to provide a consistent exercise challenge, and then freely paced exercise for the 7 minutes thereafter. It is hypothesised that [EPO] would be elevated after hypoxia and supressed after hyperoxia compared to a normoxic control, and that the distance covered during the TT_{10} would be increased after hypoxia and decreased after hyperoxia compared to normoxia.

8.3 Methods

8.3.1 Participants

Seven well-trained male runners volunteered to participate (see Table 8.1 for descriptive data). Participants reported running 5 ± 2 training sessions per week, with an average weekly mileage of 30 ± 18 miles·wk^{-1}. They were fully informed of the purposes, risks and discomforts associated with the experiment and provided written informed consent, and institutional ethical approval was issued in accordance with the Declaration of Helsinki 1975 (revised 2013). For full participant inclusion and exclusion criteria see General Methods section 3.2.

Table 8.1: Anthropometric and physiological data

Descriptive	**Participant**
Age (yr)	29 ± 5
Height (cm)	181.2 ± 6.4
Weight (kg)	72.9 ± 7.9
Sum of 8 skinfolds (mm)	62.6 ± 25.1
Body Fat (%)	13.0 ± 4.5
5K Personal Best	18:04 ± 01:53
tHbmass (g)	958 ± 94
Rel. tHbmass (g·kg^{-1})	13.2 ± 1.0
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	64.4 ± 5.7
Lactate Threshold (km·h^{-1})	13.0 ± 1.9
Lactate Turnpoint (km·h^{-1})	14.9 ± 2.0

8.3.2 Experimental design

A single-blind, randomised controlled design (Latin square) ensured there was no order effect. Each participant performed experimental trials under three conditions: control (normoxia, CON), hypoxic exposure (HYPO), and hyperoxic exposure (HYPER). Six laboratory visits were completed over 30 ± 6 days, involving one preliminary testing session, two familiarisation sessions of the TT_{10} and the three subsequent experimental trials. An overview of the experimental design is provided in Figure 8.1. Briefly, each experimental trial comprised four phases: 30 min rest to record baseline measurements, 2 h of resting passively whilst breathing a different oxygen percentage, a further 3 h of rest (Figure 8.1 – Part A), followed by a standardised warm-up and the TT_{10} (Figure 8.1 – Part B). Experimental trials were separated by a minimum of seven days (8 ± 2 days) to prevent possible carryover effects from previous testing (MacKenzie et al. 2008). Laboratory conditions were similar throughout all assessments (19.2 ± 0.9ºC temperature and 55.4 ± 5.4% relative humidity).

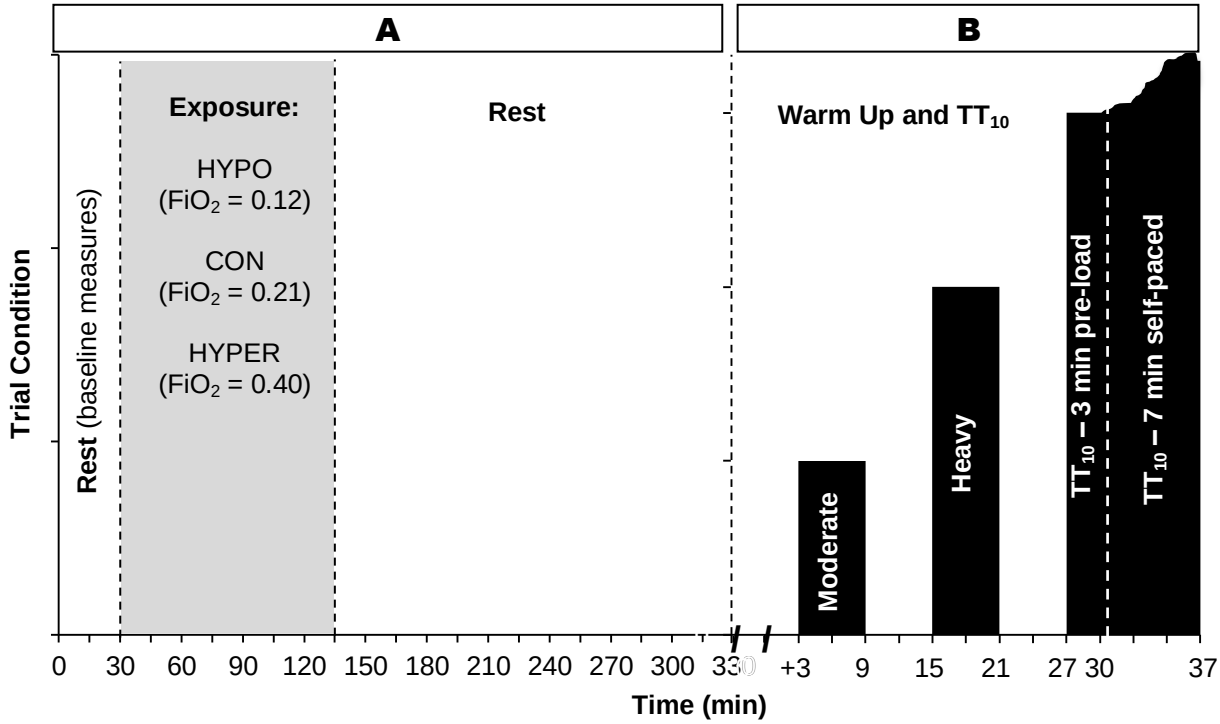


Figure 8.1: Overview of the experimental trial. Part A consists of the 2 h exposure to three different levels of FiO_2 followed by a 3 h rest period and Part B consists of the standardised warm up followed by the TT_{10}.

8.3.3 Experimental procedures

8.3.3.1 Preliminary testing

At the start of the preliminary testing, body composition was measured as previously described in the General Methods (Section 3.3). All measured were taken once.

Participants then performed a submaximal and maximal running assessment for the determination of lactate threshold (LT), lactate turnpoint (LTP) and $\dot{V}O_{2max}$. For a full description of the equipment and protocol see section 3.4. Results were used to calculate running speeds required for the TT_{10}. Finally, tHbmass, BV and PV was determined using the o-COR-method (Schmidt and Prommer 2005; Prommer and Schmidt 2007). The protocol and equipment used was previously described (see section 3.7). Haematocrit and [Hb] were collected from venous blood samples and determined using a Pentra ES 60 (Horiba Medical; Kyoto, Japan).

8.3.3.2 Familiarisation

Participants completed two separate familiarisation sessions of the TT_{10} (see *Experimental Trial*). During these familiarisation trials participants controlled treadmill speed throughout the TT_{10}, using a custom made larger increase/decrease button (see Figure 8.2), starting at a speed of 95% $v\dot{V}O_{2max}$, approximately equivalent to 3 km pace (Londeree 1986). Participants were instructed to cover the furthest distance possible over 10 min, with only a countdown clock displaying remaining time. These

sessions acted to familiarise participants with the study procedures and equipment, and to minimise any learning effects for subsequent trials (Currell and Jeukendrup 2008). The TEM of distance covered in the TT_{10} over the two familiarisation sessions was determined (Hopkins 2000) and calculated at 0.97%.

8.3.3.3 Experiential trial

Participants arrived at the laboratory between 08:00–09:00 to minimise the effects of variations in circadian rhythms (Atkinson and Reilly 1996) and diurnal variations in EPO (Klausen et al. 1993; Klausen et al. 1996). After a 10 min period of quiet rest, baseline measures of [Hb], Hct, HR, arterial oxygen saturation from pulse oximetry (SpO_2; Nonin 2500, Nonin Medical Inc., Plymouth, MN, USA), Lake Louise Questionnaire (LLQ) (Roach et al. 1993b), rating of perceived exertion (RPE; scale 6–20) and dyspnoea- respiratory discomfort (D-RPE) were recorded (Wilson and Jones 1991). A pre-exposure venous blood sample was also taken into one 2 mL BD Vacutainer® Plus Plastic K_2 EDTA Tubes (BD Vacutainer®; New Jersey, USA) from the antecubital fossa for determination of plasma [EPO]. EPO concentrations were corrected for changes in venous PV (Dill and Costill 1974). After baseline measurements were recorded, participants rested passively in CON, HYPO or HYPER for 2 h. Measures of HR and SpO_2 were recorded every 15 min during this period with LLQ and D-RPE responses monitored every 30 min. Following each exposure participants rested for a further 3 h during which water was consumed *ad libitum* and an isocaloric meal was provided. During this period HR, LLQ, D-RPE and SpO_2 were then recorded every 60 min.

Participants were exposed to a 2 h period of each condition, delivered using a hypoxic generator (McKinley Altitude Simulator, Higher Peak Performance). The generator was connected to a 15 L reservoir, allowing constant monitoring of oxygen and carbon dioxide values through sample tubing linked to a gas analyser (Servomex 1440, Crowborough, UK). The reservoir had a separate output pipe connected to tight-fitting masks using uni-directional non-rebreathing valves (Sporting Edge UK Ltd, Basingstoke, UK), allowing inspiration of hypoxia with an expired air exit. An identical system was used for the normoxic (control) condition but a 'sham' generator was used that delivered normoxic air and, for HYPER the outlet pipe was connected to the generators exhaust that delivered hyperoxic air. HYPO, CON and HYPER was undertaken 3 h prior to the start of exercise. Participants were blinded to each condition during all sessions.

The TT_{10} was performed on a calibrated motorised treadmill (PPS Med, Woodway USA Inc., Waukesha, WI, USA), set at a 1% treadmill gradient. All phases commenced from a rolling start to account for inconsistencies that could be encountered when rapidly accelerating the treadmill from stationary to the desired speed. The treadmill display panel was covered during each trial, so participants were only able to see a countdown clock that displayed the remaining time. Participants did not receive any verbal encouragement. The TT_{10} was preceded by a standardised warm up period, optimising the $\dot{V}O_2$ response to subsequent high intensity exercise (Pringle et al. 2003). The warm-up consisted of an initial 3 min baseline rest phase, a transition to 6 min of moderate intensity running [determined from preliminary testing and calculated as the running speed at 80% of $\dot{V}O_2$ at lactate threshold (LT; 10.4 ± 1.5 $km \cdot h^{-1}$)], a 6 min passive rest period, a transition to 6 min of heavy intensity

running [calculated as the running speed half way between LT and LTP (13.9 ± 2.0 km·h^{-1})] and finally a 6 min passive rest period (Figure 8.1 – Part B). The TT_{10} commenced with an initial 3 min preload phase at a fixed speed [calculated as each participant's average speed over the two familiarisation TT_{10} (16.1 ± 1.6 km·h^{-1})], followed by the remaining 7 min of the TT_{10} aiming to cover the furthest distance possible self-paced.

Participants were able to self-select their speed using a custom-made panel mounted on the treadmill in front of them (see Figure 8.2). Running speed and distance were continually recorded (Woodway Treadmill Control, Woodway USA Inc., Waukesha, WI, USA). Participants were given no indication of their completion distance and received no feedback on performance during or after the TT_{10}. Pulmonary gas exchange was continually measured, and RPE (Borg 1970) recorded at regular intervals throughout exercise testing.

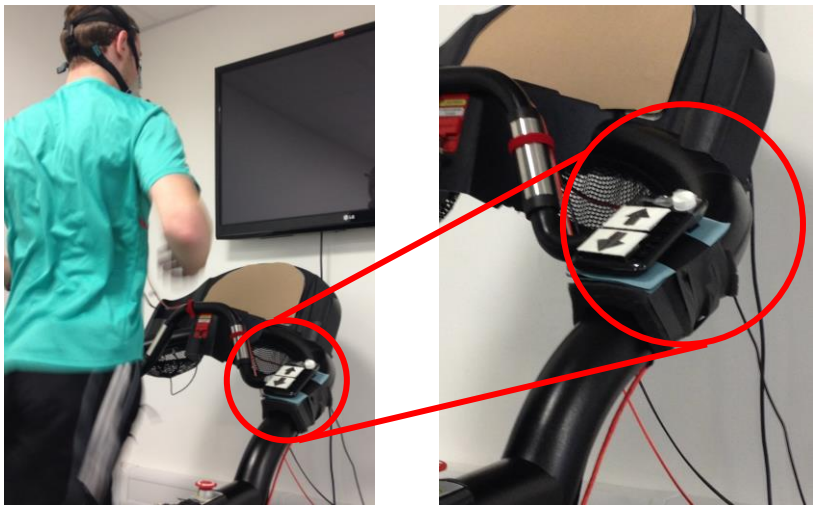


Figure 8.2: Participant completing the TT_{10} (left) with custom made self-selecting speed dial (right)

8.3.4 Statistical analyses

All outcome variables were assessed for normality and sphericity prior to further analysis. Data were analysed in three sections: exposure, haematology and performance trial. The HR, SpO_2, FiO_2, D-RPE and LLQ measured during the 2 h exposure were averaged and a one-way, repeated measures ANOVA was used to detect differences. One-way, repeated-measures ANOVA were used to detect differences between conditions in distance covered during the TT_{10}, Δ% in plasma volume (PV) and Δ% in blood volume (BV). Two-way, repeated-measures ANOVA (exposure*time) were used to test for differences in average speed, HR, RPE, oxygen consumption ($\dot{V}O_2$), ventilation ($\dot{V}E$) and oxygen cost during the performance trial. Where appropriate, Bonferroni-adjusted pairwise comparisons revealed where differences occurred. Data were analysed using SPSS (Version 20, SPSS Inc., Chicago, Illinois, USA). Effect size of main effects and interactions were presented as partial eta squared (η^2), while differences between two related samples were evaluated through Cohen's d in accordance with Lakens (2013). All data were presented as means ± SD.

8.4 Results

8.4.1 Exposure

Table 8.2 shows the physiological variables measured during the 2 h passive exposure to HYPER, CON and HYPO. Differences were found in SpO_2 (F = 144.853, P = 0.001, η_p^2 = 0.96), HR (F = 11.311, P = 0.002, η_p^2 = 0.65) and FiO_2 (F = 964.047, P = 0.001, η_p^2 = 0.99) but not D-RPE (F = 1.713, P = 0.238, η_p^2 = 0.22) or LLQ (F = 2.362, P = 0.136, η_p^2 = 0.28).

Table 8.2: Variables monitored during the 2 h exposure to HYPER, CON and HYPO

	HYPER	**CON**	**HYPO**
FiO_2	0.398 ± 0.022 * †	0.208 ± 0.002	0.118 ± 0.001 *
$FiCO_2$	0.0004 ± 0.0001	0.0009 ± 0.0001	0.0011 ± 0.0002
SpO_2 (%)	99 ± 0 * †	98 ± 1	78 ± 5 *
HR ($b \cdot min^{-1}$)	48 ± 6 †	53 ± 10	61± 9 *
D-RPE	0.2 ± 0.4	0.1 ± 0.2	0.4 ± 0.7
LLQ	0.2 ± 0.4	0.2 ± 0.5	0.5 ± 0.7

* Denotes significant difference (P < 0.05) from CON, † denotes significant difference (P < 0.05) from HYPO

8.4.2 Haematology

There were no differences between pre-exposure [EPO] measurements (see Table 8.3). The alterations in FiO_2 yielded significant changes in [EPO] across the exposure period in each condition (F = 30.698, P = 0.001, ηp2 = 0.84). For the CON and HYPER conditions, [EPO] decreased by 20 and 29% respectively; for the HYPO condition it increased by 11%. Consequently, the post-exposure, pre-TT [EPO] was significantly higher (P = 0.014) in HYPO than in HYPER (see Figure 8.3).

Table 8.3: Changes in absolute [EPO] as a result of HYPER, CON and HYPO exposures prior to the TT_{10}.

	Pre-exposure	**Pre-TT_{10}**
HYPER	9.76 ± 2.62	7.52 ± 1.84 *
CON	9.58 ± 3.58	8.04 ± 2.67 *
HYPO	9.78 ± 2.35	11.11 ± 2.91 *

* denotes difference (P < 0.05) from pre-exposure to pre-TT_{10}

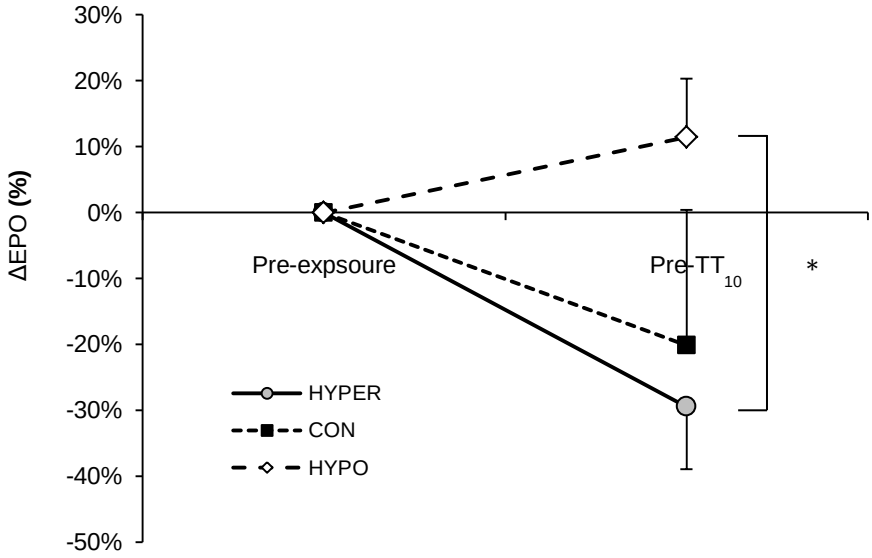


Figure 8.3: Percentage change in [EPO] as a result of HYPER, CON and HYPO exposures prior to commencing the TT_{10}. (denotes significant difference between HYPO and HYPER; P < 0.05)*

Figure 8.4 illustrates the [Hb] (**A**) and Hct (**B**) measured pre-exposure and pre-TT_{10}. For [Hb] there was no interaction effect between exposure*time (F = 1.658, P = 0.231, η_p^2 = 0.22), or main effect of exposure (F = 1.547, P = 0.253, η_p^2 = 0.21), however, there was an effect on [Hb] over time (F = 18.665, P = 0.005, η_p^2 = 0.76). Bonferroni comparison identified differences between pre-exposure [Hb] and pre-TT_{10} [Hb] in HYPO (P = 0.018), CON (P = 0.020) and HYPER (P = 0.002). For Hct there was no interaction effect between exposure*time (F = 1.999, P = 0.178, η_p^2 = 0.25), or main effect of exposure (F = 2.125, P = 0.162, η_p^2 = 0.26), however, there was an effect on Hct over time (F = 15.072, P = 0.008, η_p^2 = 0.72). Bonferroni comparison identified differences between pre-exposure Hct and pre-TT_{10} Hct in HYPER (P = 0.005), CON (P = 0.018) but not HYPO (P = 0.051).

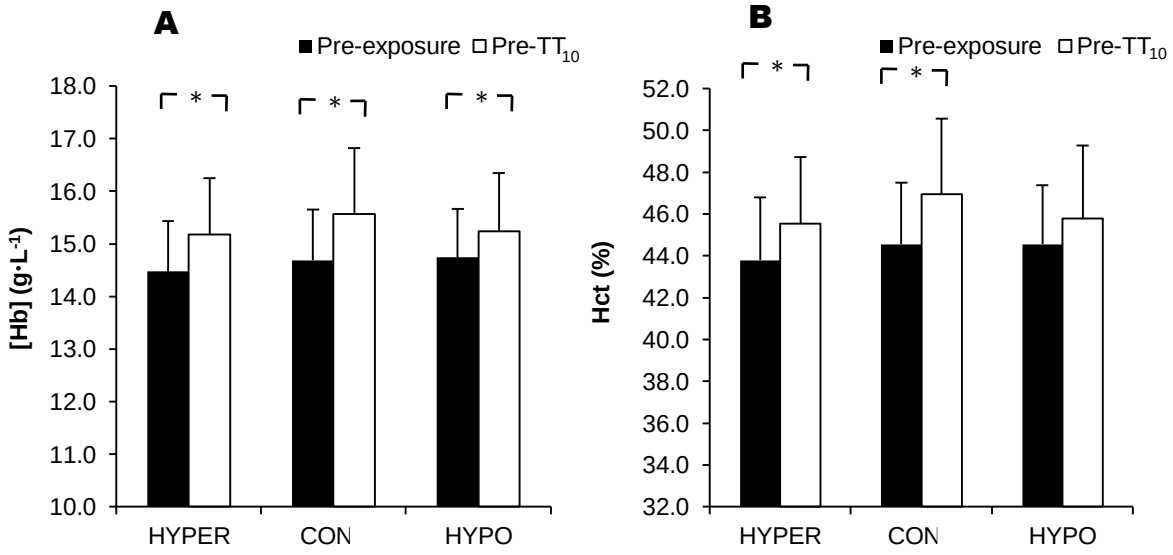


*Figure 8.4: [Hb] (**A**) and Hct (**B**) measured pre-exposure and pre-TT_{10}. * Denotes significant difference (P < 0.05) between time points*

There were no differences in the mean change in PV (F = 1.738, P = 0.217, η_p^2 = 0.23) after HYPER (-7.5 ± 3.9%), CON (-9.4 ± 7.7%) and HYPO (-5.3 ± 5.0%). There were no differences in the mean change in BV (F = 1,701, P = 0.224, η_p^2 = 0.22) after HYPER (4.8 ± 2.5%), CON (6.0 ± 5.0%) and HYPO (3.3 ± 2.8%).

8.4.3 Performance trial

The total distance covered during the TT_{10} was 2742 ± 281 m, 2724 ± 279 m and 2726 ± 277 m, in the HYPER, CON and HYPO trials respectively. No difference was found (Figure 8.5; F = 3.097, P = 0.082, η_p^2 = 0.34). Four out of the seven participants completed the furthest distance in the HYPER trial, with one in the HYPO trial, one in the CON trial and one completed the same distance in the HYPER and HYPO trials. There was no order effect

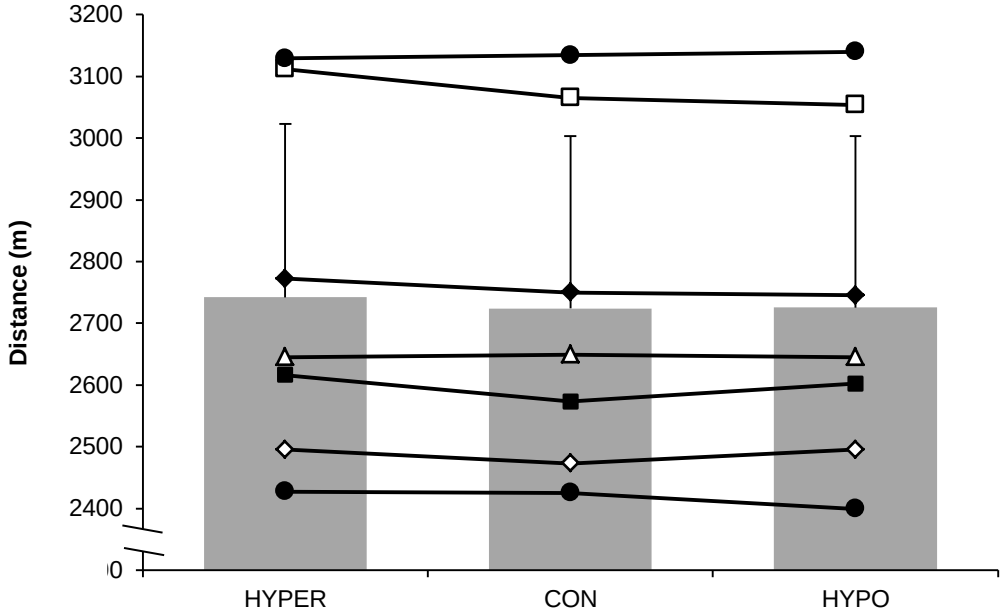


Figure 8.5: Distance covered during the TT_{10} after HYPER, CON and HYPO exposures. The bars represent the mean distance covered and the lines represent the individual participant data.

Figure 8.6 illustrates the average speed during the TT_{10}. There was no interaction effect between exposure*time (F = 1.346, P = 0.199, η_p^2 = 0.74), however, there was an effect on the average speed over time (F = 7.036, P = 0.001, η_p^2 = 1.00) and between exposures (F = 4.584, P = 0.033, η_p^2 = 0.66).

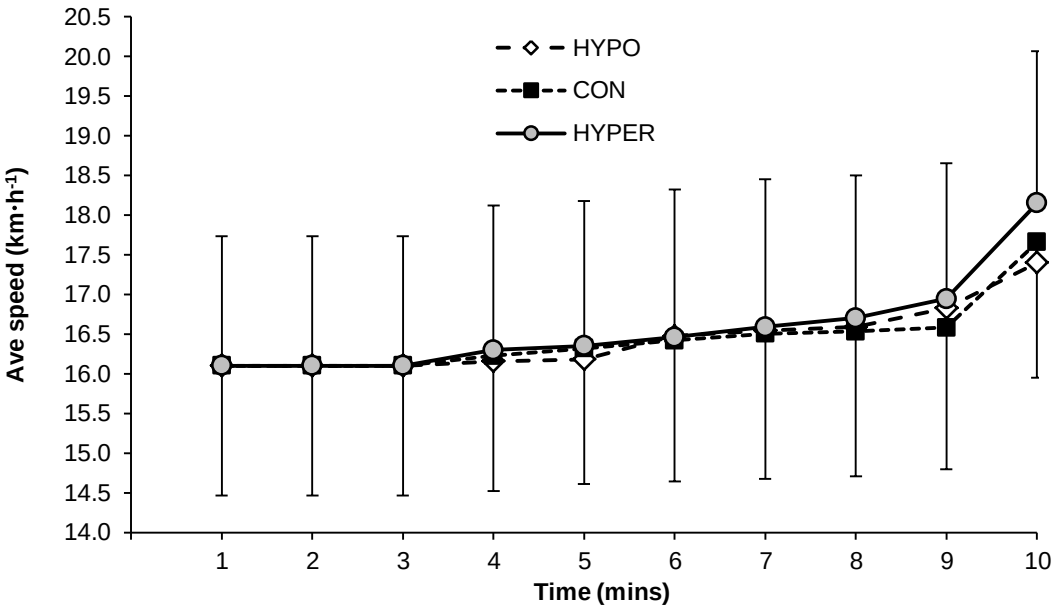


Figure 8.6: Average speed (km·h^{-1}) during each minute of the TT_{10}

There was no main effect of exposure on RPE (F = 0.276, P = 0.764, η_p^2 = 0.04) and no interaction effect between exposure*time on RPE (F = 2.043, P = 0.172, η_p^2 = 0.25). There was, however, a main effect of time from RPE measured post 3-min pre-load to RPE measures post-TT_{10} (F = 44.660, P = 0.001, η_p^2 = 0.88) in all three exposures (see Table 8.4).

Table 8.4: Changes in RPE measured after the 3-min pre-load of the TT_{10} and at the end of the full TT_{10}.

	Post 3-min pre-load	**Post-TT_{10}**
HYPER	15 ± 2	18 ± 2
CON	15 ± 2	18 ± 3
HYPO	14 ± 1	18 ± 2

There was a main effect on HR over time (F = 84.105, P = 0.001, ηp2 = 0.93), but not between exposures (F = 0.064, P = 0.938, ηp2 = 0.01), or an interaction effect between exposure*time (F = 0.811, P = 0.684, ηp2 = 0.12). There was no interaction effect found between exposure*time on $\dot{V}O_2$ (F = 0.754, P = 0.748, ηp2 = 0.51), nor a main effect between exposures (F = 0.056, P = 0.946, ηp2 = 0.06), however, a main effect over time (F = 87.404, P = 0.001, ηp2 = 1.00) was found.

There was a main effect on $\dot{V}_E$ over time (F = 96.335, P = 0.001, ηp2 = 0.94), but not between exposures (F = 0.336, P = 0.709, ηp2 = 0.06), or an interaction effect between exposure*time (F = 1.389, P = 0.152, ηp2 = 0.19). There was no interaction effect found between exposure*time on oxygen cost (F = 0.714, P = 0.790 ηp2 = 0.11), nor a main effect between exposures (F = 0.303, P = 0.619, ηp2 = 0.05), however, a main effect over time (F = 13.634, P = 0.001, ηp2 = 0.64) was found. Bonferroni comparison identified differences (P > 0.05) from 1 min over time in HR, $\dot{V}O_2$, $\dot{V}_E$ and oxygen cost after HYPO, CON and HYPER (see *Figure* 8.7).

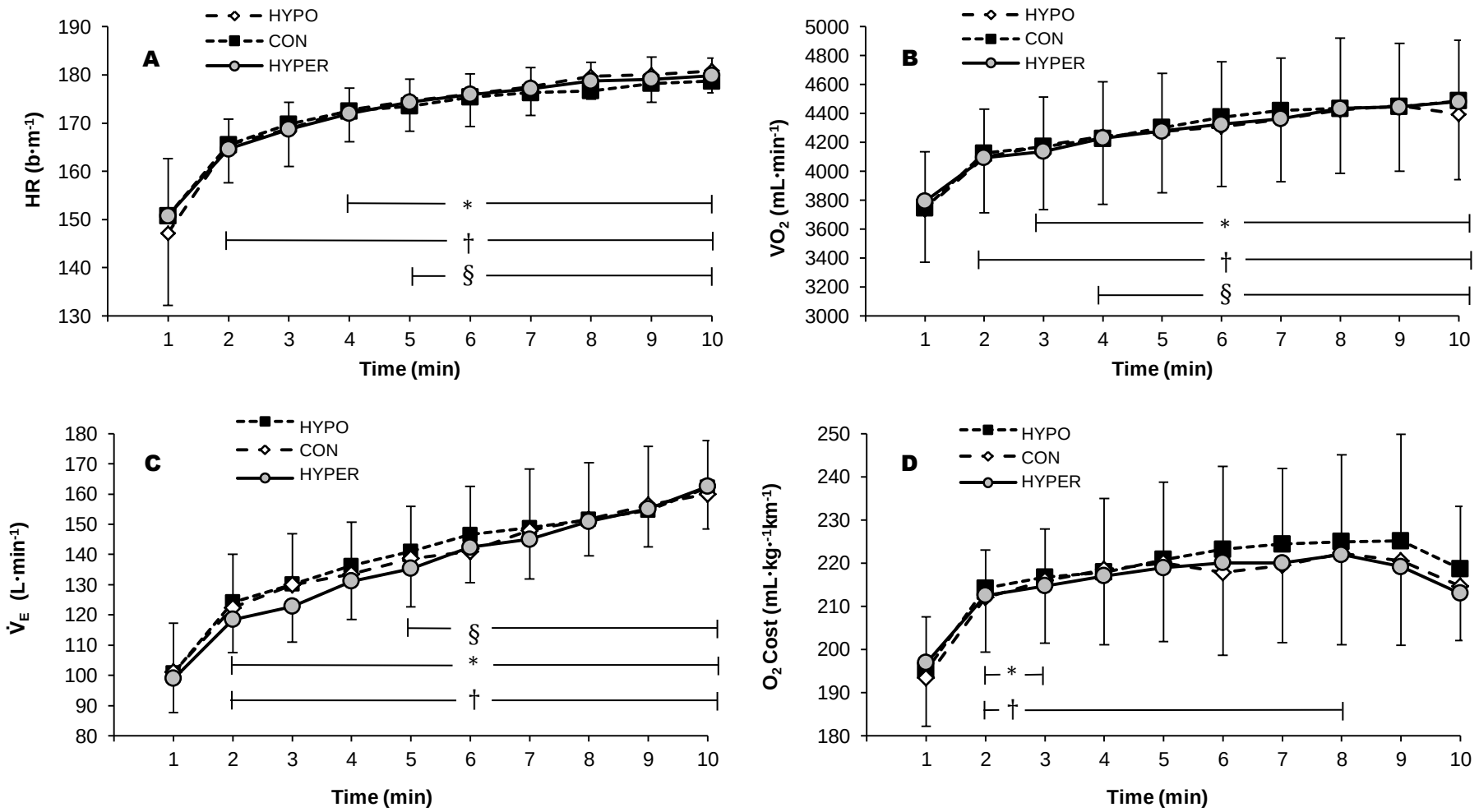


*Figure 8.7: Physiological variables measured during the TT_{10}. * denotes significant difference (P < 0.05) from 1 min in CON, † denotes significant difference (P < 0.05) from 1 min in HYPO and § denotes significant difference (P < 0.05) from 1 min in HYPER.*

8.5 Discussion

In accordance with Chapter 7 (Study 4), the present study found a 2 h exposure at FiO_2 of 0.118 (simulated altitude of ~4,800 m) was sufficient enough to increase [EPO] ($P < 0.05$), compared to a normoxic placebo control (FiO_2: 0.208). In additional an FiO_2 of 0.398 was sufficient enough to decrease [EPO] ($P < 0.05$). The novel finding of the present study was that distance covered in a TT_{10} was not affected by changes in endogenous [EPO], either by its elevation or reduction. Also, there was no effect of increased [EPO] on any of the physiological variables measured during the TT_{10}. These findings indicate that acutely elevated endogenous [EPO] does not enhance endurance performance in events lasting 10 minutes - a duration typical of competitive middle-distance races.

8.5.1 EPO concentration

The 2 h passive exposure to hypoxia (FiO_2: 0.118) significantly increased [EPO] above baseline values by 11 ± 9% 3 h post-exposure. This is in accordance with previous research where a passive exposure of >2 h at >4,000 m increased [EPO] by ~50% (Knaupp et al. 1992), ~36% (Rodríguez et al. 2000) and ~13% (Wahl et al. 2013). In the present study, HYPO exposure caused a decrease in SpO_2 ($P < 0.05$) from 98 ± 1% to 78 ± 5%, which was in agreement with the findings of Chapter 7. The increase in [EPO], however, was not as substantial as found in Chapter 7, where a 2 h exposure at FiO_2 of 0.125 and 0.115 increased [EPO] by ~42 and ~51%, respectively. It is possible that this is due to the individual sensitivity to hypoxia that affects EPO production (Chapman et al. 1998; Ge et al. 2002).

Hyperoxia, on the other hand, causes an acute decrease in EPO (Balestra et al. 2004; Balestra et al. 2006; Debevec et al. 2011; Keramidas et al. 2011). The studies found that normoxic hyperoxia (40-100% O_2) for 2 h produced a nadir in [EPO] at ~3 h post-exposure, followed by a zenith back to baseline levels around 8 h. Similarly, the present study found a 2 h passive exposure to hyperoxia (FiO_2: 0.398) significantly decreased [EPO] by 29 ± 9% at 3 h post-exposure. Heart rate was significantly lower during the HYPER (48 ± 6 b·min^{-1}) exposure than HYPO (61 ± 9 b·min^{-1}), but not the CON trial (53 ± 10 b·min^{-1}). There were no differences in D-RPE or LLQ as a result of any of the exposures and thus, did not cause dyspnoea-respiratory discomfort, or symptoms of acute mountain sickness.

No study has previously examined the impact of acutely (>2 h) increased endogenous [EPO] by normobaric hypoxia on middle-distance endurance performance; the majority of research into the role of chronically elevated [EPO] and both submaximal and maximal endurance performance has come from administering exogenous rhEPO. Studies that investigated chronic administration of rhEPO have found improvements in $\dot{V}O_{2max}$ and time to exhaustion (Birkeland et al. 2000), increased $\dot{V}O_{2max}$ and lowered submaximal HR (Russell et al. 2002), and increased $\dot{V}O_{2max}$ and prolonged time to exhaustion at 80% of maximal workload (Thomsen et al. 2007), but all attributable to changes in the haematology of the athlete. Less is understood about the role of acutely elevated EPO (endogenous or exogenous) in the days to hours before exercise.

In a study with human participants, Rasmussen et al. (2010) found that sustained low concentrations of rhEPO (3 months x 5,000 IU) were not high enough to cross the BBB compared to

short-term, higher concentrations (3 days x 30,000 IU). The study found that high-dose EPO treatment increased ventilation and arterial saturation during exercise, however, this did not enhance exercise performance or influence cognitive function in tasks undertaken before exercise (Rasmussen et al. 2010). Schuler et al. (2012) injected mice with 2000 IU of rhEPO 4-6 h before a maximal incremental exercise test. Plasma EPO was elevated to ~40 U·mL^{-1} compared to the control group (0 U·mL^{-1}), which resulted in a non-significant trend for improved $\dot{V}O_{2max}$ and time to exhaustion, but no differences found in HR. Interestingly, brain [EPO] was significantly elevated, showing the plasma [EPO] was sufficient high to penetrate the BBB (Schuler et al. 2012). The findings of the present study are in agreement with previous research, whereby increased [EPO] does not have a positive effect on endurance performance, although it should be noted that in this present work that normobaric hypoxia resulted in a smaller magnitude of [EPO] increase and it is not known whether endogenous [EPO] crossed the BBB.

8.5.2 Performance trial

There was no difference in the distance covered during the TT_{10} after HYPER, CON or HYPO. It is plausible that the increase in [EPO] was not sufficient enough to cross the BBB (although this was not measured) and, therefore had no meaningful physiological or neurological effects. Further, the significant increase in [EPO] as a result of HYPO caused no differences in HR, $\dot{V}O_2$, $\dot{V}_E$ or oxygen cost during the TT_{10}. Also, there were no differences in RPE during the TT_{10}. The increase in [EPO], after HYPO (11 ± 9%), was lower than previous studies when exposed to >2 h normobaric or hypobaric hypoxia (Knaupp et al. 1992; Rodríguez et al. 2000; Wahl et al. 2013). However, there was a significant interaction effect between the three exposures. As with the studies by Schuler et al. (2012) and Rasmussen et al. (2010) the acute increase in [EPO] did not alter PV, BV, [Hb] or Hct, and therefore an erythropoietic effect is unlikely to have occurred .

Previously, Chapman et al. (1998) divided athletes into 'responders' and non-responders' to altitude training based on a greater than mean improvement in 5,000 m time after LHTL. In the HYPO trial of the present study, six of the seven participants increased [EPO], however only three participants had an increase greater in [EPO] than the mean (11 ± 9%). The change in [EPO] ranged from -2% to +24% and this change could not be explained by the decrease in SpO_2 as a result of normobaric hypoxia. In comparison to CON, four of the seven participants covered a greater distance in the TT_{10}, however there was no relationship between Δ[EPO] and change in distance covered during the TT_{10} in the HYPO group. Only one for the four participants with a greater increase in [EPO] than the mean, covered a greater distance in the HYPO trial compared to CON. Schuler et al. (2012) speculated that the high plasma EPO level facilitated the penetration of the BBB and that EPO administration could positively affect self-perception and motivation while reducing physical pain, as well as mechanisms of central fatigue for a given workload. EPO has been found to exert neuroprotective and neurotrophic actions on the brain (Brines and Cerami 2005), modulating the cognitive and neural processing of emotional information 1 week after rhEPO administration, much like an anti-depressant drug (Miskowiak et al. 2007b). The present study did not find a main effect improvement in endurance

performance, in a time trial lasting 10 minutes - a duration typical of competitive middle-distance races, despite a hypoxia-induced increase in [EPO].

8.5.3 Limitations

The present study used a pre-loaded time trial to assess endurance performance. Performance can be influenced, both internally and externally, by physical, technical, mental and tactical variables (Currell and Jeukendrup 2008). This particular time trial was set to time rather than distance, which endurance athletes might not be as familiar with, although a time trial is a more logically valid tool than a time to exhaustion test (Currell and Jeukendrup 2008). The TT_{10} began with a rolling start at 95% of their $v\dot{V}O_{2max}$ to account for inappropriate pacing strategies. A custom made larger acceleration button was made and two familiarisation tests took place to ensure there was no learned effect during the experimental trials. The TEM between the familiarisation trails was 23 m or 0.97%. It is also possible that the distance covered during the TT_{10} was not sensitive enough to the small changes in [EPO], or the duration of the TT was insufficient for [EPO] to determine exercise performance, which differs between running events (Rabadán et al. 2011).

A large inter-individual variability in EPO has previously been reported in response to acute moderate and high simulated altitudes (Ge et al. 2002), this variability may have contributed to the TT_{10} performance. Furthermore although the participants completed a similar weekly training volume there was a wide range in both $\dot{V}O_{2max}$ (range: 56.5 to 73.2 mL·kg·min^{-1}) and 5,000 m personal best time (14:55 to 19:55 min:sec). This could have accounted for the marked individual variability in EPO response, which has previously been debated (Berglund et al. 1988; Schmidt et al. 1991; Schwandt et al. 1991). Endurance trained athletes and normal sedentary participants might have different activation of their hypothalamic-pituitary axis and sympathico-adrenal systems with related effects on diurnal EPO production (Jelkmann 1986). Rasmussen et al. (2010) who also found no improvement in performance after a high dose of EPO, suggesting that participant training status might have affected the outcome of the performance test.

8.5.4 Future directions

The present study attempted to increase endogenous [EPO] to a level that would improve endurance performance in a TT_{10}. Previous research has administered rhEPO, which is illegal; therefore normobaric or hypobaric hypoxia is the only alternative method. Assuming that the 'hypoxic dose' in the present study was not sufficient enough to cross the BBB, further investigations should consider what dose of simulated hypoxia is required. It is possible that duration of the time trial was not sufficient enough for elevated [EPO] to have a significant effect on performance. The physiological determinants of middle- and long-distance events are different (Rabadán et al. 2011), therefore performing with elevated [EPO] may only affect events with harder cognitive demands. Erythropoietic response to an acute exposure of normobaric hypoxia has also been investigated as a means of identifying athletes who may respond to altitude training (Friedmann et al. 2005a). The study found the variability in the increase in total haemoglobin mass and in sea level performance after training at

moderate altitude could not be predicted by the EPO response to acute hypoxic exposure. Further research is needed to identify the causes of individual variation in response to acute and chronic exposures to hypoxia.

8.6 Conclusion

In conclusion, 2 h of normobaric hypoxia exposure acutely increased endogenous [EPO] 3 h post-exposure, but had no measureable effect on $TT_{10.}$ This suggests that any ergogenic effect of changes in endogenous [EPO] is likely only through its chronic elevation and thus primarily as a result of increased oxygen-carrying capacity by increased tHbmass in the blood, i.e. achievable only through sustained and prolonged exposures to altitude (Gore et al. 2013). The present study was the first to show that, despite a 30% increase of [EPO] above CON and 40% above HYPER, TT_{10} was not improved in well-trained, male endurance runners. It is possible that the increase in endogenous [EPO] was not sufficient enough to cross the BBB and have a neurological effect on short-duration endurance performance, although this was not measured. Whilst there was increase in [EPO] on average across the group, there was marked individual variation in the magnitude of this increase. The individual responses to a hypoxia were explored previously in Chapter 7 (Study 4) and help contextualise the findings. The findings of the present study do not support the notion that endurance performance is enhanced when competed with elevated endogenous [EPO]. The findings also suggest poor performance on return from an altitude training camp may not be associated with the previously reported nadir in [EPO] (Garvican et al. 2012; Pottgiesser et al. 2012).

CHAPTER 9

9 PREDICTING AN ATHLETE'S PHYSIOLOGICAL AND HAEMATOLOGICAL RESPONSE TO CLASSIC ALTITUDE TRAINING USING HYPOXIC SENSITIVITY METHODS

9.1 Abstract

Purpose: Elite endurance runners frequently utilise LHTH altitude training to improve sea level performance, despite equivocal findings. This may be explained by the 'hypoxic dose' and/or the individual differences in response to an altitude exposure. This study had three aims; Part A, to test if 4-weeks of LHTH enhance physiological capacity and tHbmass in elite endurance runners; Part B, to map out the time course of tHbmass and erythropoietin concentration ([EPO]) post-LHTH and Part C, to predict the haematological and physiological responses of LHTH using the Richalet Hypoxic Sensitivity Test (HST). **Methods:** Twelve elite runners completed a 4-week altitude training camp (~2,300 m; ALT) and a further five endurance runners completed similar training at SL (CON). All participants visited the laboratory once for preliminary testing (PRE), including a treadmill test to determine lactate threshold (LT), lactate turnpoint (LTP), $\dot{V}O_{2max}$ and the oCOR-method to determine tHbmass. Both groups repeated the PRE testing post-altitude or post-training (POST-2). The ALT group completed additional post-altitude testing sessions (POST-1, -2 and -3) to determine [EPO] and tHbmass and a HST (PRE and POST-2). **Results:** Part A found a difference ($P < 0.05$) within ALT, but not CON from PRE to POST-2 in average LT (6.1 ± 4.6% vs. 1.8 ± 4.5%) and LTP (5.4 ± 3.8% vs. 1.1 ± 3.2%), respectively. No difference was found within ALT or CON in $\dot{V}O_{2max}$ (2.7 ± 3.5% vs. -3.3 ± 6.3%) or tHbmass (1.9 ± 2.9% vs. -0.1 ± 3.3%), respectively. Part B found no change in mean tHbmass post-ALT, however, [EPO] was lower ($P < 0.05$) at POST-1. In Part C the HST revealed desaturation at rest (ΔSp_r) and hypoxic ventilatory response at rest (HVR_r) predicted individual changes in tHbmass. Further, hypoxic cardiac response at rest (HCR_r) predicted individual changes in $\dot{V}O2max$. **Conclusions:** In conclusion, 4-weeks of LHTH enhanced some physiological determinants of endurance performance, but not mean tHbmass compared to CON amongst elite endurance runners. The time course analysis found no changes in tHbmass, however [EPO] decreased immediately on return from altitude. Although there was no significant change, at group level, in mean tHbmass or mean $\dot{V}O_{2max}$ the individual changes were predicted by HST indices.

9.2 Introduction

The proposed physiological benefits of altitude training have been widely debated (Jacobs 2013; Wilber 2013). The primary goal of altitude training is to improve the oxygen carrying capacity of the blood (primarily through an increase in RBCs) and improve oxygen utilisation at the muscle (Chapman and Levine 2007). The trigger for this is a reduction in atmospheric pressure that causes a reduction in inspired oxygen pressure, and therefore SaO_2 (Mazzeo 2008). To facilitate the eventual restoration of normal blood oxygen content and maintain tissue oxygenation, the reduced SaO_2 elicits production of erythropoietin (EPO) (Ge et al. 2002), which then causes an increased RBC mass and tHbmass (Garvican et al. 2011b; Saunders et al. 2013; Rasmussen et al. 2013). If this increase in tHbmass is large enough, $\dot{V}O_{2max}$ will in turn increase (Levine and Stray-Gundersen 1997; Schmidt and Prommer 2010; Robach et al. 2012) and this may improve performance in endurance exercise such as cycling and running (Bassett and Howley 2000; Jones and Carter 2000).

The threshold altitude for a sustained increase in EPO concentration ([EPO]) is ~2,200 m (Weil et al. 1968), which is a consequence of a substantial change in the partial pressure of oxygen (PO_2) to prevent enough oxygen binding to blood and a movement from the flat upper part of the oxyhaemoglobin dissociation curve to the steep portion (~92% to 88% SaO_2). Subsequent research has suggested that sea level endurance running performance (of 800-10,000 m distance), as well as determinants of endurance performance (e.g. fractional utilisation of $\dot{V}O_{2max}$, running economy and $\dot{V}O_{2max}$) and haematological markers (e.g. tHbmass) are improved if the correct hypoxic "dose" (a minimum of 4 weeks at 2,000-2,500 m with a daily dose of >14 hours) is achieved with the LHTL method (Rusko et al. 2004; Bonetti and Hopkins 2009; Millet et al. 2010; Gore et al. 2013; Chapman et al. 2014a). However, there is still a belief from some researchers that the scientific justification for the benefits of altitude training is not as strong as the general perception of its benefit (Lundby et al. 2012).

The proposed optimal 'hypoxic dose' has been employed with endurance runners using the LHTH method with varying degrees of success. Levine and Stray-Gundersen (1997) found red cell volume was significantly increased (~9%), along with $\dot{V}O_{2max}$ (~4%), as well as improvements in 5,000 m time trial performance (~2%) after four weeks of living at 2,500 m and training at 2,500–2,700 m in collegiate athletes. At a lower altitude, for national-standard distance runners living at 1,800 m and training at 1,700–2,200 m for three weeks, Garvican-Lewis et al. (2015) reported increases in tHbmass of ~3% compared to baseline. These findings are supported by Frese and Friedmann-Bette (2010) who also found increases in tHbmass (~5%) after 20 days at 1,300 m followed by 22 days at 1,650 m, and separated by 19 days at sea level in international junior and national level 400-800 m runners. Similarly, a meta-analysis by Gore et al. (2013) reported tHbmass (measured using the oCOR-method) increased at approximately 1.1% per 100 h of LHTH (>2100 m). However, Rasmussen et al. (2013) argued that exposure time needs to be longer than 4 weeks at an altitude of <3,000 m for red cell expansion.

Bailey et al. (1998) also showed no changes in maximal heart rate, ventilation ($\dot{V}_E$), running economy or lactate threshold (LT) in highly-trained to elite middle- and long-distance runners despite

reductions in blood lactate concentration (B[La]) at submaximal running speeds after four weeks LHTH at 1,500–2,000 m, as well as no changes in $\dot{V}O_{2max}$ after four weeks LHTH at 1,640 m. Other investigations using elite runners and a control group have found no change in sea level $\dot{V}O2max$ or time trial performance after LHTH (Adams et al. 1975; Svedenhag et al. 1991). Despite the conflicting findings elite British endurance runners regularly attend LHTH training camps at 2,000–2,500 m (see Section 4.5)

The 'eliteness' of the study participants should also be considered. Well-trained (Levine and Stray-Gundersen 1997), elite to well-trained (Garvican-Lewis et al. 2015) and highly-trained (Frese and Friedmann-Bette 2010) athletes have been studied, which according to Lundby et al. (2012) can have an effect on the responsiveness to a given stimulus. The conflicting findings from LHTH altitude training research in runners has been associated with an inadequate hypoxic dose (Bailey et al. 1998; Gore et al. 1998), lack of individual adjustment of training intensity (Friedmann et al. 2005b), the timing of sea level testing (Dick 1992), proximity to a physiological or performance ceiling (Robach and Lundby 2012) and inter-individual variability of EPO production in response to hypoxia (Friedmann et al. 2005a), potentially linked to the training status as mentioned above.

Due to varying individual responses to hypoxia, not every single athlete benefits from altitude training (Friedmann-Bette 2008). Chapman and colleagues (1998) first reported the individual variation in response to altitude training and associated this with measured changes in plasma [EPO]. In a retrospective analysis, they found that those well-trained collegiate runners who improved their 5,000 m time (-36.6 ± 12.0 s vs. +24.0 ± 16.2 s) post-altitude training also elicited a greater increase in [EPO] after 30 hours at 2,500 m (152 ± 6 vs. 134 ± 10%), which remained significantly elevated after 14 days at 2,500 m (134 ± 31 vs. 117 ± 27). The magnitude of EPO response at altitude could be influenced by several factors such as individual differences in hypoxic ventilatory drive, oxygen half-saturation pressure of haemoglobin, or sensitivity to hypoxia at the point of EPO release (Chapman et al. 1998), and it is possible these factors may be genetically inherited traits (Scoggin et al. 1978). In this group of collegiate athletes the changes in EPO could not be explained by pulse oximetry as both the responders (80 ± 4%) and non-responders (80 ± 5%) found no difference between groups in SaO_2 during a steady-state run at a simulated altitude of 2,700 m.

Individual variation in EPO, SaO_2, and hypoxic ventilatory response (HRV) to acute and chronic exposure to altitude and hypoxia have been reported (Chapman et al. 1998; Ge et al. 2002; Friedmann et al. 2005a; Savourey et al. 2007; MacKenzie et al. 2008). Despite this variation, many studies have neglected to attempt to understand an individual athlete's tolerance to hypoxia. Human hypoxic tolerance refers to the oxygen sensing mechanisms, which tell the organism when the problem arises and initiate the whole hypoxia response cascade (Hochachka et al. 1998). The reported individual variation in athlete hypoxia responses is thought to play a role in the underlying adaptions that result from an altitude training camp. Chapman (2013) believed that pulmonary gas exchange and specifically SaO_2 (or SpO_2 when measured by oximetry) maintenance plays a pivotal role in predicting the decline in exercise performance at different altitudes. Individuals who are least able to maintain SaO_2 usually end up being the ones with the largest drop in $\dot{V}O_{2max}$, which is a result of greater pulmonary gas exchange limitations (Chapman 2013) and secondary to a combination of inadequate

hyperventilation, greater ventilation-perfusion (V/Q) mismatch or diffusion limitations (Dempsey and Wagner 1999; Hopkins 2006).

The ability to predict which elite endurance athletes would best respond to altitude training would be a useful pre-altitude camp screening tool (Chapman et al. 2010), however, the pre-screening of athletes training at altitude has received relatively little attention (Friedmann et al. 2005a; Chapman et al. 2010). Predicting the susceptibility to acute mountain sickness (AMS) on the other hand has been well researched, most typically in mountaineers sojourning to extreme altitudes (above 5000m) (e.g. Bärtsch et al. 2002; Burtscher et al. 2004; Savourey et al. 2007; Burtscher et al. 2008; Richalet et al. 2012; Wagner et al. 2012). Studies have reported that a lower hypoxic ventilatory response (HVR) predicted AMS in mountaineers (Richalet et al. 1988) and SaO_2 measured after 20 to 30 min of a hypoxic exposure and prolonged exposures are good predictors of AMS susceptibility (Burtscher et al. 2004; Burtscher et al. 2008). Conversely, other studies (Milledge et al. 1988; Milledge et al. 1991; Hohenhaus et al. 1995; Savourey et al. 1995; Bärtsch et al. 2002) have failed to find an association between HVR and AMS.

The individual differences in the tolerance to hypoxia and/or the ability to acclimatise may explain the altitude-dependent increase of the AMS (Burtscher et al. 2008). Most research dealing with individual response to hypoxia have recorded the ventilatory and cardiovascular responses for prediction (Burtscher et al. 2008), therefore the use of these metrics may be appropriate to test on an elite athlete population attending a LHTH altitude training camp. A short, minimally invasive hypoxic sensitivity test (HST) has been utilised (Richalet et al. 1988; Richalet et al. 2012) and found a relationship between history of severe AMS and lower ventilatory and cardiac responses. The relationship between the outcome measures of the HST (e.g. SaO_2 and HVR), and typical changes in altitude training measures (e.g. $\dot{V}O_{2max}$ and tHbmass) have yet to be investigated.

The primary aim of the present study was to investigate the effect of 4-weeks of LHTH at ~2,300 m on physiological determinants of performance and tHbmass in elite endurance runners (*Part A*). The study also aimed to establish the time course of haematological markers post-LHTH (*Part B*) and finally assess the predictive ability of the Richalet HST against the hypoxia-induced changes in physiological and haematological markers (*Part C*). Three primary hypotheses were evaluated: that LHTH will improve physiological capacity and tHbmass in elite endurance runners (*Part A*); that the time course of haematological markers post-altitude will effect concomitant changes in physiological determinants of endurance performance (*Part B*) and; that there will be a relationship between Richalet HST indices and the haematological response of endurance runners after a LHTH altitude training camp (*Part C*).

9.3 Methods

9.3.1 Participants

British Athletics selected 16 elite middle- and long-distance runners to attend a LHTH altitude training camp (ALT). The participants had represented Great Britain at junior, Under 23 or senior level in either cross-country or track running. All athletes presented injury and illness free at the start of the study; however, four were unable to complete the full study duration due to injury or illness and withdrew. A further six nationally competitive middle- and long-distance runners were also recruited to form a SL control (CON) group; one participant withdrew through injury. The anthropometric and physiological characteristics of the experimental and control group are outlined in Table 9.1. All participants were sea level residents and had not been to altitude above 1,500 m in the previous three months. The institutional ethics committee approved all protocols, procedures and methods, with participants completing medical questionnaires and written informed consent following the principles outlined by the Declaration of Helsinki, as revised in 2013.

In the 3-4 months before the study period, all of the athletes were engaged in national and international track racing. Six weeks before the commencement of the study, all athletes had an end of year break (~14 days) and had been rebuilding their training for one month before the study period. All ALT athletes were UKAD registered, had completed the relevant "whereabouts" documentation and were therefore, subject to potential random anti-doping control before and during the study period. Participants were screened for iron status (Ferritin: 91 ± 66 ng·mL^{-1}) two weeks prior to taking part in the altitude training camp or training period. If a participant's ferritin was below 30 ng·mL^{-1} they were instructed to take one capsule of Ferrous Fumerate (305 mg) 3 times per day for the experimental testing period (one recorded incidence). All other participants were instructed to continue with their normal iron supplementation programme.

Table 9.1: Anthropometric and physiological characteristics of all study participants. Athlete performance time (season's best during the 2014/15 season) is expressed as percentage (%) of the male and female British record time (as of Aug 2015) in their primary event of the 2014/15 season (800 – 10,000 m).

	ALT		**CON**	
Sex	Male	Female	Male	Female
N	7	5	4	1
Age (yr)	22 ± 3	25 ± 5	24 ± 3	20
Body Mass (kg)	67.3 ± 5.5	57.0 ± 3.3	67.6 ± 4.0	57.6
Height (cm)	179.1 ± 4.8	169.0 ± 3.9	183.8 ± 6.8	168.3
Sum of 8 skinfolds (mm)	50.4 ± 10.4	73.6 ± 23.1	47.4 ± 13.0	52.5
Body Fat (%)	11.7 ± 2.4	13.7 ± 2.7	9.4 ± 2.5	10.0
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	72.1 ± 8.5	65.0 ± 3.5	71.9 ± 2.8	67.1
tHbmass (g)	909.2 ± 98.1	678.2 ± 70.4	898.9 ± 61.9	638.7
tHbmass (g·kg^{-1})	13.7 ± 1.8	11.7 ± 1.3	13.3 ± 0.3	11.1
Performance time (%)	94.0 ± 1.3	94.7 ± 2.3	92.4 ± 0.7	95.4
Weekly Training Volume (miles)	56.3 ± 19.0	46.6 ± 12.6	40.9 ± 20.9	46.4

9.3.2 Experimental design

The experimental design is illustrated in Figure 9.1. Twelve runners travelled to Iten, Kenya (2,300 m above SL) for 4 weeks to live and train at the same altitude. All participants visited the laboratory on two occasions prior to travelling for physiological testing, including a HST, and a further tHbmass and blood test. There were 8 ± 5 d between baseline tHbmass and venous blood tests. For logistical reasons each participant could not be tested on one day so it was spread across a 2-week period. There were 10 ± 4 d between the physiological testing and departure to Kenya. Upon arriving back at SL participants completed three post-ALT testing sessions. POST-1 consisted of tHbmass and blood test, POST-2 repeated the physiological testing and POST-3 repeated the tHbmass and blood test. Again for logistical reasons, each post-ALT tHbmass and blood test could not be measured on one day. Post-ALT testing was spread across a 3-week period, which is outline in Table 9.2. The SL (Loughborough; 43 m) CON group completed the same physiological testing and tHbmass testing before a block of training (PRE) and post-training (POST-2). There were 61 ± 8 days and 51 ± 4 days between PRE and POST-2 in the CON and ALT group, respectively.

Table 9.2: Timing of collection of tHbmass and [EPO] data in Part B within the ALT group only.

Days between post-ALT testing		
POST-1 to POST 2	**POST-2 to POST-3**	**POST-1 to POST-3**
10 ± 3 d	7 ± 4 d	17 d ± 3 d
(Range: 7 to 14 d)	(Range: 3 to 14 d)	(Range 13 to 22 d)
Days at SL for post-ALT testing		
POST-1	**POST-2**	**POST-3**
1 ± 2 d	11 ± 3 d	18 ± 2 d
(Range: 0 to 5 d)	(Range: 7 to 15 d)	(Range: 15 to 22 d)

9.3.3 Training distribution

Participants were given training advice, however, as the same individual did not coach each athlete their programmes were not identical. Training was predominantly characterized by a typical distribution of intensity and duration of an elite endurance runner (Seiler 2010). Using the zones described by Seiler (2010) ~85% of the training was low intensity (below LT/VT_1), and ~15% was high intensity interval training (above LT/VT_1). The training consisted of weeks 1-3, 48 ± 17 miles, weeks 4-7, 50 ± 17 miles and weeks 8-10, 44 ± 15 miles. During each week 2-3 training sessions above LTP/VT_2 were completed. Training data were recorded in training diaries and GPS watches depending on the athlete's preference. The ALT athletes were instructed to record their training 3-weeks pre- and 3-weeks post-altitude training. The CON athletes were instructed to continue training as they would during the winter phase of the season. Training data were collected for 8-10 weeks.

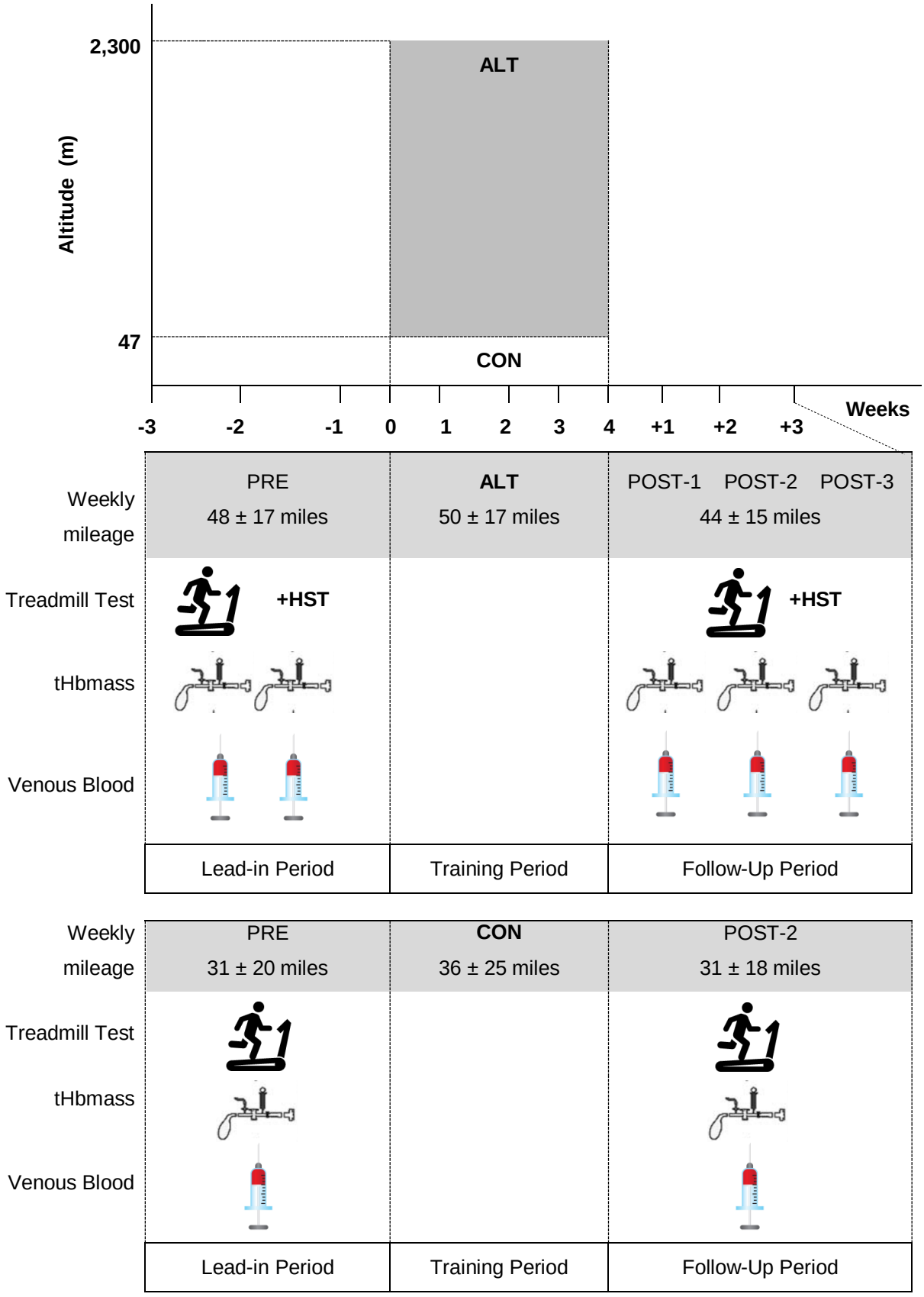


Figure 9.1: Schematic outline of testing and training before, during and after the 4-week altitude training camp (ALT) and sea level training block (CON). Physiological and haematological measures were taken in the lead-in period before the camp (PRE) and during the follow-up period (POST-1, POST-2 and POST-3). The schematic is split into two sections on the same timeline including the weekly mileage completed.

9.3.4 Physiological testing

All participants (ALT and CON) completed a full series of physiological tests consisting of submaximal and maximal treadmill testing and tHbmass testing. The ALT group completed further haematological testing and the HST.

Before exercise, body mass, stature and body composition was measured as previously described in the General Methods (see section 3.3). Participants then performed a submaximal and maximal running assessment (Shaw et al. 2015) for the determination of the $\dot{V}O_2$–speed relationship and $\dot{V}O_{2max}$. The protocol and equipment used has been previously described (see section 3.4).

Following this tHbmass was determined using the oCOR-method (Schmidt and Prommer 2005; Prommer and Schmidt 2007). The protocol and equipment used was previously described (see section 3.7). A Pentra ES 60 (Horiba Medical; Kyoto, Japan) was used to determine Hct and [Hb] from venous blood samples.

9.3.5 Haematology and biochemistry

Venous blood was collected via venepuncture of an antecubital vein in the forearm. Plasma [EPO] was quantified by ELISA plate (BioVendor R&D; Oxford Biosystems Ltd, UK). All ELISAs were processed according to the manufacturer's instructions and all blood sampling methodology is previously described (see section 3.11.4.2)

9.3.5.1 Hypoxic sensitivity test

The HST was completed ~2 h after the treadmill testing to allow the participants to recover. All ALT participants completed a HST as previously described (Richalet et al. 2012; Bourdillon et al. 2014) before and after the altitude training camp. The modified HST consisted of four 4-min phases: rest in normoxia (RN), rest in hypoxia (RH), exercise in hypoxia (EH) and exercise in normoxia (EN). For a typical physiological response to the HST see Figure 9.2.

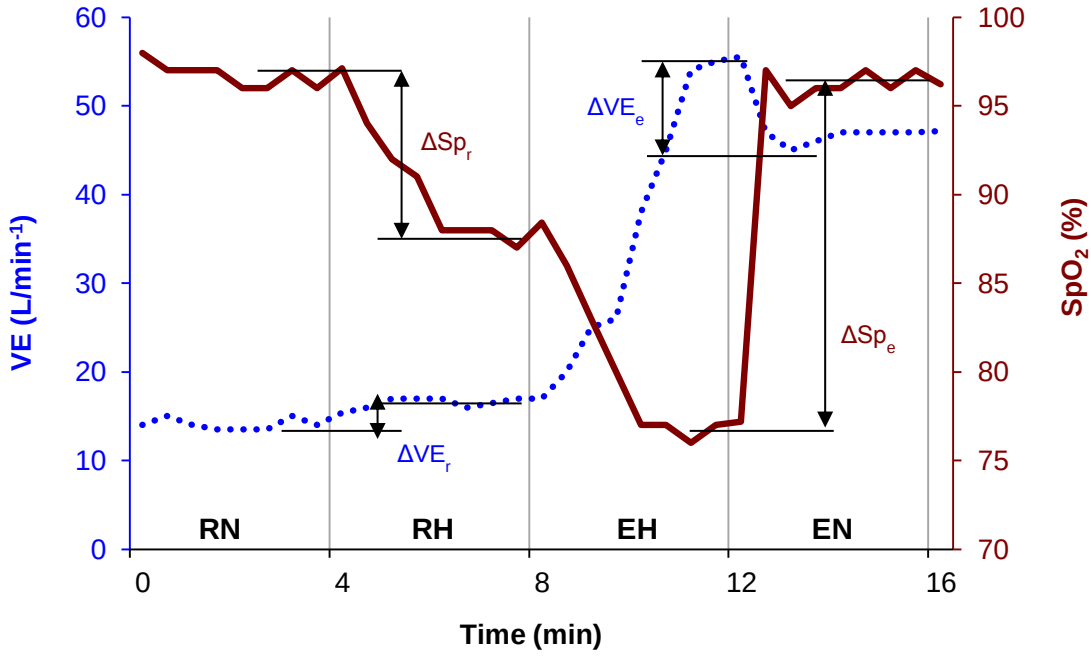


Figure 9.2: A typical physiological response from the Hypoxic Sensitivity Test (Richalet et al. 2012). RN = rest in normoxia; RH = rest in hypoxia; EH = exercise in hypoxia; EN = exercise in normoxia; Hypoxia was generated by breathing a normobaric hypoxic gas mixture (Hi-FiO_2: 0.115). Dotted blue line = VE and solid red line = SpO_2. ΔSp_r = change in desaturation at rest; ΔSp_e = change in desaturation during exercise; ΔVE_r = change in pulmonary ventilation at rest; ΔVE_e = change in pulmonary ventilation during exercise.

Minute ventilation (VE; L·min^{-1}) was continually measured during the HST via an automated open circuit metabolic cart (Oxycon Pro, Carefusion, San Diego, Calif., USA). A two-way non-breathing valve (Hans Rudolph Inc., Kansas City, MO, U.S.A.) was attached to the end of the mask and turbine to control the FiO_2 that the participants breathed. With the valve closed the participant breathed the ambient air and with the valve open the participants breathed the hypoxic air from the altitude generators (McKinley Altitude Simulator, Higher Peak Performance). The two generators were set at different levels and filled two separate Douglas bags. The bags were switched in order to breathe the different FiO_2.

First, participants rested (RN) whilst standing on the treadmill for 4-min breathing normoxic air, the valve was then opened and the participants rested for a further 4 min breathing hypoxic air (RH). The treadmill was started and participants walked uphill (5.8 ± 0.4 km·h^{-1} and 7.5 ± 0.7% gradient) whilst breathing hypoxic air (EH) for 4 min. The valve was then closed and the participants continued walking for a final 4 min whilst breathing normoxic air (EN). The walking speed and gradient for the exercise phases was the same and fixed to attain a heart rate (HR) at around 120–140 bpm. A Wrist Pulse Oximeter MD300W (ChoiceMMed; Hong Kong) measured SpO_2 continuously and HR was also measured continuously (Polar H1 heart rate sensor with FT3 wristwatch; Kempele, Finland). Ventilation, HR and SpO_2 responses during the last 30 seconds of each phase were used to characterise subjects' sensitivity to hypoxia (Richalet et al. 2012) by calculating:

- Desaturation at rest: $\Delta Sp_r = Sp_{rn} - Sp_{rh}$ (%)
- Desaturation at exercise: $\Delta Sp_e = Sp_{en} - Sp_{eh}$ (%)
- Ventilation changes at rest: $\Delta VE_r = VE_{rh} - VE_{rn}$ ($L \cdot min^{-1}$)
- Ventilation changes at exercise: $\Delta VE_e = VE_{eh} - VE_{en}$ ($L \cdot min^{-1}$);
- Hypoxic ventilatory response at rest: $HVR_r = (VE_{rh} - VE_{rn})/\Delta Sp_r/BW \times 100$ ($L \cdot min^{-1} \cdot kg^{-1}$)
- Hypoxic ventilatory response at exercise: $HVR_e = (VE_{eh} - VE_{en})/\Delta Sp_e/BW \times 100$ ($L \cdot min^{-1} \cdot kg^{-1}$)
- Hypoxic cardiac response at rest: $HCR_r = (HR_{rh} - HR_{rn})/\Delta Sp_r$ ($beats \cdot min^{-1} \cdot \%$)
- Hypoxic cardiac response during exercise: $HCR_e = (HR_{eh} - HR_{en})/\Delta Sp_e$ ($beats \cdot min^{1} \cdot \%$),

Where Sp = oxygen saturation, HR = heart rate, VE = ventilation, BW = body weight, r = rest, e = exercise, n = normoxia and h = hypoxia.

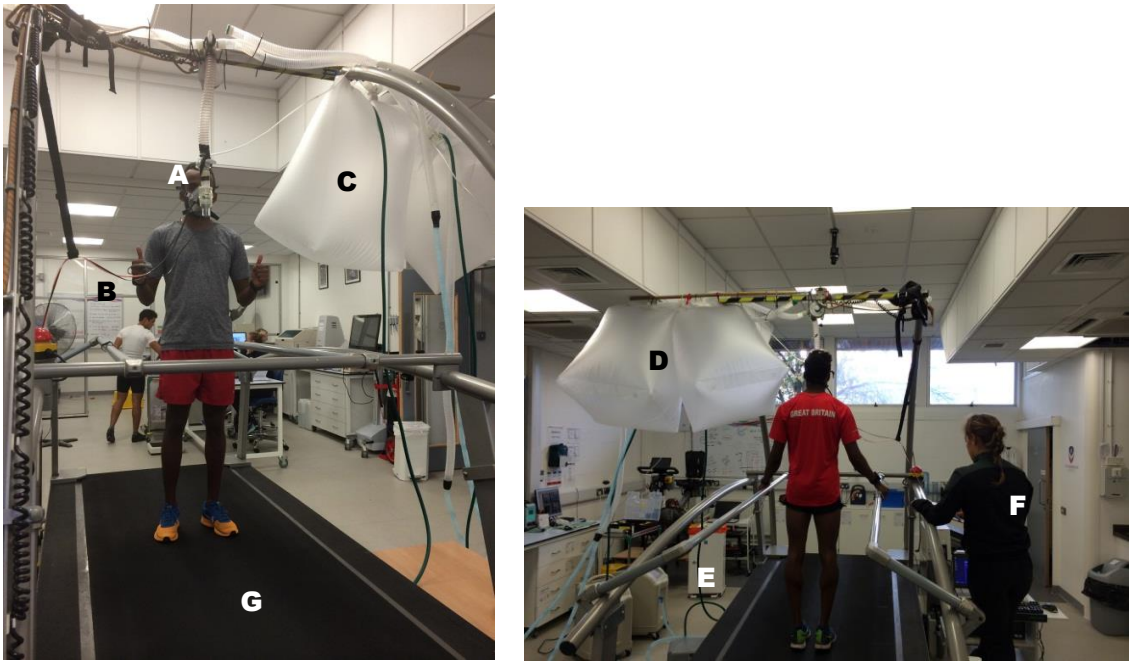


Figure 9.3: The modified HST in action. The left side photo shows the participant connected to the facemask (A) with sensor cables going to the open circuit metabolic cart (B) and the tubing to the Douglas bags (C). The right side photo shows the two Douglas bags (D) and hypoxic generators below (E). The experimenter (F) is measuring SpO_2 and HR. The participants spend the full 16-min on the treadmill (G) either standing or walking.

9.3.6 Statistical analyses

For all statistical analyses and further discussion of the results the male and female participants in each group (ALT and CON) were pooled. Due to injury, illness and availability not all participants were able to complete every testing session during the experimental testing period, which is outlined in Table 9.3. The data collected from the study was split into three investigations to answer the three aims. Where appropriate, Bonferroni-adjusted pairwise comparisons revealed where differences occurred.

Part A used a mixed measures ANOVA on LT, LTP, $\dot{V}O_{2max}$, BF%, sum8 and tHbmass measured PRE and POST-2 for the ALT (n = 11/12) and CON group (n = 5).

Part B used one-way repeated measures ANOVA on tHbmass, BV, PV and [EPO]. For the statistical analysis only the eight participants who completed at three post measurements for tHbmass, BV and PV data were included and for [EPO] on seven participants were included. Linear regression and subsequent correlation analysis were used to determine the relationships with in the ALT group.

Part C used stepwise multiple regression analysis for the eight dependant variables of the HST (ΔSp_r, ΔSp_e, ΔVE_r, ΔVE_e, HVR_r, HVR_e, HCR_r and HCR_e) with the percentage change in tHbmass at POST-1 and the percentage change in $\dot{V}O_{2max}$ at POST-2. Twelve participants were used for the model of tHbmass and ten participants were used for the model of $\dot{V}O_{2max}$, as two of the participants were unable to complete the post-ALT treadmill testing due to injury.

The following classification system refined by Hopkins et al. (2009) was used to interpret the magnitude of the relationship: trivial 0.0–0.1; small 0.1– 0.3; moderate 0.3–0.5; large 0.5–0.7; very large 0.7–0.9; almost perfect 0.9–1; and perfect 1. All data were recorded as mean ± SD, with significance accepted at $P < 0.05$.

Table 9.3: Final participant numbers for results and statistical analysis (✓= data collected, ✘= no data collection due to injury or illness)

Athlete	Submax	Max	BC	Hb-1	Hb-2	HST	Submax	Max	BC	Hb-1	Hb-2	Hb-3	HST
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✘	✓	✓
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✘
7	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
8	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✘	✓
9	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✘	✓
10	✓	✓	✓	✓	✓	✘	✓	✓	✓	✓	✓	✓	✘
11	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✘	✓
12	✓	✘	✓	✓	✓	✓	✓	✘	✓	✓	✓	✓	✓
N =	12	11	12	12	12	11	12	11	12	12	11	9	10
1	✓	✓	✓	✓			✓	✓	✓		✓		
2	✓	✓	✓	✓			✓	✓	✓		✓		
3	✓	✓	✓	✓			✓	✓	✓		✓		
4	✓	✓	✓	✓			✓	✓	✓		✓		
5	✓	✓	✓	✓			✓	✓	✓		✓		
N =	5	5	5	5			5	5	5		5		

Submax = Submaximal treadmill test (LT, LTP); Max = Maximal treadmill test ($\dot{V}O_{2max}$); BC = Body Composition (BF%, sum8); Haematological = Hb (tHbmass, BV, PV and [EPO]) and Hypoxic Sensitivity = HST (ΔSp_r, ΔSp_e, ΔVE_r, ΔVE_e, HVR_r, HVR_e, HCR_r and HCR_e).

9.4 Results

9.4.1 Part A: Physiological responses to LHTH

There were no differences between baseline measurements of ALT and CON groups in $\dot{V}O_{2max}$ (P = 0.612), LT (P = 0.981), LTP (P = 0.908), RE (P = 0.573), tHbmass (P = 0.658) and sum of 8 skinfolds (P = 0.142), there was however a difference found in body fat % (P = 0.022).

*ALT had no effect on average $\dot{V}O_{2max}$ (F = 0.003, P = 0.959, η_p^2 = 0.05) from PRE to POST-2, however an interaction however an interaction effect was found between time*condition (F = 5.023, P = 0.042, η_p^2 = 0.26). In the ALT group the ALT group $\dot{V}O_{2max}$ increased by 2.7 ± 3.5%, and in the CON group $\dot{V}O_{2max}$ decreased by 3.3 ± 6.3% (see*

*Figure 9.4A). There was a main effect of time on LT (F = 10.440, P = 0.006, η_p^2 = 0.41) and LTP (F = 11.782, P = 0.004, η_p^2 = 0.44), however there was no interaction effect on LT between time*condition (F = 2.858, P = 0.112, η_p^2 = 0.16). In the ALT group LT increased by 6.1 ± 4.6%, and in the CON group LT increased by 1.8 ± 4.5% (see*

*Figure 9.4C). An interaction effect was found on LTP between time*condition (F = 5.050, P = 0.040, η_p^2 = 0.25), with LTP increasing by 5.4 ± 3.8% and 1.1 ± 3.2% in the ALT and CON, respectively (see*

Figure 9.4D).

*There was no effect of time on tHbmass (F = 1.577, P = 0.228, η_p^2 = 0.10) and there no interaction effect was found interaction effect was found between time*condition (F = 1.377, P = 0.259, η_p^2 = 0.08). Total Hbmass increased by 1.9 increased by 1.9 ± 2.9% and 0.1 ± 3.3% from PRE to POST-2 in the ALT and CON group, respectively (see*

Figure 9.4B). Table 9.4 illustrates the HR and B[La] measured during the treadmill testing in the ALT and CON groups at PRE and POST-2. ALT had no effect on average HR or B[La] at LT, LTP or $\dot{V}O_{2max}$ from PRE to POST-2 or compared to CON. Differences were found (F = 21.187, P = 0.010, η_p^2 = 0.84) between ALT and CON within PRE and POST-2, however there was no interaction effect.

*Table 9.4: Heart rate (HR) and blood lactate (B[La]) responses in ALT and CON groups from PRE to POST-2. * denotes significant difference (P<0.05) between conditions.*

	ALT		**CON**	
	PRE	**POST-2**	**PRE**	**POST-2**
HR at LT (b·min^{-1})	159 ± 8	162 ± 10	161 ± 7	167 ± 7
HR at LTP (b·min^{-1})	170 ± 10	174 ± 10	172 ± 7	176 ± 7
HR_{max} (b·min^{-1})	183 ± 12	183 ± 10	190 ± 4	188 ± 4
B[La] at LT (mmol·L^{-1})	1.3 ± 0.5	1.1 ± 0.2	1.4 ± 0.5	1.3 ± 0.3
B[La] at LTP (mmol·L^{-1})	2.6 ± 0.5	2.4 ± 0.4	2.4 ± 0.8	2.6 ± 0.5
$B[La]_{max}$ (mmol·L^{-1})	8.2 ± 2.5	8.0 ± 2.1	10.4 ± 2.0 *	10.5 ± 1.7 *

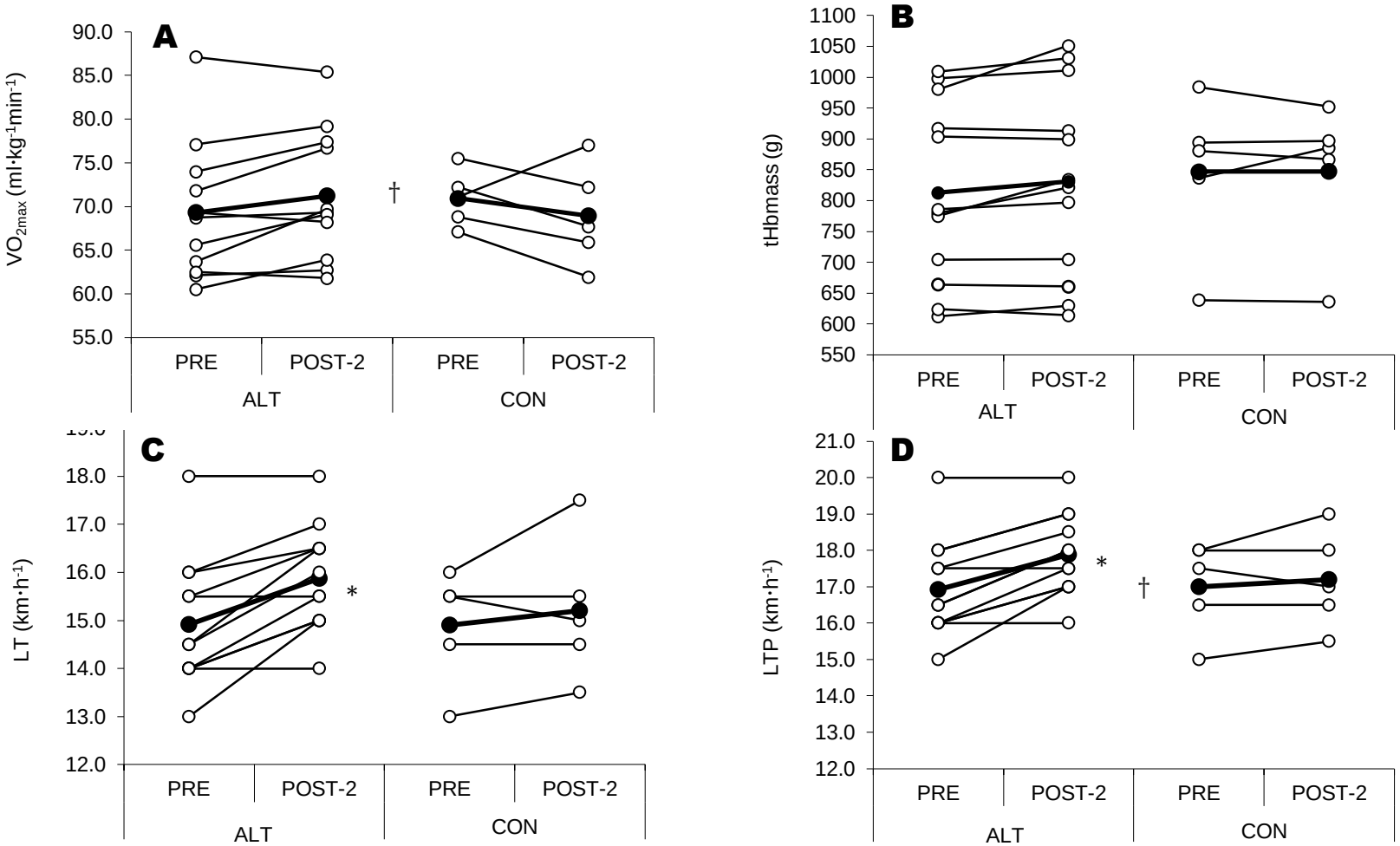


*Figure 9.4: Individual (open circle) and mean (closed circle) differences from PRE to POST-2 in $\dot{V}O_{2max}$ (Plot A), tHbmass (Plot B), LT (Plot C), LTP (Plot D) from ALT and CON groups. * denotes main effect of time and † denotes interaction effect of time*condition.*

9.4.2 Part B: Time course of tHbmass and [EPO] in response to LHTH

The baseline tHbmass, BV, PV and haematological markers, of the eight participants who completed all of the post-altitude training data collection, are displayed in Table 9.4. The CON group was not included in the time course analysis.

Table 9.5: tHbmass and haematological markers at baseline of the eight participants who completed all of the post-altitude training data collection

Baseline Value	**N**	**ALT**
tHbmass (g)	8	835 ± 162
tHbmass ($g \cdot kg^{-1}$)	8	12.8 ± 1.7
BV (ml)	8	6167 ± 1044
PV (ml)	8	3648 ± 600
[EPO] ($mUl \cdot mL^{-1}$)	7	6.6 ± 2.6

The time course of changes in tHbmass, BV and PV observed in response to altitude training is shown in

Figure 9.5A, B and C, respectively. There were no differences found in tHbmass (F = 2.060, P = 0.136, η_p^2 = 0.23), BV (F = 0,183 P = 0.906, η_p^2 = 0.08) or PV (F = 0.066, P = 0.977, η_p^2 = 0.01). There was a main effect of time on [EPO] (F = 4.486, P = 0.016, η_p^2 = 0.43). Mean [EPO] decreased from 6.6 ± 2.6 to 3.7 ± 1.0 $mUl \cdot mL^{-1}$ at POST-1, before increasing back towards baseline level at POST-2 (4.9 ± 2.0 $mUl \cdot mL^{-1}$) and POST-3 (5.4 ± 1.6 $mUl \cdot mL^{-1}$).

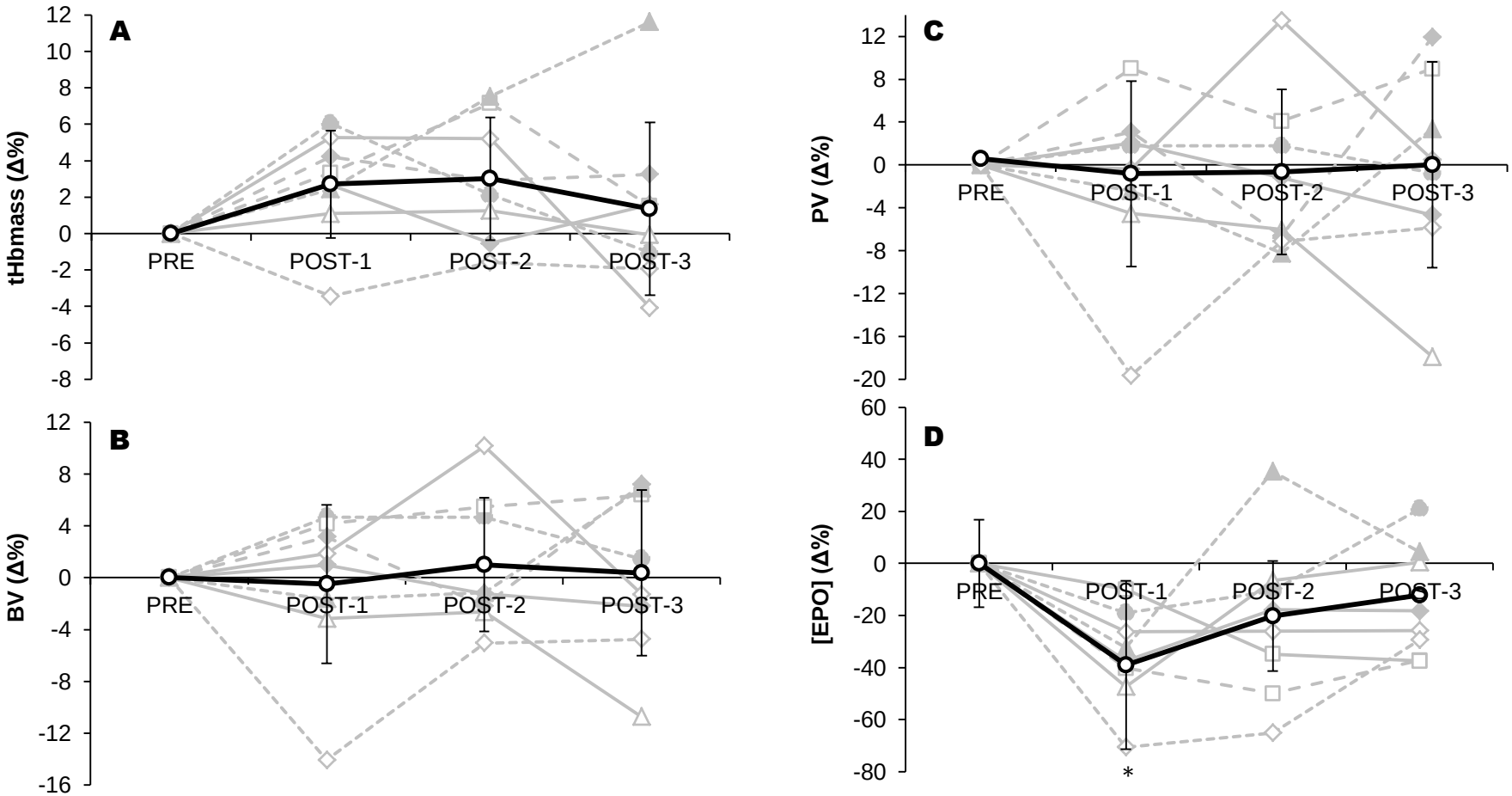


*Figure 9.5: Mean (black line) and individual (grey lines) data for percentage change (Δ) in tHbmass (Plot A), BV (Plot B), PV (Plot C) and [EPO] (Plot D). * denotes significant difference (P<0.05) from PRE.*

9.4.3 Part C: Hypoxic Sensitivity Test

The main parameters of SpO_2, $\dot{V}_E$ and HR during the four phases of the HST are summarised in Figure 9.6A, B and C, respectively. During each phase of the HST the significant differences between SpO_2, VE and HR are labelled on Figure 9.6. From PRE to POST-2 HR was significantly lower (P = 0.046) during the EH phase of the HST (134 ± 10 b·min^{-1} to 125 ± 10 b·min^{-1}). There were no other differences found from PRE to POST-2 in SpO_2, VE or HR.

Figure 9.7 illustrates the changes in the HST indices as a result of exercise (from resting in hypoxia to exercising in hypoxia) and also the effect of time on the indices measured PRE and POST-2 ALT. There was a main effect of exercise on ΔSp (F = 36.663, P = 0.001, η_p^2 = 0.80), but no effect over time (F = 0.284, P = 0.607, η_p^2 = 0.03) and no interaction effect between exercise*time. The ΔVE also found a main effect of exercise (F = 12.90, P = 0.006, η_p^2 = 0.59), but no effect over time (F = 1.509, P = 0.250, η_p^2 = 0.14) and no interaction effect between exercise*time (F = 1.023, P = 0.338, η_p^2 = 0.10). There was a similar trend for the HVR with a main effect of exercise (F = 6.769, P = 0.029, η_p^2 = 0.43), but no effect over time (F = 2.011, P = 0.190, η_p^2 = 0.18) and no interaction effect between exercise*time (F = 1.418, P = 0.264, η_p^2 = 0.14). Finally, the HCR was no different as a result of exercise (F = 1.891, P = 0.202, η_p^2 = 0.17), time (F = 0.430, P = 0.528, η_p^2 = 0.05) or the interaction between exercise*time (F = 0.707, P = 0.422, η_p^2 = 0.07).

A stepwise multiple regression was run to predict post-ALT change in tHbmass (ΔtHbmass) from ΔSp_r (r = 0.576), ΔSp_e (r = 0.519), ΔVE_r (r = 0.496), ΔVE_e (r = 0.415), HVR_r (r = 0.774), HVR_e (r = 0.201), HCR_r (r = -0.673) and HCR_e (r = 0.016). The first predictor variable to enter the model was HVR_r; the second and final predictor variable to enter the model was ΔSp_r. These variables predicted ΔtHbmass, F(2,9) = 14.323, P = 0.002, with an adjusted R^2 of 0.708 and standard error of the estimate of 1.584. The general form equation to predict ΔtHbmass (%) = 0.494 + (1.730 * HVR_r) + (0.259 * ΔSp_r).

Further to this, another stepwise multiple regression was run to predict post-ALT change in $\dot{V}O_{2max}$ ($\Delta\dot{V}O_{2max}$) ΔSp_r (r = -0.141), ΔSp_e (r = 0.314), ΔVE_r (r = 0.578), ΔVE_e (r = 0.539), HVR_r (r = 0.608), HVR_e (r = 0.418), HCR_r (r = -0.683) and HCR_e (r = 0.224). The only predictor variable to enter the model was HCR_r. The variable predicted $\Delta\dot{V}O_{2max}$, F(1,8) = 6.994, P = 0.030, with an adjusted R^2 of 0.400 and standard error of the estimate of 2.978. The general form equation to predict $\Delta\dot{V}O_{2max}$ (%) = 5.131 – (1.795 * HCR_r)

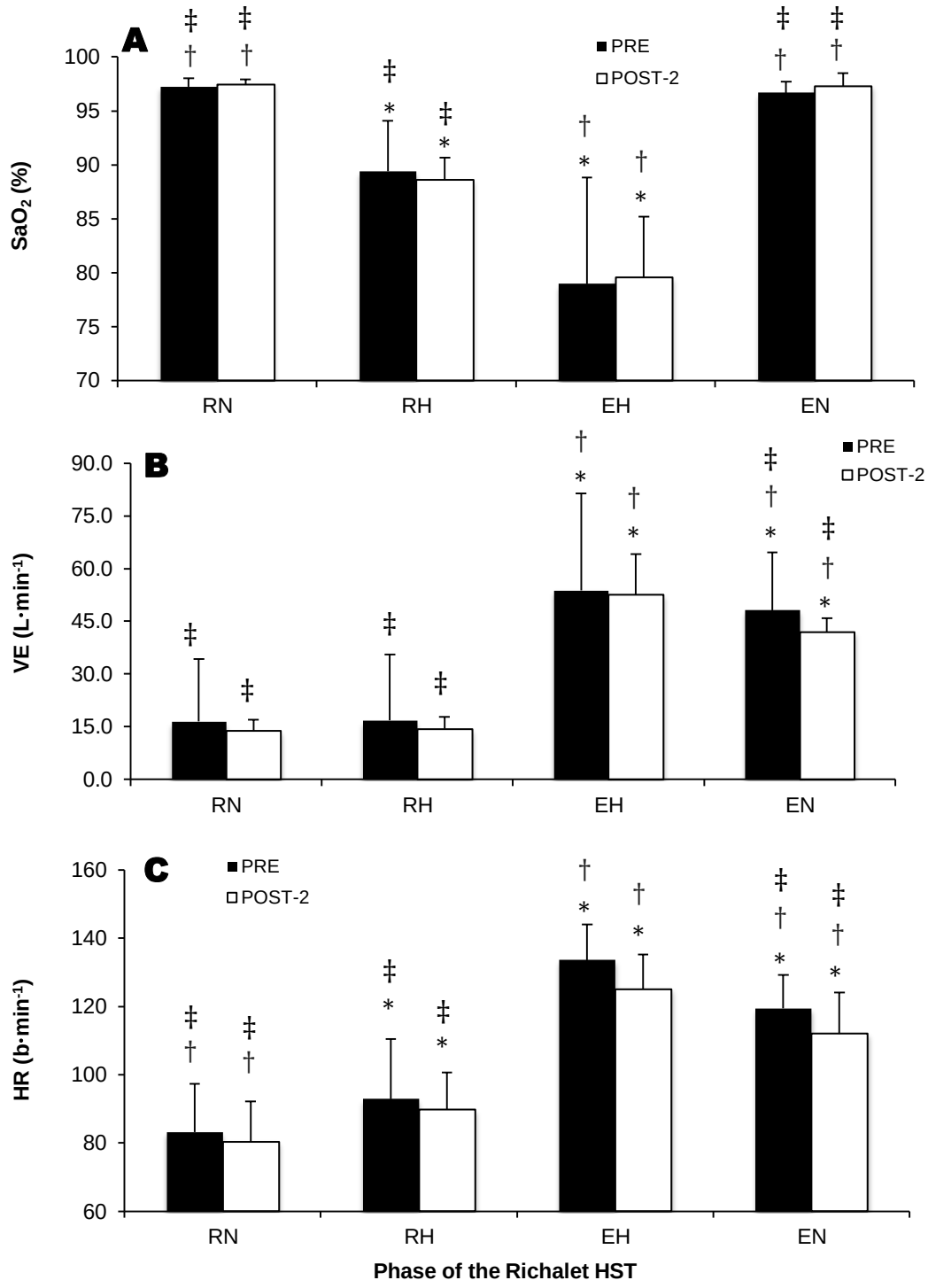


*Figure 9.6: Changes in SpO_2 (Plot A), VE (Plot B) and HR (Plot C) during the four phases of the Richalet hypoxic sensitivity test. * denotes difference from rest in normoxia (RN) (P < 0.05), † denotes difference from rest in hypoxia (RH) (P < 0.05) and ‡ denotes difference from exercise in hypoxia (EH) (P < 0.05) within PRE (black bar) and POST-2 (white bar).*

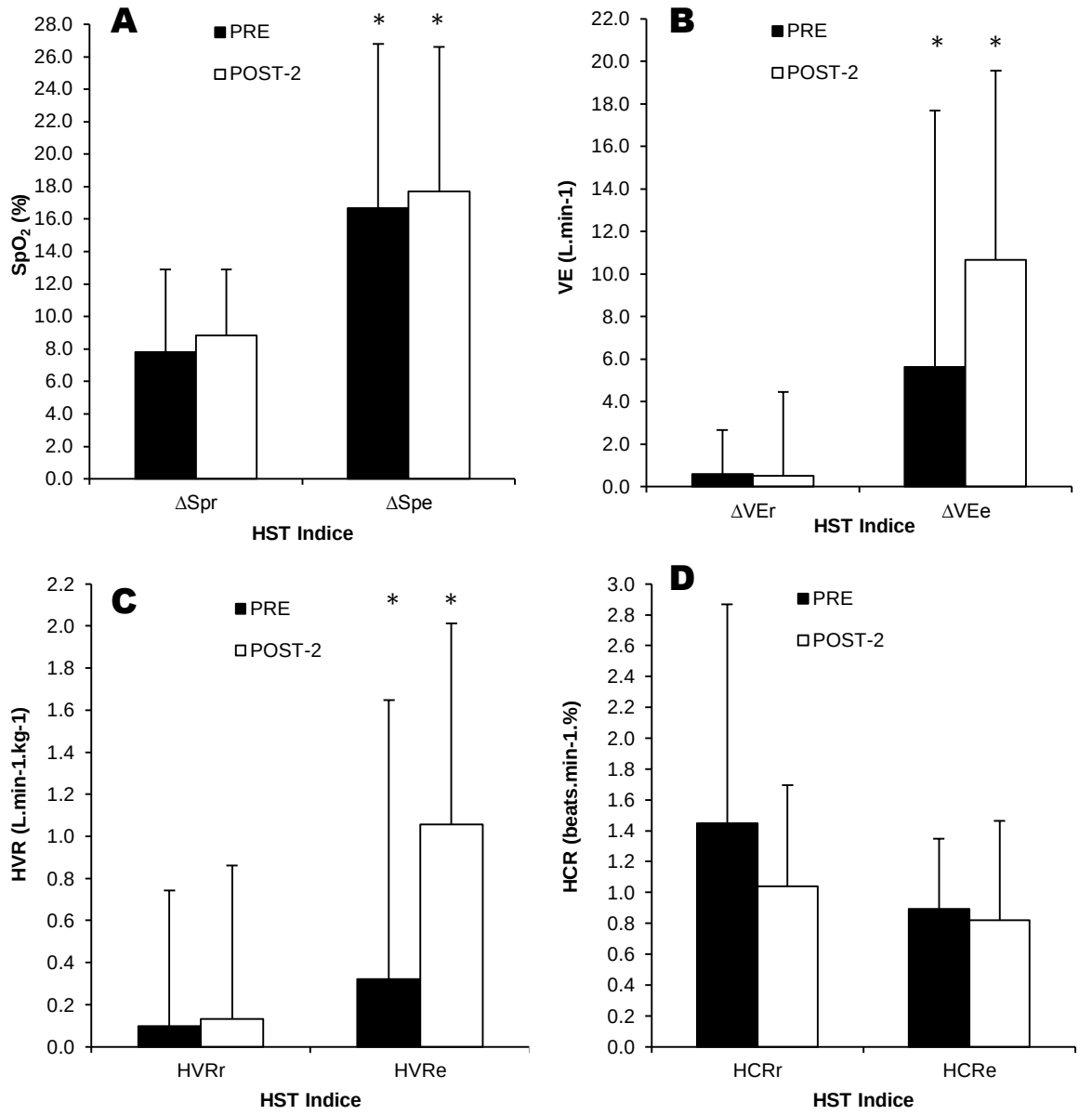


*Figure 9.7: Hypoxic Sensitivity Test (HST) indices to characterise the participants sensitivity to hypoxia (Richalet et al. 2012). * denotes difference from rest to exercise within each of the indices (P < 0.05). Desaturation at rest = ΔSp_r and desaturation at exercise = ΔSp_e (Plot A); ventilation changes at rest = ΔVE_r and ventilation changes at exercise = ΔVE_e (Plot B); hypoxic ventilatory response at rest = HVR_r and hypoxic ventilatory response at exercise = HVR_e (Plot C); hypoxic cardiac response at rest = HCR_r and hypoxic cardiac response during exercise = HCR_e (Plot D).*

9.5 Discussion

The present study set out to investigate three aims. *Part A* demonstrated that 4 weeks of LHTH altitude training at ~2,300 m was not sufficient enough to induce a significant main effect increase in tHbmass in elite distance runners compared to CON, although eight out of twelve in the ALT group presented an increase in tHbmass. The LHTH period was, however, sufficient enough to increase $\dot{V}O_{2max}$, LT and LTP. *Part B* found there was no change in tHbmass measured a three time points post-ALT, however there was a significant decrease in [EPO] at 1 ± 2 day post-ALT. *Part C* found from PRE to POST-2 there were few differences in HST parameters or indices measured as a result of LHTH. However, this work is the first to show that the pre-training camp HST indices of HVR_r and ΔSp_r may predict subsequent changes in tHbmass (R^2 = 0.708; P < 0.05), and that and HCR_r may predict changes in $\dot{V}O_{2max}$ (R^2 = 0.400; P < 0.05).

9.5.1 Part A: Physiological and haematological responses to LHTH

The underlying mechanisms behind the effects of hypoxic training are widely debated, with the popular view that altitude training may lead to an increase in haematological capacity, however this may not be the only factor involved in the improvement of endurance performance (Millet et al. 2010). LHTH was unable to show a significant increase in tHbmass after 4 weeks at ~2,300 m from PRE to POST-2. In the present study, tHbmass was determined with a TEM of 1.0%. Although the mean increase in tHbmass was statistically no different to CON, LHTH did increase tHbmass by 1.9 ± 2.9% (813 ± 146 g to 831 ± 156 g) compared 0.1 ± 3.3% (847 ± 128 g to 847 ± 122 g) in CON. It should be noted that there was a marked individual variation (range: -1.7 to 7.1%) with eight out of twelve in the ALT group eliciting an increase in tHbmass (>2.0% in 6 athletes). Hence, the observed increase is likely to be a true physiological change.

The findings of the present study are in agreement with previous studies in endurance runners measuring tHbmass with the oCOR-method (see Table 9.6). Frese and Friedmann-Bette (2010) and Robertson et al. (2010b) reported high individual variation in tHbmass response to natural LHTH and simulated LHTL. The wide inter-individual variation in endurance performance in response to altitude training is thought to be associated with the erythropoietic response (Chapman et al. 1998), however Friedmann et al. (2005a) found that the variability in the increase in tHbmass is not predicted by the EPO response to acute hypoxic exposure. In high altitude natives there are five adjustable hypoxia response systems that form a common basis for the complex physiology of hypoxia tolerance: 1) the carotid body oxygen sensor, 2) pulmonary vasculature, 3) vascular oxygen sensors, 4) kidney oxygen sensors and 5) metabolic pathways (Hochachka et al. 1999). These systems also exist in lowlanders, but are modulated at different set points or a different threshold (Hochachka et al. 1999). As a result, it is difficult to identify the mechanism for the variable response of tHbmass to hypoxia. Although it should be noted only one of these mechanisms directly controls the production of tHbmass (erythropoiesis).

Table 9.6: Changes in tHbmass as a result of natural and simulated altitude training methods in runners.

Study	Altitude	Duration	Method	Outcome
Natural Altitude				
The present study	**2,300 m**	**4 weeks**	**LHTH**	**1.9% increase**
Garvican-Lewis et al. (2015)	1,800 m	3 weeks	LHTH	3.0% increase *
Frese and Friedmann-Bette (2010)	1,300 m	20 days	LHTH	4.0% increase
Frese and Friedmann-Bette (2010)	1,650 m	22 days	LHTH	2.0% increase
Simulated Altitude				
Robertson et al. (2010c)	~2,200 m	3 weeks 14 h·day^{-1}	LHTL+H	3.6% increase *
Robertson et al. (2010a)	~2,200 m	3 weeks 14 h·day^{-1}	LHTL	2.8% increase *
Saunders et al. (2009)	~2,900 m	12 weeks (5 d·wk^{-1} of ~9 h·d^{-1}). Total: ~400 h 46 ± 8 nights (415±75 h)	LHTL	3.8% increase

* denotes difference ($P < 0.05$)

According to numerous reviews (Rusko et al. 2004; Wilber 2007a; Friedmann-Bette 2008; Millet et al. 2010; Gore et al. 2013) four weeks at 2,300 m should be a sufficient enough 'dose' to increase tHbmass in elite athletes. Despite the present study not consistently finding this in participants, on average improvements in LT, LTP and $\dot{V}O_{2max}$ were found. Comparison between altitude training studies is difficult as previous studies investigating LHTH altitude training in runners does not always describe the time during the season or measure the same physiological and haematological markers. The extent to which an athlete may benefit from altitude training will differ according to their general and specific training focus (i.e. between types of endurance training; and between different periods of the training year) (Millet et al. 2010). The present altitude training camp took place at the beginning of the preparation phase of the season, therefore the focus was mainly on 'aerobic base' work where the intensity of training lower or equal to LT (Seiler and Kjerland 2006). This will ultimately have affected the outcomes of the physiological markers. The athletes in the present study completed ~85% of training equal to or below LT, which is reflected in the improvements in LT and LTP.

Likewise, with the variation in tHbmass response there was a wide variety of response in $\dot{V}O_{2max}$ (range: -2.0% to 8.6%), LT (range: 0% to 13.3%) and LTP (range: 0% to 11.8%). The variability in performance may be associated with the timing of the performance test after altitude exposure (Robertson et al. 2010c). Although the present study did not conduct a performance test, the treadmill testing during POST-2 was completed at 10 ± 3 d at sea level. A meta-analysis by Bonetti and Hopkins (2009) found that by manipulating the study characteristics, such as the degree of altitude, number of days of exposure, and the test day (after altitude exposure) the chance of an enhancement of maximal endurance power output increased from 1.6% to 5.2% with LHTH. This highlights the importance of understanding the optimal time at sea level following altitude training when interpreting performance

tests, although the mechanisms for this variation are unclear. Chapman et al. (2014a) believed that individual rates of decay in haematological, biomechanical, and ventilatory adaptations were central to the understanding.

The ALT group found a mean improvement of 2.7% in $\dot{V}O_{2max}$, which was significantly greater than the CON group. In studies undertaking the classic LHTH method in highly trained to elite middle- and long-distance runners the findings are contradictory. A significant 4% improvement was found in $\dot{V}O_{2max}$ after 4 weeks of LHTH at 2,500 m (Levine and Stray-Gundersen 1997) and an non-significant 2.5% improvement was found after 4 weeks at 1,740 m (Gore et al. 1997). There were however, no improvements in $\dot{V}O_{2max}$ after 3 weeks at 2,300 m (Adams et al. 1975) and after 2 weeks at 2,000 m (Svedenhag et al. 1991). In a well-trained population, 4.2% improvements in $\dot{V}O_{2max}$ were found after 17 days at 3,090 m (Dill and Adams 1971). Individual variation, training status and achieving an adequate 'hypoxic dose' appear to play a key role in enhancing $\dot{V}O_{2max}$ as a result of LHTH. $\dot{V}O_{2max}$ is determined by the oxygen supply of the blood and by the oxygen consumption of the skeletal muscle (Schmidt and Prommer 2010). Therefore, an increase in tHbmass should result in an increase in $\dot{V}O_{2max}$. Conversely, in the present study there was little relationship between change in tHbmass and change in $\dot{V}O_{2max}$ (r = 0.32).

The fractional utilisation of the $\dot{V}O_{2max}$ during endurance exercise is an important determinant of endurance performance and this is linked to markers of blood lactate accumulation during exercise, such as the LT or LTP (Costill et al. 1973). The measurement of LT and LTP provide useful information on endurance performance potential and the extent of the physiological adaptations to a period of training (Jones and Carter 2000). The ALT group improved both LT and LTP by 6.1% (14.9 to 15.9 km·h^{-1}) and 5.4% (16.9 to 17.9 km·h^{-1}), respectively, compared to CON. There is limited comparison in submaximal physiological responses to LHTH in elite runners, however, Bailey et al. (1998) reported improvements in running velocity of 7.7% (16.7 to 18.1 km·h^{-1}) at 2 mmol·L^{-1} and 10.8% (21.5 to 24.1 km·h^{-1}) at 4 mmol·L^{-1} after 4 weeks at 1,500 – 2,000 m. These findings are somewhat comparable to the present study.

The non-haematological mechanisms of improved sea-level performance after hypoxic exposure have been reviewed (Gore et al. 2007) and it is clear that these may contribute to improved endurance performance in the absence of increased tHbmass. A rightward shift in the lactate-power profile indicates that an athlete is able to run at a higher speed for the same or reduced lactate accumulation, and typically leads to improved endurance performance (Amann et al. 2006). Both LT and LTP were significantly improved as a result of ALT, with LTP in comparison to CON. The transport of lactate in skeletal muscle is facilitated by two known monocarboxylate transporters, MCT1 and MCT4, therefore, an increase in MCT1 and MCT4 protein expression could minimize perturbations in intracellular pH (Gore et al. 2007). Zoll et al. (2006) found an increase in the muscle mRNA concentration of MCT1 (44%) in nine well-trained runners after six weeks of training (12 x 24- to 40 min sessions) under hypoxic conditions (3000-m simulated altitude) compared with a control group. Although, Clark et al. (2004) reported no change in MCT1 and MCT4 protein abundance in skeletal muscle, despite a decrease in lactate rate of appearance, in well-trained individuals after 20 nights of simulated moderate LHTL. The participants in the present study exercised at altitude, which further

activates the lactate/H+ transport system (Juel et al. 2003), therefore it is possible that the hypoxic stimulus was adequate to increase MCT1 and MCT4 protein expression.

The ALT group tended to produce less lactate at LT and LTP compared to CON (see Table 9.4). In addition to transport of lactate and H+, the ability of skeletal muscle to buffer H+ is important for pH regulation and changes in acid–base status have been proposed as a potential mechanism for improved performance after altitude exposure (Svedenhag et al. 1997). Gore et al. (2001) demonstrated that sleeping at altitude and training at sea level (23 nights of sleeping at simulated altitude of 3,000 m) increased muscle buffering, however, this finding was not repeated in a subsequent study from the same research group (Clark et al. 2004). Increased muscle buffering capacity is thought to be caused by hyperventilation raising the alveolar PO_2, which leads to respiratory alkalosis, a decrease in PCO_2 and a decreased H+ level, therefore a corresponding increase in pH (Hansen et al. 1967). The magnitude of the adaptations appears to be dependent upon the severity of the hypoxic stimulus, with higher altitudes reporting greater improvements. It is therefore difficult to ascertain if four weeks at 2,300 m is a sufficient enough 'dose' without directly measuring the transport of lactate or muscle buffering capacity.

9.5.2 Part B: Time course of tHbmass and [EPO] in response to LHTH

Chapman et al. (2014a) believed that the altitude-mediated increases in tHbmass and therefore enhanced oxygen enhanced oxygen delivery are one of the most important factors behind any improvement in sea level endurance endurance exercise performance. Clark et al. (2009) found tHbmass to increase by ~3% at the end of 21 days 21 days simulated LHTL with tHbmass remaining elevated at one and seven day's post-LHTL. Robertson et al. (2010b) Robertson et al. (2010b) found a ~3% increase in tHbmass after 21 days simulated LHTL, which was maintained for 6 maintained for 6 days at sea level. Garvican et al. (2012), however, found an initial increase of ~3% from baseline from baseline after 21 days natural LHTL with tHbmass then decreasing to 1.5% after two and ten days at SL. Other days at SL. Other studies also found tHbmass to drop by ~3% after nine days a sea level (Pottgiesser et al. 2012) and al. 2012) and ~5% after 16 days a SL (Heinicke et al. 2005). The present study is the first to measure the time course the time course of tHbmass (see

Figure 9.5A) after four weeks of natural LHTH at ~2,300 m, in elite endurance runners. After 1 ± 2 days at SL tHbmass was elevated by 2.7 ± 2.9%, compared to baseline. After 11 ± 3 days tHbmass remained elevated at 3.0 ± 3.4%, however, by18 ± 2 days tHbmass had then decreased to 1.4 ± 4.7% above baseline.

The mean increase in tHbmass was smaller in magnitude than expected, with previous studies finding increases ranging from 6-9% as a result of LHTH (Friedmann-Bette 2008). A meta-analysis by Gore et al. (2013) suggested a 2 week LHTH camp (336 h) may be sufficient to increase tHbmass by a mean of ~3% and by at least 1% for 97.5% of athletes. Based on the 4 week (672 h) LHTH camp of the present study increases of ~6% would be expected. It is difficult to make direct comparison between studies describing the time-course of tHbmass post-altitude training as the studies utilised different altitude training methods (i.e. simulated LHTL or natural LHTH), different 'hypoxic doses' (i.e. 24 hours per day or 14 hours per day), using different athletes (i.e. swimmers, runners or cyclists) of varying standards (i.e. well-trained or elite) and different oCOR-method procedures (i.e. volume of CO

administered or experimenter variation). The 'hypoxic dose' has been extensively reviewed (Levine and Stray-Gundersen 2006; Wilber et al. 2007; Wilber 2007b; Chapman et al. 2014a; Garvican-Lewis et al. 2016b) with the present study fulfilling the recommendations.

Unfortunately, EPO was not measured during the present altitude training camp due to safety concerns and availability of equipment. Prior research has typically found [EPO] increases by 40 to 60% after two days of hypoxic exposure and by the end of a 21 day altitude training camp was close to baseline levels (Clark et al. 2009; Robertson et al. 2010b; Garvican et al. 2012; Pottgiesser et al. 2012). After this [EPO] then declined by -25 to -45% after two days at sea level, before gradually increasing back towards baseline after two more weeks (Clark et al. 2009; Robertson et al. 2010b; Garvican et al. 2012; Pottgiesser et al. 2012). Recently, Hauser et al. (2015) confirmed there was a similar [EPO] response after ~230 h of hypobaric and normobaric hypoxia in well-trained triathletes reporting a -40% and -51% decline in [EPO], respectively, from Pre- to Post-LHTL. The present study found [EPO] was significantly lower than the pre-altitude measurement (-39.0 ± 16.7%) after 1 ± 2 days at sea level. After 11 ± 3 days at sea level [EPO] increased slightly (-20.1 ± 32.4%) but did not rise above the pre-altitude training values after 18 ± 2 days at sea level (-12.1 ± 21.2%). The observations of a sharp decline in [EPO] immediately on return to SL are the first to be shown after a natural LHTH altitude training camp at ~2,300 m.

There is increasing evidence that EPO not only stimulates RBC production but is crucial for the maintenance as well (Eckardt and Kurtz 2005), which is supported by Trial and Rice (2004) who reported that the withdrawal of EPO leads to the destruction of young red cells. Garvican et al. (2012) found that the immediate decline in [EPO] on return to sea level was accompanied by an initial decline in tHbmass. In the present study tHbmass remained elevated for 11 days, after which it steadily began to decline, which is in agreement with (Clark et al. 2009; Robertson et al. 2010b). Although there did not appear to be a relationship ($r = 0.20$) between the change in tHbmass and a decrease in [EPO]. A decrease in tHbmass on return from altitude to sea level has also been demonstrated by Prommer et al. (2009) who found that when natural altitude dwellers reside at sea level for sustained durations and a steady reduction in tHbmass occurs, suggesting that removal of the altitude stimulus results in an acclimatisation to the normoxic environment (Garvican et al. 2012). Rice et al. (2001) demonstrated a rapid down-regulation of tHbmass on descent from high altitude showing a relationship between descending from altitude and decreases in tHbmass, a phenomenon termed neocytolysis. Neocytolysis is the selective destruction of young erythrocytes, which is stimulated by a sudden drop in [EPO] upon descent to sea level (Rice et al., 2001). Although the athletes in the current study only descended from a moderate altitude it is possible that the decline in [EPO] prevented the maintenance of tHbmass for a longer duration of time or further increases in tHbmass.

Erythropoiesis is inversely related to oxygen availability and serum EPO concentration normally determines the rate of RBC production (Eckardt and Kurtz 2005). Despite this Friedmann et al. (2005b) and Clark et al. (2009) reported there to be no relationship between the change in EPO in the initial days of an altitude training camp and the change in tHbmass as a result of three weeks of altitude training. It has been suggested that the lack of increase in tHbmass is due to insufficient iron stores preventing efficient erythropoiesis (Stray-Gundersen et al. 1992), or possibly years of

endurance training in elite athletes causing polycythaemic hypervolemia, therefore reaching a maximum with no potential for a further increase (Gore et al. 1998). Further to this, Garvican et al. (2007) demonstrated [EPO] response of an athlete to a simulated altitude is not consistent and possibly modulated by training and fatigue. It is not surprising that an increase in tHbmass does not occur in every athlete returning from altitude, however establishing an individual athlete's response to a hypoxic stimulus and the regulation of EPO may help to increase the likelihood of success.

9.5.3 Part C: Hypoxic sensitivity test

Individual differences in response to altitude have been reported in both elite athletes undertaking altitude training camps (Chapman 2013) and mountain climbers ascending to high altitude (Schneider and Bernasch 2002). As a result, the individual variation in physiological responses to altitude training (Friedmann et al. 2005a; Friedmann et al. 2005b) and the susceptibility, severity and prediction of AMS have been investigated (Schneider and Bernasch 2002; Burtscher et al. 2004; Burtscher et al. 2008). The risk of AMS has been linked to the severity of the hypoxic exposure and the efficiency of the physiological adaptive responses, the ventilatory response to hypoxia and the decrease in SaO_2, at rest or exercise (Richalet et al. 2012). The present study attempted to utilise these principles to predict which athletes would increase tHbmass and $\dot{V}O_{2max}$ as a result of a 4-week LHTH altitude training camp at ~2,300 m.

The Richalet HST, which quantifies the cardiorespiratory response to acute hypoxia (FiO_2 = 0.115) during rest and exercise at an intensity corresponding to a heart rate of ~130 beats·min^{-1} in normoxia, has previously been shown to predict susceptibility to AMS (Richalet et al. 2012), but not predict the performance decrement of a 15 km time trial in severe hypoxia (FiO_2 = 0.11) (Bourdillon et al. 2014). The observations from regression analysis in the present study were that ΔSp_r (>8%) and HVR_r (< 0.11 L·min^{-1}·kg^{-1}) predicted the increase in tHbmass. Further, HCR_r (< 1.38 beats·min^{-1}·%) predicted the increase in $\dot{V}O_{2max}$ after ALT. The findings suggest that athletes who experience greater desaturation at rest and lesser HVR at rest are likely to produce a greater increase tHbmass, however, it should be noted that there will be a level of desaturation from which homeostasis cannot be sustained therefore an athlete would not be able to live and train at that altitude. Interestingly, these physiological indices from the HST were very similar to those that predicted AMS in individuals ascending to altitude above 3,500 m. C-statistics suggested that ΔSp_e (0.879) and HVR_e (0.769) significantly increased the discrimination accuracy of a severe high-altitude illness (SHAI) risk prediction model (Richalet et al. 2012). The authors believed that individuals presenting a low response to hypoxia and low SaO_2 during exercise will be exposed to more severe hypoxemia during physical activity at altitude and will be more prone to develop high altitude illness.

The role of SaO_2 in normoxia and hypoxia has been previously studied in relation to exercise at altitude. Chapman et al. (1999) reported well-trained athletes that desaturate more at $\dot{V}O_{2max}$ in normoxia, had a greater reduction in $\dot{V}O_{2max}$ at acute normobaric hypoxia (~1,000 m), a relationship which was also found by Mollard et al. (2007). Further to this, the maintenance of SaO_2 during race pace running at sea level has also been shown to demonstrate less performance impairment at altitude

(Chapman et al. 2011). SaO_2 is the end product of both pulmonary ventilation and gas exchange (Beidleman et al. 2014) and the ventilatory response to exercise in hypoxia is correlated to SaO_2 (Benoit et al. 1995), therefore individuals with a strong ventilatory response to acute hypoxia are able to alleviate a fall in arterial oxygen content by increasing ventilation (Chapman et al. 2010). The present study has found that a greater decrease in SaO_2 at rest and lesser HVR at rest during the HST are both associated with an enhanced tHbmass as a result of altitude training.

Further to this, it is well established that arterial hypoxemia is the fundamental stimulus for EPO production in the kidney (Maxwell et al. 1990), therefore factors that regulate and control arterial oxygen content at the oxygen sensing cells in the kidney, such as renal blood flow, haemoglobin concentration, and SaO_2, could be the "upstream" source of variability consistently seen in EPO release to a fixed altitude (Berglund 1992; Ge et al. 2002). It is therefore reasonable to assume that those individuals who desaturate more at altitude should ultimately have a greater increase in tHbmass. A moderate negative correlation ($r = -0.54$) was found between average SpO_2 measured across the duration of ALT and the percentage change in tHbmass measure at POST-1. The average SpO_2 measured was not able to explain all of the variance in change in tHbmass, with other factors such as injury/illness (Wachsmuth et al. 2013), iron status (Garvican et al. 2011a) and 'hypoxic dose' (Chapman et al. 2014a) often playing a role.

The degree of pulmonary gas exchange limitations during exercise contribute to the magnitude of the decline in $\dot{V}O_{2max}$ at altitude (Chapman 2013). In addition, Gavin et al. (1998) demonstrated that a reduced hyper-ventilatory response to maximal exercise exhibited a greater hypoxic arterial desaturation and a greater decline in aerobic capacity with hypoxia. Using the HST the present study found a correlation ($r = 0.71$) between those athletes with a greater change in VE (ΔVE_e) during the HST and those with a greater change in SpO_2 (ΔSp_e). The change in VE (ΔVE_e) was also correlated ($r = 0.78$) with an increased HVR_e during the HST. The increases in ventilation in hypoxia are critical to minimizing the reduction in alveolar PO_2 (and therefore SaO_2) as inspired PO_2 is reduced with increasing altitude (Dempsey et al. 2013). However, this evolutionary response aims to acclimatise an individual to survive at altitude yet those athletes with a lesser HVR_r and greater ΔSp_r went on to have a greater increase in tHbmass. Additionally, highly trained endurance athletes classically display blunted hypoxic ventilatory responses compared to untrained, age matched controls (Harms and Stager 1995). It would appear that in order to achieve the greatest possible increase in tHbmass athletes need a reduced hyperventilation and greater desaturation in response to hypoxia, but not so much so that the athlete does not acclimatise to altitude they are training at and therefore impairing their training.

Chapman et al. (2010) questioned the role of the HVR, alveolar PO_2, and the lung in general on the magnitude of the EPO release at moderate altitude in elite athletes, as individual increases in EPO were not primarily related to peripheral chemoresponsiveness. The substantial inter-individual variability in EPO (Ge et al. 2002; MacKenzie et al. 2008) and ventilatory (Sahn et al. 1977) responses to acute hypoxia make it difficult to understand the relationship between the two. HVR may change from one exposure to another due to various factors, such as previous exposures to high altitude, state of health, nutrition, sleep, or exercise (Burtscher et al. 2004) and genetic differences in hypoxia sensing

and hypoxic response pathways play a defining role in the wide variation in EPO response (Chapman et al. 2010). Although on this occasion indices of the HST predicted changes in tHbmass and $\dot{V}O_{2max}$, the HST should be completed before each exposure to altitude to obtain the most accurate results for all athletes.

9.5.4 Limitations

A meta-analysis by Bonetti and Hopkins (2009) revealed the post-exposure testing day had a significant effect on exercise performance and $\dot{V}O_{2max}$. Power output in endurance athletes was enhanced (1.8%) after ~2.5 days, followed by impairment (-1.5%) at 5 days, enhancement (1.4%) at 17 days and impairment (-2.3%) at 33 days, after LHTH. A similar trend has been reported in tHbmass in elite swimmers through the course of a season who found a small drop in tHbmass immediately after the return from altitude, an elevated plateau for the following 10 days, a sustained gain of 50 % after ~3 weeks, and values that return to baseline after ~5 weeks (Wachsmuth et al. 2013). The present study aimed to track the changes in tHbmass and [EPO] post-altitude training, however, was unable to test the participants on the same day due to the location of the athletes. As a result there was some variability between testing days on return from altitude. Equally the post-ALT treadmill testing was completed after 11 ± 3 days at sea level. Based on the meta-analysis by Bonetti and Hopkins (2009) some participants may have been in a period of enhancements and some impairment.

The present study was unable to control for iron supplementation during the testing period. Six out of the twelve participants had been supplementing iron prior to attending the altitude training camp and continued during and after the camp. One participant was shown to have a ferritin score below 30 $ng \cdot mL^{-1}$ and was instructed to take one capsule of Ferrous Fumerate (305 mg) 3 times per day for the experimental testing period. The remaining five participants did not supplement iron. The influence of pre-altitude ferritin levels on the tHbmass response to prolonged altitude exposure is currently unclear (Govus et al. 2015). Stray-Gundersen et al. (1992) found red cell volume did not improve following four weeks of moderate altitude (2,500 m) exposure in iron deficient runners, however Ryan et al. (2014) found female subjects exposed to 5,000 m for 16 days increased their tHbmass despite low pre-altitude serum ferritin levels. Athletes are typically recommended to ingest a daily oral iron supplement to facilitate altitude adaptations, and to help maintain iron balance, however there is some debate as to whether athletes with otherwise healthy iron stores should be supplement iron (Garvican-Lewis et al. 2016a). In the present study there was no significant relationship between pre-altitude serum ferritin and change in tHbmass.

9.5.5 Future directions

The present study only observed physiological and haematological variables in response to one altitude training camp at the beginning of an athletics season. More sophisticated altitude training programmes tend to utilise multiple training camps, at multiple locations with mixed altitude training methods throughout an entire season. It would be beneficial to track changes in haematology and

endurance performance during the entire season, paying particular attention to the athletes training load and how this influences the resultant adaptations. Although the participants in the present study were instructed to follow a similar training programme, as different coaches advised them, there was some variation in the training they competed. In addition to tracking tHbmass for an entire season the efficacy of using additional hypoxic exposures to maintain [EPO] and tHbmass on return to sea level could be investigated. It has been shown that relatively brief periods of severe hypoxia are sufficient to increase EPO concentrations significantly (Study 4; Chapter 7), therefore periods of intermittent hypoxia could result in enough EPO release to prevent neocytolysis and preserve the haematological acclimatization response for a longer time, thereby expanding the window for optimal competition; however, this would need to be examined directly (Chapman et al. 2014b).

9.5.6 Conclusions

Part A found 4-weeks of classic LHTH altitude training at ~2,300 m resulted in improvements in LT, LTP and $\dot{V}O_{2max}$, but not tHbmass measured after 11 ± 3 days at sea level, in elite middle- and long-distance runners. Although a main effect was not found, eight of the twelve participants increased tHbmass greater than the TEM. *Part B* found no significant changes from baseline in tHbmass measured post-LHTH on three occasions, in eight of the athletes; there was however substantial individual variation. Further analysis found [EPO] to be significantly decreased after 1 ± 2 days at sea level and after 18 ± 2 days [EPO] was still below baseline measures. Finally, in *Part C,* stepwise multiple regression analysis showed the HST indices of HVR_r and ΔSp_r predicted changes in tHbmass (R^2 = 0.708; P < 0.05) and HCR_r predicted changes in $\dot{V}O_{2max}$ (R^2 = 0.400; P < 0.05). The Richalet HST could provide a useful and novel pre-screening tool to predict the response of an athlete prior to attending an altitude training camp.

The findings from this study may help elite athletes and coaches to understand that individual responses to altitude play an important role in acclimatisation and potential endurance adaption. The data derived from the Richalet HST may be effective in prescribing individualised altitude training strategies.

CHAPTER 10

10 GENERAL DISCUSSION

The general discussion will firstly summarise the rationale and overarching aims of the thesis, and the principal findings from each experimental chapter, whilst referring to the study hypothesis throughout. Secondly, the main findings from the thesis relating to the 'hypoxic dose', individual variation in physiological and haematological responses to hypoxia and hypoxic tolerance will be discussed. The general discussion will then show how the findings of the thesis have contributed to the ever-growing body of research surrounding optimising altitude training adaptations in elite endurance runners. Finally, the practical applications of the research, limitations and future research questions arising from the data presented in the experimental chapters will be discussed.

10.1 Principal Findings

The first experimental study (Chapter 4) utilised a mixed methods approach to survey the opinions of elite athletes and coaches from British Athletics and sought the altitude training experiences of physiology practitioners working with endurance sports. Semi-structured interviews were conducted on physiology practitioners and it was found that there were successes associated with altitude training including athletes winning medals after altitude training camps in major championships and the use of sophisticated methods such as the oCOR-method to measure tHbmass. However, there were evident concerns, including methods to maximise adaptions in athletes who are 'low-responders' and the use of hypoxic exposures before and after altitude training camps to optimise the response. The survey revealed that there were differences between athlete and support staff in altitude training practices and perceptions, therefore hypothesis 1a was rejected and hypothesis 1b was accepted (see Table 10.1). As expected when surveying a group of elite endurance athletes and support staff, a high proportion had previously engaged in altitude and hypoxic training and rated it as a very important part of their training regime. The methods utilised by the athletes and advised by the coaches were primarily similar to those recommended by peer-reviewed research (Wilber 2007a; Millet et al. 2010). The challenges documented within the open-ended questions of the survey provided priority and scope to direct subsequent altitude training research questions.

The findings of Study 1 (Chapter 4) identified that the measurement of tHbmass, and therefore the oCOR-method, was as an important tool when assessing the success of an altitude training camp. The EIS has three regional sites, with three Radiometer™ hemoximeters, where practitioners use the oCOR-method to measure an athlete's tHbmass. Therefore, Study 2 (Chapter 5) compared the measurement of tHbmass with the oCOR-method using different Radiometer™ hemoximeters. No differences were found between tHbmass or BV measured using the five different hemoximeters, therefore both study hypotheses (see Table 10.1) were accepted. Bland-Altman analysis showed there was closer, i.e. better, agreement between the three newer ABL80 hemoximeters compared to the, older, OSM3 device. For the measurement of %HbCO (the key metric when quantifying tHbmass) the

ABL80 hemoximeters were found to be less variable, therefore, a minimum of triplicate measures were required to reduce analyser error to ≤1% compared to 5 replicates with the older machine. It is therefore, possible for athletes to undertake the oCOR-method across different sites and ABL80 hemoximeters, with confidence in the comparative reliability of the measures.

Study 3 (Chapter 6) assessed the reliability of the oCOR-method, this time investigating the influence of changes in CO bolus on the measurement of tHbmass. Previously, an inadequate dose of CO was believed to contribute to test-retest error during the oCOR-method and further, studies using the oCOR-method were not consistent with the CO bolus they administered. The TEM of the oCOR-method in Chapter 6 (using 1.0 mL·kg^{-1} of CO) was 0.8% (± 2.3%). Small, but significant differences were found in tHbmass when quantified using a high (1.4 mL·kg^{-1}) CO bolus (776 ± 148 g) when compared to a low (0.6 mL·kg^{-1}) bolus (791 ± 149 g), or a medium (1.0 mL·kg^{-1}) bolus (788 ± 149 g), therefore the study hypothesis (see Table 10.1) was rejected. With the newer and less variable ABL80 hemoximeters, a bolus of 0.6 to 1.0 mL·kg^{-1} provides sufficient precision, whilst being within safety margins. Further to this experimenters must be consistent with the bolus of CO administered in repeat testing.

Prior knowledge of an athlete's individual physiological response to a simulated hypoxic exposure may be valuable when the aim is to maximise haematological adaptions. Study 4 (Chapter 7) investigated the optimal normobaric hypoxic exposure to elicit the greatest response in EPO, whilst limiting physiological stress to the participants. The study revealed that a normobaric hypoxic exposure of two hours at an FiO_2 of 0.115-0.125 (4,200-4,800 m) was sufficient to increase EPO production, which peaked 2 h post-exposure and was maintained up to 4 h post-exposure (accepting hypothesis 4a, see Table 10.1). Pro-inflammatory cytokines (IL-6 and TNFα) were measured to represent physiological stress from hypoxia and no differences were found during each trial [FiO_2: ~0.135 (3,600 m), FiO_2: ~0.125 (4,200 m) and FiO_2: ~0.115 (4,800 m)]. Additionally, baseline levels of IL-6 and TNFα did not inhibit the production of EPO (accepting hypothesis 4b, see Table 10.1). Despite a dose-response relationship between severity of hypoxia and SpO_2 (FiO_2: ~0.135 = 87 ± 3%, FiO_2: ~0.125 = 83 ± 1% and FiO_2: ~0.115 = 76 ± 3%) there was no relationship between degree of desaturation and peak [EPO] production. This finding further emphasises the marked individual variability in response to hypoxia.

Study 5 (Chapter 8) sought to ascertain the influence of endogenous EPO on time trial performance in well-trained endurance runners. Using the methodology established in the previous study (Study 4; Chapter 7), performance in a 10 min pre-loaded time trial (TT) was measured three hours after two hours of hypoxia (FiO_2 of 0.118). In addition to the hypoxic trial, a control trial of normoxia (FiO_2 of 0.208) and hyperoxia (FiO_2 of 0.398; to supress EPO) were also included. Despite the hypoxic trial increasing [EPO] by ~30% above the control group and ~40% above the hyperoxic group (therefore, accepting the hypotheses 5a and 5b, see Table 10.1), there was no difference in distance covered over a 10 min pre-loaded TT (rejecting hypothesis 4c). Moreover, there were no differences is RPE, HR, $\dot{V}O_2$, $\dot{V}_E$ or running economy during the TT between trials. These results could be explained by an insufficient 'hypoxic dose' that did not increase [EPO] to the magnitude that it would cross the blood-brain barrier (BBB) (Jelkmann 2005). There was also an individual variability in the [EPO]

response to each trial. The present study found that endurance performance (over ~3,000 m) was not affected by increased endogenous [EPO] in well-trained runners. The role of EPO on endurance performance, independent to increased tHbmass and oxygen carrying capacity, requires further research.

The final experimental study (Chapter 9) examined the effect on a LHTH altitude training camp on: endurance performance determinants, the time course of tHbmass and [EPO] and the use of a pre-screening tool to predict physiological/haematological adaptation in elite middle- to long-distance runners. Firstly, the study revealed that 4 weeks of LHTH at 2,300 m was sufficient enough to increase mean LTP and $\dot{V}O_{2max}$, but not LT and tHbmass compared to a sea level control group (accepting hypothesis 6a but rejecting hypothesis 6b, see Table 10.1). Across all physiological determinants response to LHTH showed a large variation. Secondly, the study found there was no difference in mean tHbmass measured at three time points (Day 1 ± 2, day 11 ± 3 and day 18 ± 2) post-LHTH compared to baseline (rejecting hypothesis 6c), however, [EPO] was found to be substantially lower 1 day post-LHTH (accepting hypothesis 6d). Thirdly, pre-screening athletes with a hypoxic sensitivity test (HST) was demonstrated to be a useful tool in predicting which athletes increased tHbmass and $\dot{V}O_{2max}$, therefore accepting hypothesis 5 and 6. Indices derived from the HST, such as change in oxygen saturation (at rest) and hypoxic ventilatory response (at rest), correlated with an increase in tHbmass. The findings of the study agree with previous research that there is high variation in individual pulmonary and haematological responses to hypoxia, and pre-screening methodologies could assist practitioners and coaches to understand how athletes might respond to an altitude training camp.

10.2 Hypothesis

Table 10.1: Hypotheses for each study chapter presented in this thesis.

Hypothesis	Accepted	Rejected
Study 1 (Chapter 4): Altitude and hypoxic training in endurance running: Perceptions of elite athletes and support staff		
a) There will be no difference between athlete and support staff altitude training practices		✓
b) There will be difference between athlete and support staff perceptions of altitude training	✓	
Study 2 (Chapter 5): Comparison of total haemoglobin mass measured with the optimised carbon monoxide rebreathing method using different radiometer™ hemoximeter		
a) There will be a difference in tHbmass when measured with different radiometer™ hemoximeters		✓
Study 3 (Chapter 6): The influence of carbon monoxide bolus on the measurement of total haemoglobin mass using the optimized co-rebreathing method		
a) There will be no difference in tHbmass when administering different bolus of carbon monoxide		✓
Study 4 (Chapter 7): Time course of endogenous erythropoietin, IL-6 and TNFα in response to acute hypoxic exposures		
a) [EPO] will increase following a 2 h normobaric hypoxic exposure in accordance with severity of hypoxia.	✓	
b) Basal levels of IL-6 and TNFα would inhibit the production of [EPO]		✓
Study 5 (Chapter 8): Influence of endogenous erythropoietin on time trial performance in endurance runners		
a) [EPO] will increase after hypoxia compared to a normoxic control	✓	
b) [EPO] will decrease after hyperoxia compared to a normoxic control.	✓	
c) Distance covered during a 10 min pre-loaded time trial will be greater when [EPO] is elevated		✓
Study 6 (Chapter 9): Predicting an athlete's physiological and haematological response to classic altitude training using hypoxic sensitivity methods		
a) LHTH altitude training will improve physiological capacity in elite endurance runners	✓	
b) LHTH altitude training will increase tHbmass in elite endurance runners		✓
c) Total Hbmass will display a variable response on return from LHTH altitude training		✓
d) [EPO] will be supressed on return from LHTH altitude training	✓	
e) There will be a relationship between HST indices and post-LHTH changes in $\dot{V}O_{2max}$	✓	
f) There will be a relationship between HST indices and post-LHTH changes in tHbmass	✓	

10.3 The oCOR-method

The measurement of tHbmass with the oCOR-method has become common practice in altitude and hypoxic training research as evidenced by a recent meta-analysis by Gore et al. (2013), citing 17 studies using the method since 2008. The original CO rebreathing method (Burge and Skinner 1995) was optimised in 2005 (Schmidt and Prommer 2005) and since then the method has been examined by researchers from around the world. The oCOR-method is subject to a combination of biological and analytical variability (Gore et al. 2005), which can be initiated by the participant, experimenter or equipment. A variety research studies have investigated the carbon monoxide kinetics and timing/site of blood sampling (Gore et al. 2006a; Prommer and Schmidt 2007; Garvican et al. 2010a), the biological stability and variation in tHbmass (Eastwood et al. 2008; Garvican et al. 2010b; Eastwood et al. 2012c; Eastwood et al. 2012b; McLean et al. 2013), the effect of training, injury and illness (Steiner and Wehrlin 2011b; Eastwood et al. 2012a; Gough et al. 2012a; Gough, Clare Elizabeth, Sharpe, K., Garvican, L. A., Anson, J.M., Saunders, P.U., Gore 2013) and the different methodology and quality control measures (Steiner and Wehrlin 2011a; Gough et al. 2011; Durussel et al. 2013b; Naef et al. 2015). Study 2 (Chapter 5) and Study 3 (Chapter 6), respectively, have added to this important body of research, comparing the analysers used to quantify %HbCO and investigating the influence of CO bolus administered during the oCOR-method.

All methods of estimating RCV or tHbmass are subject to error, and a meta-analysis of raw data demonstrated a typical error for CO rebreathing of 2.2% (90% confidence limits of 1.4% to 3.5%) for measures taken 1 day apart (Gore et al. 2005). A more recent meta-analysis estimated the analytical error for tHbmass to be 2.0% (95% confidence limits of 1.8% to 2.3%) (Gore et al. 2013). The present thesis produced a TEM of 1.2% for tHbmass over 23 duplicate measures. The reduction of both biological and analytical error is important when interpreting the success of an altitude training camp and also inferring the effect it might have on endurance performance. Gore et al. (2013) reported that a 2-week classic camp (336 h) might be sufficient to increase tHbmass by a mean of ~3% and by at least 1% for 97.5% of athletes, which is barely higher than the typical error for the measure. However, given that a worthwhile increase in performance for an elite athlete is of the order of 0.3–0.4% (Pyne et al. 2004; Smith and Hopkins 2011), small but meaningful changes in an athletes physiology might be enough to improve race performance (Gore et al. 2013). This is supported by the findings of Schmidt and Prommer (2010) who stated that a change in tHbmass by 1 g causes a change in $\dot{V}O_{2max}$ by approximately 4 mL·min^{-1}. Crucially for the present thesis it was fundamental that coaches, athletes and practitioners had confidence in the precision of the oCOR-method when measuring tHbmass before and after an altitude training camp, as this was referenced as a priority area in the questionnaire of Study 1 (Chapter 4).

10.4 Optimisation of altitude training adaptation

10.4.1 The application of altitude training

To the authors knowledge there is currently no model describing the application of altitude and hypoxic training by elite athletes. Wilber (2007a) introduced a model of altitude training methods,

subsequently modified by Millet et al. (2010) (see Figure 2.5 in Chapter 2.4.1). The authors described the different methods of altitude training with no specific indication of the 'hypoxic dose' (height, duration and daily exposure) and what affect they might have on the athlete. Truijens and Rodríguez (2011) produced a summary of the purported physiological mechanisms involved in the use of hypoxia for performance enhancement based on the work of Rodríguez et al. (2007) (see Figure 2.6 in Chapter 2.4.2). The detailed summary provides an insight into some of the key physiological and haematological responses, acclamatory processes and potential adaptations that may result from altitude training. In this instance the final outcome is an improvement in swimming performance, however the summary presented is relevant to all endurance sports, and the final outcome could be termed an improvement in 'performance velocity or power'. This terminology is used by Joyner and Coyle (2008) in their model (see Figure 2.4 in Chapter 2.3) of 'the multiple physiological factors that interact as determinants of performance velocity or power output'.

Many of the physiological factors described in the model by Joyner and Coyle (2008) are also stated in the Truijens and Rodríguez (2011) model of proposed physiological adaptions arising from altitude training. Several aspects related to endurance performance may be altered by hypoxic exposure and training including increases in erythrocyte volume, maximal aerobic exercise capacity, capillary density, and economy (Sinex and Chapman 2015). There is also a growing body of evidence to suggest that non-haematological adaptions (Gore et al. 2007) are possible as a result of altitude training and hypoxic exposures, therefore altitude training may affect more than just the oxygen carrying capacity of the blood.

Figure 10.1 illustrates a basic flow chart of the application of altitude training in elite endurance sport. The figure provides an overview of the six key stages illustrated in the order of which they are likely to occur. There is a vast quantity of research that underpins the implementation of altitude training methods. A large proportion of research has focused on 'Stage 1' of the model, the implementation of the 'hypoxic dose' (Levine and Stray-Gundersen 2006; Wilber et al. 2007; Wilber 2007b; Chapman et al. 2014a; Garvican-Lewis et al. 2016b). Stages 3-4 have also been previously reviewed with regard to acute and chronic responses to moderate and high altitude (Hoppeler and Vogt 2001; Bartsch and Saltin 2008; Mazzeo 2008; Naeije 2010). 'Stage 5' of the model has been investigated in two parts; *Part A* the desired haematological adaptations (Ashenden et al. 1999; Friedmann et al. 2005a; Clark et al. 2009; Pottgiesser et al. 2009; Gough et al. 2012b; Garvican-Lewis et al. 2013; Bonne et al. 2014); and *Part B* the desired physiological adaptations (Saunders et al. 2004b; Schmitt et al. 2006; Saunders et al. 2009b; Robach et al. 2012; Menz et al. 2015). And lastly, researchers have also shown that altitude training may improve race performance ('Stage 6') in elite middle- and long-distance runners (Saunders et al. 2009c; Fudge et al. 2011).

It should be noted that studies have also failed to show improvements after altitude training (Gore et al. 1998; Ashenden 1999; Ashenden et al. 2000; Julian et al. 2004; Faulhaber et al. 2010; Siebenmann et al. 2012; Nordsborg et al. 2012). Interestingly, there is limited research that has investigated 'Stage 2' of the model, hypoxic sensitivity or tolerance in elite endurance runners.

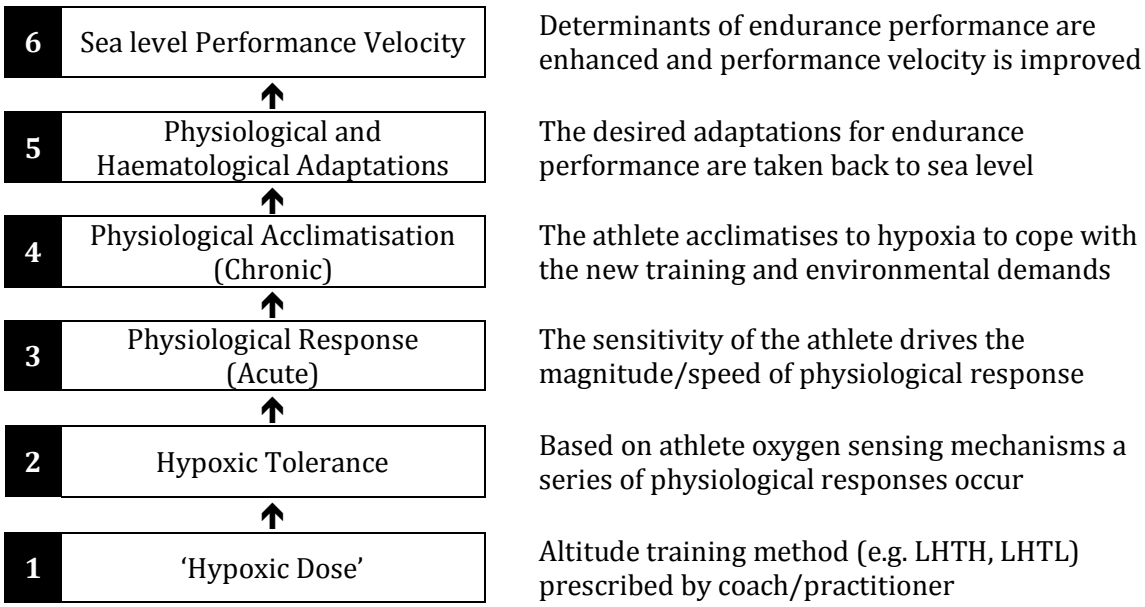


Figure 10.1: A flow chart of the application of altitude training

Altitude training is not a universally accepted method to improve sea level endurance performance, as it does not appear to affect all athletes positively. Possible reasons for a lack of physiological or performance improvement have been previously suggested, e.g. an already elevated RCV (Lundby et al. 2012), the inability to maintain sufficiently high work rates and training velocities (Jacobs 2013), the lack of a proper control group (to rule out a placebo effect) (Lundby and Robach 2016), insufficient iron stores (Debevec and Mekjavić 2013) and individual variation in the response to altitude training (Sinex and Chapman 2015). Of all of these factors it is the latter that has received the least attention and could arguably have the biggest impact on the success of an altitude training camp. The individual variation in response to hypoxic training has been associated with genetic differences in hypoxia sensing and hypoxic response pathways (Chapman et al. 2010). This issue has been raised in the literature, with the use of pre-screening tools as a potential solution (Chapman et al. 2010; Sinex and Chapman 2015). Using Figure 10.1 as a model of current altitude training application the 'hypoxic dose' is the starting point, however without a prior understanding of an individual's hypoxic tolerance, the blanket application of the 'hypoxic dose' is highly likely to show a varied response.

The findings from the present thesis will be discussed to further understand the role of the 'hypoxic dose', individual variation and hypoxic tolerance in endurance runners.

10.4.2 The 'hypoxic dose'

Altitude training, both in natural and simulated conditions, has been established as an effective means to improve oxygen transport, tHbmass and $\dot{V}O_{2max}$, given sufficiently high 'doses' of elevation and exposure duration (Sinex and Chapman 2015). Table 10.2 outlines the optimal 'hypoxic dose' as reported by a series of reviews. In the case of Rasmussen et al. (2013) and Gore et al. (2013) the 'dose' is in relation to an increase in RCV or tHbmass, respectively. Furthermore, Clark et al. (2009) and

Garvican et al. (2012) concluded that tHbmass increases at a mean rate of 1%·100 h^{-1} of exposure to response to three weeks of simulated LHTL (3,000 m) and natural LHTH (2,750 m), respectively.

Altitude (in meters) and time (in hours) are the most readily accepted metrics for quantifying the optimal 'hypoxic dose' for an altitude training camp. Recently, Garvican-Lewis et al. (2016b) attempted to combine these two measures to quantify 'hypoxic dose' in 'kilometer hours'. Data from 21 separate studies revealed that in order to achieve an increase in tHbmass of 5%, 1500 kilometer hours would need to be accumulated (see red circle on Figure 10.2). Implementing the new metric for 'hypoxic dose', the present study accumulated 1580 kilometer hours, which should have been sufficient enough to increase tHbmass by ~5%. However, after 1 ± 2 days at sea level the mean increase in tHbmass was 1.8 ± 3.2, with only two athletes showing an increase in tHbmass of more than 5%. This finding further highlights the individual variation in tHbmass in response to the same 'hypoxic dose'.

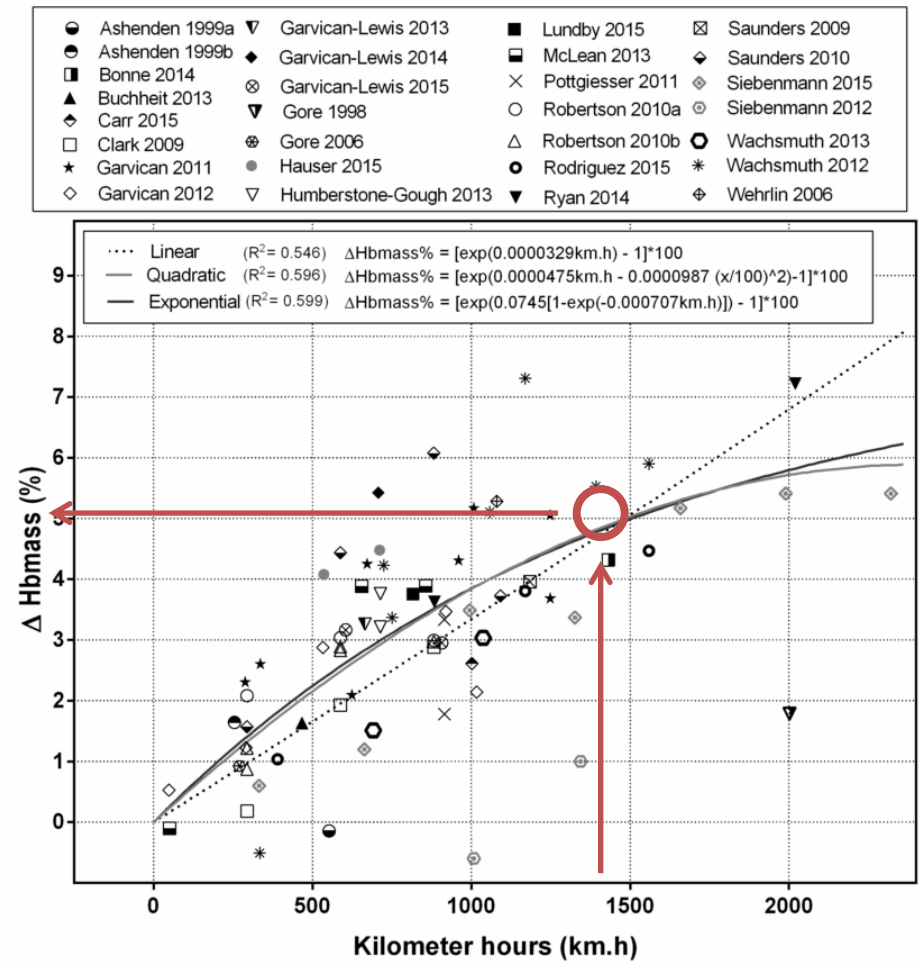


Figure 10.2: Estimates of the change in tHbmass relative to kilometer hours (km.h) of hypoxic exposure accumulated. (Garvican-Lewis et al. 2016b)

Table 10.2: Proposed 'hypoxic dose' sufficient enough to increase tHbmass by a significant or meaningful amount

Author	**Method**	**Altitude (m)**	**Duration (weeks)**	**Hours**	**'Hypoxic dose' (km·h)** Garvican-Lewis et al. (2016)
Rusko et al. (2004)	Simulated LHTL	2,000 – 2,500	3	12 h·day^{-1}; Total: 252 h	504 - 630
Wilber et al. (2007)	Natural LHTH	2000 – 2,500	4	24 h·day^{-1}; Total: 672 h	1344 – 1680
	Simulated LHTL	3,000	4	12-16 h.day-1; Total: 336-448 h	1008 - 1344
Rasmussen et al. (2013)	Natural LHTH	4,000	2	24 h·day^{-1}; Total: 336 h	1344
	Natural LHTH	3,000	4	24 h·day^{-1}; Total: 672 h	2016
Gore et al. (2013)	Natural LHTH	2,500	2	24 h·day^{-1}; Total 336 h	1008
	Simulated LHTL	3,000	3	16 h·day^{-1}; Total 504 h	1512
Present Study	**Natural LHTH**	**2,350**	**4**	**24 h·day^{-1}; Total: 672 h**	**1580**

The authors do admit there are limitations to this model. The influence of training conducted at altitude on the overall hypoxic response should be considered and the model had focused solely on increasing tHbmass during an altitude sojourn; not considering non-haematological adaptations (Garvican-Lewis et al. 2016b). Ultimately the benefit derived from hypoxic training depends on the balance of achieving haematological adaptations while reaching adequate training volume and intensity (Chapman and Levine 2000). Furthermore, the Garvican-Lewis et al. (2016b) 'hypoxic dose' metric does not account for a possible "altitude-threshold" (Wehrlin et al. 2016), which is associated with the s-shape of the oxyhaemoglobin saturation curve, whereby at altitudes above ~2,000 m the desaturation of athletes would occur on the steeper part of the curve resulting in more substantial increases in [EPO] (~90% at 2,400 m compared with ~30% at 1,800 m after 24 h) (Chapman et al. 2014a). The Garvican-Lewis et al. (2016b) metric also does not consider the large inter-subject variability in the physiological responses to a given 'hypoxic dose' and therefore the magnitude of the stimulus rather than the altitude elevation should instead be considered (Millet et al. 2016). A metric based upon hypoxic sensitivity, which considers desaturation levels in normoxia (exercise-induced arterial hypoxemia) or in hypoxia may be more beneficial.

The largest single altitude training study attempting to define the optimal 'hypoxic dose' for sea level performance sea level performance enhancement was by Chapman et al. (2014a). In this study, 48 collegiate track and cross and cross country runners were matched by sex, training history, $\dot{V}O_{2max}$, and 3-km time and assigned to live at four to live at four different altitudes (1,780 m, 2,085 m, 2,454 m or 2,800 m) for four weeks. Prior to altitude training, altitude training, slight adjustments in group assignment were made so that the mean 24-h EPO response to a response to a simulated altitude of 2,454 m was similar between groups. All participants performed daily supervised daily supervised training at the same altitude and location (between 1,250 and 3,000 m), regardless of the subjects' the subjects' assigned living altitude. The primary findings from the study are shown in Table 10.3 (immediate effect of a four week altitude training camp) and Table 10.4 (two weeks post effect of a four week altitude training camp). The authors concluded that living at 2,085 and 2,454 m was an optimal living altitude for producing improvements in RCV, $\dot{V}O_{2max}$ and sea level performance. In Chapter 9 (Study 6) of the present thesis living and training at an altitude of 2,350 m was unable to show a mean increase either tHbmass or $\dot{V}O_{2max}$ (see

Figure 9.4), however eight out of twelve participants did show an increase in tHbmass and $\dot{V}O_{2max}$ by varying magnitudes.

*Table 10.3: Immediate effect of a four week altitude training camp at different altitudes on performance (3,000 m time trial), $\dot{V}O_{2max}$ and RCM * denotes significant difference (P<0.05)*

	1,780 m	2,085 m	2,454 m	2,800 m
3,000 m TT	No change	Improved *	Improved *	No change
$\dot{V}O_{2max}$	No change	Improved *	Improved *	No change
RCM	Improved *	Improved *	Improved *	Improved *

*Table 10.4: Two weeks post effect of a four week altitude training camp at different altitudes on performance (3,000 m time trial), $\dot{V}O_{2max}$ and RCM * denotes significant difference (P<0.05)*

	1,780 m	2,085 m	2,454 m	2,800 m
3,000-m TT	No change	Improved *	Improved *	No change
$\dot{V}O_{2max}$	No change	Improved *	Improved *	Improved *
RCM	No change	No change	No change	No change

The measurement of EPO pre- and post-altitude training and the monitoring SaO_2 during sleep was a novel aspect of the study. SaO_2 values obtained during sleep over the course of the altitude camp were consistently lower (~94-95%) in the two highest living groups compared to the lowest living groups (~96-97%). Although, all four altitude groups significantly increased [EPO] over sea level baseline at the 24-h time point and RCM was ~6% higher in all four altitude groups after return to sea level. This was despite the [EPO] in the 1,780 m group returning to baseline in 72 hours, while it remained elevated in the other higher altitude groups. Therefore, it is possible that changes in RCM are independent of the athlete's degree of desaturation and differences in [EPO], at least as determined from a single measure taken early in the morning. The present study recorded an average morning SaO_2 of 94 ± 1%, which was not correlated with percentage change in tHbmass measured within 1 ± 2 days at sea level (R^2 = 0.29, P > 0.05). Chapman et al. (2014a) also found 3,000 m TT performance and $\dot{V}O_{2max}$ measured immediately post was only improved in the 2,085 and 2,454 m groups yet RCM was increased in all four groups, suggesting haematological factors may not be a causal factor.

Chapman et al. (2014a) reported individual variability in changes in [EPO] measured in an altitude chamber pre-altitude chamber pre-camp vs. [EPO] measured after 24 hours at the same altitude in the field (Figure 10.3). Not only does this highlight the inherent difficulty in trying to predict the EPO response at altitude using a short-term chamber exposure but also other individual responses (e.g. hypoxic tolerance) to altitude living or training could have influenced the amount of change in performance after the altitude camp (Chapman et al. 2014a). Figure 10.4 shows the wide variation in pre- to post-altitude training changes in $\dot{V}O_{2max}$ and 3,000 m TT performance at the four different living altitudes. Similar results can be seen in

Figure 9.4 of Chapter 9 (Study 6).

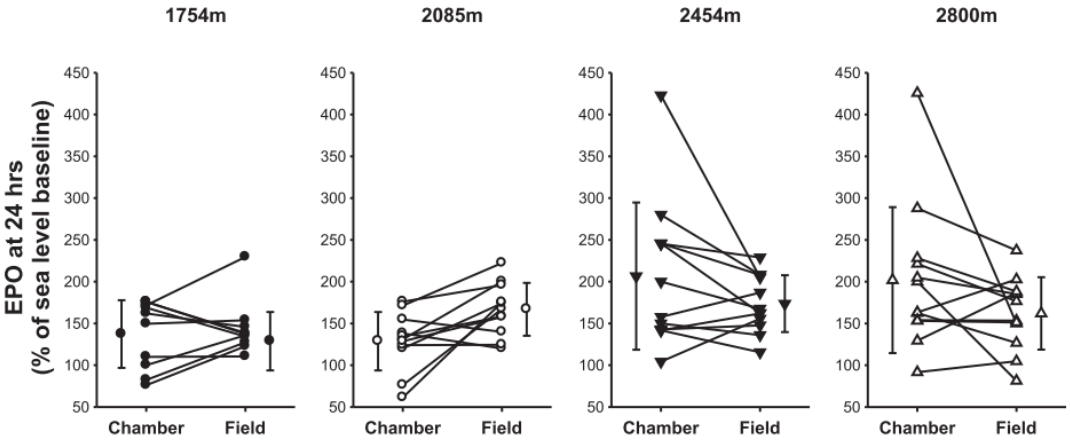


Figure 10.3: [EPO], expressed as a percentage of SL, pre-altitude baseline, after 24 h in a hypobaric chamber vs. the same altitude in the field.

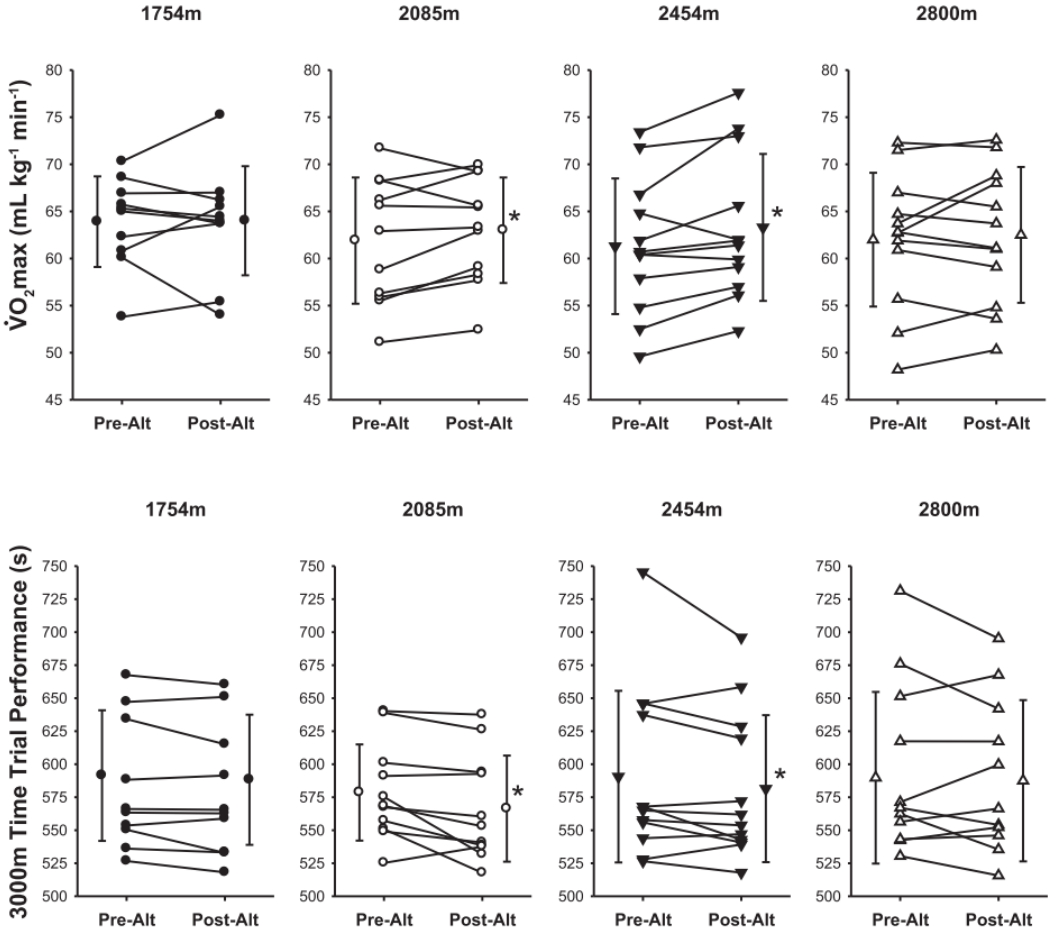


Figure 10.4: $\dot{V}O_{2max}$ (top) and 3,000-m time trial performance (bottom). Values are from measures completed at SL and pre- and post-altitude training.

The findings of Chapman et al. (2014a) highlight the role of an adequate 'hypoxic dose' in improving performance, physiological and haematological markers, however, as with previous studies the large individual variability observed makes it difficult to draw any meaningful conclusions from mean data

values. The individual variation in the results from Chapter 9 (Study 6) emphasises the need for improved measurement of physiological and haematological variables around an altitude training camps to ensure an appropriate altitude of a training camp is utilised and opportunities to intervene during a camp are taken. These considerations may increase the chances of success for more of the training group. Further research is required to understand why there is such variance in individual physiological responses to hypoxia, with variables such as, level of desaturation, EPO and HVR key to establishing the hypoxic tolerance of the individual.

10.4.3 Individual variation to hypoxic exposures

Individual responses to a particular training intervention typically show considerable variation, including particularly 'high responders' and particularly 'low responders' for different training response parameters (Mann et al. 2014). Chapman et al. (1998) were the first to investigate the reported wide variance in performance after a traditional altitude training camp systematically. During a retrospective analysis, Chapman et al. (1998) characterised athletes from a previously published study (Levine and Stray-Gundersen 1997) as 'responders' and 'non-responders' based on improvements in 5,000 m run time post-altitude training. Although the authors demonstrated novel findings in that there was a more augmented initial EPO response at 2,500m in the 'responders' and a greater reduction in interval-training velocity in the 'non-responders', they inadvertently categorised athletes into two distinctive groups. As a result of dichotomising the population, many coaches and athletes believed that they fell into one of these two groups and would either benefit from altitude training or not. It is more likely that the athletes in this study were on a continuum from 'high responders' to 'low responders' to the hypoxic stimulus, which has been previously eluded to in response to heat adaptation (Taylor 2014). Taking this into consideration the design of a more bespoke altitude training strategy may increase the chances of a positive outcome.

Many of the findings from the present thesis also showed individual variability in response to both acute simulated hypoxic exposures and chronic natural altitude. Study 4 (Chapter 7) investigated the time course of [EPO] after two hours of normobaric hypoxia at three different fractions of inspired oxygen (0.135, 0.126 and 0.115) to simulate three different altitudes (3,600, 4,200 and 4,800 m). The participants in the study rested passively in an altitude chamber whilst serial blood samples were taken and SpO_2 was measured every 15 minutes. Figure 10.5A illustrates the individual change in SpO_2 from pre-hypoxia to the average recorded during the two hour exposure. The red, dark blue and black lines show the mean ΔSpO2 from the 4,800 m (-23 ± 3%), 4,200 m (-14 ± 1%) and 3,600 m (-11 ± 2%) trials, respectively. The pink, light blue and grey lines show the individual participant ΔSpO_2 from the 4,800 m, 4,200 m and 3,600 m trials, respectively. Overall each participant responded similarly, with mean significant differences found between the groups.

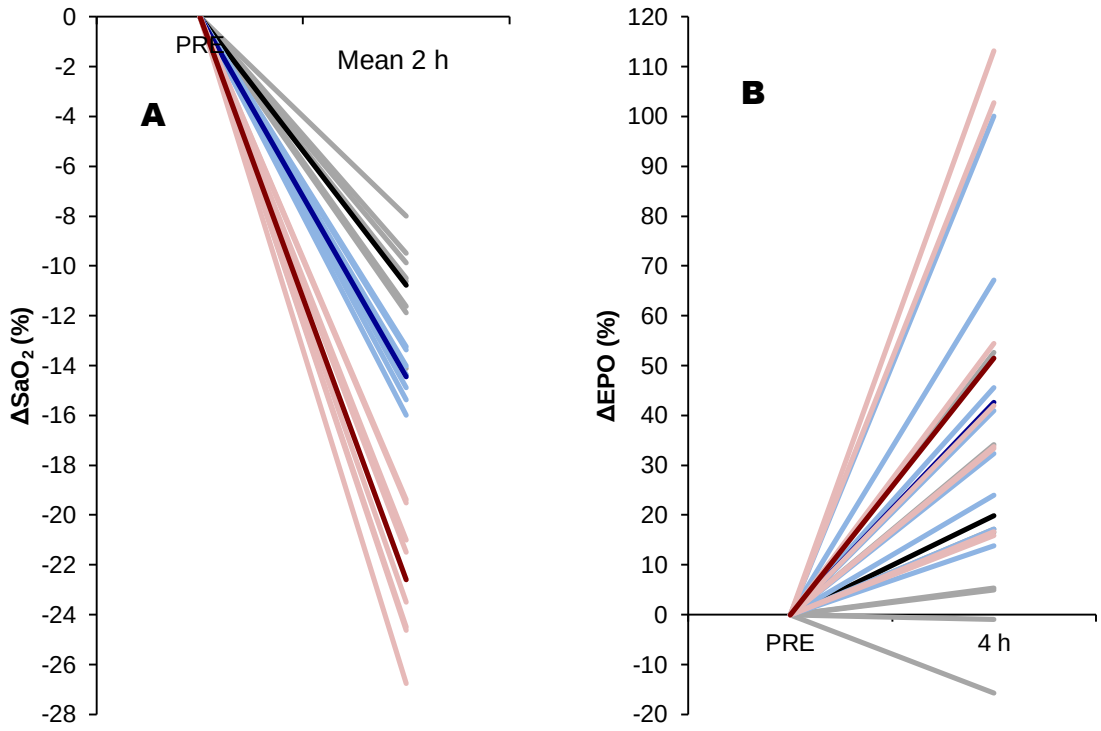


Figure 10.5: Chapter 7 (Study 4) percentage change of individual ΔSaO2 (A) and ΔEPO (B) before (PRE) and two hours after (4 h) acute hypoxic at FiO2 = 0.135 (grey), FiO2 = 0.126 (blue) and FiO2 = 0.115 (pink).Darker lines indicate mean response for each group

Despite the response of SpO_2 being somewhat predictable, as a result of acute hypoxia, the [EPO] response showed considerable within group variation. From pre-hypoxia to two hours post-hypoxia significant increases in [EPO] in the 4,200 m (43 ± 29 %) and 4,800 m (52 ± 37%) groups were evident but not the 3,600 m (20 ± 24 %) group, although there was no significance found between groups. Figure 10.5B clearly shows the spread of pink, light blue and grey lines, indicating that desaturation has little influence on individual [EPO] response.

The findings from Study 5 (Chapter 8) also indicate a distinct individual variability in EPO response that cannot be explained by SpO_2 measured during the acute normobaric hypoxic exposures. The aim of the study was to complete an endurance performance test (10 min pre-loaded time trial) whilst EPO was either elevated or supressed. This was achieved with two hours exposures at three different fractions of inspired oxygen (0.118, 0.208 and 0.394) to simulate three different conditions (hypoxia, normoxia and hyperoxia). Figure 10.6 A shows the mean ΔSpO_2 from the hypoxic (-20 ± 4%), normoxic (-1 ± 1%) and hyperoxic (0 ± 0%) trials, with the black, dark blue and red lines, respectively. The pink, light blue and grey lines show the individual participant ΔSpO_2. The three conditions were sufficient enough to induce a mean change in [EPO] of +11 ± 9%, -20 ± 20% and -29 ± 9%, although there was no significant difference found from baseline (PRE) to three hours post-exposure (5 h). The was however a difference ($P < 0.05$) in Δ[EPO] found between the hypoxic and hyperoxic trial showing there was a difference in endogenous [EPO] between groups but not within groups.

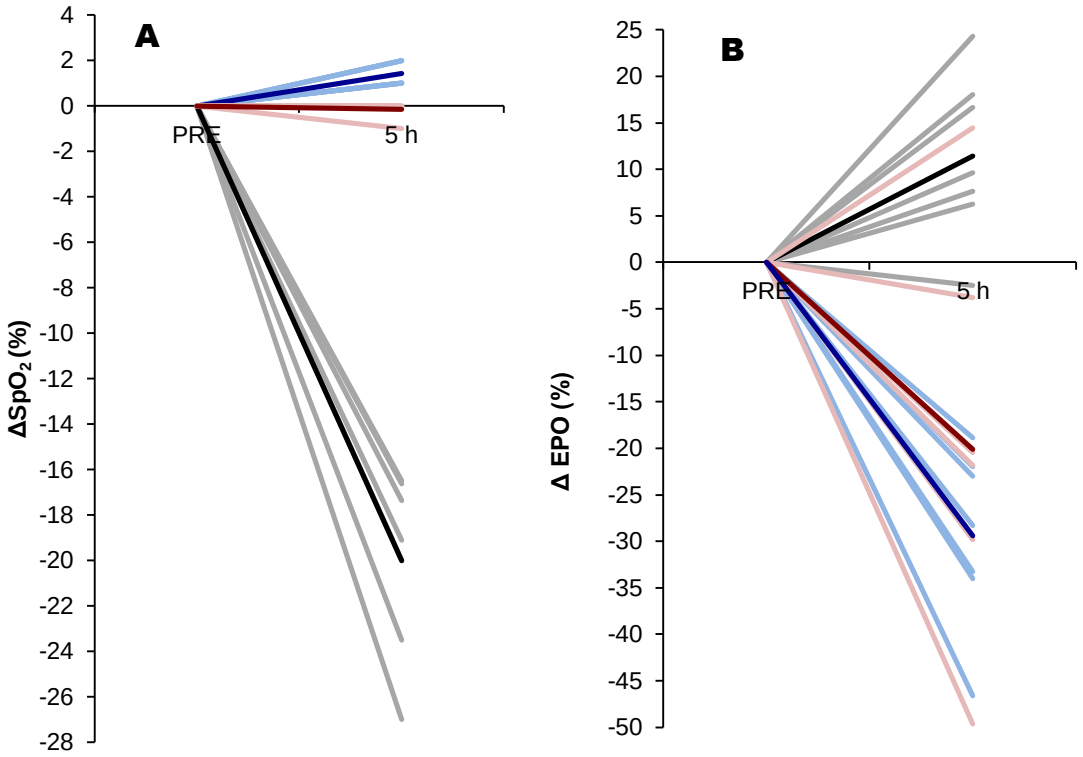


Figure 10.6: Chapter 8 (Study 5) percentage change of individual ΔSaO2 (A) and ΔEPO (B) before (PRE) and three hours after (5 h) acute hypoxic at FiO2 = 0.118 (grey), FiO2 = 0.394 (blue) and FiO2 = 0.208 (pink). Darker lines indicate mean response for each group.

Friedmann et al. (2005b) also measured the EPO response after a four hour exposure to normobaric hypoxia (FiO_2 0.15; ~2,500 m) in elite junior swimmers, finding EPO significantly increased by 58 ± 41% (range: 10–185%). The study also reported a highly variable individual response from athletes after a hypoxic exposure, however SpO_2 was not reported. A unique aspect of this study was that EPO was also measured on day 1 and day 2 of the altitude training camp (2,100–2,300 m) increasing by 51.8 ± 45.6%. Furthermore, tHbmass was measured after the three week altitude training camp, which significantly increased by 6%, on average, with a wide inter-individual variability observed. Interestingly, the EPO increase after 4 h exposure to normobaric hypoxia was significantly correlated with the acute EPO increase during altitude training, however no significant correlation was found between the acute EPO increase at altitude and the change in tHbmass (see Figure 10.7B). The authors hypothesised those athletes who respond to training at moderate altitude with a large acute EPO increase might be identified with the help of short exposure to equivalent normobaric hypoxia; however EPO response did not predict changes in tHbmass (see Figure 10.8B).

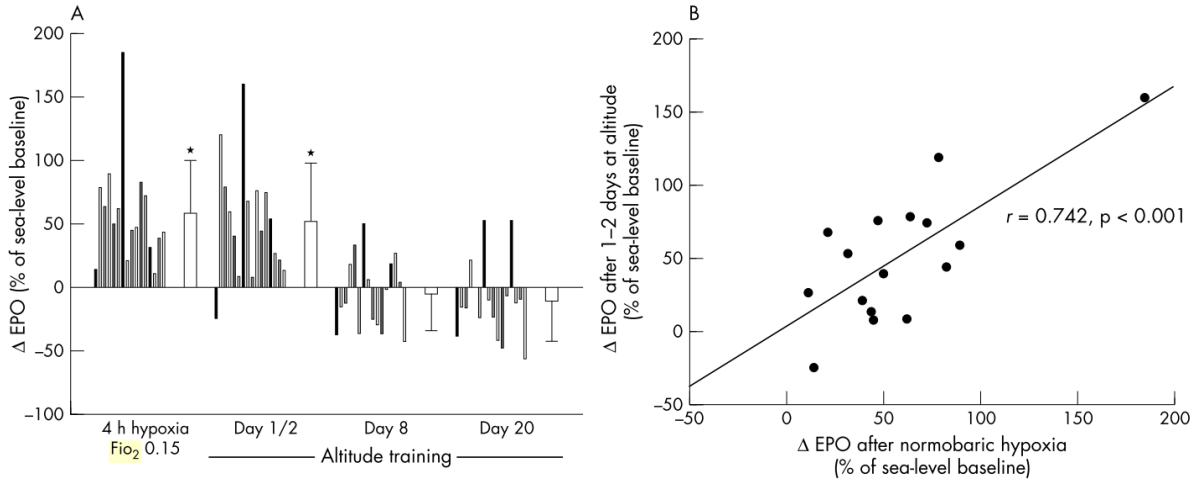


Figure 10.7: Friedmann et al. (2005b) changes in EPO after 4 h exposure to normobaric hypoxia and during altitude training (A) and correlation between the EPO response to 4 h exposure to normobaric hypoxia and the acute EPO response during altitude training (B).

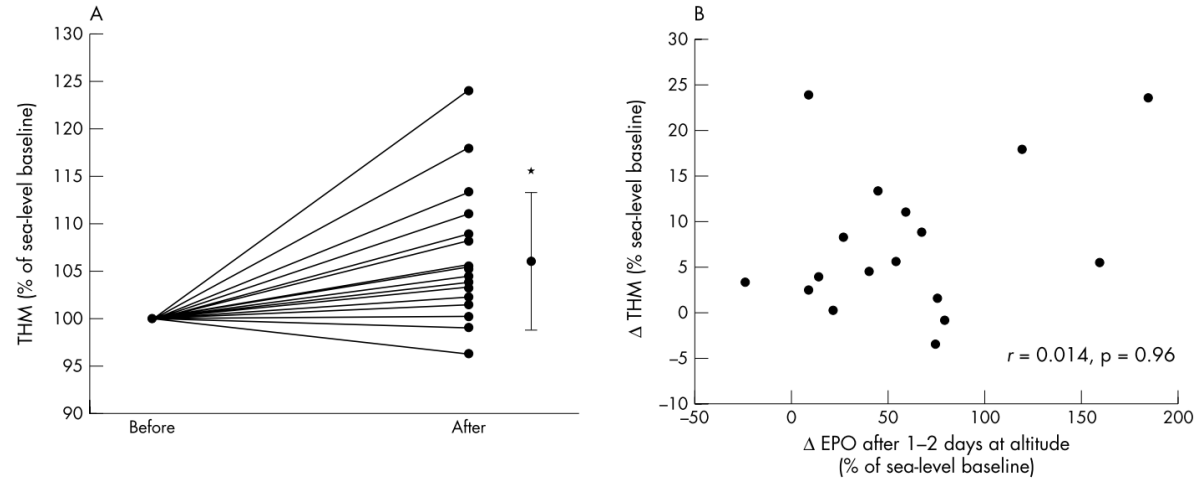


Figure 10.8: Friedmann et al. (2005b) changes in THM (tHbmass) after altitude training (A) and correlation between the acute EPO response during altitude training and the change in THM (tHbmass) after altitude training (B).

Figure 10.9 illustrates the mean and individual responses from Study 6 (Chapter 9). The results were in concordance with other studies that have measured tHbmass and $\dot{V}O_{2max}$ and found a wide inter-individual variation in response to a LHTH altitude training camp (Friedmann et al. 2005a; Wachsmuth et al. 2013; Bonne et al. 2014; Garvican-Lewis et al. 2015). Sinex and Chapman (2015) recently identified several factors that are associated with individual responses to hypoxia including, 1) iron status, since iron is needed for the production of Hb and erythrocytes, 2) EPO production, as it plays a role in determining how RBC volume and tHbmass change, and 3) pulmonary gas exchange, since athletes who do not adequately maintain SaO_2 during high intensity exercise show a greater impairment in endurance performance. There are very few studies that have attempted to understand the cause of the individual variation in response to altitude training but instead focussing the attention on mean response of the group and speculating to an insufficient 'hypoxic dose'. It is possible that by

categorising an athlete as a high or low responder on a continuum, and not a group, practitioners could increase the chances of a positive response.

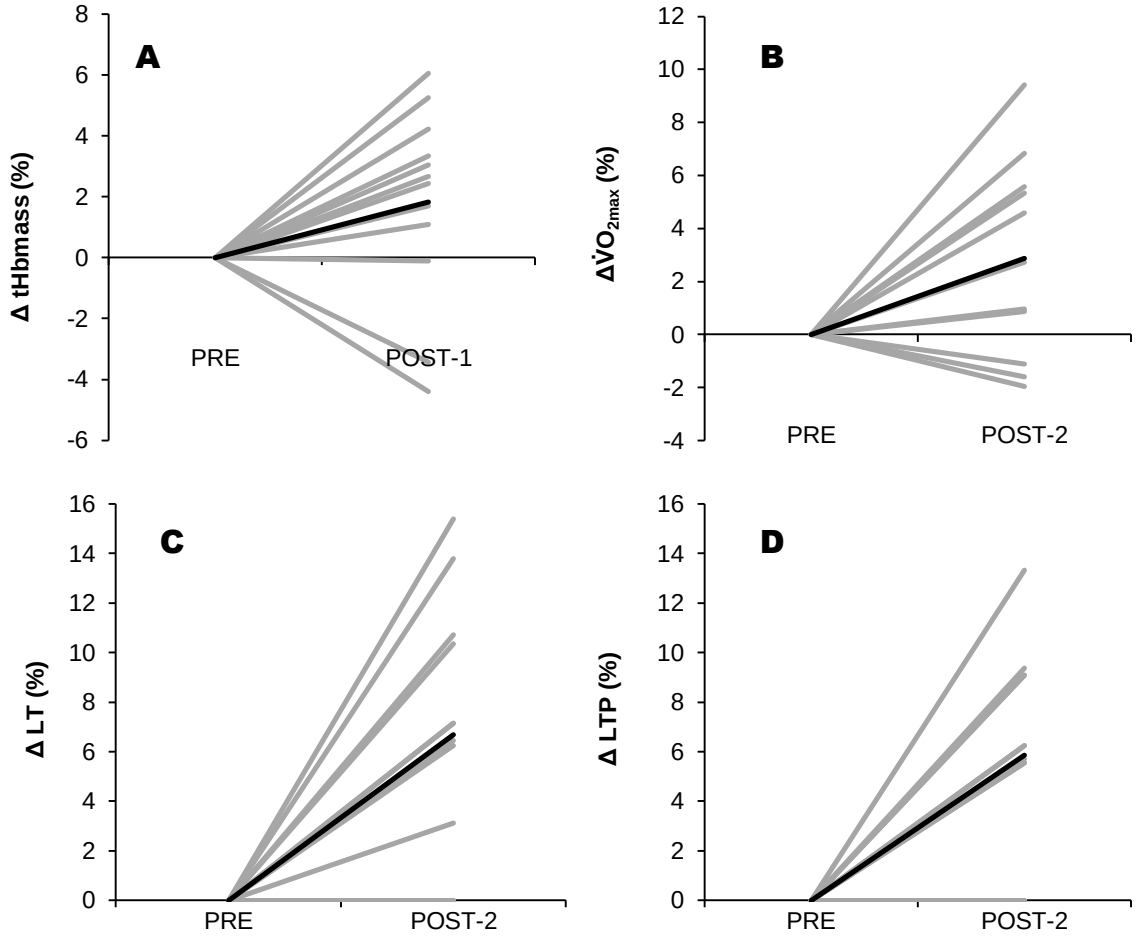


Figure 10.9: Chapter 9 (Study 6) individual (grey line) and mean (black line) responses of tHbmass (A), $\dot{V}O_{2max}$ (B), LT (C) and LTP (D) after four weeks of LHTH altitude training at 2,300 m. Results are expressed as percentage change from PRE.

Chapman et al. (2010) investigated the relationship between hypoxic ventilatory response (HVR) and the magnitude of EPO release with acute exposure to moderate altitude (~2,500 m) in elite distance runners. The study found that EPO was significantly increased (92 ± 70%) from pre-altitude sea level baseline to 20 hours at 2,500 m. Individual ΔEPO showed a wide variability ranging from −20 to 415%. There was no correlation found between the HVR and acute increase in EPO, either when ΔEPO was expressed in absolute terms (r=−0.17) or relative terms (r=−0.12). The authors stated that individual increase in EPO concentration in elite endurance athletes is not primarily related to peripheral chemoresponsiveness, in this case HVR, and suggested that factors downstream from the lung, such as the hypoxic sensitivity of the EPO producing cells of the kidney and/or the oxygen delivery to oxygen consumption ratio in the kidney, may be the primary sources of the individual variation of the acute EPO response to moderate altitude. Unfortunately SaO_2 was not measured in this study, as it is believed that renal blood flow, [Hb], and SaO_2, could be the upstream source of variability consistently

seen in EPO release to a fixed altitude (Ge et al. 2002). Arterial oxyhaemoglobin saturation in hypoxia is controlled in large part by the hypoxic ventilatory response, which is mediated primarily by the peripheral chemoreceptors in the carotid bodies (Weil et al. 1970).

The findings of the present thesis have also shown that changes in EPO are independent of changes in SaO_2. Baseline peripheral chemoresponsiveness is not a universal mediating factor determining the magnitude of acute EPO release at moderate altitude CH. Further to this there does not appear to be a simple linear relationship between punctual EPO measurements during altitude training and the increase in tHbmass FR. The hypoxic tolerance model (see Figure 2.12) states that the hypoxic ventilatory response and EPO response are initiated by different oxygen sensing mechanisms, which may explain why no relationship has been detected between the measurement of HVR and EPO. Understanding hypoxic tolerance could provide further insight into the challenge of predicting an athlete's response to altitude training.

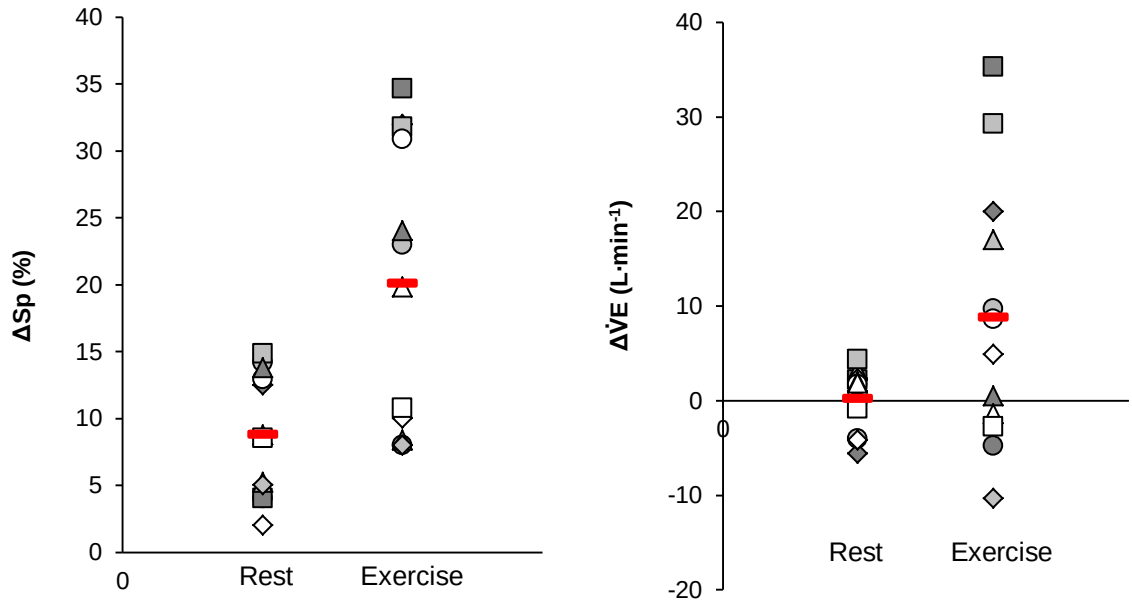
10.4.4 Hypoxic tolerance

During acute altitude exposure in lowlanders, acute hypoxia-induced adaptations are initiated by five oxygen sensing, signal transduction pathways (Hochachka et al. 1998), which have been previously outlined in Section 2.5 and Figure 2.12. The summary is based largely on studies of individuals indigenous to the Andes (Quechuas and Aymara) and the Himalayas (Sherpas and Tibetans), where the hypoxia response systems aim to help to compensate for oxygen shortage, initiate adjustments in lung perfusion, initiate angiogenesis in the heart, up regulating tHbmass and alter expression rates of hypoxia-sensitive genes for metabolic enzymes and metabolite transporters (Hochachka et al. 1998). Hypoxic tolerance logically occurs at 'Stage 2' of the application of altitude training model, as without a 'hypoxic dose' or stimulus ('Stage 1') there is no subsequent cascade of events. A recent review advised that that identification of the causes of individual variation and strategies to either pre-screen athletes before altitude training would be worthwhile additions to the altitude and hypoxic training literature (Sinex and Chapman 2015). With this in mind, before the 'hypoxic dose' is set for an endurance athlete, should their hypoxic tolerance be tested to indicate if their physiological characteristics involved in responses to hypoxia are likely to be more 'conservative' or 'adaptable'?

The present study used the Richalet hypoxic sensitivity test (HST) (Richalet et al. 1988; Richalet et al. 2012) to assess changes in SaO_2, VE and HVR and HCR. For acclimatization to high altitude and mountaineering performance, a strong HVR (i.e., large increase in ventilation) is thought to be a factor for success (Bourdillon et al. 2014), therefore it is possible that those athletes with a weak HVR would be unable to replicate sea level training intensities. Although the HST only examined one of the five oxygen sensing mechanisms (the carotid body), it is a key mechanism. There is a link between HVR and desaturation at altitude and the subsequent decrease in $\dot{V}O_{2max}$ at altitude (Chapman et al. 2011). Arterial oxyhemoglobin saturation in hypoxia is controlled in large part by the HVR, which is mediated primarily by the peripheral chemoreceptors in the carotid bodies (Weil et al. 1970), which was thought to have an important regulatory role in erythropoiesis (Berglund 1992). Consequently, the 'hypoxic dose' and training intensity completed at altitude are crucial to achieving successful

adaptations (i.e. increase in tHbmass or $\dot{V}O_{2max}$) as the altitude must be high enough to desaturate the athlete to a level that a substantial amount of EPO is produced, but not too high that $\dot{V}O_{2max}$ is compromised and the athlete cannot training optimally. Study 6 (Chapter 9) found that ΔSpO_2 at rest and HVR during exercise successfully predicted changes in tHbmass after LHTH. There was individual variation in each of the indices of the HST (see Figure 10.10); however with the goal of predicting an individual athlete's response to hypoxia and therefore, adapting their individual altitude training programme, mean responses are not as important.

The role of a HST would need to be used in conjunction with other measures to further optimise altitude training adaptions and improve sea level performance. As previously discussed the oxygen sensing in the kidney can be measured through the EPO response to an acute hypoxic exposure. The magnitude of the individual EPO response may ultimately determine the level of haematological adaptation (Chapman et al. 1998). Although Friedmann et al. (2005b) found there was no relationship between EPO response after 1-2 days at 2,500 m and change in tHbmass measured after altitude training, the timing of the tHbmass measurement may have been influential. The measurement was taken at 10 days post-altitude, during which a mild neocytolysis may have occurred (Rice et al. 2001b; Garvican et al. 2012) Additionally, degree of acclimatization, rate of ascent, age, obesity, and level of exertion are factors which influence the prevalence of individual cases of AMS (Schneider and Bernasch 2002). There is also evidence to suggest that with continued exposure of lowlanders to hypoxia, acclimation processes (e.g. increase the hypoxia sensitivity of the HVR and maintain erythropoiesis and up-regulation of tHbmass) can occur at different rates (Hochachka et al. 1998). Altitude training is a multi-dimensional process of which the outcomes can be enhanced with knowledge of individual hypoxic tolerance.



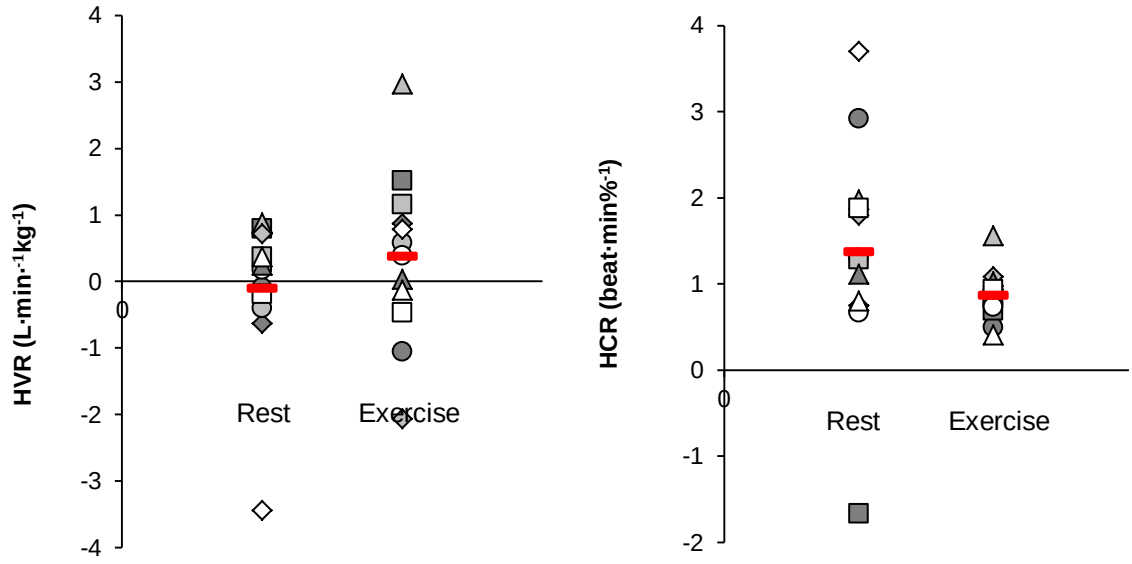


Figure 10.10: Mean (red line) and individual responses (shapes) at rest and during exercise of the calculated Richalet HST indices.

The use of a pre-screening tool to determine hypoxic tolerance would put 'Stage 2'of the basic altitude training model at 'Stage 1'. Predicting which athletes may best respond erythorpoietically and haematologically to moderate altitude exposure may be useful to support staff (Chapman et al. 2010). Currently, coaches and athletes should consider that altitude training will be effective for many, but not all participants, and perhaps experiment with altitude training at non-critical periods of the competitive season and should follow generally established recommendations, such as easing into training after traveling to higher elevations while initial acclimatization takes place (Sinex and Chapman 2015). Prior knowledge of how an athlete will respond to hypoxia may increase the chances of an effective altitude training camp, reduce the trial and error method of finding out how an athlete might respond and may reduce the time spent acclimatising during an altitude training camp.

The current focus of altitude training research is on the 'hypoxic dose' and desired sea level adaptation not the hypoxic tolerance of the individual athlete, which appears to drive the physiological response. Numerous studies are attempting to quantify the 'hypoxic dose' to maximise the sea level adaptation with little or no understanding of how individual athletes will respond to the 'hypoxic dose' itself. Pre-screening athletes to assess their tolerance to hypoxia can individualise the 'hypoxic dose' (i.e. the altitude training method), therefore increasing the chances of enhanced sea level adaptations and therefore improving race performance. Figure 10.11 introduces a proposed model of the application of altitude training for elite endurance athletes. The new model aims to first optimise the athletes response and then the altitude training method. The model is split into the same six stages, with 'Stage 1' and 'Stage 2' switched around. 'Stage 1' illustrates the concept of a hypoxic tolerance continuum. Depending on how each athlete responded to the HST will place them on the continuum and help coaches and practitioners to prescribe the correct 'hypoxic dose'. On occasions when training group are all likely to live and train at the same altitudes the model highlights the options of external strategies to optimise the acclimatisation process and subsequent adaptions. These strategies will be discussed in greater detail in the practical applications (Section 10.6) and future research (Section 10.8).

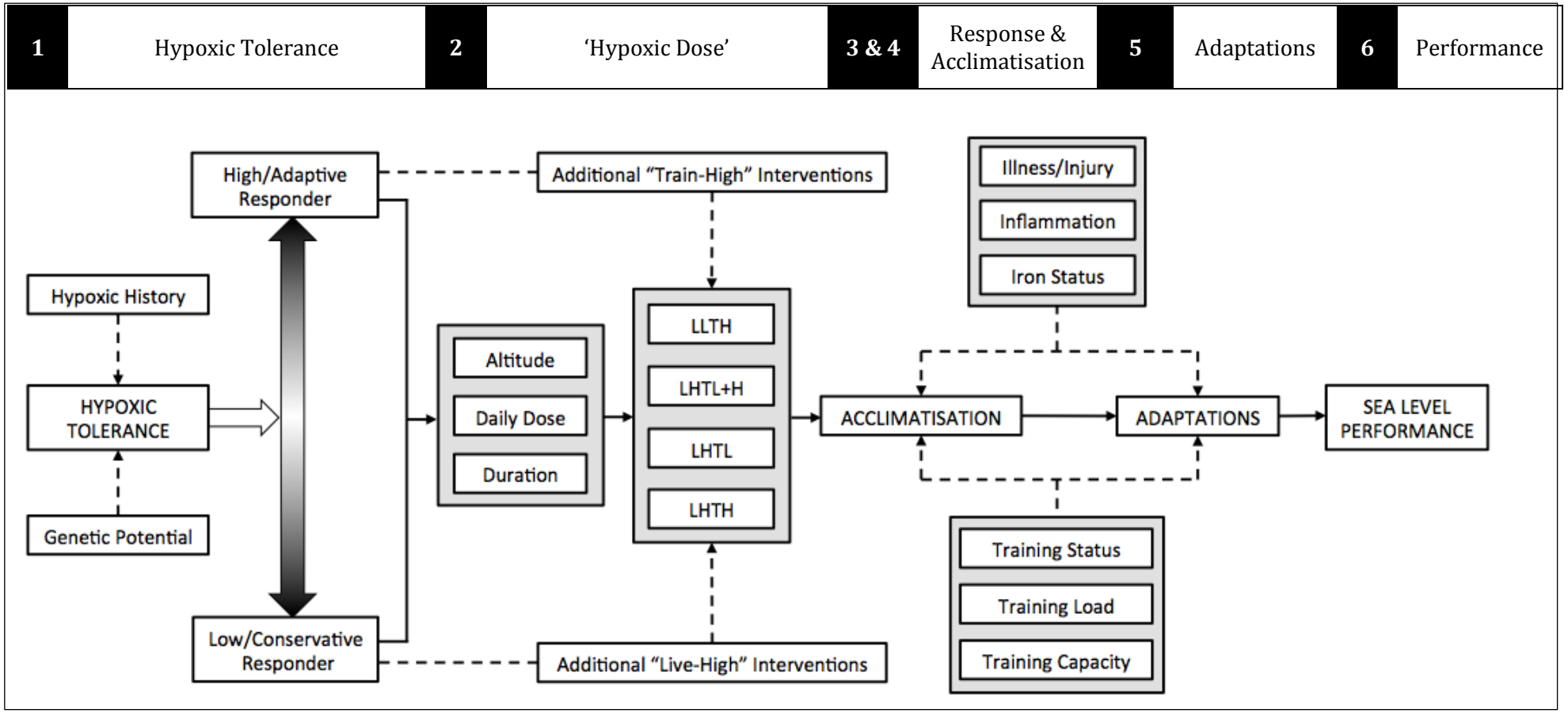


Figure 10.11: Proposed model of altitude training application. The new stages 1-6 are shown above the model. Dashed lines indicate external influences on the outcome of each stage

10.5 Performance

The present thesis did not measure race performance in Study 6 (Chapter 9) due to the time of the season and the type of training that was completed during the altitude training camp. The most common measurements during altitude training camps and research studies are tHbmass, $\dot{V}O_{2max}$ and field based performance tests. However, there are many challenges associated with assessing athlete performance after altitude training including; measurement precision, the typical error of a measure, the motivation to perform and simulating performance in the laboratory or field, compared with actual racing (Gore 2014). Using a physiological determinant of performance model significant linear regression relationships were observed for LT, LTP, $\dot{V}O_{2max}$ and $v\dot{V}O_{2max}$ versus various distance of time trial performance (Morgan et al. 1989; Noakes et al. 1990; Yoshida et al. 1993; Jones and Doust 1998; Stratton et al. 2009; McLaughlin et al. 2010). As a result it does appear feasible that performance can be predicted from measured physiological determinants of performance.

Performance in middle-distance runners has been determined using allometic models (Ingham et al. 2008). Ingham et al. (2008) identified the curvilinear power-function ratio model $(\dot{V}O_{2max} \cdot ECON^{-0.71})^{0.35}$ as the most important determinant of 800-m and 1500-m running performance, explaining 95.9% of the variance in running speed. Applying the determinants of performance measured in Study 6 (Chapter 9) to the Ingham et al. (2008) performance prediction model, LHTH improved middle-distance performance by 1.37% ± 2.61%. Within the control group using the same model a 0.64 ± 2.94 decrement in performance was found. Although this is an indirect measurement of performance, the results were comparable to previously reported performance improvements after altitude training of 1.4% (Fudge et al. 2011) and 1.9% (Saunders et al. 2009c). In track running races, Hopkins et al. (2001) demonstrated that the coefficient of variation for performance (time) is ~1%. Although it would be difficult to state that the LHTH training camp from Study 6 had improved performance explicitly, there is evidence to suggest that in some individuals this would have been the case.

Interestingly, very few studies have been able to achieve improvements in tHbmass, $\dot{V}O_{2max}$ and performance tests in the same study, often assigning no change in mean response yet not reporting a wide individual variation. However, this has not stopped research groups and physiology practitioners continually sending large groups of athletes on altitude training camps to the same altitude for the same duration of time. Again, the athletes return and the studies conclude that altitude training did not on average improve endurance performance, although individuals may have improved. As a result, despite a comprehensive physiological rationale some researchers believe any improvements in performance from an altitude training camp are as a result of a placebo effect (Siebenmann et al. 2012; Lundby et al. 2012; Lundby and Robach 2016). It is more likely that a lack in any improvement is as a result of an insufficient 'hypoxic dose', inadequate preparation, a poorly prescribed training programme or inaccurate measurements techniques. Prior knowledge of an athlete's hypoxic tolerance has the potential to target some of these areas.

10.6 Practical Applications

The studies conducted in this thesis were intended to add to the growing body of research into altitude and hypoxic training in elite endurance runners and crucially provide elite athletes and coaches with additional tools to optimise altitude training adaptions on return to sea level. Today, altitude training is regarded by many as an indispensable factor in the success of the elite endurance athlete (Flaherty et al. 2016). The use of altitude training is based on the rationale that the physiological and haematological adaptions as a result of acclimatisation can be exploited to boost sea level performance (Green 2000). However, the exact mechanisms underpinning the physiological and haematological adaptations remain to be fully elucidated, this is despite much scientific investigation (Flaherty et al. 2016). Aside from the physiological challenges associated with living and training at altitude there are logistical challenges. In reality, the modality, height and duration of altitude exposure is a trade-off between conflicting needs of athletes including safety, time efficiency, training quality, competition schedules and ability to travel (Garvican-Lewis et al. 2016b). Furthermore the costs, quality training partners, multiple altitudes in one venue, reliability of measurement techniques and the risk off illness can be added to the complications.

The present thesis has attempted to address many of these concerns. The analysis of questionnaire data from Study 1 (Chapter 4) revealed that altitude and hypoxic training is considered important to elite athletes and support staff from British Athletics. The perceptual questions raised concerns around maintaining training intensity, loss of sleep quality and homesickness from athletes and coaches alike. Crucially, for the direction of the thesis and application of any findings from the subsequent studies, the questionnaire was able to identify key challenges and questions presented by athletes and support staff. These challenges centred around: confidence in the measurement of tHbmass with the oCOR-method; the use of additional hypoxic exposures to improve the acclimatisation process and extend the competition period post-altitude; reducing the incidence of overtraining as a result of poor prescription of training load; and improved understanding of individual response to altitude and how they can be optimised. The findings of the survey ensured that each study had a practical outcome and use for elite athletes and coaches.

The questionnaire revealed that there was an awareness of the importance of oxygen carrying capacity to endurance runners and that an increase in RBCs would aid this. Study 2 was the first to establish that the inter-machine differences in the new Radiometer™ ABL80 CO-OX Flex hemoximeter were notably smaller than the old OSM3 analyser. The study also found that the required number of replicate measures to achieve an error of <1.0% was less for the new ABL80 (triplicates) than the old OSM3 (quintuplicates). Practitioners and researchers using the oCOR-method can be more confident of the replicates required to yield a low error and there is a good agreement between ABL80 analysers.

Furthermore Study 3 investigated the influence of CO bolus on the measurement of tHbmass using the oCOR-method. Using the new ABL80 hemoximeter Study 3 was the first to determine that there was a difference in tHbmass measured with the oCOR-method when administering bolus of CO. The application of a CO bolus between 0.6 and 1.0 ml·kg^{-1} produced similar results, however, using a 1.4 ml·kg^{-1} bolus tHbmass was significantly less than when using 1.0 ml·kg^{-1} (−1.4%) and 0.6 ml·kg^{-1} (−1.9%). The findings of the study are important for researchers interpreting results that have used

different bolus of CO and for research groups that might have more than one Radiometer™ hemoximeter across different sites. For the EIS and any further research in the thesis a consistent CO bolus of 1.0 ml·kg^{-1} was deemed appropriate across different sites as it provided sufficient precision and was within safety margins.

Researchers have highlighted the timing of returning to sea level for competition after altitude training as an area of importance (Chapman et al. 2014b). This area was also emphasised in the Study 1 questionnaire as a challenge for athletes and support staff. Additional hypoxic exposure had been suggested as an intervention to maintain EPO and therefore tHbmass (Pottgiesser et al. 2012), however the EPO and inflammatory response of an acute exposure to normobaric hypoxic had not been determined. Study 4 (Chapter 7) found that normobaric hypoxic exposure of two hours at a FiO_2 of 0.125-0.115 (~4,200-4,800 m) was sufficient to increase EPO production (>50%), whilst not increasing markers of inflammation (IL-6 and TNFα). The study also highlighted the large inter-individual variation in EPO production and that there was no relationship between level of desaturation and EPO production, which has previously been discussed.

Dick (1992) suggested that athletes should either compete immediately on return (Day 0) or wait for at least two weeks (Day 15 to 24); although the authors stressed that the individual response is varied even amongst athletes who work with the same coach. Additional hypoxic exposures may alleviate or delay the effects of neocytolysis by re-establishing EPO levels and therefore extending the optimal competition window. The findings of Study 4 also underlined the need to understand an athlete's individual response to hypoxia prior to attending an altitude training camp. Individual differences in EPO production play a role in determining how RBC volume and tHbmass change in response to altitude and hypoxic training (Sinex and Chapman 2015), which could be used to determine the optimal' hypoxic dose'.

Although EPO acts primarily to stimulate the production of mature RBCs, the local production of endogenous EPO in brain, in a hypoxia dependent manner, suggests that EPO may act to provide neuroprotection (Noguchi et al. 2007). It is therefore possible that EPO not only plays a role in the maintenance of tHbmass on return to sea level but also acts in a non-haematopoietic manner. Study 5 (Chapter 8) found that despite an increase in [EPO] as a result of acute hypoxia, there was no improvement in endurance performance in well-trained runners. Also the participants with the greatest increase in [EPO] did not have the greatest improvement in 10_{TT}, therefore acutely elevated endogenous [EPO] does not appear to affect middle-distance endurance performance. The findings suggest acute hypoxia prior to competing in endurance running events, would not be an appropriate pre-conditioning strategy. However, further research on the optimal 'hypoxic dose' and with a more elite athletic population is required.

The final study found that in an elite sample of middle- and long-distance runners there was considerable individual variation observed in haematological and physiological responses to living and training at moderate altitude. These findings support the need for an individualised approach to altitude training with regard to the altitude 'dose' and also an appropriate training programme for elite athletes. The present thesis investigated the use of a hypoxic sensitivity test (HST) to measure an athlete's hypoxic tolerance. The time efficient and non-invasive test has previously been shown to

predict AMS in mountaineers. Transferring this method to an elite athletic population and a walking modality, the HST a relationship between changes tHbmass and $\dot{V}O_{2max}$ after four weeks of LHTH. Specifically, HVR_r and ΔSp_r predict changes in tHbmass (R^2 = 0.708) and HCR_r predicts changes in $\dot{V}O_{2max}$ (R^2 = 0.400). The HST could provide practitioners and coaches with a novel pre-screening tool to predict the response of an athlete prior to attending an altitude training camp. This would assist coaches in setting an adequate 'hypoxic dose', establishing an appropriate acclimatisation strategy, prescribing the correct training intensities and identify which of their athletes might be at risk of returning to sea level and not improving performance.

It should be noted that although quantifying an athlete's hypoxic tolerance might increase the chances of a successful altitude training camp, there are other factors that should also be considered. Previous research has shown that optimal ferritin and iron stores are important for positive adaptions (Govus et al. 2015; Garvican-Lewis et al. 2016a), and increased incidences of injury/illness have been shown to inhibit adaptions (Bailey et al. 1998; Wachsmuth et al. 2013). Additionally, the benefit derived from altitude training depends on the balance of achieving haematological adaptations while achieving adequate training volume and intensity (Sinex and Chapman 2015). The extent to which an athlete may benefit from different methods of altitude training will differ according to both their general and specific training focus (Millet et al. 2010).

Taking these into consideration, Table 10.5 introduces some key pre-screening markers that could be utilised to increase the chance of improving sea level endurance performance after altitude training. The 'green' section on Table 10.5 and Figure 10.12 indicate the target for a higher chance of success. The topics within the table show either physiological responses or the desired physiological state that athletes should present themselves in before they attend an altitude training camp. The 'red' section indicates a lower chance of success. Success would be measured in terms of increase tHbmass, increased $\dot{V}O_{2max}$ or improved sea level race performance. Figure 10.12A illustrates what a hypothetical athlete with a low chance of success would look like. The athlete would be preparing for the altitude training camp with low ferritin, repeated illness and a poor training status. The HST would reveal that the athlete would have an increased HVR, and therefore smaller decrease in SaO_2, and also an increased HCR. This would reveal that the athlete is likely to have a conservative response to hypoxia and be more tolerant of the environmental conditions. Subsequently, the hypoxic stimulus may not be severe enough to drive hypoxia-induced adaptions. Figure 10.12B illustrates what a hypothetical athlete with a high chance of success due to a more adaptable response to hypoxia, with a blunted HVR and HCR and therefore greater reduction in SaO_2. The athlete is free from illness, has optimal ferritin stores and a more elevated erythropoietic response to altitude.

It is unlikely that the perfect scenario of athlete B would exist and as successful response to altitude training is multifaceted. Chapman et al. (1998) previously categorised athletes as either 'responders' or 'non-responders' to altitude training, however it is more likely that there is a response continuum where an athlete would sit somewhere between being a high and low responder. Being at either end of the continuum might not result in a successful altitude training camp, as a higher responder might be more sensitive when living at altitude (increased ΔSaO_2), however they may be unable to complete the high intensity training required of endurance athletes as their desaturation is

too great. In this example the athlete may require an acute intervention, for specific high intensity training sessions, to reduce the degree of desaturation that is limiting their performance at altitude (further suggestions to be made in Section 10.8). A hypoxic tolerance test, like the Richalet HST, would give an insight into the athlete's physiological response to hypoxic and therefore specific interventions could be put in place to ensure adaptions are maximised. The test would be repeated at the start and end of the season, firstly as elite athletes who have had previous hypoxic exposure better adapt to subsequent hypoxia (Pugliese et al. 2014) and secondly to assess of the seasonal altitude training strategy.

Table 10.5: Pre-screening markers prior to undertaking altitude training

		ΔEPO	Ferritin/Iron Status	ΔSaO_2 (Rest)	ΔHVR (Rest)	ΔHCR (Rest)	Training Status	Symptoms of Illness
Dark green	**Higher chance of success**	Higher	Optimal	Higher	Lower	Lower	Good	Less
Light green								
Yellow								
Orange								
Red	**Lower chance of success**	Lower	Low	Lower	Higher	Higher	Poor	More

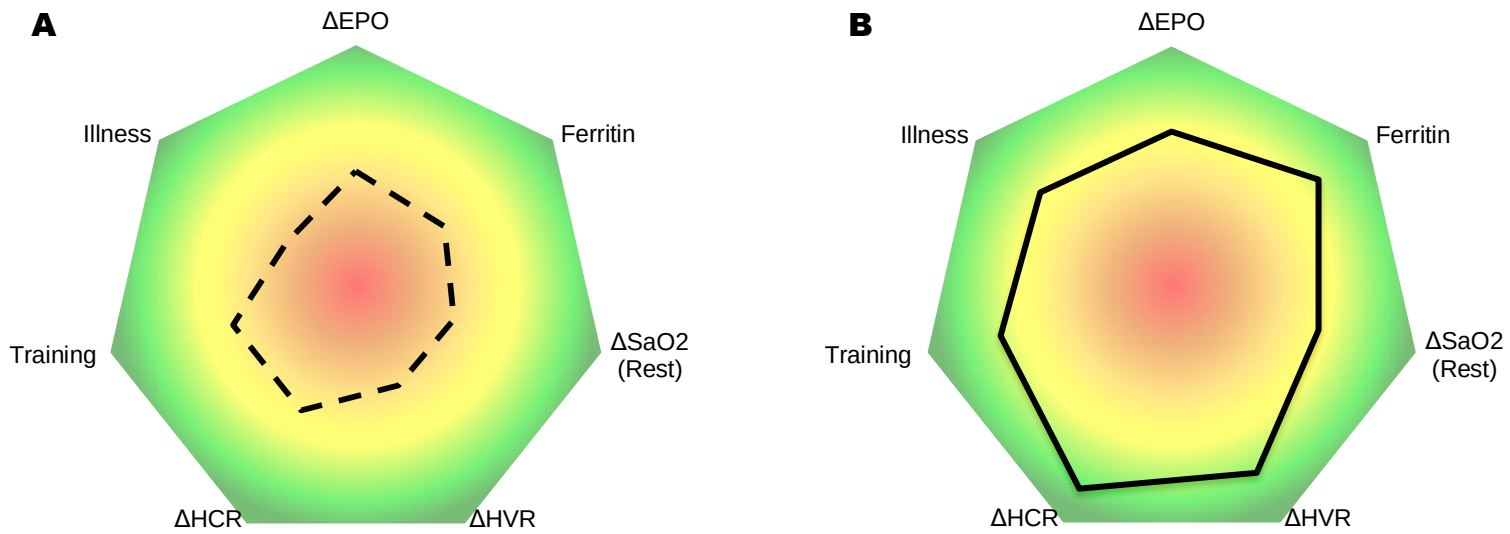


Figure 10.12: Graphical representation of pre-screening markers identified to enhance sea level performance. Chart A depicts an athlete with a lower chance of improving sea level performance. Chart B depicts an athlete with a higher chance of improving sea level performance.

10.7 Limitations

Although the present thesis has taken all possible measures to ensure the development of valid and robust tools, techniques and methods were taken throughout all experimental chapters, the findings of the thesis should be considered in the light of the following primary limitations:

- The questionnaire administered in Study 1 (Chapter 4) was distributed to elite athletes known to the British Athletics Head of Endurance. As a result the questionnaire would not have been sent to sub-elite endurance athletes who might not have had the opportunity to utilise altitude and hypoxia. The opinions of those endurance athletes who had not utilised altitude training were also not included as the sample size was not large enough to draw any meaningful conclusions. The primary aims of the study were to establish the efficacy of the methods used by elite British endurance runners and quantify the real life challenges of runners utilising altitude and hypoxia. In both incidences the input of athletes who had not used altitude training was not necessary.
- The control group in Study 6 (Chapter 9) only included five athletes compared to 12 in the LHTH group. Additionally, the control group had participants who had not represented Great Britain at age group, cross country or track level and completed less weekly mileage during their training period; as a result it would be incorrect to term them as 'elite'. However, there were no differences in physiological determinants of performance or haematological markers between both groups. As the study was not measuring race performance and their physiological characteristics were similar the control group was deemed appropriate.
- The training volume and intensity was not controlled or prescribed by the experimenters during the testing periods. Participants were instructed to maintain their normal weekly training volume. In Study 5 (Chapter 8) the participants were instructed to maintain their normal training programme during the five weeks testing period. During Study 6 (Chapter 9) the athletes were given guidance on training intensity, however as they were preparing for different competitions and coached by different coaches their training volume was not controlled. All athletes had completed a minimum of three weeks training prior to the testing period, therefore any increments in training volume were gradual and baseline fitness would have stabilised.
- Some of the studies are underpowered and as a result effect sizes (η_p^2) have been included so the reader can interpret the findings. For the latter studies of the thesis challenges in participant recruitment resulted in low sample sizes. Study 5 (Chapter 8), for example, required participants to run 5K in less than 20 min and be available for eight visits to the laboratory, with experimental trials lasting up to seven hours. Study 6 (Chapter 9) recruited participants from an invited British Athletics training camp; therefore there was a limited pool to choose from. Subsequent injury and illness to athletes resulted in a high dropout rate. Further studies with a larger sample size would provide greater statistical confidence in testing the hypotheses; extending the approach to other populations would test the generalisability of the results.

10.8 Future Directions

The application of traditional altitude training methods to an elite athletic population have received a great deal of attention (Wilber 2007b; Millet et al. 2010; Sinex and Chapman 2015). Most recently, Sinex and Chapman (2015) suggested that the identification of the causes of individual variation and strategies to either pre-screen athletes before altitude training and/or measures to turn non-responders into responders would be worthwhile additions to the altitude and hypoxic training literature. It is the opinion of the present thesis that this area is vitally important. With the understanding that altitude training, both in natural and artificial/simulated conditions, has been established as an effective means to improve oxygen transport, RBC volume and $\dot{V}O_{2max}$, given sufficiently high 'doses' of elevation and exposure duration (Sinex and Chapman 2015), attention should not only focus on how to pre-screen athletes but strategies to intervene with the physiological responses. The present thesis has established that the Richalet HST can identify which athletes are likely to improve tHbmass and $\dot{V}O_{2max}$ based on changes in SaO_2, HVR and HCR at either rest or during exercise.

Hypoxic sensitivity and the degree of an individual desaturation have been identified as primary factor that impacts performance at altitude. Running time trial performance is impaired to a greater extent among athletes who operate at a lower SaO_2 (less than approximately 92% at $\dot{V}O_{2max}$ in normoxia) compared to athletes who maintain SaO_2 (Chapman et al. 2011) and within the trained athlete population, greater declines in $\dot{V}O_{2max}$ in hypoxia have been found in athletes who substantially decrease arterial SaO_2 during high intensity exercise in normoxia (Chapman and Emery 1999). There are various strategies that have been suggested to reduce desaturation at altitude, including supplementation of nitrate (Vanhatalo et al. 2011; Masschelein et al. 2012; Puype et al. 2014; Muggeridge et al. 2014; Arnold et al. 2015; Bakker et al. 2015; Shannon et al. 2016), inspiratory muscle training (Lomax 2010) and remote ischemic preconditioning (Berger et al. 2015). For athletes who are highly responsive to hypoxia these strategies may be appropriate to use during high intensity training session, whilst still gaining the benefits of living at altitude.

The use of a hypoxic tolerance test may also indicate which athletes require a more stringent acclimatisation strategy. Pre-acclimatisation to an altitude training camp could come from a heat based protocol (White et al. 2014) or additional hypoxic exposures (Fulco et al. 2013; Chapman et al. 2013b). Additional hypoxic exposures have also been suggested during at altitude training camps to provide an added stimulus (Brocherie et al. 2015), which may be a suitable strategy for athletes with a low sensitivity to hypoxia. Finally, hypoxic exposures that acutely increase endogenous [EPO], such as those in Study 4 (Chapter 7), may provide enough additional hypoxia to prevent the sudden decrease in EPO and tHbmass when returning to sea level after altitude training. Rodríguez et al. (2000) previously found that 3 weeks of intermittent hypoxia at rest (90 min per day, three times a week, simulating an altitude of 4,000-5,000 m) led to an effective stimulation of erythropoietic adaptions. A strategy such as this implemented after an altitude training camp requires further investigation in a controlled research design. The model of altitude training application described in Figure 10.11 provides a starting point at which the responsiveness of an athlete can either determine the 'hypoxic

dose' of an altitude training camp or if this isn't possible my help to indicate alternative strategies that can be implemented by coaches and practitioners.

Further studies would include the use of the athlete '*Altitude Training Profile*' (Table 10.5 and Figure 10.12) prior to attending an altitude training camp. The study design would require participants to complete the '*Altitude Training Profile*' as part of their pre-altitude physiological testing and screening. Athletes would then be divided into a control group and intervention group based on their 'hypoxic sensitivity'. 'Hypoxic sensitivity would be determined by changes in SaO_2, HVR and HCR rest. Those athletes with a low 'hypoxic sensitivity' would undertake additional interventions (as outlined above). Pre- and post-measures of changes in $\dot{V}O_{2max}$ and tHbmass would be utilised to establish physiological and haematological adaptations to traditional LHTH altitude training.

CHAPTER 11

11 CONCLUSION

This thesis investigated the use of additional hypoxic exposure to optimise physiological and haematological adaptions to traditional LHTH altitude training. Indices of the Richalet HST have been shown to be an effective tool to predict changes in tHbmass and $\dot{V}O_{2max}$ in elite British endurance runners. The thesis initially identified that British endurance runners were completing altitude training methods according to peer reviewed research, however, both athletes and coaches revealed concerns around the individual optimisation of physiological and haematological adaptions on the return to sea level after altitude training camps. Subsequently, a series of experimental studies established the following:

- The oCOR-method is more precise when using the new Radiometer™ ABL80 CO-OX Flex hemoximeter, and requires triplicate samples to yield a measurement error of >1%. Furthermore, administering a CO bolus of 1.0 ml·kg^{-1} is sufficient to measure tHbmass accurately, therefore alleviating any concerns from athletes and coaches on the validity and reliability of the method.
- Two hours of normobaric hypoxia at a FiO_2 of 0.125-0.115 (~4,200-4,800 m), whilst at rest, is sufficient enough to increase [EPO] by on average ~50%. This increase does not cause any further stress to the individual, in the form of circulating pro-inflammatory cytokines. It is important to note that there was a large inter-individual variability in the [EPO] response, which challenges the effectiveness of utilising acute hypoxic exposures on return from altitude training to maintain haematological adaptions.
- Moreover, although two hours of normobaric hypoxic increases endogenous [EPO], this does not have an effect on endurance performance (10 min pre-loaded time trial) compared to the suppression of [EPO] with normobaric hyperoxia. The substantial decrease in [EPO] on return from altitude training has been suggested as a reason for variable endurance race performance when tHbmass and $\dot{V}O_{2max}$ have been increased. The findings of Study 5 suggest endogenous [EPO] does not affect endurance performance.
- Previous research has stated the scientific ground on which altitude training is recommended is not solid enough (Lundby et al. 2012). Four weeks of LHTH was unable to prove that endurance performance (LT, LTP and $\dot{V}O_{2max}$) or tHbmass were increased conclusively on average when compared to a control group. The study observed marked inter-individual variability in tHbmass and $\dot{V}O_{2max}$, with improvements ranging from +6.7 to -1.7% and +8.6 to -2.0%, respectively. Individual physiological responses to a HST were able to explain the changes in tHbmass and $\dot{V}O_{2max}$. The HST indices of HVR_r and ΔSp_r; and HCR_r were correlated with changes in tHbmass and $\dot{V}O_{2max}$, respectively.

Athlete hypoxic tolerance should play a pivotal role in any future prescription of LHTH altitude training. Prior knowledge of how an athlete is likely to respond can aid coaches and practitioners to

implement the correct 'hypoxic dose', design optimal training programmes and ensure the acclimatisation process is maximised. Future research should consider other additional interventions to enhance both the 'living' and 'training' element of altitude training including, ergogenic aids and additional hypoxic exposures.

CHAPTER 12

12 REFERENCES

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CHAPTER 13

13 APPENDICIES

Appendix 1:

The reliability and stability of tHbmass measured using the oCOR-method

Introduction

Athletes typically present themselves in varying states of hydration status when arriving at the laboratory for testing. The effect of hypohydration and hyperhydration on the measurement tHbmass using the oCOR-method has received little attention. Body hydration status (hypohydration or hyperhydration) can modify the vascular fluid compartments (Sawka 1992; Freund et al. 1995). Haematocrit and [Hb] are relatively crude measures of blood characteristics that can be affected by fluid shifts from hydration status, body posture and exercise mode (Harrison 1985). Both chronic and acute training and altitude exposure have been shown to shift plasma volume (PV) and as a result blood volume (BV). The effect of these shifts on the measurement of tHbmass with the oCOR-method has not been investigated. Hydration status can alter blood flow (Sawka et al. 2000), which may result in a diminished quality of the capillary blood sample used for analysis on the hemoximeter, this intern may affect the tHbmass measurement.

Most critically, tHbmass is used to accurately assess the absolute BV and PV, therefore, it is critical that the validity of the measurement is established (i.e. BV and PV are not influenced by their own acute changes). Fundamental to the correct interpretation and understanding of these compensatory mechanisms (i.e. tHbmass changes in response to acute or chronic changes in PV), is identifying the effect of PV changes on the accuracy and stability of the ΔHbCO and tHbmass measurement itself, a question not previously addressed in the literature. The purpose of the investigation was to examine the robustness of the oCOR-method when tested under different states of hydration to determine the affect this had on the measurement of tHbmass. We hypothesised that the tHbmass would be independent of the hydration status of the participant.

Methods

Participants

Seven physically active participants (6 males and 1 female) age 26 ± 4 yr, height 178 ± 7 cm, weight 75.8 ± 12.7 kg and body fat 11 ± 3 % volunteered to participate. Participants were involved in regular exercise at least 3 times per week and were required to maintain their normal moderate training regimen (Eastwood et al. 2008). Participants refrained from any strenuous activity, alcohol and caffeine during the previous 24 hours. All trials were completed between the hours of 08:00 and 10:00 to ensure that any fluctuations in body mass were limited. The trials were completed in 19 ± 5; according to Eastwood *et al* (2012a) tHbmass remains stable following 40 days of moderate physical activity. On arrival to the laboratory participants provided a urine sample, which was analysed for urine specific gravity (Refractometer; Atago, USA), urine colour [Colour Chart; Armstrong(Armstrong 2000)] and urine osmolality (Pocket PAL-OSMO Osmocheck™; Vitech Scientific Ltd, West Sussex, UK). Participants then weighed themselves nude (Adams Equipment, Model GFK 150; Milton Keynes, UK). To establish 'normal' or 'baseline' body mass nude post morning void body mass measurements were taken on five consecutive mornings. Cheuvront et al. (2004) suggested a minimum of three measurements is necessary to ascertain a valid baseline

body mass as daily variation in adults was quantified as 0.51 ± 0.2 kg. and Richardson et al. (2009a) has previously used seven consecutive measurements. Changes in body mass was calculated with the Shirreffs (2003) equation.

Experimental Design

The experimental protocol consisted of each subject visiting the laboratory on four separate occasions to complete four oCOR-breathing trials in a randomised order, in the following conditions: hypohydrated (HYPO), euhydrated (EU) and hypohydrated (HYPER). On the five consecutive days (Cheuvront et al. 2003) leading up to the testing period for the study, participants were required to weigh themselves nude to provide an average baseline body mass from which euhydration was established.

Experimental Procedures

Euhydrated Trial: Participants arrived at the laboratory and provided a urine sample and nude body mass was measured to determine euhydration. Participants were considered to be euhydrated if they conformed to 3 out of 4 following conditions: urine osmolality (Uosm) > 400 mOsm·L^{-1}, urine specific gravity (Uspg) >1.012, urine colour (Ucol) between 1-3 and body weight change ± 1% difference from average baseline body mass. If participants arrived in a dehydrated state then fluids were consumed until their urine measures conformed to the criteria.

Hypohydrated Trial: Participants arrived at the laboratory on the evening before (15 h) they were required to complete the oCOR-breathing method to complete the HYPO protocol. Participants provided a urine sample and nude body mass. After being seated for 20 min, fingertip capillary blood samples, for the measurement of [Hb] and Hct were taken. As previously suggested (Richardson et al. 2009b), participants then completed 1 h of high intensity running (~75% HR_{max}) whilst wearing warm clothing (hat, gloves, jumper, trousers). Nude body mass was measured after 60 minutes, if a minimum of 1 ± 0.2% of body mass was lost, exercise ceased; if this stipulation was not met nude body mass was measured every 15 minutes until 1 ± 0.2% of body mass was lost. All participants were then restricted from drinking fluids for the 15 hours preceding the oCOR-method. Upon arrival to the laboratory the following day a urine sample and nude body mass were recorded. If 2 ± 0.2% body mass loss occurred the oCOR-breathing procedures were followed. If the participants body mass loss

*Hyperhydrated Trial: P*articipants drank 750 mL of water throughout the evening before the test. After eating breakfast, participants then drank an additional 500 mL of water, which was finished before they arrived at the laboratory. Upon arrival at the laboratory, participants voided, before nude body mass was recorded. Uspg, Uosm and Ucol were measured from the urine sample. After being seated for 20 min, baseline fingertip capillary blood samples, for the measurement of [Hb] and Hct were taken before ingesting the high sodium beverage. Participants then completed the hyperhydration protocol, which involved consuming a high sodium beverage (10 mL·kg^{-1} body mass, high Na^+, 164mmol Na^+/L, 7.72g of sodium-citrate with 4.5g NaCl, 253 mOsm·kg^{-1}), chilled to 4°C (Sims et al. 2007). The beverage was measured into seven equal portions, of which one was consumed every 10 min and starting two hours before commencing the oCOR-method. During this drinking period, participants walked approximately 1 min every 20 min to limit venous pooling. They remained seated for 20 min before each fingertip capillary blood sampling. [Hb] and Hct was measured pre-beverage, 20 min post-beverage and one hour after consuming the high sodium beverage. The oCOR-method was then completed.

CO-rebreathing procedures

Total Hbmass, BV and PV were measured using the o-COR-method (Schmidt and Prommer 2005; Prommer and Schmidt 2007). A CO bolus of 1.0 mL·kg^{-1} body weight was administered and rebreathed for 2 min. Triplicate fingertip capillary blood samples, for determination of carboxyhaemoglobin concentration (%HbCO), were taken before the start of the rebreathing procedure and at 6 and 8 min after the CO bolus was administered. Blood

samples were measured immediately in triplicate using an ABL80 CO-OX Flex hemoximeter (Radiometer™; Copenhagen, Denmark). Total Hbmass was calculated from the mean change in HbCO before and after rebreathing CO. Under normal conditions (1.0 mL·kg^{-1} of CO; euhydrated) the TEM (± 95% confidence intervals) for tHbmass, BV and PV, were 0.8% (± 2.3%), 2.2% (± 6.0%) and 3.8% (± 10.5%), respectively.

Statistical Analysis

Data were assessed for normality and sphericity and adjusted where necessary using the Huynh-Feldt method. The reliability of the oCOR-method (using 1.0 mL·kg^{-1} of CO) was evaluated using typical error of measurement (TEM) (Hopkins 2000). Differences in tHbmass, BV, PV and ΔHbCO were evaluated with a one-way repeated measure ANOVA. For the comparison of body mass, haematological and urinary indices as a result of manipulation of hydration status, a one-way ANOVA was completed. All statistical tests were completed using SPSS Statistics 20 (International Business Machines Corp., Armonk, New York). Significance was accepted at $p < 0.05$. Values are reported as mean ± SD unless otherwise indicated.

Results

There were no differences in tHbmass [F (2, 12) = 0.852, p = 0.451)], BV [F (2, 12) = 1.267, p = 0.317)] or PV [F (2, 12) = 2.368, p = 0.136)] when the oCOR-methods were completed under different states of hydration. There were also no significant difference in Δ%HbCO [F (2, 12) = 0.294, p = 0.75)].

Table 13.1: oCOR-method measures from different bolus of CO and hydration statues

	Hydration Status			
	HYPO	EU_1	EU_2	HYPER
ΔHbCO (%)	5.9 ± 0.7	5.9 ± 0.6	5.9 ± 0.6	6.0 ± 0.5
tHbmass (g)	757 ± 135	769 ± 138	771 ± 145	768 ± 149
BV (mL)	5164 ± 925	5215 ± 798	5218 ± 849	5343 ± 929
PV (mL)	2913 ± 524	2878 ± 427	2962 ± 524	3049 ± 523

Euhydration. Participants were considered to be euhydrated as their urinary indices and body mass (see Table 13.2) were within the previously stated requirements. All participants were also within 1% of their five day pre-experimental body mass measurements prior to completing the oCOR-method.

Hypohydration. The HYPO protocol elicited a change in body mass of 2.0 ± 0.6% from pre-run to pre-oCOR-method. Table 13.2 illustrates the differences in haematological and urinary markers as a result of the HYPO protocol. Significant differences were found between mean EU and HYPO in body mass (P = 0.006), Uspg (P = 0.001), Uosm (P = 0.001) and Ucol (P = 0.001) but not Hct (P = 0.727) or [Hb] (P = 1.000). The HYPO protocol caused marginal changes in BV (0.0 ± 9.6%) and PV (0.2 ± 10.9) from pre-run to pre-oCOR-breathing, however, there was a large amount of individual variability (see Figure 13.1).

Hyperhydration. The HYPER protocol elicited a change in body mass of 0.3 ± 0.3% from pre-sodium loading to pre-oCOR-method. Table 13.2 illustrates the differences in haematological and urinary markers as a result of the HYPER protocol. The HYPER protocol also caused a 2.3 ± 5.3% increase in BV and 4.9 ± 5.7% increases in PV, again there was a large amount of individual variability (see Figure 13.1). Significant differences were found between mean EU and HYPER in Uspg (p = 0.014) and Uosm (p = 0.019) but not body mass (p = 0.055), Ucol (p = 0.071), Hct (p = 0.156) or [Hb] (p = 0.160).

Table 13.2: Mean (± SD) body mass, urinary indices and haematological markers before the dehydration and hyperhydration protocols and before the euhydrated oCOR-breathing method

	HYPO		**EU_1**	**EU_2**	**HYPER**	
	Pre-DE	*Pre-oCOR*	*Pre-oCOR*	*Pre-oCOR*	*Pre-HYP*	*Pre-oCOR*
Body Mass (kg)	75.7 ± 12.6	74.2 ± 12.6 *	75.7 ± 12.6	76.3 ± 13.0	75.9 ± 12.9	75.7 ± 12.7
Uspg	1.008 ± 0.005	1. 026 ± 0.004 *	1.004 ± 0.002	1.010 ± 0.003	1.007± 0.003	1.011 ± 0.003 *
Uosm ($mOsm \cdot L^{-1}$)	317± 192	945 ± 138 *	172 ± 70	224 ± 92	260 ± 109	404 ± 116
Ucol	3 ± 1	8 ± 1 *	1 ± 1	2 ± 1	2 ± 1	3 ± 1
Hct (%)	43.5 ± 3.0	43.4 ± 3.4	44.1 ± 3.0	43.3 ± 2.5	44.5 ± 2.5	42.9 ± 1.5
[Hb] ($g \cdot dL^{-1}$)	14.7 ± 1.0	14.7 ± 1.2	14.8 ± 0.9	14.7 ± 1.0	14.7 ± 1.0	14.3 ± 14.3

* Indicates significant difference ($p < 0.05$) from EU_1

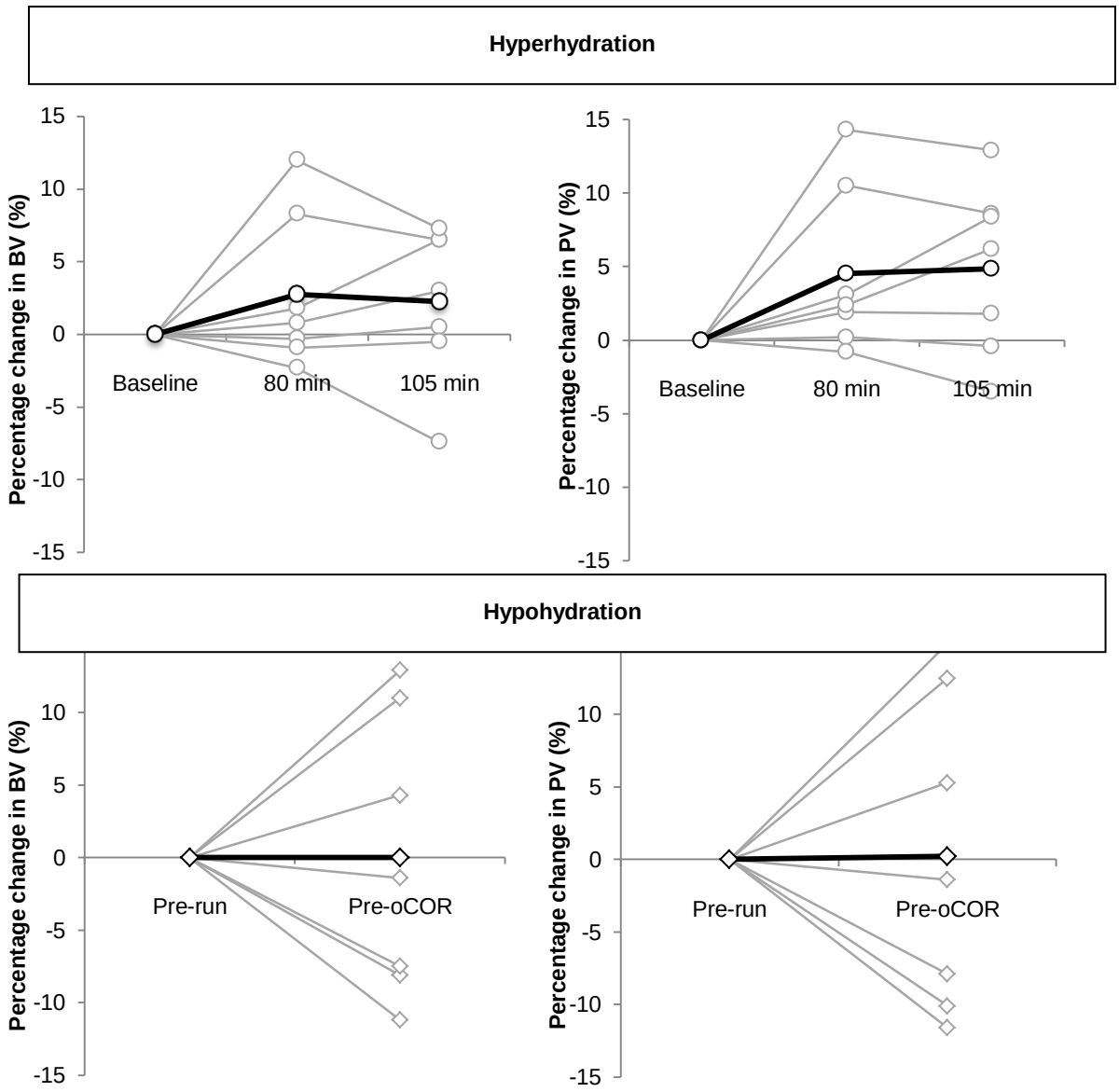

Figure 13.1: Individual variation of BV (left) and PV (right) as a result of HYPER and HYPO. Grey lines represent individual data and black lines represent mean data.

Discussion

Altitudes training camps and hypoxic exposures have been found to increase tHbmass by between 3-10%, depending on the altitude strategies used (Fudge et al. 2012). The oCOR-method is routinely used in elite sport to detect these marginal gains in tHbmass, of which the method is subject to an error of ~2.2% (Gore et al. 2005). The present studies established the stability of the measurement of tHbmass under different states of hydration. The stability of the measurement of tHbmass is not affected by the hydration status of the athlete when undertaking the oCOR-breathing method.

Blood volume represents the sum of erythrocyte volume and PV, with erythrocyte volume and PV changing independently of each other to alter BV (Sawka et al. 2000). Erythrocyte volume is regulated by the hormone EPO and PV is regulated by the extracellular fluid (ECF) volume. Both of these vascular volumes are continually changing in elite athletes and during periods of physiological testing athletes will arrive presenting different levels of erythrocytes and plasma, and therefore BV. The effect of hypohydration and hyperhydration on BV, PV and EV is well documented (Sawka et al. 2000), however, less is known about how these conditions effects

the outcome measures of the oCOR-method. It was hypothesised that hypohydrating the body to ~2% body mass loss and hyperhydrating the body according to Sims et al. (2007b) would not produce any differences in the measurement of tHbmass. No difference was found ($p > 0.05$) in tHbmass; therefore, practitioners could use the oCOR-method even if an athlete is in a state of hypohydration or hyperhydration.

Measures of blood parameters and urinary indices can offer significant information in the accurate assessment of hydration status partly due their simplicity in terms of sample collection and analysis (Kavouras 2002). Dehydration can result in an increase in osmolality and a decrease in PV (hyperosmotic hypovolaemia). Despite the present studies HYPO protocol causing significant decrements in body mass and urinary indices, suggesting that the HYPO protocol was severe enough to dehydrate the participants, there were no percentage changes in BV, PV or EV. Similarly, in a study by Heaps et al. (1994) dehydration to ~2.5% did not reduce BV. It should be noted that there was large individual variation to the HYPO protocol. The HYPO protocol incorporated sweat loss and fluid restriction to cause dehydration and body mass loss. Sweat loss will result in a reduction of total body water, which at low levels of dehydration will be primarily from the extracellular fluid compartments. With a low level of hypohydration induced in the present study is it feasible that the status of the participant (i.e. heat acclimatisation, physical training, hydration or sex) may have affected the movement of total body water and therefore PV and BV.

The HYPER protocol caused an increase in BV of ~2% and in PV of ~5%, which is in agreement with previous studies (Sims et al. 2007b; Sims et al. 2007a). Equally, the HYPER had large individual variation, which may have be due to individual responses to dietary sodium, with some individuals exhibiting a sodium sensitive phenotype and others a sodium-resistant phenotype (Jones 2004; Weinberger 2004). As a result for some participants the sodium loading had the desired effect of expanding PV and therefore BV but for others it did not. In the present study participants adopted a consistent posture for at least 20 min before blood sampling, as it is critical for a reliable measurement of Hct or [Hb] to derive tHbmass, PV and BV (Gore et al. 2005), however it is still possible that the fluctuation found could be as a result of measurement errors in Hct and [Hb] as had previously been suggested (Harrison et al. 1982). Irrespective of the large variation the measurement of tHbmass was not affected by either HYPER or HYPO suggesting that the oCOR-breathing method is robust enough to measure tHbmass even if athletes arrive for testing in either of these states of hydration.

Conclusion

When testing elite athletes on a regular basis consistency is crucial and the most accurate and safe measure would be deemed appropriate. Total Hbmass also remained stable under different states of hydration, this is essential as athletes are often tested when hypohydration or hyperhydration. The present study has reaffirmed the stability of tHbmass measured with the oCOR-method.

Appendix 2: Semi-Structured Interview Plan

Semi-structure interview with _____

EIS Physiologists working with _____ in _____

Purpose:

The purpose of this interview is to explore the altitude/hypoxic training methods that are currently taking place with _____ in _____. The findings of the interview may shape the future of my research into optimising the altitude/hypoxic training methods of elite endurance athletes. The findings of the interview will also determine the questions posed in a survey to coaches and athletes on altitude training methods.

Topics:

Perceptions: • What do coaches think about altitude • What do athletes think about altitude • Do they value altitude? • Do they believe altitude beneficial?	Current methods utilised: • Optimal altitude • Optimal duration • Daily 'dose' of altitude • Based upon research? • Frequency of exposure to altitude? • Camps • Tents
Do you have a strategy or model you use? • Pre-competition • In competition	How do you measure the success of altitude? • PB's • Medals/competition • Physiological measures
Natural vs simulated altitude? • Which do you use? • Does a combination of the two work?	Unanswered questions or themes with regards to altitude training • What would you like to know • Potential research area's • Difficulties? • What is stopping you using altitude • Do the athletes moan about altitude

Question	Check?	Notes
In your opinion, or for the coaches you work with, what would you identify as being the single most successful use of altitude/hypoxia?		
What altitude/hypoxic methods do you currently use? Optimal altitude Optimal duration Daily 'dose' of altitude		
What research have you based these methods upon? LHTL etc. Author, Study etc. Previous Experience Coaches input		
How often do the swimmers use altitude/hypoxia? How many times per year Camps or Tents Living or just sleeping		
Do you have an altitude programme or model that you follow? Yearly Pre-competition In competition		
How do you measure the success of the programme/model? PB's, Medals/competition Physiological enhancements		
What are your views on natural vs. simulated altitude? Preferred use Combination of the two		
What would you like to know about altitude/hypoxia? Future areas of research that would benefit you Problems you have encountered Unanswered questions		
Given all you've said, what are your three highest priorities for altitude/hypoxia use?		

Appendix 3: Pilot survey feedback

Athlete

Good questions, although the answer choices in some of the sections were a little confusing. It sometimes seemed like they were there to catch you out, given what you had answered to the previous question. 10 minutes to complete. Electronic version is fine; I think others would find this easy to fill in.

Practitioner

Some of the questions were tricky in that were they asking what you were currently doing, or what you would like to do? As a science practitioner in a sport dictated by coaches, your preferences are often overridden by other factors, and although you have different goals in mind you end up having to compromise. It flows fine and takes about 20-25 minutes to complete. I think online is better than a paper version and yes, with a little clarity on some of the questions they should be filled in adequately

Athlete

It would be nice not to have to answer questions I've already rules out as irrelevant -- for example if you've never been to an altitude camp it's a bit frustrating to be required to answer a question about your routine at altitude that doesn't even have an 'irrelevant' option. Ditto the repeated altitude tent questions.

Pg. 7 is confusing, since 'good' is not always at the same end of the scale, so you need to read the questions with care. There are a few double negative questions.

I feel that the questionnaire would be easier to deal with if it were separated into 'camps' and 'generators', since they're very different things. I've never been to an altitude camp, and I seem to have to repeat that very frequently, or just lie about it to get onto the next page.

It only took about 10 minutes, and most of the questions seemed relevant. On line is probably easier than doing a paper version and having to return it. I'd think it would be filled in accurately, other than some of the frustrations from its assumption that you have both been to an altitude camp and used a generator-based system.

Practitioner

It's very comprehensive. Here are my thoughts:

- It flows nicely, categories are clear
- A bit too long for an athlete.
- You assume all athletes have the same level of input as UKA to their altitude training and education e.g. rowers won't have the decision to plan it into 'their' annual plan it's a team directive. You train to be 'selected' to go.
- You use athletics terminology e.g. 'run' rather than generic terms. Rowers won't go for a 'run'!
- I'd also gather data about who uses it and how in a rehab setting – this would need to be a separate category
- Not all sports will have same EIS practitioner advising e.g. Rowing will be NGB doc/physiologists/combination or EIS and NGB
- Not all athletes will know the difference between EIS and NGB practitioners (or care so long as they get support!)

Appendix 4: Perception analysis topics, sub-topics and questions (Athlete)

Topic	Sub-topic	Question
Understanding	Understanding	I do not understand why I am on an altitude training camp/using hypoxic exposures
		My coach has explained the benefits of altitude/hypoxic training to me
		I know why I have been sent to altitude to train
	Pre-camp education	I am educated as to why I am going to altitude to train
		My coach does not give me instructions on how to alter my training at altitude/during hypoxia
		I am given advice on how to best cope with altitude/hypoxia
	Planning	I am given advice on how to best cope with altitude/hypoxia
		Altitude training/hypoxic exposure is not beneficial to my long term performance
		I will plan my altitude training camps/hypoxic exposure around competitions
Training	Training	My training programme is not affected whilst I am at altitude/exposed to hypoxia
		My quality of training improves whilst I am at altitude/exposed to hypoxia
		I cannot train as hard at altitude/hypoxia as I can at sea level
	Intensity	I find it easy to maintain my sea level training intensity at altitude/hypoxia
		I am able to produce my sea level training sessions at altitude/hypoxia
		I am unable to produce the same training volume whilst at altitude/hypoxia
	Development	I do not feel that altitude/hypoxia has developed me as an athlete
		My training has taken a backwards step since using altitude/hypoxia
		I am training harder than ever before partly as a result of altitude/hypoxic training
Lifestyle	Lifestyle	I miss my family and friends whilst I am at an altitude training camp
		I am disappointed with the facilities at the altitude training camps
		I do not miss any home comforts that I would get if I was in the UK
	Enjoyment	I do not enjoy going on the altitude training camps/hypoxic exposures
		I feel the altitude training camps/hypoxic exposures are a worthwhile experience
		I have fun with the other athletes whilst on an altitude training camp/hypoxic exposures
	Other Influences	Other athletes have told me about altitude/hypoxia
		I have heard that many of the athletes on the circuit are using altitude/hypoxia
		I am aware that other athletes are using altitude/hypoxia
Physiology	Physiology	I find it difficult to catch my breath whilst at altitude/during hypoxia
		I do not notice any difference in my breathing rate at altitude/during hypoxia
		I find myself short of breath whilst training at altitude/during hypoxia
	Sleep	When I am at altitude/exposed to hypoxia I feel I am not consuming enough good food
		My sleep pattern remain the same whilst at altitude/exposed to hypoxia
		I struggle to get to sleep when I am at altitude/exposed to hypoxia
	Nutrition	My eating habits remain the same whilst I am altitude/exposed to hypoxia
		I continue to consume a normal diet whilst at altitude/exposed to hypoxia
		When I am at altitude/exposed to hypoxia I feel I am not consuming enough good food
Performance	Performance	My performance improved as a result of altitude/hypoxia
		I ran a PB or SB as a result of altitude/hypoxia
		I have been unable to perform as well as previously as a result of altitude/hypoxia
	Return to altitude	I would be happy to altitude/hypoxia in the future
		I don't think that altitude/hypoxia have a future in my training programme
		I have felt that altitude/hypoxic training was extremely beneficial and I would like to return
	Post-Altitude	I am not given feedback by my coach as to the success of altitude/hypoxia
		The physiologist explains if I have made any improvements after altitude/hypoxia
		I undertake a series to performance measures when I return from altitude

Appendix 5: Perception analysis topics, sub-topics and questions (Support Staff)

Topic	Sub-topic	Question
Understanding	Pre-camp education	I educated my athletes as to why they are using altitude/hypoxia to train
		I do not give my athletes instructions on how to alter their training at altitude/hypoxia
		I give advice on how to best cope with altitude/hypoxia
	Understanding	I understand why my athlete is using altitude/hypoxia
		I have explained the benefits of altitude/hypoxic training to my athlete
		I know why my athlete is training at altitude/hypoxia
Training	Planning	Altitude/hypoxic training is an important part of my athletes yearly plan
		Altitude/hypoxic training is not beneficial to my athletes long term performance
		I will plan altitude training camps/hypoxic exposures around competitions
	Training	My athlete's training programme is not affected whilst using altitude/hypoxia
		My athlete's quality of training improves whilst using altitude/hypoxia
		My athlete can train as hard using altitude/hypoxia as they would at sea level
Performance	Post-Altitude	I give my athlete feedback as to the success of altitude/hypoxia
		The physiologist explains to my athlete if they have made any improvements as a result of altitude/hypoxia
		My athlete undertakes a series to performance measures after altitude/hypoxia
	Performance	My athletes performance usually improves on after using altitude/hypoxia
		My athlete usually runs a PB or SB as a result of altitude/hypoxic training
		My athlete has been unable to perform as well as usual as a result of altitude/hypoxic training
Development	Return to altitude	I would be happy for my athlete use altitude/hypoxia in the future
		I don't think that altitude/hypoxia have a future in my athletes training programme
		I have felt that altitude/hypoxic training was extremely beneficial and I would like my athlete to use it again
	Development	I do not feel that altitude/hypoxia has developed my athlete
		My athletes training has taken a backwards step since using altitude/hypoxia
		My athlete is training harder than ever before partly as a result of altitude/hypoxic training

Appendix 6: Conventional content analysis of athlete's responses to the open-ended question: In your opinion and experience, what do you consider the main benefits of using altitude/hypoxia and/or training at altitude? Please be as specific or as broad as you wish to be. If you think there is no benefit then say so.

Athlete	Tier 1	Tier 2	Tier 3	Tier 4
Benefits of altitude training	Life style	Training camp effect	Other athletes	• "Simply being in a training camp environment, surrounded by other athletes, so it was much easier to do the extra bits of work around training" • "Training with other athletes and the increased amount of rest gained is also a real benefit" • "I also enjoy training with different people which is another benefit of going on any training camp"
			• "Ability to completely focus in an environment where the whole focus is on running"	
		No distractions	• "Having a regimented routine helped with having a good lifestyle conducive to training well and hard" • "Increase in mental toughness focus on training/lifestyle more than ever no distractions" • "The benefits of altitude camps and trip are great for getting away and putting in the quality training in a different environment where you can focus on the job in hand" • "Away from distractions and can focus easier on training and recovering" • "I enjoy the opportunity of being able to train away from distractions and with like-minded athletes" • "Give a clear period of time to focus on training and put a consistent block of training in place away from normal distractions." • "Time to concentrate solely on training. No distractions"	
		Facilities	• "Similarly the facilities are really good at the venues I've been to (Iten and Font Romeu) in terms of it being very easy to go to the gym and do things that sometimes are difficult at home, either because of life getting in the way"	
		Rest and recovery	• "Also the camps ensure that you get the recovery time in that is well needed" • "As general camp note I also have more time to recover/put my feet up by being away from home life distractions"	
		Location	• "Altitude venues are typically places that you can go to focus 100% on training: Not just the actual altitude, there are usually great training venues, perfect weather, fellow elite athletes, coaches and a high level of focus that you may not always have in everyday life, especially as a non-full time athlete." • "It breaks up the winter, gets you somewhere warm and a focused environment with many other athletes to train with. Specifically in Kenya is a beautiful environment, endless trails right on your doorstep."	
	Physiological	Aerobic	Training load	• "Most importantly though, it enables me to work hard without stressing my legs as much, I get a better aerobic benefit while not running as fast for steady and long runs, whilst the same is true for sessions." • "Provides a demand and challenge on your body that I haven't experienced before" • "I can get a really good base, and good volume of training, without as much stress on the legs, and feel I can keep the quality of training up if we make sure training is adapted for altitude" • "The main benefits of altitude for me are being able to gain an additional physiological stimulus without having to increase the volume/intensity of training"
			Aerobic base	• "Develop endurance and enhance my aerobic base." • "Particularly pre-season or in the early part of a marathon build up altitude camps are great for gaining strength and base fitness" • "when I have been training at altitude, I like returning and knowing that I will get an oxygen boost, and that I can run faster at sea level" • "I find I can get a lot steadier aerobic running in, which helps build a big aerobic base… I also find a lot more time to recover in between sessions and runs"
			Haematology	• "Having the extra red blood cells is the primary benefit, as when back at sea level, your ability to train at a higher intensity allows for further adaptation and improvement to take place" • "My blood data responds well to continued exposure to altitude." • "My blood levels also always improved going from base line 44% to 48/49%." • "Increase in red blood cells on return" • "From increased blood levels are essential to improve endurance performance" • "Increasing haemoglobin mass help my performance, in particular find swimming easier post altitude as air is restricted." • "Training at altitude has meant my body has made responses to deal with the lack of oxygen, and increase red bloody cell count, meaning when I return back to sea level, more red blood cell count for more O_2."

			Other	• "It boosts fitness very quickly." • "Improved aerobic economy/ capacity. - feel better when back at sea level" • "Improved running economy and ability to burn fat as a fuel source"
		Body weight	• "I always lost a little bit more weight at altitude 0.5k- which for me was also another benefit" • "Lean body from altitude training."	
		Recovery	• "I also noticed an improvement in fitness/recovery when using the altitude tent." • "Ability to stress my physiological system in a different way with increased recovery time" • "Recovery at sea level is also enhanced"	
	Returning to sea level	Performance	Race	• "The majority of my best performances have come after a spell of high altitude training" • "I have always come away from altitude training in my best racing form." • "I also have always seen performances improve on my return from altitude." • "I have run well and secured significant PB's after using altitude and certainly feel fitter on my return from each trip"
			Training / Racing	• "These improvements can be seen both physically in the training/racing I do when I return " • "I feel that four to six months after returning from altitude I feel the benefits of altitude as a result of improved level of training/racing and also mental confidence that results." • "Possible physiological benefit for return to sea level competition" • "I feel that I am often fitter (compared with if I had not attended a camp) after altitude and my performances are better"
			Effort	• "Increased effort perception whilst at altitude also benefits performance / decreases perceived effort at sea level
			Timing	• "The last benefit is the short-term effect I get when I return; I've raced really well on returning from altitude, sometimes 2-3 days afterwards and sometimes 2-3 weeks afterwards. I always feel good at some point in the 3-4 weeks after I get back to sea level and can use that to race really well" • "When I first come down I felt great then rubbish for about 3 weeks then had my best races ever so that's how it works for me"
		Training	Intensity	• "The incredible feeling you get when you return, just gets you through that first week of acclimatisation. You feel great when you return and could run for ever." • "I do feel the body has to work harder during sessions and that you come back fitter as a result of this" • "Main benefits for me it that training is harder and more effective so "worth" more than at sea level for the same amount of mileage"
			• "…You could feel your respiratory system struggling to come to terms with the change in oxygen intake! This on return to sea level... Makes it surely only an advantage to your training programme…"	
	Yearly programme	Development	• "It's now becoming an essential training method in the yearly plan" • "Overall I think that regular altitude exposure has been a key factor in my recent successes and positive development. I have been using altitude since 2008 (18 years old) and have attended 1 or 2 camps per year. Again this may be something which I will look into further."	
		Full time	• "Opportunity to experience training as full time athlete" • "Partly it enables me to train full-time - which I'm not always able to be at sea level"	
		Additional Support	• "Opportunity to spend time with other athletes, coaches and physiologists" • "Camp attitude, positivity, constant medical is also a huge benefit."	
		Psychology	• "Psychological benefits are also immense as you feel that you have an advantage over others and that you know you have improved x amount due to the altitude compared to regular training at sea level." • "When I am going through tough time training at sea level, I often remind myself of my altitude experiences, which helps me keep my focus and pull through the sessions. This is because I tell myself, "if I can do it in Kenya, I can do it at home"." • "Mentally to know that I have gone some way to matching my African and best prepared non-African rivals when standing on the starting line of a major competition" • "Being exposed to such an elite environment also has psychological benefits, as they force you to live and train like an elite athlete for a prolonged period of time, which is then transferred into your day-to-day living when back home."	
			Physiology	• "I think the main benefit is a mental advantage if your sport scientist shows you that you have become X amount better purely down to the altitude, you will believe that you are stronger and faster than before and race more aggressively"
	Repeat Visits	• "Every time I went back to altitude I adapted quicker and gained the benefits sooner. I used the altitude tents to complement this and again helped my adaption when I'm back living at altitude"		

Appendix 7: Conventional content analysis of athlete's responses to the open-ended question: In your opinion and experience, what do you consider the main drawbacks of using altitude/hypoxia and/or training at altitude? Please be as specific or as broad as you wish to be. If you think there are no drawbacks then say so.

Athlete	Tier 1	Tier 2	Tier 3	Tier 4
Drawback of altitude training	Training	Over training	• "The possibility of overtraining at altitude. By this I mean falling into the trap of doing too much too quickly upon arrival and getting altitude sick (I have experienced this before)." • "Possibility of over-training" • "I've also almost over-trained at altitude, this wasn't a result purely of being at altitude - more because of being at a full-time training camp, but I think the altitude would have made the consequences worse if I hadn't have got away with it." • "Sometimes you can get yourself into a hole if you're not careful," • "The potential to over train if not monitored properly which can lead to under performance." • "Risk of over training and fatigue." • "Over cooking the training. Not necessarily overtraining, but being careful and not hitting sessions too hard as recovery can be effected and this can have a severe knock on effect. I have had a couple of poor races after altitude due to trying to run 'PB sessions' rather than just bagging them."	
		Undertraining	• "I do also worry that having to back off training during the acclimatisation phases and on returning to sea level can disrupt the flow of your training when compared to sea level."	
		Location	Injuries	• "I do tend to get niggles and lower leg problems from the uneven and hilly terrain that typically occurs with altitude camps." • "Different environment can be harder to adjust to if you aren't used to this (terrain, underfoot conditions = injury),"
			Racing	• "Some locations don't have flat routes for quality tempo running, particularly important to me in the lead up to races." • "Perhaps closer to the outdoor track season it would be difficult to do the more event-specific track workouts that require a proper track."
		Pacing	• "It can be difficult to set up specific race conditions and hard to sometimes know the significance of the workouts being done, especially if using a combination of altitude heights. E.g. Training for a marathon, doing long periods at marathon pace is very difficult at altitude." • "'Easy' training run/rides are difficult to come by at high altitude" • "Decreased intensity of sessions compared to at sea level may slow progression" • "I would be weary of using somewhere as high as Iten for specific marathon preparation-partly due to the rough terrain and partly due to the fact that I would struggle to run at my goal marathon pace for extended periods of time."	
		Recovery	• "Increased injury risk due to harder to recover. Lack of energy between training sessions. General higher demand on body"	
	Return to sea level	Training	• "The time taken to adjust and come down from altitude is time which could be used training at sea level" • "It reduces my power and speed for several weeks after returning." • "I have previously visited Iten and Font Romeu but found my immediate performance on return suffered. I found it difficult to replicate the intensity and speed as I would at sea level which left me feeling very "slow" when I returned" • "I didn't recover enough between training block and subsequently became heavily fatigued. (Not sure how much the altitude played a part of this)" • "I also once came back having done a lot of steady running, mileage and tempo running and was pretty sluggish on my return, this was fine for the time of the season I was in - start of cross-country"	

		Racing		• "Timing return so as not to be too tired from traveling for races" • "As I had 5 weeks to sharpen before the big races, but coming down for races 2 days after my return, and also in the track season," • "Getting the timing right so that the best performance is achieved as a result of using the altitude." • "These include short term performances upon return (sometimes hard to get right)," • "I suppose it's possible I could target a big race after altitude, and find that I don't feel amazing, because even though I always have periods where I feel brilliant in the 3-4 weeks after I get back equally I have a few days (not always the same) where I feel pretty awful. I've only ever raced badly once on my return and it wasn't a big race, but it could happen another time I'm sure." • "Getting the timing right before competition."
	Lifestyle	Location		• "Some altitude training camps are too basic and 3rd world" • "It can take a bit of getting used to. I find Boulder a lot easier to adjust to than Iten, but I have only been to Iten once so that might be partly why, and because it is higher and the lifestyle changes are bigger."
		Health risks		• "Also going to Iten there are more health risks (e.g. Malaria, parasites, stomach bugs)" • "I have also struggled with illness on my return from previous altitude trips. The dust in Iten aggravated my asthma and I also got Shingles as a result of over-training in Font Romeu" • "Travel, disease, diet, in some cases facilities"
		Work		• "Availability-i.e. Taking 3 weeks off work twice yearly difficult"
		Personal		• "No physical support from my partner, he is very important, and it's hard being away from him." • "Being away from my family" • "Going away from home." • "Long time away from home. This has an effect on finances/job/studies" • "Making communication with family difficult when I'm feeling home sick." • "Missing family & friends"
	Physiology	Low iron		• "I have also suffered from low iron levels and real increased fatigue after returning from altitude and disruption of my menstrual cycle" • "Getting low iron stores"
	Other	Tent sleeping		• "Lastly I've used a tent at sea level before and didn't sleep very well, it got really hot and I felt it was doing me more harm than good."
		Logistical	Weather	• "Travel and occasional bad weather." • "The risks of going into a climate that may not be conducive to the training that is required i.e. Snow, ice, cold air."
				• "I also found the pre summer camp in font Romeu was too cold to be able to do the faster sessions I felt I needed to do at that time in order to prepare for the summer."
			Cost	• "Cost. Can't afford to buy an altitude tent and struggle to get time off work." • "Altitude camps can be expensive" • "The cost of flights to most locations in the winter is expensive."
		None		• "I don't think there are any drawbacks really" • "However, I always believe that when I return to sea level I will feel great again, so I don't consider this a drawback" • "No drawbacks as long as you get it right and don't overcook it when you first get there." • "But I feel the benefits hugely over power the one drawback!" • "I didn't have any negative opinions on my trip" • "…so I think this outweighs the drawbacks!"

Appendix 8: Conventional content analysis of coaches' responses to the open-ended question: In your opinion and experience, what do you consider the main benefits of using altitude/hypoxia and/or training at altitude? Please be as specific or as broad as you wish to be. If you think there are no drawbacks then say so.

Coach	Tier 1	Tier 2	Tier 3
Benefits of altitude training	Training	Long term	• "I feel if any British endurance athletes wants to be world class they have to use altitude, but in saying that just a six week block is no use, I feel they need to live more up at altitude for longer period of time, and you need to keep top up trips throughout racing programme, or tents etc. used as part of this" • "Very good for facilitating volume training during winter build phase"
		Distractions	• "An opportunity to work in an environment with athletes where there are few other distractions."
		Environment	• "Training Environment/ Group Ethos/Relaxing atmosphere"
		Location	• "An altitude camp can also include challenging terrain and an all-round good training environment which are beneficial in themselves and it can be a good break from training at home and sleeping in an altitude tent." • "Excellent training environment."
	Return to sea level	Performance	• "Short-term preparation for big races (although I have used for this purpose only on a few occasions - and with mixed results thus far)." • "Every athlete has different demands and response... Testing and evaluating to clarify best options early in an athlete's development will be useful for future major champs...i.e. 2day, 10day or 18-21day down cycles."
		Physiology	• "Combination of altitude and heat exposure promotes physiological adaptations that can enhance competition performance on return to sea level"
		Training	• "Combination of altitude and heat exposure promotes physiological adaptations that can enhance competition performance on return to sea level"
	Physiology	Oxygen carrying capacity	• "By completing all their training at altitude during a camp the athletes stimulate their oxygen carrying systems to a greater adaptation which benefits them both in the short and long term. " • "Hb mass" • "Greater O2 transport"
		Aerobic	• "An increase in aerobic qualities, a chance to focus specifically on developing the aerobic system over others." • "For my athlete an 800/1500m runner training and sessions have to be carefully planned so as to get the maximum aerobic benefit whilst still maintaining muscular power." • "Primarily use altitude to develop a base and/or return to aerobic conditioning mid-season" • "Conditioning" • "The early winter camp that we use has been very successful in building a very good aerobic base, in fact in the 5 weeks last year we had the same shift in his physiology that we normally get across the whole winter" • "Primarily coaching 800m and 1500m events I have used altitude as a means to develop aerobic conditioning in athletes as a boost to training performed later at sea level. It is the quality of this later event specific training that is key to performance. Much clearer benefit for longer events."
		Recovery	• "The athlete can train hard but at a slower pace thus creating less strain on joints and better recovery." • "Recovery" • "Recovery"
		Additional	• "After trips to altitude vo2 max and running economy have measurably improved"

			• "I feel that there are several benefits in addition to the obvious physiological benefits" • "Long-term investment in improving physiological parameters." • "Muscle buffering" • "Ischaemic tolerance" • "Ability to exercise in Hypoxic conditions" • "Breath regulation"
		Placebo	• "Mental benefits to athletes who believe in what they are doing and gaining as a result of being at altitude." • "Placebo effect"
	Additional comments		• "I think it has a place but only for some athletes not all." • "Our experience has been positive, altitude is a valuable component of our programme and one which we intend to continue" • "Repeated exposures facilitate a more rapid acclimation to altitude, enabling training to be progressed more rapidly."

Appendix 9: Conventional content analysis of coaches' responses to the open-ended question: In your opinion and experience, what do you consider the main drawbacks of using altitude/hypoxia and/or training at altitude? Please be as specific or as broad as you wish to be. If you think there are no drawbacks then say so.

Coach	Tier 1	Tier 2	Tier 3
Drawback of altitude training	Training	Overtraining	• "Drawbacks is overtraining at high altitude, and recovery strategies up there"
			• "Also if the athlete's over-reach prior to or during a camp then it is very difficult for them to recover to normal while still at altitude."
		Coach	• "Athlete trains remote from coach" • "Optimal benefits come from prolonged exposure 3-4 weeks, can be difficult to support the athlete for the entire duration."
		Recovery	• "The other bad results occurred when I took a group to Font Romeu and they did their recovery runs around where they were staying in the town. This was far too hilly and the amount of downhill running resulted in some of the runners developing tight legs and not being recovered enough for sessions"
		Training camp effect	• "Training camp effect can also be an issue, adapting training to fit in with group."
		Intensity	• "Control of intensity of sessions, athlete trains at a lower intensity." • "Disrupted training when travelling overseas, and giving an 'easy week' to start the camp - some race pace efforts are missed." • "The main problem we have experienced is Coach's pushing on too hard, once they realize that the swimmer can physically complete the set work/density, and then suffering the consequences either later in the camp or upon return to sea level"
		Athlete development	• "It can be difficult for less well developed athletes to keep their faster training at a sufficiently high level. There is a greater risk of respiratory illness and poor recovery from illness in general"
	Lifestyle	Travel	• "Travel boredom " • "When I took an athlete to Boulder, came back to UK then took him to St Moritz 2 and a half weeks later for a 2 week period. It seemed that our bodies reacted to the number of changes in the timeframe and both my athlete and myself were fatigued on returning to sea level" • "Long travel to/from Iten, change in diet on arrival and return from altitude"
		Personal	• "Time away from family limitation of quality work" • "The environment and diet in Kenya are not to my athletes liking."
		Illness/Injury	• "The entire value of several weeks training can be negated by lack of enlightened attention to a niggling injury or minor illness."

			• "Risk of illness"
		Costs	• "Limited by finance and time away" • "For most of my athletes, the key issue is cost and access to Camps with adequate recourse to medical and paramedical support services" • "Costs and being away from home environment for some athletes/coaches" • "Expense logistics"
		Other	• "Boredom at remote camps"
	Physiology	Nutrition	• "Change in nutritional intake" • "GI problems on return adapting to richer foods."
		Other	• "Poorer recovery" • "Stress hormones higher" • "Variability of response"
		Sleep	• "Disturbed sleep during intermittent exposures due to the number of timing of sessions. Logistics of setting the systems up" • "Sleep quality" • "Disrupted sleep when using tents, and/or the unknown of how much acid-base buffering is impacted by sleeping in hypoxia for part of every 24 h period - it's all too easy for the Coach to blame the hypoxic tent when the swimmer is training poorly.- whether it is true or not is very difficult to gauge."
		Weight loss	• "Weight loss during camps,"
		Altitude tents	• "Altitude tents were not available then but I think using one in the period between trips would have stopped the adverse reaction."
		Physiologist	• "Monitoring responses on initial periods at altitude [access to physiologist,] hydration etc." • "Not being sure whether it is going to add value"
	Planning	Personal	• "The time that they need to be exposed to it for the purpose that we are using it at the moment means that he has to plan university work very well and be prepared to spend 4-5 weeks in Kenya."
		• "The drawbacks are that if you get it wrong, a whole seasons racing can be ruined. The coach has to really understand their individual athlete and monitor their training extremely closely." • "No1 concern is being away from the 'HPC' support services and personnel if unable to allocate into cost of camp. " • "There is a reduction in some areas of quality work which can be done, but this is easily tempered by appropriate planning. I feel that the maximum benefit can be gained from exposure three times a year and as the group do not have access to this we are unable to gain the full benefits of altitude, making a single camp less beneficial, finding a system that met the needs of the athletes."	
		Returning to sea level	• "There is always some risk in planning when to race after an altitude camp and it is not always possible to plan things precisely around all the athletes' races." • "Some unknowns around multiple usage and how long to leave between"

Appendix 10: Letter and information emailed to athletes, coaches and practitioners to explain the rational of the survey.

Dear coach/athlete,

As an individual involved in high performance sport we value your thoughts. The EIS physiology team is conducting a review of altitude training methods and strategies, through an on-line survey to be sent out to athletes, coaches and practitioners (link is below).

This review is designed to investigate altitude/hypoxic training methods used by elite endurance athletes and their coaches, and to explore perceptions and opinions of the broader altitude/hypoxic training area. Central to this review is finding out exactly what facilities, equipment and technical support athletes currently have access to (or will want future access to), and as to how these strategies are utilised. The outcomes of the investigation will shape the direction, strategy and investment from the EIS, as well as helping establish optimal altitude/hypoxic training methods, and bringing guidance to how we can best support these individuals and National Governing Bodies.

The survey takes approximately 10-15 minutes to complete, is anonymous, and for ease and simplicity, it is embedded in a web page which can be completed on any device with internet access, including an iPhone, Blackberry, iPad etc. If you are able to complete the questionnaire it would be greatly appreciated. The survey is online at the following link: English Institute of Sport Altitude and Hypoxia survey.

By completing the questionnaire you are consenting to your results being used as part of Gareth Turner's PhD thesis. In accordance with the Data Protection Act 1988 all data obtained from the survey will remain anonymous, be saved and stored on an encrypted hard drive. Data will be retained until the conclusion of the research project and if necessary used to be written up in a peer-reviewed journal once the thesis is completed in 2014. The findings of the survey will also be used for a chapter in the PhD and for an internal report for the EIS.

Many thanks for taking the time to read this email. Please feel free to forward this onto other athletes, coaches and practitioners (medical and science) that you feel would be interested in contributing.

Sincerely and regards,

Gareth Turner, Jamie Pringle, Barry Fudge, Stephen Ingham

Gareth Turner | English Institute of Sport | PhD Research Studentship (Hypoxic and Altitude Training in Endurance Athletes) | University of Brighton
Jamie Pringle, PhD | Lead Physiologist (Central Region) English Institute of Sport | British Triathlon
Barry Fudge, PhD | Senior Physiologist, English Institute of Sport | UK Athletics
Stephen Ingham, PhD | Head of Physiology, English Institute of Sport

English Institute of Sport, East Midlands Region, EIS/Loughborough Performance Centre, Loughborough University, Loughborough, Leicestershire, LE11 3TU

Appendix 11: Evidence of communication with the relevant ethics committees, informed consent and subject information document

Study	Title	Level of Ethics	Approved by
1	Altitude and hypoxic training in endurance running: Perceptions of elite athletes and support staff	University of Brighton Tier 1 Ethics Approval	Supervisors
2	Comparison of total haemoglobin mass measured with the optimised carbon monoxide rebreathing method using different radiometer™ hemoximeter	University of Brighton Tier 2 Ethics Approval - FREGC/35/12	College Research Ethics Committee
3	The influence of carbon monoxide bolus on the measurement of total haemoglobin mass using the optimized co-rebreathing method	University of Brighton Tier 2 Ethics Approval - FREGC/35/12	College Research Ethics Committee
4	Influence of endogenous erythropoietin on time trial performance in endurance runners	University of Brighton Tier 2 Ethics Approval - ESREGC/33/14	College Research Ethics Committee
5	Time course of endogenous erythropoietin, IL-6 and TNFα in response to acute hypoxic exposures	Loughborough University Ethics Approval - R14-P104	HPSC Research Committee
6	Predicting an athlete's physiological and haematological response to classic altitude training using hypoxic sensitivity methods	University of Brighton Tier 2 Ethics Approval - ESREGC/33/14	College Research Ethics Committee

Further email evidence of Education and Sport Faculty Research Ethics and Governance Committee research proposals and correspondence of acceptance can be provided upon request. These include Control of Substances Hazardous to Health (COSHH) proposals and participant information sheets. Evidence of medical questionnaires and informed consent are provided below.

University of Brighton

CHELSEA SCHOOL OF SPORT

MEDICAL QUESTIONNAIRE

Name: .....
Age: .....

Are you in good health ? Yes/No
If no, please explain:
How would you describe your present level of activity?
Vigorous: < once per month
once per month
2-3 times per week
4-5 times per week
> 5 times per week

Have you suffered from a serious illness or accident ?Yes/No
If yes, please give particulars:

Do you suffer, or have you ever suffered from:

Asthma	Yes	No
Diabetes	Yes	No
Bronchitis	Yes	No
Epilepsy	Yes	No
High blood pressure	Yes	No

Are you currently taking medication ? Yes/No
If yes, please give particulars:

Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? Yes/No

If yes, please give particulars:

Have you, or are you presently taking part in any other laboratory experiment ? Yes/No

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to participate in the study if they:

- are unsure of the test protocol and the possible risks and discomforts designated on the subject information sheet;
- the answers given on the medical questionnaire or informed consent form do not meet the required criteria;
- are pregnant;
- have been admitted to the hospital due to any form of carbon monoxide poisoning in the last 6 months
- have suspended training due to joint or muscle injury;
- have been verified, or documented as having any blood carried infections (Hepatitis, HIV), are diabetic or obese (Body Mass Index>30), or have a known history of haematological, cardiac, respiratory, or renal disease;
- have a known history of mountain sickness or have experienced gastrointestinal problems, severe headache or faintness/dizziness when entering altitudes greater than 1500m;
- have a known history of heat stroke or other heat induced illness;
- have symptoms of nausea or light-headedness to needles, probes or other medical-type equipment;
- have known anal problems such haemorrhoids, fissures and anal bleeding; and
- have been to altitudes above 1,600 m or travelled via aircraft in the 8 wks leading up to data collection.

DECLARATION

I ..... hereby volunteer to be a participant in experiments/investigations during the period commencing 2012

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has fully informed me of, and I have understood, the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Signature of Subject ..... Date.....

Signature of Experimenter ..... Date .....

University of Brighton

SCHOOL OF SPORT AND SERVICE MANAGEMENT

PHYSIOLOGY INFORMED CONSENT

Title of Study: The precision of the carbon monoxide rebreathing method to determine haemoglobin mass

DECLARATION

I …… hereby volunteer to take part in this research, which is to investigate **the effect of hydration status and carbon monoxide bolus on the CO-rebreathing method**.

The principal investigator has explained to my satisfaction the purpose of the experiment and the possible risks involved.

I have had the principles and the procedure explained to me and I have also read the participant information sheet. I understand the principles and procedures fully.

I am aware that I will be required to:

- Exercise for one hour 15 hours before the testing procedure on 5 occasions
- Consume a high sodium beverage on one occasion
- Complete the CO-rebreathing method on 6 separate occasions
- Have fingertip blood samples taken

I understand how the data collected will be used, and that any confidential information will normally be seen only by the researchers and will not be revealed to anyone else

I understand that I am free to withdraw from the investigation at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

I agree that should I withdraw from the study, the data collected up to that point might be used by the researcher for the purposes described in the information sheet.

I understand that the results of the study can be made known to me.

Furthermore, if I am a student, I am aware that taking part, or not taking part in this experiment, will neither be detrimental to, nor further my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the investigators regarding safety, subject only to my right to withdraw declared above.

Signature of Subject: .....

Date: .....

Signature of Investigator: .....

Date: .....