Physiological and performance adaptations to altitude and hypoxic training.

Submitted by Ben Alaric Holliss to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Sport and Health Sciences in April 2014.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: …………………………………………………………..

ACKNOWLEDGEMENTS

This doctoral thesis would not have been achievable without the support from numerous colleagues, friends and family, to all of whom I am incredibly grateful.

With the experience and expertise of my two academic supervisors, Professor Andrew Jones and Dr Charles Pedlar, I was in safe hands from day one. This supervisory team helped me to achieve the balance of scientific rigour and applied practice, and I am grateful to them for their substantial role in shaping both my academic and professional career. Andy and Charlie – thank you for all your support throughout the ups and downs – without your joint approach I would have been quite lost.

My thanks go to the examiners, Professor Yannis Pitsiladis and Dr Stephen Bailey – I really appreciated the time that you put into this process – this thesis is undoubtedly a better read following your recommendations. Thank you.

In addition to my formal supervisors, I received a great deal of help and support from a number of fellow scientists at the University of Exeter and St Mary's University College. In particular, thanks to Dr Anni Vanhatalo, Dr Jon Fulford, James Kelly, Len Parker Simpson, Dr Weerapong "Tony" Chidnok, Katie Lansley and Richard Burden – you all selflessly volunteered your time and energy to ensure that the various experiments within this thesis came to fruition – none of this would have been possible without you – thank you so much!

In addition to those physically involved in this work, my thanks go to Professor Craig Williams, Dr Richard Winsley and all the other academic staff at the School of Sport and Health Sciences, St Luke's College, for going above and beyond the call of duty during my years at the University of Exeter – you all make Exeter, and in particular St Luke's, a wonderful place in which to learn.

Importantly, I would like to specifically thank all those who acted as participants in the enclosed experimental chapters – whether you are an elite athlete or, like me, an averagely fit individual, you have all taught me a great deal – thank you for your time and energy!

I have received much support from my past and present employers during this doctoral research programme – the English Institute of Sport, and British

As well as it having been an academic journey, this PhD has also been a physical one – with writing having been undertaken in trains rumbling through Thai paddy fields, campsites and beaches in Tuscany, mountain huts in Slovenia, Colorado's Garden of the Gods, the Spanish Sierra Nevada and French Pyrenean mountains, hotel rooms overlooking the Monte Carlo Mediterranean coastline, tea shops in the Peak District and Dartmoor, and more swimming pools than I care to remember. All in all, this thesis has been quite a journey – I hope it provides a thought-provoking read to any interested scientist.

ABSTRACT

INTRODUCTION: There have been few well controlled altitude and hypoxic training studies to date. This thesis investigated the effects of altitude and (sham controlled) intermittent hypoxic training (IHT) on exercise capacity, and the associated physiological adaptations. METHODS: Chapter 3 investigated how living and training at 2320 m or at sea level affected total haemoglobin mass (tHb) and race performance in highly trained swimmers. Chapter 4 investigated how IHT or normoxic training affected cardiopulmonary variables and the incremental exercise limit of tolerance (T-Lim), in highly trained runners. Chapter 5 investigated how single-legged IHT or normoxic training affected phosphorus-31 nuclear magnetic resonance spectroscopy assessed muscle energetics. RESULTS: In Chapter 3, tHb increased significantly more after altitude (+0.6 ± 0.4 g·kg^{-1}, or +4.4 ± 3.2%) than after sea level (+0.03 ± 0.1 g·kg^{-1}, or +0.3 ± 1.0%), but the changes in swimming performances were not different between groups, and there were no correlations between tHb and performance changes. In Chapter 4, submaximal heart rate in normoxia decreased significantly more after IHT than after normoxic training (-5 ± 5 vs. -1 ± 5 b·min^{-1}), and submaximal $\dot{V}O_2$ in hypoxia significantly decreased, only after IHT. T-Lim in hypoxia significantly increased post-IHT, but there were no between group differences. In Chapter 5, the phosphocreatine recovery time constant was speeded significantly more in the IHT compared to the normoxic trained leg, when tested in hypoxia (-25 ± 8% vs. -13 ± 6%), but not in normoxia (-16 ± 15% vs. -9 ± 10%). CONCLUSIONS: Altitude training likely increases tHb, but this is not necessarily associated with improved athletic performance. IHT may induce other non-haematological adaptations; potentially an enhanced skeletal muscle oxidative capacity, but evidence for exercise capacity gains is lacking. The precise underlying causes for these adaptations require further investigation, as does any translation to athletic performance.

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DEFINITIONS

LH+TH   Live high, train high (both at physical altitude)

LH+TH+TL   Live high (at physical altitude), train high and low

LH+TL   Live high (at physical altitude), train low for all exercise

Simulated LH+TL Live high (at simulated altitude), train low for all exercise

IHT   Intermittent hypoxic training

IHE   Intermittent hypoxic exposure (at rest)

F_IO_2 Fraction of inspired oxygen

Hb   Haemoglobin

tHb   Total haemoglobin mass

RBC   Red blood cell

RCM   Red (blood) cell mass

BV   Total blood volume

RCV   Red (blood) cell volume

PV   Plasma volume

Epo   Erythropoietin

rHuEpo   Recombinant human erythropoietin

^{31}P-MRS   Phosphorus-31 nuclear magnetic resonance spectroscopy

PCr   Phosphocreatine

[PCr]-τ   Phosphocreatine recovery time constant

INTRODUCTION

Since the discovery of oxygen (O_2) by the combined work of Joseph Priestly, Carl Wilhelm Scheele, and Antoine Laurent Lavoisier, during 1772-1777 (Jones & Poole, 2005), the role of this chemical element in metabolism has been the topic of much attention. One of the most prominent realisations has been the dependence of O_2 by 'respiratory pigments' (McMunn, 1884), later termed cytochromes (Keilin, 1925), in the energy-producing biochemical process of oxidative phosphorylation (Keilin, 1966). While much of the focus has been on the fundamental biochemistry of these cellular pathways (see Papa *et al.* (2012) for a recent review), *in vivo* research into their functional significance has also markedly progressed.

The maximal capacity of oxidative phosphorylation has most commonly been (indirectly) quantified as the maximum pulmonary O_2 uptake ($\dot{V}O_2$max), which is determined by: a) factors that determine the rate of O_2 transport from the environment to the mitochondria; and b) biochemical factors that are unrelated to O_2 transport, involving substrate availability and enzymatic activity (Wagner, 1996). In the exercising human, $\dot{V}O_2$max is primarily limited by the (mainly diffusive) delivery of O_2 from the lungs to the exercising muscles (Bassett & Howley, 2000). As such, given that in endurance athletic sporting endeavours, a high $\dot{V}O_2$max is a key determinant of performance (Jones, 2006), strategies aiming to enhance O_2 delivery have been adopted by athletes worldwide. When O_2 availability is particularly low, for example upon exposure to altitude, it's delivery to the exercising skeletal musculature becomes even more of a prominent endurance performance limitation (Wehrlin & Hallen, 2006).

At the Mexico City 1968 Olympics, held at an elevation of 2420 m, it was overwhelmingly clear that those athletes who competed in endurance events, who had undertaken a phase of altitude pre-acclimatisation were the most successful (Waddell, 1970). As such, it was acknowledged that some form of adaptation that was beneficial to endurance performance at altitude had occurred (Stiles, 1974). Ever since, altitude training has been regularly used by athletes worldwide, sometimes as pre-acclimatisation for subsequent competition at altitude, but more often with the aim of enhancing subsequent sea level performances (Millet *et al.*, 2010). The evidence to date is not

conclusive, but a comprehensive meta-analysis by Bonetti & Hopkins (2009) reported that when appropriately conducted, various altitude and hypoxic strategies result in maximal aerobic power output enhancements in non-elite athletes. Moreover, acclimatisation to physical altitude was reported to elicit maximal aerobic power output improvements of up to 4% in the elite athlete (Bonetti & Hopkins, 2009). Accordingly, there is general agreement that if well timed and practiced, altitude and hypoxic training strategies can result in small, but potentially meaningful performance gains, and that the magnitude of these gains likely depends on the participants' baseline fitness status.

In this respect, it is important to differentiate between fitness, training and competitive status. Throughout this thesis, participants of the discussed investigations are broadly separated into three categories: i) untrained (those who may undertake physical exercise in their leisure time, up to 4 sessions·week^{-1}, but do not participate in a structured exercise training program); ii) moderately trained (those who routinely undertake structured exercise training and competition for a specific sport, and may take part in competitions up to a regional level); or iii) highly trained (those who train specifically for a single sport, at a national or international level).

Altitude and hypoxic interventions remain popular, both in terms of their use in the purported enhancement of athletic performance, and also in terms of the understanding of the physiological processes that underpin any such exercise capacity changes. While much has been learnt, a great deal of uncertainty still remains with regard to the wide array of physiological adaptations that take place; in particular the effects of altitude and hypoxia in highly trained athletes.

Contribution made by papers in the context of the approved field of study:

Chapter 3 is currently being peer-reviewed in an international journal.

Chapter 4 was accepted for publication in the Journal of Strength and Conditioning Research in January 2014 (Holliss *et al.*, 2014).

Chapter 5 was accepted for publication in the Journal of Applied Physiology in January 2013 (Holliss *et al.*, 2013) – see Appendix 8.

CHAPTER 1:

Literature Review and PhD Rationale

1.1 Physical effects of altitude and hypoxia

At sea level, barometric pressure is ~760 mmHg (normobaria), and the fraction of inspired O_2 (F_IO_2) is ~0.209, which results in a partial pressure of inspired O_2 (P_IO_2) of ~149 mmHg, in accordance with the below equation (Wilber, 2004):

$P_IO_2 = (P - 47 \text{ mmHg}) \cdot F_IO_2$

Where: P_IO_2 = partial pressure of inspired O_2, P = barometric pressure, 47 mmHg = the reduction in partial pressure of O_2 (PO_2) due to water vapour in the upper airways, and F_IO_2 = the fraction of inspired O_2.

By the time the O_2 has diffused from the alveoli into the pulmonary arteries, this initial P_IO_2 of 149 mmHg has been reduced due to gas mixing to a partial pressure of arterial O_2 (P_aO_2) of ~100 mmHg. This differs at altitude, as although the F_IO_2 remains at a constant 0.209, barometric pressure decreases with increasing elevation above sea level, termed hypobaria. For example, at 2320 m above sea level, barometric pressure is reduced to ~582 mmHg, which results in a P_IO_2 of ~112 mmHg, and a P_aO_2 of ~64 mmHg (Wilber, 2004). This P_IO_2 reduction of ~75% can also be achieved at sea level by inhaling a normobaric hypoxic inspirate; in this instance air with an F_IO_2 of 0.157, thereby simulating altitude, at a barometric pressure of ~760 mmHg.

Whether there are significant differences in the physiological effects of hypobaric hypoxia (i.e. physical altitude) or normobaric hypoxia (i.e. simulated altitude) is the topic of much debate (Millet *et al.*, 2012a; Millet *et al.*, 2012b; Millet *et al.*, 2012c; Mounier & Brugniaux, 2012a; Mounier & Brugniaux, 2012b; Mounier & Brugniaux, 2012c), but in both circumstances, there is a reduced P_IO_2. This causes reduced diffusion differences at each step of the 'O$_2$ cascade', so the O_2 diffusion rate is consistently lower then when exposed to normoxia, thus causing a reduction in arterial O_2 content (C_aO_2) and a state of biological hypoxia (Wilber, 2004).

1.2   Physiological effects of altitude and hypoxia

Human skeletal muscle mitochondrial oxidative capacity has been shown to exceed O_2 delivery capacity by approximately 50% (Boushel *et al.*, 2011). This is not the case for the majority of other mammals, as most are thought to conform to biological symmorphosis; that is, O_2 delivery being matched to mitochondrial oxidative capacity (Taylor & Weibel, 1981). Thus O_2 delivery capacity is one of the key limiting factors to human skeletal muscle maximal oxidative capacity (Wagner, 1996), which is further exaggerated in situations of biological hypoxia (Wagner, 2010). Under such circumstances, the human body adapts via a wide array of physiological mechanisms, to enhance O_2 transport and/or utilisation, with the goal of re-establishing sea level C_aO_2.

1.2.1   Hypoxia sensing and cardiorespiratory responses

As the circulation time from the lungs to the carotid artery is ~6 s at rest, shorter than that to central areas (Prabhakar, 2001), the lower PO_2 is first detected by chemoreceptors in the carotid bodies, as they can respond before central areas are even exposed (Prabhakar, 2001; Smith *et al.*, 2006). Subsequent carotid body driven alterations in respiratory control provide the 'first defence' against biological hypoxia, resulting in elevated minute ventilation ($\dot{V}_E$). This phenomenon is quantifiable as the hypoxic ventilatory response (HVR) (Mou *et al.*, 1995), which acutely increases the alveolar air O_2 tension (P_AO_2), despite an unchanged hypoxic stimulus (Richard & Koehle, 2012; Townsend *et al.*, 2005). In addition to the P_AO_2 increase, the elevated $\dot{V}_E$ also causes respiratory alkalosis, due to the increased CO_2 exhalation leading to a reduced alveolar air CO_2 tension (P_ACO_2), and therefore a reduced arterial blood CO_2 tension (P_aCO_2). Renal reabsorption of filtered bicarbonate (HCO_3^-) decreases, with the net result being an increased HCO_3^- excretion, in an attempt to match the reduction in PCO_2 and H^+ (West *et al.*, 2013). Nevertheless, with $\dot{V}_E$ remaining elevated, the peripheral and central chemoreceptors' sensitivity to CO_2 increases during the first few hours and days of acclimatisation to hypoxia, so this respiratory alkalosis does not acutely attenuate the hyperventilation stimulus (West *et al.*, 2013). Thus, stimulation of the respiratory centre is maintained, termed the hypercapnic ventilatory response (HCVR) (Ainslie *et al.*, 2003).

A further near-immediate response to the hypoxic stimulus is the hypoxic pulmonary vasoconstriction response (HPVR), whereby the reduced P_AO_2 and mixed venous blood O_2 tension ($P_{mv}O_2$) causes vasoconstriction of the pulmonary arterial and arteriole smooth muscle (al-Tinawi *et al.*, 1994). The outcome is a redirection of blood flow to allow greater perfusion to better oxygenated areas of the lungs, for example in patients suffering from chronic obstructive pulmonary disease (Voelkel *et al.*, 2013). However, at altitude the entire respiratory tract is hypoxic, so an overall increase in pulmonary vascular resistance occurs, resulting in pulmonary hypertension (Sylvester *et al.*, 2012). This, in combination with enhanced sympathetic activity causing increased cardiac output ($\dot{Q}$) and systemic blood pressure (BP), enables enhanced blood flow and thus O_2 delivery to active tissues (Hainsworth *et al.*, 2007).

While the fast-acting HVR and the HCVR are effective at increasing $\dot{V}_E$, and the increases in $\dot{Q}$ and BP due to the HPVR and elevated sympathetic activity are effective at increasing blood flow to active tissues, biological hypoxia still persists during sustained exposure to moderate to high altitudes, and thus other chronic adaptations are required in order to increase C_aO_2.

1.2.2   Hypoxia-inducible factors

Hypoxia-inducible factors (HIF's) are transcription factors that respond to changes in PO_2. In the presence of plentiful O_2, hypoxia-inducible factor 1α (HIF-1α) is subjected to rapid proteasomal hydroxylation by prolyl hydroxylase domain proteins (PHD's) and factor-inhibiting HIF (FIH), both involving O_2-dependant enzymatic reactions (Torbett & Friedman, 2009). As such, this ubiquitination and degradation of HIF-1α is inhibited by biological hypoxia (Wang *et al.*, 1995). In these instances, HIF-1α stabilises and dimerises with the constitutively expressed hypoxia-inducible factor 1β (HIF-1β), to form the stable heterodimeric protein, hypoxia-inducible factor 1 (HIF-1) (Kaelin & Ratcliffe, 2008). HIF-1 drives expression of a multitude of hypoxia-sensitive genes that encode numerous growth factors and hormones, for example; vascular endothelial growth factor (VEGF), hepcidin, transferrin, erythropoietin (Epo), and peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) (Hochachka & Rupert, 2003; Semenza, 2009). As such, the sustained HIF-1

activity during periods of biological hypoxia results in a range of adaptive responses that enhance O_2 delivery and/or utilisation (Wang *et al.*, 1995).

1.2.3   High altitude populations

To understand the numerous physiological adaptations resulting from biological hypoxia and the associated HIF-1 transcriptional activity, it is useful to consider how high altitude populations have adapted to offset the stresses of chronic living in hypobaric hypoxic environments. Today's residents of the Tibetan and Andean highlands (3,500-4,500 m) are descendants of colonisers who arrived at most 25,000 and 11,000 years ago, respectively. Interestingly, they have adapted to the hypoxic stress via different mechanisms, thus providing researchers with an informative 'natural experiment' (Beall, 2007). The main similarity is that both Tibetan and Andean highland natives have normal basal metabolic rates, implying that their functional adaptations do not require a greater O_2 cost (Mazess *et al.*, 1969). However, compared to Andeans, Tibetans have elevated resting ventilatory rates and a more vigorous response to O_2 fluctuations, as depicted by a greater HVR (Beall, 2007). To compensate, even when controlling for confounding factors such as iron deficiency, abnormal haemoglobin, and differing recruitment and analytical methods, male Andeans' haemoglobin concentrations ([Hb]) are significantly greater than that of Tibetans (19.2 g/dL vs. 15.6 g/dL, respectively) (Beall *et al.*, 1998). This results in Andean's having a C_aO_2, as assessed at 4000 m, of ~24 mLO$_2$/100mL blood, which is higher than the C_aO_2 of ~21 mLO$_2$/100mL reported for high altitude natives living at sea level (Beall, 2006). In comparison, even when accounting for the Tibetan's increased ventilation, C_aO_2 is still low (~19 mLO$_2$/100mL), as neither arterial O_2 saturation (S_aO_2) or [Hb] are higher than their sea level counterparts (Beall, 2006; Beall *et al.*, 1997). As such, Andeans are thought to have overcompensated in terms of resting C_aO_2 measurements, whereas Tibetans have undercompensated, so are considered to be in a state of consistent hypoxia; Tibetan's must have adapted via different mechanisms (Beall, 2007). Indeed, the vasodilator, nitric oxide (NO) is known to be higher in the exhaled air of Tibetans, with the outcome being a higher blood flow through the pulmonary vasculature, and thus a faster rate of O_2 delivery into the pulmonary capillaries (Beall *et al.*, 2001). Similarly, Tibetans have a denser capillary network, allowing improved tissue perfusion compared with Andeans

(Hoppeler *et al.*, 2003). Finally, there is evidence that Tibetans have fewer mitochondria than Andeans, and because there is no less an oxidative demand (Mazess *et al.*, 1969), some authors argue that Tibetans' mitochondria have evolved to use O_2 more efficiently (Kayser *et al.*, 1996).

It is not the purpose of this text to further discuss such evolutionary physiology in altitude dwellers, but it is clear from these between-population differences that a range of adaptations to biological hypoxia occur, likely mediated to a great extent by innate genetic variation (Zoll *et al.*, 2006). Therefore, in any case of adaptations to hypoxia, there will likely be significant inter-individual variation.

1.2.4   Haematological adaptations

In instances of biological hypoxia, assuming that renal O_2 utilisation does not decrease, and that haemodynamics and renal blood flow are relatively constant, the C_aO_2 reduction causes a decrease in renal PO_2 (Ge *et al.*, 2002). This hypoxic state immediately causes sustained HIF appearance and transcriptional activity, with one outcome being elevated synthesis and release of the glycoprotein hormone Epo by peritubular capillary lining cells of the renal cortex (Klein *et al.*, 2009). This causes detectable increases in blood Epo concentration ([Epo]) within 90-120 min of environmental hypoxic exposure (Eckardt *et al.*, 1989), which peaks within 24-48 h and then declines thereafter (Abbrecht & Littell, 1972; Garvican *et al.*, 2012). In this manner, the kidneys are the predominant Epo producer during adulthood, although the liver is also a site of Epo synthesis during foetal development (Eckardt *et al.*, 1992) and in extreme hypoxia (Tan *et al.*, 1992).

This increase in Epo production leads to elevated circulating [Epo], detected by burst-forming unit-erythroids (BFU-E), located within the bone marrow. These are the most immature cells restricted specifically to the erythroid cell line, they are highly proliferative, and give rise to many colony-forming unit-erythroid cells (CFU-E) (Stephenson *et al.*, 1971). The raised [Epo] inhibits apoptosis in these erythroid progenitor cells, which then differentiate into proerythroblasts, which are also highly sensitive to Epo (Koury & Bondurant, 1990). Further differentiation occurs, involving accumulation of haemoglobin (Hb), decreases in cell size, nuclear condensation and, finally, enucleation, before the erythroblasts

are released into the bloodstream as reticulocytes (Gifford *et al.*, 2006). These immature cells circulate for 1-2 d, as they finally differentiate into mature fully functional RBC's, which have a lifespan of ~120 d (Gifford *et al.*, 2006).

In conjunction with enhanced erythropoiesis, the importance of plasma volume (PV) contraction on total blood volume and C_aO_2 maintenance has been demonstrated. After eight healthy but untrained males underwent five weeks of oral iron supplementation and recombinant human Epo (rHuEpo) treatment at sea level, Lundby *et al.* (2007b) reported a significant ~11% [Hb] increase, and a significant ~16% C_aO_2 increase. Interestingly, these authors estimated that this C_aO_2 increase was 62% due to an increase in total haemoglobin mass (tHb), and 38% due to a reduction in PV, with the contribution from PV estimated at 54% after 11 weeks (Lundby *et al.*, 2007b). Furthermore, this research group have also investigated the chronology of such adaptations: showing that elevated [Epo] firstly decreases the renal proximal tubular reabsorption rate and the glomerular filtration rate, causing a PV reduction, and then augments erythropoiesis, leading to tHb increases (Olsen *et al.*, 2011). Furthermore, upon cessation of rHuEpo treatment, [Hb] is rapidly normalised, firstly due to a restoration in PV, then red cell mass (RCM) contractions (Lundby *et al.*, 2008a; Olsen *et al.*, 2011), likely as a result of Epo decreasing below a threshold, and in doing so initiating neocytolysis (Alfrey *et al.*, 1997). Although these studies induced higher circulating [Epo] than would naturally occur at moderate altitude, and participants were non-athletic, the mechanistic findings remain valid, as a PV contraction during sustained exposure to moderate altitude in trained athletes is a well know phenomenon (Dill *et al.*, 1974).

In this manner, hypoxia induced elevations in circulating [Epo] result in significant haemoconcentration and a greater number of circulating RBC's, which collectively increase the O_2 carrying capacity of the blood, bringing C_aO_2 back towards normative sea level values (Beall, 2007). This (haematological) downstream HIF-1 transcription pathway is the one to have received the most attention in the field of hypoxia research, largely due to: i) the aforementioned higher [Hb] in Andean highland natives compared to their lowland counterparts (Arnaud *et al.*, 1979; Beall *et al.*, 1998); ii) the obvious effects of rHuEpo administration and/or RBC infusions on exercise performance (Berglund &

Ekblom, 1991; Brien *et al.*, 1989); and iii) the relative ease of tHb and/or RCM quantification (Schmidt & Prommer, 2005).

In accordance with these mechanisms, the traditionally held theory is that exposure to 2000-2500 m altitude for at least 22 $h \cdot d^{-1}$, for four weeks or longer, results in a significant tHb increase in already well trained athletes (Levine & Stray-Gundersen, 1997; Wilber *et al.*, 2007). Considering that total blood volume (BV) remains relatively stable during this phase of haemoconcentration and erythropoiesis (Lundby *et al.*, 2007b), the elevated circulating Hb causes an enhanced C_aO_2. This results in a greater O_2 diffusion gradient at the active tissues and a greater arteriovenous O_2 difference, causing an increased $\dot{V}O_2$max, in accordance with the Fick principle (Mathews & Singh, 2008).

In their classic study, Levine & Stray-Gundersen (1997) assessed haematological changes via the Evans blue dye dilution technique before and after 39 moderately trained middle distance runners spent four weeks' living and training at either 2500 m altitude or at sea level. These authors reported significant increases in red cell volume (RCV) (~9%) and $\dot{V}O_2$max (~5%) in the altitude groups, but not in the sea level control group (Levine & Stray-Gundersen, 1997), providing the basis for numerous further investigations.

However, around this time issues with the quantification of erythropoiesis confounded results of many altitude training studies, for example Ingjer & Myhre (1992) drew firm conclusions regarding haematological adaptations in highly-trained cross-country skiers who underwent three weeks of altitude training, solely based on RBC concentration indices, e.g. [Hb] and haematocrit (HCT). Even though these authors attempted to control for the effects of exercise, nutrition, posture and environmental temperature on PV (Kargotich *et al.*, 1998), it is clear that significant haemoconcentration does occur in response to an altitude sojourn and/or increase in Epo. Indeed, even when these factors are carefully controlled, there is significant haemoconcentration, for example, a 9% PV reduction after 9 d at 4100 m altitude (Sawka *et al.*, 1996). Lundby & Robach (2010) specifically investigated this notion by injecting eight untrained participants with Epo for 15 weeks, and found that while the treatment increased [Hb] in all participants, this increase was mainly due to an increased tHb in approximately half the participants, but in the remaining participants the

change was due to a decreased PV. Regardless, simple RBC concentration indices have commonly been used to quantify erythropoietic changes, which is problematic given simultaneous effects on PV. A solution to this problem is the use of dilution techniques to estimate RCM or tHb, using Evans blue dye (el-Sayed *et al.*, 1995), radio-labelled chromium (Wennesland *et al.*, 1959), or carbon monoxide (CO) gas rebreathing. The CO-rebreathing technique was first described by Myhre *et al.* (1968), and later optimised by Burge & Skinner (1995) and Schmidt & Prommer (2005). Only studies having used these techniques to quantify haematological change should be considered in relation to altitude acclimatisation, due to their greatly enhanced reliability and validity in comparison to RBC concentration indices.

Heinicke *et al.* (2005) found that when six highly trained male biathletes lived at 2050 m for three weeks, [Epo] significantly increased until day four, and by the end of the three weeks, tHb had significantly increased, by ~9%. A similar RCV increase of ~9% was reported by Robach *et al.* (2006b) after highly trained swimmers underwent just two weeks of living in normobaric hypoxia for 16 $h \cdot d^{-1}$, while training at sea level. This considerable RCV increase is somewhat surprising, given the shorter intervention duration compared to many other similar studies, and the 8 $h \cdot d^{-1}$ spent in normoxia. This may be at least partially explained by the RCV assessment methods being somewhat erroneous, for example one participant appeared to have experienced a RCV increase of ~32%, which would seem physiologically unlikely (Gore & Hahn, 2005). Nevertheless, the reported RCV increases may have been in part due to the relatively high simulated altitude of 2500-3000 m. Recently, Wachsmuth *et al.* (2013) performed one of the very few studies to have monitored haematological adaptations in response to repeated altitude sojourns. 21 highly trained swimmers lived and trained for 3-4 weeks at 2320 m, three times over two years, and overall there was a mean ~7% tHb increase, which showed reasonable repeatability (Wachsmuth *et al.*, 2013).

While results from these empirical studies provide important detail regarding individual responses and underlying adaptations to altitude and hypoxia, by combining results from numerous studies, some of the 'noise' from inter-individual participant variation is reduced, and overall outcomes become clearer. In a review and analysis of the available literature, Schmidt & Prommer

(2008) estimated that on average, tHb gains of 6.5% occur when sea level residents are exposed to ≥2500 m altitude, for ≥14 $h \cdot d^{-1}$, for ≥3 weeks. However, with the optimised CO-rebreathing technique for tHb estimation only being publicised in 2005 (Schmidt & Prommer, 2005), as well as additional methodological developments since (Prommer & Schmidt, 2007; Steiner & Wehrlin, 2011; Ulrich *et al.*, 2011), many of the more recent investigations into the haematological effects of altitude using these properly controlled methods in highly trained participants are showing more modest gains.

Garvican *et al.* (2012) investigated Epo and tHb changes in 13 highly trained cyclists who performed three weeks of living at 2760 m and training between 1000-3000 m for 2-6 $h \cdot d^{-1}$ (n = 8) or at sea level (n = 5). After 2 d at altitude, Epo had increased by 64 ± 19%, and after 19 d at altitude, tHb had increased on average by 3.5%; whereas Epo and tHb remained unchanged in the sea level group (Garvican *et al.*, 2012). This rather modest tHb increase is a deviation from much of the previously published literature, especially given that participants spent 18-22 $h \cdot d^{-1}$ at this relatively high altitude of 2760 m, in addition to the training at 1000-3000 m (Garvican *et al.*, 2012). Similarly, Gough *et al.* (2012) found that regardless of whether elite swimmers underwent three weeks of resting in normobaric hypoxia for 14 $h \cdot d^{-1}$ (altitude simulation of ~3000 m), or living and training at a physical altitude of 2135-2320 m, on average both interventions induced ~4% tHb increases. And finally, Robertson *et al.* (2010b) found that in middle distance runners, three weeks of living and intermittently training at a normobaric hypoxic altitude simulation of ~2200 m elicited significant tHb increases, on average +3.6% (Robertson *et al.*, 2010b).

All the available literature suggests that hypoxia induced Epo increases are highly variable between individuals (Chapman *et al.*, 1998). Furthermore, Friedmann *et al.* (2005) found that acute changes in Epo are not necessarily related to tHb changes. Indeed, even when Epo is elevated in response to brief repeated hypoxic exposures, increased red blood cell (RBC) production does not necessarily follow (Ashenden *et al.*, 2000; Gore *et al.*, 2006). This is likely because the 'hypoxic dose' of ≥2500 m altitude, for ≥14 $h \cdot d^{-1}$, for ≥3 weeks, as outlined by Schmidt and Prommer (2008), is not achieved by such intermittent exposures. The 'hypoxic dose' is defined as the exposure duration in terms of

the number of $h \cdot d^{-1}$ and the number of total days, as well as the altitude and/or hypoxia severity (Wilber *et al.*, 2007).

Gore *et al.* (1998) investigated the effects of 31 d at 2690 m altitude on erythropoiesis and performance in eight elite male cyclists. While there was a significant post-altitude improvement in cycling performance, these authors did not observe any changes in Epo or tHb, and suggested that this was likely due to initial tHb values being close to their natural physiological limits (Gore *et al.*, 1998). More recently, Siebenmann *et al.* (2012) also found no significant erythropoietic enhancement in a group of 10 elite cyclists or triathletes, after they spent 16 $h \cdot d^{-1}$ in normobaric hypoxia (altitude simulation of ~3000 m), for four weeks, compared to a normoxic control group. Both the interventions employed by Gore *et al.* (1998) and Siebenmann *et al.* (2012) surpassed the suggested required hypoxic dose (Schmidt & Prommer, 2008), and in both studies, participants were highly trained, elite athletes – all actively competing at national or international levels. As such, it may be that athletes with an already high tHb are less susceptible to further erythropoiesis, although caution must be adopted here, as it is clear that a course of rHuEpo treatment does induce significant erythropoiesis (Durussel *et al.*, 2013). Therefore it is expected that erythropoiesis would ensue at some magnitude of altitude, but potentially not in response to the moderate altitudes that athletes tend to be exposed to (Wilber *et al.*, 2007), especially if baseline tHb is already high (Robach & Lundby, 2012).

Furthermore, Pottgiesser *et al.* (2009) investigated whether three weeks of living and training at 1816 m resulted in enhanced erythropoiesis in seven elite male cyclists. After the altitude sojourn there were no significant differences in tHb (mean 2.6% increase), RCV, PV, BV, or any RBC concentration variables. Similarly, Saunders *et al.* (2004) found that after 10 elite male endurance runners underwent four weeks of living for 9-12 $h \cdot night^{-1}$ for 5 $nights \cdot week^{-1}$, at a simulated altitude of 2000-3100 m, tHb remained statistically unchanged (mean 1.6% increase), and was not different to normoxic control group participants (Saunders *et al.*, 2004). Participants in both these studies were highly trained, elite athletes; German national cycling team under 23 y category in Pottgiesser *et al.* (2009), and national or international runners with a mean $\dot{V}O_2max$ of 73.0 ± 2.8 $mL \cdot kg^{-1} \cdot min^{-1}$ in Saunders *et al.* (2004). As such, again, it

could be argued that the already high pre-intervention tHb values left minimal scope for further enhancement (Gore *et al.*, 1998). However, it seems more likely that the lack of tHb change was due to an insufficient hypoxic dose. Indeed, Pottgiesser and colleagues (2009) broadly concurred with Schmidt and Prommer (2008) in their conclusion that an altitude at or above 2100 m is required to elicit significant erythropoietic responses.

In another more recent comprehensive meta-analysis, Rasmussen *et al.* (2013) only assessed studies that directly measured blood compartments, then carried out a Monte Carlo Simulation on the pooled data set. These authors estimated that to elicit RCV increases of 5% and 10%, athletes would need to be exposed for 24 h·d^{-1} to 2500 m altitude for 29 and 37 d, respectively (Rasmussen *et al.*, 2013). These hypoxic doses are considerably greater than previous guidelines (Schmidt & Prommer, 2008; Wilber *et al.*, 2007), and are in disagreement with Levine & Stray-Gundersen (1997), who reported a 9% RCV gain after 28 d at 2500 m. Part of this discrepancy likely comes from the significantly worse coefficient of variation (CV) for RCV assessed via the Evans blue dye dilution technique (~7%), compared to tHb or RCM assessed via CO-rebreathing (~2%) (Gore *et al.*, 2005). As such, Gore and colleagues recently published a similar meta-analysis, but which only included studies that directly measured blood compartments via the optimised CO-rebreathing technique (Gore *et al.*, 2013). According to this latter meta-analysis and associated linear mixed modelling, a tHb increase of 5% on average requires ~20 d continuous altitude exposure (Gore *et al.*, 2013), i.e. substantially less than the 29 d for a 5% RCV increase, as estimated by Rasmussen *et al.* (2013).

In summary, although significant haematological adaptations to altitude and hypoxia are reported, the expected magnitude of any such change is still in question, and is perhaps less than reported by early studies, e.g. Levine & Stray-Gundersen (1997). This is likely because: i) RBC concentration indices have been replaced with more advanced haematological assessment techniques that have inherently lower error margins; ii) modern techniques are generally less complex and invasive (e.g. radio-labelled chromium vs. CO-rebreathing), thus allowing more laboratories worldwide to undertake the technique, and more frequent assessments to be carried out, providing an enhanced overall understanding of haematological changes; and iii) instead of

investigating haematological responses in collegiate level and/or moderately trained individuals (Levine & Stray-Gundersen, 1997), much of the recent research has assessed highly trained athletes (Garvican *et al.*, 2012; Gore *et al.*, 1998; Pottgiesser *et al.*, 2009; Siebenmann *et al.*, 2012), who unsurprisingly seem to show less exaggerated haematological gains. Furthermore, cases where significant haematological adaptations are not apparent, but sea level athletic performance subsequently improves (Gore *et al.*, 1998), and where tHb increases without a concomitant $\dot{V}O_2$max increase (Robach *et al.*, 2012), suggest that factors other than erythropoiesis transpire.

1.2.5 Non-haematological adaptations

In an elegant study by Garvican *et al.* (2011), 11 highly trained female cyclists spent 16 h·d^{-1} living in normobaric hypoxia (altitude simulation of ~3000 m), while training in normobaric normoxia. After 14 nights, participants were split into either a response group (n = 5), in which tHb continued to be free to adapt, or a clamp group (n = 6), in which tHb was maintained at baseline levels by means of phlebotomy. At the end of the intervention, tHb increased by ~5% in the response group, and after blocking a ~4.5% increase in the clamp group, tHb was unchanged. While endurance cycling performance improved in the response group, but not in the clamp group, the (significant) improvements in anaerobic cycling performance were not different between groups (Garvican *et al.*, 2011). This confirms that a) as per the traditional view, changes in tHb influence the aerobic contribution to high intensity exercise, and b) accelerated erythropoiesis is not the sole mechanism by which hypoxic exposure at rest improves athletic performance (Garvican *et al.*, 2011). Therefore, other, non-haematological, adaptations to altitude and/or hypoxia may be equally if not more important than accelerated erythropoiesis (Gore & Hopkins, 2005). Such adaptations, also mediated by HIF-1, may include enhanced angiogenesis (Berra *et al.*, 2000), mitochondrial biogenesis (Geiser *et al.*, 2001; Vogt *et al.*, 2001), H^+ buffering capacity (Mizuno *et al.*, 1990) and glycolytic capacity (Semenza, 2009), as well as other non-erythropoietic effects of Epo, such as an improved perception of physical condition (Ninot *et al.*, 2006) and an increased mitochondrial oxidative phosphorylation capacity (Plenge *et al.*, 2012).

1.2.5.1   Angiogenesis

Angiogenesis, the expansion of the capillary network, enhances tissue vascularity, thereby improving exchange properties between blood and tissue, most notably concerning O_2, CO_2 and glucose (Prior *et al.*, 2004). The hypoxic induced enhanced HIF-1 expression is thought to activate multiple genes that encode angiogenic growth factors and cytokines, the most established and seemingly potent of which is VEGF (Semenza, 2009). Indeed, VEGF messenger ribonucleic acid (mRNA) levels are dramatically increased within a few hours of exposing cell cultures to hypoxia, and return to baseline levels when normoxic conditions are resumed (Shweiki *et al.*, 1992).

In an early hypoxic training study, eight competitive cyclists trained for 60-90 min, 4-5 sessions·week^{-1}, for 3-4 weeks, in either normobaric normoxia, or at a hypobaric hypoxic altitude simulation of ~2300 m (Terrados *et al.*, 1988). Although muscle biopsy derived morphology results did not show any statistically significant differences, probably due to the small participant numbers (n = 4 in each group) and the varied individual responses, the number of capillaries per unit muscle area increased in all participants in the hypoxic group (mean change +15%), whereas this was not the case in the normoxic group (mean change -8%) (Terrados *et al.*, 1988). Similarly, in a larger scale, thorough training study, Vogt *et al.* (2001) had a group of 30 untrained males undertake five cycling sessions·week^{-1} for a total of six weeks, either at a high or low exercise intensity, in either normobaric normoxia or a normobaric hypoxic altitude simulation of 3850 m. In the high intensity hypoxic group, VEGF mRNA increased significantly, as did capillary density, assessed via muscle biopsy, but this was not the case in the low intensity hypoxic group, or in the normoxic groups. This is perhaps due to a combination of the (low) O_2 sensing, as well as the high intensity exercise causing shear and/or mechanical stress, which are known to result in exaggerated angiogenesis (Prior *et al.*, 2004). While these results are informative, unfortunately Vogt *et al.* (2001) did not report the capillary density per unit cross-sectional area, so it is difficult to estimate to what extent overall muscle vascularisation was altered. Nevertheless, these links between hypoxia, HIF-1, VEGF mRNA and enhanced angiogenesis have been repeatedly shown *in vitro* (Forsythe *et al.*, 1996; Ladoux & Frelin, 1993; Terrados *et al.*, 1988; Vogt *et al.*, 2001). *In vivo* results are sparse though, and

the physiological implications of increased VEGF mRNA and capillary density has not been thoroughly investigated.

Phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P-MRS) can accurately estimate the phosphocreatine (PCr) concentration within skeletal muscle tissue (McMahon & Jenkins, 2002). Assuming that prior exercise intensity has not been severe enough to induce a reduction in intracellular pH, the rate of PCr resynthesis, quantified as the PCr recovery time constant ([PCr]-т), is considered to be a robust measure of mitochondrial respiration, thus representing maximal skeletal muscle oxidative capacity (Conley *et al.*, 2000; McMahon & Jenkins, 2002; Taylor *et al.*, 1983). Using ^{31}P-MRS, Haseler *et al.* (1999) showed that [PCr]-т was slower when O_2 availability was restricted, and in contrast, [PCr]-т was quicker in hyperoxia. Thus it appears that O_2 delivery to the active tissue is inadequate in environmental hypoxia (albeit using a rather extreme simulated altitude of approximately 6000 m by Haseler and colleagues), so it would seem logical that, in agreement with Vogt *et al.* (2001), after a sufficient hypoxic dose, angiogenesis would occur to enhance O_2 delivery. In contrast, a similar study to that by Vogt and colleagues did not find a significant increase in VEGF mRNA after six weeks' exercise training at a simulated altitude of ~3000 m, even though HIF-1α mRNA was significantly elevated (Zoll *et al.*, 2006). Whether the augmenting effects of endurance exercise training on angiogenesis is further enhanced by hypoxic exposure, either at rest or during exercise, remains to be conclusively demonstrated.

1.2.5.2 Mitochondrial biogenesis

As well as enhanced O_2 transport via erythropoietic and vascular pathways, the observed increases in V̇O$_2$max after hypoxic exposures could also be at least partially attributable to an increased O_2 utilisation by the active tissue.

In the first study to assess muscle energetic responses to short-term hypoxic training, Kuno *et al.* (1994) reported a significantly shorter [PCr]-т, assessed via ^{31}P-MRS, after a hypobaric hypoxic training intervention, indicative of an enhanced maximal oxidative capacity. The hypoxic dose of 60 min, twice per day, for four consecutive days (at a simulated altitude of ~2000 m), was well below any threshold by which erythropoiesis might occur (Rasmussen *et al.*, 2013). As such, this greater oxidative capacity was likely caused by either

enhanced angiogenesis, thereby increasing O_2 transport to the mitochondria, or alternatively a greater utilisation of O_2 by the mitochondria themselves, due to an increase in the total number or mass of skeletal muscle mitochondria. These suppositions were highlighted in the aforementioned investigation by Vogt *et al.* (2001), who found that subsarcolemmal mitochondrial density significantly increased after six weeks of high- and low-intensity hypoxic training (+130% and +100%, respectively), but not after high- or low-intensity normoxic training (+1% and -13%, respectively). Comparable results have been confirmed by the same Swiss research group, using a similar intermittent hypoxic training intervention (Geiser *et al.*, 2001). In addition, PGC-1α, which stimulates mitochondrial biogenesis in response to physical exercise (Jornayvaz & Shulman, 2010), is likely more active in response to hypoxic exercise (Geiser *et al.*, 2001; Vogt *et al.*, 2001), and the subsequent increase in mitochondrial O_2 consumption has been shown to result in further intracellular hypoxia (O'Hagan *et al.*, 2009).

However, the physiological effects of sustained living in hypoxia are not consistent. Robach *et al.* (2012) found that *in vitro* muscle maximal oxidative capacity was not significantly improved after endurance trained athletes were exposed to a normobaric hypoxic altitude simulation of ~3000 m for ≥16 $h \cdot d^{-1}$ for four weeks, in comparison to a double blinded normoxic control group. In this circumstance, however, resting in hypoxia has been shown to result in enhanced muscle blood flow, that serves to counteract C_aO_2 reductions (Heinonen *et al.*, 2010), and therefore total muscle O_2 delivery and muscle PO_2 remain largely unaltered (Calbet *et al.*, 2009; DeLorey *et al.*, 2004). As such, it is then unsurprising that only resting in moderate hypoxia does not enhance skeletal muscle oxidative morphology (Robach *et al.*, 2012); instead, an intensive (O_2 demanding) exercise stimulus is likely required.

In support of this view, Levett and colleagues from the Caudwell Xtreme Everest Research Group assessed muscle biopsy ultrastructure of lowland natives during a 10 week sojourn to high altitudes, including a Mount Everest summit attempt for some participants (Levett *et al.*, 2011). After 19 d at 5300 m, there were no detectable changes in mitochondrial content, however, after 66 d at or above 6400 m, total mitochondria and subsarcolemmal mitochondria densities significantly decreased, on average by -21% and -73%, respectively.

Body mass also significantly decreased, on average by 9%, indicating a degree of lean muscle atrophy, but as mitochondria densities were calculated per muscle fibre volume, anthropometric changes would not have confounded these morphological results. Additionally, PGC-1α levels had significantly decreased, on average by -35%, suggestive of a down-regulation of mitochondrial biogenesis during this sustained 66 d hypoxic sojourn (Levett *et al.*, 2011). Whilst this is a high altitude study, key findings may be applicable to the responses in athletes to moderate altitude.

As Hochachka & Somero (2002) summarise, "hypo-metabolism is a widespread hypoxia defence response" (p.136), so it is perhaps unsurprising that prolonged exposure to extremely hypoxic conditions down-regulates mitochondrial biogenesis (Hoppeler *et al.*, 2003; Levett *et al.*, 2011; Robin *et al.*, 1984). However, the effects of short-term and/or intermittent exposures to moderate degrees of hypoxia on mitochondrial biogenesis are not yet fully understood.

1.2.5.3 H^+ buffering capacity

Although a variety of other influences exist, the accumulation of H^+ during high intensity exercise poses one limiting factor to maintained skeletal muscle force production (Allen *et al.*, 2008). Therefore, an improved capacity to buffer H^+ has been proposed to enhance high intensity exercise performance (Messonnier *et al.*, 2007). Furthermore, it is logical to postulate that the relatively reduced oxidative and greater anaerobic contribution during exercise in hypoxia compared to normoxia (Balsom *et al.*, 1994), may elicit specific anaerobic adaptations, potentially related to the defence of acid-base balance.

Juel *et al.* (2003) reported that RBC monocarboxylate transporter 1 (MCT1) content increased linearly with time spent at 4100 m altitude in healthy but untrained sea level natives. As MCT1 is a key transporter of lactate and H^+ (Messonnier *et al.*, 2007), this suggests a hypoxia induced enhanced capacity for lactate and H^+ to be transported out of muscle cells to plasma, and from plasma into RBC's, which may elicit enhanced H^+ buffering (Juel *et al.*, 2003). These results are further supported by Boning *et al.* (2001), who reported that the transport of lactate and H^+ across cell membranes was positively influenced by altitude acclimatisation involving a Himalayan expedition to 2800-7600 m, and Ullah *et al.* (2006), who showed that monocarboxylate transporter 4 (MCT4)

gene expression was significantly increased in cell cultures that were incubated with a gas mixture of 1% O_2, 5% CO_2 and 94% N_2. Taken together, these results suggest that hypoxic exposure can improve intracellular H^+ buffering, which may be beneficial for maintaining skeletal muscle force production during high-intensity exercise. None of these studies investigated effects in trained athletes, however.

The overwhelming methodological issue with this field of research is the invasive nature of quantifying H^+ buffering capacity, especially in trained athletes, who are seldom at ease with taking time out of training to undergo muscle biopsies. As such, there is a paucity of data in athletes. Clark *et al.* (2004) reported that muscle MCT1 and MCT4 content remained unchanged after highly trained athletes spent 20 nights in normobaric hypoxia (simulating ~2650 m altitude). However, participants only exercised in normoxia, and it may be that high intensity exercise is required to stimulate an improved H^+ buffering capacity. Indeed, Zoll *et al.* (2006) reported a significant MCT1 mRNA expression increase after highly trained endurance runners exercised intermittently for six weeks in normobaric hypoxia. Additionally, Mizuno *et al.* (1990) found that H^+ buffering capacity significantly improved in muscle biopsies of 10 highly trained cross-country skiers after they spent two weeks living at 2100 m and training at 2700 m. This may have been due to direct MCT effects, but equally it should not be overlooked that Hb itself provides a potent buffer against H^+ accumulation: the Bohr-Haldane effect allows deoxygenated Hb to carry dissolved CO_2 as carbaminohaemoglobin, thereby acting to reduce tissue acidosis (Grant, 1982). As such, in addition to the previously discussed improved O_2 transport, any tHb increase resulting from sustained hypoxic exposure will likely have beneficial effects on systemic H^+ buffering capacity. Despite results from these few studies, given the limited research to date, and the relatively indirect assessment methods of the research that has occurred, whether altitude and/or hypoxic interventions influence H^+ buffering capacity to a sufficient extent as to enhance exercise performance is currently unknown.

1.2.5.4   Glycolytic capacity

There is considerable evidence that chronic exposure to high altitude causes a decreased reliance on lipids, and an enhanced reliance on carbohydrate for

metabolic energy production. Using highly sensitive stable isotope tracer methods, Brooks *et al.* (1991) showed that acute exposure to 4300 m altitude causes increased resting glucose utilisation. Furthermore, both Brooks *et al.* (1991) and Roberts *et al.* (1996) showed that three weeks of continuous exposure to 4300 m caused decreased lipid and increased carbohydrate utilisation during submaximal exercise. Whether similar effects occur in highly trained athletes, who tend to have a greater capacity to oxidise fats during exercise compared to untrained individuals (Holloszy & Coyle, 1984), has been questioned. Roels *et al.* (2007) exposed endurance trained athletes to three weeks of intermittent training (15 sessions, each lasting 60 to 90 min) in either normobaric normoxia or hypoxia (altitude simulation of 3000 m), and found that the hypoxic training stimulus enhanced the mitochondrial preference for glucose metabolism. Taken together, these results suggest that hypoxic exposure may alter substrate preference, even in highly trained athletes who are only exposed to a moderate severity of hypoxia. Given that glucose is the most O_2 efficient substrate (Brooks *et al.*, 1991), it would seem logical that any such increased metabolic reliance on glucose would be of direct benefit to exercise efficiency and/or capacity. This may not be the case for events that significantly reduce liver and muscle glycogen stores, as a more preferential usage of glucose may lead to earlier glycogen depletion, but in any case this direct link has not been conclusively demonstrated (Roels *et al.*, 2007).

1.2.5.5   Non-erythroid effects of Epo

As well as the erythropoietic actions governed by Epo, wider-reaching effects have recently been investigated, largely due to the potential for positive health consequences of rHuEpo dosages for numerous clinical conditions; see Arcasoy (2008) for a review. As well as the potential for physiological enhancement, Miskowiak *et al.* (2008) reported significantly improved cognitive function just 3 and 7 d after healthy males were administered a single 40,000 IU dose of rHuEpo. Considering that there was no detectable change in RBC concentration indices, this study suggests that rHuEpo has a direct neurological action (Miskowiak *et al.*, 2008). Similarly, one study assessing the effects of six weeks of rHuEpo dosages in endurance trained males reported positive influences on perceived physical condition and strength, and increased clinically classified self-esteem (Ninot *et al.*, 2006). Specifically in relation to sports

performance, while the effects of Epo on skeletal muscle has not shown any significant positive impact on angiogenesis (Lundby *et al.*, 2008b), eight weeks of regular rHuEpo dosages have resulted in significant positive influences on muscle mitochondrial capacity (Plenge *et al.*, 2012).

Clearly the potentially positive effects of Epo for athletic performance reach far further than simply those of new RBC formation (Boning *et al.*, 2011), and considering it is known that altitude and hypoxic training does increase circulating concentrations of Epo (Eckardt *et al.*, 1989; Garvican *et al.*, 2012), all adaptation pathways must be considered. That said, it is currently unconfirmed whether any non-erythropoietic effects of Epo may be responsible for increases in exercise performance in highly trained athletes (Lundby & Olsen, 2011).

1.2.6 Summary of the mechanisms associated with altitude and hypoxic exposure

Whilst the wealth of research interest, using numerous modern analytical techniques, are helping researchers to understand the key mechanisms, there is still much debate as to whether performance enhancement resulting from altitude and/or hypoxic exposure is primarily due to haematological (Levine & Stray-Gundersen, 1997; Levine & Stray-Gundersen, 2005) or non-haematological adaptations (Gore *et al.*, 2007; Gore & Hopkins, 2005). These questions warrant further investigation.

1.3   Methods and performance efficacy of altitude and hypoxia

Given the above adaptive responses, there has been considerable interest in the potential for athletic performance enhancement resulting from hypoxic stimuli. However, when assessing the efficacy of an intervention in relation to performance enhancement, defining what is a meaningful change is a complex task (Gore, 2014), as highly trained athletes who are nearing their genetic performance limit may only experience marginal gains (Ahmetov & Rogozkin, 2009). Perhaps the most striking and recent real world examples of this come from the London 2012 Olympic Games, when the sum of the differences between 1st and 2nd place in the men's and women's longest distance swimming, cycling and running races totalled just 0.35 s (0.3%). Additionally, Pyne *et al.* (2004) reported that in highly trained swimmers, the CV between major competitions during an Olympic year was just 0.8%, and suggested that interventions that enhance performance by as little as 0.4% would substantially increase the chances of winning an international standard medal (Pyne *et al.*, 2004). With margins as small as 0.3-0.4%, the rationale for performance targeted altitude and hypoxia interventions is clear.

There are now a wide variety of altitude and hypoxic strategies in use, each targeting different mechanisms, with varying degrees of evidence supporting their performance efficacy. Strategies range in hypoxic dose (Wilber *et al.*, 2007) from living and training for 24 $h \cdot d^{-1}$ for numerous weeks at moderate altitude (Gore *et al.*, 1998), to repeated 5 min exposures to normobaric hypoxia at rest, while living at sea level (Hamlin & Hellemans, 2007). Figure 1.1 summarises the most commonly used altitude and hypoxic interventions, in order of their hypoxic dose. The subsequent text discusses the performance efficacy of each type of intervention, with tables at the end of each section acting to summarise all the discussed empirical research.

Hypoxic dose	Type of exposure
LARGE (24 h·d^{-1})	**Live high, train high (LH+TH)** Traditional altitude camp, spending all day at moderate altitude (1500-3000 m), typically for 2-4 weeks.
	Live high, train high and train low (LH+TH+TL) Traditional altitude camp; spending most of each day at moderate altitude (1500-3000 m), with some exercise performed at lower altitudes, or while breathing a hyperoxic inspirate to simulate being at lower altitudes.
	Live high, train low (LH+TL) Traditional altitude camp; spending most of each day at moderate altitude (1500-3000 m), with all exercise performed at lower altitudes, or while breathing a hyperoxic inspirate to simulate being at lower altitudes.
	Simulated live high, train low (simulated LH+TL) Sleeping in a normobaric or hypobaric hypoxic tent or room, typically simulating moderate altitude (1500-3000 m), while training at or near sea level (usually spending 10-16 h·d^{-1} in the hypoxic environment).
	Intermittent hypoxic training (IHT) Performing some exercise training in a normobaric or hypobaric hypoxic chamber, or while breathing a normobaric hypoxic inspirate, while living at sea level.
SMALL (<1 h·d^{-1})	**Intermittent hypoxic exposure (IHE)** Short phases of passive rest in a normobaric or hypobaric hypoxic chamber, or while breathing a normobaric hypoxic inspirate, while living at sea level.

Figure 1.1: A summary of the most common altitude and hypoxia methods, in descending order of their 'hypoxic dose'.

1.3.1 Performance efficacy of traditional altitude training camps

Traditionally altitude training camps involved acclimatisation to moderate altitude, usually by living and undertaking normal exercise training at 1500-3000 m, for 2-4 weeks, termed 'live high, train high' (LH+TH) (Wilber *et al.*, 2007). Research interest in LH+TH interventions increased in the lead up to and after the Mexico City 1968 Olympic Games, as it was held at an elevation of 2420 m. Daniels & Oldridge (1970) investigated the effects of six weeks' LH+TH at 2300 m, spread intermittently over a 10 week period in highly trained middle distance runners, and reported impressive performance gains, including personal best times for all six participants, and a World Record in the one mile race. Similarly, Dill & Adams (1971) reported significant improvements in the limit of tolerance (T-Lim) during incremental treadmill tests, after six highly trained middle distance runners undertook LH+TH for 17 d at 3090 m. In the previously detailed study by Mizuno *et al.* (1990), two weeks of LH+TH (living at 2100 m and training at 2700 m) was reported to significantly improve T-Lim in 10 highly trained cross-country skiers. Beneficial effects on performance after altitude training were not universally reported in this era though. For example, Buskirk *et al.* (1967) found that after six highly trained middle distance runners undertook 4-5 weeks of LH+TH at 4000 m, $\dot{V}O_2$max values were comparable, and running performances were worsened compared to pre-altitude. Similarly, Svedenhag *et al.* (1997) found that LH+TH at 1900 m for 29 d did not elicit any significant performance gains in highly trained cross-country skiers (mean $\dot{V}O_2$max = 77.9 and 71.8 mL·kg^{-1}·min^{-1} for males and females, respectively). Importantly, as all these studies were observational in their design (Buskirk *et al.*, 1967; Daniels & Oldridge, 1970; Dill & Adams, 1971; Mizuno *et al.*, 1990; Svedenhag *et al.*, 1997), they did not include control groups, so whether it was the hypoxic stimuli or simply an intensified period of training that resulted in performance changes is not known.

Interestingly, Buskirk *et al.* (1967) suggested that after the 4-5 weeks of LH+TH at 4000 m the runners were relatively detrained as a result of the reduction in their (absolute) training intensity. Indeed, when undertaking all exercise training at altitude, absolute exercise intensity (power output or velocity) is reduced compared to the equivalent exercise at sea level, due to the lesser metabolic

contribution from oxidative sources; a lower "O_2 flux" (Wilber, 2013a). As such, Levine & Stray-Gundersen (1992) proposed an original approach whereby athletes live at moderate altitude (1500-3000 m) and perform their exercise training at or near sea level, termed 'live high, train low' (LH+TL), or using a combination of exercise training at moderate altitude and near sea level, termed 'live high, train high and low' (LH+TH+TL). These authors then carried out what is now the most commonly cited altitude training study to date (Levine & Stray-Gundersen, 1997).

After four weeks of sea level training, 39 collegiate level middle distance runners underwent four weeks of either LH+TH (living and training at 2500 m), LH+TL (living at 2500 m, training at 1250 m), or sea level living and training. 5000 m time trial performance significantly improved after LH+TL (on average by 1.4%), and this performance improvement was significantly greater than that of both the LH+TH and the sea level control groups (Levine & Stray-Gundersen, 1997). It was suggested that the LH+TL participants trained at a low enough altitude to maintain O_2 flux and interval training velocity near sea-level values, thereby preserving muscle structure and function specific to 5000 m race pace (Chapman *et al.*, 1998). It is somewhat perplexing though, that in addition to 5000 m performances worsening in the LH+TH group, performances were on average ~3% slower after sea level training. Possible causes of this worsened performance could be a nocebo effect, due to these participants having not been selected for one of the assumed beneficial altitude groups, or that these participants were overly fatigued during post-intervention testing. Although the worsened performances were not significant, they would have contributed to the significant difference between groups. Nevertheless, this report provided the stimulus for numerous LH+TH and LH+TL investigations since.

In their "double case study", Wehrlin & Marti (2006) assessed two highly trained 29 y old male runners who for 26 d lived at 2456 m for 18 h·d^{-1}, while training at 1800 m (LH+TH+TL). The first participant's 5000 m time 1 d after altitude improved by 3.0%, and by 1.3% 27 d later at the World Championships, compared to his previous best time. In the second participant, marathon performance was 0.2% slower at the World Championships than his best time, although the authors argued that these performances were incomparable due to the differing relief of the routes and pacing strategies employed. Similarly, to

further investigate whether the LH+TL induced performance enhancement from their previous study (Levine & Stray-Gundersen, 1997) would apply to highly trained athletes, Stray-Gundersen *et al.* (2001) exposed 26 national and international standard runners to 27 d living and low intensity training at 2500 m, while performing high intensity interval training at 1250 m (a LH+TH+TL design). These authors reported significantly enhanced running performances post-altitude (on average by 1.1% during a 3000 m race), which was comparable to the 1.4% performance improvement during a 5000 m race in their previous study (Levine & Stray-Gundersen, 1997). These results are also in broad agreement with Wehrlin *et al.* (2006), who reported significantly improved 5000 m running performances (mean +1.6%) in moderately trained athletes after 24 d of living at 2500 m for 18 h·d^{-1} while performing low and medium intensity training at 1800 m, and high intensity training at 1000 m. Again, there were no control groups in Stray-Gundersen *et al.* (2001) or Wehrlin & Marti (2006), and the control group in Wehrlin *et al.* (2006) did not perform comparable exercise assessments. As such, although reports of (ecologically valid) race performance gains in the magnitude of 1.1-1.6% do constitute a meaningful change, it is difficult to discern to what extent the LH+TH+TL induced biological hypoxia caused these changes, as opposed to a training camp or placebo effect (Bonetti & Hopkins, 2009).

To address this issue, Dehnert *et al.* (2002) carried out a well designed study whereby 21 moderately trained triathletes (baseline mean cycling $\dot{V}O_2$max of ~61 mL·kg^{-1}·min^{-1}) undertook similar training at 800 m for 14 d, and either lived at this same altitude (control group), or at 1956 m for 13-15 h·d^{-1} (LH+TL group). While T-Lim during an incremental speed treadmill test improved in 6 of 10 LH+TL participants, compared to only 2 of 10 in the control group, the between group differences did not reach statistical significance (P = 0.068). This lack of a significant performance change is perhaps not surprising though, as the hypoxic dose of 13-15 h·d^{-1} at 1956 m for just 14 d was considerably less than previous reports whereby participants spent most of each day at ~25000 m for 24-48 d (Levine & Stray-Gundersen, 1997; Stray-Gundersen *et al.*, 2001; Wehrlin & Marti, 2006; Wehrlin *et al.*, 2006).

In order to maximise the hypoxic dose, as well as due to certain logistical constraints causing travel to lower altitudes for LH+TL to be problematic,

LH+TH interventions are still commonly used. Wachsmuth *et al.* (2013) controlled for a variety of covariates, and concluded that after highly trained swimmers undertook 3-4 weeks of LH+TH at 2320 m, competitive race performances were not significantly improved at 0-14 d or 15-24 d post-altitude, but after spending between 25-35 d at sea level, performance did significantly improve, on average by +0.8%. While this may seem a relatively small margin, the study's statistical power was strong because data were included from three altitude camps over a 2 y period, in 45 highly trained swimmers (27 of which competed at the Beijing 2008 Olympic Games). Similarly, Bonne *et al.* (2014) found that swimming test performance (4 x 50 m anaerobic efforts and a 3000 m aerobic time trial) improved by effectively the same extent immediately after 3-4 weeks of LH+TH or sea level living and training. The intervention was somewhat complex, as 7 of the 10 LH+TH group participants spent an initial 7 d living at 3094 m (Leadville, Colorado, USA), before being joined by the 3 other LH+TH group participants for a further 21 d at 2130 m (Flagstaff, Arizona, USA). It would have been insightful to have also quantified performance in competitive races, and to have assessed any such performance changes at more time points post-altitude, for example in the 25-35 d period (Wachsmuth *et al.*, 2013).

Bailey *et al.* (1998) found that four weeks of LH+TH at 1500-2000 m resulted in slower maximum running speeds in a standardised training session (at 20 d post-altitude), and no improvement in maximum endurance performance, compared to a sea level control group. These results must be interpreted with a degree of caution though, as 10 participants were found to be iron deficient, despite daily oral iron supplementation, and the frequency of upper respiratory and/or gastrointestinal tract infections increased markedly during the altitude camps, whereas there were no reports of any such illness in the sea level control group (Bailey *et al.*, 1998).

In the study to have used perhaps the most highly trained group of participants to date, Gore *et al.* (1998) reported exercise test data of eight male cyclists (mean $\dot{V}O_2peak$ = 81 mL·kg^{-1}·min^{-1}), all of whom had been Olympic or World gold medallists and/or senior or junior level World Record holders, before and after LH+TH for 31 d at 2690 m. While there was no single time point post-altitude when total work during a 4000 m cycling time trial had significantly improved, when the best of the three time trials at 4, 9 and 21 d post-altitude

were compared to pre-altitude, there was a significant performance improvement (mean +4.3%). Conversely, as the mean reduction for each individual's worst post-attitude time trial performance was not significantly different from pre-altitude (mean -2.4%), Gore and colleagues took this to indicate meaningful performance gains. In this study, injuries and a gastrointestinal illness during the third and fourth week at altitude reduced the training load of most of the group for 3-4 d (Gore *et al.*, 1998). Interestingly, Wachsmuth *et al.* (2013) demonstrated that in seven swimmers who reduced their training load due to illness or injury at altitude, tHb gains were significantly attenuated compared to those swimmers who remained injury and illness free (mean tHb increase of ~2% vs. ~7%, respectively). If a similar illness / injury mediated blunting of tHb occurred in participating athletes in the reports by Gore *et al.* (1998) and Bailey *et al.* (1998), this may partially explain the inconsistency or lack of post-altitude performance changes.

In their meta-analysis, Bonetti & Hopkins (2009) concluded that for "elite athletes", maximal endurance power output enhancement was "possible" after LH+TL, but that conversely, $\dot{V}O_2$max reductions were also "possible" after LH+TH. Specifically, these authors calculated that when appropriately conducted, acclimatisation to physical altitude elicits up to 4% maximal endurance power output improvements in elite athletes (Bonetti & Hopkins, 2009). When this is considered in context with the premise that a meaningful change in competitive performance may be as small as 0.3% (see Section 1.3), it is clear why numerous athletes worldwide regularly undertake some form of altitude training. However, while some studies have been well planned and executed, using sea level control groups, very few have assessed post-altitude performances at important competitions, in highly trained athletes, when baseline physiological variables are already near optimum levels. The LH+TL paradigm in particular is lacking a sound evidence base, given that the original report by Levine and Stray-Gundersen (1997) is the only study that has included a sea level control group to have found a performance benefit from LH+TL to date (please see the above critique of this paper). This area of physical altitude training clearly warrants further research.

Table 1.1 summarises the key research that has been discussed in this section in relation to the performance efficacy of traditional altitude training camps.

Table 1.1: Summary of the referenced literature concerning the performance efficacy of traditional altitude training camps.

Study	Hypoxic exposure type and dose	Participant no. and training status	Key performance outcomes	Key considerations and/or limitations
Bailey *et al.* (1998)	LH+TH (living and training at 1500-2000 m) or sea level living and training, for 28 d.	23 highly trained middle distance runners (14 in the LH+TH group and 9 in the sea level control group).	Significantly slower mean running velocity during a standardised track training session after LH+TH than before (mean -2% change), compared to no such change in the sea level control group.	In the LH+TH group, 10 were iron deficient, and the frequency of infectious illnesses increased. There were no such incidences in the control group.
Bonne *et al.* (2014)	LH+TH (living and training at 3094 m for 7 d, then at 2130 m for a further 21 d) or sea level (training camp) living and training for 28 d.	20 highly trained swimmers (10 in the LH+TH group, 7 of which spent the first 7 d at 3094 m, and 10 in the sea level control group).	Swimming performances during a repeated anaerobic test set (4 x 50 m at maximum effort, each interspersed by 10 s rest), and a 3000 m time trial improved within the LH+TH and the sea level control group, but did not significantly differ between groups.	Performance was only assessed training test sets, and not via any race performances. The additional 7 d at 3094 m for certain participants adds confusion to the study design.
Buskirk *et al.* (1967)	LH+TH (living and training at 4000 m) for 28-35 d.	6 highly trained middle distance runners.	$\dot{V}O_2max$ values were comparable, and running performances were worsened.	No control group.
Daniels & Oldridge (1970)	LH+TH (living and training at 2300 m) intermittently for 42 d as four separate camps over a 70 d period.	6 highly trained middle distance runners.	$\dot{V}O_2max$ improved, on average by +5%, and all 6 participants improved their personal best 1 mile or three mile times, including a 1 mile World Record.	No control group.
Dehnert *et al.* (2002)	LH+TL (living at 1956 m for 13-15 $h \cdot d^{-1}$ while training at 800 m) or near sea level living and training (at 800 m) for 14 d.	21 moderately trained triathletes (11 in the LH+TH group and 10 in the near sea level control group).	T-Lim during an incremental treadmill test improved in 6 of 10 LH+TL group participants, compared to only 2 of 10 control participants, but the between group differences were not significant ($P = 0.07$).	Hypoxic dose may have been too small to induce significant erythropoiesis.
Dill & Adams (1971)	LH+TH (living and training at 3090 m) for 17 d.	6 highly trained middle distance runners.	$\dot{V}O_2max$ improved, on average by +4%, and T-Lim during an incremental gradient and speed test significantly improved, on average by +24% (range 10% to 44%).	No control group.
Gore *et al.* (1998)	LH+TH (living at 2690 m and training at 1850 m – 4578 m) for 31 d.	8 highly trained male track cyclists.	The best of the 4000 m time trials at 4, 9 and 21 d post-altitude were on average 4% better than pre-altitude.	No control group. Injuries and illness during the 3rd and 4th week at altitude reduced the training load.

Table 1.1 (continued)...

Study	Hypoxic exposure type and dose	Participant no. and training status	Key performance outcomes	Key considerations and/or limitations
Levine & Stray-Gundersen (1997) and Chapman *et al.* (1998)	LH+TH (living and training at 2500 m), LH+TL (living at 2500 m, training at 1250 m) or sea level living and training, for 28 d.	39 moderately trained runners (13 in each of the LH+TH, LH+TL and sea level control groups).	Competitive 5000 m running performances were significantly faster after LH+TL, on average by +1.4%, and this improvement was significantly greater than that of both the LH+TH and the control groups.	Control group 5000 m time trial performances got worse after training, which would have impacted the between-group statistical analyses.
Mizuno *et al.* (1990)	LH+TH (living at 2100 m and training at 2700 m) for 14 d.	10 highly trained cross-country skiers.	T-Lim during an incremental gradient treadmill test significantly improved, on average by +17%.	No control group.
Stray-Gundersen *et al.* (2001)	LH+TH+TL (living at 2500 m, performing high intensity training at 1250 m) for 27 d.	26 highly trained middle distance runners.	Competitive 3000 m running performances were significantly faster, on average by +1.1%, after LH+TH+TL.	No control group.
Svedenhag *et al.* (1997)	LH+TH (living and training at 1900 m) for 29 d.	7 highly trained cross-country skiers.	Neither $\dot{V}O_2$max nor T-Lim during an incremental gradient and speed treadmill test significantly improved after LH+TH.	No control group.
Wachsmuth *et al.* (2013)	LH+TH (living and training at 2320 m) for 21-28 d.	25 highly trained swimmers.	Competitive swimming race performances were significantly faster, on average by +0.8%.	Results were converted to a scalar system ("FINA Points"), which adds greater uncertainty.
Wehrlin & Marti (2006)	LH+TH+TL (living and performing low intensity training at 2456 m, and performing high intensity training at 1800 m) for 26 d.	2 highly trained distance runners (5000 m and marathon specialists).	Competitive 5000 m running performance was faster (+3%) in one participant and marathon performance was relatively unaltered (-0.2%) in the other participant.	No control group.
Wehrlin *et al.* (2006)	LH+TH+TL (living at 2500 m, while performing low and medium intensity training at 1800 m, and high intensity training at 1000 m) for 24 d.	10 moderately trained orienteer athletes.	Competitive 5000 m running performances were significantly faster, on average by +1.6%.	Although they included a control group for haematological comparisons, they did not perform comparable pre- and post-altitude exercise tests.

LH+TH = live high, train high; LH+TH+TL = live high, train high and train low; LH+TL = live high, train low; T-Lim = limit of tolerance.

1.3.2   Performance efficacy of simulated altitude camps

On the basis of reports of performance enhancement subsequent to traditional altitude training, a group of Finnish researchers led by Dr Heikki Rusko devised a novel approach to simulate LH+TL, without the logistical constraints of travelling up and down mountains. This was achieved by pumping controlled volumes of nitrogen gas into a closed room, thereby reducing the ambient F_IO_2, creating a normobaric hypoxic environment (Rusko *et al.*, 1995). As the physical consequences of exposure to normobaric compared to hypobaric hypoxia are similar, i.e. a resultant C_aO_2 reduction, and that the human ability to sense this biological hypoxia is similar regardless of barometric pressure, it has been argued that the physiological effects are similar (Mounier & Brugniaux, 2012a; Mounier & Brugniaux, 2012b; Mounier & Brugniaux, 2012c). Most notably, the Epo response was shown to be similar between hypobaric and normobaric hypoxic exposure (Laitinen *et al.*, 1995). However, this view is far from unanimous (Millet *et al.*, 2012a; Millet *et al.*, 2012b; Millet *et al.*, 2012c), and it is clear that the degree of erythropoiesis is not the only important deciding factor by which to differentiate these two hypoxic methods. For example, Hemmingsson & Linnarsson (2009) showed that exhaled nitric oxide (NO) is lower in hypobaric hypoxia than in the equivalent degree of normobaric hypoxia. Given the possibility of raised NO levels enhancing oxidative function (Bailey *et al.*, 2009; Larsen *et al.*, 2011; Vanhatalo *et al.*, 2011), this is one mechanism suggesting that outcomes from hypobaric hypoxic exposure may differ to outcomes from normobaric hypoxic exposure (Kayser, 2009), although this remains to be seen in highly trained athletes, and at moderate (1500-3000 m) as opposed to high (>3000 m) altitude simulations.

In an early pilot study, Mattila & Rusko (1996) reported that simulated LH+TL for 11 d (18 $h \cdot d^{-1}$ at a normobaric hypoxic altitude simulation of ~3000 m) resulted in significant Epo increases and time trial performances in five competitive cyclists, although being a pilot study they did not have a control group. The more comprehensive study by Brugniaux *et al.* (2006) investigated the effects of simulated LH+TL for 26 d in highly trained male middle-distance runners (14 $h \cdot d^{-1}$ at a normobaric hypoxic altitude simulation of 2500-3000 m while training and spending the remaining 10 $h \cdot d^{-1}$ at 1200 m altitude). Both

$\dot{V}O_2max$ and T-Lim improved to a greater extent after LH+TL compared to a control group who spent 26 d living and training at 1200 m. It is noteworthy that baseline running $\dot{V}O_2max$ assessed at 1200 m altitude (63 mL·kg^{-1}·min^{-1}) was rather low for these highly trained athletes, who were all capable of running within 9-24 s of the existing 1500 m World Record. $\dot{V}O_2max$ increased by substantial margins (~4% in the control group and ~10% in the LH+TL group), thus indicating that simulated LH+TL resulted in a degree of acclimatisation, but which may or may not have shown such improvements at sea level.

Investigators at the Australian Institute of Sport have consistently reported moderate beneficial effects of simulated LH+TL in highly trained athletes using normobaric hypoxia (Garvican *et al.*, 2011; Robertson *et al.*, 2010b; Saunders *et al.*, 2004). Notably, as previously detailed (see Section 1.2.5), Garvican *et al.* (2011) found that despite blocking a 5% tHb increase, via phlebotomy, maximal 4 min power output significantly improved in 11 highly trained cyclists after 26 nights of simulated LH+TL, compared to a normoxic control group. This performance efficacy of simulated LH+TL is not undisputed though, even within this Australian group, as Gore *et al.* (2001) reported that 23 nights of simulated LH+TL (training at 600 m, living at a normobaric hypoxic simulation of 3000 m) resulted in statistically unchanged maximal cycling performance compared to normoxic living and training. Similarly, Clarke *et al.* (2009), Robach *et al.* (2006b) and Robach *et al.* (2006a) reported no significant performance gains after simulated LH+TL in highly trained athletes.

Most recently, Neya *et al.* (2013) implemented a novel simulated LH+TL study design, whereby for 22 d collegiate level athletes (mean $\dot{V}O_2max \approx 68$ mL·kg^{-1}·min^{-1}) lived and trained at 1300-1800 m, and either spent night time (~10 h·d^{-1}) at 1300 m (control group) or at 1300 m in normobaric hypoxic enclosures set at an altitude simulation of ~3000 m. $\dot{V}O_2max$ after simulated LH+TL was reported to increase by ~9% more than in the control group, but this is somewhat misleading, as $\dot{V}O_2max$ in the control group actually worsened by ~5% (compared to a ~4% improvement in the LH+TL group). These authors proposed that the $\dot{V}O_2max$ gains were caused by the haematological enhancement in the LH+TL group. While this may have had an influence, it is likely that the lack of any F_IO_2 blinding resulted in placebo and/or nocebo effects

that influenced participants' motivation to perform well after LH+TL or poorly after control training, respectively, during the post-intervention T-Lim tests (unfortunately T-Lim was not reported). The blinding of at least the participants, and ideally also the researchers, is clearly an important aspect of any simulated LH+TL investigation. The only simulated LH+TL study to have been performed in a double-blinded manner is that by Siebenmann *et al.* (2012), who had 16 well trained athletes (mean baseline $\dot{V}O_2$max ≈ 70 mL·kg^{-1}·min^{-1}) perform cycling training for four weeks at 1200 m while living at either 1200 m (control group) or at a normobaric hypoxic simulation of 3000 m. Cycling time trial performance and $\dot{V}O_2$max tended to increase, but neither significantly differed between groups.

The literature review by Richalet & Gore (2008) concluded that simulated LH+TL is able to elicit maximal aerobic performance improvements when the exposure to hypoxia is at least over 18 d, whereas the meta-analysis by Bonetti & Hopkins (2009) calculated that in "elite athletes", maximal endurance power output "possibly" improves by ~4.0% after traditional LH+TL, compared to an "unclear" change of only ~0.6% after normobaric hypoxic simulated LH+TL. These discrepancies, as well as results from the double-blinded investigation by Siebenmann and colleagues, indicate that the performance efficacy of simulated LH+TL in highly trained athletes requires further investigation.

Table 1.2 summarises the key research that has been discussed in this section in relation to the performance efficacy of simulated altitude training camps.

Table 1.2: Summary of the referenced literature concerning the performance efficacy of simulated altitude camps.

Study	Hypoxic exposure type and dose	Participant no. and training status	Key performance outcomes	Key considerations and/or limitations
Brugniaux et al. (2006)	Simulated LH+TL for 18 nights (14 $h \cdot d^{-1}$ at 2500-3000 m, while training at 1200 m), or living and training at 1200 m (control group).	11 highly trained middle distance runners (5 in the LH+TL group and 6 in the control group).	$\dot{V}O_2max$ increased significantly more after LH+TL than in the control group (+10% vs. +4%), and the same was the case for T-Lim during a treadmill incremental gradient and speed test.	Baseline running $\dot{V}O_2max$ at 1200 m altitude was only 63 $mL \cdot kg^{-1} \cdot min^{-1}$, so changes were at least in part due to altitude acclimatisation.
Clarke et al. (2009)	Simulated LH+TL for 21 d (14 $h \cdot d^{-1}$ at 3000 m, while training and spending the remainder of each day at 600 m).	12 highly trained cyclists.	No significant $\dot{V}O_2max$ changes after LH+TL (mean change +0.4%).	No control group, no blinding of the hypoxic living condition, and no performance data reported.
Garvican et al. (2011)	Simulated LH+TL for 26 nights (living for ~16 $h \cdot d^{-1}$ at 3000 m).	11 highly trained cyclists (5 who's tHb was free to respond, and 6 who's tHb was "clamped" via phlebotomy.	Despite a 5% tHb increase being prevented via phlebotomy, maximal 4 min power output significantly improved by an equal magnitude in both groups.	
Gore et al. (2001)	Simulated LH+TL for 23 nights (9.5 $h \cdot d^{-1}$ at 3000 m while training at 600 m), or living and training at 600 m (control group).	13 highly trained multi-sport endurance athletes (6 in the LH+TL group and 7 in the control group).	Total work during a 2 min cycling TT was statistically unchanged in both groups throughout the intervention (mean -1.6% in the LH+TL group and -0.4% in the control group).	No blinding of the hypoxic living condition.
Mattila & Rusko (1996)	Simulated LH+TL for 11 nights (18 $h \cdot d^{-1}$ at 3000 m, while training at sea level).	5 moderately trained cyclists.	Mean cycling TT velocity increased significantly after LH+TL (on average by +4%).	No control group and no blinding of the hypoxic living condition.
Neya et al. (2013)	Simulated LH+TL for 21 nights (resting and training for ~14 $h \cdot d^{-1}$ at 1300-1800 m), and spending the remaining 10 $h \cdot d^{-1}$ at 3000 m or at 1300 m (control group).	14 moderately trained middle distance runners (7 in the LH+TL group and 7 in the control group).	$\dot{V}O_2max$ increased by ~4% after simulated LH+TL, and decreased by ~5% in the control group were comparable, and running performances were worsened.	T-Lim was not reported. No blinding of the hypoxic living condition.

Table 1.2 (continued)...

Study	Hypoxic exposure type and dose	Participant no. and training status	Key performance outcomes	Key considerations and/or limitations
Robach *et al.* (2006a)	Simulated LH+TL for 18 nights (11 h·d^{-1} at 2500-3500 m while training at 1200 m), or living and training at 1200 m (control group).	11 highly trained Nordic skiers (6 in the LH+TL group and 5 in the control group).	There was a trend for a worsened fixed velocity T-Lim, but changes were not significantly different within or between groups (mean change -15% in both groups).	tHb decreased by ~12% in the control group (who ingested lower iron dosages, and whose serum ferritin decreased).
Robach *et al.* (2006b)	Simulated LH+TL for 13 nights (16 h·d^{-1} at 2500-300 m while training at 1200 m), or living and training at 1200 m (control group).	18 highly trained swimmers (9 in the LH+TL group and 9 in the control group).	Changes in performance and $\dot{V}O_2$max during a controlled incremental speed swimming test were not significantly different within or between groups ($P > 0.05$).	Step test and aerobic performance measures were not specific or relevant to competition performances.
Robertson *et al.* (2010b)	Simulated LH+TH+TL for 21 nights (training for 4 sessions·week^{-1} at 2200 m while living at sea level (IHT), or at 3000 m for 14 h·d^{-1}).	17 moderately trained middle distance runners (8 in the LH+TH+TL group and 9 in the IHT group).	3000 m TT performance substantially improved after LH+TH+TL (mean -1.1%), but not after IHT (mean -0.1%).	No blinding of the hypoxic living condition.
Saunders *et al.* (2004)	Simulated LH+TL for 20 nights (9-12 h·d^{-1} at 2000-3100 m, with 2 normoxic nights·week^{-1}), or living and training at sea level (control).	23 highly trained runners (10 in the LH+TL group and 13 in the control group).	Within one month of the intervention, all participants in the LH+TL who raced ran personal or season best times over distances ranging from 1,500 m to 10,000 m, compared to only three of the 13 participants in the control group.	Small hypoxic dose (~53 h·week^{-1} in hypoxia, so ~115 h·week^{-1} in normoxia).
Siebenmann *et al.* (2012) and Nordsborg *et al.* (2012)	Simulated LH+TL for 28 nights (16 h·d^{-1} at 3000 m while training at 1200 m), or living and training at 1200 m (control group), in a double blinded design.	16 highly trained cyclists (10 in the LH+TL group and 6 in the control group).	Cycling performances during a simulated TT tended to increase in all participants (on average by ~5%), but there were no significant difference within or between groups ($P > 0.05$).	Double-blinded, placebo controlled intervention.

LH+TH+TL = live high, train high and train low; LH+TL = live high, train low; IHT = intermittent hypoxic training, T-Lim = limit of tolerance; TT = time trial; tHb = total haemoglobin mass.

1.3.3 Performance efficacy of intermittent hypoxic training

Intermittent hypoxic training (IHT) is when exercise is performed within a hypobaric or normobaric hypoxic chamber, or while breathing a normobaric hypoxic inspirate via a mouthpiece / face mask. It is not a new concept; originating from a 1930's requirement for Soviet Union pilots to be pre-acclimatised prior to flying in open cockpits to altitudes of up to 6000 m (Serebrovskaya, 2002; Streltsov, 1939). Scientists at the "secret depressurised underground training facility" within the German Democratic Republic's Kienbaum Training Centre were also investigating potential athletic performance enhancement resulting from IHT long before the concept became internationally popular (Houston & Harris, 2005). Although findings have seldom been published, and in any case many of the results would have been confounded due to extensive doping regimes (Franke & Berendonk, 1997), these reports formed a foundation for subsequent research interest.

IHT is now most commonly performed using normobaric hypoxia, so in addition to any differences in the physiological responses to normobaric compared to hypobaric hypoxia (Hemmingsson & Linnarsson, 2009; Millet *et al.*, 2012a; Millet *et al.*, 2012b; Millet *et al.*, 2012c), the lesser overall hypoxic dose (Wilber *et al.*, 2007), as well as the skeletal muscle being in an active rather than resting state, mean that different mechanisms are targeted compared to chronic hypoxia acclimatisation interventions. Indeed, resting in moderate hypoxia does not significantly reduce muscle O_2 delivery – instead, moderately intensive exercise is required to do so (Calbet *et al.*, 2009). On this basis, an early investigation by Terrados *et al.* (1988) found that work capacity was significantly increased, on average by 33%, after competitive cyclists performed IHT for ~2 $h \cdot d^{-1}$, 4-5 sessions·week^{-1}, for 3-4 weeks, at an altitude simulation of ~2300 m. This compared to a mean increase of 22% in a normoxic control group, but performance change after IHT was only significantly greater when both groups were tested in hypoxic conditions. This was similarly the case in another study by Vogt *et al.* (2001), who found that maximal cycling power output increased significantly more after six weeks of IHT (+16%) compared to normoxic training (+11%), only when tested in hypoxic conditions and when results from both the high and low intensity groups were combined. As such, although these authors

commented that IHT may be a useful athletic performance enhancing tool, neither of these two studies found performance to be enhanced at sea level.

In contrast, Dufour *et al.* (2006) reported significant performance gains in both hypoxia and normoxia, after nine moderately trained runners incorporated IHT into their usual training schedules for six weeks (24-40 min·session^{-1}, twice per week, at an altitude simulation of ~3000 m). Specifically, in normoxic test conditions, T-Lim at the pre-intervention velocity that elicited $\dot{V}O_2$max significantly improved, on average by 35%, compared to a non-significant increase of +10% in a normoxic control group (n = 9). This was also the case for results during an incremental speed test to exhaustion, whereby $\dot{V}O_2$max significantly increased after IHT (mean +5%), but not after normoxic training (mean ~1% increase). However, normoxic test results for individual participants are perplexing – for example, one IHT participant improved his $\dot{V}O_2$max by ~15%, and another improved his T-Lim by ~65%, whereas T-Lim and $\dot{V}O_2$max worsened in one of the normoxic trained participants, by ~20% and ~7%, respectively. Clearly there was a substantial degree of inter-individual variability after IHT and normoxic training (Dufour *et al.*, 2006).

In a particularly well designed and implemented study, Faiss *et al.* (2013b) had 40 moderately trained cyclists complete four weeks of repeated sprint training in normoxia (n = 20) or normobaric hypoxia (n = 20) (F_IO_2 0.146, equivalent to ~3000 m altitude). Participants were blinded to the training session F_IO_2. In a repeated sprint test to exhaustion, the IHT group performed significantly more sprints (9 vs. 13 sprints) compared to the normoxic trained group (9 vs. 9 sprints). Using a similar single-blinded study design, Galvin *et al.* (2013) also reported a significantly improved repeated running sprint ability after 33 academy rugby players completed 12 repeated sprint training sessions over four weeks in either normoxia or normobaric hypoxia (F_IO_2 0.130, equivalent to ~3900 m altitude). This area of high intensity IHT is a relatively new concept, and warrants further research.

Aside from these results, most investigations to date have found that IHT does not result in performance enhancement when tests are performed in normoxic conditions. For instance, Morton & Cable (2005), Lecoultre *et al.* (2010) and Messonnier *et al.* (2004) found that cycling IHT for four weeks did not cause any

greater performance gains than in normoxic control groups. Similarly, the previously detailed study by Robertson *et al.* (2010b) exposed moderately trained runners to three weeks of either IHT or IHT and simulated LH+TH+TL, and while there were significant $\dot{V}O_2$max improvements in both groups, 3 km time trial performance only improved in the LH+TH+TL group, and not in the IHT group (-1.1% vs. -0.1%, respectively). Roels *et al.* (2005) found that four weeks of interval training induced significant endurance performance improvements in moderately trained male athletes, but the inclusion of twice weekly IHT sessions did not elicit any greater performance gains. Lastly, while some of the highly trained cyclists who participated in the study by Ventura *et al.* (2003) may have experienced a level of over-reaching, as indicated by performance remaining unchanged after six weeks of training, there were no signs that IHT led to any more favourable adaptations than normoxic training.

Aside from the recent investigations by Faiss *et al.* (2013b) and Galvin *et al.* (2013), none of these studies (Dufour *et al.*, 2006; Lecoultre *et al.*, 2010; Messonnier *et al.*, 2004; Morton & Cable, 2005; Robertson *et al.*, 2010b; Roels *et al.*, 2005; Terrados *et al.*, 1988; 2003; Vogt *et al.*, 2001) reported that participants were blinded to the F_IO_2 during training sessions. That IHT participants were the only ones to either train in a hypoxic chamber or to receive the inspirate via face masks, while normoxic control group participants did not, is a fundamental study design flaw, and undoubtedly will have resulted in placebo and/or nocebo effects in at least some of these otherwise well executed investigations. The precise influence of the placebo effect is difficult to assess in relation to hypoxic strategies, for example Saunders *et al.* (2010) attempted to quantify how placebo and nocebo effects impacted the performance efficacy of simulated LH+TL, but by the end of the intervention the placebo group had identified their experimental condition. In a broader, sense, Clark *et al.* (2000) demonstrated the power of the placebo effect, by reporting that that in moderately-trained cyclists, mean power output during a 40 km time trial was on average 4% higher in those who were told that they were receiving a carbohydrate drink, compared to those who were told they were receiving a zero calorie placebo drink, regardless of the actual drink they ingested.

Table 1.3 summarises the key research that has been discussed in this section in relation to the performance efficacy of intermittent hypoxic training.

Table 1.3: Summary of the referenced literature concerning the performance efficacy of intermittent hypoxic training.

Study	Hypoxic exposure type and dose	Participant no. and training status	Key performance outcomes	Key considerations and/or limitations
Dufour *et al.* (2006)	IHT for 6 weeks; 2 sessions·week^{-1}, 24-40 min·session^{-1}, at an altitude simulation of ~3000 m, or normoxic training (control group).	18 moderately trained middle distance runners (9 in the IHT group, 9 in the control group).	T-Lim significantly improved in the IHT group (mean +35%, $P < 0.05$), but not in the control group (mean +10%. $P > 0.05$).	Between group interaction statistics were not reported, and participants were not blinded to the training F_IO_2.
Faiss *et al.* (2013b)	IHT for 4 weeks; 2 sessions·week^{-1}, 3 x (5 x 10 s with 20 s active recovery), 5 min between sets, at an altitude simulation of ~3000 m, or normoxic training (control group).	40 moderately trained cyclists (20 in the IHT group, 20 in the control group).	The maximum number of sprints performed during a repeated sprint test improved significantly more in the IHT group (9 vs. 13 sprints) compared to the normoxic trained control group (9 vs. 9 sprints).	To the authors knowledge this is one of only two IHT studies that has successfully blinded the participants to the training F_IO_2.
Galvin *et al.* (2013)	IHT for 4 weeks; 3 sessions·week^{-1}, 10 x 6 s sprint efforts with 30 s passive recovery), at an altitude simulation of ~3900 m, or normoxic training (control group).	30 moderately trained academy level rugby players (the numbers in each group were not reported).	The distance completed during a repeated sprint test (Yo-Yo Intermittent Recovery Level 1 test) improved significantly more in the IHT group (+33 ± 12%) compared to the normoxic trained group (+14 ± 10%) ($P = 0.002$).	To the authors knowledge this is one of only two IHT studies that has successfully blinded the participants to the training F_IO_2.
Hendriksen & Meeuwsen (2003)	Daily IHT for 10 d, 120 min·session^{-1}, at an altitude simulation of ~2500 m, or normoxic training (control group).	16 moderately trained triathletes (8 in the IHT group, 8 in the control group), 12 of which 'crossed-over' one year later.	Mean and peak power output during a 30 s Wingate test were significantly improved after IHT (on average by +4%), and these changes were significantly different to the non-existent changes after normoxic training.	It is not clear whether participants were blinded to the training F_IO_2 or not.
Lecoultre *et al.* (2010)	IHT for 4 weeks; 3 sessions·week^{-1}, 66-100 min·session^{-1}, at an altitude simulation of ~3000 m, or normoxic training (control group).	14 moderately trained cyclists (7 in the IHT group, 7 in the control group).	Mean power output during a cycling TT and maximal power output during an incremental cycling test to exhaustion significantly increased in the IHT group (+7% and +4%, respectively) and the control group (+6% and +7%, respectively), but there were no significant differences between groups.	Participants were not blinded to the training F_IO_2, so placebo and nocebo effects likely occurred in the IHT and control groups, respectively.

Table 1.3 (continued)...

Study	Hypoxic exposure type and dose	Participant no. and training status	Key performance outcomes	Key considerations and/or limitations
Messonnier *et al.* (2004)	IHT for 4 weeks; 6 sessions·week^{-1}, 120 min·session^{-1}, at an altitude simulation of 3800 m, or normoxic training (control group).	13 untrained participants (5 in the IHT group, 8 in the control group).	T-Lim during a cycling TT significantly improved in both the IHT and control groups, but there was no significant difference between groups (mean change IHT +43% vs. control +63%, $P > 0.05$).	Whether participants were blinded or not to the training condition was not reported.
Morton & Cable (2005)	IHT for 4 weeks; 3 sessions·week^{-1}, 30 min·session^{-1}, at an altitude simulation of ~2750 m, or normoxic training (control group).	16 moderately trained team sport players (8 in the IHT group, 8 in the control group).	During an incremental cycle test, both work capacity (+16% vs. +18%) and $\dot{V}O_2max$ (+7% vs. +8%) significantly increased in the IHT and control group, respectively, but differences between groups were not significant.	Only the IHT participants trained in a normobaric hypoxic chamber, so they were not blinded to the training condition.
Robertson *et al.* (2010b)	IHT for 3 weeks; 4 sessions·week^{-1}, ~65 min·session^{-1}, at an altitude simulation of ~2200 m, while living at sea level (IHT group) or at simulated 2200 m (LH+TH+TL group).	17 moderately trained middle distance runners (9 in the IHT group and 8 in the LH+TH+TL group).	3000 m TT performance substantially improved after LH+TH+TL (mean -1.1%), but not after IHT (mean -0.1%).	No blinding of the hypoxic living condition, so placebo and nocebo effects likely occurred in the LH+TH+TL and IHT groups, respectively.
Roels *et al.* (2005)	IHT for 7 weeks; 2 sessions·week^{-1}, ~60 min·session^{-1}, at an altitude simulation of ~3000 m, or normoxic training (control group).	19 moderately trained cyclists and triathletes (11 in the IHT group, 8 in the control group).	$\dot{V}O_2max$ significantly increased in the IHT group (mean +9%), but not in the control group (mean +5%). There were no significant $\dot{V}O_2max$ or performance differences between groups.	Whether participants were blinded or not to the training condition was not reported.
Terrados *et al.* (1988)	IHT for 3-4 weeks; 4-5 sessions·week^{-1}, ~120 min·session^{-1}, at an altitude simulation of ~2300 m, or normoxic training (control group).	8 highly trained cyclists (4 in the IHT group, 4 in the control group).	Incremental cycle test work capacity was significantly increased after IHT (mean +33%, $P < 0.05$), but not in the control group (mean +22%, $P > 0.05$), although the difference between groups was only significant during the hypoxic tests.	Only the IHT participants trained in a hypobaric chamber, so they were not blinded to the training condition.

Table 1.3 (continued)...

Study	Hypoxic exposure type and dose	Participant no. and training status	Key performance outcomes	Key considerations and/or limitations
Terrados *et al.* (1990)	IHT for 4 weeks; 3-4 sessions·week^{-1}, 30 min·session^{-1}, at an altitude simulation of ~2300 m, or normoxic training (control leg).	10 untrained participants, who exercised each leg separately, one in hypoxic (IHT leg) and one in normoxia.	T-Lim during constant submaximal load single-legged cycling improved significantly more after IHT (28 to 117 min; +313%) compared to normoxic training (28 to 97 min; +242%).	Whether participants were blinded or not to the training condition was not reported.
Ventura *et al.* (2003)	IHT for 6 weeks; 3 sessions·week^{-1}, 30 min·session^{-1}, at an altitude simulation of ~3200 m, or normoxic training (control group).	12 moderately trained cyclists (7 in the IHT group, 5 in the control group).	Maximum power output during an incremental cycle test to exhaustion remained statistically unchanged in both the IHT and the control group (mean change IHT +0.8% vs. control -3.6%, $P > 0.05$).	Whether participants were blinded or not to the training condition was not reported. Maximum HR was significantly lower after training in both groups, which may indicate a state of over-reaching.
Vogt *et al.* (2001)	Low or high intensity IHT for 6 weeks; 5 sessions·week^{-1}, 30 min·session^{-1}, at an altitude simulation of ~3850 m, or equivalent normoxic training.	30 untrained participants (8 in the low and high intensity control groups, 7 in the low and high intensity IHT groups).	Incremental cycle test power output increased significantly more when results from both the low and high intensity IHT groups were combined (+16%), compared to the normoxic groups (+11%), but this was only the case for the tests conducted in hypoxia, not normoxia.	Participants were not blinded to the training condition (face masks were worn only by IHT participants).

LH+TH+TL = live high, train high and train low; IHT = intermittent hypoxic training, T-Lim = limit of tolerance; TT = time trial.

1.3.4   Performance efficacy of intermittent hypoxic exposure

Intermittent hypoxic exposure (IHE) is when individuals rest while inspiring normobaric hypoxic air, or while residing within a hypobaric hypoxic chamber for short durations, and perform all other daily tasks, including exercise training, in normoxia. As participants do not exercise during IHE, $\dot{V}O_2$ is at baseline, so it is possible to expose them to more severe degrees of hypoxia than during IHT interventions, without risking extreme biological hypoxia. For example, Hamlin & Hellemans (2007) had 22 moderately trained runners perform IHE for three weeks, which consisted of 5 min intervals of breathing either normoxic air (single blinded control group) or normoxic air and normobaric hypoxic air, that decreased in F_IO_2 over the three weeks from 0.130 (~3900 m) to 0.100 (~6000 m). The IHE group improved their 3 km performance on average by 2.2%, compared to an average 0.6% improvement in the control group, but considering that the standard error for this time trial performance was 2.3% in the IHE group and 1.7% in the control group, these results are inconclusive.

Aside from the potential to enhance the HVR (Faulhaber *et al.*, 2012; Garcia *et al.*, 2000; Townsend *et al.*, 2002), which does not appear to be a useful adaptation for athletic performance (Racinais *et al.*, 2010), other studies have found no benefits of IHE (Hinckson *et al.*, 2007; Humberstone-Gough *et al.*, 2013; Tadibi *et al.*, 2007). In particular, Truijens *et al.* (2008) reported no beneficial physiological changes, including in the velocity at $\dot{V}O_2$max, within or between an IHE group or a normoxic control group. This was a comprehensive double-blinded study in 23 well trained swimmers and runners, who undertook either IHE at a hypobaric hypoxic altitude simulation of 4000-5500 m, or normobaric normoxia, for 3 h·d^{-1}, 5 d·week^{-1}, for four weeks in total (Truijens *et al.*, 2008). As such, while IHE has been commonly marketed as providing a performance enhancing stimulus (Hinckson *et al.*, 2007), this does not appear to be justified. It is likely that the duration of hypoxic exposure is simply too short to elicit any beneficial adaptations (Levine, 2002).

1.4 Summary of the limitations of the research to date and the key remaining research questions

In summary, while there have been numerous insightful studies carried out in the field of athletic performance focused altitude and hypoxic training, there are also some major limitations common to much of this research, notably:

- A lack of control groups – while case studies of highly trained participants provoke interesting discussion, these reports do not substantially add to the understanding of altitude and hypoxic interventions. For example, it is unclear whether the participating athletes in the report by Fudge *et al.* (2011) benefited from the LH+TH exposure, or simply that they completed a favourable phase of endurance training. Similarly, a lack of an appropriate control group can confound results. For example, Robach *et al.* (2006a) reported that control group participants ingested significantly less dietary iron than those in the LH+TL group, and suffered a ~12% tHb reduction.

- Interventions being implemented without blinding of the experimental condition – this is crucial. For example, the ~4% $\dot{V}O_2$max improvement in the simulated LH+TL group and the ~5% $\dot{V}O_2$max reduction in the control group reported by Neya *et al.* (2013), were likely at least partially due to placebo and nocebo effects, respectively. In addition, while the IHT induced performance enhancements reported by Faiss *et al.* (2013b) and Galvin *et al.* (2013) are in contrast to much of the related literature, they are the only reports to have successfully blinded the participants to the experimental condition.

- An insufficient hypoxic dose being used, with conclusions being extrapolated to the wider context. For example, the lack of any measurable erythropoiesis after LH+TH for three weeks at 1816 m as reported by Pottgiesser *et al.* (2009) should not be taken out of context to mean that LH+TH does not result in haematological changes. The hypoxic dose was simply too small (Rasmussen *et al.*, 2013; Schmidt & Prommer, 2008).

- The effects of altitude or hypoxia being assessed in untrained or moderately trained participants, who respond to any training intervention to a far greater extent than highly trained athletes would. For example, it is unsurprising that performance changes equivalent to the +15% $\dot{V}O_2$max and +65% T-Lim improvements in moderately trained IHT participants in the report by Dufour *et al.* (2006) were not equalled in world-class cyclists who took part in the LH+TH study by Gore *et al.* (1998). As highly trained athletes may have reached a natural physiological limit in terms of certain physiological variables (Gore *et al.*, 1998), what constitutes a worthwhile performance change is largely governed by participant's baseline competitive level.

- Methodologies to assess physiological adaptations to altitude or hypoxia being inappropriate, for example, Czuba *et al.* (2011) and Ingjer & Myhre (1992) proposing haematological alterations after IHT and LH+TH, respectively, evidenced only by RBC concentration indices, even when it is clear that PV is highly variable during hypoxic exposure (Sawka *et al.*, 1996). Similarly, investigative methodologies being not sufficiently sensitive, or reliant on *in vitro* techniques, for example Robach *et al.* (2012) concluding that neither maximal capacity of oxidative phosphorylation nor mitochondrial efficiency occur in response to LH+TL, evidenced only by a single post-intervention muscle biopsy. In order to establish whether meaningful functional changes within skeletal muscle have occurred, multiple biopsy samples (Elder *et al.*, 1982), or alternative techniques that allow a greater sample frequency are required, such as the ^{31}P-MRS measurements used by Kuno *et al.* (1994).

The challenges of undertaking any such applied scientific research should be acknowledged, especially when using a particularly select cohort of participants, such as highly trained athletes. By addressing as many of the above limitations as possible, future experiments in this field of altitude and hypoxic training will be better placed to answer the key remaining questions.

1.5   Overall PhD rationale and aims

With too few properly controlled studies having been carried out, there is still debate as to whether traditional LH+TH or LH+TL, simulated LH+TL, or IHT can enhance athletic performance capacity. Given this research inconsistency, and suggestions that this has been due to a lack of appropriate control groups and participant blinding, relatively untrained participants being assessed, insufficient hypoxic doses, and inappropriate analytical methodologies, this thesis takes such factors into consideration. The overall aim is to assess the performance efficacy of traditional LH+TH and IHT interventions, in highly trained athletes, using proper control conditions wherever possible.

Additionally, there is much still to learn as to which mechanisms are primarily responsible for any performance enhancements with LH+TH and IHT; for example it is still debated whether performance changes are primarily due to haematological (Levine & Stray-Gundersen, 2005) or non-haematological adaptations (Gore *et al.*, 2007; Gore & Hopkins, 2005).

Therefore, to further the understanding of the mechanisms associated with traditional LH+TH and IHT, both haematological and non-haematological adaptations are assessed. While highly trained participants are investigated wherever is practically possible (Chapters 3 and 4), due to their strict training regimes and a lack of available time for more thorough assessments, this is not always possible. Therefore, the effects of IHT in healthy but untrained participants is investigated in Chapter 5, thus enabling more time intensive measurements to be taken.

The specific aims of each experimental chapter are:

- Chapter 3 – Using a parallel group design, to assess changes in competitive race performance and tHb in response to three weeks of traditional LH+TH in highly trained swimmers. It was hypothesised that traditional LH+TH of an appropriate hypoxic dose, similar to that used by Wachsmuth *et al.* (2013), would result in significantly greater haematological adaptations, and improved race performance, compared to a sea level control group.

- Chapter 4 – Using a blinded parallel group design, to assess changes in incremental exercise performance, and submaximal and maximal physiological variables in response to eight weeks of IHT in highly trained runners. It was hypothesised that an IHT intervention of an appropriate hypoxic dose, similar to that used by Dufour *et al.* (2006), would result in an improved $\dot{V}O_2max$ and enhanced incremental running performance, compared to a blinded normoxic trained control group.

- Chapter 5 – Using a blinded single-legged design, to assess changes in incremental exercise performance and skeletal muscle energetics in response to three weeks of IHT in healthy but untrained participants. It was hypothesised that a single-legged IHT intervention of an appropriate hypoxic dose, similar to that used by Terrados *et al.* (1990), would result in improved ^{31}P-MRS assessed skeletal muscle oxidative function and incremental exercise performance, compared to a (blinded) normoxic trained leg.

CHAPTER 2:

General Methods

The methodologies employed specific to each investigation are detailed within the relevant experimental chapter, but there are some procedures that require further detail, especially when common to two or three of the investigations. These consist of the ethical approval process (Chapters 3, 4 and 5), heart rate monitoring (Chapters 3, 4 and 5), venepuncture and venous blood analysis (Chapters 3 and 4), normobaric hypoxia production (Chapters 4 and 5), and the measurement of pulmonary O_2 uptake in a normobaric hypoxic environment (Chapter 4). Further detail regarding these general experimental methodologies is now provided.

2.1   Ethical approval process

Common to all experimental chapters was the requirement for ethical approval prior to the commencement of participant recruitment or testing. The process for ethical approval by the University of Exeter Ethical Approval Committee distinguishes all applications into Path A or Path B applications, namely:

Path A applications will:

- Involve low ethical risk procedures and participants (i.e. ≥18 y, and healthy).

- Not involve children or vulnerable adults.

- Not involve novel exercise protocols or substantially modified protocols.

- Include only non-invasive procedures for human test subjects.

Path B applications will:

- Include all studies not covered by Path A.

As such, all three experimental chapters in this thesis followed the University of Exeter Ethical Approval Committee Path B application pathway, and all were

granted ethical approval prior to commencement of any procedures. Appendix 1 comprises the formal ethical approval certificates that were specific to each chapter, Appendix 2 comprises the participant information sheets that were specific to each chapter, Appendix 3 comprises the informed consent forms that were specific to each chapter, and Appendix 4 comprises an example of the physical activity readiness questionnaire that was used in all three chapters.

2.2   Heart rate monitoring

Common to all experimental chapters was the assessment of participants' heart rate (HR) during exercise, for the purpose of monitoring exercise training intensity, as well as to assess any changes pre- to post-training.

In Chapters 3 and 4, HR was estimated using a coded chest strap transmitter (T31, Polar Electro, Kempele, Finland), which detected the R-R time interval of the electrocardiogram signal, and transmitted this as a single heart beat signal in real-time, via telemetry. This signal was received by a wrist watch (FT1 or S610i, Polar Electro, Kempele, Finland), which was held within a ~3 m transmission range of the chest strap. The accuracy of HR measurement using this equipment, as stated by the manufacturer, is ± 1 b·min^{-1}, with a measurable range of 15-240 b·min^{-1}.

In Chapter 5, HR (as well as S_aO_2) was estimated using a fingertip optical sensor (Nonin PureLight$^{®}$ 8000FC-30, Nonin Medical Inc., Plymouth, MN), connected via a ~9 m fibre-optic cable to a pulse-oximeter (Nonin 7500FO, Nonin Medical Inc., Plymouth, MN). This apparatus transmitted red and infra-red light across the fingertip to a photodetector. The surge of arterial blood during each heart beat caused expansion and contraction of arterial vessels, detected as brief light absorption increases. In this manner, a wave-form was created, with the time interval between peaks used to estimate individual heart beats (Jubran, 1999). This table-top pulse-oximeter is designed specifically for use within the magnetic resonance environment, and was situated at a safe distance away from the magnetic field of the superconducting magnet (Gyroscan Clinical Intera, Philips Medical Systems, Best, Netherlands). The accuracy of HR measurement using this equipment, as stated by the manufacturer, is ± 3 b·min^{-1}, with a measurable range of 18-300 b·min^{-1}.

2.3 Venepuncture and venous blood analysis

Common to Chapters 3 and 4 was the withdrawal and analysis of venous blood, for the purpose of assessing haematological adaptations to the altitude and hypoxic interventions, and to screen for illness.

Time of day, the timing of any preceding exercise, nutrition and hydration status, and ambient conditions were standardised for every test. Participants rested supine for 10 min, before venous blood samples were taken from an anti-cubital vein via venepuncture (Vacutainer®, BD Diagnostics, Oxford, UK), by a National Association of Phlebotomists trained and certified practitioner. Samples were transported to a local hospital laboratory where they were analysed within 2 h for RBC count, [Hb], HCT and total white blood cell count and differentials, by an automated cell counter (ADVIA 120, Siemens AG, Erlangen, Germany). From these variables the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated. Serum ferritin concentration ([sFe]) was quantified by a chemiluminescent microparticle immunoassay (ARCHITECT Ferritin Assay, Abbott Point of Care Inc, Birmingham, UK).

2.4 Normobaric hypoxia production

Common to Chapters 4 and 5 was the production of normobaric hypoxic air during training and testing protocols.

In Chapter 4, the reduced ambient F_IO_2 was produced by a custom built hypoxic generator that was installed within the architecture of the St Mary's University College Physiology Laboratory (S3 Hypoxic System, Sporting Edge UK Ltd, Basingstoke, UK). This apparatus consisted of an air compressor which, after multiple filters removing particles down to 0.01 μm in diameter, forced ambient (normoxic) air under ~6 bar into a hollow 'sieve' type fibre membrane. This pressure was created by a variable flow restrictor situated at the membrane output. The membrane was formed by a polymer, acting as a molecular filter, allowing O_2 molecules to leak out through the membrane walls, but retaining most N molecules. The system pressure was adjusted by the flow restrictor to produce an N enriched output gas, with an O_2 content of 0.100. The ambient O_2 content in the room was automatically monitored every 5 s, and the control

system alternated the air supply between N enriched air that had an O_2 content of 0.100, and normoxic air (O_2 content 0.209), to allow any user defined F_IO_2 to be maintained within an operational range of 0.130 to 0.209. In this manner, the desired ambient F_IO_2 was achieved without the need for participants to wear face masks, thus they remained unaware of the F_IO_2.

In Chapter 5, the reduced F_IO_2 inspirate was produced by a smaller, portable hypoxic generator (Cloud 9, Sporting Edge UK Ltd, Basingstoke, UK). The unit also used an air compressor, operating at ~1 bar, which fed into one of two alternatively cycled cylinders, which instead of a polymer membrane, contained zeolite crystals. As the compressed air was applied to the cylinder, some N molecules were trapped in the crystalline structure, while some O_2 molecules passed freely through into a waste exhaust. The pressure built within the first cylinder until a switch valve diverted the supply to the second cylinder. The cylinder that was pressurised released the air to produce an N enriched gas, which formed the hypoxic inspirate, and these cyclic bursts of N enriched air continued to be produced. An adjustment feature allowed a user defined F_IO_2 to be maintained within a range of 0.145 to 0.155, and a 'sham' unit was modified that had an output of 0.209, but was identical in appearance. The output from either unit was connected via a series of valves and a reservoir to the face mask from which participants breathed through during all the exercise training and pre- and post-intervention tests, thus they remained unaware of the F_IO_2.

The accuracy of F_IO_2 production from both the installed S3 Hypoxic System (Chapter 4) and the portable Cloud 9 generator (Chapter 5), as stated by the manufacturer, was ± 0.001, and this was regularly checked using a high accuracy paramagnetic O_2 analyser (Servomex 5200, Servomex, Crowborough, UK). Whether the F_IO_2 was set at 0.160 (experimental condition in Chapter 4), 0.145 (experimental condition in Chapter 5) or 0.209 (control condition in both Chapters 4 and 5), the hypoxic generators were switched on during all training and testing to ensure complete blinding of the environmental condition. Additionally, the control panels of these generators were blocked from the view of the participants, and also from the view of the researcher who supervised the exercise testing protocols during Chapter 5.

2.5 Estimation of pulmonary O_2 uptake in a normobaric hypoxic environment

During the pre- and post-training tests in Chapter 4 the ability to accurately measure $\dot{V}O_2$ in a normobaric hypoxic environment was required. In order to use an open-circuit breath-by-breath analyser, it required adaptations of certain calibration and normal usage procedures. At the time the manufacturer of the Jäeger Oxycon-Pro® (VIASYS Healthcare, Höechberg, Germany) were unable to advise on this matter, as they themselves had never used it within a reduced F_IO_2 environment. As such, a phase of pilot testing, and then a short study was carried out to assess the reliability and validation of these novel procedures. The results were presented as an e-poster at the 2011 Congress of the European College of Sport Science in Liverpool (see Appendix 5 – 'The Jäeger Oxycon Pro® provides a reliable estimate of pulmonary oxygen uptake in normobaric hypoxia'), and form the following brief report.

2.5.1 Abstract

AIMS: To investigate the validity of $\dot{V}O_2$ estimations by the Jäeger Oxycon Pro® (OXYCON) expired air analyser in normobaric hypoxia (NH), compared with the Douglas bag method (DBM). METHODS: The OXYCON calibration procedures were adjusted to assess $\dot{V}O_2$ in NH. 10 recreationally trained male cyclists completed two identical submaximal cycling tests, in NH (F_IO_2 = 0.160 ± 0.001) and normobaric normoxia (SHAM), in a single blinded design. Tests consisted of 4 x 4 min stages at 120 W, and 4 x 4 min stages at 160 W, during which $\dot{V}O_2$ was estimated by the OXYCON and DBM. RESULTS: Statistical comparisons between the two methods in SHAM gave a CV of 5.0% at 120 W, and 3.3% at 160 W, and in NH a CV of 4.3% at 120 W, and 3.1% at 160 W. The OXYCON provided $\dot{V}O_2$ estimates that were on average 3.6 and 2.5% higher than the DBM in NH ($P > 0.05$), whereas estimates in SHAM were 6.6 and 3.4% higher than the DBM ($P < 0.05$), at 120 and 160 W, respectively. CONCLUSIONS: The OXYCON is capable of estimating breath-by-breath $\dot{V}O_2$ in ambient NH, and while results are at least as reliable as when used in SHAM, the validity is questionable as the OXYCON tends to over-estimate submaximal $\dot{V}O_2$ compared to the criterion DBM.

2.5.2 Introduction

The closed-circuit Douglas bag method (DBM) for estimating $\dot{V}O_2$ was initially designed to assess patients at rest, but even in the early 20th Century, the DBM pioneer Claude Gordon Douglas confirmed it was equally suitable for assessments during "violent muscular work, such as running" (Douglas, 1911). Since then, the DBM has been widely used during exercise testing. When human and technical error is minimised, and used in combination with a precise O_2 analyser, the DBM provides arguably the 'gold standard' approach for whole body $\dot{V}O_2$ measurement (Macfarlane, 2001).

However, more recently open-circuit breath-by-breath analysers have allowed greater insights into the physiological conditioning of athletes, for example, the assessment of $\dot{V}O_2$ kinetics (Jones *et al.*, 2006). One such analyser, the Jäeger Oxycon-Pro® (OXYCON; VIASYS Healthcare, Höechberg, Germany) provides both valid and reliable estimates of $\dot{V}O_2$ in normobaric normoxia (NN) (Carter & Jeukendrup, 2002; Foss & Hallen, 2005; Rietjens *et al.*, 2001), but to date its reliability and validity in normobaric hypoxia (NH) has not been reported.

Numerous investigations have estimated $\dot{V}O_2$ under NH conditions by administering the inspirate directly through a face mask (Dufour *et al.*, 2006; Holliss *et al.*, 2013), but with the ever increasing research and applied sport science interest in NH exposure, specifically in quantifying exercise economy and maximal aerobic capacity in athletes while exercising in NH, there is a need for breath-by-breath $\dot{V}O_2$ estimations in ambient NH. As the OXYCON is renowned for providing quality $\dot{V}O_2$ estimates in NN, there is a clear requirement to clarify how valid its $\dot{V}O_2$ estimates are in NH.

Therefore, the aim of this study was to investigate the reliability and validity of $\dot{V}O_2$ estimations by the OXYCON in NH, compared with the DBM. Additionally, this document serves to communicate the bespoke calibration adaptations to use the OXYCON in NH, which have not been reported elsewhere.

2.5.3   Materials and methods

Following institutional ethical approval, 10 male cyclists (age: 29.4 ± 7.1 y, body weight: 74.9 ± 5.2 kg), who routinely undertook exercise training for ≥ 5 h·week^{-1}, gave their written informed consent, then participated in this study.

Three identical exercise tests were performed, using an SRM cycling ergometer (SRM International, Jülich, Germany): firstly a familiarisation test, then two exercise testing protocols, in either NN (SHAM) or NH. Each test was separated by at least 48 h, and they were performed in a randomised order. The exercise testing protocol started with a 6 min warm up at 120 W, followed by 4 x 4 min stages at 120 W, 2 min at 160 W (to achieve a new $\dot{V}O_2$ steady state), and 4 x 4 min stages at 160 W, as illustrated in Figure 2.1. These relatively low work rates were selected in order to avoid any participant from exercising above their critical power, as if this had occurred the $\dot{V}O_2$ slow component would have caused an increased O_2 cost of exercise (Poole *et al.*, 1988), and therefore may have resulted in a less stable steady state.

Participants initially self-selected their cycling cadence, then maintained this constantly throughout all testing. The SRM ergometer was set in 'hyperbolic mode', thus achieving a constant work rate throughout all testing, regardless of small fluctuations in cycling cadence. Expired air was analysed during the final 60 s of each stage with the OXYCON or DBM, in a counterbalanced order.

Environmental conditions were controlled by an S3 Hypoxia, Temperature & Humidity System (Sporting Edge UK Ltd, Basingstoke, UK) allowing single blinding of the F_IO_2. During NH tests F_IO_2 was 0.160 ± 0.001, temperature was 19.9 ± 0.2 °C, and humidity was 43.5 ± 2.3%, and during SHAM tests F_IO_2 was 0.209 ± 0.001, temperature was 19.8 ± 0.3 °C, and humidity was 43.0 ± 2.7%. Environmental conditions were factored into all $\dot{V}O_2$ analyses.

Prior to all testing, to enable its use in NH, the OXYCON calibration was achieved using an adapted procedure (Figure 2.2). Briefly, during gas calibration and background zero phases of the automated OXYCON calibration, the ambient air intake and sample line were connected to a bottled normoxic air supply (BOC Special Gasses, Guildford, UK). Once calibration was completed, the gas supply was removed, and the remainder of the volume calibration and

subsequent $\dot{V}O_2$ estimations were carried out according to the manufacturer's instructions (i.e. drawing in ambient air). An alternative method whereby a capillary tube was vented to the outdoors ambient air was trialled, but the OXYCON intake pump was not powerful enough to achieve a sufficient flow rate, hence the bottled gas was required.

During DBM analyses, the fraction of expired O_2 (F_EO_2) was measured using a Servomex 5200 paramagnetic O_2 analyser (Servomex, Crowborough, UK), from the air collected via a Hans Rudolph face mask and two-way valve into 150 L Douglas Bags (Cranlea & Co, Birmingham, UK). During F_EO_2 analyses the sample air was constantly returned to the Douglas bag using a closed system, and the expired 60 s volume (V_E) was measured using a dry gas meter (Harvard Apparatus, Edenbridge, UK). The method used in NH was identical to that in SHAM, in both cases ensuring that the ambient F_IO_2 was measured with the Servomex 5200, and adjusted accordingly in the following calculation:

$$\dot{V}O_2 = (\dot{V}_I \cdot F_IO_2) - (\dot{V}_E \text{ STPD} \cdot F_EO_2).$$

Where: $\dot{V}O_2$ = pulmonary O_2 uptake, $\dot{V}_I$ = inspired minute volume, F_IO_2 = fraction of inspired O_2, $\dot{V}_E$ = expired minute volume, STPD = standardised for temperature (equivalent to 0 °C) and pressure (equivalent to 760 mmHg), and desaturated (equivalent to 0% relative humidity), and F_EO_2 = fraction of expired O_2.

The Servomex 5200, S3 Hypoxia, Temperature & Humidity System, and SRM Ergometer were calibrated according to the manufacturer's instructions prior to all testing.

Calibration equations and correlation coefficients were calculated using linear regression analyses in Excel (Microsoft UK, Reading, UK), and standard error of the estimate (SEE) and CV were computed (Hopkins *et al.*, 2009). Two-tailed students' paired t-tests were used to assess differences between the two analysers using PASW Statistics (v18.0, IBM SPSS, Portsmouth, UK), with the probability level of $P < 0.05$ being accepted as statistically significant.

Stage	Warm Up	Passive Rest	Analysis Stage One					Analysis Stage Two				
Analyser	N/A	N/A	N/A	OXYCON	DBM	OXYCON	DBM	N/A	DBM	OXYCON	DBM	OXYCON
Power (W)	120	0	120	120	120	120	120	160	160	160	160	160
Duration (mm:ss)	06:00	06:00	02:00	03:30	03:30	03:30	03:30	02:00	03:30	03:30	03:30	03:30

Figure 2.1: Experimental protocol; participants warmed up at 120 W, then after a rest period during which the apparatus was fitted, the 120 W and 160 W test stages commenced. The two "steady" stages were designed to achieve a steady state $\dot{V}O_2$ prior to analysis. The analyser order was alternated and evenly matched between each test.
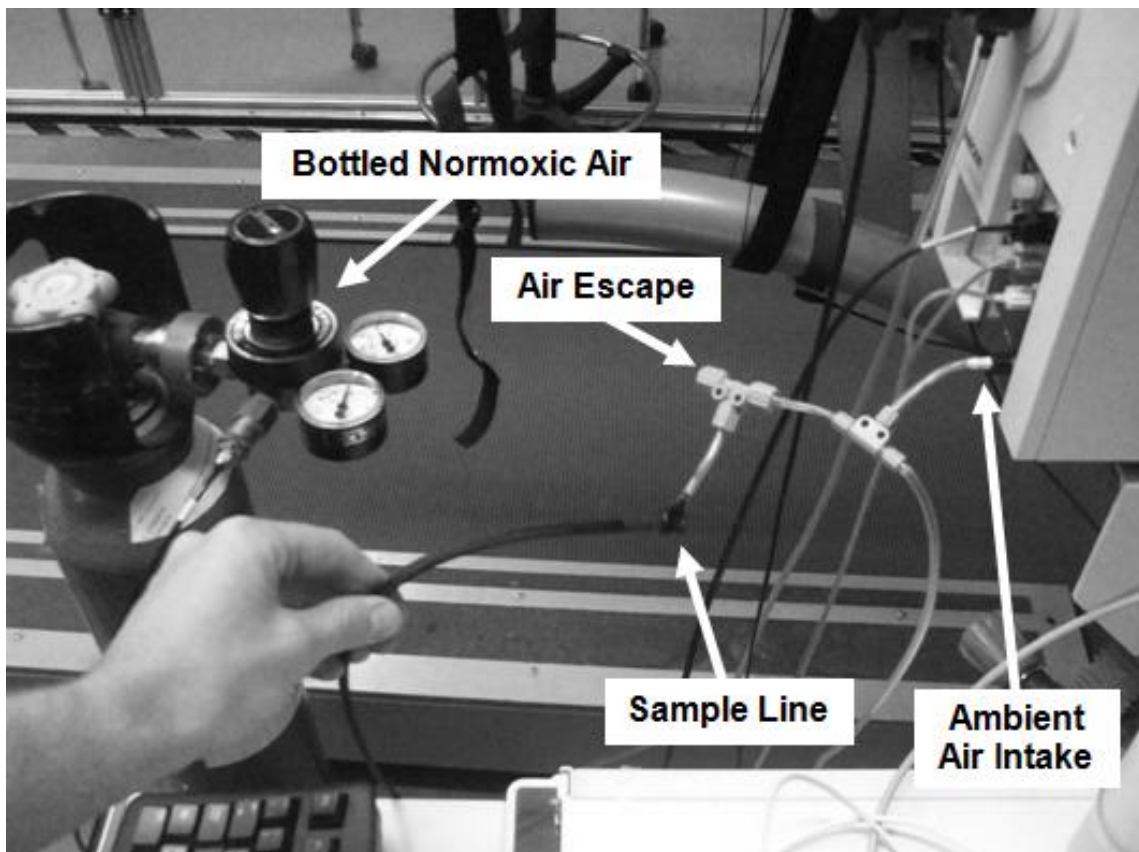
Figure 2.2: Adapted configuration to supply the OXYCON ambient air intake and sample line with bottled normoxic gas, thus allowing subsequent calibration according to the manufacturer's instructions.

2.5.4   Results

Figure 2.3 illustrates the degree of $\dot{V}O_2$ estimate agreement between the 'criterion' DBM with the OXYCON. Statistical comparisons between the two analysers in SHAM gave a CV of 5.0% at 120 W (95% likely range: 3.4 to 9.8%), $R^2 = 0.70$, and 3.3% at 160 W (95% likely range: 2.2 to 6.3%), $R^2 = 0.85$. In NH the CV was 4.3% at 120 W (95% likely range: 2.9 to 8.4%), $R^2 = 0.74$, and 3.1% at 160 W (95% likely range: 2.1 to 6.0%), $R^2 = 0.82$.

Table 2.1 shows that the OXYCON $\dot{V}O_2$ estimates were significantly higher than the DBM in SHAM at 120 and 160 W ($P < 0.05$), but not in NH ($P > 0.05$). The OXYCON provided $\dot{V}O_2$ estimates in NH that were on average 3.6 and 2.5% higher than the DBM, whereas in SHAM the OXYCON $\dot{V}O_2$ estimates were 6.6 and 3.4% higher than the DBM, at 120 and 160 W, respectively.
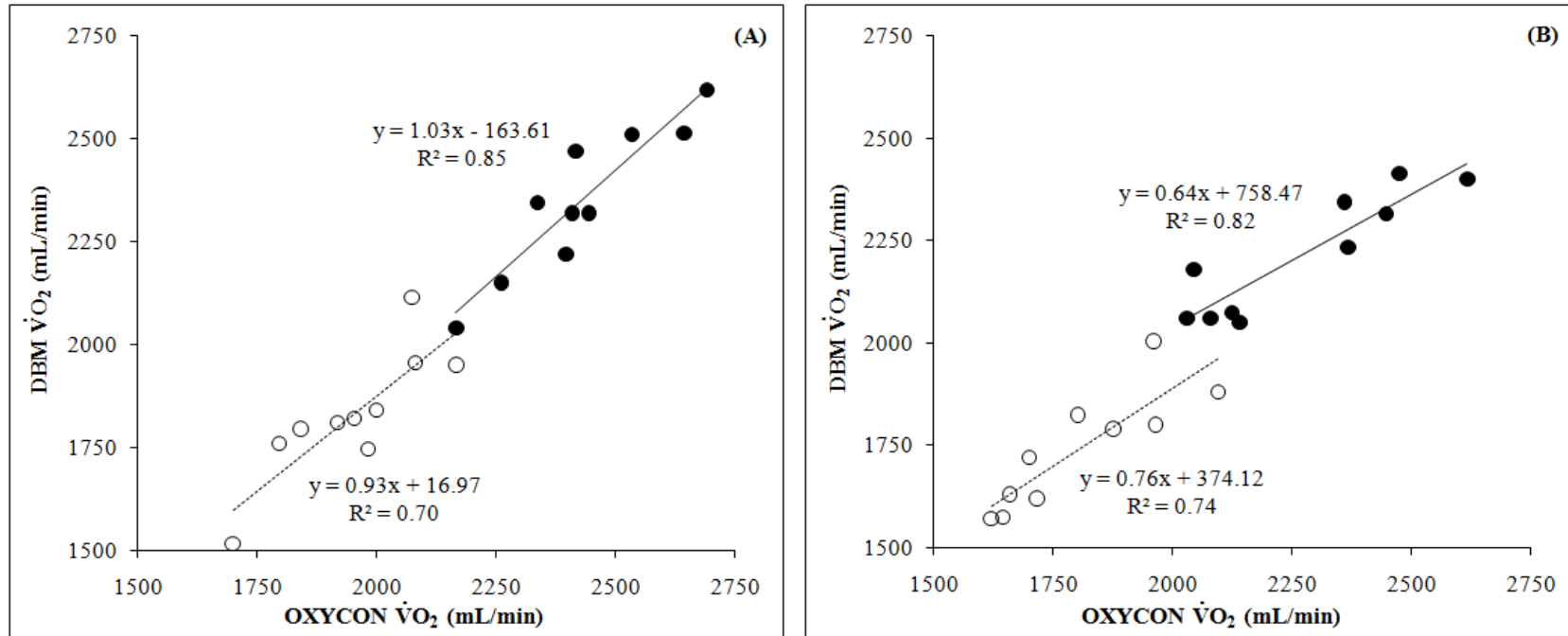
Figure 2.3: OXYCON vs. DBM in (A) SHAM, and (B) NH (○ 120 W; ● 160 W). This illustrates the agreement between OXYCON and DBM in both environments, at both work rates, and the regression equations and R^2 linearity slope for each.

Table 2.1: Mean OXYCON and DBM $\dot{V}O_2$ estimations at 120 and 160 W, in SHAM and NH.

*Significant difference between the OXYCON and DBM $\dot{V}O_2$ estimations ($P < 0.05$).

Environment / Work Rate	Mean OXYCON $\dot{V}O_2$ (mL/min)	Mean DBM $\dot{V}O_2$ (mL/min)
SHAM at 120 W	1951 ± 143 *	1831 ± 158
SHAM at 160 W	2430 ± 161 *	2351 ± 181
NH at 120 W	1804 ± 163	1742 ± 144
NH at 160 W	2269 ± 209	2214 ± 148

2.5.5   Discussion

The CV's between analysers in both environments are within those reliability CV's reported during SHAM trials by Carter & Jeukendrup (2002), where at 100 W CV's were 5.1 ± 2.1% for the DBM, and 6.5 ± 2.0% for the OXYCON, and at 150 W CV's were 3.3 ± 2.5% for the DBM and 4.7 ± 1.2% for the OXYCON. In both the present study and that by Carter & Jeukundrup (2002), higher work rates elicited more reliable estimates than lower work rates.

Data linearity in the present study were somewhat lower than the $R^2 = 0.96$ reported from NN trials by Rietjens *et al.* (2001), but much of this is likely due to the differing methodologies employed. Specifically, Rietjens and colleagues measured $\dot{V}O_2$ via the OXYCON and DBM simultaneously using a modified volume transducer housing, whereas the same face mask was used in the present study, using two separate collection devices, during two separate exercise stages. While the methods of the present study may have introduced greater variation in $\dot{V}O_2$ estimates, they were more ecologically valid, because in ordinary daily usage only one of the analysers is ever used at any one time. Furthermore, the fact that Rietjens *et al.* (2001) had highly trained participants is likely to have resulted in more consistent cadence and less variable pedalling technique, so potentially less variable $\dot{V}O_2$.

As observed in the present study, and as has been reported by others, higher work rates tend to improve the $\dot{V}O_2$ agreement between OXYCON and DBM, which may partially explain the more favourable R^2 seen by Rietjens *et al.* (2001), who used a step test to volitional exhaustion. In the present study higher work rates were purposefully avoided, due to the fact that above an individual's critical power, the $\dot{V}O_2$ slow component results in an increasing O_2 cost of exercise (Poole *et al.*, 1988), which would probably add to the variability in analyser agreement as assessed by the protocols in the present study. Again, this is in line with usual laboratory procedures, whereby exercise economy is usually assessed at exercise intensities below critical power.

In contrast to the finding that the OXYCON overestimated $\dot{V}O_2$ in both environments (see Table 2.1), Foss & Hallen (2005), in a particularly well controlled study using a step test protocol, found the OXYCON to slightly

underestimate the DBM in SHAM, but by only 0.8% ($P < 0.05$). The more favourable variability between analysers reported by Foss & Hallen (2005) was again probably due to more robust methodologies being employed, most notably as they used a computerised DBM collection system that was able to precisely control the DBM sampling time with end ventilation. This was also connected to the OXYCON to allow simultaneous $\dot{V}O_2$ assessments by both analysers, meaning that the exact same air was analysed.

Unfortunately there are no studies with which to compare the present study's OXYCON validity results in NH, but it is clear that compared to SHAM trials, the OXYCON functioned at least as reliably in NH. While this novel investigation was strengthened by the homogenous group of participants, it would be useful to further assess the validity of the OXYCON in NH with more participants, during exercise requiring higher ventilatory rates, and at a higher metabolic cost. The latter may prove problematic due to the $\dot{V}O_2$ slow component introducing further variability, therefore future related investigations in NH may use more highly trained participants with inherently high ventilatory rates, or alternatively a 'pulmonary simulator', as used by Gore *et al.* (1997). These pulmonary simulators are useful in providing detailed information regarding whether $\dot{V}O_2$ estimation errors are due to the volume transducer or gas concentration probe, thereby shedding further light on precisely how such analysers are performing. By employing these methods, confidence will be gained when estimating breath-by-breath $\dot{V}O_2$ under NH conditions, in order to calculate an athlete's $\dot{V}O_2$ kinetics, exercise economy, and $\dot{V}O_2$max.

In conclusion, when adopting the modified calibration procedures described herein, the Jäeger Oxycon-Pro[®] provides a somewhat higher estimation of $\dot{V}O_2$ compared to the gold-standard Douglas bag method, but most importantly, the resultant breath-by-breath $\dot{V}O_2$ results are reliable. Therefore, the Jäeger Oxycon-Pro[®] provides a suitable means of assessing $\dot{V}O_2$ in normobaric hypoxia, as part of a repeated measures trial.

CHAPTER 3:

Three Weeks Of Traditional Altitude Training at 2320 m Increases Total Haemoglobin Mass But Does Not Improve 200 m Race Performance In Highly Trained Swimmers

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Chapter 3 is currently being peer-reviewed in an international journal.

3.1   Abstract

PURPOSE: To investigate tHb and performance changes after altitude training in highly trained swimmers. METHODS: 11 highly trained swimmers undertook altitude training (ALT; three weeks at 2320 m), or formed a control group (CONT; three weeks at sea level). Weekly swimming volumes, categorised into four training zones, were compared between ALT and CONT. tHb was assessed via CO-rebreathing immediately before and 1, 14 and 28 d afterwards. 200 m race performances were assessed before and 25 d afterwards. RESULTS: Training volumes and proportions of training in each training zone were not significantly different between ALT and CONT ($P > 0.05$). The tHb change immediately post-ALT was significantly greater than the change immediately post-CONT (+0.6 ± 0.4 g·kg^{-1}, or +4.4 ± 3.2% vs. +0.03 ± 0.1 g·kg^{-1}, or +0.3 ± 1.0%, $P = 0.04$), but the tHb change within the ALT group was only a (non-significant) trend ($P = 0.08$). 200 m swimming race performances were faster post-intervention across both groups ($P = 0.01$), but there were no significant differences in the performance changes between the ALT and CONT groups (-0.8 ± 1.5 s, or -0.6 ± 1.2% vs. -0.4 ± 0.4 s, or -0.3 ± 0.3%, $P = 0.76$). CONCLUSIONS: Traditional altitude training resulted in some positive haematological adaptations, but this did not lead to significantly improved 200 m race performances over and above that observed after sea level training.

3.2   Introduction

A sufficient 'hypoxic dose' is required to elicit an increase in the tHb or RCV when athletes spend time at altitude (Wilber *et al.*, 2007). In a two year assessment of highly trained swimmers, Wachsmuth *et al.* (2013) reported that 3-4 week LH+TH altitude camps at 2320 m resulted in a mean ~7% tHb increase. Similarly, Gough *et al.* (2012) also reported a significant mean ~4% tHb increase after highly trained swimmers undertook three weeks of LH+TH at 2135-2320 m. However, even when erythropoietin is elevated, this is not always followed by increases in tHb or RCV (Friedmann *et al.*, 2005; Gore *et al.*, 2006; Siebenmann *et al.*, 2012). Accordingly, some researchers have found no significant tHb changes after sustained hypoxic exposure (Gore *et al.*, 1998; Robach *et al.*, 2012; Saunders *et al.*, 2004; Siebenmann *et al.*, 2012).

Given that the altitude training studies to date have all varied in terms of the subtleties of their design, it is difficult to predict the likely erythropoietic effects. As such, two recent related meta-analyses provide useful resources: Rasmussen *et al.* (2013) assessed RCV results from studies that used CO-rebreathing, plasma dye dilution, or radio-labelled albumin methods, and Gore *et al.* (2013) assessed tHb results from studies that used CO-rebreathing. The haematological responses predicted to occur after LH+TH for 21 d at 2320 m are a ~2.5% RCV increase according to Rasmussen *et al.* (2013), and a ~5% tHb increase according to Gore *et al.* (2013), both with a probability of >95%. The likely reason for these differences is the disparity in the error margins of the RCV and tHb methods. Nevertheless, given that these predictions are markedly lower than the mean tHb increase of ~7% reported by Wachsmuth *et al.* (2013), it is important that they are thoroughly tested, in highly trained athletes.

Moreover, the functional importance of any post-altitude haematological changes is also uncertain. While Schmidt & Prommer (2010) reported that a 1 g tHb increase results in a ~4 mL·min^{-1} $\dot{V}O_2$max increase, Saunders *et al.* (2013) assessed numerous data sets (n = 145), and found that post-altitude tHb changes explained less than one-sixth of the variations in $\dot{V}O_2$max.

In terms of competitive race performance, a recent study by Chapman *et al.* (2014) reported significant 3000 m time trial improvements (mean change

~+3%) after moderately trained runners lived at 2085 m, and trained between 1250 - 3000 m, for four weeks. In this same investigation, another evenly matched group undertook the same training, while living at 2800 m, but did not experience any performance improvements (mean change ~+0.1%), despite both groups achieving indistinguishable RCV increases (mean change ~+6%). As such, these authors concluded that erythropoiesis is necessary, but not alone sufficient, to improve post-altitude performance (Chapman *et al.*, 2014)

However, the study by Gough *et al.* (2012) found that performances improved more in swimmers who remained at sea level, than those who undertook altitude training. It should be noted that these "quasi-control group" participants were not physically part of this study, so there were no assessments of training loads. Similarly, although Wachsmuth *et al.* (2013) found that tHb was positively related to swimming performance over a competitive season, its role after return from altitude was unclear – performance only improved 3-4 weeks later (Wachsmuth *et al.*, 2013). One issue with this latter study is that race times were converted to Fédération Internationale de Natation (FINA) Points, to allow analyses between different strokes and races in 25 m and 50 m pools. This scalar system which gives 1000 points for the current World Record time, and greater or fewer points for proportionally faster or slower times, respectively, results in highly variable outcome data, mostly dependant on World Record progression. As such, the time-course of post-altitude competitive swimming performance changes requires further investigation.

The first aim of this study was to assess tHb and absolute race performance, and the relationship between these variables, in a group of highly trained swimmers before and after a three week LH+TH altitude camp at 2320 m, compared to a sea level control group. A second aim was to assess tHb and race performance in one highly trained female swimmer undertaking a second LH+TH altitude camp in preparation for the World Championships, held 24 d post-altitude. It was hypothesised that after the three week LH+TH intervention at 2320 m: i) tHb would increase, on average by 3-5%, and more so than in the sea level control group (Gore *et al.*, 2013; Rasmussen *et al.*, 2013), ii) race performances would improve more so than in the sea level control group (Wachsmuth *et al.*, 2013), and iii) that changes in race performance would be related to tHb changes.

3.3   Methods

3.3.1   Participants

After being granted approval from the University of Exeter Ethical Approval Committee, nine male (age: 24.6 ± 3.4 y, body weight: 76.1 ± 5.6 kg) and four female (age: 21.2 ± 1.7 y, body weight: 66.0 ± 6.0 kg) highly trained swimmers provided written informed consent, completed a physical activity readiness questionnaire, then participated in this study (mean baseline FINA Points = 817 ± 37). Participants' habitual weekly swimming durations ranged from 16-20 h, over 9-10 sessions, and 2-4 h of land exercises.

3.3.2   Experimental design

Following a three week break from training, to screen for illness and any iron deficiencies, [sFe] and FBC were measured. All participants then completed three months of sea level training.

Race performances in the participants' best 200 m event was assessed at an international standard competition within two weeks before participants were split into an altitude group (ALT; six males and two females), or a control group (CONT; three males and two females). Baseline tHb, [sFe], and FBC were measured within 48 h before the ALT group travelled to Sierra Nevada, Spain (2320 m), where they lived and trained for 21 nights, while the CONT group lived and trained at sea level. tHb, [sFe], and FBC measurements were repeated at 1, 14, and 28 d after the ALT or CONT intervention, and race performances in the same 200 m events were again assessed after 25 d.

3.3.3   Training intervention

During ALT and CONT, the total accumulated swimming distances for each training session were recorded for each participant, within four 'training zones': 1) aerobic (<3 mM blood [lactate] ([BLa])), 2) anaerobic threshold (3-5 mM [BLa]), 3) between 400 m to 100 m race pace, and 4) maximal speed. To help differentiate between these first two categories, capillary [BLa] was regularly measured during swimming training from an earlobe using a hand-held portable analyser (Lactate Pro, Akray Ltd, Kyoto, Japan).

3.3.4 Resting haematology

Time of day, hydration status, and ambient conditions were standardised. Participants rested supine for 10 min, before venous blood samples were taken from an anti-cubital vein. Samples were analysed within 3 h for FBC using an automated cell counter (ADVIA 120, Siemens AG, Erlangen, Germany) and [sFe] using a chemiluminescent microparticle immunoassay (ARCHITECT Ferritin Assay, Abbott Point of Care Inc, Birmingham, UK).

tHb was tested using the optimised CO-rebreathing method as described by Schmidt & Prommer (2005). Briefly: participants rested while seated for 15 min, after which capillary blood samples were taken in duplicate from an earlobe, and immediately analysed for carboxyhaemoglobin percentage (COHb%) (OSM-3, Radiometer Medical, Copenhagen, Denmark). Participants fully exhaled through the nose, which was then immediately closed, and then fully inhaled as a syringe filled with pure medical grade CO (BOC Special Gases, Guildford, UK), at a dose of 1.0 mL·kg^{-1} for males, and 0.8 mL·kg^{-1} for females, was emptied into the spirometer, with 3.0 L of medical grade O_2 (BOC Special Gases, Guildford, UK). Participants held this first breath for 10 s, and then breathed normally through the spirometer for 1 min 50 s. After this 2 min rebreathing phase, participants exhaled maximally to residual volume into an anaesthetic bag to enable quantification of unabsorbed CO. Quadruple COHb% measures were again taken from the earlobe at both 4 and 6 min post-CO exhalation, and mean values were calculated.

3.3.5   Data analyses

tHb was calculated using the optimised CO-rebreathing equations (Schmidt & Prommer, 2005) and software (SpiCO® Calculation Software, Blood tec, Bayreuth, Germany). The CV for this tHb methodology in the experimental laboratory during the time of this study was 2.2%. tHb was measured in duplicate (24-48 h interval) at baseline. The mean from the two tests was recorded, unless the variance was >2.2%, in which case a third test was conducted, and a mean between the two measures in closest agreement was recorded.

3.3.6 Statistical analyses

Independent samples t-tests were used to assess differences between the total and the intensity categorised weekly swimming distances. Analysis of covariance (ANCOVA) was used to assess race performance and tHb changes pre- to post-training between ALT and CONT, with the absolute pre-training values entered as covariates, to account for any baseline differences. Bonferroni pairwise comparisons were used to assess at which time point any tHb differences occurred. Pearson product-moment correlation coefficients were calculated between the changes in race performance times and tHb values.

All statistical analyses were performed using PASW Statistics (v18.0, IBM SPSS, Portsmouth, UK), with the probability level of $P < 0.05$ being accepted as statistically significant. Data sets were checked for normality of distribution prior to analyses. All data were reported as mean ± standard deviation (SD).

3.4 Results

Of the 13 participants, 11 finished the study, and two participants dropped out due to illness during the training intervention (one male from ALT, and one female from CONT). Neither the total or the intensity categorised weekly swimming training volumes were significantly different between the ALT vs. CONT groups: total 53.8 ± 2.7 vs. 51.1 ± 1.8 km·week^{-1} ($P = 0.51$); aerobic 45.8 ± 5.1 vs. 41.8 ± 0.9 km·week^{-1} ($P = 0.09$), anaerobic threshold 3.6 ± 1.7 vs. 4.5 ± 0.7 km·week^{-1} ($P = 0.13$), race pace 3.9 ± 0.8 vs. 4.1 ± 0.4 km·week^{-1} ($P = 0.17$), maximal speed 0.6 ± 0.1 vs. 0.7 ± 0.1 km·week^{-1} ($P = 1.00$).

3.4.1 Haematology

During ALT and CONT, [sFe] remained above 40 µg·L^{-1} in all participants, and the medical team had no concerns regarding the FBC red or white blood cell differential variables, which did not significantly differ from baseline. Baseline tHb relative to body weight was 13.7 ± 0.9 g·kg^{-1} (males) and 11.6 ± 0.4 g·kg^{-1} (females).

The ANCOVA revealed a significant tHb and time interaction, with the tHb change immediately post-ALT being significantly greater than the change immediately post-CONT (+4.4 ± 3.2% vs. +0.3 ± 1.0%, $P = 0.04$; see Figure

3.1). However, the within group ANCOVA only showed a (non-significant) trend for this tHb increase within the ALT group ($P = 0.08$). The only significant within group tHb changes were the decreases during the two week periods from immediately post-ALT to 14 d ($P = 0.01$) and 28 d ($P = 0.02$) post-ALT.

3.4.2 Race performance

Of those who completed the study, 200 m race performance improved in five of the seven ALT participants, and similarly in three of the four CONT participants (Table 3.1). The between-group ANCOVA revealed a significant main effect for time, i.e. when all participants were grouped together, race performances were faster post-intervention, independent of the training condition ($P = 0.01$). However, although this race performance improvement was significant within the ALT group (-0.6 ± 1.2%, $P = 0.02$), and not within the CONT group (-0.3 ± 0.3%, $P = 0.94$), there were no significant differences between the ALT and CONT groups ($P = 0.76$; see Table 3.1).
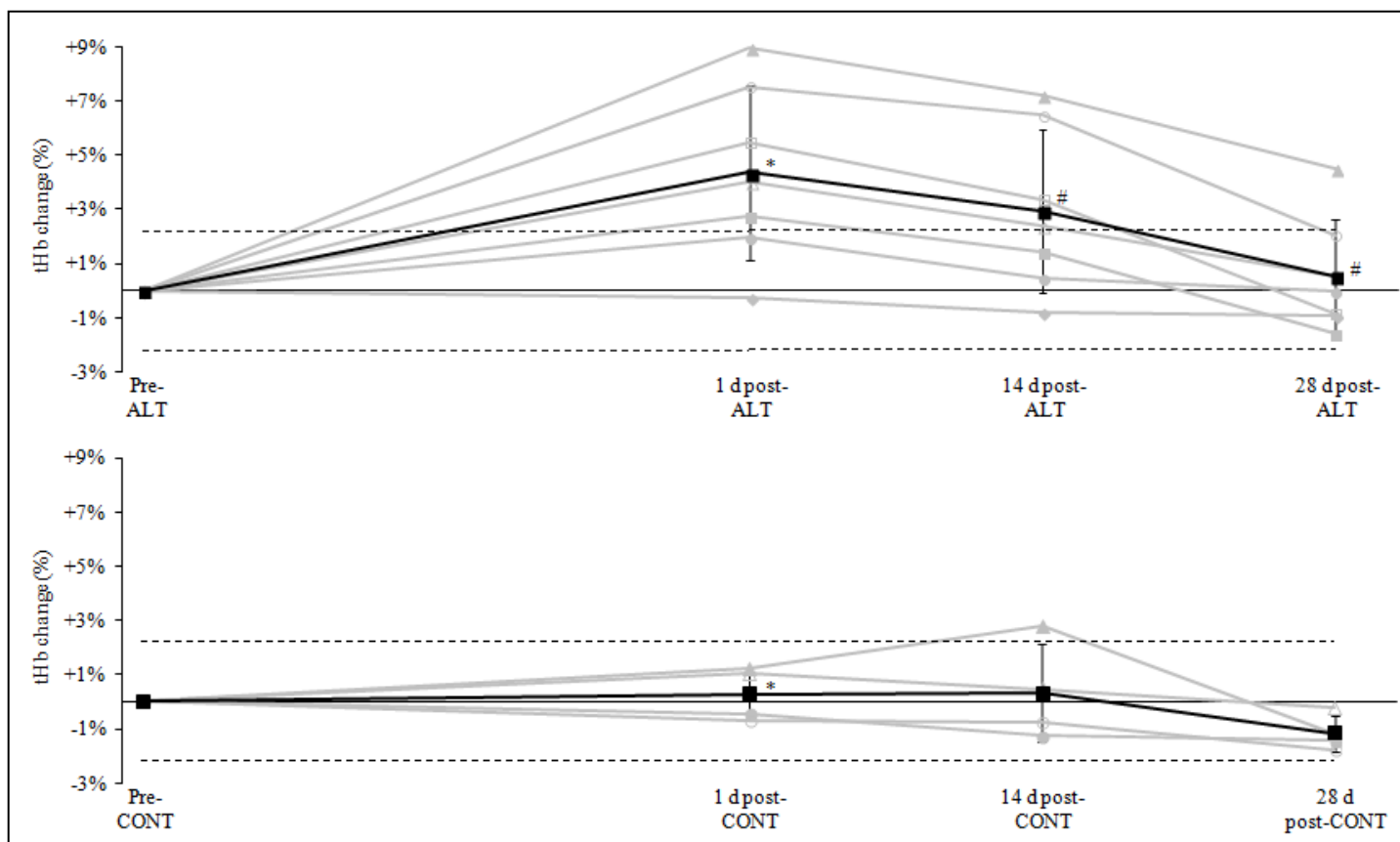
Figure 3.1: tHb percentage change from baseline to 1, 14, and 28 d post-ALT (upper panel) and CONT (lower panel). Grey lines represent individual swimmers, and black lines represent the mean change ± SD. Dotted lines represent the CV of ±2.2% for tHb. [#]Significant difference from baseline to immediately post-ALT ($P < 0.05$). *Significant difference between the ALT and CONT groups ($P < 0.05$). The tHb change immediately post-ALT was significantly greater than post-CONT ($P = 0.039$), but this within ALT group increase was not significant (+4.4 ± 3.2%, $P = 0.08$). tHb then reduced thereafter to +2.9 ± 3.0% after 14 d ($P = 0.01$) and to +0.5 ± 2.1% after 28 d ($P = 0.02$) post-ALT. There were no significant tHb changes within the CONT group.

Table 3.1: Performance changes for 200 m races, pre- to post-ALT and CONT. [#]Significant difference from pre- to post-ALT ($P < 0.05$). The mean performance improvement within the ALT group was significant ($P = 0.02$), whereas there was no such performance change within the CONT group ($P = 0.94$), but there was no significant difference between the performance changes in the ALT and the CONT groups ($P = 0.76$).

Group	Participant ID (gender)	Event	Time (pre) (mm:ss.00)	Time (post) (mm:ss.00)	Δ Time (%)
ALT	1 (♂)	200 BF	01:59.87	01:59.53	-0.3%
ALT	2 (♂)	200 BF	01:56.58	01:58.16	+1.4%
ALT	3 (♂)	200 BR	02:14.92	02:13.60	-1.0%
ALT	4 (♂)	200 BR	02:16.16	02:13.89	-1.7%
ALT	5 (♀)	200 IM	02:15.60	02:13.23	-1.7%
ALT	6 (♂)	200 FC	01:48.36	01:49.05	+0.6%
ALT	7 (♀)	200 FC	02:01.50	01:59.96	-1.3%
		MEAN ± SD			*-0.6 ± 1.2%*[#]
CONT	9 (♂)	200 FC	01:54.90	01:54.38	-0.5%
CONT	10 (♂)	200 BF	02:02.45	02:02.58	+0.1%
CONT	11 (♂)	200 FC	01:52.89	01:52.62	-0.2%
CONT	12 (♀)	200 FC	02:04.41	02:03.65	-0.6%
		MEAN ± SD			*-0.3 ± 0.3%*

♂ = male, ♀ = female, BF = butterfly, BR = breaststroke, IM = individual medley, FC = front crawl.

3.4.3   tHb and race performance correlations

There were no significant correlations between the changes in 200 m race performance and the tHb changes at 1 d or 28 d post-ALT (Figure 3.2). Likewise, when results from both the ALT and CONT groups were combined, there were no significant correlations between the changes in 200 m race performance and the tHb changes at 1 d or 28 d post-intervention (Figure 3.3).
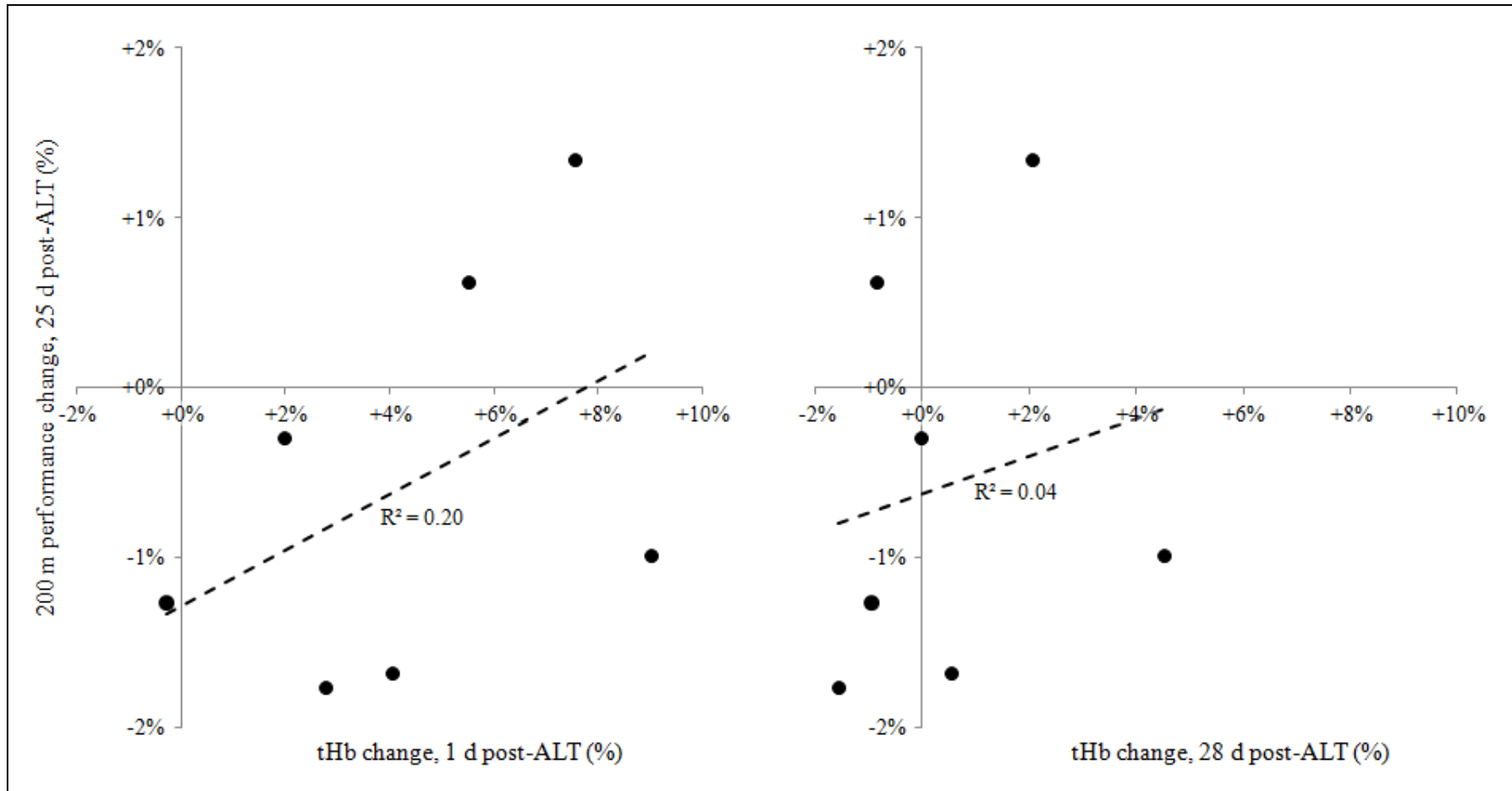
Figure 3.2: Relationship between the tHb percentage changes from baseline to 1 d post-ALT (left panel) and from baseline to 28 d post-ALT (right panel), with the 200 m race performance changes pre- to post-ALT. Data points represent the ALT group participants only. The relationships between these variables were not significant (tHb 1 d post-ALT: $R^2 = 0.20$, $P = 0.31$, tHb 28 d post-ALT: $R^2 = 0.04$, $P = 0.67$).
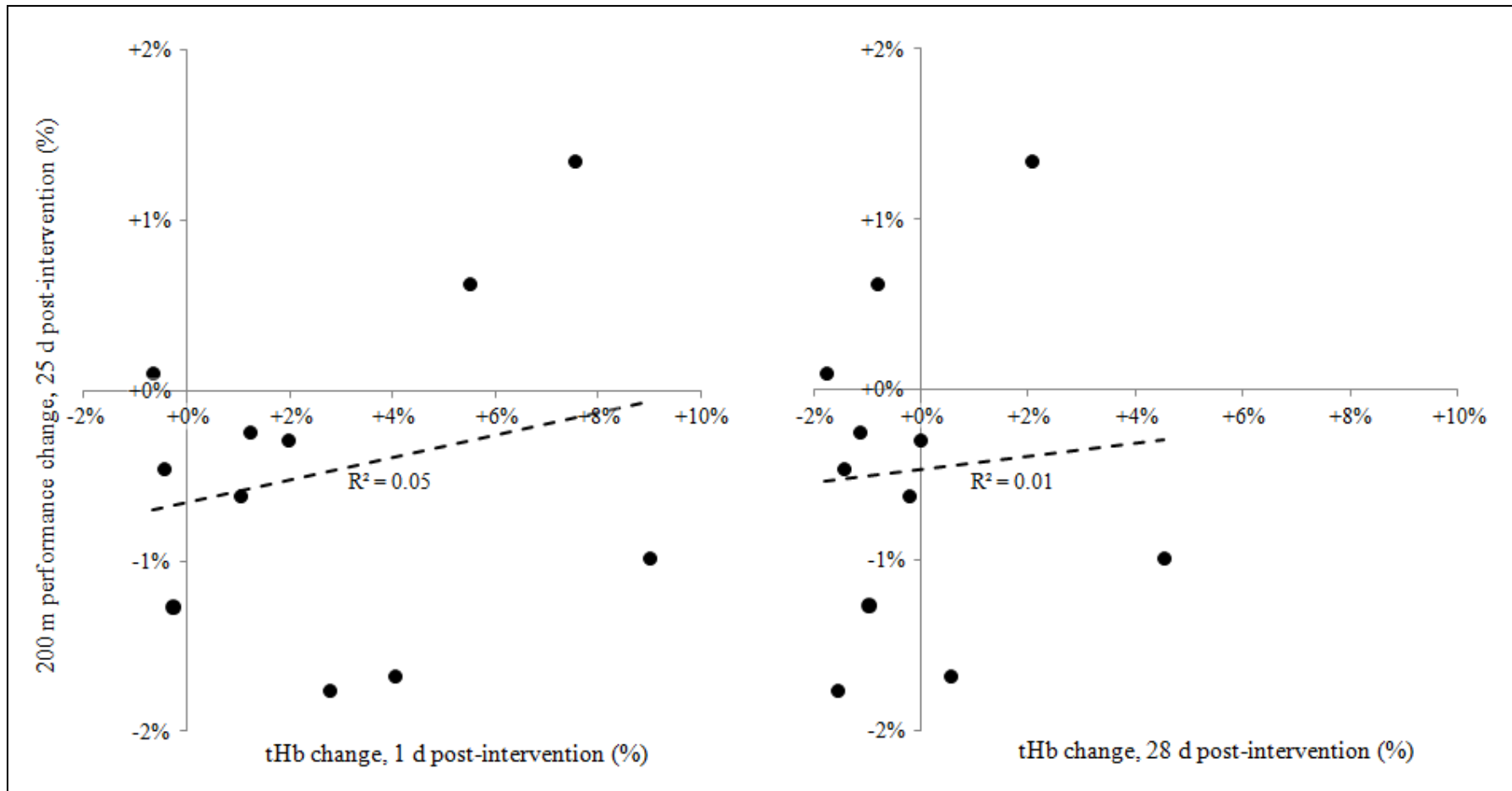
Figure 3.3: Relationship between the tHb percentage changes from baseline to 1 d post-intervention (left panel) and from baseline to 28 d post-intervention (right panel), with the 200 m race performance changes pre- to post-intervention. Data points represent both the ALT and CONT groups combined. The relationships between these variables were not significant (tHb 1 d post-intervention: $R^2 = 0.05$, $P = 0.49$, tHb 28 d post-intervention: $R^2 = 0.01$, $P = 0.82$).

3.5 Discussion

This study is one of very few to have investigated the effects of traditional altitude or sea level training on tHb and race performances in highly trained swimmers. Both groups completed training that was indifferent in terms of the total and the intensity categorised weekly volumes, with aerobic swimming being the only training zone to approach a statistically significant between group difference (ALT 45.8 ± 5.1 vs. CONT 41.8 ± 0.9 km·week^{-1}, P = 0.09). This mean 4 km (non-significant) difference is inconsequential (≤ 60 min of easy swimming spread over 7 d). The key findings were that three weeks of LH+TH resulted only in a trend for a tHb increase, which then declined to circa baseline values after 28 d at sea level (see Figure 3.1). After 25 d post-altitude there were no significant differences in the changes in 200 m race performance between the altitude and sea level groups (see Table 3.1). There were no significant correlations between the changes in tHb and the changes in 200 m race performances (see Figure 3.2 and Figure 3.3). tHb and race performance results showed considerable variability between individuals, as illustrated in Figure 3.1 and Table 3.1, respectively.

3.5.1 Haematology

At baseline, tHb was 13.7 ± 0.9 g·kg^{-1} for males and 11.6 ± 0.4 g·kg^{-1} for females, which is higher than reported for highly trained male swimmers (12.7-13.2 g·kg^{-1}) (Heinicke *et al.*, 2001; Wachsmuth *et al.*, 2013), and female swimmers (10.7 g·kg^{-1}) (Wachsmuth *et al.*, 2013), respectively. The mean 4.4% tHb increase observed immediately after ALT was significantly more than observed in the CONT group, although likely due to the inter-individual variability and the relatively small sample size (n = 7), the tHb change within the ALT group was not statistically significant (P = 0.08). These group values are comparable to data from Garvican *et al.* (2012), who reported a mean 3.5% tHb increase after 19 d at 2760 m in highly trained cyclists, and similarly to Gough *et al.* (2012), who reported a mean ~4% tHb increase after 21 nights at 2135-2320 m in highly trained swimmers. tHb changes in the present studies' ALT group were variable between individuals (-0.3 to +9.0%), which is also consistent with Garvican *et al.* (2012) (~-1 to ~+8%), and Gough *et al.* (2012) (~-3 to ~+8%).

Using a similar design to the present study, Wachsmuth *et al.* (2013) reported a mean tHb increase of ~7% after highly trained swimmers spent four weeks at 2320 m. It is plausible that the extra week at altitude was the cause of this considerably larger tHb change than observed in the present study and those detailed above (Garvican *et al.*, 2012; Gough *et al.*, 2012), but this is more likely due to results from participants that became ill or injured being excluded from the mean values. Similarly, Bonne et al. (2014) reported a mean tHb increase of 6 ± 4% after 10 highly trained swimmers undertook 3-4 weeks of LH+TH at 2130-3094 m. This greater tHb gain than observed in the present study was likely due to 7 of the 10 LH+TH participants having spent an initial 7 d at 3094 m, before a further 21 d at 2130 m; i.e. a greater hypoxic dose than in the present study (Wilber *et al.*, 2007).

Furthermore, tHb results from the present study are substantially lower than the meta-analysis derived predictions by Gore *et al.* (2013), who estimated that a 4.3% tHb gain would require only ~16 d at or above 2100 m (see Figure 6.2). However, in disagreement with these estimations, and with results from the present study, Gore *et al.* (1998) reported that traditional altitude training (31 d at 2690 m) did not increase tHb or any other indicators of erythropoiesis. Participants in this study were arguably some of the highest calibre of athletes to have been documented in any such intervention (World Champion track cyclists), so these authors proposed that they had reached their natural tHb physiological limit (Gore *et al.*, 1998). Importantly, all eight of these participants succumbed to illness during or immediately after the altitude exposure, and as Wachsmuth *et al.* (2013) demonstrated, training load reductions due to illness or injury at altitude result in significantly attenuated tHb gains.

Nevertheless, none of the participants in the present study suffered from illness or injury, yet 2-3 of the ALT group participants showed no signs of increased erythropoiesis after the LH+TH camp (i.e. tHb changes remained within the test CV of ±2.2%; see Figure 3.1). Similarly to participants in Gore *et al.* (1998), these swimmers were already highly endurance trained pre-altitude, as verified by the higher baseline tHb relative to body weight than previously reported: males 13.7 ± 0.9 g·kg^{-1} vs. 12.7-13.2 g·kg^{-1} (Heinicke *et al.*, 2001; Wachsmuth *et al.*, 2013); females 11.6 ± 0.4 g·kg^{-1} vs. 10.7 g·kg^{-1} (Wachsmuth *et al.*, 2013).

It therefore remains a possibility that there was not scope for further tHb gains in response to this altitude of 2320 m (Robach & Lundby, 2012).

3.5.2   Race performance

Even though there was a significant 200 m race performance improvement after three weeks of LH+TH, this improvement was not significantly different to the performance changes in the sea level control group (see Table 3.1). Furthermore, the correlations between the changes in tHb and 200 m race performance were not significant. This lack of a post-altitude performance change over and above the effect of sea level training is consistent with Gough *et al.* (2012), whereby the mean ~4% tHb increase did not transfer into performance gains in highly trained swimmers, at 1, 7, 14 and 28 d post-altitude. Additionally, the recent very thorough experiment by Bonne *et al.* (2014) showed no greater benefit of LH+TH than sea level training on aerobic (3000 m) and anaerobic (4 x 50 m) time trial performance, assessed within 7 d post-altitude. However, Wachsmuth *et al.* (2013) found that the mean ~7% tHb gains after LH+TH at 2320 m corresponded to significantly improved swimming performances (on average +0.8%) after 25-35 d at sea level.

Although participants in the present study were highly motivated to compete at the post-intervention competitions, this was not the primary competition of the year, so they did not complete a 2-4 week taper, as would be the case for a major international competition. If this had been the case, different performance outcomes may have occurred (Thomas *et al.*, 2008). Considering the link between tHb and $\dot{V}O_2max$ (Schmidt & Prommer, 2010), it is possible that had race performances of longer duration events been assessed (that are more dependent on $\dot{V}O_2max$), performance effects may have been different. However, the 3000 m time trial results in the study by Bonne *et al.* (2014) do not support this concept. Assessing performance in the same 200 m event for all participants allowed direct comparisons of race times, whereas Wachsmuth *et al.* (2013) used results from events of a variety of distances, and then converted all results to FINA Points for comparative purposes. This arguably inappropriate scalar method is a potential reason for the disparity in results, in that Wachsmuth *et al.* (2013) reported significant swimming performance gains after LH+TH, whereas most other authors have not.

Interestingly, there were some swimmers who achieved performance improvements in excess of 1%, despite showing no signs of increased tHb after the LH+TH altitude intervention (see Figure 3.1 and Table 3.1), in a similar manner to the report by Gore *et al.* (1998). The lack of a significant correlation between tHb and performance changes supports the notion that non-haematological factors were likely involved in performance changes, at least in some of participants (Gore *et al.*, 2007). While early investigations in untrained males (Geiser *et al.*, 2001) and in trained cyclists (Terrados *et al.*, 1988) highlighted beneficial skeletal muscle oxidative adaptations to hypoxic training, such mechanisms in response to sustained hypoxic exposure at rest were recently disputed (Robach *et al.*, 2012). Nevertheless, strong evidence is provided by Garvican *et al.* (2011), who found that four minute cycling performance significantly improved after 26 nights of simulated altitude exposure, despite the researchers blocking a ~5% tHb increase.

There are a number of experimental considerations associated with the present study. Firstly, there is a risk of Type I and Type II errors for tHb and race performance, due to the relatively small sample size. Secondly, it would have been beneficial to measure erythropoietin and $\dot{V}O_2max$, to ascertain how much of a direct impact the altitude intervention had on erythropoiesis and aerobic capacity, respectively. These assessments were not possible due to the nature of collecting this data in highly trained participants, as well as the logistical challenges of doing so at an international location. And finally, although the design of the present study was based on previous published data (Wachsmuth *et al.*, 2013), it would have been insightful to have quantified race performances over a range of event durations, and more frequently post-altitude.

3.6   Conclusion

The first hypothesis, that tHb would increase more in response to altitude compared to sea level training, is accepted, as tHb increased significantly more after LH+TH compared to the control group. The second hypothesis, that changes in swimming race performance would improve to a greater extent in the altitude compared to the control group, is rejected, as no such significant difference was observed between the two groups. And the third hypothesis, that

performance changes would be related to the changes in tHb, is also rejected, as none of these correlations were significant.

This study demonstrates that three weeks of traditional LH+TH altitude training is not an efficacious means of enhancing 200 m swimming performance. In terms of the haematological adaptations to three weeks of LH+TH, while an increased tHb occurs in most athletes, this is not a universal adaptation. Accelerated erythropoiesis is not the sole mechanism by which altitude exposure may improve performance; non-haematological adaptations are likely to occur, and are worthy of further investigation. Furthermore, as race performance changes are not necessarily related to tHb changes, it is suggested that when identifying athletes as "responders" or "non-responders" to altitude training, classifications should not be based on haematology results, but instead solely on race performances.

Link between Chapter 3 and Chapter 4

Given that Chapter 3 showed that changes in 200 m swimming performance after LH+TH altitude exposure were not necessarily due to erythropoietic gains, it is plausible that non-haematological adaptations took place. Chapter 4 further investigated this possibility by exposing highly trained athletes to an IHT intervention. In this manner, the hypoxic dose was too small to induce any substantial erythropoietic gains, so the effects of hypoxic exercise on a range of other physiological variables and maximal exercise capacity were investigated.

CHAPTER 4:

Eight Weeks of Intermittent Hypoxic Training Improves Submaximal Physiological Variables in Highly Trained Runners

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4.1 Abstract

INTRODUCTION: It is unclear whether IHT results in improvements in physiological variables associated with endurance running. METHODS: 12 highly trained runners ($\dot{V}O_2$peak 70.0 ± 3.5 mL·kg^{-1}·min^{-1}) performed incremental treadmill tests to exhaustion in normobaric normoxia and hypoxia (0.160 F_IO_2) to assess submaximal and maximal physiological variables and the T-Lim. Participants then completed eight weeks of moderate to heavy intensity normoxic training (CONT) or IHT (twice weekly 40 min runs, in combination with habitual training), in a single blinded manner, before repeating the treadmill tests. RESULTS: Submaximal HR decreased significantly more after IHT (-5 ± 5 b·min^{-1}; P = 0.001) than after CONT (-1 ± 5 b·min^{-1}; P = 0.021). Changes in submaximal $\dot{V}O_2$ were significantly different between groups (P < 0.05); decreasing in the IHT group in hypoxia (-2.6 ± 1.7 mL·kg^{-1}·min^{-1}; P = 0.001) and increasing in the CONT group in normoxia (+1.1 ± 2.1 mL·kg^{-1}·min^{-1}; P = 0.012). There were no $\dot{V}O_2$peak changes within either group, and while T-Lim improved post-IHT in hypoxia (P = 0.031), there were no significant differences between groups. CONCLUSIONS: IHT resulted in a degree of enhanced cardiovascular fitness that was evident during submaximal, but not maximal intensity exercise. PRACTICAL APPLICATIONS: These results suggest that moderate to heavy intensity IHT provides a means of improving the capacity for submaximal exercise, and may be a useful tool for pre-acclimatisation for subsequent exercise in hypoxia, but there is a lack of evidence for the improvement of endurance athletic performance at sea-level.

4.2   Introduction

Under hypoxic conditions, the reduced cellular PO_2 results in an increased activity of the 'oxy-gene' HIF-1 (Kallio *et al.*, 1999). Several HIF-1 target genes have been identified, including those encoding Epo, glucose transporters, glycolytic enzymes, and VEGF (Semenza, 1999), and there is some evidence for direct effects on mitochondrial function (Melissa *et al.*, 1997; Terrados *et al.*, 1990). As such, interventions that expose athletes to altitude and/or hypoxia for varying durations are commonly used. The traditional approach involving spending 20 $h \cdot d^{-1}$ or more for three weeks or more, at physical altitude, may enhance subsequent sea level endurance performance via haematologically and/or non-haematologically mediated improvements in O_2 transport and utilisation (Gore & Hopkins, 2005; Levine & Stray-Gundersen, 2005). An alternative approach is for athletes to breathe a hypoxic inspirate during some of their usual exercise training, while living in normoxia, termed IHT. While IHT does not provide a sufficient exposure required for complete acclimatisation (Millet *et al.*, 2010), there have been some noteworthy adaptations reported.

In an early study using highly trained cyclists, Terrados *et al.* (1988) found that 3-4 weeks of moderate and heavy intensity IHT at an altitude simulation of 2300 m resulted in significantly lower submaximal exercise [BLa] and significantly enhanced capillarisation, compared to a normoxic trained control group (CONT). Furthermore, cycling work capacity improved significantly more after IHT than CONT, when tested in hypoxia. Although work capacity improved in the IHT group on average by 33% in normoxia, this was not significantly different to the 22% improvement in CONT (Terrados *et al.*, 1988). Similarly, Roels *et al.* (2005) exposed moderately trained cyclists to seven weeks of heavy intensity IHT at an altitude simulation of ~3000 m. $\dot{V}O_2$max significantly increased by ~9% after IHT, compared to a mean increase of ~5% after CONT, but this between group difference was not statistically significant. Moreover, the increased $\dot{V}O_2$max after IHT did not correspond to a greater endurance cycling performance improvement than after CONT.

Another study, by Dufour *et al.* (2006), involved 18 moderately trained males completing six weeks of moderate intensity IHT or CONT, at the speed corresponding to the second ventilatory threshold (Beaver *et al.*, 1986). Despite

the similar training load, the IHT group experienced a significant ~5% $\dot{V}O_2$max increase, which did not change in CONT, and time to exhaustion at the $\dot{V}O_2$max velocity also improved significantly more after IHT (+35%) compared to CONT (+10%) (Dufour et $al.$, 2006). Given that hypoxic doses of <2 h·d^{-1} (Roels et $al.$, 2005) and <1 h·d^{-1} (Dufour et $al.$, 2006) are too small for erythropoiesis to have occurred (Rasmussen et $al.$, 2013), it is likely that the $\dot{V}O_2$max improvements were due to non-haematological adaptations (Gore & Hopkins, 2005).

The frequency of IHT sessions is important, with participants in the earlier study by Terrados et $al.$ (1988) dedicating the majority of their training to the 4-5 IHT sessions·week^{-1}, compared to just 2 sessions·week^{-1} in Dufour et $al.$ (2006) and Roels et $al.$ (2005). The ecological validity of the design of any such IHT intervention is paramount, in that experimental treatments aimed at enhancing athletic performance should match what is likely possible to incorporate into an athlete's training schedule.

While participants in Roels et $al.$ (2005) were "well trained cyclists", with a mean baseline cycling $\dot{V}O_2$max of 66 mL·kg^{-1}·min^{-1}, and participants in Dufour et $al.$ (2006) were moderately trained runners, with a mean baseline running $\dot{V}O_2$max of ~63 mL·kg^{-1}·min^{-1}, Terrados et $al.$ (1988) are the only authors to date to have assessed the effects of IHT in highly trained athletes (international standard cyclists with a mean baseline cycling $\dot{V}O_2$max of 70 mL·kg^{-1}·min^{-1}). Moreover, IHT participants trained in a hypobaric chamber in Terrados et $al.$ (1988), and wore face masks in Dufour et $al.$ (2006), whereas CONT participants trained in normoxic laboratories, without masks, and Roels et $al.$ (2005) did not report whether participants were blinded to the environmental treatment. A lack of blinding in these studies may have resulted in influential placebo and/or nocebo effects, which must be controlled for in order to ascertain the efficacy of IHT as a worthwhile intervention.

The purpose of this study was to investigate cardiopulmonary physiological adaptations resulting from IHT or CONT, in highly trained endurance runners, using a single-blinded research design. It was hypothesised that eight weeks of IHT would elicit greater improvements in submaximal and maximal physiological variables, and would lead to an enhanced incremental exercise T-Lim, compared to CONT.

4.3   Methods

4.3.1   Experimental approach to the problem

After a competition phase, a group of runners completed six weeks of routine training to ensure stability of basic fitness, and were then randomly allocated into an IHT group (n = 9) or a CONT group (n = 9), in a single-blinded manner. In Weeks 1 and 10 participants completed incremental exercise tests to quantify a range of submaximal and maximal physiological variables, as well as T-Lim, in a controlled laboratory environment. In between, during Weeks 2-9 participants undertook their habitual training, with two 'anaerobic threshold' runs each week replaced by 40 min IHT or CONT running sessions (16 x 40 min sessions in total over the eight weeks) (Table 4.1). These 'anaerobic threshold' runs would ordinarily be 20-60 min, between the speed corresponding to the [BLa] threshold (LT_{speed}) and [BLa] turnpoint (LTP_{speed}) (Jones, 2006; Smith & Jones, 2001) (see Section 4.3.3).

Table 4.1: Typical running training week during the eight week intervention.

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
IHT / CONT in lab (40 min) and easy run (30 min)	Intense intervals (60 min)	IHT / CONT in lab (40 min) and threshold run (50 min)	Intense intervals (50 min)	Easy run (30 min)	Intense race (15-35 min) or rest day	Easy run (75 min)

The "IHT / CONT in Lab" sessions replaced two of the participants' normal outdoors runs, thus maintaining comparable total training volumes.

4.3.2   Participants

Following approval by the University of Exeter Ethics Committee, the participating athletes were instructed as to the details of the study, and then provided written informed consent. Initially there were 18 highly trained male endurance runners who volunteered for this study. Descriptive data for the 12 participants that finished the study are detailed in Table 4.2. They were all part of the same training group, they lived in the same accommodation, and ate in the same canteen. These participants regularly competed at a national and

international level in track events ranging from 1500 m to 10,000 m, as well as in a variety of cross-country races.

4.3.3 Procedures

Incremental step test to volitional exhaustion

Tests were performed at the same time of day, on the same days of the week, in normobaric normoxia (F_IO_2 = 0.209) and hypoxia (F_IO_2 = 0.160, equivalent to ~2150 m), each separated by one day. Participants wore a chest harness for safety, due to the high speed running, and completed a discontinuous incremental test on a treadmill (ELG, Woodway, Waukesha, USA) set at a gradient of 1% to compensate for the lack of air resistance (Jones & Doust, 1996) – this was also the case for all the IHT and CONT training sessions. Each stage lasted 3 min, followed by a 15 s rest interval while a capillary blood sample was taken from the earlobe for [BLa] determination (Biosen C_Line, EKF, Magdeburg, Germany). The first stage was at 10 km·h^{-1}, with subsequent increments of 1.5 km·h^{-1} each stage. S_aO_2 and HR were quantified continuously via finger tip pulse-oximetry (BCI-Autocorr$^®$, Smiths Medical, Waukesha, US) and telemetry (S610i, Polar Electro, Kempele, Finland). These variables, as well as breath-by-breath $\dot{V}O_2$ (Oxycon-Pro$^®$, VIASYS, Höechberg, Germany) were averaged over the final 60 s of each stage, when Rating of Perceived Exertion (RPE) was also recorded using the Borg 6-20 scale (Borg, 1998). Running economy (submaximal $\dot{V}O_2$, relative to body weight) was measured at 14.5 km·h^{-1}, which was below the LT$_{speed}$ for all participants. The running speed that elicited 4.0 mM [BLa] and the LT (LT$_{speed}$) were derived using the Lactate-E Software (Newell *et al.*, 2007). The LT$_{speed}$ was defined as the final running velocity before the first sustained increase in [BLa] above baseline (Jones, 2006; Smith & Jones, 2001). In addition, for the purpose of setting the initial training treadmill speeds, the running speed that elicited the LTP (LTP$_{speed}$) was determined by two independent reviewers, defined as the final running speed before the observation of a sudden and sustained rise in [BLa], at approximately 2-5 mM (Jones, 2006; Smith & Jones, 2001). Minimum S_aO_2 and maximal HR (HRmax) were calculated as the lowest and highest 5 s rolling average, respectively, and $\dot{V}O_2$peak was calculated as the highest 30 s rolling average. Throughout all tests the participants were verbally encouraged to perform

maximally, and the treadmill was only stopped at volitional exhaustion, when the participant placed his feet either side of the treadmill belt or lifted his legs up and became suspended from the chest harness, at which time T-Lim was recorded to the nearest s. Based on the investigators experience of testing these calibre of athletes, maximal [BLa] ([BLa]max) was assessed at 2 min post-exhaustion.

Table 4.2: Descriptive characteristics of participants who completed the study.

	CONT group (n = 7)	IHT group (n = 5)	Combined groups (n = 12)
Age (y)	19.3 ± 0.6	20.2 ± 0.7	19.7 ± 0.8
Body weight (kg)	67.3 ± 6.1	66.5 ± 6.7	67.0 ± 6.1
Stature (cm)	179.0 ± 7.2	181.3 ± 8.0	180.1 ± 7.3
$\dot{V}O_2$peak in normoxia	70.7 ± 3.1	69.2 ± 4.0	70.0 ± 3.5
$\dot{V}O_2$peak in hypoxia	58.3 ± 3.7	58.2 ± 4.2	58.3 ± 3.8

Participants were split into a CONT or an IHT group. $\dot{V}O_2$peak in normoxia was measured in the laboratory at a F_IO_2 of 0.209, and $\dot{V}O_2$peak in hypoxia was measured in the same laboratory at a F_IO_2 of 0.160. Participants were successfully blinded to the environmental condition during the pre- and post-intervention tests and all training sessions.

Environmental control and calibrations

Ambient F_IO_2 and humidity were controlled by an installed S3 Hypoxic & Humidity System (Sporting Edge UK Ltd, Basingstoke, UK), switched on during all training and testing, to allow complete participant blinding. This system was accurate to within ± 0.1%, and was checked prior to and during all tests (Servomex 5200, Servomex, Crowborough, UK). During the treadmill tests, participants wore a face mask for breath-by-breath $\dot{V}O_2$ determination using the Oxycon-Pro®, which required adapted calibrations for use in hypoxia, as previously described (Holliss *et al.*, 2011) (see Section 2.4 and Appendix 5). Face masks were not worn during any of the training sessions.

IHT and CONT sessions

Each treadmill training session lasted 40 min, comprising a 5 min moderate intensity warm up at the LT_{speed}, a 30 min heavy intensity core phase at the LTP_{speed}, and a 5 min moderate intensity cool down at 1.0 $km·h^{-1}$ slower than the LT_{speed}. All speeds were set according to the pre-training test in the specific F_IO_2 environment. HR, RPE and [BLa] were recorded every 10 min during the 30 min core phase, to relativise the exercise intensity between the IHT and CONT groups. The aim was to achieve a steady state [BLa], with a rise of <2.0 mM from 10 to 30 min. Treadmill speed was adjusted within the session after any 10 min period using the following criteria: if RPE <13 or there was a [BLa] decrease, speed was increased by 0.5 $km·h^{-1}$. If RPE >17 or there was an increase in [BLa] >2.0 mM, between 10 to 30 min, speed was decreased by 0.5 $km·h^{-1}$. Participants were obstructed from viewing their exercising HR, [BLa] and treadmill speeds, to minimise the chances of them guessing the training and/or testing F_IO_2. When asked, after the final supervised exercise session, 46% of participants were correct and 54% were incorrect in their guess of which environmental F_IO_2 they had exercised in (50% of the IHT group and 43% of the CONT group were correct in their judgement, respectively), so the blinding was judged to have been successful.

Training monitoring

The running speeds during the final 10 min of each of the supervised laboratory sessions were recorded and used to assess any differences in the progression between the IHT and CONT groups over the eight week intervention. In addition, all participants wore a GPS equipped watch (Forerunner 405, Garmin Ltd, Southampton, UK) during all runs completed outside the laboratory, in order to quantify running distances. Data were analysed to investigate any differences in total running distances between groups. Table 4.1 illustrates a typical training week.

Resting haematology and nutrition

Venous blood samples were drawn from an anti-cubital vein via phlebotomy during Weeks 1, 5 and 10, to screen for illness and to ensure adequate iron stores ([sFe] ≥30 $μg·L^{-1}$). Within 2 h, samples were analysed for FBC using an

automated cell counter (ADVIA 120, Siemens AG, Erlangen, Germany) and [sFe] using a chemiluminescent microparticle immunoassay (ARCHITECT Ferritin Assay, Abbott Point of Care Inc, Birmingham, UK). Furthermore, to stress the importance of adequate dietary iron and other nutrient intake, all participants attended a 45 min nutrition seminar by an experienced sports dietician during Week 1.

4.3.4 Statistical analyses

In order to assess changes pre- to post-training, absolute changes in each dependent variable were compared between IHT and CONT groups using repeated measures analyses of covariance (ANCOVA) with mixed measures. Pre-training values for all exercise test variables were entered as covariates, to control for any baseline differences between the IHT and CONT groups. Analyses were performed using PASW Statistics (v18.0, IBM SPSS, Portsmouth, UK), with the probability level of $P < 0.05$ being accepted as statistically significant. Data sets were checked for normality of distribution prior to analyses. All data were reported as mean ± SD.

4.4 Results

Of the 18 participants who started the study, four dropped out due to illness (IHT Group n = 3, CONT Group n = 1), and two qualified for international competitions so ceased their involvement (IHT Group n = 1, CONT Group n = 1). Baseline descriptive data is detailed in Table 4.2 for the 12 'finishers' who completed all 16 supervised treadmill sessions within the planned eight week period. The mean running speed during all 16 of the laboratory training sessions was significantly slower in the IHT group compared to the CONT group (15.6 ± 0.8 vs. 17.1 ± 0.8 km·h^{-1}, $P = 0.007$). There was a significant increase in the mean running speed during Week 1 to Week 8 when results from both groups were combined (16.1 ± 1.1 to 16.9 ± 1.0, km·h^{-1}, $P = 0.033$), but there was no difference in this running speed progression between the IHT and the CONT groups (IHT 15.2 ± 0.7 to 16.0 ± 0.7 vs. CONT 16.8 ± 0.9 to 17.5 ± 0.7 km·h^{-1}, $P = 0.565$). Total weekly running distances were also not different between groups (IHT 85.1 ± 5.1 km vs. CONT 84.6 ± 5.6 km; $P = 0.776$).

4.4.1 Submaximal variables

The changes in submaximal $\dot{V}O_2$ were significantly different after IHT compared to CONT, in both normoxia ($P = 0.003$) and hypoxia ($P = 0.010$) (Figure 4.1). During normoxic tests, submaximal $\dot{V}O_2$ increased in five of the seven CONT participants (54.1 ± 2.9 to 55.2 ± 1.5 mL·kg^{-1}·min^{-1}, $P = 0.012$) and showed a tendency to decrease post-IHT, with submaximal $\dot{V}O_2$ decreasing in four out of the five IHT participants (52.0 ± 3.6 to 50.5 ± 2.7 mL·kg^{-1}·min^{-1}, $P = 0.052$). In hypoxia, submaximal $\dot{V}O_2$ did not change post-CONT (49.0 ± 1.7 to 50.3 ± 1.7 mL·kg^{-1}·min^{-1}, $P = 0.296$), but decreased in all five of the IHT participants (48.6 ± 3.5 to 46.0 ± 1.8 mL·kg^{-1}·min^{-1}, $P = 0.001$). Submaximal HR when tested in normoxia decreased significantly after both IHT (154 ± 13 to 148 ± 8 b·min^{-1}; $P = 0.001$) and CONT (160 ± 10 to 159 ± 7 b·min^{-1}; $P = 0.021$), with this difference being statistically greater after IHT ($P = 0.001$) (Figure 4.1). There were no significant submaximal HR changes within or between groups in hypoxia, and there were no significant changes within groups or differences between groups in the speed at 4.0 mM [BLa] or in submaximal S_aO_2 (Figure 4.1).
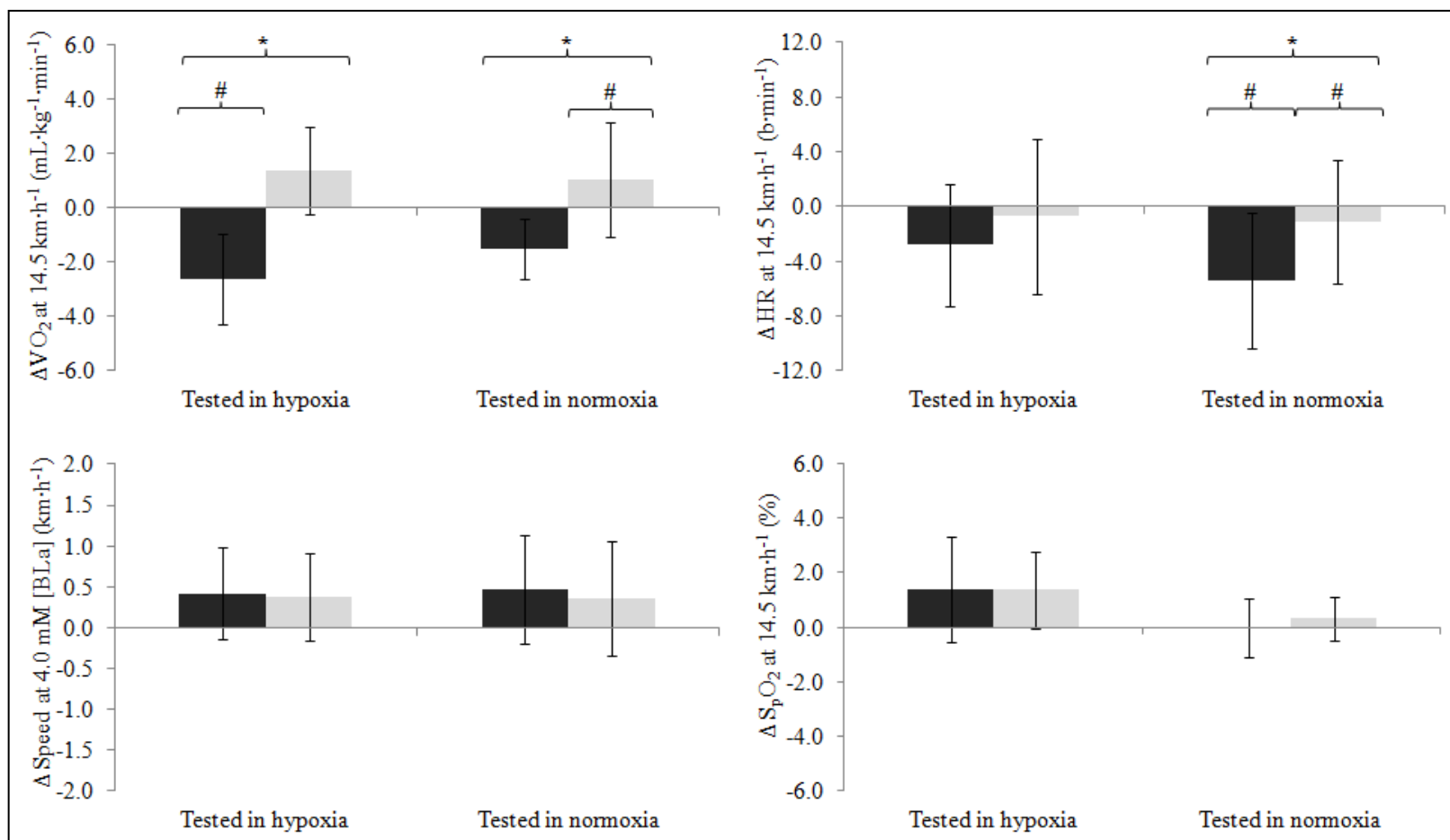
Figure 4.1: Changes in submaximal variables in the IHT group (black bars) and CONT group (grey bars). Data are presented as mean change values, with SD error bars. #Significant training effect within the IHT or CONT group ($P < 0.05$). *Significant difference between the IHT and CONT groups pre- to post-training ($P < 0.05$). $\dot{V}O_2$ at 14.5 km·h^{-1} significantly decreased post-IHT in hypoxia, and increased post-CONT in normoxia. HR at 14.5 km·h^{-1} decreased significantly more so post-IHT compared to post-CONT, when tested in normoxia.

4.4.2   Maximal variables

T-Lim remained unaltered after both CONT and IHT in normoxic conditions. During hypoxic tests there was a significant T-Lim improvement after IHT (25.0 ± 1.9 to 25.6 ± 1.1 min; P = 0.031), but not after CONT (23.7 ± 1.7 to 23.8 ± 1.9 min; P = 0.836). However, T-Lim changes were not statistically different between the IHT and CONT groups when tested in either normoxia (P = 0.463) or hypoxia (P = 0.214) (Figure 4.2).

While $\dot{V}O_2$peak in normoxia increased to a greater extent after CONT than after IHT, HRmax in hypoxia decreased to a greater extent after IHT than after CONT, and $S_aO_2$min in normoxia increased to a greater extent after IHT than after CONT (Figure 4.3), such changes were not significant within either group, in either normoxic or hypoxic test conditions. Similarly, while in hypoxic conditions there was a significant decrease in [BLa]max post-IHT (13.0 ± 4.1 to 11.4 ± 2.1 mM; P = 0.007), but not post-CONT (11.0 ± 1.9 to 10.5 ± 2.3 mM; P = 0.372) there were no significant between group differences (Figure 4.3).

4.4.3   Resting haematology

[sFe] remained above 30 µg·L^{-1} in all participants who completed the study, and the medical team had no concerns regarding the FBC red or white blood cell differential variables, which did not significantly differ from baseline, and did not differ between groups.
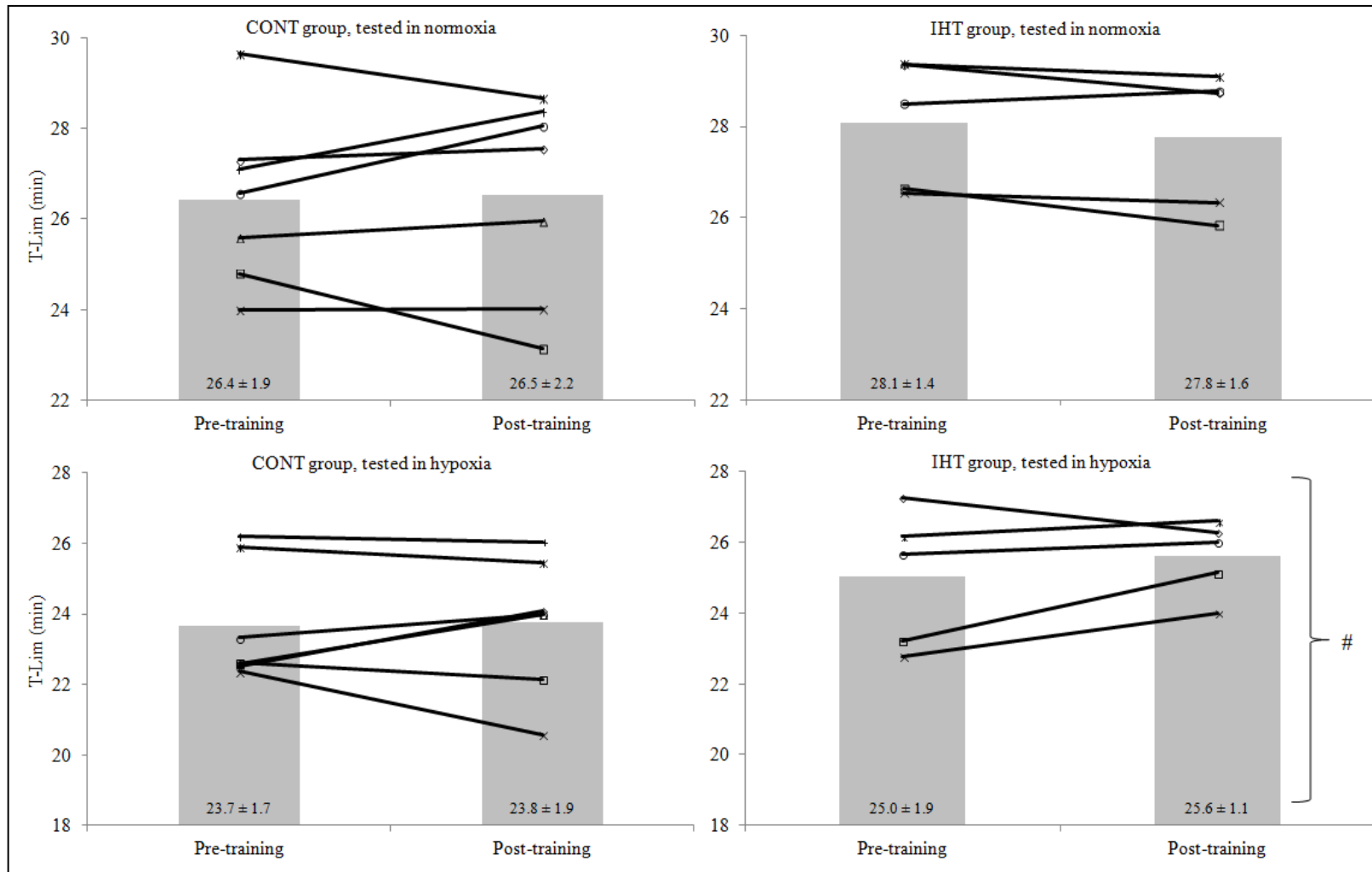
Figure 4.2: Absolute changes in T-Lim pre- to post-IHT and CONT; black lines represent individual participants, while grey bars are mean values. T-Lim in hypoxia significantly increased post-IHT. #Significant training effect within the IHT group ($P < 0.05$).
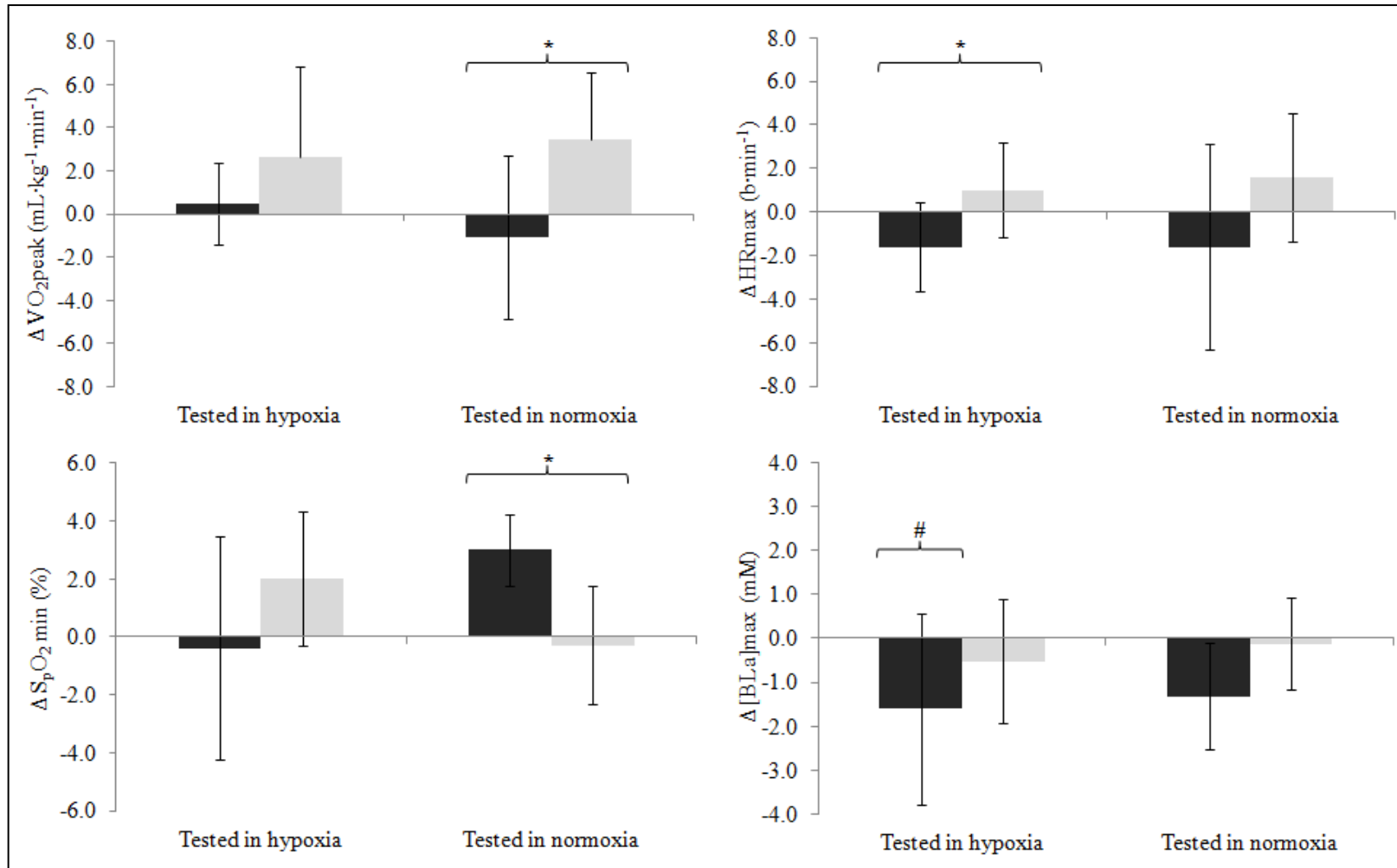
Figure 4.3: Comparisons of the changes (Δ) in maximal variables between the IHT (black bars) and CONT (grey bars) groups. Data are presented as mean values, with SD error bars. #Significant training effect within the IHT or CONT group ($P < 0.05$). *Significant difference between the IHT and CONT groups pre- to post-training ($P < 0.05$).

4.5   Discussion

This study investigated whether eight weeks of IHT would elicit improvements in submaximal and maximal physiological variables and incremental running T-Lim compared to CONT. The main findings were that i) submaximal HR reduced more after IHT, compared with CONT, ii) submaximal $\dot{V}O_2$ tended to increase after CONT and to decrease after IHT, and iii) although T-Lim after IHT improved in hypoxic test conditions, there were no T-Lim changes within either group in normoxic test conditions.

4.5.1   Submaximal variables

The greater submaximal HR reductions that were observed post-IHT (mean -4%) compared to post-CONT (mean -1%) indicate a greater cardiovascular fitness gain post-IHT. In a similar manner, Vallier *et al.* (1996) reported that submaximal HR was on average 4% lower after highly trained triathletes performed three weeks of IHT. While consistent with results from the present study, these authors used a more extreme altitude simulation (~4000 m), hypobaric rather than normobaric hypoxia, and did not include a CONT group, so it is impossible to judge to what extent the changes were due to the hypoxia, per se, or to enhanced cardiovascular fitness after exercise training. Moreover, Terrados *et al.* (1990) found that constant submaximal load HR was reduced significantly more after highly trained cyclists undertook 3-4 weeks of IHT (at an altitude simulation of ~2300 m) compared to CONT (mean -16% vs. -12%, respectively).

In the present study, five out of the seven CONT participants showed submaximal $\dot{V}O_2$ increases, which reached statistical significance in normoxia. On the contrary, of the five IHT participants, submaximal $\dot{V}O_2$ decreased in four of them in normoxia, and all five of them in hypoxia, which reached statistical significance in hypoxia. These results in conjunction with the decreased submaximal HR indicate a lower energetic cost of exercise post-IHT, and an increased energetic cost of exercise post-CONT, the latter being rather surprising, as an increase in submaximal $\dot{V}O_2$ would not be expected after eight weeks of training.

However, in the similarly designed study by Roels *et al.* (2005), these authors also reported a trend for submaximal $\dot{V}O_2$ to decrease after IHT (n = 11, -3%, tested in hypoxia), and increase after CONT (n = 11, +2%, tested in normoxia), although these differences were not statistically significant. Furthermore, Robertson *et al.* (2010b) found that submaximal $\dot{V}O_2$ remained unchanged in moderately trained middle distance runners who performed three weeks of either IHT, or IHT while living in normobaric hypoxia (3000 m simulated altitude). This study also had a number of withdrawals, meaning that there were only 12 'finishers', thus in a similar manner to the present study, the statistical power was reduced. Finally, although the aforementioned study by Dufour *et al.* (2006) showed impressive gains in maximal physiological variables and T-Lim after IHT (n = 9) compared to CONT (n = 9), these authors also reported no significant submaximal $\dot{V}O_2$ changes in either group. Given the larger sample sizes, the power of statistical analyses in Roels *et al.* (2005) and Dufour *et al.* (2006) would have surpassed that of the present study. Together with results from Robertson *et al.* (2010b), these investigations question whether the submaximal $\dot{V}O_2$ changes observed in the present study were physiologically meaningful. While the lower submaximal HR may suggest that IHT is a useful means of improving submaximal exercise endurance capacity, this has not been categorically proven, so this area warrants further research.

4.5.2   Maximal variables

It is reasonable to suggest that any reduction in the energetic cost of exercise post-IHT would likely lead to an improved T-Lim. Indeed there was a significant T-Lim increase post-IHT, when participants were tested in hypoxia. Although the measure of T-Lim in the present study can only provide a surrogate estimate of athletic performance, and changes were not statistically different between groups, the within IHT group change shows a clear trend (Figure 4.2). Based on this finding, and that from another of our recent IHT investigations, whereby muscle oxidative capacity improved in active men when tested in hypoxic conditions (Holliss *et al.*, 2013) (see Chapter 5 and Appendix 8), it is possible that IHT elicits adaptations within skeletal muscle that result in a degree of acclimatisation to subsequent hypoxia. Although a (resting) intermittent normobaric hypoxic exposure intervention has been reported not to

enhance subsequent exercise tolerance at moderate altitude (Faulhaber *et al.*, 2010), to the knowledge of the authors this has not been trialled using a similar IHT intervention to the present study, in highly trained athletes.

Results from the similar study by Dufour *et al.* (2006) differ to the present study, as after six weeks' training, T-Lim in normoxia improved in all nine IHT participants (mean +35%, approximate range +5% to +63%) compared with only five out of nine CONT participants (mean +10%, approximate range -18% to +51%). Some variability is to be expected in duration-blinded time to exhaustion tests, perhaps ~15% (Laursen *et al.*, 2007), but the changes reported by Dufour and colleagues are considerably greater, so it is suggested that the lack of F_IO_2 blinding and the resulting placebo and/or nocebo effects, were partially responsible. These researchers found no *in vitro* maximal oxidative capacity improvements after IHT compared to CONT, and concluded either that the participants had reached a mitochondrial adaptation plateau, or that IHT does not improve mitochondrial content (Ponsot *et al.*, 2006).

In contrast, studies in untrained participants by Vogt *et al.* (2001) and Geiser *et al.* (2001) reported significantly greater increases in mitochondrial and capillary densities after IHT compared to CONT. Similarly, Terrados *et al.* (1990) and Melissa *et al.* (1997) reported significant citrate synthase activity increases in the hypoxic trained legs compared to normoxic trained legs of untrained participants (using the same absolute work rates), thus indicating enhanced mitochondrial function post-IHT. Results from these studies being largely different to results in moderately trained participants (Ponsot *et al.*, 2006) indicate that baseline training status likely has an important impact on adaptive outcomes. In the present study, the participants' pre-intervention $\dot{V}O_2peak$ of 70.0 ± 3.5 mL·kg^{-1}·min^{-1} was already high, so the scope for further maximal aerobic improvements via an eight week intervention was likely to be limited.

Nevertheless, in another investigation using single-legged exercise, Bakkman *et al.* (2007) had eight untrained participants undertake IHT and CONT, at 65% of the maximal attained work rate in the specific F_IO_2 that each leg was to be trained in. Maximal power output improved similarly in both legs, regardless of training condition, and in contrast to Terrados *et al.* (1990) and Melissa *et al.* (1997), citrate synthase activity increased significantly more in the CONT leg

than in the IHT leg, in which there was no such change. These authors suggested that the lower absolute work rate in the IHT leg likely caused a reduced stimulus for mitochondrial biogenesis (Bakkman *et al.*, 2007). Furthermore, Heinonen *et al.* (2010) demonstrated that during submaximal exercise in normobaric hypoxia, elevated cardiac output provides adequately increased muscle blood flow to counteract reductions in arterial O_2 content, thus total muscle O_2 delivery remains largely unaltered. It is only during high intensity whole body exercise in hypoxia that muscle O_2 delivery is significantly reduced (Calbet *et al.*, 2009).

On this basis, the recent studies by Faiss *et al.* (2013b) and Galvin *et al.* (2013) found that repeated sprint performance improved significantly more after IHT compared to CONT, when equal absolute (maximal effort) workloads were used. As such, the IHT stimulus in the present study, whereby the relative training intensity was essentially 'clamped', meaning that IHT participants underwent lower absolute exercise workloads than CONT participants, may simply not have been sufficiently intense to stress O_2 delivery. So the lack of observed T-Lim gains in the present study, and similarly by others that used moderate to heavy intensity training (Lecoultre *et al.*, 2010; Robertson *et al.*, 2010b; Roels *et al.*, 2005; Terrados *et al.*, 1988), is perhaps not all that surprising. In the case of the moderate intensity IHT study by Dufour *et al.* (2006), again, it is suggested that the performance gains were at least in part due to placebo and nocebo effects. Moreover, these authors did not report any between group statistical analyses, meaning that the effects within the IHT and CONT group were not shown to be different.

In agreement with the present study, Ventura *et al.* (2003) also reported no significant performance improvements after six weeks' IHT in moderately trained cyclists. However, their IHT sessions were *in addition* to participants' habitual training, and these authors suggested that the lack of performance change could have been a result of over-training (Ventura *et al.*, 2003). Reductions in submaximal HR, [BLa]max, and maximal exercise capacity are commonly observed during over-training (Meeusen *et al.*, 2010), and it is possible that a similar effect occurred in the present study. The experimental design of the present study meant that training loads were maintained consistently over the eight weeks, so participants were not able to take

additional rest when feeling fatigued, as they may have done under normal circumstances. It is therefore likely that some participants experienced accumulated fatigue by the end of the intervention, which was confirmed anecdotally by the athletes and their coach, and is evidenced by the lack of T-Lim changes, the high proportion of withdrawals due to illness, and by trends towards a post-IHT reduction in normoxic $\dot{V}O_2$peak and HRmax.

It should be acknowledged that there is a risk of a Type I 'false-positive' error for submaximal HR and VO_2, due to potential issues associated with multiplicity. Equally, given the relatively small sample size and the rather wide inter-individual variation in responses (see Figure 4.2), there is also a risk of Type II 'false-negative' errors. Additionally, the discontinuous combined submaximal and maximal intensity exercise test may not have provided the most sensitive means of assessing maximal exercise capacity. Instead of using T-Lim as the sole estimate of performance, future similar studies should include a range of exercise capacity tests, including extended submaximal protocols to establish whether any changes in submaximal HR and/or $\dot{V}O_2$ lead to greater aerobic exercise tolerance, and also using more ecologically valid assessments of maximal performance capacity (i.e. actual races).

4.5.3   Practical applications

- The results of this study do not support the use of moderate intensity IHT to enhance athletic performance at sea level. The concept of using IHT as a pre-acclimatisation tool for subsequent training at altitude or in hypoxia is interesting, but requires more direct assessments.

- It is recommended that those professionals who supervise IHT interventions closely monitor individual athlete fatigue and wellbeing, and that research is carried out into the fatigue consequent to IHT in more depth, especially if equal absolute workloads are used for IHT as they would be for normoxic training.

- Recent literature suggests that IHT using severe or supra-maximal intensity exercise may provide an additional benefit on for repeated sprint athletic performance than normoxic training, but these claims must be further substantiated. In particular future research should comprise

rigorously designed, double-blinded assessments of the effects of whole body severe or supra-maximal intensity IHT in highly trained athletes, as there is a scarcity of data in this population.

4.6   Conclusion

In conclusion, while eight weeks of IHT resulted in some seemingly beneficial adaptations assessed during submaximal exercise, there were no greater exercise capacity improvements detected after IHT compared to (blinded) normoxic training.

Link between Chapter 4 and Chapter 5

The practical challenge of researching the effects of a training intervention in highly trained participants was highlighted in Chapter 4, notably the numerous withdrawals due to illness and injury, and in some cases due to performance enhancement. Aside from the training F_IO_2, attempts were made to maintain equality in the other training that participants undertook, as well as in general lifestyle factors, between the IHT and control groups, but inevitably there would have been some differences that may have impacted performance results. Nevertheless, there were signs of some (non-haematological) adaptations apparent during submaximal exercise that warranted further research. Chapter 5 utilised ^{31}P-MRS methods to assess muscle specific adaptations to IHT, in a highly controlled laboratory based study, with participants being completely blinded to the training and testing F_IO_2, and the researcher who administered the exercise tests also being blinded to the F_IO_2. Additionally, Chapter 5 took into account a relatively recent suggestion that equal absolute workloads should be used for high intensity IHT and normoxic training (Faiss *et al.*, 2013b), and also followed the trend in the current literature for using a lower F_IO_2 compared to that used in Chapter 4 (0.145 vs. 0.160).

CHAPTER 5:

Influence Of Intermittent Hypoxic Training On Muscle Energetics And Exercise Tolerance

Chapter 5 was accepted for publication in the Journal of Applied Physiology in January 2013 (Appendix 8: Holliss, B. A., Fulford, J., Vanhatalo, A., Pedlar, C. R., & Jones, A. M. (2013). Influence of intermittent hypoxic training on muscle energetics and exercise tolerance. *J Appl Physiol, 114*(5), 611-619).

5.1 Abstract

IHT is used by some athletes to elicit 'non-haematological' physiological adaptations to simulated altitude. This study investigated whether IHT would result in greater improvements in muscle energetics and exercise tolerance compared to work-matched intermittent normoxic training (INT). Nine physically-active males completed three weeks of intensive single-leg knee-extensor exercise training. Each training session consisted of 25 min of IHT (F_IO_2 = 0.145 ± 0.001) with the experimental leg and 25 min of INT with the alternate leg which served as a control. Before and after the training intervention, the participants completed a test protocol consisting of a bout of sub-maximal constant-work-rate exercise, a 24 s high-intensity exercise bout to quantify the [PCr]-τ, and an incremental test to the T-Lim. The tests were completed in normoxia and hypoxia, in both the INT and IHT legs. Muscle metabolism was assessed non-invasively using ^{31}P-MRS. Improvements in the T-Lim during incremental exercise were not significantly different between training conditions either in normoxia (INT: 28 ± 20 vs. IHT: 25 ± 9 %, $P = 0.86$) or hypoxia (INT: 21 $\pm$ 10 vs. IHT: 15 ± 11 %, $P = 0.29$). In hypoxia, [PCr]-τ was speeded slightly but significantly more post-IHT compared to post-INT (-7.3 ± 2.9 vs. -3.7 ± 1.7 s, $P < 0.01$), but changes in muscle metabolite concentrations during exercise were essentially not different between IHT and INT. Under the conditions of this investigation, IHT does not appreciably alter muscle metabolic responses or incremental exercise performance compared to INT.

5.2   Introduction

IHT, whereby athletes live at or near sea level while undertaking a portion of their training under normobaric or hypobaric hypoxia, has been suggested to be a worthwhile strategy to enhance athletic performance (Fudge *et al.*, 2012; Millet *et al.*, 2010). However, there is controversy surrounding the mechanisms of physiological adaptations to IHT, and the extent of the potential performance advantages (Levine, 2002).

Under physiological hypoxia, the O_2 homeostasis regulating transcription factor, HIF-1, is activated, initiating a range of adaptations to preserve O_2 delivery (Kallio *et al.*, 1999; Semenza, 2009), of which the best documented is the hepatic and renal release of Epo. Given sufficient 'hypoxic dose', this will result in a sustained increase in the circulating Epo concentration, and consequently increased erythropoiesis (Levine & Stray-Gundersen, 1997). This response timeline is still in question but, for example, it has been reported that after a four-week phase of resting IHE (3 $h \cdot d^{-1}$, 5 $d \cdot week^{-1}$, at a simulated altitude of 4000-5500 m), there were no significant changes in tHb, RCV or other RBC indices in comparison to a placebo group (Gore *et al.*, 2006). It is therefore not surprising that studies have failed to measure an increased tHb following IHT interventions, which use relatively short duration total hypoxic exposures (Hoppeler *et al.*, 2008; Levine, 2002; Wilber *et al.*, 2007).

A sustained high level of HIF-1 is also known to be associated with a range of other non-haematological adaptations that enhance muscle O_2 availability (Semenza, 2009). These adaptations are suggested to include enhanced tissue perfusion linked to angiogenesis (Toffoli *et al.*, 2009), improved mitochondrial efficiency and control of mitochondrial respiration (Ponsot *et al.*, 2006; Roels *et al.*, 2007), and enhanced H^+ buffering capacity (Gore *et al.*, 2001). Due to the invasive nature of assessing these variables, most studies have been restricted to low sample sizes or measures taken only during rest, such that the influence of IHT on skeletal muscle metabolism during dynamic exercise has not been comprehensively investigated.

The non-invasive technique, ^{31}P-MRS, has been utilised to assess muscle energetics during exercise in response to a range of interventions (Baguet *et*

al., 2010; Conley et al., 2000; Forbes et al., 2008; Haseler et al., 1999; Jones et al., 2008; Jones et al., 2007; Jones et al., 2009; Takada et al., 2011; Vanhatalo et al., 2011). Greater muscle oxidative capacity is reflected in faster post-exercise PCr resynthesis (Tomlin & Wenger, 2001); when muscle acidosis is avoided, the speed of [PCr] recovery provides a valid estimate of in vivo oxidative capacity (Conley et al., 2000; Haseler et al., 1999; McMahon & Jenkins, 2002; Taylor et al., 1983).

To the authors knowledge, only one study has used ^{31}P-MRS to investigate the effects of IHT on the muscle metabolic responses to exercise (Kuno et al., 1994). In that study, four combination skiers trained for 60 min, twice a day, for four consecutive days, at a simulated altitude equivalent to 2000 m (Kuno et al., 1994). The [PCr]-т was significantly faster post-IHT (mean change of -19%), but remained unchanged in the eight control participants, who undertook no training (Kuno et al., 1994). The IHT modality (running/cycling) was different to the ^{31}P-MRS test exercise modality (repeated right knee extensions), and as the control group remained sedentary, it was not possible to assess the effect of the hypoxic stimulus, per se, compared to the effects of normobaric normoxic running/cycling. However, the faster [PCr]-т suggests enhanced muscle oxidative capacity following just 8 h of IHT. If confirmed, this would provide an evidence base for the use of IHT by athletes.

The purpose of this study was therefore to investigate the muscle metabolic responses to exercise following a short, intense period of IHT. A study design was used in which participants trained one leg in normoxia (as a control; INT) and the other in hypoxia (IHT). The same exercise modality (knee extension) was used for all training and ^{31}P-MRS tests. The hypotheses were that: 1) muscle metabolic perturbation (as assessed by changes in [PCr], pH and inorganic phosphate concentration ([P_i]) would be attenuated during sub-maximal exercise); 2) [PCr] recovery kinetics following exercise would be faster; and 3) the T-Lim during incremental exercise would be extended following both IHT and INT in both normoxia and hypoxia, but that the effects would be greater following IHT.

5.3 Methods

5.3.1 Participants and experimental design

After institutional ethical approval, nine physically-active, healthy males participated in this study (age: 21.5 ± 3.7 y, body weight: 75.5 ± 11.7 kg, stature: 1.79 ± 0.03 m). Prior to testing, each participant completed a physical activity readiness questionnaire and provided written informed consent. The participants' reported habitual exercise ranged from four sessions of 45 min per week to five sessions of 90 min per week. The participants were engaged in training for a variety of recreational sports (soccer, cycling, running, rowing and hockey) and could be best described as moderately-trained.

The participants' legs were randomly allocated into the normoxic or hypoxic training group, i.e. one leg was trained while they inhaled normoxic gas (INT), and the other while inhaling hypoxic gas (IHT). All participants completed: i) one single-leg knee-extension exercise test protocol practice (described below) and, after a 48 h break, one incremental test to volitional exhaustion under hypoxic conditions, for familiarisation purposes; ii) pre-training testing, consisting of four ^{31}P-MRS test protocols, two for each leg in each of normoxia and hypoxia, iii) three weeks of intensive IHT (experimental leg) and INT (control leg); and iv) post-training testing in which the pre-training test protocols were repeated.

5.3.2 ^{31}P-MRS testing

All testing took place with the participants in a prone position inside the bore of a 1.5 T superconducting magnet (Gyroscan Clinical Intera, Philips Medical Systems, Best, Netherlands). Participants had Velcro straps securely fastened around the thighs, hips, and lower back, and the foot of the test leg was fastened to a pulley system via a padded sling. The ^{31}P-MRS test protocol then commenced. Knee-extension exercise was performed using a custom-built non-ferrous ergometer, over a distance of ~0.22 m, in time with a visual queue which coincided with MR pulse acquisition (40 pulses·min^{-1}). The protocol included a 4 min moderate-intensity exercise bout; 6 min rest; two 24 s high-intensity bouts, separated by 3 min 36 s rest; 5 min 36 s rest; and finally an incremental test to the T-Lim. The work rates applied were calculated from pilot testing undertaken during the familiarisation period. The moderate-intensity

work rate was performed at a load of 1 kg lower than that eliciting the pH threshold, and the 24 s high-intensity bouts were performed at the peak work rate achieved during the familiarisation incremental test. The duration and intensity of this 24 s exercise bout was based on a phase of pilot testing to find the optimal exercise intensity and duration to elicit a drop to 50-60% of baseline [PCr], without a concomitant reduction in intracellular pH. It is known that PCr recovery is not sensitive to differences in end-exercise [PCr] when pH is not altered (Thompson *et al.*, 1995). For the incremental exercise test, the initial resistance was 0.5 kg and this was increased by 0.5 kg every 30 s until volitional exhaustion. The [Pi]/[PCr] and pH during the incremental tests was plotted against work rate, and a pH threshold was identified, as described by Barker *et al.* (2006). Pulmonary gas exchange was not measured during the ^{31}P-MRS tests due to restrictions related to the magnetic environment and the small $\dot{V}O_2$ amplitude (and low signal-to-noise ratio) during single-legged knee-extension exercise performed in the bore of the magnet.

5.3.3 Training intervention

The participants completed three weeks of intensive IHT (experimental leg) and INT (control leg). Participants trained five times per week and thus completed 15 sessions in total over the three week training intervention. Each training session consisted of two identical 25 min phases, one in which the IHT leg was trained, and one in which the INT leg was trained (in a randomised, alternating order). In the IHT condition, the training program totalled 316 min of active IHT or 375 minutes of hypoxia inspiration including rest intervals. The training intervention was based on previous studies which have shown that: three weeks of IHT significantly improved peak power output compared to normoxic training during incremental exercise in hypoxia (Roels *et al.*, 2007); and 384 min of IHT (over six weeks) improves mitochondrial function, $\dot{V}O_2$max and endurance exercise performance compared to normoxic training (Zoll *et al.*, 2006). The training program particularly emphasised high-intensity interval training because this has been shown to be particularly effective in invoking rapid muscle metabolic adaptations and improvements in endurance fitness (Gibala & McGee, 2008; Truijens *et al.*, 2003). Indeed, Forbes *et al.* (2008) have reported that just six sessions of high-intensity training results in

significant speeding of [PCr]-τ. It was therefore anticipated that an intense, well-controlled three week training intervention would result in significant muscle metabolic adaptations which would underpin an enhanced incremental exercise test performance and that these adaptations may be greater in IHT compared to INT (Roels *et al.*, 2007; Zoll *et al.*, 2006).

After being securely fastened to the exercise apparatus, as previously described, the single-leg knee-extension exercise training commenced with 2.5 min at the work rate corresponding to the pH threshold in the IHT leg as measured in hypoxia. This was immediately followed by 2.5 min at a work rate 10% higher than that of the pH threshold, then a further 5 min at a work rate 20% above the pH threshold. After 30 s rest, high intensity interval exercise commenced. During Week One of training, this consisted of 10 x 60 s exercise bouts (with 30 s passive recovery intervals), while during Weeks Two and Three of training, this consisted of 10 x 70 s exercise bouts (with 20 s passive recovery), with the work rate being the mean of the pH threshold and the peak work rate attained during incremental exercise in the IHT leg in hypoxia. S_aO_2 and HR, which were assessed using pulse-oximetry (Nonin 7500FO, Nonin Medical Inc., Plymouth, MN), and the RPE, which was assessed with the Borg scale (Borg, 1998), were recorded after the initial 5 and 10 min of continuous exercise, and then after the 5th and 10th bout of interval exercise. Work rates were identical for each leg, regardless of F_IO_2, and were increased by 0.5 kg when RPE ≤ 15 after the 5th interval. The inspirate F_IO_2 (0.145 ± 0.001 for IHT; 0.209 ± 0.001 for INT) was checked before, during and after each training session using a Servomex 5200 Paramagnetic Analyser (Servomex, Crowborough, UK), as described below.

5.3.4   Inspired gases

The inspirate was generated by a Cloud 9 hypoxic generator (Sporting Edge UK Ltd, Basingstoke, UK), placed in the MR control room, connected to a 10 m extension pipe, which fed into a 150 L Douglas Bag (Cranlea & Co, Birmingham, UK). This acted as a reservoir and mixing chamber, and had a separate output pipe, feeding into a Hans Rudolf one-way valve (Cranlea & Co, Birmingham, UK), connected to a facemask for the participant to breathe from, with an expired air exit. Thus, the flow rate was maintained constant, and no

rebreathing of expired air occurred. The O_2 and CO_2 concentration of the inspirate was checked by a researcher in the MR control room during every test using the Servomex 5200, taking samples via a 10 m capillary tube. This analyser was calibrated prior to each use with a 16.0% O_2, 8.0% CO_2 and 76.0% N gas mix (BOC Special Gases, Guildford, UK). For all normoxic tests and training sessions, the O_2 filters were inactivated, yielding an F_IO_2 of 0.209 ± 0.000, and an F_ICO_2 of 0.0005 ± 0.0000, whereas during hypoxic tests and training sessions, an F_IO_2 of 0.145 ± 0.001, and an F_ICO_2 of 0.0004 ± 0.0000 were produced (simulating ~3000 m altitude). During testing, both the participant and the researcher administering the test were blinded to the F_IO_2, with only the researcher in the MR control room being aware of the F_IO_2. Moreover, participants were blinded to the F_IO_2 during all training sessions.

5.3.5 ^{31}P-MRS procedures

Prior to the exercise test beginning, absolute baseline concentrations of metabolites were established via a technique similar to that described by Kemp *et al.* (Kemp *et al.*, 2007) using a 6 cm ^{31}P transmit / receive surface coil. Participants were positioned within the scanner with the coil placed within the scanner bed and positioned such that the participant's quadriceps muscle was cantered directly over it and a phosphoric acid source was directly beneath it. After initially acquiring images to confirm the m. rectus femoris was positioned correctly relative to the coil, spatially localised spectroscopy was undertaken to determine the relative signal intensities obtained from the phosphoric acid source and P_i from the participant's quadriceps. On completion of the exercise protocol, and after the participant had been removed from the scanner, subsequent scans were obtained, comparing the signals obtained from the same phosphoric acid standard and an external P_i solution of known concentration. The localised voxel sampled within the external solution was of the same dimensions and distance from the coil as from the muscle previously, allowing the calculation of muscle $[P_i]$ following corrections for relative coil loading. Absolute concentrations of PCr and adenosine triphosphate (ATP) were subsequently calculated via the ratio of $[P_i]:[PCr]$ and $[P_i]:[ATP]$, respectively.

Following metabolite concentration determinations, the phosphoric acid source was removed from the scanner bed and the participants were securely fastened to the exercise apparatus, as previously described. Images were acquired to confirm the quadriceps muscle was positioned directly above the 6 cm ^{31}P coil, and participants commenced breathing the inspirate, which was continued for 30 min prior to the commencement of ^{31}P data acquisition. Initially, a number of pre-acquisition steps were carried out to optimise the signal from the muscle under investigation. Matching and tuning of the coil was performed and an automatic shimming protocol undertaken within a volume that defined the quadriceps muscle. A baseline spectrum before exercise was then acquired with long repetition time (TR = 20 s) in which the relative unsaturated peak amplitudes could be determined. Two min of further rest then followed, to acquire baseline MR sequences, after which the single-legged knee-extension exercise test protocol commenced, as previously described. During the 2 min resting baseline and the subsequent exercise protocol, ^{31}P data were acquired every 1.5 s, with a spectral width of 1,500 Hz and 1K data points. Phase cycling with four phase cycles was employed, leading to a spectrum being acquired every 6.0 s.

5.3.6 Data analyses

The acquired spectra were quantified via peak fitting, assuming prior knowledge, using the jMRUI (version 3) software package employing the AMARES fitting algorithm (Vanhamme *et al.*, 1997). Spectra were fitted assuming the presence of the following peaks: P_i, phosphodiester, PCr, α-ATP (2 peaks, amplitude ratio 1:1), γ-ATP (2 peaks, amplitude ratio 1:1), and β-ATP (3 peaks, amplitude ratio 1:2:1). Intracellular pH was calculated using the chemical shift of the P_i spectral peak relative to the PCr peak (Taylor *et al.*, 1983). [ADP] was calculated via knowledge of [P_i], [PCr], and pH values, as described by Kemp *et al.* (2001), taking into account the dependency of rate constants on pH. The oxidative ATP turnover rate (ATP-Ox) was determined based on the hyperbolic relationship between [ATP] production rate and free cytosolic [ADP], and calculated using the PCr recovery time constant determined from the 24 s bout (Lanza *et al.*, 2006; Layec *et al.*, 2009).

The 4 min moderate-intensity exercise bout values of [PCr], adenosine diphosphate ([ADP]), [P_i], [P_i]/[PCr], ATP-Ox and pH were calculated as the mean between 90 s and 210 s of exercise (i.e. 120 s total sample time, omitting the first 90 s and final 30 s). End-exercise values were quantified as the mean of the final three data points (i.e. final 18 s) prior to volitional exhaustion.

For the [PCr] values following the 24 s high-intensity exercise period, [PCr] recovery was fitted with Prism 5 software (GraphPad Software Inc, La Jolla, California) by a single exponential of the form:

$$[PCr]\text{-}\tau = [PCr]_{end} + [PCr]_{(0)}(1-e^{(-t/\tau)})$$

Where [PCr]$_{end}$ is the value at the end of exercise, [PCr]$_{(0)}$ is the difference between the [PCr] at end-exercise and when fully recovered, t is the time from exercise cessation, and τ is the time constant for the exponential recovery of [PCr]. The [PCr]-τ from each of the two 24 s exercise bouts was determined separately and the mean of the two values was then calculated.

5.3.7   Statistics

Separate repeated measures ANCOVA with mixed measures were used for each of the two test conditions (normoxic vs. hypoxic), to assess differences in changes of each ^{31}P-MRS variable ([PCr], [ADP], [P_i], [P_i]/[PCr], ATP-Ox, and pH), during the moderate-intensity exercise bouts and the incremental exercise tests to exhaustion, between the two training conditions (INT vs. IHT). The pH threshold and the T-Lim during the incremental tests were compared in the same way. Before calculating the mono-phasic [PCr]-τ from the 24 s high-intensity exercise bouts, paired samples t-tests were used to assess any differences between resting baseline and end 24 s pH. Differences between pre-training [PCr]-τ under normoxic and hypoxic conditions were also assessed using paired samples t-tests, and differences in [PCr]-τ resulting from INT vs. IHT were assessed by ANCOVA. Pre-training values were used as covariates for all ANCOVA's. Results were expressed as mean ± SD. All t-tests and ANCOVA's were performed using PASW Statistics (v18.0, IBM SPSS, Portsmouth, UK). Data sets were checked for normality of distribution prior to analyses. The probability level of *P* < 0.05 was considered to represent a significant difference.

5.4   Results

5.4.1 S_aO_2 in normoxia and hypoxia

Prior to training, during moderate intensity exercise, the hypoxic inspirate resulted in a S_aO_2 of 91 ± 1 %, compared to the normoxic inspirate which resulted in a S_aO_2 of 98 ± 1 %. There were no significant changes in these S_aO_2 values after training ($P > 0.05$).

5.4.2 ^{31}P-MRS variables during moderate-intensity exercise

The moderate-intensity exercise test results are summarised in Table 5.1 and Table 5.2. Significant overall training effects existed for most end-exercise MR variables when IHT and INT data were combined (see Table 5.1 and Table 5.2 for ANCOVA derived F and P values). However, there were no significant interactions; that is, changes resulting from IHT were not significantly different from INT. The absolute [PCr] at rest and over the final 30 s of moderate-intensity exercise were significantly reduced after both IHT and INT training (Figure 5.1), but there was no difference between the two training conditions. However, Δ[PCr] (i.e. the magnitude of PCr degradation) during exercise was reduced by both types of training and there was an interaction in hypoxia, such that IHT spared PCr utilisation to a greater extent than INT. The end-exercise pH was higher following training when participants breathed hypoxic gas but not normoxic gas (Table 5.1 and Table 5.2).

Table 5.1: ^{31}P-MRS variables measured during the moderate intensity exercise bout, while breathing the normoxic inspirate.

	Pre-training	Post-training	Pre-training	Post-training	Main effect for time		Interaction effect	
	Normoxic trained leg		Hypoxic trained leg		F	*P*	F	*P*
Baseline [PCr] (mM)	32.2 ± 3.8	31.2 ± 2.5	33.1 ± 3.8	31.8 ± 3.6	8.39	0.01	<0.001	0.95
End-exercise [PCr] (mM)	27.2 ± 5.0	26.5 ± 2.3	27.5 ± 3.8	27.2 ± 3.6	16.93	0.001 #	0.27	0.61
Δ[PCr] (mM)	5.1 ± 2.0	4.7 ± 2.6	5.5 ± 2.0	4.5 ± 1.8	1.47	0.25	3.38	0.09
End-exercise PCr (%)	83.5 ± 7.3	85.0 ± 7.7	83.2 ± 5.9	85.6 ± 4.8	5.38	0.04 #	0.08	0.78
End-exercise ATP-Ox (mM·s^{-1})	0.38 ± 0.08	0.46 ± 0.17	0.44 ± 0.12	0.50 ± 0.14	<0.001	0.96	0.33	0.58
End-exercise [ADP] (µM)	15.3 ±7.1	15.3 ± 7.4	16.4 ± 5.9	12.4 ± 3.5	6.65	0.02 #	2.28	0.15
End-exercise [P$_i$] (mM)	7.9 ± 2.3	7.2 ± 2.8	9.0 ± 2.8	6.6 ± 1.7	4.39	0.05	2.91	0.11
End-exercise [P$_i$]/[PCr]	0.31 ± 0.13	0.28 ± 0.13	0.34 ± 0.12	0.25 ± 0.06	9.97	0.01 #	1.96	0.18
End-exercise pH	7.03 ± 0.04	7.04 ± 0.04	7.03 ± 0.03	7.05 ± 0.03	2.40	0.14	0.01	0.94

Significant training effect across both training conditions ($P < 0.05$).

Δ[PCr] indicates the difference in [PCr] between baseline and end-exercise.

Table 5.2: ^{31}P-MRS variables measured during the moderate intensity exercise bout while breathing the hypoxic inspirate.

	Pre-training	Post-training	Pre-training	Post-training	Main effect for time		Interaction effect	
	Normoxic trained leg		Hypoxic trained leg		F	P	F	P
Baseline [PCr] (mM)	34.1 ± 4.4	32.5 ± 3.1	34.4 ± 4.2	32.0 ± 4.3	6.12	0.03	0.19	0.672
End-exercise [PCr] (mM)	28.5 ± 4.4	27.4 ± 3.1	28.4 ± 5.0	27.4 ± 4.6	6.54	0.02 #	<0.001	0.995
Δ[PCr] (mM)	5.6 ± 3.5	5.1 ± 1.9	6.0 ± 2.8	4.6 ± 1.6	14.20	0.002	36.07	<0.001 *
End-exercise PCr (%)	83.7 ± 9.2	84.4 ± 5.6	82.1 ± 7.9	85.2 ± 5.6	20.12	<0.001 #	0.86	0.37
End-exercise ATP-Ox (mM·s^{-1})	0.31 ± 0.08	0.37 ± 0.13	0.32 ± 0.12	0.45 ± 0.10	2.13	0.17	2.01	0.18
End-exercise [ADP] (µM)	16.1 ± 8.0	14.1 ± 6.5	16.3 ± 5.6	16.0 ± 3.4	11.32	0.01 #	1.21	0.29
End-exercise [P$_i$] (mM)	8.2 ± 2.9	6.5 ± 2.8	8.7 ± 2.5	7.1 ± 1.6	7.22	0.02 #	0.13	0.72
End-exercise [P$_i$]/[PCr]	0.30 ± 0.14	0.24 ± 0.12	0.32 ± 0.10	0.26 ± 0.05	12.54	0.003 #	0.15	0.70
End-exercise pH	7.05 ± 0.06	7.07 ± 0.04	7.03 ± 0.05	7.06 ± 0.03	23.61	<0.001 #	0.03	0.87

* Significant difference between INT and IHT legs ($P < 0.05$).

Significant training effect across both training conditions ($P < 0.05$). Δ[PCr] indicates the difference in [PCr] between baseline and end-exercise.

Figure 5.1: Mean ± SD muscle [PCr] during two minutes passive rest (baseline values), the four minute moderate-intensity exercise bout, and three subsequent minutes passive rest, pre- (●) and post-training (○). Note that absolute [PCr] at rest and over the final 30 s of exercise were significantly reduced after both IHT and INT, i.e. there was a training effect when tested under normoxia ($P = 0.001$) and under hypoxia ($P = 0.023$), but there were no significant differences between the two training conditions (no interaction effect).

5.4.3 PCr recovery kinetics

The results for PCr recovery kinetics are summarised in Table 5.3. There were no significant differences between resting pH and the pH measured after the 24 s high-intensity exercise bout, with a mean change of only -0.01 ± 0.01 units. There were no significant [PCr]-τ differences pre-training between the legs that had been selected for INT and the legs that had been selected for IHT, whether tested in normoxia (21 ± 3 vs. 20 ± 4 s, t = 0.81, P = 0.44) or hypoxia (28 ± 4 vs. 29 ± 5 s, t = -0.53, P = 0.61). As expected, before training, [PCr]-τ was significantly faster in normoxia compared to hypoxia, in both the leg that had been selected for INT (21 ± 3 vs. 28 ± 4 s, t = -4.74, P = 0.001) and the leg that had been selected for IHT (20 ± 4 vs. 29 ± 5 s, t = -5.43, P = 0.001). The [PCr]-τ was significantly reduced after both INT and IHT, under both normoxic and hypoxic test conditions (Table 5.3 and Figure 5.2). In hypoxia, the [PCr]-τ reduction was significantly greater after IHT (-7 ± 3 s) compared to after INT (-4 ± 2 s) ($F_{(1,14)}$ = 14.46, P = 0.002). Although [PCr]-τ in normoxia tended to decrease more after IHT (-3 ± 3 s) compared to INT (-2 ± 2 s), this was not statistically significant ($F_{(1,15)}$ = 2.98, P = 0.11).

Table 5.3: [PCr]-τ measured following the 24 s severe intensity exercise bout.

	Pre-training	Post-training	Pre-training	Post-training	Main effect for time		Interaction effect	
	Normoxic trained leg		Hypoxic trained leg		F	P	F	P
[PCr]-τ (s) while breathing the normoxic inspirate	20.9 ± 2.9	19.1 ± 3.2	20.1 ± 4.2	16.6 ± 3.4	3.90	0.07	2.98	0.11
[PCr]-τ (s) while breathing the hypoxic inspirate	27.8 ± 4.3	24.1 ± 3.7	28.6 ± 4.5	21.2 ± 2.7	12.55	0.003 #	14.46	0.002 *

* Significant difference between INT and IHT legs ($P < 0.05$).

Significant training effect across both training conditions ($P < 0.05$).

Figure 5.2: [PCr]-т after the 24 s severe-intensity exercise bout, pre- and post-training. Grey bars illustrate mean values; black lines illustrate individual responses. * Significant difference between INT and IHT ($P < 0.05$). # Significant effect across both training conditions ($P < 0.05$). Note that under hypoxic conditions, all tests revealed a significant overall training effect, i.e. a faster [PCr]-т after training, and under hypoxic conditions there was a significant interaction ($P = 0.002$), showing that the IHT group realised a greater [PCr]-т reduction than the INT group.

5.4.4 ^{31}P-MRS variables and exercise tolerance during incremental exercise

The incremental exercise test results are summarised in Table 5.4 and Table 5.5. There were no significant differences between the improvements in T-Lim post-IHT compared to post-INT, regardless of whether participants were tested in normoxia (122 ± 41 vs. 128 ± 67 s) or hypoxia (78 ± 54 vs. 106 ± 45 s). There was only a significant overall training effect for T-Lim under normoxic test conditions when INT and IHT data were combined (Table 5.4 and Table 5.5). There were significant overall training effects when both IHT and INT data were combined for absolute and relative [PCr] at the T-Lim in both normoxia and hypoxia (Figure 5.3), and for [ADP] and [ATP-Ox] at the T-Lim in hypoxia. There were no other significant differences in MR variables measured at the T-Lim, or at the pH threshold, during the incremental tests, between IHT and INT (Table 5.4 and Table 5.5).

Table 5.4: ^{31}P-MRS variables and exercise tolerance, measured during the incremental test while breathing the normoxic inspirate.

	Pre-training	Post-training	Pre-training	Post-training	Main effect for time		Interaction effect	
	Normoxic trained leg		Hypoxic trained leg		F	P	F	P
pH threshold (s)	424 ± 74	485 ± 57	441 ± 88	488 ± 76	6.58	0.02 #	0.20	0.66
[PCr] @ T-Lim (mM)	16.4 ± 5.2	11.3 ± 3.4	16.2 ± 5.0	10.8 ± 3.0	23.66	<0.001 #	0.16	0.70
PCr @ T-Lim (%)	49.9 ± 11.8	36.0 ± 9.8	48.4 ± 13.2	33.9 ± 8.7	12.33	0.003 #	0.16	0.69
[ADP] @ T-Lim (μM)	42.4 ± 20.7	56.4 ± 30.7	45.7 ± 19.3	59.0 ± 24.7	2.20	0.16	0.01	0.95
[P_i] @ T-Lim (mM)	15.4 ± 5.7	19.6 ± 6.0	18.5 ± 6.6	18.3 ± 6.5	2.19	0.16	2.85	0.11
[P_i]/[PCr] @ T-Lim	1.10 ± 0.72	1.95 ± 0.97	1.33 ± 0.81	1.90 ± 0.98	0.70	0.42	0.41	0.53
pH @ T-Lim	6.86 ± 0.11	6.71 ± 0.15	6.85 ± 0.19	6.76 ± 0.19	3.55	0.08	0.43	0.52
ATP-Ox @ T-Lim (mM·s^{-1})	0.67 ± 0.13	0.90 ± 0.20	0.76 ± 0.14	1.10 ± 0.19	2.42	0.14	0.27	0.12
T-Lim (s)	488 ± 63	616 ± 42	505 ± 59	627 ± 57	10.31	0.006 #	0.03	0.86

Significant training effect across both training conditions ($P < 0.05$). T-Lim indicates limit of tolerance.

Table 5.5: ^{31}P-MRS variables and exercise tolerance, measured during the incremental test while breathing the hypoxic inspirate.

	Pre-training	Post-training	Pre-training	Post-training	Main effect for time		Interaction effect	
	Normoxic trained leg		Hypoxic trained leg		F	*P*	F	*P*
pH threshold (s)	457 ± 76	486 ± 74	438 ± 79	474 ± 76	1.45	0.25	0.08	0.78
[PCr] @ T-Lim (mM)	16.5 ± 4.9	10.2 ± 2.7	17.7 ± 7.7	11.1 ± 3.7	62.0	<0.001 #	0.24	0.63
PCr @ T-Lim (%)	48.3 ± 10.7	31.1 ± 7.5	50.1 ± 17.4	34.4 ± 9.1	35.82	<0.001 #	1.02	0.33
[ADP] @ T-Lim (µM)	44.9 ± 12.5	65.9 ± 19.5	42.1 ± 16.2	67.0 ± 16.5	5.86	0.03 #	0.04	0.85
[P_i] @ T-Lim (mM)	15.9 ± 3.8	19.2 ± 7.9	17.9 ± 7.3	19.5 ± 5.9	3.48	0.08	0.04	0.85
[P_i]/[PCr] @ T-Lim	1.06 ± 0.45	2.09 ± 1.11	1.23 ± 0.86	1.98 ± 1.02	<0.001	0.98	0.63	0.44
pH @ T-Lim	6.88 ± 0.10	6.76 ± 0.13	6.85 ± 0.17	6.76 ± 0.21	0.86	0.37	0.10	0.75
ATP-Ox @ T-Lim (mM·s^{-1})	0.54 ± 0.06	0.81 ± 0.13	0.52 ± 0.19	0.88 ± 0.15	6.91	0.02 #	1.36	0.26
T-Lim (s)	509 ± 40	615 ± 45	518 ± 41	595 ± 57	2.45	0.14	1.18	0.29

Significant training effect across both training conditions ($P < 0.05$). T-Lim indicates limit of tolerance.
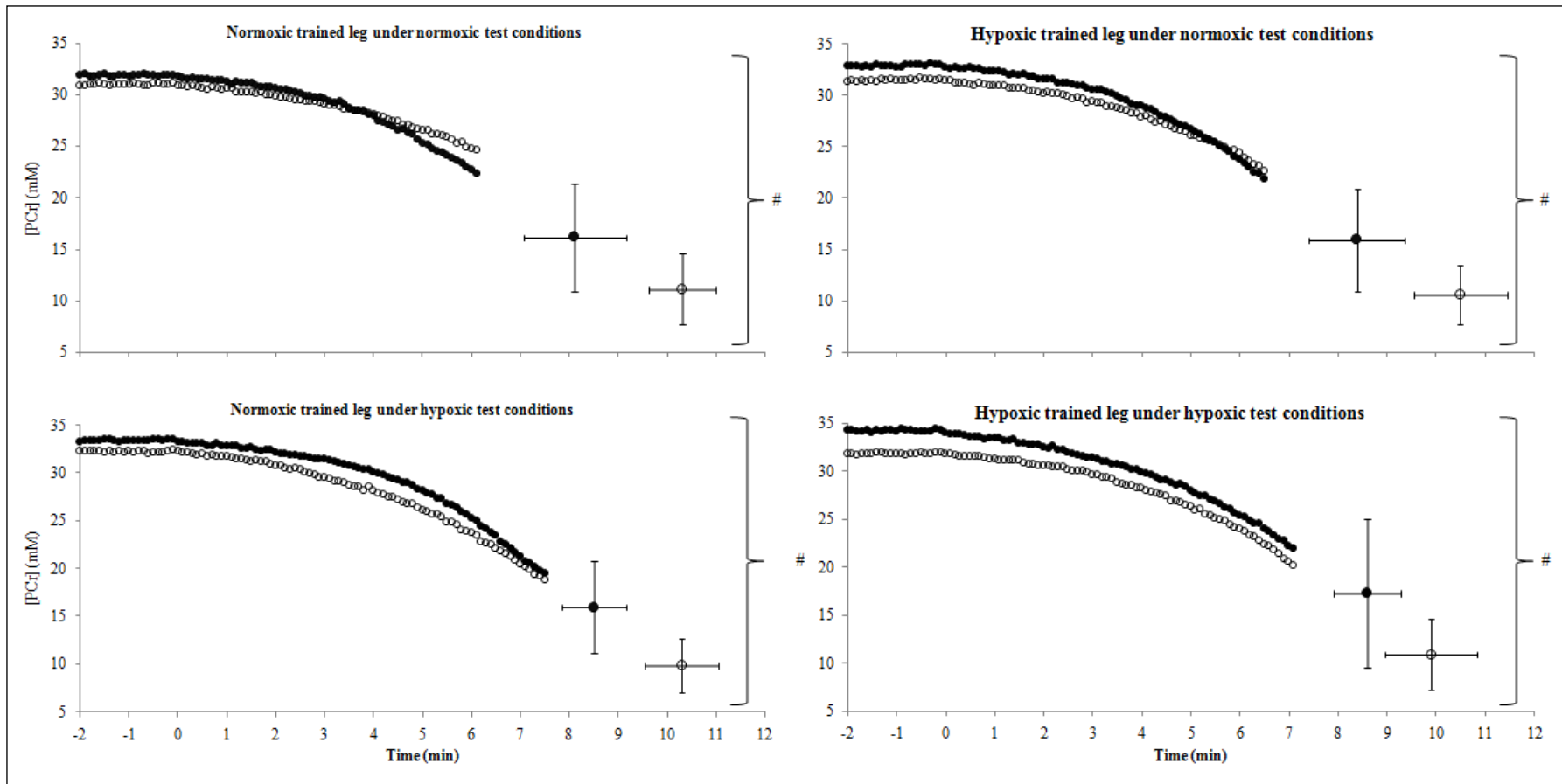
Figure 5.3: Mean [PCr] during the incremental test to exhaustion, pre- (●) and post-training (○). Circles without error bars are the mean [PCr] until a participant reached the T-Lim; circles with error bars are the mean (± SD) [PCr] and time at the T-Lim. #Significant effect across both training conditions ($P < 0.05$). Note that absolute [PCr] at the T-Lim significantly decreased post-training, under both normoxic and hypoxic test conditions ($P < 0.001$), but regardless of these overall training effects, there were no significant differences between the two training conditions (no interaction effect)

5.5   Discussion

To the knowledge of the authors, this is the first study to investigate the influence of IHT, compared to INT, on muscle energetics during exercise in normoxia and hypoxia. Overall, IHT had only limited effects on the muscle metabolic responses to exercise. During moderate-intensity exercise, the effects of training were similar between IHT and INT although, in hypoxia, Δ[PCr] was reduced to a slightly greater extent by IHT. Similarly, there were no significant differences in the effects of training on the pH threshold or any of the other ^{31}P-MRS-derived variables between IHT and INT during incremental exercise. Compared to INT, IHT resulted in a slightly but significantly faster [PCr]-τ in hypoxia and there was a tendency for there to be a similar effect in normoxia. However, changes in T-Lim during incremental exercise were not significantly different between IHT and INT either in normoxia or hypoxia.

5.5.1   PCr recovery kinetics

The pre-training differences in [PCr]-τ between normoxic and hypoxic test conditions of 7-9 s (34-42% slower) confirm that, in hypoxia, recovery from high-intensity exercise is substantially impaired. These results are similar to those of Haseler *et al.* (1999) who reported a 34% difference between [PCr]-τ measured in 0.209 FIO_2 compared to 0.100 FIO_2.

The reduction in [PCr]-τ in hypoxia following IHT was significantly greater than the reduction following INT (26% vs. 13%), suggesting that adding a hypoxic stimulus to normal training may augment the physiological adaptations that modulate the [PCr]-τ in hypoxia. The [PCr]-τ reduction of 17% post-IHT in normoxia is similar to the significant 19% change reported by Kuno *et al.* (1994) following four consecutive days of IHT (~480 min exposure). Unfortunately, that study did not include an INT control condition, such that it is not possible to determine to what extent hypoxia *per se* influenced the training adaptation above and beyond normal exercise training. However, collectively, these two studies suggest that IHT speeds muscle [PCr]-τ, which has been proposed to reflect muscle oxidative capacity (Harris *et al.*, 1976).

The high-intensity training stimulus employed in the present study is similar to that used in the study of Vogt *et al.* (2001), in which participants completed either high- or low-intensity INT or IHT for six weeks. These authors reported a significant gain in subsarcolemmal mitochondria density in the high- (+130%) and low- (+100%) intensity IHT groups, but not in the high (+1%) or low (-13%) intensity INT groups (Vogt *et al.*, 2001). Similarly, Geiser *et al.* (2001) reported that total and subsarcolemmal mitochondrial densities increased by +54% and +105%, respectively, following high-intensity IHT compared with +24% and +13%, respectively, following high-intensity INT. The results of the present study are consistent with these findings: assuming a sufficient muscle O_2 supply, an increase in mitochondrial volume would be expected to result in faster [PCr]-τ (Harris *et al.*, 1976; Haseler *et al.*, 1999; McMahon & Jenkins, 2002; Taylor *et al.*, 1983; Tomlin & Wenger, 2001), as was observed post-IHT. It is important to note, however, that the improvement in [PCr]-τ post-IHT was only different to that observed post-INT when participants were tested in hypoxia. If IHT had promoted mitochondrial biogenesis to a greater extent than INT, a significantly faster [PCr]-τ might also have been expected in normoxia. Therefore, it is likely that the faster [PCr]-τ observed after IHT was not solely due to enhanced mitochondrial biogenesis. It is known that post-exercise PCr recovery is heavily influenced by the muscle oxygenation status (Harris *et al.*, 1976; Haseler *et al.*, 1999). It may be speculated therefore that, compared to INT, IHT caused physiological adaptations that resulted in a greater enhancement of muscle O_2 delivery in hypoxia.

In hypoxia, the mitogen-activated protein kinase pathway is stimulated, enhancing the activity of HIF-1α. A range of molecular and structural changes subsequently take place (Hoppeler *et al.*, 2003) including enhanced transcriptional activation of VEGF (Ookawara *et al.*, 2002). Geiser *et al.* (2001) reported that capillary density increased significantly (by +12%) following high-intensity IHT, whereas there was no significant change following high-intensity INT. Vogt *et al.* (2001) also reported significant increases in VEGF mRNA and capillary density after high-intensity IHT by +52% and +19%, respectively, with no significant changes after low-intensity IHT, or high-intensity or low-intensity INT. Although not consistently found (Zoll *et al.*, 2006), these differences in capillary density changes between high-intensity IHT and INT (Geiser *et al.*,

2001; Vogt *et al.*, 2001) suggest that IHT may result in enhanced muscle oxygenation. If so, this would contribute to the reduced [PCr]-τ observed post-IHT in hypoxia (Haseler *et al.*, 1999). Faster [PCr]-τ following IHT would be expected to result in less fatigue and to enable better maintenance of performance during intermittent high-intensity exercise (Tomlin & Wenger, 2001). It may therefore be speculated that athletes competing in sports requiring repetitive sprints might obtain an advantage from performing IHT prior to competition, especially competitions at altitude (Faiss *et al.*, 2013a; Faiss *et al.*, 2013b; Galvin *et al.*, 2013; Millet *et al.*, 2010). It should be emphasised, however, that the faster [PCr]-τ following IHT was only statistically significant in hypoxia and that, in absolute terms, the effect was small, such that it is not certain to have functional relevance (see Section 5.5.3).

5.5.2 Muscle metabolic responses during moderate-intensity exercise

There were essentially no differences in the muscle metabolic response to moderate-intensity exercise resulting from IHT compared to INT. The only difference between conditions was that Δ[PCr] was reduced to a greater extent following IHT compared to INT when participants exercised in hypoxia. This sparing of the extent of PCr degradation suggests a lower muscle metabolic perturbation in hypoxia following IHT. The mechanistic basis for this effect is uncertain but might be linked to enhanced local muscle oxygenation (Haseler *et al.*, 1999; Wilson, 1994). However, although there were differences in Δ[PCr] following IHT and INT, the end-exercise [PCr] was not different and thus the functional significance of this change is questionable.

5.5.3 Muscle metabolic responses to incremental exercise and time-to-exhaustion

There were essentially no differences in the muscle metabolic response to incremental exercise following IHT and INT. Participants performed better in the post-training exercise tests in both normoxia and hypoxia, but there were no additional T-Lim improvements detected following IHT compared to INT.

The results from the present study are consistent with several previous studies (Geiser *et al.*, 2001; Roels *et al.*, 2005; Vogt *et al.*, 2001) in showing that, despite seemingly favourable adaptations in indices of mitochondrial function and/or capillarisation, IHT does not result in a greater improvement in exercise performance in normoxia compared to INT. Similar to the present study, using a double-blind placebo-controlled design, Truijens *et al.* (2003) added high-intensity interval training (three sessions per week for five weeks) to the programmes of trained swimmers and found that IHT was no more effective than INT in improving 100 m or 400 m time trial performance. Results from the present study contrast with those of Terrados *et al.* (1988) and Zoll *et al.* (2006) who reported that exercise tolerance was significantly increased after IHT but not INT in well-trained athletes. It is possible that differences in the reported effectiveness of IHT reflect inter-study differences in participant training status and the hypoxic 'dose' administered. It should be noted that, in the Zoll *et al.* (2006) study, face masks were used to administer the inspirate in the IHT group only, so the lack of blinding may have resulted in a performance-enhancing placebo effect. In the present study, participants remained blinded to the F_IO_2 during both training and testing (at the end of the study, only four out of the nine participants correctly guessed which leg had been trained in hypoxia) and the performance tests were conducted in a double blind manner.

5.5.4   Experimental considerations

This study employed an incremental exercise test to exhaustion to assess maximal aerobic performance, but incremental tests are less sensitive than constant-work-rate tests for assessing changes in exercise tolerance following an intervention (2009). The training intervention was relatively short (15 sessions spread over three weeks), so the hypoxic dose was rather small, so it cannot be excluded that IHT might have more effectively enhanced performance if it had been practiced for longer. The moderate training status of the participants is also an important consideration. Previous studies have suggested that differences in performance changes between INT and IHT may be more likely in highly-trained (Terrados *et al.*, 1988; Zoll *et al.*, 2006) compared to less well-trained (Geiser *et al.*, 2001; Vogt *et al.*, 2001) participants. The statistical power would have been stronger if more than the 18

treatment conditions (i.e. two legs from nine participants) was included in the study. Nevertheless, the only other IHT investigation to date to have assessed skeletal muscle adaptations via ^{31}P-MRS only had four participants (Kuno *et al.*, 1994), thus highlighting the challenge of obtaining large samples when using this resource intensive approach.

It should also be noted that the IHT and INT training sessions were completed at the same absolute intensity such that the IHT leg was trained at a slightly higher relative intensity. It is possible that this contributed to the minor differences (for example in [PCr]-τ) observed between IHT and INT. The localised muscle mass engaged by the present studies' single-legged exercise training and testing modality precluded the measurement of $\dot{V}O_2$max and did not simulate the oxidative energy demand that would be experienced during whole-body exercise. However, studies that have used separate training groups or cross-over designs are subject to the normal daily variations in participants' activities outside of the controlled training and test environments. One of the key strengths of the present investigation is the single-legged study design, which ruled out any placebo effects and allowed non-invasive interrogation and comparison of the muscle metabolic adaptations to IHT compared with INT in the same participants.

5.6   Conclusion

In conclusion, compared to INT, IHT resulted in no meaningful changes in the muscle metabolic response to moderate-intensity constant-work-rate exercise or exhaustive incremental exercise. IHT resulted in a significant reduction of [PCr]-τ in the recovery from high-intensity exercise, in hypoxia only. While the reduced [PCr]-τ in hypoxia may reflect increased muscle oxidative capacity following IHT, the practical importance of this is questionable, given that IHT was no more effective than INT in enhancing incremental exercise performance either in hypoxia or normoxia.

CHAPTER 6:

General Discussion and Conclusion

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6.1   General discussion

The aims of this thesis were to investigate changes in competitive race performance and tHb in response to a three week LH+TH intervention in highly trained swimmers, to investigate changes in physiological variables during submaximal and maximal exercise in response to an eight week IHT intervention in highly trained runners, and to investigate changes in skeletal muscle energetics and incremental exercise performance in response to a three week single-legged IHT intervention in healthy but untrained participants.

When designing these investigations consideration was given to the limitations within the existing altitude and hypoxia research to date, namely that:

- Control groups are often not appropriately matched to experimental groups, or are not used at all. Appropriate control groups were used in Chapters 3 and 4, and the single-leg design in Chapter 5 allowed the normoxic trained leg to be used as the 'control' condition.

- Participants are often not blinded to the experimental condition, meaning that influential placebo and/or nocebo effects may occur. While it was not possible to blind participants to the LH+TH in Chapter 3, participants were completely blinded to the F_IO_2 during all training sessions in Chapters 4 and 5. Furthermore, both the participants and the researchers were blinded to the F_IO_2 throughout all exercise testing in Chapters 4 and 5.

- Some interventions expose participants to an insufficient hypoxic dose. Although the ~500 h at 2320 m altitude in Chapter 3 equals or surpasses the hypoxic dose of noteworthy studies in this field (Chapman *et al.*, 2014; Gore *et al.*, 2013; Robach *et al.*, 2006b; Wachsmuth *et al.*, 2013), some recent reports suggest an increase in the required hypoxic dose (Rasmussen *et al.*, 2013), compared to conventional views (Schmidt &

Prommer, 2008; Wilber *et al.*, 2007), and certainly this requirement is far from being universally accepted. Similarly, the hypoxic dose of any IHT intervention is arguably very small, and while the 640 min spread over eight weeks in Chapter 4 was primarily based on the study by Dufour *et al.* (2006), this IHT 'dose' is a highly contentious topic.

- The results in untrained participants or recreational athletes are extrapolated to suggest implications for highly trained athletes, whereas participants in Chapters 3 and 4 were highly trained athletes, and while participants in Chapter 5 where not highly trained, the results were not suggested to specifically relate to highly trained athletic populations.

- Inappropriate or not sufficiently sensitive methodologies are often used, whereas: i) Chapter 3 is one of very few investigations to have assessed tHb (using the optimised CO-rebreathing protocol) in highly trained athletes (see Section 3.3.4); ii) novel methodologies were developed to assess breath-by-breath $\dot{V}O_2$ in a hypoxic environment in Chapter 4 (see Section 2.5); and iii) the ^{31}P-MRS techniques used in Chapter 5 provided detailed *in vivo* quantification of skeletal muscle energetics, at an effective sample frequency of 6.0 s, during moderate and severe exercise and recovery – which had never been previously assessed in relation to IHT (see Section 5.3). It is important here to acknowledge that all such assessments of physiological, biochemical or haematological variables are essentially limited by issues of day-to-day biological variation. However sensitive a particular test may be, if the variable of interest changes a great deal on a daily basis, the ability to detect a true change is limited. Novel means of overcoming these issues are suggested in Section 6.2.

The common theme throughout the preceding experimental chapters was the exposure of participants to varying degrees of hypoxia. In Chapter 3, LH+TH for three weeks at an altitude of 2320 m resulted in highly trained swimmers experiencing significant tHb increase compared to a sea level control group, but did not result in a greater 200 m race performance gain as assessed 28 d later. Importantly, this was not the case for all swimmers – some swimmers did not

show any tHb changes, yet swimming performances improved, and there were no correlations between the changes in tHb and 200 m race performances. Therefore, these results suggest that non-haematological adaptations likely occurred in at least some of these swimmers. As such, Chapter 4 sought to investigate these non-haematological adaptations, by exposing highly trained runners to two IHT sessions·week^{-1} for eight weeks, at an altitude simulation of ~2100 m. This IHT intervention elicited some physiological adaptations observed during submaximal exercise, but there were no more beneficial changes at maximal intensity exercise compared to that in a normoxic control group. Then, Chapter 5 also investigated non-haematological adaptations to IHT, and in order to specifically target the hypoxic stimulus on skeletal muscle, a single-legged IHT intervention was used (15 IHT sessions spread over three weeks, at an altitude simulation of ~3000 m). The ^{31}P-MRS techniques employed in this final experimental chapter had never before been applied to an environmental condition-blinded sham-controlled IHT study. While this regular IHT may have enhanced the muscle oxidative capacity, as evidenced by a faster [PCr]-τ during hypoxic tests, the practical importance is questionable, given that there were no greater improvements in incremental exercise performance or other ^{31}P-MRS variables compared to the normoxic trained leg.

By acknowledging and rectifying some of the aforementioned limitations with past research, the experimental designs of the preceding chapters were strengthened, in particular by using properly blinded control groups / conditions, using novel and sensitive techniques, and by assessing responses in trained athletes where possible. The resultant data is therefore more robust, so findings regarding the physiological effects due to altitude and hypoxic exposure can be discussed with a greater degree of certainty than in many studies to date.

6.1.1   Physiological effects of altitude and hypoxia

The experimental chapters quantified a range of variables that have been proposed to be influenced by varying degrees of hypoxic exposure. As stated, these are broadly categorised as being related to haematological or non-haematological adaptations, both of which are now discussed.

6.1.1.1   Haematological adaptations

Chapter 3 illustrated that LH+TH altitude training for three weeks at 2320 m resulted in a significant tHb increase, on average a 4.1% greater increase immediately after altitude compared to sea level training. The inter-individual response was extremely varied though; increasing in five of the seven participants, but remaining unchanged in two others. The within group mean change was +4.4%, range -0.3 to +9.0% (see Figure 3.1). These responses are similar to those reported by Garvican *et al.* (2012), whereby LH+TH+TL for three weeks at 2760 m (living) and 1000-3000 m (training) resulted in a greater tHb increase than in a sea level control group (mean difference between groups ± 90% confidence limits (CL) = +3.3%, ± 3.4%). However, these changes are substantially less than results reported by Levine & Stray-Gundersen (1997), whereby LH+TH for four weeks at 2500 m resulted in a significant ~9% RCV increase. Importantly, both of these studies also reported significant [Epo] increases; peaking after 24-48 h at altitude (Garvican *et al.*, 2012; Stray-Gundersen *et al.*, 1995). Unfortunately it was not possible to quantify [Epo] in Chapter 3, so it cannot be ruled out that the altitude of 2320 m elicited an insufficient degree of biological hypoxia (in particular renal tissue hypoxia) to induce the erythropoietic response.

To investigate this concept, Ge *et al.* (2002) exposed untrained volunteers to a variety of simulated altitudes, from 1780 m to 2800 m, using a hypobaric hypoxic chamber. [Epo] was significantly elevated after 6 h at all altitude simulations, but only continued to increase up until 24 h at simulated altitudes of 2454 m and 2800 m, and not at 1780 m or 2085 m (Ge *et al.*, 2002). These results indicate that short-term acclimatisation (involving the HVR, HCVR and HPVR, as detailed earlier in Section 1.3.1) may restore renal tissue oxygenation and attenuate the rise in Epo at lower altitudes, but that at altitudes of ~2454 m and higher, these responses are inadequate. Clearly there will be a substantial degree of inter-individual variation, and the altitude threshold for more sustained Epo elevations will not be exact, but the altitude of 2320 m used in Chapter 3 does fall within this lower boundary. If the biological hypoxia was insufficient to induce any significant erythropoietic response, this would explain why some individuals did not respond haematologically to the LH+TH stimulus.

However, even when six highly trained male athletes lived at 2050 m, i.e. a ~12% lower altitude than used in Chapter 3, Heinicke *et al.* (2005) reported that [Epo] significantly increased up until day 4, and tHb significantly increased after three weeks, on average by ~9%. More specifically to the experimental design of the present study, Wachsmuth *et al.* (2013) investigated the effects of LH+TH for 3-4 weeks, also in highly trained swimmers, at the same location as used during Chapter 3 (Centro Alto Rendimiento Deportivo in Sierra Nevada, Spain, at an altitude of 2320 m). These authors reported a significant [Epo] increase up until the 10th day at altitude (which diminished thereafter) and a significant tHb increase by the end of the intervention (on average +7%). In accordance with these results, assuming that the hypoxic dose in Chapter 3 was sufficient, the question still remains as to why some individuals did not experience tHb increases after the altitude exposure.

As previously detailed, the LH+TH investigations by Gore *et al.* (1998) and Pottgiesser *et al.* (2009), and the double-blinded placebo controlled simulated LH+TL investigation by Siebenmann *et al.* (2012), also failed to show any haematological increments in highly trained, elite athletes who were actively competing at international levels. This fitness status of participating athletes may be fundamental to the reasons as to why some athletes do not respond haematologically to LH+TH altitude exposure. Accordingly, even the results from the most commonly cited altitude training study by Levine & Stray-Gundersen (1997) have recently been questioned. Robach & Lundby (2012) calculated the tHb of the participating athletes using the PV and [Hb] data from Levine & Stray-Gundersen (1997) and the HCT data from the same experiment, reported by Chapman *et al.* (1998). BV and tHb were thus calculated using the below equations (see Figure 6.1):

$$BV = PV / HCT \qquad (\text{Thomsen } et\ al., 1991)$$

$$tHb = BV \cdot [Hb] \qquad (\text{Thomsen } et\ al., 1991)$$

Where: BV = blood volume, PV = plasma volume, HCT = haematocrit, tHb = total haemoglobin mass, and [Hb] = haemoglobin concentration.

Robach & Lundby (2012) reported that the participant's baseline tHb relative to body weight was only ~9.8 g·kg^{-1} in the study by Levine & Stray-Gundersen (see Figure 6.1). Importantly, it must be considered that the mean RCV results used to estimate these tHb results encompassed data from both males (n = 9) and females (n = 4), and also that there may be a degree of error involved with these retrospective calculations, especially given the reliance of a stable HCT, so the real tHb values *could* have been marginally higher. Nevertheless, this estimation of ~9.8 g·kg^{-1} is the lowest of any publication to have been cited in this thesis, and is substantially lower than the combined baseline results for males (n = 8) and females (n = 3) of 13.1 g·kg^{-1} in Chapter 3. According to a comprehensive meta-analysis encompassing the tHb results of 611 participants, a similarly low baseline tHb (~9.9 g·kg^{-1}) was established for "moderately trained" females (with a $\dot{V}O_2$max range of just 48 – 57 mL·kg^{-1}·min^{-1}). These recent calculations highlight that despite the 4-6 week lead in phase of sea level training, it would appear that the collegiate level athletes recruited by Levine & Stray-Gundersen (1997) did not possess the haematological characteristics associated with a highly developed endurance capacity. Therefore the significant ~9% RCV increase is largely unsurprising, but such substantial adaptations are less likely to occur in more highly trained athletes.

As such, it could be that tHb gains only occur in those "athletes" with an initial low baseline tHb (Robach & Lundby, 2012), and much of the literature confusion as to whether haematological gains do (Levine & Stray-Gundersen, 2005) or do not (Gore & Hopkins, 2005) occur is mostly due to the wide range of baseline performance and fitness level of the participants (see Figure 6.1).
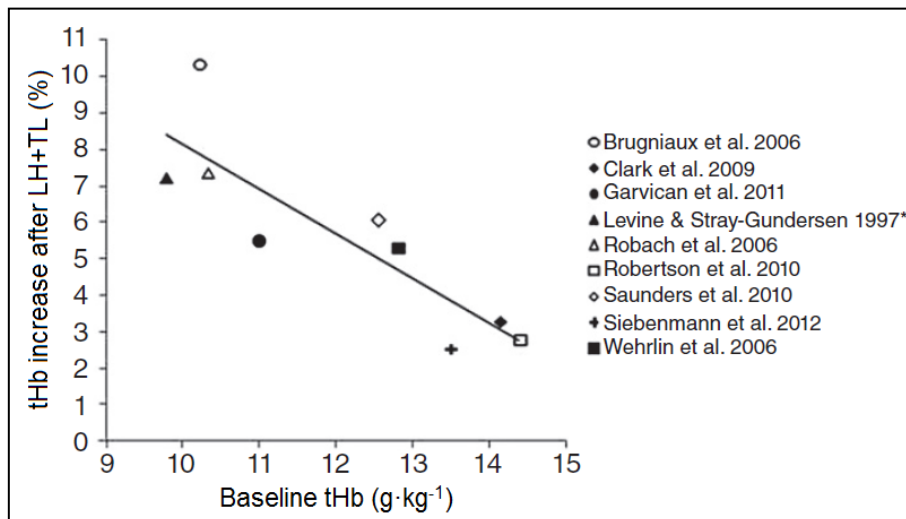
Figure 6.1: The relationship between baseline body weight adjusted tHb and the percentage tHb increase after various LH+TL interventions (Brugniaux *et al.*, 2006; Clark *et al.*, 2009; Garvican *et al.*, 2011; Levine & Stray-Gundersen, 1997; Robach *et al.*, 2006b; Robertson *et al.*, 2010a; Saunders *et al.*, 2010; Siebenmann *et al.*, 2012; Wehrlin *et al.*, 2006). This figure has been adapted from Robach & Lundby (2012) with permission from the authors. Note that the relationship is significant ($R^2 = 0.74$, $P < 0.01$), such that those participants who have lower baseline tHb are most likely to experience the greatest tHb gains.

With this in mind, in relation to the results from Chapter 3, it must be considered that the participants' tHb relative to body weight of 13.7 ± 0.9 g·kg^{-1} (males) and 11.6 ± 0.4 g·kg^{-1} (females) were substantially higher than the means of 12.7-13.2 g·kg^{-1} and 10.7 g·kg^{-1} as reported for other highly trained male and female swimmers, respectively (Heinicke *et al.*, 2001; Wachsmuth *et al.*, 2013). Furthermore, some of the participants in Chapter 3 were previous Commonwealth and World Champions. These factors illustrate that these swimmers were highly trained, elite athletes, with well developed endurance capacities. As such, it is perhaps unsurprising that some of these participants did not experience any further haematological gains; in agreement with Gore *et al.* (1998), probably due to initial tHb values being close to the natural physiological limit, with little scope for further change, at least not in response to this moderate altitude of 2320 m. Although the meta-analysis based guidelines for RCV and/or tHb expansions at altitude may provide a useful resource (Gore *et al.*, 2013; Rasmussen *et al.*, 2013), it is imperative that attention is given to the highly variable responses for each individual athlete (see Figure 6.2).
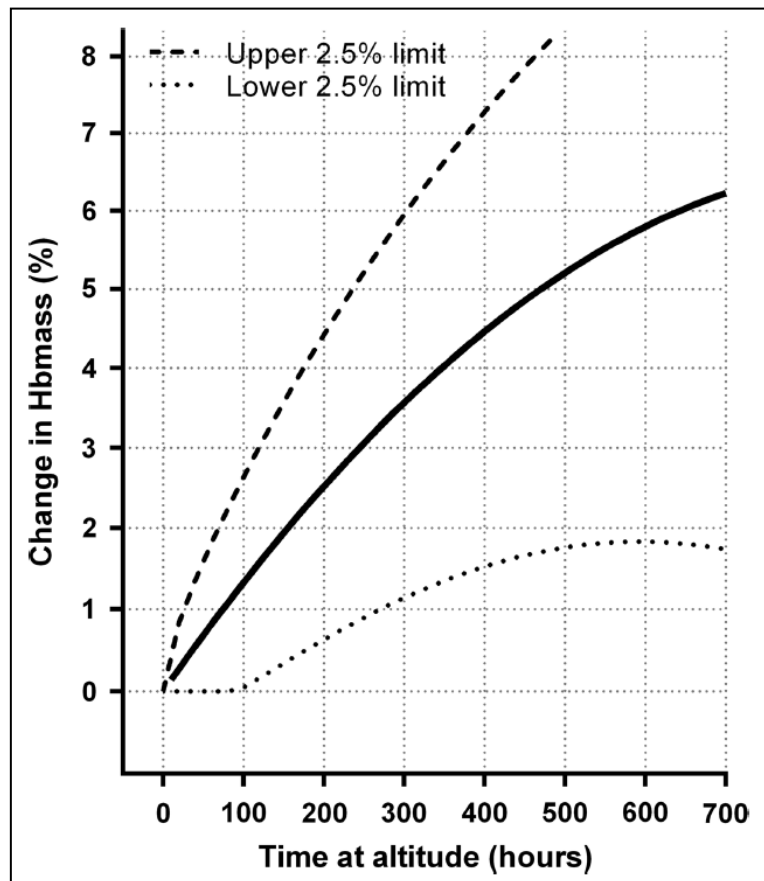
Figure 6.2: Estimated median and between-participant 'true' tHb changes in response to LH+TL and traditional altitude training. The solid line depicts the meta-analysis-based quadratic fitted model, and the dashed lines represent the upper and lower 95% individual response limits (where the lower limit was estimated to be negative, it has been truncated at zero). Adapted from Gore *et al.* (2013) with permission from the authors. Note that the tHb change from baseline depicted by the lower 95% individual response limit is consistently <2%, in agreement with the tHb results for some participants in Chapter 3 of this thesis (see Figure 3.1).

If the occurrence of a substantial haematological gain is dependent on the baseline tHb and state of aerobic conditioning (Robach & Lundby, 2012), it is imperative that when identifying athletes as "responders" or "non-responders" to altitude or hypoxic training, classifications should be based not on haematological variables, but instead on competitive race performance. It is plausible that those athletes with an already high tHb at baseline would require a more severe hypoxic stimulus (Ge *et al.*, 2002), or indeed that these athletes simply will not experience substantial tHb gains (Gore *et al.*, 1998), and instead alternative adaptations may occur.

6.1.1.2   Non-haematological adaptations

Given the lack of an association between the change in performance and tHb in Chapter 3 (see Figure 3.2 and Figure 3.3), it is suggested that non-haematological changes may have occurred, which positively influenced performance after the LH+TH intervention, at least in some participants. Similarly, the IHT interventions employed in Chapters 4 and 5 would not have induced any significant haematological alterations, due to an insufficient hypoxic dose (Gore *et al.*, 2013; Rasmussen *et al.*, 2013; Schmidt & Prommer, 2008; Wilber *et al.*, 2007). As such, an array of non-haematological adaptations to altitude and hypoxia must be considered in relation to results from these experimental chapters.

6.1.1.2.1   *Angiogenesis and mitochondrial biogenesis*

As previously discussed (Section 1.2.4), an increased tHb is suggested to augment maximum cardiac output ($\dot{Q}max$) and/or arteriovenous O_2 difference (Schmidt & Prommer, 2010), resulting in an enhanced O_2 delivery to the exercising skeletal muscles, and a greater O_2 utilisation, evident as an improved $\dot{V}O_2max$ (Levine & Stray-Gundersen, 1997; Levine & Stray-Gundersen, 2005). Alternatively, a greater peripheral vascular network may also enhance muscle O_2 delivery (Terrados *et al.*, 1988), and a greater mitochondrial mass may also enhance overall O_2 utilisation (Geiser *et al.*, 2001; Vogt *et al.*, 2001).

Chapter 5 exercised and tested energetic adaptations within quadriceps muscles of moderately trained participants, and owing to the single-legged design, it was possible to specifically isolate the hypoxia induced adaptations at the peripheral level. On average, [PCr]-τ was speeded up approximately twice as much in the IHT leg compared to the control leg, when tested in both hypoxia (IHT -26% vs. control -13%), and normoxia (IHT -17% vs. control -9%), but these differences only reached statistical significance in hypoxic test conditions ($P = 0.002$), not in normoxia ($P = 0.11$) (see Figure 5.2). Using ^{31}P-MRS (and without skeletal muscle biopsies) it was not possible to discern precisely how these [PCr]-τ changes occurred after IHT; only that PCr recovery was speeded up, indicating an overall increased muscle oxidative capacity (Harris *et al.*, 1976). Nevertheless, the differences between the results of tests while

participants breathed an F_IO_2 of 0.209 and 0.145 provide some further insight. While chronic exercise causes an increased skeletal muscle mitochondrial mass (Hood, 2009), if an enhanced mitochondrial biogenesis had occurred in the IHT leg compared to the control leg, a similarly significant [PCr]-т reduction under normoxic test conditions would have likely been measured.

It is noteworthy that Hoppeler *et al.* (1990) reported significant decreases in muscle fibre cross-sectional area (-10%) and mitochondrial volume (-25%) after an eight week mountaineering expedition to high altitudes, but this was mirrored by a sparing of muscle capillary density, leading to a significant reduction in the muscle fibre volume per capillary. This finding of a decrease in mitochondrial mass but a relatively improved O_2 supply after prolonged altitude expeditions has also been shown in response to simulated (high) altitude exposure (MacDougall *et al.*, 1991). It is important to note that the pronounced muscle atrophy reported after many such extreme hypoxic interventions may not solely be due to the hypoxia-induced down-regulated oxidative metabolism (Chaudhary *et al.*, 2012), but at least partially due to sustained periods of relative inactivity or low absolute intensity exercise (Edwards *et al.*, 2010), and changes in habitual nutritional intake (Rose *et al.*, 1988).

With more application to athletic populations, Robach *et al.* (2012) reported that neither mitochondrial content (quantified as CS activity) nor mitochondrial function (quantified as O_2 flux per unit of mitochondrial content) was altered when highly trained cyclists undertook four weeks of simulated LH+TL or normoxic living and training, in a double-blinded, placebo-controlled manner. These results should be considered in relation to findings that in untrained humans, the skeletal muscle mitochondrial oxidative capacity exceeds O_2 delivery capacity by ~50% during normoxic cycling exercise (Boushel *et al.*, 2011), indicating that O_2 delivery limits muscle oxidative capacity. It therefore seems illogical that mitochondrial mass would increase in response to an even more restricted muscle O_2 supply than normal, i.e. a state of "hypo-metabolism" (Hochachka & Somero, 2002; Howald & Hoppeler, 2003). At the other end of the spectrum, enhanced (cerebral) mitochondrial biogenesis has been observed when rats were exposed to plentiful O_2 (Gutsaeva *et al.*, 2006).

In relation to Chapter 5, while severe changes such as those observed after sustained hypoxic exposures (Hoppeler *et al.*, 1990; MacDougall *et al.*, 1991; Robach *et al.*, 2012) would not be expected after three weeks of IHT, a less exaggerated response may have occurred. The reduced P_aO_2 during the hypoxic training sessions may have attenuated the typical (normoxic) exercise-induced mitochondrial mass increase (Hood, 2009). This theory is in opposition to conclusions by Kuno *et al.* (1994), who proposed that the quicker [PCr]-τ after 8 h of IHT over 4 d was due to an enhanced mitochondrial function. However, as was also the case in Chapter 5, these authors had no way of categorically distinguishing between changes in mitochondrial mass and capillarisation. Certainly the reduced P_aO_2 and therefore muscle O_2 extraction under hypoxic conditions would have exaggerated the O_2 delivery requirement to the skeletal muscle (Heinonen *et al.*, 2010). As the hypoxic dose was too small to initiate a tHb change (Gore *et al.*, 2013), and indeed any such systemic adaptation would have equally influenced the normoxic trained legs, it is speculated that the reduced [PCr]-τ may have been achieved via a greater degree of angiogenesis after IHT compared to normoxic training, rather than via an increased mitochondrial mass. In order to be more definitive, results from studies that have assessed muscle ultrastructure directly via biopsies must be considered.

The experimental design by Terrados *et al.* (1990) is comparable to Chapter 5, as untrained participants undertook single-legged IHT (while in a hypobaric hypoxic or normobaric normoxic chamber), exercising each leg for 30 min, 3-4 d·week^{-1}, for four weeks. The key findings were that CS activity increased significantly more in the IHT leg compared to the control leg, but capillary density remained unaltered in both legs (Terrados *et al.*, 1990). This CS increase may reflect an enhanced oxidative capacity, but the lack of a capillarisation change is in contrast to the angiogenesis linked speculations based on the results from Chapter 5. These capillarisation results also conflict with the study by Geiser *et al.* (2001), who reported a significant capillary density increase (mean change +12%) after six weeks of IHT, but not after normoxic training (mean change -0.3%). Such findings were also replicated in a separate study by the same Swiss research group (Vogt *et al.*, 2001), who reported that HIF-1 and VEGF mRNA, and capillary density (mean change +19%) significantly increased after high-intensity whole body IHT, but not after

equivalent normoxic training (mean change -4%). As increases in VEGF mRNA correlate with HIF-1 mRNA changes (Gustafsson *et al.*, 1999), these results suggest that the capillary density changes were likely caused by HIF-1 and VEGF increases. Furthermore, in contrast to their single-legged study (detailed above), Terrados *et al.* (1988) reported that CS activity was unchanged, and the number of capillaries per muscle fibre tended to increase after 3-4 weeks of whole body IHT (mean change +15%), but not after normoxic training (mean change -8%), in competitive cyclists.

Although the studies by Vogt *et al.* (2001) and Geiser *et al.* (2001) also both reported that subsarcolemmal mitochondrial mass increased significantly more after IHT (+130% and +105%, respectively) compared to normoxic training (+1% and +13%, respectively), no such changes have been reported in moderately or highly trained participants. Indeed, the study by Terrados *et al.* (1990) used male participants without any prior history of regular physical training. Conversely, the investigation by Ponsot *et al.* (2006) (for details see the discussion in Section 1.3.3 regarding Dufour *et al.* (2006), which was part of the same study) showed that after moderately trained runners completed six weeks of IHT, the *in vitro* estimate of muscle maximal oxidative capacity remained unaltered. Similarly, after moderately trained cyclists exercised in normobaric normoxia, or hypoxia at an altitude simulation that matched that used during Chapter 5 (~3000 m / F_IO_2 ~0.146), Faiss *et al.* (2013b) reported significant decreases in the mRNA concentrations of PGC-1α and mitochondrial transcription factor A (mtTFA), indicating an attenuated stimuli for mitochondrial biogenesis (Faiss *et al.*, 2013b).

Taken together, findings from the available literature suggest that IHT may result in mitochondrial biogenesis only in untrained individuals, and may result in enhanced angiogenesis in those individuals who are more highly trained. Importantly though, although the [PCr]-т results from Chapter 5 are suggestive of enhanced angiogenesis or mitochondrial mass, the magnitude of these changes is questioned by the fact that there were no other ^{31}P-MRS derived variables to respond. If extensive angiogenesis or mitochondrial mass enhancements had occurred, a significantly greater improvement in the rate of oxidative ATP turnover would also have been expected, but this was not the

case, whether tested in normoxia (IHT leg +14% vs. control leg +21%, *P* = 0.58) or hypoxia (IHT leg +41% vs. control leg +19%, *P* = 0.18).

Although the findings reported in Chapter 5 are in agreement with the majority of the literature, i.e. that IHT can augment oxidative capacity changes within the skeletal muscle, likely caused by angiogenesis, and perhaps also by a degree of mitochondrial biogenesis, there is not a unified agreement as to the key mechanisms. Further detailed investigation is required into the responses in highly trained participants, using ecologically valid training interventions (i.e. interventions that would be possible for highly trained athletes to use), with outcome variables being assessed via reliable methodologies (see Section 6.2).

6.1.1.2.2   *Submaximal exercise economy*

Results from Chapter 4 showed a significantly lower submaximal HR after IHT, thus indicating an enhanced cardiovascular fitness. These results are consistent with Vallier *et al.* (1996), who reported a 3-4% submaximal HR reduction in highly trained triathletes after three weeks of IHT, and also with Terrados *et al.* (1990), who reported that submaximal HR was reduced significantly more after highly trained cyclists undertook 3-4 weeks of IHT, compared to a normoxic control group (mean -16% vs. -12%, respectively). However, further comparisons are not possible, as other analogous IHT studies did not report submaximal HR data (Dufour *et al.*, 2006; Geiser *et al.*, 2001; Melissa *et al.*, 1997; Robertson *et al.*, 2010b; Roels *et al.*, 2005; Terrados *et al.*, 1988; Ventura *et al.*, 2003; Vogt *et al.*, 2001).

Regardless of any submaximal HR change, the submaximal $\dot{V}O_2$ (exercise economy) changes observed in Chapter 4 were highly variable; decreasing after IHT when tested in hypoxia (mean change -5.7 ± 3.4% or -2.6 ± 1.7 mL·kg^{-1}·min^{-1}; *P* = 0.001), but increasing after normoxic training when tested in normoxia (mean change +1.9 ± 3.9% or +1.1 ± 2.1 mL·kg^{-1}·min^{-1}; *P* = 0.012) (see Figure 4.1). This lack of a consistent exercise economy change following IHT is common across much of the comparable literature. Roels *et al.* (2005) found that after seven weeks of IHT there was a trend for a submaximal $\dot{V}O_2$ decrease when tested in hypoxia (n = 11, mean change -2.9%), whereas after normoxic training there was a trend for a submaximal $\dot{V}O_2$ increase when tested

in normoxia (n = 11, mean change +2.1%), but these differences were not statistically significant within or between groups. Unfortunately these authors did not conduct submaximal exercise tests in normoxia for the IHT group, but in any case these results are in agreement with Chapter 4. Similarly, in their six week IHT intervention, Dufour et al. (2006) found that submaximal $\dot{V}O_2$ measured in normoxia did not significantly change within their IHT group (n = 9, mean change -3.4% at 12 km·h^{-1} and -0.2% at 18 km·h^{-1}) or their control group (n = 9, mean change -2.2% at 12 km·h^{-1} and -2.8% at 18 km·h^{-1}), and there were no significant between group differences. Lastly, in the particularly well controlled study by Robertson et al. (2010b), the changes in submaximal $\dot{V}O_2$ were not significantly different between the highly trained runners who undertook three weeks of IHT (n = 9, mean change +2.8%) or three weeks of IHT while sleeping in normobaric hypoxia (n = 8, mean change +2.9%). Thus, it would appear that there is minimal, if any, evidence that IHT positively influences exercise economy, at least in terms of submaximal $\dot{V}O_2$.

However, the economy results from the study by Robertson et al. (2010b) conflict with most other LH+TL studies (i.e. sustained hypoxic exposure) from this Australian research group, which have shown significant improvements (submaximal $\dot{V}O_2$ reductions). Saunders et al. (2004) found that 20 d of simulated LH+TL (sleeping at a simulated altitude of 2000-3100 m, training at a physical altitude of 600 m) resulted in significantly reduced submaximal $\dot{V}O_2$ (mean change -3.3%) in a group of highly trained middle distance runners (see Table 1.2). These results compared to no such change in a group who lived at a physical altitude of 1570 m and trained at 1500-2000 m (mean change -0.3%), or in a sea level control group (mean change +0.6%). This (significant) running economy change would appear to be meaningful, especially in such a highly trained cohort (mean pre-intervention $\dot{V}O_2$max = 73 ± 4 mL·kg^{-1}·min^{-1}).

As part of Chapter 3 it would have been insightful to have also assessed submaximal $\dot{V}O_2$ during swimming, but unfortunately this was not possible due to the technological and logistical challenges of doing so in a swimming pool. Largely because of these challenges, the only study to have measured exercise economy in highly trained swimmers in relation to a hypoxic intervention is that by Truijens et al. (2008), who found that submaximal $\dot{V}O_2$ did not change pre- to

post-intervention, but they used IHE (at rest), for only 3 h·d^{-1}, so results should not be extrapolated to hypoxic exercise (IHT) or sustained LH+TL. As such, further research would be informative in establishing the effects, if any, of traditional and simulated altitude training on swimming economy.

While similar results have been reported in other studies by this Australian research group, i.e. ~3% submaximal $\dot{V}O_2$ decreases after sustained LH+TL (Gore *et al.*, 2001; Humberstone-Gough *et al.*, 2013; Saunders *et al.*, 2009), Lundby *et al.* (2007a) pooled together results from three similar studies from a US based research group (Levine, 2002; Levine & Stray-Gundersen, 1997; Stray-Gundersen *et al.*, 2001), and concluded that exercise economy remains unchanged following sustained hypoxic exposure. Although the meta-analysis is statistically powerful, it should be noted that the methodologies employed by the Australians are particularly stringent (e.g. triple repeats of submaximal exercise tests to ensure low typical error of measurement), so their results cannot be discounted. One key difference is the sample population heterogeneity: the Australian studies consistently assessed highly trained athletes, whereas the US studies included moderately trained (collegiate level) participants, which may have resulted in different adaptations to the hypoxia. With these leading research groups being at a clear disagreement, the effects of sustained LH+TL on exercise economy in highly trained athletes is presently uncertain.

These above discussed studies (Dufour *et al.*, 2006; Gore *et al.*, 2001; Humberstone-Gough *et al.*, 2013; Levine, 2002; Levine & Stray-Gundersen, 1997; Lundby *et al.*, 2007a; Robertson *et al.*, 2010b; Roels *et al.*, 2005; Saunders *et al.*, 2004; Saunders *et al.*, 2009; Stray-Gundersen *et al.*, 2001), as well as the incremental exercise tests in Chapter 4, only assessed exercise economy via pulmonary $\dot{V}O_2$ estimations, whereas more direct measures of skeletal muscle energetics should also be considered. This is especially the case, considering that Chapter 5 (see Section 5.5.1 and Figure 5.2) and others (Geiser *et al.*, 2001; Kuno *et al.*, 1994; Terrados *et al.*, 1990; Terrados *et al.*, 1988; Vogt *et al.*, 2001) suggest an IHT induced enhanced oxidative capacity, potentially due to altered angiogenesis and/or mitochondrial biogenesis (see Section 6.1.1.2.1).

During the moderate intensity exercise tests in Chapter 5, Δ[PCr] (i.e. the magnitude of PCr degradation) decreased significantly more in the IHT leg compared to the normoxic trained control leg, when tested in hypoxic conditions (mean changes: IHT -23%, control -9%, $P < 0.001$) (see Table 5.2), and showed a tendency to do the same when tested in normoxic conditions (mean changes: IHT -18%, control -8%, $P = 0.09$) (see Table 5.1). With the absolute exercise test intensities being equal for both legs, these results show that IHT spared PCr utilisation to a greater extent than normoxic training. Thus it could be argued that this PCr sparing is representative of an enhanced energetic economy, at least in hypoxic test conditions. If so, changes in at least some of the other ^{31}P-MRS derived variables would have been expected, in particular variables related to oxidative phosphorylation. However, while trends were apparent, as previously detailed (see Section 5.4.2 and Section 5.4.4, and Table 5.1 and Table 5.2), there were no significant between-leg differences in the reduction in the rate of oxidative ATP turnover. This was the case in both the hypoxic tests (mean changes: IHT +41% vs. control +19%, $P = 0.18$) and the normoxic tests (mean changes: IHT +14% vs. control +21%, $P = 0.58$), indicating that Δ[PCr] changes were unlikely caused by ameliorations in oxidative metabolism.

Ponsot *et al.* (2006) reported skeletal muscle morphology and ultrastructure data of those participating athletes who underwent biopsies after six weeks of IHT. This intervention (twice per week IHT, 24-40 min·session^{-1}, at an altitude simulation of ~3000 m) significantly increased the sensitivity of mitochondrial respiration (oxidative adenosine triphosphate (ATP) production) to adenosine diphosphate (ADP), which represents an improved coupling between ATP demand and supply pathways (Hochachka *et al.*, 2002). This is known to improve homeostasis of glycolytic metabolites, such as lactate, adenosine monophosphate, ADP and PCr (Matheson *et al.*, 1991), and may have delayed the dominance of anaerobic energy production during incremental exercise (Ponsot *et al.*, 2006), thus resulting in more efficient and fatigue resistant submaximal exercise. Conversely, Robach *et al.* (2012) found that highly trained cyclists' mitochondrial efficiency (respiratory control ratio and leak control coupling) was unaltered in response to four weeks of LH+TL. However, unlike the duplicate tHb measurements carried out by Robach *et al.* (2012),

conducted in order to minimise analytical error, these changes in mitochondrial metabolism reported by Ponsot *et al.* (2006) and Robach *et al.* (2012) were quantified via single post-intervention muscle biopsy samples. This is problematic, particularly as 3-5 biopsy samples are required to achieve acceptable error margins when differentiating between type I and type II fibres (Elder *et al.*, 1982), which given their different morphological properties, will have substantial implications on *in vitro* energetic assessments.

The recent investigation by Faiss *et al.* (2013b) performed all muscle biopsies and analytical techniques in triplicate, and in agreement with Ponsot *et al.* (2006), also reported no signs of any mitochondrial oxidative capacity gains post-IHT (on the contrary, these authors reported decreased PGC-1α and mtTFA mRNA). Moreover, these authors did report an enhanced capacity for pH regulation and glycolysis after IHT, exemplified by significantly increased carbonic anhydrase 3 (CA3) and MCT4 mRNA, but not after normoxic training (Faiss *et al.*, 2013b). The participants in this study undertook a repeated sprint protocol, so the exercise intensity was substantially higher than the majority of the previously discussed IHT studies (Dufour *et al.*, 2006; Geiser *et al.*, 2001; Kuno *et al.*, 1994; Ponsot *et al.*, 2006; Robertson *et al.*, 2010b; Roels *et al.*, 2005; Terrados *et al.*, 1990; Terrados *et al.*, 1988; Vogt *et al.*, 2001), as well as Chapter 4 and Chapter 5, which used longer duration exercise intervals. In this respect, it is possible that if the exercise intensity is sufficiently high, IHT may induce an enhanced capacity for anaerobic metabolism (Faiss *et al.*, 2013b), and perhaps a sparing of anaerobic energy reserves (Ponsot *et al.*, 2006).

In summary, given that the mechanistic results from Chapters 4 and 5 were highly variable, and these studies detailed above (Dufour *et al.*, 2006; Robertson *et al.*, 2010b; Roels *et al.*, 2005) showed no obvious signs of a beneficial effect of IHT on whole body oxidative exercise economy, it could be concluded that any such effects are negligible, at least in highly trained athletes. The suggestion by Faiss *et al.* (2013b) that high intensity IHT may induce specific skeletal muscle adaptations that are beneficial to anaerobic exercise metabolism, certainly warrants further investigation.

One common limitation with all these investigations, including Chapters 4 and 5, is the relatively small experimental group sample sizes, which may have

resulted in inadequate statistical power – for example, in Chapter 5 there was a trend for a greater oxidative ATP turnover after IHT (mean change +41%) vs. normoxic training (mean change +19%), but the differences were not statistically significant ($P = 0.18$). Whether a greater statistical power would have resulted in a significant difference remains unknown, so it is important that larger scale studies are conducted. It would be sensible for a unified intervention design to be agreed in order for a valid meta-analysis to be calculated, specifically focussing on IHT. The debate continues as to whether utilising a greater hypoxic dose than IHT (i.e. LH+TH, LH+TL and LH+TH+TL) elicits significant and meaningful exercise economy changes. In a similar manner to the IHT research, an experimental design must be arranged, importantly including a measurement of the fitness status of participants, such as aerobic capacity or performance, before any conclusions may be drawn.

6.1.1.2.3   *H^+ buffering*

During intense exercise, the lactate dehydrogenase catalysed conversion of pyruvate into lactate concomitantly accepts H^+, thereby reducing the pool of free H^+ that results from elevated anaerobic glycolysis (Robergs *et al.*, 2004). As such, an increased [BLa] appearance may indirectly represent an enhanced capacity to buffer H^+ (Kayser *et al.*, 1993). As part of Chapter 4, the effects of eight weeks of IHT on [BLa] during submaximal and exhaustive exercise was assessed. While there were no significant changes in the running speed at the [BLa] threshold, or at a fixed [BLa] of 4.0 mM, there was a significant reduction in the peak [BLa], as assessed 2 min post-exhaustion, in the IHT group in hypoxic conditions only (13.0 vs. 11.4 mM, $P = 0.01$), compared to no such change in the control group (11.0 vs. 10.5 mM, $P = 0.86$). This may suggest either a trend for reduced [BLa] production or an impaired ability to buffer [BLa] after IHT, but the between group difference was not statistically significant, so results are inconclusive. Terrados *et al.* (1988) reported a similar outcome in that [BLa] at near maximal exercise capacity decreased after highly trained cyclists completed 3-4 weeks of IHT or normoxic training, but the difference between groups was only significant when these cyclists were tested in hypoxic conditions. There are clearly a multitude of other factors acting on these

processes, so these rather indirect and arguably invalid estimations of H^+ buffering require more detailed assessment, using more precise markers.

It should be noted, that during the data collection phase of Chapter 4 the researchers attempted to quantify capillary blood pH, via biosensor potentiometry assessed H^+ concentration, using a hand-held analyser (i-STAT®, Abbott Point of Care Inc., Maidenhead, UK). However, even though the researchers sought to induce localised earlobe hyperaemia via means of a tetracaine cream (Ametop Gel, Smith & Nephew Healthcare Ltd, Hull, UK), in order to sample arterialised capillary blood (Pitkin *et al*., 1994), due to the participants being in an exhausted state, and the cold laboratory conditions, obtaining a sufficient sample volume in a timely manner was not possible. As such, the capillary blood pH data set was incomplete, so could not be included in any analysis. Instead, studies that have specifically assessed arterial blood and/or muscle H^+ and/or pH, or measurements of skeletal muscle ultrastructure and morphology must be considered.

Zoll *et al*. (2006) found that six weeks of IHT resulted in increased expression of skeletal muscle CA3 (mean change +74%) and MCT1 (mean change +44%) mRNA. These mRNA variables were not significantly altered within the normoxic trained group (CA3 mean change ~+10%, MCT1 mean change ~+5%), but unfortunately the between group statistical comparisons were not reported. Furthermore, these results are weakened by the fact that only one biopsy sample was analysed per participant. Nevertheless, Faiss *et al*. (2013b) also reported that after four weeks of high intensity IHT, a significant 74% increase in the expression of skeletal muscle CA3 mRNA occurred, compared to a non-significant ~15% reduction after normoxic training. Unlike the investigation by Zoll *et al*. (2006), these researchers conducted skeletal muscle biopsies and analyses in triplicate, which substantially strengthens their results. While these mRNA results demonstrate that IHT may elicit a greater *potential* for pH regulation, compared to normoxic training, they did not directly asses H^+ buffering capacity.

The ^{31}P-MRS techniques used in Chapter 5 allowed the estimation of intracellular pH every 6.0 s during exercise and post-exercise recovery, calculated using the chemical shift of the Pi spectral peak relative to the PCr

peak (Taylor *et al.*, 1983). None of the pH related variables (pH during moderate intensity exercise, pH threshold during incremental exercise and pH at volitional exhaustion) were altered significantly more by hypoxic training (7.5 h total hypoxic exposure spread over 21 d), compared to normoxic training. Conversely, Kuno *et al.* (1994) reported that 8 h of IHT spread over four consecutive days (2 h·d^{-1}) led to significantly higher ^{31}P-MRS assessed intracellular pH during fixed intensity exercise. Similarly, Mizuno *et al.* (1990) reported that the *in vitro* H$^+$ muscle buffering capacity (*β*m) significantly improved in 10 highly trained cross-country skiers, after two weeks of living at 2100 m and training at 2700 m. Caution must be adopted here, as the results from Chapter 5 are in clear agreement with these studies (Kuno *et al.*, 1994; Mizuno *et al.*, 1990), as a significant intracellular pH threshold improvement was apparent in both legs after the three week intervention, but, importantly, this was irrespective of the training F$_I$O$_2$. As neither Kuno *et al.* (1994) or Mizuno *et al.* (1990) included normoxic control groups, their suggestions of enhanced H$^+$ buffering capacity are unsubstantiated – these results may simply demonstrate that exercise training, per se, improves pH regulation.

Using a more sustained hypoxic intervention, Gore *et al.* (2001) reported that in 13 moderately trained participants, 23 nights of simulated LH+TL significantly increased duplicate assessed *β*m (mean change +18%), compared to a non-significant change in a normoxic control group (mean change +1%). While these results may imply an enhanced *in vivo* H$^+$ buffering capacity (*β*$_{in-vivo}$), the muscle biopsy samples taken immediately after 2 min of severe intensity cycling showed no significant [H$^+$] or *β*$_{in-vivo}$ changes (Gore *et al.*, 2001). This is in agreement with part of the double-blinded, placebo-controlled study by Siebenmann *et al.* (2012), detailed earlier (see Section 1.2.4 and Table 1.2). These authors reported that *β*m remained unchanged in highly trained cyclists after both 28 nights of 16 h·d^{-1} simulated LH+TL, as well as in a normoxic control group (Nordsborg *et al.*, 2012). In accordance with a lack of *β*$_{in-vivo}$ enhancement, both of these studies reported a lack of short-duration high-intensity exercise performance improvements: mean and peak power output during 30 s sprint cycling was unaltered in Nordsborg *et al.* (2012), and total work during 2 min maximum effort cycling was unaltered in Gore *et al.* (2001).

Given that contrary to traditional belief, intracellular lactate and H^+ accumulation may not be the most influential factor limiting skeletal muscle force generation (Allen *et al.*, 2008), it is perhaps unsurprising that Gore *et al.* (2001) reported an 18% βm improvement, but 2 min maximum effort (anaerobic) performance remained unaltered. Taken together, results from Chapters 4 and 5, and findings from the literature to date, do not support the hypothesis that IHT or sustained hypoxic exposure result in significantly enhanced H^+ buffering capacity. Whether the increased expression of skeletal muscle CA3 mRNA after the novel repeated sprint IHT intervention used by Faiss *et al.* (2013b) causes concurrent $\beta_{\text{in-vivo}}$ improvements remains to be seen.

6.1.2 Efficacy of altitude and hypoxia to enhance performance

In addition to the above detailed physiological adaptations, exercise performance was assessed in relation to traditional LH+TH altitude training (Chapter 3), and indirect estimates of performance (incremental time to exhaustion) were assessed in relation to whole body IHT (Chapter 4), and single-legged IHT (Chapter 5).

6.1.2.1   Performance efficacy of traditional altitude training

In Chapter 3, the three week LH+TH intervention resulted in 200 m swimming race performance changes that were on average 0.3% faster than in the control group. However, the ANCOVA statistics showed that LH+TH did not elicit an additional benefit on 200 m race performance over and above that caused by sea level training (see Section 3.4.2 and Table 3.1). This is logical, as the mean performance difference of -0.3% is within the typical within-participant race-to-race SD for highly trained swimmers of ± 0.8% (Pyne *et al.*, 2004).

A comparable study to Chapter 3 is that of Wachsmuth *et al.* (2013), in which 25 highly trained swimmers lived and trained for 3-4 weeks at 2320 m, on three separate occasions over a 2 y period. After 25-35 d at sea level, race performances significantly improved, on average by +23 FINA Points, or in terms of race time, an improvement of approximately -0.8% (Wachsmuth *et al.*, 2013). Importantly, there were no such performance changes detected at 0-14 d or 15-24 d post-altitude. Compared to Chapter 3, this study was based on a far

greater number of participants and training camp / competition repetitions, but the conversion of race times to FINA Points is problematic, due to it being a scalar system, based on the current World Record, so could have at least partially confounded the results. Another investigation to have assessed the effects of LH+TH at similar altitudes to Chapter 3, also in highly trained swimmers, is that by Gough *et al.* (2012), who reported that competitive race performances did not change following three weeks at 2135-2320 m. Specifically, 28 d after return to sea level, race performances in the LH+TH group were on average (± 90% CL) 0.2 ± 0.9% slower, leading the authors to conclude that any performance effects were "unclear" – see Appendix 7 for an explanation of some of the statistical techniques used by Gough *et al.* (2012).

Although the assessments of swimming performance capacity in the study by Bonne et al. (2014) only included 'in training' test sets, and no actual race results, the outcomes are supportive of the findings from Chapter 3 and those by Wachsmuth *et al.* (2013) and Gough *et al.* (2012), i.e. swimming performance does not improve immediately upon return from LH+TH altitude training to a greater extent than after sea level training. A common feature with Chapter 3 and these three other studies (Bonne *et al.*, 2014; Gough *et al.*, 2012; Wachsmuth *et al.*, 2013), is that performance was assessed during the middle of the competitive season, at relatively low priority competitions and/or during training sessions, without participants undertaking a prior taper. While the complexities of a taper may add to performance variances (Mujika *et al.*, 1995), assessing race performances at the targeted competition for that season would minimise alternative sources of error such as an accumulation of fatigue, and varying levels of effort and motivation to succeed.

In addition to these four swimming investigations, a number of others have assessed the effects of traditional altitude training on alternative endurance-based linear sports, although surprisingly most have not included control groups (see Table 1.1). The comprehensive investigation reported by Levine & Stray-Gundersen (1997) and Chapman *et al.* (1998) showed that 5000 m running performance was significantly faster after four weeks of LH+TL (mean 5000 m time decrease of 1.4%). However, race performances got worse in both the LH+TH group and the sea level control group (mean 5000 m time trial increases

of ~0.6% and ~2.9%, respectively). As such, it is unsurprising that the between group difference was statistically significant, but this may be more influenced by the worsened performance in the non-LH+TL groups rather than the improved performance in the LH+TL group, per se.

The finding that LH+TH may result in attenuated competitive race performances remains a concern for any athlete undertaking such an intervention, as a decrease in absolute training intensity and/or an increase in accumulated fatigue and incidences of illness are clearly undesirable. This latter problem has been widely observed: two of the seven LH+TH participants in Svedenhag *et al.* (1991) withdrew from the study due to being diagnosed with gastroenteritis, Bailey *et al.* (1998) found that the frequency of infectious illnesses significantly increased during LH+TH, whereas there were no such incidences during sea level living and training, and Gore *et al.* (1998) reported that all their participants became ill during or immediately after a four week LH+TH intervention, so training load was substantially reduced (see Table 1.1). Whether the altitude is the *cause* of any such increased illness incidence is debatable, but this is certainly a factor to be considered when implementing altitude interventions.

Contrary to popular belief, there is a dearth of rigorously designed studies to have investigated the effects of physical LH+TH or LH+TL altitude exposure. Aside from the one study by Levine and Stray-Gundersen (1997), there are no other experiments to have found beneficial performance effects of LH+TL using physical altitude, when compared to a sea level control group. A recent 'pro/con' debate well summarises the key arguments in this field (Jacobs, 2013a; Jacobs, 2013b; Wilber, 2013a; Wilber, 2013b). It is fundamental to note that even in the 'pro' argument by Wilber (2013a), aside from the Levine and Stray-Gundersen (1997) publication (see the above critique), the cited studies in support of LH+TL having a beneficial impact on performance either: i) did not include a control group (Stray-Gundersen *et al.*, 2001; Wehrlin & Marti, 2006), ii) included a control group which did not complete post-intervention performance tests (Wehrlin *et al.*, 2006), or iii) was based on a duplicated data set from the three years of data collection (1994, 1995 and 1996) during the Levine and Stray-Gundersen (1997) experiments (Chapman *et al.*, 1998). These studies must be

appropriately cited, in order for the risk to be minimised of perpetuating a concept that may in fact not turn out to be based on sound evidence.

Given the continued reliance on these training paradigms, additional research attention is clearly warranted. As blinding participants to the environmental treatment is impossible, the most useful means of investigating the effects of LH+TH is to assess competitive race performances at competitions that are deemed important by the athletes themselves (to avoid any nocebo effects of sea level control training). Wehrlin & Marti (2006) did so in their case study of a male runner, who undertook 26 d of LH+TH+TL, and subsequently improved his 5000 m personal best time by -1.3% at the World Championships. In a similar manner, Appendix 6 details a case study of one female participant from the LH+TH intervention in Chapter 3, illustrating clear performance gains after three weeks of LH+TH (-2.3% (-22.8 s) in a 1500 m front crawl race and the gold medal at in the 10 km open water race at the FINA World Championships). Much of the continued interest in the use of traditional altitude training for athletic performance enhancement is because of these types of case study results. In order for properly controlled studies to provide meaningful answers as to the associated performance efficacy, highly trained participants must complete repeated cross-over interventions, thus acting as their own sea level control groups, and again, with performance assessed after the usual taper, at major international competitions.

In addition, a novel statistical approach, as described by Allen et al. (2014), that derives individual quadratic trajectories from a mixed linear model, may prove useful in establishing whether any intervention (e.g. altitude training), has improved athletic performance over and above what would ordinarily be expected. Other novel statistical techniques have been used to assess changes in relatively small samples of highly trained athletes. For example the above discussed study by Gough *et al.* (2012) used an approach that involved magnitude based inferences; founded on the location of the confidence interval, in relation to threshold values for a (pre-defined) substantial effect (Hopkins *et al.*, 2009). This alternative statistical approach allows dictation of the 'smallest worthwhile change' for the specific variable and population in question, so has been suggested to prove useful when assessing effects in highly trained

athletes (Hopkins *et al.*, 2009). As such, this approach was also computed for the tHb and performance results from Chapter 3 (see Appendix 7), but (encouragingly) the interpretation and outcomes were essentially indifferent regardless of the statistical method, so the traditional null hypothesis-based approach was used for consistency throughout this thesis.

6.1.2.2   Performance efficacy of IHT

The effects of IHT and blinded condition normoxic training on incremental exercise performance (T-Lim) were assessed in Chapters 4 and 5. The interventions were dissimilar with regard to: a) the study design – a placebo controlled group trial in Chapter 4, and single-legged trial in Chapter 5; b) the hypoxic dose – 640 min spread over eight weeks at an altitude simulation of ~2100 m in Chapter 4, and 450 min spread over three weeks at an altitude simulation of ~3000 m in Chapter 5; and c) the participants' training status – highly trained athletes in Chapter 4 and physically active but non-trained participants in Chapter 5. The resultant T-Lim changes were similar in both Chapters 4 and 5 though – neither IHT intervention resulted in enhanced maximal exercise capacity compared to (blinded condition) normoxic training:

- In Chapter 4 there was a significant T-Lim increase within the IHT group when tested in hypoxia (mean ± SD: +2.4 ± 4.3%), but there were no significant differences between the groups in either test condition.

- In Chapter 5, although T-Lim improved in all participants and conditions, in the hypoxic and normoxic trained legs (range +2% to +76%), there was only a significant training effect in normoxic test conditions when the IHT and control leg results were combined. There were no significant T-Lim differences between the hypoxic and normoxic trained legs.

This finding of a lack of an IHT induced endurance performance enhancement is common (see Table 1.3). Robertson *et al.* (2010b) reported that 3000 m running performance was not significantly improved after nine moderately trained runners completed three weeks of IHT. Lecoultre *et al.* (2010) reported that mean power output during cycling tests to exhaustion improved by similar magnitudes after moderately trained cyclists completed four weeks of IHT

(+7%) or normoxic training (+6%). Morton & Cable (2005) reported that work capacity during incremental cycling to exhaustion improved similarly after moderately trained team sport players completed four weeks of IHT (+16%) or normoxic training (+18%). And finally, while mean power output during a 10 min cycling time trial significantly improved after 19 moderately trained athletes undertook four weeks of moderate to high intensity IHT (+5.2 ± 3.9%), Roels *et al.* (2005) reported that equivalent normoxic training provided an indifferent ergogenic effect (+5.0 ± 3.4%).

On the contrary, some authors have reported beneficial endurance performance effects of IHT, at least in untrained participants (Terrados *et al.*, 1990; Vogt *et al.*, 2001). However, such effects should not be assumed to occur in highly trained athletes, as far less pronounced performance changes would likely manifest when baseline training status is already high. For example, one study reported a mean T-Lim improvement of +313% post-IHT (Terrados *et al.*, 1990). These authors also investigated the effects of combined sustained moderate and high intensity interval IHT in highly trained cyclists, and concluded that performance improved in the IHT group to a greater extent than after normoxic training (Terrados *et al.*, 1988). When the results are assessed in depth though, the performance increments after IHT were only statistically different to the normoxic trained control group when tested in hypoxic conditions.

More recently, Dufour *et al.* (2006) reported noteworthy performance gains in moderately trained endurance runners, after an IHT stimulus similar to that used in Chapter 4 (see Table 1.3). However, as discussed in Section 1.3.3, the more than 3-fold greater T-Lim improvement in the IHT compared to the control group was in part due to a ~65% increase in one of the IHT participants, and a ~20% decrease in one of the control group participants. This variability is more than expected (Laursen *et al.*, 2007), for example the range for incremental exercise T-Lim in highly trained runners during Chapter 4 was -9% to +8%. Potential reasons for this variability were not discussed by these authors, but in addition to the lack of F_IO_2 blinding resulting in placebo and/or nocebo effects, which are known to be highly influential (Bonetti & Hopkins, 2009), there are a range of other factors that are seldom reported, that may significantly influence each individual's performance, for example: diet quality and calorific intake, sleep

quality and quantity, psychological well-being and the intrinsic desire to continue the study, the volume and intensity of training outside the laboratory, over-reaching, and illness / infections. In addition to these variances, unlike the ANCOVA statistics reported in Chapters 4 and 5, Dufour and colleagues failed to report any 'between group' statistics; only the significance of changes within the IHT and control groups were detailed. As such, it is impossible to judge the performance efficacy of the Dufour *et al.* (2006) IHT intervention.

The overwhelming limitation with these studies is that they all failed to blind the participants to the training F_IO_2, as either face masks were used in the IHT groups only (Dufour *et al.*, 2006; Lecoultre *et al.*, 2010; Vogt *et al.*, 2001), or participants in IHT groups were the only ones to exercise within a hypoxic chamber (Morton & Cable, 2005; Terrados *et al.*, 1988). Furthermore, authors commonly fail to report whether participants were blinded to the F_IO_2 (Hendriksen & Meeuwsen, 2003; Messonnier *et al.*, 2004; Roels *et al.*, 2005; Terrados *et al.*, 1990; 2003), and given the logistical challenges of doing so, it is therefore assumed that in these instances, successful participant blinding did not occur. Specifically in relation the study by Robertson *et al.* (2010b), IHT participants may have experienced influential nocebo effects, as in addition to the IHT stimulus, other participants were also exposed to a LH+TH+TL intervention. This lack of F_IO_2 blinding considerably diminishes the value of performance results from these otherwise well executed investigations, and may be a key reason for many of the discrepancies between studies. Indeed, when participants were successfully blinded to the F_IO_2 during Chapter 4 and 5, the results showed no significant performance gains. Furthermore, the rather misleading report of significant endurance performance gains after moderate intensity IHT, as reported by Dufour *et al.* (2006), have not been replicated elsewhere. Specifically, the investigation by Ventura *et al.* (2003) used an almost identical IHT intervention to that used by Dufour and colleagues (see Table 1.3), and found that maximum power output during an incremental cycling test to exhaustion was not statistically improved in the IHT group (mean change +1% in normoxia and -2% in hypoxia). Furthermore, in the normoxic trained control group, maximal power output tended to decrease in normoxic tests (mean change -4%), and was significantly decreased in hypoxic tests (mean change -8%), and maximum HR during the post-intervention exercise tests was

also significantly lower than pre-intervention (Ventura *et al.*, 2003). In a similar manner to the postulation in Chapter 4 (see Section 4.5.2), these authors suggested that the training stimuli induced an accumulation of fatigue that prevented performance gains. Participants in Ventura *et al.* (2003) had just finished their competitive season, whereas participants in Chapter 4 had only completed six weeks of training after a summer break, but both scenarios appeared to have resulted in an accumulation of fatigue. Participants may not have been sufficiently accustomed to the sustained moderate to high intensity laboratory based training sessions.

There have been reports of anaerobic performance gains post-IHT, first documented using a well-controlled parallel group study by Meeuwsen *et al.* (2001), then 1 y later in the subsequent 'cross-over' using the same group of moderately trained triathletes (Hendriksen & Meeuwsen, 2003). These authors found that IHT for 120 min·session^{-1}, 1 session·d^{-1}, for 10 d, resulted in significant ~4% increases in mean and peak power attained during a maximal effort 30 s Wingate cycling test, compared to no such changes after normoxic training (Hendriksen & Meeuwsen, 2003). These anaerobic gains occurred despite the relatively low intensity training; just 60-70% of the HR reserve. Thus, in absolute terms, the normoxic trained participants undertook a higher training intensity, so it is somewhat surprising that the Wingate test data showed a mean 0% change for mean and peak power after normoxic training. Again, the lack of proper blinding of the training condition likely led to influential nocebo effects, but unfortunately as anaerobic performance tests were not conducted in Chapter 4 or 5, the results are not comparable.

As far as the author is aware, aside from Chapter 4 and 5, only two other IHT studies to date have successfully blinded the participating athletes to the training F_IO_2 (Faiss *et al.*, 2013b; Galvin *et al.*, 2013). Faiss *et al.* (2013b) had 40 moderately trained cyclists undertake four weeks of high intensity interval training within a normobaric hypoxic chamber (15 x 10 s maximum effort sprints with 20 s to 5 min active recovery intervals, twice per week), while breathing a 0.146 (IHT group) or 0.209 (normoxic control group) F_IO_2 inspirate. Even though participants were successfully blinded to the training F_IO_2 (95% of the IHT group participants and 85% of the control group participants thought that their training

was performed in hypoxia), the maximum number of sprints achieved during a repeated sprint ability test improved significantly more in the IHT group (9 vs. 13 sprints) compared to the normoxic group (9 vs. 9 sprints). As this performance test involved a 1:2 work:rest ratio (repetitions of 10 s maximum effort, followed by 20 s rest), the outcome of an enhanced exercise tolerance post-IHT may be due to an enhanced [PCr]-τ, as observed in hypoxic tests during Chapter 5. In the study by Galvin *et al.* (2013), all participants breathed from face masks during high intensity interval training (12 sessions spread over four weeks, each consisting of 10 x 6 s efforts interspersed with 30 s of passive recovery), but while the IHT group participants breathed a 0.130 F_IO_2 inspirate, the single-blinded control group breathed normoxic air (F_IO_2 0.209). In a similar manner to findings by Faiss *et al.* (2013b), repeated sprint ability (YoYo Intermittent Recovery Test Level 1) improved significantly more so in the IHT compared to the control group (mean +33% vs. +14%, *P* = 0.002).

Considering the above discussed lack of evidence for the performance efficacy of moderate intensity IHT (Lecoultre *et al.*, 2010; Morton & Cable, 2005; Robertson *et al.*, 2010b; Roels *et al.*, 2005), if athletes choose to undertake IHT as part of their routine training, they are advised to experiment with a repeated sprint protocol such as those used by Faiss *et al.* (2013b) and Galvin *et al.* (2013). In particular, those athletes who compete in sports that rely on a superior repeated sprint ability may benefit from such an intervention.

6.1.3   Experimental considerations

As with all empirical research, the three studies that comprise this thesis are subject to a number of experimental considerations. Some limitations have been highlighted within each chapter, but there are a number of issues common to multiple chapters that warrant further detail.

Sample size

A common limitation across all three experimental chapters is the relatively small sample size for each of the experimental treatments. While 13, 18 and 10 participants started Chapters 3, 4 and 5, respectively, there were some drop-outs during the training interventions, resulting in only 11, 12 and 9 'finishers', respectively. Importantly though, as Chapter 5 used a single-legged training intervention, the normoxic trained leg effectively acted as a control group, so the statistical power was arguably equal to having 18 participants complete a parallel group trial. Nevertheless, these relatively small sample sizes would have reduced the statistical power of each investigation. Here, it is important to note that these experimental chapters were all longitudinal training studies, and in order to achieve the above samples, each experiment was repeated twice, using the same methodological protocols, with different participants. As such, the data collection phases (particularly the supervised training) of each chapter were both logistically challenging and time consuming; lasting 16 weeks, 20 weeks and 10 weeks, for Chapters 3, 4 and 5, respectively. Furthermore, the participants in Chapters 3 and 4 were highly trained athletes, i.e. a specialist population, and hence it was difficult to recruit more numbers.

Performance tests

Part of the strength of Chapter 3 was the ecologically valid assessment of performance via competitive swimming races in these highly trained athletes. This differed to Chapter 4, whereby performance in highly trained runners was only estimated via treadmill-based incremental tests to exhaustion. While such tests in laboratory conditions provide a reliable estimate of exercise capacity (Weltman *et al.*, 1990), constant load tests have been shown to be somewhat more reliable: CV's of 0.9% vs. 0.6%, respectively (Hopkins *et al.*, 2001), and in

particular when testing highly trained runners, field-based performance tests are even more reliable (Hopkins *et al.*, 2001). A combination of field-based and laboratory-based performance assessments, similar to those used previously for swimmers (Robach *et al.*, 2006b) and runners (Chapman *et al.*, 2014), in both Chapters 3 and 4, would have provided more detailed information regarding the performance efficacy of LH+TH and IHT, respectively.

Furthermore, in addition to the aerobic requirement during an incremental exercise test to exhaustion, there is a substantial anaerobic contribution (Bertuzzi *et al.*, 2013), with the relative aerobic/anaerobic contribution being largely dependent on the specifics of the protocol, for example the increment and total test duration, the increment intensity, and the proportion of total muscle mass being exercised. This was exemplified in Chapter 5, as there was only a significant training effect for T-Lim under normoxic test conditions when the IHT and normoxic trained leg data were combined (see Section 5.4.4). A more informative means of quantifying performance would have been to use two distinct tests: i) a constant moderate or heavy intensity test to exhaustion, to quantify aerobic fitness; and ii) a severe or supra-maximal intensity test, to quantify anaerobic and/or repeated sprint capacity. This latter high intensity test is especially warranted given suggestions of an IHT induced improved mean power output during 30 s efforts (Hendriksen & Meeuwsen, 2003) and a skeletal muscle shift from aerobic to anaerobic glycolytic activity (Faiss *et al.*, 2013b).

Training specificity

Apart from the twice weekly supervised laboratory sessions, the running training intervention in Chapter 4 was prescribed by the athlete's coaches (6 or 7 sessions·week^{-1}), resulting in varying exercise intensities being practiced, from moderate to supra-maximal efforts. The twice weekly laboratory training involved sustained submaximal effort (heavy intensity) running, while breathing a hypoxic or normoxic inspirate, whereas performance was assessed by means of an incremental speed test to exhaustion. Thus, the performance outcome, T-Lim, required participants to tolerate running speeds that were substantially faster than all the supervised treadmill training speeds. Similarly, the training intervention in Chapter 5 consisted of a combination of sustained moderate intensity and repeated very heavy to severe intensity efforts, whereas

performance was also assessed by means of an incremental resistance based test to exhaustion. Again, the performance outcome, T-Lim, was determined by the participants' ability to tolerate the high intensity loads for substantially longer durations than used during training efforts. In contrast, the blinded IHT studies by Faiss *et al.* (2013b) and Galvin *et al.* (2013) used identical repeated sprint based protocols for the training intervention as used during performance tests, and reported favourable adaptations after IHT. The lack of specificity between the training and testing intensity in Chapters 4 and 5 may have limited the ability to detect any beneficial performance outcomes. However, results from both a submaximal and maximal test would be expected to improve if aerobic power increases, due to a delayed onset of anaerobic metabolic contribution.

Intravascular compartments

The key mechanistic focus of Chapter 3 was the effect of moderate altitude on haematological variables, which required a sufficient frequency of tHb data to ensure proper reliability (a minimum of five CO-rebreathing tests per participant). In order to minimise the degree of invasive testing, venepuncture was not performed at all of these laboratory visits. Accordingly, to avoid the results being confounded by spurious data generated by the only available hand-held analyser (HemoCue®, HemoCue Ltd, Dronfield, UK), recently reported to vary by ~5% from gold-standard measurements (Rudolf-Oliveira *et al.*, 2013), it was not possible to calculate intravascular compartments (RCV, PV and total BV). Quantifying these additional variables could have been insightful, especially given the known marked effects of Epo on plasma volume (Lundby *et al.*, 2007b).

6.2   Future research

Taking into consideration the results, implications and limitations of the three experimental chapters, as well as those of the discussed related literature, there are a number of key questions that warrant further research:

i.   What effect does sustained living in hypobaric or normobaric hypoxia have on tHb and sea level athletic performance? With normobaric hypoxic apartments becoming increasingly accessible, it is now possible to expose athletes to 12-16 $h \cdot d^{-1}$ of normobaric hypoxia throughout entire training seasons. In addition, while long-term living at physical altitudes is common in endurance athletics, particularly in East African runners (Wilber & Pitsiladis, 2012), it is uncommon in other sports. Whether an endurance swimmer, for example, would benefit from living and training at moderate altitudes for entire training seasons, is an important area for future research. Would these athletes acclimatise enough to be able to exercise at race pace velocities without becoming overly fatigued?

ii.   Does a whole body severe or supra-maximal intensity IHT intervention improve sprint or repeated sprint performance in highly trained athletes? Faiss *et al.* (2013b) demonstrated this effect in moderately trained male cyclists, as did Galvin *et al.* (2013) in academy level rugby players, but there have been no similar studies in highly trained athletes.

iii. Do hypoxic or altitude interventions improve specific factors that are known to limit muscle force production? If lactate and/or H^+ buffering are not the most influential causes of fatigue during high intensity exercise (Allen *et al.*, 2008), more relevant variables should be assessed in relation to altitude and hypoxic interventions, for example the failure of sarcoplasmic reticulum Ca^{2+} release (Lamb, 2009) and a reduced sarcolemmal K^+ gradient (Sejersted & Sjogaard, 2000).

iv.   What are the effects of LH+TH or IHT on skeletal muscle energetics in highly trained athletes? By using modern methods such as ^{31}P-MRS, it is possible to assess muscle energetics without the invasive procedures that may have previously limited athlete's involvement in such studies.

v. Instead of only using conventional physiological / biochemical / haematological markers, which are inherently susceptible to substantial day-to-day variation, alternative more sensitive approaches should also be implemented. Since the completion of the Human Genome Project in 2003, much research attention has been given to the discovery of genetic variants associated with athletic performance (Bray *et al.*, 2009). While the majority of this research to date has been restricted to the candidate gene approach, more recently genome wide association studies have been conducted, thus allowing simultaneous testing of multiple genes (Pitsiladis *et al.*, 2013). Indeed the micro-array technology based assessments of genomics, transcriptomics, proteomics and metabolomics, collectively termed the 'omics' cascade (Reichel, 2011), are providing exciting new progressions for the detection of rHuEpo abuse and blood doping (Pitsiladis *et al.*, 2014). There is a wealth of ongoing research attempting to identify the specific gene variants of an elite athlete (Ahmetov & Rogozkin, 2009), and while single nucleotide polymorphisms specifically related to beneficial adaptations in response to altitude and hypoxia have not yet been discovered, attempts have begun (Jedlickova *et al.*, 2003). Undoubtedly this field of research will bring a wealth of new insights in the very near future.

In addition to these above research questions, the following key principles should be considered when planning future altitude and hypoxia research:

i. Quantify performance at competitive events or by means of ecologically valid field tests, rather than by means of laboratory based assessments. By doing so the participant's motivation and desire to succeed will be greater (Hopkins *et al.*, 2001), and the issue of estimating the effect of alterations in exercise test results on real sports performance is avoided.

ii. Wherever possible ensure that the mode and intensity of the training intervention is sufficiently specific to the performance test protocol.

iii. Although it is challenging to assess the effects of hypoxia on highly trained athletes in a double-blinded manner, this design is imperative in the generation of unbiased results. As far as the authors are aware,

Chapter 4 of this thesis is the only investigation to date that has exposed highly trained athletes to a single-blinded IHT intervention (Faiss *et al.* (2013b) and Galvin *et al.* (2013) used double- and single-blinded designs, respectively, but participants were only moderately trained).

iv. To reduce the issue of individual athlete variability leading to Type I and Type II statistical errors, mainly due to small sample sizes, alternative study designs to parallel group trials should be used. For example, cross-over studies such as that by Hendriksen & Meeuwsen (2003), single legged training studies such as Chapter 5 of this thesis and those studies by Bakkman *et al.* (2007) and Terrados *et al.* (1990), and large scale studies, for example Brothers *et al.* (2007), who assessed acclimatisation to moderate altitude in 2147 military recruits. For traditional altitude interventions, repeated cross-over designs should be used, with performance assessed at international competitions, thus avoiding placebo and/or nocebo effects (Bonetti & Hopkins, 2009).

v. As the optimised CO-rebreathing protocol involves numerous stages (see Section 3.3.4), the possibility of calculating erroneous tHb data is substantial, so all assessments should be performed in duplicate.

vi. As per the above point, more sensitive 'precision medicine' biomarkers that are less influenced by day-to-day variation should be adopted (Pitsiladis *et al.*, 2013), instead of only assessing traditional physiological / biochemical / haematological variables.

6.3   Practical implications

The outcomes from all three of the experimental chapters within this thesis have implications that inform recommended practices regarding the use of altitude and hypoxic strategies in athletic populations.

From Chapter 3, it is clear that traditional altitude training (LH+TH) for 21 d at 2320 m is sufficient to elicit a substantial tHb increase in highly trained athletes, at least in terms of the group response. According to these results, assuming no illnesses or injuries occur, ~70% of highly trained athletes who undertake such a LH+TH intervention should expect to realise a tHb increase, whereas ~30% of highly trained athletes will likely experience either no tHb change or a tHb decrease (see Figure 3.1). Importantly, changes in tHb and competitive race performances are not firmly related, so when identifying athletes as "responders" or "non-responders" to altitude training, in particular when making decisions as to their participation in any future altitude training interventions, judgements should not be based on haematology results, but instead on performance during training and racing.

The results from Chapter 4 do advocate using moderate to heavy intensity IHT interventions to aid subsequent normoxic athletic endurance performance. Alternatively, some recent related literature (Faiss *et al.*, 2013b; Galvin *et al.*, 2013) suggests that IHT involving severe or supra-maximal intensity exercise may provide an additional benefit for repeated sprint performance (see Section 6.1.2.2), but these claims must be further substantiated in relation to competitive sporting events. An additional implication from Chapter 4 is that coaches and support staff should closely monitor individual athlete's fatigue and wellbeing during any IHT interventions.

One further potential use for IHT interventions is as a pre-acclimatisation tool for subsequent exercise in hypoxia: in Chapter 4, T-Lim was increased in hypoxia after IHT. Moreover, in Chapter 5, [PCr]-τ was speeded up in hypoxia following IHT, which reflects an improved muscle oxidative capacity. Accordingly, this would be expected to result in less fatigue and to enable better maintenance of performance during intermittent high-intensity exercise in hypoxia or at altitude. It may therefore be speculated that athletes competing in sports requiring

repetitive sprints might obtain an advantage from performing IHT prior to competitions at altitude (see Section 5.5.1).

In addition, the results from Chapter 5 demonstrated that when non-specifically trained but healthy males breathed air at an F_IO_2 of 0.145 (simulating ~3000 m altitude), [PCr]-т was 34-42% slower compared to when they breathed normoxic air. Although these exact [PCr]-т data should not be equally applied to highly trained athletes, the basic premise remains. When individuals perform repeated bouts of severe or supra-maximal intensity exercise in a hypoxic environment (e.g. during an altitude training camp or during IHT), additional recovery durations should be allowed in order to maintain peak forces or velocities.

Overall, this thesis advises that:

a) For those sports where aerobic capacity is a key performance determinant, traditional altitude training should be used as an out-of-competition season aerobic stimuli. This specific purpose of any such intervention must be stated in advance (i.e. if the aim is an aerobic stimuli, ensure that coaches and athletes do not expect anaerobic gains).

b) Unless an array of valid and reliable methods of assessing non-haematological adaptations to altitude training are also employed (see Section 6.1.1.2), in addition to the more commonly discussed haematological changes, the justification for measuring tHb is lacking. If only RBC haematology is quantified, coaches and athletes may overemphasise the importance of these values, then question why performance has not improved, when tHb has increased.

c) For athletes who are due to compete at altitude, high-intensity IHT is applicable as a pre-acclimatisation strategy. The exercise modality should be specific to their competitive event, as should the absolute exercise intensity (power output or velocity).

d) For repeated-sprint dominant sports that are contested either at sea level or at altitude (e.g. football, rugby, hockey and tennis), high intensity interval type IHT should be used to enhance the recovery between competitive bouts, and therefore improve overall power or velocity.

6.4   General conclusions

Although cases of individuals having experienced substantial performance gains after traditional LH+TH altitude training and IHT were observed, none of the parallel group assessments within the three experimental chapters of this thesis support the performance efficacy of traditional LH+TH altitude training or IHT. This was the case for competitive race performance assessed in highly trained participants (Chapter 3), and for indirect estimates of performance in both highly trained participants (Chapter 4) and in relatively untrained participants (Chapter 5).

In terms of the physiological adaptations caused by traditional altitude training, Chapter 3 demonstrated that three weeks of LH+TH was sufficient to elicit a significant haematological increase in highly trained swimmers. On the contrary, tHb and performance changes were not correlated, with some participants experiencing performance improvements despite no tHb changes, and vice-versa. In accordance with these results and the related literature, it is suggested that baseline endurance fitness status likely has a substantial impact on the degree of haematological gains in response to traditional altitude training, and therefore only race performance results (and not haematological variables) should be used to classify whether an athlete has "responded" to the intervention.

In terms of the physiological adaptations caused by IHT, Chapter 4 demonstrated that in highly trained runners, IHT resulted in a reduced submaximal HR, representative of an improved cardiovascular fitness, but that running economy and $\dot{V}O_2$max remained largely unaltered. Also in relation to IHT, this time in relatively untrained participants, Chapter 5 demonstrated that skeletal muscle oxidative capacity was improved by IHT (depicted by a faster [PCr]-т), at least when tested in hypoxic conditions. However, this was not the case during normoxic exercise tests, and as the numerous other ^{31}P-MRS assessed variables did not show significantly different responses to IHT compared to normoxic training, any functional impact is questionable.

Although the experimental chapters within this thesis were well controlled, it is clear that numerous studies, even some that are highly cited in the literature,

have substantial limitations in their experimental designs (e.g. a lack of blinding of the experimental condition and/or a lack of comparable control groups), analysis (e.g. non-sensitive analytical methods being used), and reporting (e.g. a lack of between group statistics being communicated). Future altitude and hypoxic research should: i) ensure that wherever possible participants are blinded to the experimental condition; ii) use at least single-blinded, but ideally double-blinded parallel group or cross-over research designs; iii) employ precise analytical techniques that are sufficiently sensitive and robust enough to overcome day-to-day variation; and iv) investigate the effects in highly trained participants, with performance being assessed at competitions with substantial intrinsic worth.

This thesis contributes to the understanding of the athletic performance efficacy and some of the associated physiological mechanisms related to altitude and hypoxic interventions.

APPENDICES

Appendix 1: University of Exeter certificate of ethics approval (Chapter 3)

UNIVERSITY OF
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SPORT AND HEALTH SCIENCES

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   +44 (0) 1392 722896 (Education Enquiries)
f +44 (0) 1392 724726
e sihs-school-office@exeter.ac.uk
w sihs.exeter.ac.uk

Certificate of Ethical Approval

Title: 2012/99 Changes in haematology and performance following three weeks altitude training in elite swimmers
Applicants: Mr Ben Holliss, Dr Charles Pedlar

The proposal was reviewed by a Representative on the Committee.

Decision: The proposal was approved from MARCH 2012 to JULY 2012

Signature: Date: 12 - 11 - 12

Name/Title of Ethics Committee Reviewer: Dr. D. Wilkerson

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

Appendix 1: University of Exeter certificate of ethics approval (Chapter 4)

UNIVERSITY OF
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Web www.sshs.ex.ac.uk

Certificate of Ethical Approval

Proposal A1 (25/2/09)
Title:Intermittent hypoxic training intervention
Applicants: Professor Andrew Jones with Mr Richard Burden (English Institute of Sport,
St Mary's Twickenham) and Mr Ben Holliss (Research Student)

The proposal was reviewed by the Chair of the Committee.

Decision: The proposal was approved from January 2009 to May 2009.

Signature: *Joanne R Welsman* Date: 19/1/09

Name/Title of Chair: Dr J Welsman

*Your attention is drawn to the attached paper which reminds the researcher of
information that needs to be observed when Ethics Committee approval is given.*

The Times Higher
University
of the Year
2007/08

Appendix 1: University of Exeter certificate of ethics approval (Chapter 5)

UNIVERSITY OF
EXETER

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Certificate of Ethical Approval

Proposal 5 (24/02/10)
Title: Oxidative adaptations to intermittent hypoxic exercise at the muscle using [31]
P-MRS
Applicant: Prof Andy Jones (Staff) with Dr Anni Vanhatalo (Staff) and Ben Holliss
(PG Research Student)

The proposal was reviewed by the Ethics Committee on 24/02/2010 and following amendments has been approved until November 2010

Signature: _AWTaylor_   Date: 15/3/10

Name/Title of Ethics Committee Reviewer: Prof. Adrian Taylor

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

Appendix 2: Participant information sheets (Chapter 3)

ENGLISH INSTITUTE OF SPORT

Total Haemoglobin mass test
Athlete information

What's it for?

A key adaptation to being at altitude in an increased production of red blood cells, and hence an increased oxygen carrying capacity of the blood. This laboratory test measures the total mass of haemoglobin in your red blood cells along with the total blood volume. The test is used before and after trips to altitude (or simulated altitude) and at periodic points in the athlete's season.

The English Institute of Sport and the Australian Institute of Sport are the two of the leading organisations across the world who are undertaking this procedure with elite sports performers.

What happens in the test?

The test involves a very quick and simple breathing procedure along with pre-and post fingertip blood samples. The method involves a short 2 minute period of breathing a mixture of oxygen and (a very small volume of) carbon monoxide. The picture to the right shows an athlete undertaking this process. Fingertip blood samples are taken pre and post, and a final venous blood sample is collected. All the testing is seated and at rest. You will, however, need to be seated for approximately 15 minutes before the test starts, and preferably have not completed any exercise in the preceding 15 minutes before that.

Risks

There are minimal risks associated with this protocol, although the athlete should be aware that fingertip and venous blood samples are used. The volume of carbon monoxide is very small and harmless, although the test is not suitable for those with pre-disposing disease or anaemia, or for pregnant or breastfeeding women. We would not recommend that the athlete competes or undertakes intense exercise in the 3 hours after the test, but normal aerobic exercise is beneficial and suggested.

Benefits

This test will give a very accurate indication of your aerobic capacity from the oxygen carrying capacity of the blood. For the athlete venturing to altitude, knowing this information both pre- and post-exposure allows an altitude strategy to skilfully fine-tuned and for future trips to be expertly planned. The precision and reliability of the measurement is very good, and the turnaround of results is quick – within 10 minutes of completing the test.

Contact details

•Jamie Pringle, Lead Physiologist, English Institute of Sport, Loughborough; 07507 ######
•Clare Lobb, British Swimming Performance Scientist; 07919 ######
•Ben Holliss, Physiologist, British Swimming/English Institute of Sport; 07900 ######
•Lizzie Wraith, Physiologist, British Swimming, English Institute of Sport; 07884 ######

ENGLISH INSTITUTE OF SPORT   EXCELLENCE IN SPORT SCIENCE & MEDICINE   **BRITISH Swimming**

Appendix 2: Participant information sheets (Chapter 4)

PARTICIPANT INFORMATION

Physiological adaptations and performance changes resulting from an eight week intermittent hypoxic training period

What is the aim of the project?
This study is being conducted as part of a PhD in Exercise Physiology, and is designed to find out whether an eight week simulated altitude training period has a beneficial effect on running performance, and what physiological changes result.

What types of participants are needed?
We require well trained middle- to long-distance athletes (age 18-26 years) who are free from any major illnesses, disease or injuries, and who are able to commit to the eight week training period and testing protocols as detailed below.

What will participants be asked to do?
For an eight week period, two 'threshold' runs that are normally completed outside will be performed under close supervision on a treadmill in the English Institute of Sport Human Performance Laboratory (St. Mary's University College, Twickenham). Half the participants will perform these treadmill runs in normoxic (sea level), and the other half in hypoxic (~2250 m simulated altitude) conditions. Exercise intensity (running speed) will be based on the pre-training performance tests, and set level relative to each individual's maximal heart rate in that environment. As such, the relative exercise intensity will be identical between all individuals, and neither group will know which environment they are exercising in.

To quantify any physiological and/or performance changes in both environments, one week before, and one week after training, two identical 'step tests' will be conducted; one in normoxic and one in hypoxic conditions, as timetabled below:

Week 1:   Hypoxic step test[1]
            Normoxic step test[1]
            Analysis of body composition, lung function and iron status

Weeks 2-9: Two treadmill running sessions per week

Week 10:   Hypoxic step test[1]
            Normoxic step test[1]
            Analysis of body composition, lung function and iron status

[1]These tests will involve participants running at gradually increasing speeds until voluntary exhaustion, while blood lactate, heart rate and expired air are monitored.

Clearly such running exercise performed at challenging speeds may involve some discomfort, although this will likely only represent the feelings of exertion that you typically experience in a training session. However, if at any time, you decide that you no longer want to take part in this project (for any reason), you will be allowed to withdraw without disadvantage to yourself.

What data or information will be collected and what use will be made of it?
One week before, and one week after the training period, we will analyse your body composition (via skinfold thickness), lung function (via rested respiratory tests) and full blood count and iron status (via a rested blood sample). Additionally, during the exercise step tests we will be monitoring expired air (using a face mask), heart rate (using a chest strap monitor), and blood oxygen content (using a pulse oximeter clipped to a finger). During the two training runs each week, only heart rate (via a chest strap) and blood lactate (via a small earlobe blood sample) will be monitored. All participants will be loaned a Garmin GPS watch and be required to monitor every run outside the laboratory using this device for the duration of the study.

All data collected will be treated with confidentiality, and under no circumstances will names or personal details be included in the write up, either for this PhD or for any publication(s). In addition, all individuals' data will be made available to participants in a practical report format, and this feedback will be discussed with both the athletes and coaches.

What if participants have any questions?
If you have any questions about this project, please feel free to contact either:

Ben Holliss, MSc
ben.holliss@eis2win.co.uk
07944 ######

or

Charles Pedlar, PhD
charles.pedlar@eis2win.co.uk

This project has been reviewed and approved by the Ethics Committee of the School of Sport and Health Sciences, University of Exeter.

Appendix 2: Participant information sheets (Chapter 5)

UNIVERSITY OF
EXETER

SCHOOL OF SPORT &
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EX1 2LU

Muscular adaptations to simulated altitude training

Thank you for showing an interest in this project. Please read this info carefully before deciding whether to participate. If you are keen, it would be great to have you involved, but if you decide not to there will be no disadvantage to you and we thank you for considering it.

What is the Aim of the Project?
The main aim is to find out how breathing air containing low levels of oxygen during exercise ('simulated altitude training') affects adaptations in the muscle, and performance. This has never been assessed using magnetic resonance spectroscopy, which is completely non-invasive. We hope to understand precisely how altitude training affects the working muscles and exercise performance, and therefore judge whether athletes should use such training.

What Type of Participants are Needed?
Male or female participants in the age range between 18 – 35 years old, who lead an active lifestyle. You should be free of any athletic injury or other condition which may make it difficult for you to perform leg-extension exercise.

What will Participants be Asked to Do?
Should you agree to take part in this project, you will be asked to visit the lab for the following purposes, over a six week period between 11th October – 21st November 2010:
- Week one (11th - 15th October):
    - Familiarisation with the exercise and testing protocol (less than 1 hour)
- Week two (18th – 22nd October):
    - 4 x exercise tests to exhaustion in the magnet scanner (1-2 hours each)
- Weeks three until five (25th October – 14th November):
    - 5 x single leg training sessions per week, repeated for each leg
      (25 minutes per leg, therefore 50 minutes total exercise time per session)
- Week six (15th – 21st November):
    - 4 x exercise tests to exhaustion in the magnet scanner (1-2 hours each)

Exercise Tests
All exercise tests will be conducted in the Peninsula MRI Unit, on St Luke's Campus. You will be asked to lie on your stomach inside the scanner during set-up, for approximately 30 minutes, after which you will be asked to contract one leg in time with a visual cue. The initial intensity will feel easy, for 2 x 4 minutes, after which you will perform an intense bout for 24 seconds. You will then be asked to re-commence single leg contractions, carrying out a step test to maximal effort. Specifically, the intensity will get harder every 30 seconds until volitional exhaustion. You will have 6 minutes rest between each of these bouts.

During all this exercise testing, you will be breathing either normal or low oxygen air, through a face mask, and will not be told which air you are breathing. This will not induce any side effects, and you will likely not even realise which air you are breathing.

<u>Training Sessions</u>
All training sessions will be conducted in the Physiology Lab in the Richards Building using the same leg-extension set-up as for the exercise tests. You will exercise one leg at a time, while breathing normal or low oxygen content air during each. Each session will consist of:
- 5 minutes warm up
- 10 minutes steady state exercise
- 5 x 1 minute intervals, with 1 minute recovery between each
    = 25 minutes per leg, therefore a total of 50 minutes exercise per session

The only discomfort felt will only be that associated with short-duration intense exercise.

Can Participants Change their Mind and Withdraw from the Project?
You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind. You may also request that any information collected from you to date be destroyed or deleted and not used either now or in the future.

What Data or Information will be Collected and What Use will be Made of it?
Data from all tests will be collected and analysed anonymously, via a coding system. As a participant, you will have access to all your own data, and once the entire data set has been analysed, you will be given feedback and interpretation as to the study outcomes. The data will be used to inform our knowledge of simulated altitude training. Results of this project may be published but any data included will in no way be linked to any specific participant. You are most welcome to request a copy of the results of the project/copy of any transcripts etc should you wish. The data collected will be securely stored in such a way that only those mentioned below will be able to gain access to it. There are no plans to destroy the data set after a set period of time, as there is nothing particularly personal being collected, and it would be useful to have this on record to help inform future work.

What if Participants have any Questions?
If you have any questions about our project please feel free to contact:

Ben Holliss (Email: ben.holliss@eis2win.co.uk, Tel: 07900 ######)
Professor Andrew Jones (Email: A.M.Jones@exeter.ac.uk)

This project has been reviewed and approved by the Ethics Committee of the School of Sport and Health Sciences, University of Exeter.

Appendix 3: Informed consent forms (Chapter 3)

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UNIVERSITY OF EXETER

Changes in haematology and performance following three weeks altitude training in elite swimmers

INFORMED CONSENT FORM FOR PARTICIPANTS

I have read and understood the Information Sheet concerning this project, and all my questions have been answered satisfactorily. I understand that I am free to request further information at any stage.

I understand that:

1. My participation in the project is entirely voluntary; I am free to withdraw from the project at any time without any disadvantage or prejudice;
2. The data will be destroyed at the conclusion of the project, but any raw data on which the results of the project depend on will be retained in secure storage;
3. In the case of publication of collected data my anonymity will be preserved;
4. There are some risks associated with performing exercise to exhaustion and all-out sprint exercise as outlined in the participant information sheet;
5. There are some risks associated with the carbon monoxide breathing total haemoglobin mass technique, as outlined in the participant information sheet;
6. I will have a sterile needle inserted in an anticubital vein by a phlebotomy trained person who is a member of staff and a post-graduate student;
7. A small quantity (< 200 ml for each visit) of blood will be collected in sterile tubes;
8. The blood will only be analysed for the parameters stated in the information sheet, and will not be stored beyond the duration required for analyses (less than one week);
9. All used blood waste will be disposed of by incineration.

I agree to take part in this project.

.... (Signature of participant)

.... (Date)

This project has been reviewed and approved by the Ethics Committee of the School of Sport and Health Sciences

Appendix 3: Informed consent forms (Chapter 4)

School of Sport and Health Sciences

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Physiological adaptations and performance changes resulting from an eight week intermittent hypoxic training period

INFORMED CONSENT FORM FOR PARTICIPANTS

I have read and understood the Information Sheet concerning this project, and all my questions have been answered satisfactorily. I understand that I am free to request further information at any stage.

I understand that:

1. My participation in the project is entirely voluntary; I am free to withdraw from the project at any time without any disadvantage or prejudice;

2. The data will be destroyed at the conclusion of the project, but any raw data on which the results of the project depend on will be retained in secure storage;

3. In the case of publication of collected data my anonymity will be preserved;

4. There are some risks associated with performing incremental exercise to exhaustion, as outlined in the participant information sheet;

5. I will have a sterile needle inserted in an anticubital vein by a phlebotomy trained person who is a member of staff and a post-graduate student;

6. A small quantity (< 200 ml for each visit) of blood will be collected in sterile tubes;

7. The blood will only be analysed for the parameters stated in the information sheet, and will not be stored beyond the duration required for analyses (less than one week);

8. All used blood waste will be disposed of by incineration.

I agree to take part in this project.

.... (Signature of participant)

.... (Date)

This project has been reviewed and approved by the Ethics Committee of the School of Sport and Health Sciences

Appendix 3: Informed consent forms (Chapter 5)

UNIVERSITY OF EXETER

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Oxidative adaptations to intermittent hypoxic exercise at the muscle using ^{31}P-MRS

INFORMED CONSENT FORM FOR PARTICIPANTS

I have read and understood the Information Sheet concerning this project, and all my questions have been answered satisfactorily. I understand that I am free to request further information at any stage.

I understand that:

1. My participation in the project is entirely voluntary;

2. I am free to withdraw from the project at any time without any disadvantage;

3. The data will be destroyed at the conclusion of the project, but any raw data on which the results of the project depend on will be retained in secure storage;

4. There are some risks associated with performing incremental exercise to exhaustion and all-out sprint exercise as outlined in the information sheet;

5. In the case of publication of collected data my anonymity will be preserved;

6. The data will be stored securely and any personal information will be disposed of by the principal investigator after a period of five years has elapsed from data collection.

I agree to take part in this project.

.... (Signature of participant)

.... (Date)

This project has been reviewed and approved by the Ethics Committee of the School of Sport and Health Sciences

Appendix 4: Example physical activity readiness questionnaire

SCHOOL OF SPORT AND
HEALTH SCIENCES

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Telephone +44 (0)1392 262896
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Web   www.ex.ac.uk/sshs

SSHS General Health Questionnaire

Your Name: ....

Your Date of Birth: ....

Male / Female (please circle)

Your Height: ....   Your Weight: ....

Your Address: ....

....

....

Your Phone No.: ....

Name of person responsible for study: ....

Please read the following carefully and answer as accurately as possible. The questions are designed solely to determine whether the proposed exercise is appropriate for you. Your answers will be treated as strictly confidential. If you have any doubts or difficulties with any of the questions please contact the person responsible for the study.

1.	Have you seen your doctor in the last 6 months?	YES	NO
2.	Are you currently taking any prescription medications?	YES	NO
3.	Has a doctor ever said you have heart trouble?	YES	NO
4.	Do you ever feel chest pain when you undertake physical activity?	YES	NO
5.	Do you ever feel faint or have spells of dizziness?	YES	NO
6.	Do you experience unreasonable breathlessness?	YES	NO
7.	Do you take heart medications?	YES	NO
8.	Has a doctor ever said you have epilepsy?	YES	NO
9.	Has a doctor ever said you have diabetes?	YES	NO
10.	Has a doctor ever said you have asthma or other lung disease?	YES	NO
11.	Do you have a bone, joint or muscular problem which may be aggravated by exercise?	YES	NO

		YES	NO
12.	Do you have any form of injury?	YES	NO
13.	Has a doctor ever said you have high blood pressure?	YES	NO
14.	Has a doctor ever said you have high cholesterol?	YES	NO
15.	Do you have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)?	YES	NO
16.	Do you smoke, or have you quit smoking in the last 6 months?	YES	NO
17.	Do you get more than 30 minutes of physical activity on at least 3 days per week?	YES	NO
18.	If you are female, are you pregnant?	YES	NO

On how many days each week do you exercise for more than 30 minutes?

On average, how many minutes do you exercise for on each of these days?

What type of exercise do you most regularly take part in?

I have completed the questionnaire to the best of my knowledge and any questions that I have raised have been answered to my full satisfaction.

Signed: ....

Date: ....

UNIVERSITY OF
EXETER

SCHOOL OF SPORT AND
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Web   www.ex.ac.uk/sshs

Current Health Status Questionnaire for males up to 45 years of age and females up to 55 years of age

This form is to be used in conjunction with the SSHS General Health Questionnaire.

It is to be completed in the laboratory prior to the commencement of the exercise test.

		YES	NO
1.	Have you suffered from a viral illness in the last two weeks?		
2.	Have you eaten within the last hour?		
3.	Have you consumed alcohol within the last 24 hours?		
4.	Have you performed exhaustive exercise within the last 48 hours?		
5.	Is there anything to your knowledge that may prevent you from successfully completing the tests that have been outlined to you?		

I have completed the questionnaire to the best of my knowledge and any questions that I have raised have been answered to my full satisfaction.

Signed:...

Date: ....

Appendix 5: Conference abstract

(Congress of the European College of Sport Science, Liverpool, 2011)

The Jäeger Oxycon Pro® provides a reliable estimate of pulmonary oxygen uptake in normobaric hypoxia (see Section 2.5 for the full manuscript).

EUROPEAN COLLEGE OF SPORT SCIENCE
SPORTS SCIENCES:
NEW HORIZONS FROM A WORLD HERITAGE CITY
16th annual Congress of the ECSS | 6th - 9th July 2011, Liverpool – UK
Hosted by The Research Institute for Sport and Exercise Sciences
Liverpool John Moores University

Validity of the Jäeger Oxycon-Pro® Expired Air Analyzer in Normobaric Hypoxia

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INTRODUCTION: When utilising a high precision O_2 analyser, the closed-circuit Douglas Bag Method (DBM) arguably provides the 'gold standard' estimation of oxygen uptake (VO_2) (Macfarlane, 2001). However, open-circuit breath-by-breath analysers give greater insight into the physiological conditioning of athletes, so are now commonplace in exercise science laboratories worldwide. One such unit, the Jäeger Oxycon-Pro® (JOP) provides valid and reliable estimates of VO_2 in normobaric normoxia (NN) (Foss & Hallen, 2005), but there are no published data regarding the performance of the JOP in normobaric hypoxia (NH). PURPOSE: To investigate the validity of the JOP in NH. METHODS: 10 physically active males (29.4 ± 7.1 y, 74.9 ± 5.2 kg) completed two identical cycling tests; one in NH (fraction of inspired oxygen (F_IO_2) = 16.0 ± 0.1 %), and one in NN (F_IO_2 = 20.9 ± 0.1 %), separated by 1-2 days, in a randomized order. The JOP was calibrated using a modified procedure, involving feeding the ambient air intake with bottled NN air. Participants cycled at 120 W for 4 consecutive 4 minute stages, while VO_2 was estimated from expired air using the JOP and DBM. VO_2 was estimated over 60 s intervals, twice by each analyzer, in an alternating, randomized order, then repeated at 160 W. Correlation coefficients were estimated using linear regression analyses, and standard error of the estimate and coefficient of variation (CV) were calculated. RESULTS: Linear regression analyses revealed VO_2 agreement between the 'criterion' DBM with the JOP estimations: correlation coefficients in NN were R^2 = 0.70 at 120 W, and 0.85 at 160 W, and in NH R^2 = 0.74 at 120 W, and 0.82 at 160 W. The statistical comparison between the JOP and DBM in NN gave a CV at 120 W of 5.0% (95% likely range: 3.4 to 9.8%), and at 160 W a CV of 3.3% (95% likely range: 2.2 to 6.3%). In NH, the CV was calculated at 120 W as 4.3% (95% likely range: 2.9 to 8.4%), and at 160 W as 3.1% (95% likely range: 2.1 to 6.0%). CONCLUSION: The JOP performed well in both NN and NH, providing a more valid estimation of VO_2 in NH than in NN. Further investigations should be conducted to establish this validity at higher ventilatory rates, either using participants with greater lung capacities, or using an 'automated pulmonary simulator' (Gore et al, 1997).
REFERENCES:
Foss, O. & Hallen, J. (2005). Int. J. Sports Med. 26(7): 569-75. Gore, C.J., Catcheside, P.G., French, S.N., Bennett, J.M., & Laforgie, J. (1997). Med. Sci. Sports Exerc. 29(8): 1095-103. Macfarlane, D.J. (2001). Sports Med. 31(12): 841-61.

APPENDIX 6: Case study of a female World Champion swimmer

BACKGROUND / METHODS

As part of the experiments involved in Chapter 3, one female member of the altitude group ('Swimmer 7') competed in a 1500 m front crawl race within two weeks before and at 25 d after the three week LH+TH intervention at 2320 m (in addition to the 200 m races that all participants performed). Six months later, Swimmer 7 undertook a second LH+TH camp, which was identical in design to the first. Swimmer 7 was an open water swimmer, so comparing finish times between the main races of interest was inappropriate due to varying environmental conditions. Instead, finish position in the 10 km open water race at the FINA World Championships, performed 24 d after the second altitude camp, was compared to finish position at the previous year's FINA World Open Water Swimming Championships, when Swimmer 7 did not use altitude training. To further quantify changes in swimming cardiovascular fitness, Swimmer 7 completed an aerobic swimming test twice per week for two weeks before, during, and for four weeks after the second altitude camp. This comprised 4 x 100 m front crawl with 75 s allowance for each repetition, targeting 71 s per 100 m (if either the third or fourth repetition was >0.5 s outside the range of 70.5-71.5 s, results were void). HR was recorded after the fourth 100 m (FT1 & T31, Polar Electro, Kempele, Finland).

RESULTS

Swimmer 7 experienced a -1.3% (-1.5 s) and -2.3% (-22.8 s) improvement in 200 m and 1500 m front crawl races, respectively, from before to after the second altitude camp. After 24 d at sea level following the second altitude camp, Swimmer 7 won the 10 km open water race at the FINA World Championships, out of 50 race finishers, in a time of 2:01:58. As the below figure illustrates, this was an improvement of +7 finish positions compared to 1 y earlier, at the FINA World Open Water Swimming Championships. This improvement was only equalled by one other 'world top 10' competitor, and not surpassed by others. In addition, Swimmer 7's HR during the submaximal swimming test set significantly decreased from pre to post-altitude (151 ± 3 b·min^{-1} to 135 ± 3 b·min^{-1}, P = 0.01).
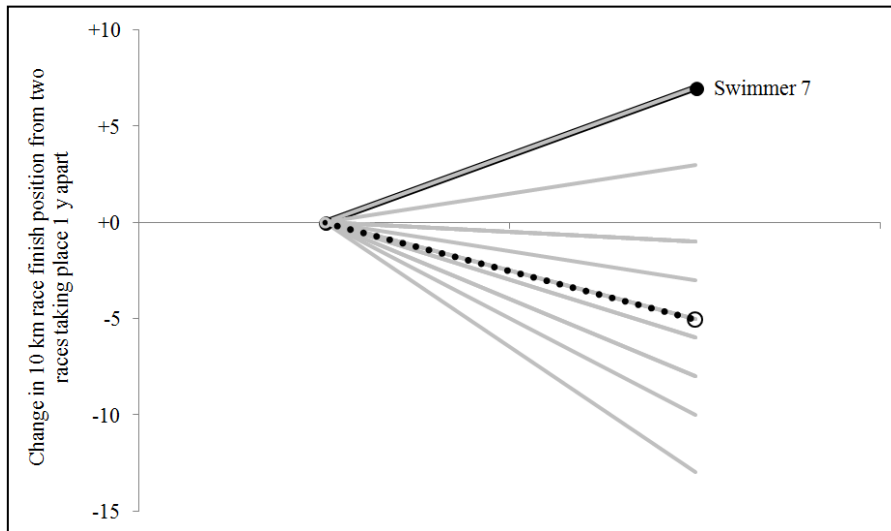
The change in 10 km race finish positions in the top 10 finishers at the FINA World Open Water Swimming Championships, to the FINA World Championships, held 1 y later. The +7 position improvement by Swimmer 7 (solid black line) was equalled by only one of the other top 10 finishers, but not surpassed. The mean change (dashed line) in this group was -3 ± 5 positions.

Swimmer 7's baseline tHb relative to body weight was 11.1 g·kg^{-1} before the first, and 11.5 g·kg^{-1} before the second altitude camp. The below figure illustrates that at no time did Swimmer 7's change in tHb exceed the 2.2% CV.
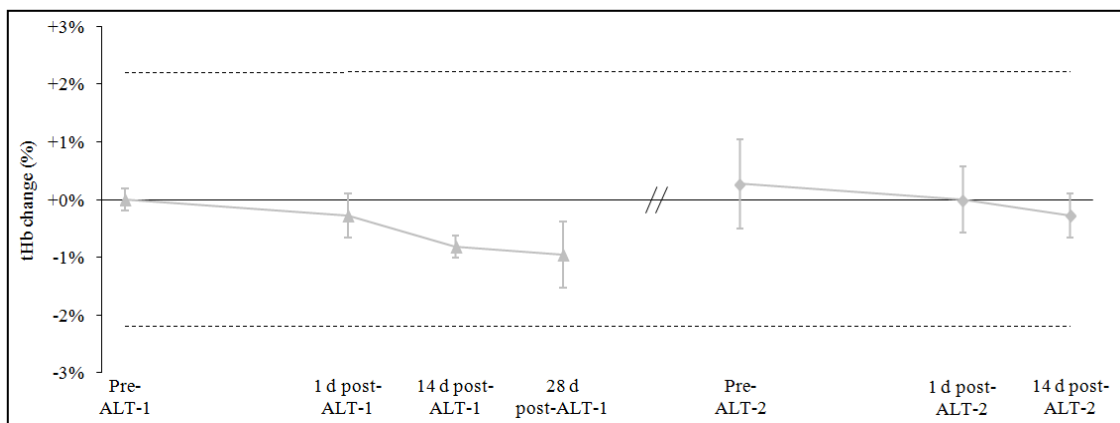
tHb percentage change from baseline in the case study of Swimmer 7, who completed two separate three week altitude training camps (ALT and ALT-2), interspersed with six months of sea level training. Data are the mean of duplicate tHb tests (± SD). "Pre-ALT-1" and "Pre-ALT-2" were time points within 48 h of travelling to altitude. It is clear that at no point did Swimmer 7's tHb change by a greater margin than the CV of 2.2% (depicted by the dotted lines).

DISCUSSION

Swimmer 7 is an example of one participant whose race performances improved after the LH+TH interventions. This swimmer achieved -1.3% (-1.5 s) and -2.3% (-22.8 s) improvements in 200 m and 1500 m front crawl race times, respectively, 25 d after the first altitude camp. In addition, six months later, 24 d after the second altitude camp, Swimmer 7 won the 10 km open water race at the FINA World Championships, having finished in 8th place the previous year (this progression compared favourably to the other competitors).

These performance improvements occurred despite Swimmer 7 showing no signs of increased tHb after either altitude camp, in a similar manner to participants in Gore *et al.* (1998). Similarly to participants in that study, Swimmer 7 was already highly endurance trained pre-altitude, as verified by her higher baseline tHb relative to body weight than previously reported (11.5 g·kg^{-1} vs. 10.7 g·kg^{-1}) (Wachsmuth *et al.*, 2013). Therefore, it is probable that there was not scope for further tHb gains in response to this hypoxic dose of three weeks at 2320 m altitude (Robach & Lundby, 2012).

A placebo effect cannot be ruled out, but the significant reduction in submaximal HR during the swimming test sets indicated an improved overall cardiovascular fitness, and thus it can be speculated that non-haematological adaptations favouring improved post-altitude race performances likely took place, in accordance with findings by Garvican *et al.* (2011).

CONCLUSIONS

One highly trained female swimmer did not experience tHb changes after two similar LH+TH altitude training camps, yet cardiovascular fitness and race performances were substantially improved, culminating in a victory at the FINA World Championships. This case study demonstrates that physiological adaptations to altitude training likely occur that are undetectable via haematological assessments, and overall highlights the importance of closely assessing results in individual athletes, as well as group effects.

APPENDIX 7: Alternative statistical analyses of data from Chapter 3

BACKGROUND

On the advice of a reviewer from an international journal, an alternative to traditional probability-based statistics was computed for the results from Chapter 3. For consistency of techniques within this thesis, traditional statistics were presented in Chapter 3, but this alternative approach is shown below, as computed for the tHb and race performance data before and after three weeks of LH+TH at 2320 m (see Section 3.3).

STATISTICAL METHODS

This contemporary statistical approach involved the use of magnitude-based inferences to detect effects of practical importance in highly trained athletes (Hopkins *et al.*, 2009). Analysis of the effects between the altitude and sea level control groups were performed with log-transformed race performance and tHb data, to account for non-uniformity of error (Hopkins *et al.*, 2009). Threshold values for assessing magnitudes of differences between groups were: trivial <0.2; small 0.2-0.6; moderate 0.6-1.2; large >1.2 (Hopkins *et al.*, 2009). Standardised effects between groups were expressed as mean ± 90% CL, with percentage probabilities calculated to establish whether the true effect was substantially positive or negative. These probabilities were used to make a qualitative probabilistic mechanistic inference about the effect, using the scale: <0.5%, almost certainly not; <5%, very unlikely; <25%, unlikely, probably not; 25-75%, possibly; 75-95%, likely; 95-99.5%, very likely; >99.5%, most likely (Hopkins *et al.*, 2009).

The smallest worthwhile change for tHb was ± 2.73%, calculated as Cohen's smallest standardised effect size (0.2 x the between-participant SD) (Hopkins *et al.*, 2009). The smallest worthwhile change for race performances was ± 0.24%, calculated as 0.3 x the typical race-to-race SD of 0.8% (Pyne *et al.*, 2004).

Within group results are reported as mean ± SD, while between-group results are reported as mean ± 90% CL.

RESULTS

Haematology: The mean (± SD) within group tHb change immediately after altitude training was +4.3 ± 2.7% ("small"), which reduced to +2.9 ± 2.7% after 14 d ("trivial"), and +0.6 ± 1.9% after 28 d ("trivial") at sea level. The mean within group tHb change immediately after sea level training was +0.4 ± 0.5% ("trivial"), then +0.4 ± 1.4% after 14 d ("trivial"), and -1.1 ± 0.5% after 28 d ("trivial"). The net effect of altitude on tHb was (mean ± 90% CL) +4.0 ± 2.2% immediately post-intervention ("likely, small"), then +2.5 ± 2.4% after 14 d at sea level ("possibly, trivial"), and +1.7 ± 1.5% after 28 d at sea level ("likely, trivial").

Race performance: The mean (± SD) 200 m race performance changes of -0.6 ± 1.2% after altitude training, and -0.3 ± 0.3% after sea level training were deemed "unclear". The net effect of altitude training on 200 m race performance was (mean ± 90% CL) -0.3 ± 0.9% ("unclear").

DISCUSSION AND CONCLUSIONS

While the above contemporary statistical approach may be useful when trying to establish the value of a particular intervention in terms of 'real-world' effects, specific to that population, encouragingly the outcome statistics in this example did not particularly differ to the traditional statistical assessment.

The 200 m race performance changes were considered to be "unclear" (i.e. no substantial effect) by the contemporary approach, and the ANCOVA showed no significant difference between the altitude and sea level groups (P = 0.76). Similarly, the "likely small" tHb increase after altitude compared to sea level training was considered a significant effect (P = 0.04).The only difference between the two statistical techniques, is that the tHb increase within the altitude group was considered to be "small" by the contemporary approach, whereas the within group ANCOVA reported a non-significant effect (P = 0.08). The contemporary approach was able to compare the effect size (+4.4 ± 3.2%) to what was pre-determined to be the smallest worthwhile change (± 2.73%), and therefore judged that there was a small effect, whereas the traditional statistical approach simply labelled this effect as being non-significant.

See Hopkins *et al.* (2009) for a description of this contemporary approach.

Appendix 8: Published version of Chapter 5

Holliss, B. A., Fulford, J., Vanhatalo, A., Pedlar, C. R., & Jones, A. M. (2013). Influence of intermittent hypoxic training on muscle energetics and exercise tolerance. *J Appl Physiol, 114*(5), 611-619.

Influence of intermittent hypoxic training on muscle energetics and exercise tolerance

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Holliss BA, Fulford J, Vanhatalo A, Pedlar CR, Jones AM. Influence of intermittent hypoxic training on muscle energetics and exercise tolerance. *J Appl Physiol* 114: 611–619, 2013. First published January 10, 2013; doi:10.1152/japplphysiol.01331.2012.—Intermittent hypoxic training (IHT) is sometimes used by athletes to enhance nonhematological physiological adaptations to simulated altitude. We investigated whether IHT would result in greater improvements in muscle energetics and exercise tolerance compared with work-matched intermittent normoxic training (INT). Nine physically active men completed 3 wk of intensive, single-leg knee-extensor exercise training. Each training session consisted of 25 min of IHT (F_{IO_2} 14.5 $\pm$ 0.1%) with the experimental leg and 25 min of INT with the alternate leg, which served as a control. Before and after the training intervention, subjects completed a test protocol consisting of a bout of submaximal constant-work-rate exercise, a 24-s high-intensity exercise bout to quantify the phosphocreatine recovery time constant ([PCr]-τ), and an incremental test to the limit of tolerance. The tests were completed in normoxia and hypoxia in both INT and IHT legs. Muscle metabolism was assessed noninvasively using ^{31}P-magnetic resonance spectroscopy. Improvements in the time-to-exhaustion during incremental exercise were not significantly different between training conditions either in normoxia (INT, 28 $\pm$ 20% vs. IHT, 25 $\pm$ 9%; $P = 0.86$) or hypoxia (INT, 21 $\pm$ 10% vs. IHT, 15 $\pm$ 11%; $P = 0.29$). In hypoxia, [PCr]-τ was speeded slightly but significantly more post-IHT compared with post-INT (-7.3 ± 2.9 s vs. -3.7 ± 1.7 s; $P < 0.01$), but changes in muscle metabolite concentrations during exercise were essentially not different between IHT and INT. Under the conditions of this investigation, IHT does not appreciably alter muscle metabolic responses or incremental exercise performance compared with INT.

normobaria; altitude; metabolism; performance; magnetic resonance spectroscopy

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INTERMITTENT HYPOXIC TRAINING (IHT), whereby athletes live at or near sea level while undertaking a portion of their training under normobaric or hypobaric hypoxia, has been suggested to be a possibly worthwhile strategy to enhance athletic performance (6, 27). However, there is controversy surrounding the mechanisms of physiological adaptations to IHT, and the extent of the potential performance advantages (24).

Under physiological hypoxia, the O_2 homeostasis–regulating transcription factor, hypoxia-inducible factor 1α (HIF-1α) is activated, initiating a range of adaptations to preserve O_2 delivery (18, 33), of which the best documented is the hepatic and renal release of erythropoietin (EPO). Given sufficient hypoxic dose, this will result in a sustained increase in the

circulating EPO concentration and, consequently, increased erythropoiesis (25). The response timeline is still in question but, for example, it has been reported that after a 4-wk phase of resting intermittent hypoxic exposure (3 h/day, 5 days/wk, at a simulated altitude of 4,000–5,500 m), there were no significant changes in total hemoglobin mass, red cell volume, or other red cell indices compared with a placebo group (10). It is therefore not surprising that studies have failed to measure an increased total hemoglobin mass following IHT interventions, which use relatively short-duration total hypoxic exposures (13, 24, 45).

In addition to hematological effects, a sustained high level of HIF-1α is also known to be associated with a range of other adaptations that enhance muscle O_2 homeostasis (33). These adaptations include enhanced tissue perfusion linked to angiogenesis (38), improved mitochondrial efficiency and control of mitochondrial respiration (29, 32), and enhanced hydrogen ion (H$^+$) buffering capacity (9). Due to the invasive nature of assessing these variables, most studies have been restricted to small sample sizes or resting measures, such that the influence of IHT on skeletal muscle metabolism during dynamic exercise has not been comprehensively investigated. The noninvasive technique, ^{31}P magnetic resonance spectroscopy (^{31}P-MRS) has been utilized to assess muscle energetics during exercise in response to a range of interventions (1, 4, 5, 12, 15–17, 34, 42). Greater muscle oxidative capacity is reflected in faster postexercise phosphocreatine (PCr) resynthesis (39); when muscle acidosis is avoided, the speed of [PCr] recovery provides a valid estimate of in vivo oxidative capacity (4, 12, 26, 35).

To our knowledge, only one study has used ^{31}P-MRS to investigate the effects of IHT on the muscle metabolic responses to exercise (21). In that study, four combination skiers trained for 60 min twice a day for 4 consecutive days at a simulated altitude equivalent to 2,000 m (21). The PCr recovery time constant ([PCr]-τ) was significantly faster post-IHT (mean change of -19%) but remained unchanged in the eight control participants who undertook no training (21). The IHT modality (running/cycling) was different from the ^{31}P-MRS test exercise modality (repeated right-knee extensions), and because the control group remained inactive, it was not possible to assess the effect of the hypoxic stimulus, per se, compared with the effects of normobaric normoxic running/cycling. However, the faster [PCr]-τ suggests enhanced muscle oxidative capacity following just 8 h of IHT. If confirmed, this would provide a strong evidence base for the use of IHT by athletes.

The purpose of this study was therefore to investigate the muscle metabolic responses to exercise following a short, intense period of IHT. We used a study design in which

subjects trained one leg in normoxia (as a control; INT) and the other in hypoxia. The same exercise modality (knee extension) was used for all training and ^{31}P-MRS tests. We hypothesized that *1)* muscle metabolic perturbation (as assessed by changes in [PCr], pH, and inorganic phosphate concentration, expressed as [P$_i$], would be attenuated during submaximal exercise); *2)* [PCr] recovery kinetics following exercise would be faster; and *3)* the time-to-exhaustion during incremental exercise would be extended following both IHT and INT in both normoxia and hypoxia, but that the effects would be greater following IHT.

METHODS

Participants and experimental design. After institutional ethical approval, nine physically active, healthy men participated in this study (mean ± SD: age, 21.5 ± 3.7 yr; body mass, 75.5 ± 11.7 kg; stature, 1.79 ± 0.03 m). Prior to testing, each participant completed a physical activity readiness questionnaire and provided written informed consent. The participants' reported habitual exercise ranged from four sessions of 45 min duration per week to five sessions of 90 min duration per week. The participants were engaged in training for a variety of recreational sports (soccer, cycling, running, rowing, and hockey) and could be best described as moderately trained.

Participants' legs were randomly allocated into the normoxic or hypoxic training group [i.e., one leg was trained while they inhaled normoxic gas (INT); the other was trained while inhaling hypoxic gas (IHT)]. All participants completed *1)* one single-leg knee-extension exercise test protocol practice (described below) and, after a 48-h break, one incremental test to volitional exhaustion under hypoxic conditions, for familiarization purposes; *2)* pretraining testing, consisting of four ^{31}P-MRS test protocols, two for each leg in each of normoxia and hypoxia, *3)* 3 wk of intensive IHT (experimental leg) and INT (control leg); and *4)* post-training testing in which the pretraining test protocols were repeated.

^{31}P-MRS testing. All testing took place with the subjects in a prone position inside the bore of a 1.5 T superconducting magnet (Gyroscan Clinical Intera, Philips Medical Systems, Best, Netherlands). Participants had Velcro straps securely fastened around the thighs, hips, and lower back, and the foot of the test leg was fastened to a pulley system via a padded sling. The ^{31}P-MRS test protocol then commenced. Knee-extension exercise was performed using a custom-built nonferrous ergometer over a distance of ∼0.22 m in time with a visual queue that coincided with magnetic resonance (MR) pulse acquisition (40 pulses/min). The protocol included a 4-min moderate-intensity exercise bout; 6 min of rest; two 24-s high-intensity bouts separated by 3 min and 36 s of rest; then a 5-min 36-s rest and, finally, an incremental test to the limit of tolerance. The work rates applied were calculated from pilot testing undertaken during the familiarization period. The moderate-intensity work rate was performed at a load of 1 kg lower than that eliciting the pH threshold, and the 24-s high-intensity bouts were performed at the peak work rate achieved during the familiarization incremental test. The duration and intensity of this 24-s exercise bout was based on a phase of pilot testing to find the optimal exercise intensity and duration to elicit a drop to 50–60% of baseline [PCr] without a concomitant reduction in intracellular pH. It is known that PCr recovery is not sensitive to differences in end-exercise [PCr] when pH is not altered (37). For the incremental exercise test, the initial resistance was 0.5 kg, and this was increased by 0.5 kg every 30 s until volitional exhaustion. The [P$_i$]/[PCr] and pH during the incremental tests was plotted against work rate, and a pH threshold was identified, as described by Barker et al. (2). We did not measure pulmonary gas exchange during the ^{31}P-MRS tests due to restrictions related to the magnetic environment and the small V̇o$_2$ amplitude (and low signal-to-noise ratio) during single-legged knee-extension exercise performed in the bore of the magnet.

Training intervention. The participants completed 3 wk of intensive IHT (experimental leg) and INT (control leg). Participants trained 5 times per week and thus completed 15 sessions in total over the 3-wk training intervention. Each training session consisted of two identical 25-min phases, one in which the IHT leg was trained, and one in which the INT leg was trained (in a randomized, alternating order). In the IHT condition, the training program totaled 316 min of active IHT or 375 min of hypoxia inspiration, including rest intervals. We based our training intervention on previous studies showing that 3 wk of IHT significantly improved peak power output compared with normoxic training during incremental exercise in hypoxia (30); and 384 min of IHT (over 6 wk) improves mitochondrial function, V̇o$_{2max}$, and endurance exercise performance compared with normoxic training (47). The training program particularly emphasized high-intensity interval training because this has been shown to be particularly effective in invoking rapid muscle metabolic adaptations and improvements in endurance fitness (8, 40). Indeed, Forbes et al. (5) have reported that just six sessions of high-intensity training results in significant speeding of [PCr]-τ. We therefore anticipated that an intense, well-controlled, 3-wk training intervention would result in significant muscle metabolic adaptations that would underpin an enhanced incremental exercise test performance, and that these adaptations may be greater in IHT compared with INT (30, 47).

After being securely fastened to the exercise apparatus as previously described, the single-leg knee-extension exercise training commenced with 2.5 min at the work rate corresponding to the pH threshold in the IHT leg as measured in hypoxia. This was immediately followed by 2.5 min at a work rate 10% higher than that of the pH threshold, then a further 5 min at a work rate 20% above the pH threshold. After a 30-s rest, high-intensity interval exercise commenced. During *Week 1* of training, this consisted of 10 · 60-s exercise bouts (with 30-s passive recovery intervals), while during *Week 2* and *Week 3* of training this consisted of 10 × 70-s exercise bouts (with 20 s of passive recovery), with the work rate being the mean of the pH threshold and the peak work rate attained during incremental exercise in the IHT leg in hypoxia. Arterial O$_2$ saturation (Sa$_{O_2}$) and heart rate (HR), which were assessed using pulse-oximetry (Nonin 7500FO, Nonin Medical, Plymouth, MN); and the rating of perceived exertion (RPE), which was assessed with the Borg scale (3), were recorded after the initial 5 and 10 min of continuous exercise, and then after the fifth and tenth bouts of interval exercise. Work rates were identical for each leg regardless of F$_{IO_2}$, and were increased by 0.5 kg when RPE > 15 after the fifth interval. The inspirate F$_{IO_2}$ (14.5 ± 0.1% for IHT; 20.9 ± 0.0% for INT) was checked before, during, and after each training session using a Servomex 5200 Paramagnetic Analyzer (Servomex, Crowborough, UK) as described below.

Inspired gases. The inspirate was generated by a Cloud 9 hypoxic generator (Sporting Edge, Basingstoke, UK), placed in the MR control room, connected to a 10-m extension pipe, which fed into a 150-liter Douglas Bag (Cranlea & Co, Birmingham, UK). This acted as a reservoir and mixing chamber, and had a separate output pipe feeding into a Hans Rudolf one-way valve (Cranlea & Co) connected to a face mask from which the subject could breathe, with an expired air exit. Thus the flow rate was maintained constant, and no rebreathing of expired air occurred. The O$_2$ and CO$_2$ concentration of the inspirate was checked by a researcher in the MR control room during every test using the Servomex 5200, taking samples via a 10-m capillary tube. This analyzer was calibrated prior to each use with a 16.0% O$_2$, 8.0% CO$_2$, and 76.0% N gas mix (BOC Special Gases, Guildford, UK). For all normoxic tests and training sessions, the O$_2$ filters were inactivated, yielding an F$_{IO_2}$ of 20.9 ± 0.0% and an F$_{ICO_2}$ of 0.05 ± 0.00%, whereas during hypoxic tests and training sessions, an F$_{IO_2}$ of 14.5 ± 0.1% and an F$_{ICO_2}$ of 0.04 ± 0.00% were produced (simulating ∼3,000 m altitude). During testing, both the subject and the researcher administering the test were blinded to the F$_{IO_2}$, with only the researcher in the MR control room being aware of the F$_{IO_2}$. Moreover, subjects were blinded to the F$_{IO_2}$ during all training sessions.

^{31}P-MRS procedures. Prior to the exercise test beginning, absolute baseline concentrations of metabolites were established via a technique similar to that described by Kemp et al. (19) using a 6-cm ^{31}P transmit/receive surface coil. Subjects were positioned within the scanner with the coil placed within the scanner bed and positioned such that the subject's quadriceps muscle was centered directly over it and a phosphoric acid source was directly beneath it. After initially acquiring images to confirm that the m. rectus femoris was positioned correctly relative to the coil, spatially localized spectroscopy was undertaken to determine the relative signal intensities obtained from the phosphoric acid source and P_i from the subject's quadriceps. On completion of the exercise protocol and after the subject had been removed from the scanner, subsequent scans were obtained to compare the signals obtained from the same phosphoric acid standard and an external P_i solution of known concentration. The localized voxel sampled within the external solution was of the same dimensions and distance from the coil as from the muscle previously, allowing the calculation of muscle P_i following corrections for relative coil loading. Absolute concentrations of PCr and ATP were subsequently calculated via the ratio of $[P_i]:[PCr]$ and $[P_i]:[ATP]$, respectively.

Following metabolite concentration determinations, the phosphoric acid source was removed from the scanner bed and the subjects were securely fastened to the exercise apparatus as previously described. Images were acquired to confirm the quadriceps muscle was positioned directly above the 6-cm ^{31}P coil, and subjects commenced breathing the inspirate, which was continued for 30 min prior to the commencement of ^{31}P data acquisition. Initially, a number of preacquisition steps were carried out to optimize the signal from the muscle under investigation. Matching and tuning of the coil was performed and an automatic shimming protocol undertaken within a volume that defined the quadriceps muscle. A baseline spectrum before exercise was then acquired with long repetition time (TR = 20 s) in which the relative unsaturated peak amplitudes could be determined. Two minutes of further rest then followed to acquire baseline MR sequences, after which the single-legged knee-extension exercise test protocol commenced, as previously described. During the 2-min resting baseline and the subsequent exercise protocol, ^{31}P data were acquired every 1.5 s, with a spectral width of 1,500 Hz and 1K data points. Phase cycling with four phase cycles was employed, leading to a spectrum being acquired every 6.0 s.

Data analyses. The acquired spectra were quantified via peak fitting, assuming prior knowledge, using the jMRUI (version 3) software package employing the AMARES fitting algorithm (41). Spectra were fitted assuming the presence of the following peaks: P_i, phosphodiester, PCr, α-ATP (two peaks, amplitude ratio 1:1), γ-ATP (two peaks, amplitude ratio 1:1), and β-ATP (three peaks, amplitude ratio 1:2:1). Intracellular pH was calculated using the chemical shift of the P_i spectral peak relative to the PCr peak (35). [ADP] was calculated via knowledge of $[P_i]$, [PCr], and pH values as described by Kemp et al. (20), taking into account the dependency of rate constants on pH. The oxidative ATP turnover rate (ATP-Ox) was determined on

the basis of the hyperbolic relationship between [ATP] production rate and free cytosolic [ADP], and calculated using the PCr recovery time constant determined from the 24-s bout (22, 23).

The 4-min moderate-intensity exercise bout values of [PCr], [ADP], $[P_i]$, $[P_i]/[PCr]$, ATP-Ox, and pH were calculated as the mean between 90 s and 210 s of exercise (i.e., 120 s total sample time, omitting the first 90 s and final 30 s). End-exercise values were quantified as the mean of the final three data points (i.e., final 18 s) prior to volitional exhaustion.

For the [PCr] values following the 24-s high-intensity exercise period, [PCr] recovery was fitted with Prism 5 software (GraphPad, La Jolla, CA) by a single exponential of the form:

$$[PCr]_{(t)} = [PCr]_{end} + [PCr]_{(0)}(1 - e^{(-t/\tau)})$$

where $[PCr]_{end}$ is the value at the end of exercise, $[PCr]_{(0)}$ is the difference between the [PCr] at end-exercise and when fully recovered, t is the time from exercise cessation, and τ is the time constant for the exponential recovery of [PCr]. The [PCr]-τ from each of the two 24-s exercise bouts was determined separately and the mean of the two values was then calculated.

Statistics. Separate repeated-measures ANCOVA with mixed measures were used for each of the two test conditions (normoxic vs. hypoxic) to assess differences in changes of each ^{31}P-MRS variable ([PCr], [ADP], $[P_i]$, $[P_i]/[PCr]$, ATP-Ox, and pH) during the moderate-intensity exercise bouts and the incremental exercise tests to exhaustion, between the two training conditions (INT vs. IHT). The pH threshold and the time-to-exhaustion during the incremental tests were compared in the same way. Before calculating the monophasic [PCr]-τ from the 24-s high-intensity exercise bouts, paired-samples *t*-tests were used to assess any differences between resting baseline and end 24-s pH. Differences between pretraining [PCr]-τ under normoxic and hypoxic conditions were also assessed using paired-samples *t*-tests, and differences in [PCr]-τ resulting from INT vs. IHT were assessed by ANCOVA. Pretraining values were used as covariates for all ANCOVAs. Results are expressed as mean $\pm$ SD. All *t*-tests and ANCOVAs were performed using PASW Statistics (v18.0, IBM SPSS, Portsmouth, UK), and the probability level of $P < 0.05$ was considered to represent a significant difference.

RESULTS

Sa_{O_2} in normoxia and hypoxia. Prior to training during moderate-intensity exercise, the hypoxic inspirate resulted in an Sa_{O_2} of 91 $\pm$ 1% compared with the normoxic inspirate, which resulted in an Sa_{O_2} of 98 $\pm$ 1%. There were no significant changes in these Sa_{O_2} values after training ($P > 0.05$).

^{31}P-MRS variables during moderate-intensity exercise. The moderate-intensity exercise test results are summarized in Tables 1 and 2. Significant overall training effects existed for most end-exercise MR variables when IHT and INT data were

Table 1. *^{31}P-MRS variables measured during the moderate-intensity exercise bout while breathing the normoxic inspirate*

	Normoxic trained leg		Hypoxic trained leg		Main effect for time		Interaction effect	
	Pretraining	Post-training	Pretraining	Post-training	F	P	F	P
Baseline [PCr] (mM)	32.2 ± 3.8	31.2 ± 2.5	33.1 ± 3.8	31.8 ± 3.6	8.39	0.01	<0.001	0.95
End-exercise [PCr] (mM)	27.2 ± 5.0	26.5 ± 2.3	27.5 ± 3.8	27.2 ± 3.6	16.93	0.001*	0.27	0.61
Δ[PCr] (mM)	5.1 ± 2.0	4.7 ± 2.6	5.5 ± 2.0	4.5 ± 1.8	1.47	0.25	3.38	0.09
End-exercise PCr (%)	83.5 ± 7.3	85.0 ± 7.7	83.2 ± 5.9	85.6 ± 4.8	5.38	0.04*	0.08	0.78
End-exercise ATP-Ox (mM/s)	0.38 ± 0.08	0.46 ± 0.17	0.44 ± 0.12	0.50 ± 0.14	<0.001	0.96	0.33	0.58
End-exercise [ADP] (µM)	15.3 ± 7.1	15.3 ± 7.4	16.4 ± 5.9	12.4 ± 3.5	6.65	0.02*	2.28	0.15
End-exercise $[P_i]$ (mM)	7.9 ± 2.3	7.2 ± 2.8	9.0 ± 2.8	6.6 ± 1.7	4.39	0.05	2.91	0.11
End-exercise $[P_i]/[PCr]$	0.31 ± 0.13	0.28 ± 0.13	0.34 ± 0.12	0.25 ± 0.06	9.97	0.01*	1.96	0.18
End-exercise pH	7.03 ± 0.04	7.04 ± 0.04	7.03 ± 0.03	7.05 ± 0.03	2.40	0.14	0.01	0.94

Δ[PCr] indicates difference in [PCr] between baseline and end-exercise. *Significant training effect across both training conditions ($P < 0.05$).

Table 2. ^{31}P-MRS variables measured during the moderate-intensity exercise bout while breathing the hypoxic inspirate

	Normoxic trained leg		Hypoxic trained leg		Main effect for time		Interaction effect	
	Pretraining	Post-training	Pretraining	Post-training	F	P	F	P
Baseline [PCr] (mM)	34.1 ± 4.4	32.5 ± 3.1	34.4 ± 4.2	32.0 ± 4.3	6.12	0.03	0.19	0.672
End-exercise [PCr] (mM)	28.5 ± 4.4	27.4 ± 3.1	28.4 ± 5.0	27.4 ± 4.6	6.54	0.02†	<0.001	0.995
Δ[PCr] (mM)	5.6 ± 3.5	5.1 ± 1.9	6.0 ± 2.8	4.6 ± 1.6	14.20	0.002	36.07	<0.001*
End-exercise PCr (%)	83.7 ± 9.2	84.4 ± 5.6	82.1 ± 7.9	85.2 ± 5.6	20.12	<0.001†	0.86	0.37
End-exercise ATP-Ox (mM/s)	0.31 ± 0.08	0.37 ± 0.13	0.32 ± 0.12	0.45 ± 0.10	2.13	0.17	2.01	0.18
End-exercise [ADP] (μM)	16.1 ± 8.0	14.1 ± 6.5	16.3 ± 5.6	16.0 ± 3.4	11.32	0.01†	1.21	0.29
End-exercise [Pi] (mM)	8.2 ± 2.9	6.5 ± 2.8	8.7 ± 2.5	7.1 ± 1.6	7.22	0.02†	0.13	0.72
End-exercise [Pi]/[PCr]	0.30 ± 0.14	0.24 ± 0.12	0.32 ± 0.10	0.26 ± 0.05	12.54	0.003†	0.15	0.70
End-exercise pH	7.05 ± 0.06	7.07 ± 0.04	7.03 ± 0.05	7.06 ± 0.03	23.61	<0.001†	0.03	0.87

*Significant difference between INT and IHT legs ($P < 0.05$). †Significant training effect across both training conditions ($P < 0.05$). Δ[PCr] indicates difference in [PCr] between baseline and end-exercise.

combined (see Tables 1 and 2 for ANCOVA-derived F and P values). However, there were no significant interactions; that is, changes resulting from IHT were not significantly different from INT. The absolute [PCr] at rest and over the final 30 s of moderate-intensity exercise were slightly but significantly reduced after both IHT and INT training (Fig. 1), but there was no difference between the two training conditions. However, Δ[PCr] (i.e., the magnitude of PCr degradation) during exercise was reduced by both types of training and there was an interaction in hypoxia, such that IHT spared PCr utilization to a greater extent than INT. The end-exercise pH was higher following training when subjects breathed hypoxic gas but not normoxic gas (Tables 1 and 2).

PCr recovery kinetics. The results for PCr recovery kinetics are summarized in Table 3. There were no significant differences between resting pH and the pH measured after the 24-s

high-intensity exercise bout, with a mean change of only $-0.01 ± 0.01$ units. There were no significant [PCr]-τ differences pretraining between the legs that had been selected for INT and the legs that had been selected for IHT, whether tested in normoxia (21 ± 3 s vs. 20 ± 4 s; $t = 0.81$, $P = 0.44$) or hypoxia (28 ± 4 s vs. 29 ± 5 s; $t = 0.53$, $P = 0.61$). As expected, before training, [PCr]-τ was significantly faster in normoxia compared with hypoxia in the legs that had been selected for INT (21 ± 3 s vs. 28 ± 4 s; $t = 4.74$, $P = 0.001$) and the legs that had been selected for IHT (20 ± 4 s vs. 29 ± 5 s; $t = -5.43$, $P = 0.001$). The [PCr]-τ was significantly reduced after both INT and IHT under both normoxic and hypoxic test conditions (Table 3 and Fig. 2). In hypoxia, the [PCr]-τ reduction was significantly greater after IHT ($-7 ± 3$ s) compared with after INT ($-4 ± 2$ s) [$F_{(1,14)} = 14.46$, $P = 0.002$]. Although [PCr]-τ in normoxia tended to decrease more
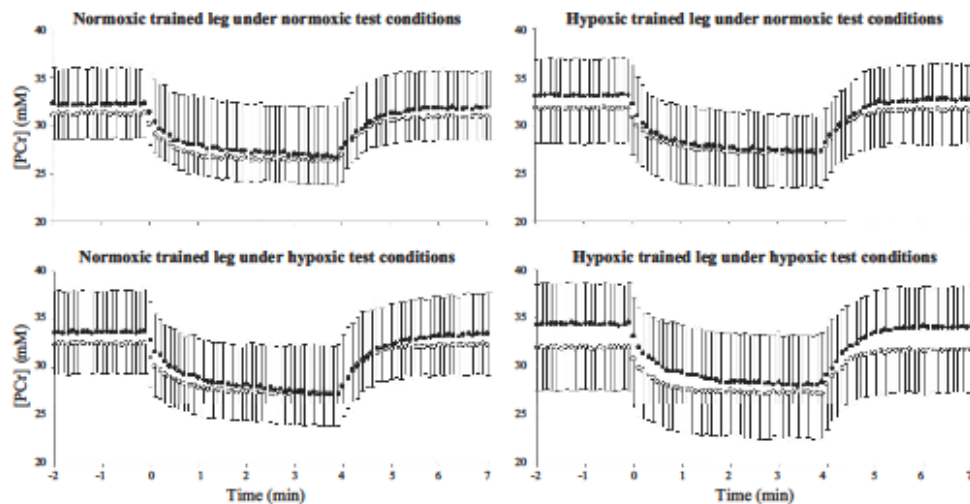
Fig. 1. Mean ± SD muscle [PCr] during 2-min passive rest (baseline values); the 4-min moderate-intensity exercise bout; and 3 subsequent min of passive rest pretraining (♦) and post-training (○). Note that absolute [PCr] at rest and over the final 30 s of exercise were significantly reduced after both IHT and INT [i.e., there was a training effect when tested under normoxia ($P = 0.001$) and under hypoxia ($P = 0.023$) but there were no significant differences between the two training conditions (no interaction effect)].

Table 3. *[PCr]-τ measured following the 24-s high-intensity exercise bout*

	Normoxic trained leg		Hypoxic trained leg		Main effect for time		Interaction effect	
	Pretraining	Post-training	Pretraining	Post-training	F	P	F	P
[PCr]-τ (s) while breathing the normoxic inspirate	20.9 ± 2.9	19.1 ± 3.2	20.1 ± 4.2	16.6 ± 3.4	3.90	0.07	2.98	0.11
[PCr]-τ (s) while breathing the hypoxic inspirate	27.8 ± 4.3	24.1 ± 3.7	28.6 ± 4.5	21.2 ± 2.7	12.55	0.003†	14.46	0.002*

*Significant difference between INT and IHT legs ($P < 0.05$). †Significant training effect across both training conditions ($P < 0.05$).

after IHT (-3 ± 3 s) compared with INT (-2 ± 2 s), this was not statistically significant ($F_{1,15} = 2.98, P = 0.11$).

³¹P-MRS variables and exercise tolerance during incremental exercise. The incremental exercise test results are summarized in Tables 4 and 5. There were no significant differences between the improvements in time-to-exhaustion post-IHT compared with post-INT regardless of whether subjects were tested in normoxia (122 ± 41 s vs. 128 ± 67 s) or hypoxia (78 ± 54 s vs. 106 ± 45 s); indeed there was only a significant overall training effect for time-to-exhaustion under normoxic test conditions when INT and IHT data were combined (Tables 4 and 5). There were significant overall training effects when both IHT and INT data were combined for absolute and relative [PCr] at the limit of tolerance in both normoxia and hypoxia (Fig. 3), and for [ADP] and [ATP-Ox] at the limit of tolerance in hypoxia. There were no other significant differences in MR variables measured at the limit of tolerance or at the pH threshold during the incremental tests between IHT and INT (Tables 4 and 5).

DISCUSSION

To our knowledge, this is the first study to investigate the influence of IHT compared with INT on muscle energetics

during exercise in normoxia and hypoxia. Overall, IHT had only limited effects on the muscle metabolic responses to exercise. During moderate-intensity exercise, the effects of training were similar between IHT and INT, although in hypoxia, Δ[PCr] was reduced to a slightly greater extent by IHT. Similarly, there were no significant differences in the effects of training on the pH threshold or any of the other ³¹P-MRS-derived variables between IHT and INT during incremental exercise. Compared with INT, IHT resulted in a slightly but significantly faster [PCr]-τ in hypoxia and there was a tendency for there to be a similar effect in normoxia. However, changes in time-to-exhaustion during incremental exercise were not significantly different between IHT and INT either in normoxia or hypoxia.

PCr recovery kinetics. The pretraining differences in [PCr]-τ between normoxic and hypoxic test conditions of 7–9 s (34–42% slower) confirm that, in hypoxia, recovery from high-intensity exercise is substantially impaired. Our results are similar to those of Haseler et al. (12) who reported a 34% difference between [PCr]-τ measured in 21% F_{IO_2} compared with 10% F_{IO_2}.

The reduction in [PCr]-τ in hypoxia following IHT was significantly greater than the reduction following INT (26% vs.
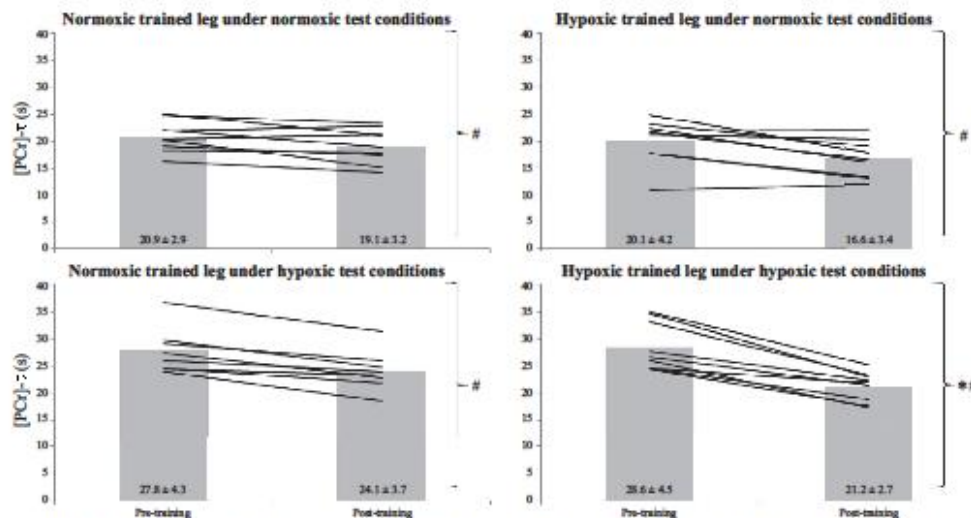
Fig. 2. [PCr]-τ after the 24-s severe-intensity exercise bout pre- and post-training. Gray bars illustrate mean values; black lines illustrate individual responses. *Significant difference between INT and IHT ($P < 0.05$); #significant effect across both training conditions ($P < 0.05$). Note that under hypoxic conditions, all tests revealed a significant overall training effect (i.e., a faster [PCr]-τ after training, but most importantly, under hypoxic conditions there was a significant interaction ($P = 0.002$), showing that the IHT group realized a greater [PCr]-τ reduction than the INT group).

Table 4. ^{31}P-MRS variables and exercise tolerance measured during the incremental test while breathing the normoxic inspirate

	Normoxic trained leg		Hypoxic trained leg		Main effect for time		Interaction effect	
	Pretraining	Post-training	Pretraining	Post-training	F	P	F	P
pH threshold (s)	424 · 74	485 57	441 88	488 · 76	6.58	0.02*	0.20	0.66
[PCr] at T-Lim (mM)	16.4 ± 5.2	11.3 — 3.4	16.2 — 5.0	10.8 ± 3.0	23.66	<0.001*	0.16	0.70
PCr at T-Lim (%)	49.9 ± 11.8	36.0 = 9.8	48.4 = 13.2	33.9 ± 8.7	12.33	0.003*	0.16	0.69
[ADP] at T-Lim (μM)	42.4 ± 20.7	56.4 ± 30.7	45.7 ± 19.3	59.0 ± 24.7	2.20	0.16	0.01	0.95
[Pi] at T-Lim (mM)	15.4 ± 5.7	19.6 = 6.0	18.5 ± 6.6	18.3 ± 6.5	2.19	0.16	2.85	0.11
[Pi]/[PCr] at T-Lim	1.10 ± 0.72	1.95 ± 0.97	1.33 — 0.81	1.90 ± 0.98	0.70	0.42	0.41	0.53
pH at T-Lim	6.86 · 0.11	6.71 0.15	6.85 0.19	6.76 · 0.19	3.55	0.08	0.43	0.52
ATP-Ox at T-Lim (mM/s)	0.67 · 0.13	0.90 0.20	0.76 0.14	1.10 · 0.19	2.42	0.14	0.27	0.12
T-Lim (s)	488 · 63	616 42	505 59	627 · 57	10.31	0.006*	0.03	0.86

T-Lim indicates limit of tolerance. *Significant training effect across both training conditions ($P < 0.05$).

13%), suggesting that adding a hypoxic stimulus to normal training may augment the physiological adaptations that modulate the [PCr]-τ in hypoxia. The [PCr]-τ reduction of 17% post-IHT in normoxia is similar to the significant 19% change reported by Kuno et al. (21) following 4 consecutive days of IHT (~480 min exposure). Unfortunately, that study did not include an INT control condition, such that it is not possible to determine to what extent hypoxia per se influenced the training adaptation above and beyond normal exercise training. However, collectively, these two studies suggest that IHT speeds muscle [PCr]-τ, which has been proposed to reflect muscle oxidative capacity (11).

The high-intensity training stimulus we employed is similar to that used in the study by Vogt et al. (43), in which subjects completed either high- or low-intensity INT or IHT for 6 wk. These authors reported a significant gain in subsarcolemmal mitochondria density in the high- (~130%) and low- (~100%) intensity IHT groups, but not in the high- (~1%) or low- (~13%) intensity INT groups (43). Similarly, Geiser et al. (7) reported that total and subsarcolemmal mitochondrial densities increased by ↑54% and 105%, respectively, following high-intensity IHT compared with 24% and ↑13%, respectively, following high-intensity INT. The results of the present study are consistent with these findings: assuming a sufficient muscle O_2 supply, an increase in mitochondrial volume would be expected to result in faster [PCr]-τ (11, 12, 26, 35, 39), as was observed post-IHT. It is important to note, however, that the improvement in [PCr]-τ post-IHT was only different from that observed post-INT when subjects were tested in hypoxia. If IHT had promoted mitochondrial biogenesis to a greater extent

than INT, a significantly faster [PCr]-τ might also have been expected in normoxia. Therefore, it is likely that the faster [PCr]-τ observed after IHT was not solely due to enhanced mitochondrial biogenesis. It is known that postexercise PCr recovery is heavily influenced by the muscle oxygenation status (11, 12). It may be speculated therefore, that compared with INT, IHT caused physiological adaptations that resulted in a greater enhancement of muscle O_2 delivery in hypoxia.

In hypoxia, the mitogen-activated protein kinase pathway is stimulated, enhancing the activity of HIF-1α. A range of molecular and structural changes subsequently take place (14), including enhanced transcriptional activation of vascular endothelial growth factor (VEGF) (28). Geiser et al. (7) reported that capillary density increased significantly (by ↑12%) following high-intensity IHT, whereas there was no significant change following high-intensity INT. Vogt et al. (43) also reported significant increases in VEGF mRNA and capillary density after high-intensity IHT by ~52% and ~19%, respectively, with no significant changes after low-intensity IHT or high-intensity or low-intensity INT. Although not consistently found (47), these differences in capillary density changes between high-intensity IHT and INT (7, 43) suggest that IHT might result in enhanced muscle oxygenation. If so, this may contribute to the reduced [PCr]-τ observed post-IHT in hypoxia (12). Faster [PCr]-τ following IHT would be expected to result in less fatigue and to enable better maintenance of performance during intermittent high-intensity exercise (39). It may therefore be speculated that athletes competing in sports requiring repetitive sprints might obtain an advantage from performing IHT prior to competition, especially at

Table 5. ^{31}P-MRS variables and exercise tolerance measured during the incremental test while breathing the hypoxic inspirate

	Normoxic trained leg		Hypoxic trained leg		Main effect for time		Interaction effect	
	Pretraining	Post-training	Pretraining	Post-training	F	P	F	P
pH threshold (s)	457 ± 76	486 ± 74	438 ± 79	474 ± 76	1.45	0.25	0.08	0.78
[PCr] at T-Lim (mM)	16.5 · 4.9	10.2 · 2.7	17.7 · 7.7	11.1 · 3.7	62.0	<0.001*	0.24	0.63
PCr at T-Lim (%)	48.3 · 10.7	31.1 · 7.5	50.1 · 17.4	34.4 · 9.1	35.82	<0.001*	1.02	0.33
[ADP] at T-Lim (μM)	44.9 · 12.5	65.9 · 19.5	42.1 · 16.2	67.0 · 16.5	5.86	0.03*	0.04	0.85
[Pi] at T-Lim (mM)	15.9 ± 3.8	19.2 ± 7.9	17.9 ± 7.3	19.5 ± 5.9	3.48	0.08	0.04	0.85
[Pi]/[PCr] at T-Lim	1.06 ± 0.45	2.09 ± 1.11	1.23 ± 0.86	1.98 ± 1.02	<0.001	0.98	0.63	0.44
pH at T-Lim	6.88 ± 0.10	6.76 ± 0.13	6.85 ± 0.17	6.76 ± 0.21	0.86	0.37	0.10	0.75
ATP-Ox at T-Lim (mM/s)	0.54 ± 0.06	0.81 ± 0.13	0.52 ± 0.19	0.88 ± 0.15	6.91	0.02*	1.36	0.26
T-Lim (s)	509 ± 40	615 ± 45	518 ± 41	595 ± 57	2.45	0.14	1.18	0.29

T-Lim indicates limit of tolerance. *Significant training effect across both training conditions ($P < 0.05$).

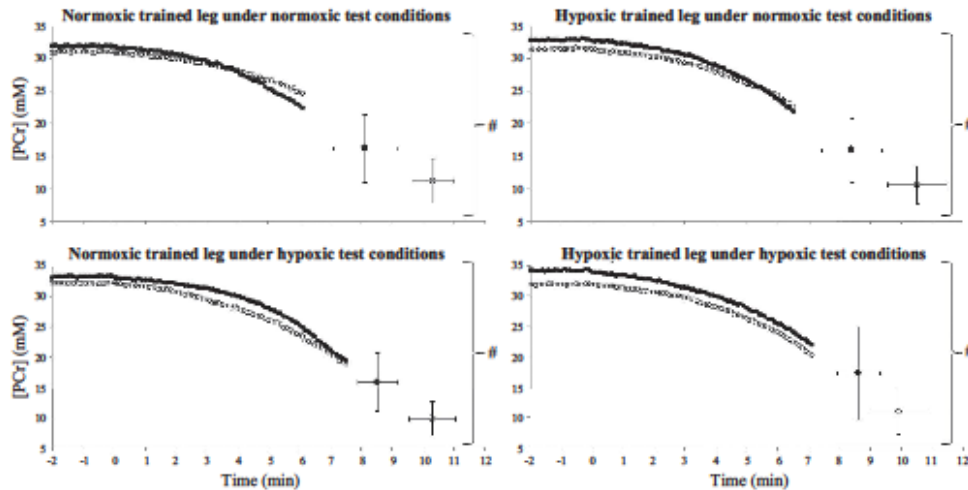Intermittent Hypoxic Training and Muscle Energetics · Holliss BA et al.

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Fig. 3. Mean [PCr] during the incremental test to exhaustion pretraining (♦) and post-training (○). Circles without error bars are the mean [PCr] until a participant reached the limit of tolerance; circles with error bars are the mean ± SD [PCr] and time at the limit of tolerance. #Significant effect across both training conditions ($P < 0.05$). Note that absolute [PCr] at the limit of tolerance was significantly lower post-training under both normoxic and hypoxic test conditions ($P < 0.001$), but regardless of these overall training effects, there were no significant differences between the two training conditions (no interaction effect).

altitude (27). It should be emphasized, however, that the faster [PCr]-τ following IHT was only statistically significant in hypoxia and that, in absolute terms, the effect was small, such that it is not certain to have functional relevance (see *Muscle metabolic responses to incremental exercise and time-to-exhaustion*).

Muscle metabolic responses during moderate-intensity exercise. There were essentially no differences in the muscle metabolic response to moderate-intensity exercise resulting from IHT compared with INT. The only difference between conditions was that Δ[PCr] was reduced to a greater extent following IHT compared with INT when subjects exercised in hypoxia. This sparing of the extent of PCr degradation suggests a lower muscle metabolic perturbation in hypoxia following IHT. The mechanistic basis for this effect is uncertain but might be linked to enhanced local muscle oxygenation (12, 46). However, although there were differences in Δ[PCr] following IHT and INT, the end-exercise [PCr] was not different and thus the functional significance of this change is questionable.

Muscle metabolic responses to incremental exercise and time-to-exhaustion. There were essentially no differences in the muscle metabolic response to incremental exercise following IHT and INT. On average, the subjects were able to sustain exercise longer after training in both normoxia and hypoxia, but in hypoxia, the intersubject variability precluded the attainment of statistical significance. Importantly, there was no additional improvement in time-to-exhaustion following IHT compared with INT.

Our results are consistent with several previous studies (7, 31, 43) in showing that, despite seemingly favorable adaptations in indices of mitochondrial function, capillarization, or both, IHT does not result in a greater improvement in exercise

performance in normoxia compared with INT. Similar to the present study, using a double-blind placebo-controlled design, Truijens et al. (40) added high-intensity interval training (three sessions per week for 5 wk) to the programs of trained swimmers and found that IHT was no more effective than INT in improving 100-m or 400-m time trial performance. Our results contrast with those of Terrados et al. (36) and Zoll et al. (47) who reported that exercise tolerance was significantly increased after IHT but not INT in well-trained athletes. It is possible that differences in the reported effectiveness of IHT reflect interstudy differences in subject training status and the total hypoxic dose administered. It should be noted that in the Zoll et al. (47) study, face masks were used to administer the inspirate in the IHT group only, so the lack of blinding may have resulted in a performance-enhancing placebo effect. In the present study, subjects remained blinded to the $F_{I_{O_2}}$ during both training and testing (at the completion of the study, 4 out of 9 subjects correctly guessed which leg had been trained in hypoxia) and the performance tests were conducted in a double blind manner.

Experimental considerations. Our study employed an incremental exercise test to exhaustion to assess maximal aerobic performance, but we recognize that incremental tests are less sensitive than constant-work-rate tests for assessing changes in exercise tolerance following an intervention (44). Also, the training intervention was relatively short (3 wk and 15 sessions), and we cannot exclude the possibility that IHT might have more effectively enhanced performance if it had been practiced for longer. The moderate training status of our subjects is also an important consideration. Previous studies have suggested that differences in performance changes between INT and IHT may be more likely in highly trained (36,

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47) compared with less well-trained (7, 43) subjects. It should also be noted that the IHT and INT training sessions were completed at the same absolute intensity such that the IHT leg was trained at a slightly higher relative intensity. It is possible that this contributed to the minor differences (for example, in [PCr]-τ) observed between IHT and INT. The localized muscle mass engaged by our single-legged exercise training and testing modality precluded the measurement of $\dot{V}o_{2max}$ and did not simulate the oxidative energy demand that would be experienced during whole-body exercise. However, studies that have used separate training groups or cross-over designs are subject to the normal daily variations in subjects' activities outside of the controlled training and test environments. One of the key strengths of the present investigation is the single-legged study design, which ruled out any placebo effects and allowed noninvasive interrogation and comparison of the muscle metabolic adaptations to IHT compared with INT in the same subjects.

In conclusion, compared with INT, IHT resulted in no meaningful changes in the muscle metabolic response to moderate-intensity constant-work-rate exercise or exhaustive incremental exercise. However, in hypoxia only, IHT resulted in a small but statistically significant reduction of [PCr]-τ in the recovery from high-intensity exercise. Although the reduced [PCr]-τ in hypoxia may reflect increased muscle oxidative capacity following IHT, the practical importance of this is questionable, given that IHT was no more effective than INT in enhancing incremental exercise performance either in hypoxia or normoxia.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: B.A.H., J.F., A.V., C.R.P., and A.M.J. conceived and designed research; B.A.H., J.F., and A.V. performed experiments; B.A.H., J.F., and A.V. analyzed data; B.A.H., J.F., A.V., and A.M.J. interpreted results of experiments; B.A.H. prepared figures; B.A.H., J.F., and A.V. drafted manuscript; B.A.H., J.F., A.V., C.R.P., and A.M.J. approved final version of manuscript; J.F., A.V., C.R.P., and A.M.J. edited and revised manuscript.

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