ACCLIMATISATION, DE-ACCLIMATISATION AND RE-ACCLIMATISATION TO HYPOXIA

by

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ABSTRACT

World-wide, increasing numbers of individuals repeatedly alternate between low and high altitude for work and play. There is a general impression that acquired acclimatisation status persists for some time following return to sea level and that subsequent altitude tolerance is improved by previous hypoxic experience. However, it is unknown whether previous exposure to high altitude fundamentally alters the process of hypoxic re-acclimatisation (RA). My Doctoral research employed a number of approaches to investigate potential differences between the processes of initial acclimatisation (IA) and RA. The time course and mechanisms of hypoxic de-acclimatisation (DA) were explored to determine the time domains across which the retention of previous acclimatisation status might facilitate RA. Cross-sectional and longitudinal field studies were conducted to compare functional outcomes, cardiorespiratory function in rest and exercise and haematological responses throughout IA and RA in high-altitude trekkers. Results indicated that clinical outcomes and trekking performance were improved in RA but with limited physiological evidence of underlying improvements in hypoxic compensation. The haematological response to hypoxia was slightly greater in RA than IA, prompting further investigation of haematological RA in an animal model. Three paradigms of RA were used to examine the effect of IA and DA duration on the process of haematological RA in mice exposed to normobaric hypoxia. Despite altered erythropoietic control in RA treatments, the resulting haematological responses were generally consistent between IA and RA with no evidence of improved responses in RA. In fact, haematological acclimation was impaired in one RA treatment, possibly due to reduced availability of nutrients required for haemoglobin synthesis following an extended period of IA and upregulated erythropoiesis. Given the lack of physiological explanation for improved functional outcomes in RA, non-physiological mechanisms were pursued. Interviews with altitude-experienced individuals identified a perception that prior altitude experience leads to reduced altitude-induced anxiety and improved psychological tolerance of sensations associated with altitude exposure. Although physiological aspects of hypoxic re-acclimatisation merit further investigation, it is possible that improved psychological tolerance of high altitude contributes to the improved functional outcomes in RA that are reported here and elsewhere.

PREFACE

The work presented in Appendix III has been published as:

MacNutt MJ and AW Sheel (2008) Performance of evacuated blood collection tubes at high altitude. High Alt Med Biol 9: 235-7.

The full text of this article is included in the dissertation with permission from Mary Ann Libiert, Inc. I identified the research question, designed the study, completed all aspects of data collection and analysis and wrote the manuscript. Dr. Sheel provided financial support and offered improvements to the manuscript.

Work presented in CHAPTERS 3 and 4 was conducted with approval from the UBC Clinical Research Ethics Board (Certificate #H07-01400). This work was also approved by the Nepal Health Research Council.

Work presented in CHAPTER 5 was conducted with approval from the UBC Animal Care Committee (Certificate #A05-1077). This application for ethical approval was originally submitted by collaborators and I was added to the application with Ammendment A005. The protocol was later modified and the number of research animals increased in Amendments A006 and A007.

Work presented in CHAPTER 6 was conducted with approval from the UBC Behavioural Research Ethics Board (Certificate #H07-01456).

Preliminary data were collected as part of the proposed research described in APPENDIX I. This work was approved by the UBC Clinical Ethics Research Board (Certificate #H06-00266).

The work presented in Appendix IV was conducted with approval by the UBC Clinical Research Ethics Board (Certificate #H06-00252).

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$_a$	arterial
$_A$	alveolar
A_E	area under the exercise measures vs. workload curve
ALT_{max}	maximum altitude attained
AMS	acute mountain sickness
ANOVA	analysis of variance
A_R	area under the recovery measures vs. workload curve
BFU-E	erythroid burst forming unit
BL	baseline
BMI	body mass index
BP	blood pressure
C	concentration
CB	carotid body
cDNA	complimentary deoxyribonucleic acid
CFU-E	erythroid colony forming unit
CIH	chronic intermittent hypoxia
CNS	central nervous system
CO_2	carbon dioxide
CSF	cerebrospinal fluid
CV	coefficient of variation
CVAH	cardiovascular acclimation *or* acclimatisation to hypoxia
CVDH	cardiovascular de-acclimation *or* de-acclimatisation from hypoxia
d	day(s)
DA	de-acclimation *or* de-acclimatisation
DBP	diastolic blood pressure

DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
EPO	erythropoietin (protein)
Epo	erythropoietin gene
ET	end-tidal
f_H	heart rate
F	fraction
g	gram(s) *or* units of gravitational force
G	gauge
GO	Gokyo village
GoRI	Gokyo Ri
h	hour(s)
HAH	haematological acclimation *or* acclimatisation to hypoxia
Hb	haemoglobin
HbA	haemoglobin A (adult form)
HbF	haemoglobin F (foetal form)
Hct	haematocrit
HCVR	hypercapnic ventilatory resposne
HDH	haematological de-acclimation *or* de-acclimatisation from hypoxia
HIF-1α	hypoxia-inducible factor (1α isoform)
HRV	heart rate variability
HVR	hypoxic ventilatory response
$_I$	inspired
IA	initial acclimation *or* initial acclimatisation
IH	intermittent hypoxia
iHVR	isocapnic hypoxic ventilatory response

ISH	intermittent sustained hypoxia
La	lactate
LLQ	Lake Louise Questionnaire
LTF	long term facilitation
MAP	mean arterial pressure
mCAFT	modified Canadian Aerobic Fitness Test
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
min	minute(s)
mo	month(s)
mRNA	messenger ribonucleic acid
NB	Namche Bazaar
NS	not statistically significant
O_2	oxygen
OD	optical density
P_{50}	partial pressure at which Hb is 50% saturated with O_2
P_{ATM}	atmospheric pressure
PBS	phosphate buffered saline
P_{CO_2}	partial pressure of carbon dioxide
PCR	polymerase chain reaction
pHVR	poikilocapnic hypoxic ventilatory response
P_{max}	maximal power output
P_{O_2}	partial pressure of oxygen
PV	plasma volume
qPCR	real time (quantitative) polymerase chain reaction

RA	re-acclimation or re-acclimatisation
RBC	erythrocyte count
RC	reticulocyte count
RER	respiratory exchange ratio
rHuEPO	recombinant human erythropoietin
RI	recovery index
RM ANOVA	repeated measures analysis of variance
RNA	ribonucleic acid
RPE	rate of perceived exertion
rpm	revolutions per minute
RSL	return to sea level
RT	reverse transcription *or* reverse transcriptase
s	second(s)
S_aO_2	arterial oxyhaemoglobin saturation, measured directly
SBP	systolic blood pressure
sd	standard deviation
se	standard error
SL	sea level
S_pO_2	arterial oxyhaemoglobin saturation, measured by pulse oximeter
sTfR	soluble transferrin receptor
$T_{<3000m}$	time (number of days) since last exposure above 3000 m
$T_{>3000m}$	time (number of nights) spent above 3000 m
UBC	University of British Columbia
UNBC	University of Northern British Columbia
$\dot{V}CO_2$	carbon dioxide production
$\dot{V}A$	alveolar ventilation

$\dot{V}_E$	expired minute ventilation
$\dot{V}_I$	inspired minute ventilation
$\dot{V}_{O_2}$	oxygen consumption
VAH	ventilatory acclimati on *or* acclimatisation to hypoxia
VDH	ventilatory de-acclimation *or* de-acclimatisation from hypoxia
$\dot{V}_{O_{2\text{-max}}}$	maximal oxygen consumption
W	watts
w	week(s)
WMRS	White Mountain Research Station
$\Delta_{altitude}$	percent change from BL to the average response measured across testing points at altitude

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CHAPTER 1. INTRODUCTION

In 2004 I spent several months travelling in Nepal and Tibet, experiencing for the first time the powerful effects of high-altitude exposure that I had learned about in undergraduate physiology classes. Following a 3-w trek in the Annapurna region of Nepal, I planned to spend a few days in the Kathmandu Valley before heading back into the mountains for another month of trekking and mountaineering in the Khumbu and Gokyo Valleys near Mount Everest. The plan was to minimise time spent at low altitude in an attempt to carry forward the altitude tolerance I had gained while acclimatising on the Annapurna Circuit. However, as a result of several factors (a sprained ankle, a nation-wide general strike, and a decision to walk an additional 6 d from Jiri to Lukla rather than spend $75 on a 30-min flight), more than a month had passed between leaving high altitude and arriving there again. En route to the Everest region, two questions came to mind. Would I still be somewhat acclimatised from the first trek or had I completely de-acclimatised while at low altitude for a month? Even if I had to re-acclimatise from scratch, would my body "remember" what it had recently done and respond more quickly during the second exposure? The answers were not in my Lonely Planet guidebook (Armington 2001). In fact, the answers were still elusive when I later consulted the scientific literature, and I realised I had stumbled upon a fascinating set of questions on which to base my Doctoral research.

1.1 Hypoxic acclimatisation

The high-altitude environment presents a severe physiological challenge in the form of hypobaric hypoxia. Oxygen lack at altitudes as low as 2000 m can cause dyspnoea, headache, sleep disturbances, nausea and loss of appetite. At higher altitudes, these symptoms of acute mountain sickness (AMS) can

be accompanied by high-altitude cerebral or pulmonary oedema. Fortunately, the risk of developing these conditions, which range from unpleasant to potentially fatal, can be virtually eliminated by ensuring a slow ascent rate to allow acclimatisation to take place (West 2004). Over time, exposure to hypoxia or altitude elicits a myriad of compensatory changes in haematology and respiratory, cardiovascular and renal physiology (Monge and Leon-Velarde 1991). This acclimatisation serves to enhance the transport of oxygen along the cascade from the environment to the working tissues, thereby partially compensating for the reduced atmospheric oxygen tension. The acclimatisation process has been studied rigorously in humans and other animals with immense clinical and academic interest in understanding the time course of acclimatisation for each organ system (Pugh 1962, Dempsey et al 1974, Schoene et al 1984, Cogo et al 1997, Houston 1997, Peacock and Jones 1997, Richalet et al 1999, Hupperets et al 2004).

The vast majority of research on acclimatisation to hypoxia has examined individuals with no prior or recent experience with the stimulus. However, in the Andes, the Himalaya and the Rocky Mountains many people alternate between extended periods at high altitude (hypoxia) and at sea level (normoxia) for work and recreation (West 1988, Gunga et al 1996, Richalet et al 2002a, Heinicke et al 2003). For trekkers and mountaineers, periods of acclimatisation to altitude are often interrupted by days, weeks or months in normoxic or less hypoxic conditions. Athletes may also repeatedly experience periods of intermittent hypoxic exposure through live-high-train-low programs designed to improve endurance performance (Richalet and Gore 2008, Stray-Gundersen and Levine 2008). The existing body of literature may not accurately reflect the acclimatisation process in altitude-experienced subjects and an understanding of how physiological responses to hypoxia may differ between initial and subsequent exposures is necessary.

1.1.1 Assessing acclimatisation status

Assessment of any number of variables related to ventilatory, cardiovascular, or haematological physiology can provide information about an organism's acclimatisation status. Among the most informative are a handful of variables used to assess integrative hypoxic compensation and general hypoxia tolerance.

One of the most commonly-assessed markers of acclimatisation status is the degree to which arterial oxygenation is protected in the face of reduced environmental O_2 availability. Arterial oxygenation can be measured as the partial pressure (P_aO_2), saturation (S_aO_2) or concentration (C_aO_2) of oxygen in arterial blood and serves as an integrative marker of hypoxic compensation brought about by complex changes in ventilatory control, blood flow distribution, gas exchange and more. Arterial oxygenation is attractive for use as a marker of acclimatisation because of its integrative nature but also because of the ease with which it can be indirectly measured. Although direct measures of P_aO_2, S_aO_2 or C_aO_2 are considered the gold standard and have been employed even near the top of Mt. Everest (Grocott et al 2010), the invasive nature of assessing arterial blood gases makes the measurement best-suited to laboratory experiments. However, indirect assessment of arterial oxyhaemoglobin saturation (S_pO_2) by pulse oximetry is simple, non-invasive and aptly suited for repeat (or continuous) assessment in field conditions. Although not without its problems (Ralston et al 1991), pulse oximetry has proven to be a acceptable proxy for direct measures and is used universally in both laboratory and field studies of hypoxic acclimatisation.

The degree to which an individual is functionally or clinically impacted by exposure to hypoxia is also indicative of hypoxia tolerance. Hypoxic tolerance improves with acclimatisation and is quantified by reductions in AMS symptoms and increases in submaximal exercise capacity. Again, these measures are both indicative of overall well-being, integrating the physiological response to hypoxia of virtually every organ system. The assessment of AMS is by brief questionnaire and clinical assessment (Hackett and Oelz 1992) and is limited to use in humans. Exercise capacity can be evaluated in both humans and other animals using standardised laboratory- or field-based tests to assess maximal or submaximal aerobic performance.

1.2 Hypoxic de-acclimatisation

A defining feature of the process of acclimatisation to high altitude (or any other stimulus) is that morphological and physiological compensatory changes are reversible upon removal of the hypoxic stimulus (Wilson and Franklin 2002). Mechanisms of de-acclimatisation (DA) do not necessarily involve a simple reversal of acclimatisation and the understanding of the time course of DA from high altitude is

far from complete. However, it appears that the majority of ventilatory and haematological and adjustments are normalised within weeks of leaving the hypoxic environment (see CHAPTER 2 for review).

1.3 Hypoxic re-acclimatisation

There are multiple claims that previous exposure to high altitude confers an advantage during subsequent re-exposure. Although no data were presented, Mani (1990) stated:

> We have abundant empirical field evidence in support of the favourable role of previous experience in rapid acclimatisation.

Furthermore, the same author claimed:

> The acclimatisation, which develops in sea-level man on the ascent of a high mountain, naturally disappears on his return to the plains. He is yet left with some kind of "memory" of high-altitude life he had experienced, so that during his next ascent he suffers much less acutely from high-altitude sickness than on his first ascent... He also becomes acclimatised *[sic]* sooner on the second occasion... ... it is also possible for him to reach a higher elevation.

Although the lack of data to support his assertions is dubious, Dr. Mani (a prolific high-altitude entymologist) had extensive experience travelling with research teams between high and low altitude, and his perception of improved re-acclimatisation (RA) apparently reflects those of prominent physiologists in the field of high-altitude medicine and biology. According to Ward and colleagues (2000c), there is a "strong impression" within the research community that individuals with previous experience at high altitude acclimatise better than those who are altitude-naïve, and that the ability to acclimatise is improved with each subsequent exposure (Hultgren 1997, Ward et al 2000b). In addition to these anecdotal reports, several researchers have presented empirical evidence that individuals with previous experience at altitude will have improved responses on subsequent re-exposure.

1.3.1 Evidence of improved acclimatisation in individuals with previous altitude experience

Milledge and colleagues (1983) compared acclimatisation profiles of men who had been recently and repeatedly exposed to high and extreme altitudes to those of men with no recent altitude experience. Although responses to altitude were similar in both groups, altitude-experienced subjects achieved ventilatory acclimatisation more rapidly than inexperienced subjects. Similarly, Koller and colleagues

(1991a) showed that subjects who had been acclimatised to >4000 m roughly three weeks earlier were able to achieve similar alveolar gas tensions without hyperventilating to the same extent as altitude-inexperienced subjects. The former group also showed reduced oxygen consumption and a less marked activation of the cardiovascular system, suggesting an overall more energetically-economical response to acute hypoxia in altitude-experienced subjects. Recent altitude exposure is also associated with reduced AMS, increased mountaineering success and faster climbing times during subsequent expeditions (Bircher et al 1994, Schneider et al 2002, Pesce et al 2005, Tsianos et al 2006) but these associations are not always present (O'Connor et al 2004). However, individuals who experience significant illness, substantially impaired exercise performance and poor climbing success are unlikely to return to high altitude on subsequent expeditions. As such, individuals with repeated previous exposure to altitude likely represent a self-selected group that is more likely to escape clinical and functional impairment at altitude. Hence, evidence for improved hypoxic RA from cross-sectional studies should be interpreted with caution.

1.3.2 Evidence of improved responses to acute hypoxia in individuals with recent exposure

Others have used longitudinal studies to compare responses to acute hypoxic exposure before and after a period of acclimatisation to high altitude. Following 16 d at 4300 m, participants exhibited higher S_pO_2, haemoglobin concentration ([Hb]), and haematocrit (Hct) and reduced AMS scores during re-exposure after 8 d DA (Lyons et al 1995). Following 18 d at 4300 m, submaximal exercise during acute re-exposure elicited reduced heart rate (f_H) and blood lactate concentration ([La]) and increased S_aO_2, indicating substantial retention of acclimatisation after 8 d DA (Beidleman et al 1997). About 3 d after an expedition to Mt. Everest (8848 m), climbers exhibited higher resting and exercise S_pO_2 in acute hypoxia, as compared to during hypoxic exposure before acclimatisation (Savourey et al 1994). Following a different expedition to Mt. Everest, climbers exhibited increased minute ventilation ($\dot{V}_E$) and increased O_2 extraction upon hypoxic re-exposure after 5 d DA (Savourey et al 1996). The same research group studied the responses to acute hypoxic exposure before and after a 3-w expedition in the Andes (up to 6768 m). Climbers exhibited elevated [Hb], Hct, resting S_pO_2 and C_aO_2 and decreased P_aCO_2 in acute hypoxia for up to 1 w after the expedition (Savourey et al 2004). Compared to before the expedition,

hypoxic exercise f_H was reduced, and S_pO_2 and muscle oxygenation were increased 1 mo after a climb to 8032 m (Usaj and Burnik 2009, Usaj 2010).

Clearly, there are multiple reports of improved altitude tolerance during acute re-exposure to hypoxia indicating that previous acclimatisation status is partially retained for at least several days following return to sea level. It is possible that returning to high altitude while still partially acclimatised would provide a head-start on the acclimatisation process, but these studies did not involve hypoxic re-exposures of sufficient duration to examine changes in the process itself.

1.3.3 Evidence of progressive acclimatisation with repeated exposure to high altitude

Several clinical and experimental models of intermittent hypoxia have been examined extensively. Many paradigms exist with alternating exposure to hypoxia and normoxia over minutes (as in sleep-disordered breathing), hours (as in live high-train low athletes), days (as in workers at high-altitude mines and observatories), weeks (as in mountaineers), or months (as in the military or seasonal altitude workers). Although the literature seems to have adopted the blanket term of chronic intermittent hypoxia (CIH) to describe these exposures, the physiological responses to different paradigms are highly varied. Of most relevance to my research questions are models that involve exposures of days, weeks or months. It is my personal preference that such exposure paradigms are referred to as intermittent exposure to sustained hypoxia (ISH) to distinguish from the much-studied model of CIH that characterises the intermittent hypoxaemia of sleep-disordered breathing. Apart from the obvious difference in the time domains of exposures, another key difference between these paradigms is that hypercapnia accompanies the intermittent hypoxaemia of sleep-disordered breathing while other models of ISH elicit hypocapnia. The physiological implications of this discrepancy on ventilatory control, and cerebral and peripheral vascular regulation are substantial (reviewed in Dempsey et al 2010).

There are several reports of progressive acclimatisation in individuals who commute between high and low altitude for work. After 1 d at altitude, Chilean soldiers exposed to ISH (11 d at 3550 m, 3 d at SL) for 6 mo had a lesser reduction in $\dot{V}O_{2\text{-max}}$ and a higher [Hb] and C_aO_2 than altitude-naïve soldiers

(Prommer et al 2007). In workers at the Collahuasi mine in northern Chile, both $\dot{V}_E$ and S_pO_2 were increased during exercise after 12 mo ISH (7 d at 3800-4600 m, 7 d at SL; Richalet et al 2002a). After 4 d at 4500 m, another group of mine workers exposed to ISH (7d at 4500 m, 7 d at SL) for 36 mo had reduced altitude illness and resting f_H, and increased resting and exercise S_pO_2 compared to those exposed for the first time (Farias et al 2006). Workers on the Qinghai-Tibet railroad also showed progressive decreases in the incidence and severity of AMS and increases in resting S_pO_2 across 5 y of ISH (7 mo at 4500 m, 5 mo at SL; Wu et al 2009). As with research presented in the previous section, these findings suggest that hypoxia tolerance is partially retained from one exposure to the next, and progressive improvements are made possible because the acclimatisation process has a head start with each re-exposure. Timing of shift changes is critical to determining whether altitude tolerance will be retained. AMS scores in observatory staff on Mauna Kea are reduced during altitude shifts (5 d at 2800-4200 m) that are separated by 5 d at SL but not 45 d (Brown 1989). In Canadian mine workers in Kyrgyztan, S_pO_2 at altitude was not improved after 1 y of ISH (4 w at 3700-4200 m, 4 w at SL; Sarybaev et al 2003). However, even with brief DA periods, the finding of improved hypoxia tolerance with ISH is not universal. In Chilean mine workers, re-exposure resulted in substantial AMS even after at least 12 y of ISH (4 d at 3550 m, 3 d at SL; Brito et al 2007) and a longitudinal study showed no reduction in AMS scores across 2.5 y of ISH (7 d at 3800-4600 m, 7 d at SL; Richalet et al 2002a).

The retention of previous acclimatisation status is also likely responsible for progressive increases in [Hb] and Hct that are reported over time in long-term ISH, since DA periods are too short to allow haematological variables to normalise before re-exposure to altitude (Richalet et al 2002a, Heinicke et al 2003, Siques et al 2006). However, a normal erythropoietic response occurs with each re-exposure to high altitude after 5 y ISH (10 d at 3600-4500 m, 4 d at SL; Gunga et al 1996), 6 mo ISH (11 d at 3550, 3 d at SL; Heinicke et al 2003) and 22 y ISH (3.5 d at 3550, 3.5 d at SL; Heinicke et al 2003). Thus, despite the progressive improvement in hypoxia tolerance reported in several studies, these studies provide no evidence of changes in the acclimatisation process itself.

1.3.4 Evidence that prior hypoxic exposure fundamentally changes the acclimatisation process

To date there has been no direct comparison of the time course and magnitude of compensatory changes that occur during an initial acclimatisation (IA) versus RA to high altitude. However, data from two studies of acclimation and re-acclimation to intermittent hypoxia suggest that the processes of IA and RA might be different.

Katayama and colleagues (2005a) compared the profiles of ventilatory changes during IA and RA to an IH paradigm (1 $h \cdot d^{-1}$ at ~4700 m for 10 d) separated by 30 d DA. Significant differences were reported between the first and second series of exposures, with increased $\dot{V}E$ and S_pO_2, and decreased end-tidal CO_2 ($P_{ET}CO_2$) in the first few days of the RA series. However, decreased $P_{ET}CO_2$ and increased S_pO_2 were evident on the first day of re-exposure, again suggesting that differences between IA and RA were driven by retention of previous acclimation and not by changes in the acclimation process.

The same cannot be said about a study of haematological acclimation and re-acclimation to IH. In rabbits exposed to IH (6 $h \cdot d^{-1}$ at ~6000 m for 30 d), Jain and colleagues (1978) reported a larger [Hb] response that occurred more rapidly during RA than IA following 30 d DA, despite normalisation of [Hb] before re-exposure. The increased haematological response in RA was attributed to a greater haemoconcentration, which was implicated in the death of 25% of animals during the second series of exposures. No deaths occurred during IA, leading the authors to conclude that re-exposure to hypoxia "imposes a more severe stress than experienced during acclimation *[sic]*". It is unclear whether this detrimental response is unique to the paradigm of intermittent hypoxia examined; re-exposure to acute and chronic sustained hypoxia has elicited both increases (Singh et al 1988, Savourey et al 2004) and decreases (Singh et al 1990, Lyons et al 1995) in plasma volume relative to an initial exposure. Thus the effects of previous acclimatisation on the haemoconcentration component of RA remain unclear.

There is more consistent evidence that the erythropoietic component of haematological acclimatisation is altered by previous hypoxic exposure. In two separate experiments, Savourey and colleagues reported a blunted [EPO] response to hypoxia after an extended expedition in the Himalayas (1998) and the Andes

(2004). However, there is evidence of increased sensitivity of erythroid precursors to the hormone indicated by an increased reticulocyte response post-expedition, despite reduced [EPO] (Savourey et al 2004). Much earlier work also supports an increased sensitivity to EPO following hypoxic exposure; the erythropoietic response to exogenous EPO was greater in mice made similarly polycythaemic by hypoxic exposure versus transfusion (Okunewick et al 1969, Okunewick and Fulton 1970).

The effects of changes in erythropoietic responses to acute hypoxia have not been followed through and observed during acclimation or acclimatisation to a more lengthy hypoxic exposure. However, Wu and colleagues (2009) stated that [Hb] was consistently higher in ISH than altitude-naïve workers, even though [Hb] returned to baseline during the 5 mo DA period at SL. Unfortunately no data were presented and no mechanisms examined; nonetheless this report provides the best available evidence that the process of haematological acclimatisation is fundamentally altered by previous hypoxic exposure.

1.3.5 Mechanisms of improved hypoxia tolerance during re-exposure

Though results are not universal, there is a relatively consistent finding that previous exposure to high altitude leads to greater hypoxic compensation and improved clinical and functional outcomes during re-exposure. It appears there are two basic processes by which previous hypoxic exposure might confer an advantage during re-exposure. Although these processes likely operate on slightly different time scales, there may be overlap and they are not necessarily mutually exclusive. Available data to support each potential mechanism is summarised below.

1. If a relatively short amount of time has passed since the previous hypoxic exposure, some degree of the acclimatised phenotype is retained. The amount of retention likely depends on the degree of initial acclimatisation and the duration of the DA period before re-exposure. The retention of altitude tolerance from previous acclimatisation has been demonstrated by improved physiological responses to acute re-exposure (Lyons et al 1995, Savourey et al 1996, Beidleman et al 1997, Savourey et al 2004) and better functional outcomes during sustained re-exposure (Bircher et al 1994, Schneider et al 2002, Pesce et al 2005, Tsianos et al 2006). Upon re-exposure to high altitude, recently-acclimatised individuals seem to

have a head start on the acclimatisation process (Richalet et al 2002b, Wu et al 2009). Several models of ISH lead to progressive increases in hypoxic compensation and altitude tolerance over months and years of repeated exposure. This "head start" phenomenon clearly contributes to improved hypoxia tolerance during re-exposure, but it is not known how long acclimatisation status is retained during de-acclimatisation. In addition, it is not clear whether the head start phenomenon can account for all incidences of improved hypoxia tolerance in individuals with previous experience at high altitude.

2. It is possible that the process of hypoxic acclimatisation might be fundamentally altered by previous exposure, even if sufficient time has passed to allow all aspects of the acclimatised phenotype to return to baseline before hypoxic re-exposure. The process of acclimatisation demonstrates that some physiological responses to hypoxia are not fixed but are flexible, or plastic. The idea that acclimatisation to hypoxia may be altered in individuals who have acclimatised in the past suggests that the process itself might be plastic. This concept of plastic plasticity, or metaplasticity, has been described primarily in the neuroscience literature and is demonstrated when an initial experience with a stimulus alters the response's capacity to show plasticity with subsequent perturbations (Abraham and Bear 1996, Byrne 1997, Kim and Yoon 1998, Abraham 1999, Mitchell and Johnson 2003). It has been suggested that metaplasticity could also apply to other systems and integrated processes like acclimatisation (Powell and Garcia 2000) and might account for improved hypoxic re-acclimatisation over the longer term. There is limited evidence that some aspects of re-acclimation to IH might be both improved and impaired relative to an initial acclimation (Jain et al 1978, Katayama et al 2005a). However, to date there has been no assessment of the process of re-acclimatisation to sustained hypoxia. The potential role of metaplasticity in effecting improved hypoxia tolerance during extended re-exposure to high altitude is unknown.

1.4 Research objectives and investigative approaches

The majority of relevant works have examined the effects of previous altitude exposure on acute responses to hypoxia or on functional outcomes, hypoxia tolerance and physiological acclimatisation status at a single point during extended re-exposure. The effects of prior exposure on the actual process of

acclimatisation - the time course, magnitude and mechanisms of compensatory adjustments – remain unexplored. Therefore, the overarching questions guiding my Doctoral research were:

- Is the process of hypoxic acclimatisation facilitated by previous exposure to the stimulus?
- If so, is improved re-acclimatisation mediated by the retention of previously-acquired hypoxia tolerance?
- Or, is the process of acclimatisation fundamentally altered by previous exposure hypoxia?

The primary research objective was to explore these questions by conducting the first direct comparison of the processes of acclimatisation and re-acclimatisation to sustained hypoxia.

A secondary objective was to explore the time domains of facilitated re-acclimatisation by addressing the following questions:

- How long is hypoxia tolerance retained following acclimatisation?
- Is the acclimatisation process altered by previous hypoxic exposure, even after all aspects of the acclimatised phenotype have been completely reversed?

A tertiary objective was to explore other contributing factors:

- Are there non-physiological explanations for improved altitude tolerance following previous acclimatisation to hypoxia?

A number of diverse approaches were undertaken to address the above research questions. Specific hypotheses are outlined in each research chapter.

- The processes of ventilatory and haematological DA from hypoxia were thoroughly examined. Data collected as part of my Doctoral research were incorporated with previously published data to create the only known review of the timelines and mechanisms of hypoxic DA. Implications for hypoxic RA were considered. This review can be found in CHAPTER 2.

- A comprehensive set of experiments were designed to examine the process of RA following short- and longer-term DA. Functional hypoxia tolerance and physiological compensation for hypoxia were to be examined to determine whether RA was facilitated by previous acclimatisation. Typical assessment of ventilatory, cardiovascular and haematological acclimatisation was to allow comparisons between IA and RA and reveal potential mechanisms of improved RA. Furthermore, early acclimation responses to hypoxic re-exposure were also to be examined following DA periods of up to a year. This project was approved by my Supervisory Committee and preparation occupied much of the first 2.5 y of my Doctoral program. Unfortunately, this proposed work was cancelled for logistical reasons during the early stages of data collection. Details of the proposed methodology can be found in APPENDIX I.

- A cross sectional study of high-altitude trekkers was conducted to build on earlier findings of improved functional outcomes in individuals with previous altitude exposure. Here, the effects of prior altitude exposure on physiological acclimatisation status were also evaluated. Furthermore, attempts were made to correlate the dose of altitude pre-exposure with functional and physiological markers of acclimatisation. Results of this study are discussed in CHAPTER 3.

- A group of high-altitude trekkers were intensely monitored as they repeated the same 10-d trek from Lukla to Gokyo Ri (5340 m) separated by a 10-d DA period in Kathmandu (1300 m). This data set allows the first direct comparison of the time course and magnitude of compensatory changes throughout IA and RA. This novel experiment is presented in CHAPTER 4.

- A comprehensive experiment to investigate the process of IA, DA and RA under controlled laboratory conditions was designed. This investigation was to compare physiological and functional hypoxia tolerance in rats exposed and re-exposed to normobaric hypoxia and explore the physiological mechanisms underlying the integrative responses throughout IA and RA. Unfortunately, after ~6 mo of study design and preparation, this project was also abandoned in the very late stages of planning. Details of the proposed methodology can be found in APPENDIX II.

- The haematological responses to IA, DA and RA were examined under controlled laboratory conditions using a mouse model. Collected data allow the first detailed comparison of the processes of haematological acclimation and re-acclimation to sustained hypoxia. Furthermore, the durations of IA and DA were manipulated to investigate the effects on the time course and magnitude of responses during RA. These experiments are presented in CHAPTER 5.

- Mountaineers and mountain guides with extensive experience at very high altitude were interviewed about their perceptions of the acclimatisation process and whether or not it has changed over years of repeated exposure. These conversations instigated an investigation of the substantial contribution of non-physiological factors on real and perceived altitude tolerance. In particular, the role of psychological altitude tolerance in determining outcomes during hypoxic RA is discussed in CHAPTER 6.

As part of the research process, a number of methodological studies were undertaken, either to aid in selecting data collection methods for my Doctoral research, to assist in interpretation of dissertation results or to improve methodologies for future studies. Two of these studies are included in the dissertation to demonstrate the rigour of the above investigations.

- The use of evacuated blood collection tubes at high altitude can be problematic and potentially dangerous. The performance of Vacutainer® tubes was evaluated at a range of altitudes in order to develop recommendations to clinicians and researchers about their use at altitude (APPENDIX III).

- Hypoxic exercise testing is a routine part of both field studies of acclimatisation and laboratory studies of acclimation. A cycling challenge was designed that could be administered repeatedly without eliciting a training response and that would provide repeatable measures of cardiorespiratory and metabolic responses to hypoxic exercise. Details of the resultant exercise protocol and data on the intra-individual repeatability of exercise responses are presented in APPENDIX IV.

1.5 Notes to the reader

1.5.1 Acclimation vs. acclimatisation

Hypoxic acclimation and acclimatisation represent physiologically equivalent processes involving the time-dependent acquisition of hypoxia tolerance brought about by numerous temporary physiological adjustments (Wilson and Franklin 2002). Acclimatisation refers to the process when it occurs in a human or other animal that is naturally exposed to the hypoxia of high altitude. An individual who is chronically exposed to intermittent or sustained hypoxaemia due to a clinical condition also undergoes acclimatisation to the pathological stimulus. Conversely, acclimation refers to the process as it occurs during artificially exposure to hypoxia. In most studies of acclimation, altitude is simulated using a hypobaric chamber or with mixtures of low oxygen gas. Acclimation and acclimatisation are often misused in the scientific literature and the distinction is not always entirely clear. When humans or other animals are transported to a high-altitude field station for experimental purposes do they undergo acclimation or acclimatisation? Similarly, when climber-scientists ascend the highest peaks in the world for the purpose of obtaining blood gas measurements, are they acclimated or acclimatised? Although an excellent topic for a debate of semantics, the label is physiologically irrelevant. Therefore, throughout the dissertation, exposure to natural altitude is considered to elicit acclimatisation regardless of the reason for the exposure. Exposure to simulated altitude is discussed as eliciting acclimation. Every attempt is made to correctly characterize a hypoxic exposure but when studies of acclimation and acclimatisation are considered together or the conditions of an exposure unclear, the term acclimatisation is used as default.

1.5.2 Equivalency of hypoxic exposures

Throughout the dissertation, all hypoxic exposures are described in terms of equivalent terrestrial elevation for the purpose of simplifying comparisons across studies. Wherever necessary, hypobaric exposures were converted by solving the formula given by West (1996):

$$P_B \text{ (Torr)} = \exp(6.63268 - 0.1112h - 0.00149h^2)$$

for h, where h is altitude in km.

For exposures to hypoxic hypoxia, fractions of inspired oxygen (F_IO_2) were first converted to P_B using the formula:

$$P_B = 47 + 713*(F_IO_2/0.2093)$$

before converting P_B to equivalent terrestrial altitude using West's model.

These conversions are not accurate for a few reasons. First, P_B at any terrestrial altitude depends on geographic location; at a given altitude, P_B will be higher when closer to the equator and lower when closer to the poles. Thus, altitude cannot be calculated from P_B without taking latitude into consideration. However, changes in P_B with the season and weather patterns are of similar magnitude to discrepancies with latitude, making it erroneous to assume P_B from altitude when current P_B values are not given. Furthermore, although it has long been argued that a given P_IO_2 is physiologically equivalent regardless of whether the hypoxic stimulus is hypo- or normobaric, a growing body of literature suggests that this is not the case and that hypobaria may represent a more severe physiological stimulus (reviewed in Conkin and Wessel 2008). Although research is underway to develop a method for determining physiologically equivalent normo- and hypobaric exposures, no adequate model is currently available. Thus there is no correct way to express all hypoxic exposures in the same units. However, the majority of data discussed in this dissertation are not substantially affected by small changes in P_B and most comparisons are made within (and not between) studies where hypoxic severity is always expressed in the same manner. Thus, to simplify general comparisons between studies for the reader, all hypoxic exposures have been converted to approximate equivalent terrestrial altitude. Throughout the dissertation, any exposure to simulated hypobaric or hypoxic hypoxia is indicated by preceding the approximate altitude with ~ (i.e. ~4300 m).

CHAPTER 2. VENTILATORY AND HAEMATOLOGICAL DE-ACCLIMATISATION FOLLOWING HYPOXIC EXPOSURE: A COMPREHENSIVE REVIEW OF TIMELINES AND MECHANISMS

2.1 Introduction

Hypoxic acclimatisation is a multifaceted process involving countless structural and functional changes in the respiratory (Bisgard and Forster 1996), haematological (Grover and Bärtsch 1996), and cardiovascular systems (Mirrakhimov and Winslow 1996). Acclimatisation is defined by its reversibility (Wilson and Franklin 2002) and when the hypoxic stimulus is removed, compensatory adjustments and hypoxia tolerance are lost through the process of de-acclimatisation (DA). Compared to the vast body of work examining the physiological responses to acute and sustained hypoxia, the process of hypoxic DA has received little attention. This is reflected in the following statements, taken from the recent literature:

> The effect of acclimatisation probably falls off exponentially with time over perhaps 2-3 weeks, though some feel there is some residual benefit even after months at sea level. (Ward et al 2000c)

> Furthermore, the surprisingly large protective carry-over effect from previous exposures observed in this study highlights our lack of knowledge regarding de-acclimatisation. (Bärtsch et al 2004)

> The same characteristics return toward pre-expedition values with different, mostly unknown, dynamics than during acclimatization. (Usaj 2010)

> The rate at which altitude de-acclimatisation occurs has not been well-studied. (Muza et al 2010)

In fact, many data have been collected about the time course and mechanisms of DA, either as a primary research objective or (more commonly) as a secondary objective during a study of acclimatisation. Thus, the above statements do not reflect a lack of data about hypoxic DA; rather, they reflect the fact that the substantial accumulation of DA data has yet to be assembled and synthesised. Therefore, the primary objective of this review was to conduct a comprehensive survey of the DA literature and compile available data to:

1) describe the time course of hypoxic DA

2) examine potential mechanisms of hypoxic DA

3) explore potential implications of the time course and mechanisms of DA for subsequent re-acclimatisation to hypoxia

This review will focus on the process of hypoxic DA in the human but will be heavily supplemented with available data from various animal models. Wherever possible, the reader will be referred to reviews and syntheses of the current knowledge of hypoxic acclimatisation.

2.1.1 Why study de-acclimatisation?

In addition to theoretically complementing the current appreciation of hypoxic acclimatisation, understanding the process of DA has practical relevance to many interest groups. Hypoxaemia can be induced by environmental (high altitude) or pathological (cardiovascular or respiratory disease) conditions and normoxaemia can be restored when an individual returns to sea level (SL) or has a medical condition effectively treated. In fact, the hypoxaemic stimulus does not have to be completely eliminated in order for DA to occur; return to a lower altitude or partial correction of pathology will also result in the loss of acclimatory changes over time. Thus, DA most obviously affects those who alternate between extended periods at higher and lower altitudes. Even within a single expedition, mountaineers might ascend and descend several times in an attempt to maximise acclimatisation and minimise high-altitude deterioration (Powell and Garcia 2000). Though rarely discussed in the literature, an appreciation for the time course of DA can be just as critical in designing safe and effective acclimatisation schedules as ensuring that ascent rates are adequately slow. Military personnel (Singh et al 1990, Heinicke et al 2003), high-altitude porters (Basnyat and Litch 1997), and workers in high-altitude mines (Gunga et al 1996, Richalet et al 2002a) and research stations (Powell and Garcia 2000) also commonly commute between high and low altitudes, partially acclimatising and de-acclimatising with each shift change. For these groups, a better understanding of DA could inform trip-planning and shift-scheduling to improve success, productivity, health and safety.

DA also occurs following intermittent exposure to hypoxia. Endurance athletes commonly incorporate a live high-train low program whereby they spend 8–20 h·day^{-1} in a hypoxic environment and complete high intensity training in normoxic (or less hypoxic) conditions (Stray-Gundersen and Levine 2008). For these individuals, the concern is not with the DA of hypoxia tolerance per se; rather, the goal is to design training schedules such that the aspects of hypoxic acclimatisation that improve SL performance will be retained until race day. In mountaineers, recent acclimatisation improves climbing performance and clinical outcomes during subsequent expeditions (Richalet et al 1992, Schneider et al 2002, Tsianos et al 2006). As a result, much work has been dedicated to the development of pre-acclimatisation protocols for use before sojourns to high altitude (Benoit et al 1992, Savourey et al 1994, Beidleman et al 2004). Intermittent hypoxia (IH) paradigms involving exposures ≥1.5 h·day-1 at ≥4000 m for ≥6 d can effectively elicit ventilatory and haematological acclimatisation (reviewed in Muza 2007). However, given the limited understanding of DA following IH, Muza and colleagues (2010) recommended that travel to high altitude occurs "as soon as possible" after completing a pre-acclimatisation protocol.

A number of pathological conditions also result in various paradigms of chronic intermittent hypoxia (CIH) exposure in those afflicted. Individuals with sleep apnoea can experience hundreds of hypoxaemic episodes each night (Chiang 2006) and, in cardiovascular (Mortara et al 1996) and respiratory disease (Weitzenblum and Chaouat 2001), individuals who are chronically hypoxaemic intermittently become even more hypoxaemic upon exertion or with disease exacerbation. While it is known that the effects of sustained pathological hypoxaemia can be reversed with treatment (Dammann et al 1961, Ramirez et al 1968, Kobayashi et al 1994), DA following pathological CIH has not been investigated.

Finally, an improved understanding of DA could better inform study design for researchers investigating the physiological effects of hypoxic exposure. A number of studies have assumed that 2 mo DA should allow a complete reversal with so much confidence that measurements of minute ventilation ($\dot{V}_E$) and blood flow taken post-exposure are assumed to represent pre-exposure values (Dempsey et al 1979, Jansen et al 2002). Similarly, Hansen and Sander (2003) collected "baseline" data 4-6 months post-exposure for comparison with changes in sympathetic activation during acclimatisation. However,

without thoroughly appreciating the time course and mechanisms involved in DA, such risky assumptions could have a profound influence on the interpretation of results.

2.1.2 De-acclimatisation: a time-dependent process

Like acclimatisation, DA occurs in a time-dependent manner; acclimatory changes and acquired hypoxia tolerance are not lost immediately upon return to sea level (RSL) but disappear gradually over time. Several researchers have made anecdotal observations that hypoxia tolerance is retained for some time after RSL following acclimatisation to high altitude. During the Silver Hut Expedition, Pugh (1962) observed that after spending several months at 5790 m, altitude tolerance was not impaired in an individual who de-acclimatised for 2 w at low altitude before returning to high altitude. However, two other individuals forfeited all acquired altitude tolerance after a 3-mo DA period at low altitude. Although altitude tolerance was assessed subjectively in the above cases, other works have objectively evaluated hypoxia tolerance during DA. This has been accomplished by assessing arterial oxygenation and severity of acute mountain sickness (AMS) during hypoxic re-exposure after various durations of DA.

Following a 50-d expedition to Mt. Everest (8848 m), Savourey and colleagues (1996) reported that arterial oxyhaemoglobin saturation (S_aO_2) was higher during both hypoxic rest and exercise at 5 d but not at 1 mo RSL, suggesting that hypoxia tolerance was lost some time between these testing points. Following a slightly shorter expedition (41 d at altitudes up to 7600 m), arterial oxygenation during hypoxic exercise was no better compensated at 7-12 d RSL than before acclimatisation (Boning et al 2001). Following 18 d at 4300 m, the acclimatisation-induced improvement in S_pO_2 during hypoxic exercise was retained 65-92% after 8 d, suggesting that substantially more time would be needed for hypoxia tolerance to be completely lost (Beidleman et al 1997). Indeed, Usaj and Burnik (2009) reported that S_pO_2 in hypoxic exercise was still elevated 1 mo after an expedition to 8032 m. Lyons and colleagues (1995) reported a reduction in AMS scores during hypoxic exposure at 8 d RSL compared to before an acclimatisation period of 16 d at 4300 m. However, AMS scores were similarly high in the first few days of every high-altitude shift in mine workers who alternated between SL and 4500 m every 7 d for 2.5 y (Richalet et al 2002a). In fact, AMS remained a regular occurrence at the beginning of high-altitude shifts

even after at least 12 y of alternating between 4 d at 3550 m and 3 d at SL (Brito et al 2007).

Clearly, hypoxia tolerance is partially retained after leaving the high-altitude environment but the duration and degree of its persistence is not consistent. Muza and colleagues (2010) postulated that hypoxia tolerance will endure for a period that is proportional to the degree of acclimatisation acquired during the initial exposure. However, improved hypoxia tolerance is a highly integrative measure brought about by a myriad of underlying physiological changes. A more thorough understanding of functional DA could be accomplished by examining the time courses and mechanisms underlying the loss of distinct acclimatory changes. Thus, the bulk of this review will consist of a comprehensive examination of what is known about DA of the ventilatory and haematological responses to hypoxia, arguably the two most important aspects of hypoxic acclimatisation (Bisgard and Forster 1996, Grover and Bärtsch 1996).

2.2 Ventilatory acclimatisation and de-acclimatisation

The process of ventilatory acclimatisation to hypoxia (VAH) is characterised by progressive increases in $\dot{V}_E$ resulting in increases and decreases in the partial pressure of arterial, alveolar and end-tidal oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}), respectively (Bisgard and Forster, 1996). These changes are brought about by increases in ventilatory chemosensitivity to both hypoxia and hypercapnia, involving structural and biochemical changes at the central and peripheral chemoreceptors. VAH has been widely investigated and comprehensive reviews of the process, its time domains and underlying mechanisms are available (Bisgard and Forster, 1996, Schoene, 1997, Powell et al, 1998, Lahiri et al, 2000, Smith et al, 2001, Duffin and Mahamed, 2003, Powell, 2007). Ventilatory DA from hypoxia (VDH) is defined as the persistent hyperventilation that continues beyond removal of the hypoxic stimulus (Bisgard and Neubauer 1995). Although few investigations have examined VDH as a primary objective, numerous studies of VAH have included assessment of respiratory function on return to normoxia. Thus VDH has been repeatedly demonstrated in humans, other mammals (Olson and Dempsey 1978 - rat, Dempsey et al 1979 - pony, Smith et al 1986 - goat) and birds (Powell et al 2004 - duck).

2.2.1 Time course of ventilatory de-acclimatisation

2.2.1.1 Hyperventilation

During VDH, above-normal $\dot{V}_E$ is accompanied by increases in P_{O_2} and decreases in P_{CO_2} relative to pre-exposure. P_{CO_2} reflects the relationship between metabolic rate and alveolar ventilation ($\dot{V}_A$) and has frequently been used to quantify ventilatory acclimatisation (Bisgard and Forster 1996). The progress of VDH is also better defined by the normalization of P_{CO_2} (rather than $\dot{V}_E$ itself), as illustrated by data from Dempsey and colleagues (1979 - pony). Following 36 h at 4300 m, $\dot{V}_E$ returned to baseline (BL) within 1 h RSL, but $\dot{V}_A$ was elevated for at least 24 h due to a reduction in dead space ventilation following hypoxia. Since P_{CO_2} reflects effective ventilation, the timeline of VDH is examined in terms of P_{CO_2} wherever possible and supplemented with available $\dot{V}_E$ data. VDH is considered complete when these variables have returned to pre-exposure values.

Insight into VDH can be gained from data collected a century ago. Ward (1908) demonstrated in one individual that $P_{ET}CO_2$ was below normal immediately upon RSL but had returned to BL within 1.5 d RSL following 7 d at 4530 m. In a second individual $P_{ET}CO_2$ approached BL values only after 6 d RSL. In a subsequent study, an individual who had been spending six months each year at 4300 m was monitored for several months following descent to 1800 m. Although no pre-exposure data were available, P_ACO_2 continued to increase upon RSL for over two months before plateauing at a normal value of 37-39 mmHg (Schneider 1913). Although anecdotal in nature, these reports provided a framework for the later development of ideas about VDH.

A comprehensive list of studies that report resting P_{CO_2} or $\dot{V}_E$ data during VDH is presented in TABLE 2.1. Each study's contribution to understanding the timeline of VDH depends on the timing of post-exposure measurements. In several cases, investigations were terminated before P_{CO_2} or $\dot{V}_E$ returned to BL values, and in others hyperventilation had ceased by the time of first post-exposure measurement. Each of these studies identifies either a lower (i.e. Goldberg et al 1992: > 1 h) or upper limit (i.e. Singh et al 2003: < 7 d) of the time required for complete ventilatory de-acclimatisation. However, the most informative works included multiple post-exposure measurements that bookended the return to BL,

TABLE 2.1 Summary of studies that measured resting normoxic minute ventilation ($\dot{V}E$) or arterial (P_a), alveolar (P_A) or end-tidal (P_{ET}) CO_2 tension during de-acclimatisation from hypoxia. Unless otherwise noted, studies used human subjects. For laboratory exposures to hypobaric or hypoxic hypoxia, the estimated equivalent terrestrial elevation is given in parentheses.

Reference	Exposure	Variable	VDH Time	Comments
Ward 1908	7 d at 4500 m	$P_{ET}CO_2$	<1.5 d or >5 d	2 individuals assessed separately
Schneider 1913	6 mo at 4300 m	P_ACO_2	~1 mo	n = 1; time to plateau (no BL data)
Houston and Riley 1947	32-d simulated ascent to 6700 m	P_aCO_2	> 4 d	33% below BL at final test
Astrand 1954	5 d at 4000 m	P_ACO_2	>7 d	n = 1, 5% below BL at final test
Forster et al 1971	45 d at 3100 m	$P_{ET}CO_2$	< 7 d	
Olson and Dempsey 1978	14 d at 4300 m	P_aCO_2	< 24 h	rat, measured in hyperoxia
Dempsey et al 1979	3-5 d at 4300 m	P_aCO_2	12 – 24 h	
Dempsey et al 1979	36 h at 4312 m	P_aCO_2	12 – 24 h	pony
Masuyama et al 1986	2.5-mo expedition to Kanchenjunga, max 7800-8586 m	P_aCO_2	> 35 – 40 d	10% below BL at final test
Goldberg et al 1992	7 d at 447 mmHg (4267m)	P_aCO_2	> 1 h	26% below BL at final test
Sato et al 1992	5 d at 3810 m	$P_{ET}CO_2$	3 – 5 d	
Malconian et al 1993*	40-d simulated ascent of Everest (8848 m)	P_ACO_2	> 3 d	28% below BL at final test
Sato et al 1994	12 d at 3810 m	$P_{ET}CO_2$	> 4 d	9% below BL at final test
Savouery et al 1996	41 or 62-d expedition to Everest (8848 m)	$P_{ET}CO_2$	< 5 d	
Boning et al 1997	40 d expedition to Broad Peak (max 7600 m)	P_aCO_2	> 10-11 d	10% below BL at final test
Robach et al 2000	7 d at 4350 then 3-d simulated ascent of Everest (8848 m)	$\dot{V}E$	< 1 - 3 d	
Bhaumik et al 2003	10-d trek to 5500 m	$P_{ET}CO_2$	< 4 – 5 d	
Hansen and Sander 2003	4 w at 5260 m	P_aCO_2	<3 d	
Singh et al 2003	60 d at 3500 m then 70 d at 5800 m	P_aCO_2	< 7 d	
MacNutt et al (CHAPTER 4)	10-d trek to 5360 m	$\dot{V}E$	< 3 d	

*Minute ventilation data reported in Schoene et al. 1990.

identifying both an upper and lower limit for the time required for complete VDH (Dempsey et al 1979: 12-24 h, Sato et al 1992: 3-5 d).

VDH was examined following hypoxic exposures of very different durations (1.5 to 130 d) and severities (3100 to 8848 m). These exposures elicited hyperventilatory responses during VAH such that $\dot{V}_E$ or P_{CO_2} changed 18 (Forster et al 1971) to 150% (Schoene et al 1990) from BL to the end of exposure. The majority of studies found that these changes reversed back to BL within 1 w RSL, with complete VDH as early as 12-24 h following a 3-5 d exposure to 4300 m (Dempsey et al 1979). Rapid DA usually occurred even with long exposures to very high and extreme altitudes. Following a 31-d simulated ascent of Mt. Everest (~8848 m, Operation Everest III), resting $\dot{V}_E$ returned rapidly to normal after only 1-3 d RSL (Robach et al 2000) and after a 50-d field expedition to the same mountain, P_aCO_2 was back to BL within 5 d RSL (Savourey et al 1996). However, a few studies reported that VDH occurred more slowly. Boning and colleagues (2001) found that P_aCO_2 was still 3 mmHg below BL at 10-11 d RSL after a 4-w expedition to 4900-7600 m on Broad Peak. Following a 40-d simulated ascent of Mt. Everest, (~8848 m, Operation Everest II), average P_ACO_2 was still more than 10 mmHg below BL at 3 d RSL and did not normalize until 10 d RSL in the single individual studied beyond 3 d post-exposure (Malconian et al 1993). However, this participant had been removed from the chamber early due to illness so it is uncertain whether this datum demonstrates a normal, healthy timeline for VDH. Even slower VDH was reported following an expedition to Kanchenjunga (8586 m), where participants spent more than 40 d above 5500 m (Masuyama et al 1986); P_aCO_2 was still 4 mmHg below BL after 35-40 d RSL. Although participants were not assessed at any earlier time points, it is difficult to dispute this finding since all five climbers demonstrated a drop in P_aCO_2 from pre- to post-expedition (range 1-6 mmHg, 3-16% of BL values).

As suggested by Muza and colleagues (2010), it is intuitive to presume the altitude or duration of exposure, or the magnitude of initial acclimatory change would influence the subsequent rate of VDH. Indeed, the results of some comparable studies suggest that such patterns might exist. After 5 d at 3810 m, $P_{ET}CO_2$ was below BL at 3 d RSL but normalised within 4-7 d RSL (Sato et al 1992). With a longer exposure (12 d) to the same altitude, VDH occurred somewhat more slowly, with $P_{ET}CO_2$ still 3 mmHg

(9%) below BL at 4 d RSL (Sato et al 1994). However, of the 20 studies described in TABLE 2.1, a narrow window for the time required to complete VDH could be determined in only a handful of cases. Thus, it was impossible to determine if there were correlations between time to complete VDH and any of the above variables. The rate of ventilatory DA has also been described relative to the rate of changes that occur during VAH and it is generally suggested that hyperventilation is switched off at approximately the same rate as it is switched on during acclimatisation (Powell et al 1998). Sufficient data were available from six studies to compare the time course of VAH and VDH (FIGURE 2.1). It is clear that both processes involve a rapid rate of early change that gradually slows to a plateau but data could not be confidently modelled to determine the rates of VAH and VDH.

It is not only resting ventilation that is altered by recent exposure to hypoxia. Green and colleagues (2000) showed an upward shift in $\dot{V}_E$ vs. workload during a progressive exercise test 3-5 d after RSL from a 21-d expedition to Denali (6194 m). Although Savourey and colleagues (1996) found that $P_{ET}CO_2$ during exercise returned to pre-exposure levels as quickly as it did at rest, this was not the case in two other studies. At 1-3 d RSL exercise $\dot{V}_E$ remained significantly above BL despite normal $\dot{V}_E$ at rest (Robach et al 2000). And although Astrand and colleagues (1954) found complete DA of resting $\dot{V}_E$ within 1 d RSL, exercise $\dot{V}_E$ was still elevated until 5-9 d RSL. Savourey and colleagues (1996) also reported that $\dot{V}_E$ during hypoxic rest and exercise remained elevated after normoxic $\dot{V}_E$ had normalised. Taken together, these data suggest that even after hyperventilation during normoxic rest has ceased, subtle alterations in ventilatory control persist that are masked under normal conditions but become apparent when the body is stressed to control ventilation in times of altered oxygen supply or demand. It is possible that many studies therefore underestimate the time required to complete all aspects of VDH.

2.2.1.2 Chemosensitivity

2.2.1.2.1 Hypoxic ventilatory response

Underlying the time-dependent hyperventilation during VAH is a gradual increase in respiratory system sensitivity to hypoxia (Bisgard and Neubauer 1995). Hypoxic sensitivity remains elevated for some time after RSL and has even been shown to increase beyond high-altitude values during VDH (Schoene et al

1990). Increased hypoxic sensitivity led to lower P_{CO_2} and higher oxyhaemoglobin saturation (S_aO_2 or S_pO_2) higher during acute hypoxia for up to 9 d post-altitude (Savourey et al 2004, Merz et al 2006).

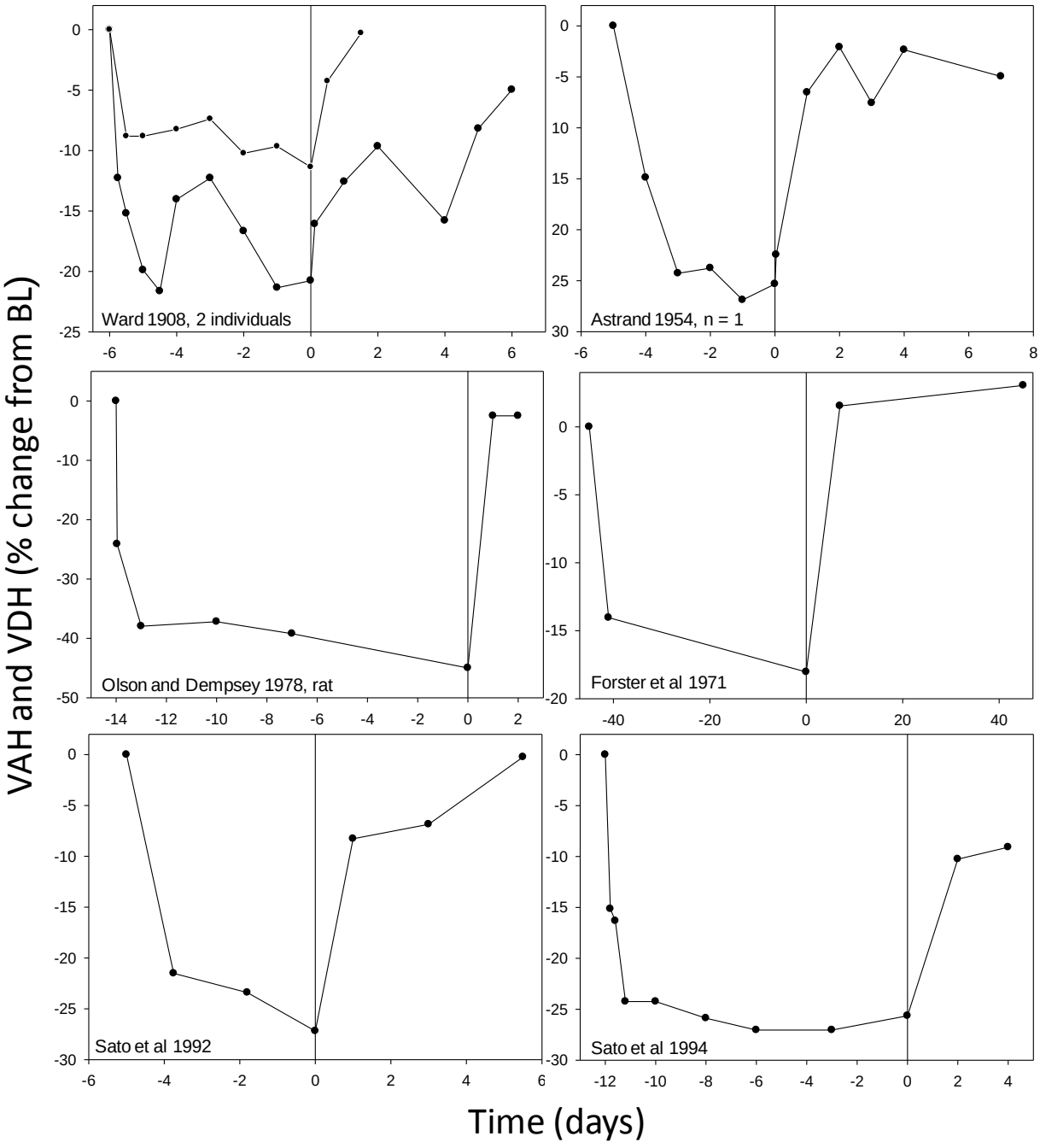


FIGURE 2.1 Comparison of the time course of ventilatoy acclimatisation to hypoxia (VAH) and de-acclimatisation from hypoxia (VDH). Data are presented as relative change from baseline (BL). Vertical line on each plot represents the cessation of hypoxia. Error bars were omitted for simplification. Details of each hypoxic exposure can be found in TABLE 2.2. Note the scales of both axes are different for each panel.

Sensitivity eventually returns to pre-exposure levels and has even been normalized by corrective surgery following years of pathologically-induced hypoxaemia (Edelman et al 1970, Blesa et al 1977, Kobayashi

et al 1994). At rest, peripheral O_2 sensitivity is assessed by measuring the hypoxic ventilatory response (HVR). HVR can be determined a number of ways (isocapnic vs. poikilocapnic; step vs. progressive exposure) and different methods should not generally be compared to one another (Powell 2006). However, the same HVR protocol was used pre- and post-altitude within each of the following studies so findings about the timeline of HVR during VDH can be discussed together.

Data on HVR during VDH is usually accompanied by measures of normoxic $\dot{V}_E$ so the return of HVR to BL can be examined in terms of post-exposure hyperventilation. Although some studies have shown that HVR returns to BL at the same rate as $\dot{V}_E$ or P_aCO_2 (Sato et al 1992, Bhaumik et al 2003), this is not always the case. While normoxic $\dot{V}_E$ had normalized by 7 d RSL following 45 d at 3100 m, HVR remained almost triple the BL value at this time point and was still almost double after 45 d RSL (Forster et al 1971). Moreover, despite the persistent elevation in HVR at 45 d RSL, $P_{ET}CO_2$ was actually higher than at BL. Opposite to this, Sato and colleagues (1994) reported that HVR was back to BL at 4 d RSL but normoxic hyperventilation persisted at this time. And the longest report of persistent hyperventilation following altitude exposure (P_aCO_2 10% below BL at 35 - 40 d RSL) was accompanied by an HVR that had reversed to the point that it approached being significantly below BL ($p = 0.05$, Masuyama et al 1986). Though there does not appear to be a correlation between time needed for complete VDH and HVR, this may not be surprising. Given that the CO_2 drive to breathe dominates in normoxic conditions, it is more logical to anticipate a link between normoxic $\dot{V}_E$ and hypercapnic sensitivity during VDH.

2.2.1.2.2 Hypercapnic ventilatory response

Likely triggered by the hypocapnia that accompanies chronic hypoxic exposure, CO_2 sensitivity of the respiratory system also increases during VAH. Hypercapnic ventilatory response (HCVR) can also be measured a number of ways and the response has a threshold and sensitivity that together determine $\dot{V}_E$ at a given CO_2 and H^+ stimulus. Threshold and sensitivity do not always change together as shown during the DA of HCVR following two weeks at 3810 m (Kellogg 1963). In this case, sensitivity returned to BL immediately upon RSL but threshold gradually increased back to normal by about 23 d RSL. As a result, the P_aCO_2 needed to drive $\dot{V}_E$ to 26 $L \cdot min^{-1}$ remained numerically below BL for more than 20 d RSL,

although no statistical analyses were performed.

Several studies report that HCVR returns to BL during VDH at the same rate as $\dot{V}_E$ or P_{CO_2} (Forster et al1971, Schoene et al 1990, Sato et al 1992). However, there is little evidence to suggest that elevated HCVR correlates any better with hyperventilation during VDH than does increased HVR. Sato and others (1994) reported that HCVR was normal after only 2 d RSL whereas $P_{ET}CO_2$ was below BL for more than 4 d RSL. And in a single individual, HCVR had returned to BL by 3 d RSL while P_ACO_2 remained low for more than a week (Astrand 1954). Similarly, HCVR was no longer elevated at 35-40 d RSL and could therefore not underlie the hyperventilation that persisted for so long post-expedition (Masuyama et al 1986). Furthermore, although the group mean returned to BL within 7 d RSL, Forster and others (1971) reported that HCVR in a single individual was elevated 293% for at least 45 d. This did not appear to affect $\dot{V}_E$ and there was no correlation between HCVR and $\dot{V}_E$ during VDH in this or any other study that provided sufficient raw data to complete the re-analysis (Astrand 1954, Masuyama et al 1986).

2.2.1.3 pH

Ventilation is a powerful means for acid-base regulation (Milsom 1990) and changes in P_{CO_2} that occur during VAH and VDH can have a large impact on pH. By 7 d at 4300 m, the rat is able to compensate pH virtually back to normal (7.45 vs. 7.43 - Olson and Dempsey 1978). However, humans tend to remain alkalotic throughout prolonged hypoxic exposure of many weeks (Ward et al 2000a) and pH is normalised only upon return to normoxic conditions. Even with prolonged and severe hypoxic exposures, blood pH has frequently been recorded at pre-exposure levels by the first testing point back at SL, whether that be at 7 d (Grassi et al 1996, Boning et al 1997), 4 d (Houston and Riley 1947), or 3 d (Hansen and Sander 2003) RSL. With more frequent measurement, other researchers have shown that normalization of pH can occur even more rapidly. Though still elevated at 1 h RSL after 7 d at 4267 m (Goldberg et al 1992) blood pH reached normal values within 1 h (pony) and 2 h (human) RSL after 36 h or 3-5 d, respectively, at 4300 m (Dempsey et al 1979). In both of these cases however, pH continued to decrease below normal, rendering study participants significantly acidotic by 24 h RSL. In the same pattern, Kellogg (1963) reported blood pH to be normal immediately on RSL but becoming persistently

acidic for at least 23 d after 2 w at 3810 m.

2.2.2 Mechanisms of ventilatory de-acclimatisation

Although hyperventilation during VAH importantly preserves P_aO_2 to maintain oxygen delivery to the brain and metabolically active tissues, the persistent hyperventilation of VDH serves no such purpose. Instead, it might be considered an unproductive, energetically costly after-effect of chronic hypoxia, mediated by the lingering mechanisms that induced the hyperventilatory response during VAH. Several potential explanations are explored below.

2.2.2.1 Long term facilitation

It is tempting to consider long term facilitation (LTF) as a factor contributing to the persistent hyperventilation of VDH. LTF has been documented in the anaesthetised rat (Bach and Mitchell 1996) and decerebrate cat (Eldridge and Gill-Kumar 1980) such that phrenic motor output remained elevated for up to 30 min after removal of either an intermittent hypoxic exposure or direct electrical stimulation of the carotid sinus nerve. Although "long-term" by designation, $\dot{V}_E$ remains elevated for minutes to hours in LTF (Feldman et al 2003), not days. Also, while LTF is known to be a serotonin-dependent mechanism (Bach and Mitchell 1996), VDH has been shown to occur normally in serotonin-depleted rats (Olson 1987). Finally, to date, LTF has only been demonstrated when electrical or hypoxic stimulation has been intermittent (Dwinell et al 1997, Baker and Mitchell 2000), and does not appear to occur in awake humans (McEvoy et al 1996). In summary, LTF cannot be considered responsible for VDH.

2.2.2.2 CO_2-sensitive mechanisms

It has been suggested that VAH (and VDH) may occur partly because of the mechanical effect of prolonged hyperventilation during chronic hypoxia and could relate to stretch receptors in the lung or chest wall (Dempsey et al 1975). In fact, as occurs following VAH, $\dot{V}_E$ was persistently elevated following 24 h artificial hyperventilation that occurred in the absence of hypoxia but still resulted in hypocapnia (Brown et al 1948). Persistent spontaneous hyperventilation was attributed to the marked increase in CO_2 sensitivity that resulted from the intervention. There is other evidence that CO_2-sensitive

mechanisms may be involved in VDH. In the goat, VDH does not occur unless animals are allowed to become hypocapnic and alkalotic during hypoxic exposure (Bisgard et al 1986, Engwall and Bisgard 1990). However, Howard and Robbins (1995b, 1995a) demonstrated that VDH occurred in humans who were maintained isocapnic during sustained hypoxic exposure. It was argued for some time that respiratory acidosis on return to normoxia could be the stimulus for persistent hyperventilation and that $\dot{V}_E$ would gradually return to normal as cerebrospinal fluid (CSF) $[H^+]$ is compensated (Crawford and Severinghaus 1978). However there is ample data to suggest that the that this is not the case: hyperventilation actually tends to fall off as CSF pH decreases (summarized in Dempsey et al 1979), suggesting that pH during VDH follows $\dot{V}_E$ and not the other way around. Thus, CO_2 sensitivity and CSF pH do not appear responsible for VDH.

2.2.2.3 Peripheral chemosensitivity

An abundance of evidence demonstrates that changes in peripheral chemosensitivity are primarily responsible for VAH (Bouverot and Bureau 1975- dog, Forster et al 1976 - pony, Smith et al 1986 - goat). In numerous rat studies, changes in carotid body (CB) ultrastructure during chronic exposure to hypoxia have been repeatedly described, with clear documentation of substantial CB enlargement (McGregor et al 1984), hypertrophy (Pequignot et al 1984) and hyperplasia of chemosensory Type I (glomus) cells (Wang et al 2008), and increased vascularisation of the CB (Kusakabe et al 2004). While most reports conclude that CB size and vascularity have statistically returned to BL within 1 w RSL, regression seems to occur gradually for up to 4 – 8 weeks (Kusakabe et al 2004, Matsuda et al 2006) and newly proliferated Type I cells survive for at least 1 mo post-hypoxia (Wang et al 2008). However, though CB sensitivity remains heightened post-hypoxia, no increase in CB afferent activity was reported upon RSL (Bisgard 1990 - goat). Hyperventilation in humans persisted during VDH even after CB activity was inhibited with dopamine (Pedersen et al 2000); and Vizek and colleagues (1987) demonstrated that cats continued to hyperventilate after cessation of hypoxic exposure, even after carotid sinus nerve section (though to a lesser extent than intact animals). Thus, although clearly involved in the process of VAH, the role of the carotid body in VDH is certainly disputable.

2.2.2.4 Central nervous system integration of peripheral input

Although central nervous system (CNS) hypoxia alone does not lead to VAH (Weizhen et al 1992 - goat), CNS integration of peripheral input is enhanced during VAH (Dwinell and Powell 1999 - rat). In fact, it now seems that output from central chemoreceptors might be mediated by signals from the periphery (Takakura et al 2006 - rat). In a recent discussion of new paradigms of ventilatory control, it is suggested that interactions between sensitized peripheral and central chemoreceptors are in large part responsible for the persistent hyperventilation of VDH (Smith et al 2010). With little evidence to counter this hypothesis, modulated CNS integration of input from both types of chemoreceptors is currently the best candidate explanation for persistent hyperventilation during VDH.

2.2.3 Implications for ventilatory re-acclimatisation to hypoxia

Although VDH occurs relatively rapidly following return to normoxia, it is possible that structural and functional changes that occur during VAH can affect the process during subsequent exposure to hypoxia. Bach and Mitchell (1996) presented evidence for metaplasticity in the ventilatory control system and the concept has been thoroughly reviewed more recently (Mitchell et al, 2001, Feldman et al, 2003, Mitchell and Johnson, 2003). Powell (1998) explained the implications of ventilatory metaplasticity, stating that "some mechanisms last long enough to affect future ventilatory responses to hypoxia, indicating 'memory' or functional plasticity in the ventilatory control system". Thus it is theoretically possible that VAH may be altered by previous exposure, but the time domains and magnitude of any carry-over are completely unknown. The only known empirical evidence of altered VAH during re-exposure comes from a study using IH (Katayama et al 2005a). Participants were exposed to hypoxia ($F_IO_2 = 0.12$) for 1 $h \cdot d^{-1}$ for 10 consecutive days and the IH series was repeated after a 30-d washout. VAH began earlier in the RA series of exposures as indicated by lower $P_{ET}CO_2$ and higher S_pO_2 compared during IA. More data are required to determine whether a similar phenomenon occurs with exposure and re-exposure to sustained hypoxia.

2.3 Haematological acclimatisation and de-acclimatisation

Haematological acclimatisation to hypoxia (HAH) is defined as the time-dependent increase in O_2

carrying capacity brought about by progressive increases in circulating haemoglobin concentration ([Hb]). During HAH, initial increases in [Hb] are caused by a rapid diuresis and reduction in plasma volume (PV; Swenson et al 1995). Erythropoiesis is also initiated almost immediately: erythropoietin (EPO) production is upregulated by hypoxia inducible factor (HIF-1α), triggering the differentiation of erythroid stem cells in the bone marrow and the release of reticulocytes into the circulation (Hopfl et al 2003). Newly-recruited reticulocytes mature into adult erythrocytes, leading to relatively linear increases in erythrocyte count (RBC), red cell volume (RCV), haematocrit (Hct) and [Hb]. These variables plateau when HAH has reached a steady state appropriate to the altitude or severity of hypoxia (Mylrea and Abbrecht 1970). The time courses and underlying mechanisms of these changes have been examined at length and are discussed in several reviews (Grover and Bärtsch 1996, Isbister 1997, Weidemann and Johnson 2009). If the hypoxic stimulus is removed, these variables will gradually return to normal by the process of haematological de-acclimatisation from hypoxia (HDH). HDH has been observed following various paradigms of sustained hypoxic or high-altitude exposure in low altitude natives as well as in high-altitude natives travelling to SL (Merino 1950, Reynafarje 1968). HDH has also been described following surgical correction of chronic pathological hypoxaemia (Kobayashi et al 1994).

2.3.1 Time course of haematological de-acclimatisation

2.3.1.1 Haemoglobin and haematocrit

The gradual normalization of RBC and Hct have been frequently reported following cessation of hypoxia. However, since oxygen transport is directly affected by [Hb], the timeline of HDH is primarily discussed here in terms of this variable and Hct data are included in supplement. Likely due to the methodological ease of blood sampling compared to measurement of $\dot{V}_E$ or P_{CO_2}, a great deal more data exist on HDH than VDH. In addition, many studies include serial measurement of [Hb] and/or Hct upon RSL, allowing a more thorough examination of the time course of HDH.

TABLE 2.2 summarises data from 40 published studies of HDH. In addition, unpublished data collected during experiments presented in CHAPTERS 4 and 5 are included. Studies were heterogeneous in terms of exposure duration (7 d to 13 mo), hypoxic severity (1700 to 8848 m) and species studied (human, mouse,

TABLE 2.2 Summary of available data on haematological de-acclimatisation from hypoxia (HDH). Unless otherwise noted, studies used human subjects and measured [Hb]. Times to complete HDH (HDH Time) marked with an asterisk were interpolated or extrapolated from a linear model of [Hb] or Hct over time. For laboratory exposures to hypobaric or hypoxic hypoxia, the estimated equivalent terrestrial elevation is given in parentheses.

Reference	Exposure	HDH Time	Comments
Ward 1908	7 d at 4500 m	7 d*	n = 1
Schneider 1913	6 mo at 4300 m	47 d*	n = 1; time to plateau (no BL data)
Merino 1950	18-21 d at 4540 m	<4-5 weeks	
Reissmann et al 1951	12 w at 6000 m	37 d*	dog
Astrand 1954	5 d at 4000 m	10 d*	n = 1; Hct; still 6% elevated at 6 d
Pace et al 1956	70 d expedition to Makalu, max 7000 m	28 d*	still 10% elevated at 17 d
Dill et al 1969	2-6 w at 3800 m	1 d	
Hannon et al 1969	10 w at 4300 m	<2 w	Hct
Okunewick et al 1969	3-4 w at 380 mmHg (~5500 m)	11 d*	Hct; mouse
Buderer and Pace 1972	6 mo at 3800 m	51 d*	monkey
ResSL et al 1974	12 w (5 $d \cdot w^{-1}$), 8 $h \cdot d^{-1}$ at 310 mmHg (~7000 m)	<70 d	rat; intermittent hypoxia
Huff et al 1975	16 d at 300 mmHg (~7300m)	8 d*	mouse; still 13% elevated at 6 d
Leach et al 1977	3 w at 10% O_2 (~6600m)	< 6 w	Hct; rat
Smith et al 1979	90 d at 380 mmHg (~5500 m)	>10 d	mouse, no data given
Jain et al 1978	30 d of 6 $h \cdot d^{-1}$ at 350 mmHg (6100 m)	9 d*	rabbit; intermittent hypoxia
Kay et al 1980	4 w at 380 mmHg (~5500m)	<6 w	rat
Kentera and Susic 1980	4 w at 240 mmHg (~8500m)	4 w	Hct; rat
Marki et al 1982	5 w at 433 mmHg (~4500 m)	43 d*	rabbit; still 10% elevated at 28 d
Fried and Reid 1984	2 w at 380 mmHg (~5500m)	1 mo	Hct; rat
Masuyama et al 1986	2.5 mo expedition to Kanchenjunga, max 7800-8586 m	<35-40 d	
Stutte et al 1986	4 w at 370 mmHg (~6000m)[1]		
Ferretti et al 1990	10 w expedition to Everest (8848 m)	> 3 w	still 14% above BL, cannot extrapolate

[1]Manuscript claimed the exposure was to 543 mmHg or 6000 m, however these are not equivalent. Given the large haematological response exhibited by the animals it is more likely that the given altitude was correct and the exposure was actually to ~370 mmHg.

TABLE 2.2 (continued)

Reference	Exposure	HDH Time	Comments
Poiani et al 1990	10 d at 10% O_2 (~6600m)	14 d*	Hct; rat
Lyons et al 1995	16 d at 4300 m	> 6 d	still 14% above BL, cannot extrapolate
Ponchia et al 1995	4 w expedition to Pumori (7135 m)	16 d*	still 7% above BL at 14 d
Grassi et al 1996	5 w at 5050 m	< 1 w	
Gunga et al 1996	7d at 3600 m	1 d	Hct
Harik et al 1996	3 w at 380 mmHg (~5500 m)	<3 w	Hct; rat
Savourey et al. 1996	41 or 62 d expedition to Everest (8848 m)	61 d*	data for two groups of climbers combined
Beidleman et al 1997	18 d at 4300 m	12 d*	still 5% above BL at 8 d
Boning et al. 1997	40 d expedition to Broad Peak (max 7600 m)	>10-11 d	still 13 %, cannot extrapolate
Svedenhag et al 1997	1 mo at 1900 m	1 d	
Green et al 2000	21 d expedition to Denali (6194 m)	>3-5 d	still 5% above BL, cannot extrapolate
Robach et al 2000	7 d at 4350 then 31d simulated Everest ascent (8848 m)	<1-3 d	simulated ascent of Everest
Pichiule and LaManna 2002	3 w at 380 mmHg (~5500 m)	25 d*	Hct; rat
Hansen and Sander 2003	31 d at 5260 m	< 3 d	
Singh et al 2003	60 d at 3500 m then 70 d at 5800 m	<7 d	
Robach et al 2004	7 d at 4350 m	3 d*	still 3% above BL at 2 d
Savourey et al 2004	3 w expedition to Huascaran (6768 m)	> 9 d	still 7% above BL, cannot extrapolate
Singh et al 2004	9-13 mo at 57-6100 m	> 90 d	still 13% above SL control group (no BL data)
Schobersberger et al 2005	3 w at 1700 m	<7-10 d	
Risso et al 2007	53 d expedition to Dhaulagiri (8167 m)	< 6 d	Hct
Zubieta-Calleja et al 2007	2 w at 3100 m	21-23 d	Hct; time to plateau (no BL data)
MacNutt et al (CHAPTER 4)	10 d trek to 5360 m	14 d*	1st DA; mean of individually determined values
MacNutt et al (CHAPTER 4)	10 d trek to 5360 m	13 d*	2nd DA; mean of individually determined values
MacNutt et al (CHAPTER 5)	2 w at 12% O2 (~4500 m)	7 d*	mouse
MacNutt et al (CHAPTER 5)	8 w at 12% O2 (~4500 m)	14 d*	mouse

rat, dog, pig-tailed monkey, and rabbit). Both normo- and hypobaric exposures were included as were exposures to both constant and progressive hypoxia. Two studies involved exposure to intermittent, rather than continuous hypoxia (Ressl et al 1974, Jain et al 1978). These exposures substantially exceeded the threshold suggested by Muza (2007) as necessary to elicit HAH and were included for completeness. Hypoxic stimulation was sufficient to elicit HAH responses such that [Hb] or Hct increased 6-93% above pre-exposure values. Following return to normoxia, HDH resulted in a decrease in [Hb] or Hct back toward, or even below BL (Svedenhag et al 1997), but the time required for complete HDH was variable. As with VDH, several studies report that HDH was either already completed at the first measurement upon RSL or was not yet complete at the final post-hypoxia assessment. However, given the repeatable linearity of HDH, sufficient data were available from 28 studies to confidently model the HDH response ($R^2 = 0.94 \pm 0.04$), calculate the rate of DA (HDH rate) and estimate the time required to return [Hb] or Hct numerically to BL (HDH time) by interpolation or extrapolation.

HDH time varied across studies, with [Hb] and Hct returning to BL in as little as 1 d (Dill et al 1969, Gunga et al 1996, Svedenhag et al 1997) or as long as 51 d (Buderer and Pace 1972) or 61 d (Savourey et al 1996). HDH occurred at a mean rate of 3.8 ± 1.0 (range 0.2 to 23) % of BL values per day, with [Hb] declining by 0.05 to 3.6 $g \cdot dL^{-1}$ and Hct falling 0.8 to 4.0 percentage points each day. Of interest, Hct declined at a similar rate (1.7 percentage points$\cdot day^{-1}$) following transfusion-induced polycythaemia (Gurney et al 1961), suggesting that similar mechanisms might be responsible for the removal of excess erythrocytes from the circulation, regardless of the reasons for plethora. HDH times and rates were calculated based on data from 13 animal studies and 15 human studies. Animals were exposed to significantly higher altitudes (5700 vs. 4300 m; $p = 0.01$) and demonstrated a greater HAH response ([Hb] or Hct increased 43 vs. 18%; $p < 0.001$) but exhibited similar HDH rates and times compared to humans. Thus, all available data were considered together when assessing factors that correlate with HDH time and rate.

HDH time correlated with a metric of exposure dose (approximate area under the curve of altitude vs. time; $r = 0.74$, $n = 28$, $p < 0.001$) and with the magnitude of the initial acclimatisation response ($r = 0.61$, $n = 25$, $p = 0.001$). As might be expected, these two variables also correlated with each other ($r = 0.37$, n

= 35, p = 0.01). There was a modest correlation between HDH time and HDH rate (r = -0.52, n= 23, p = 0.01), but the rate of HDH did not correlate with exposure dose or with the magnitude of HAH response. Instead, HDH rate seems to reflect the acclimatisation phase during which the hypoxic stimulus was removed. Mice exposed to ~4500 m for either 2 or 8 w demonstrated a similar increase in [Hb], but animals with the longer hypoxic exposure required 14 instead of 7 d for complete HDH to occur (see CHAPTER 5). Similarly, HDH rate was significantly faster for hypoxic exposures that lasted ≤ 2 w (n = 7 studies; 0.4 ± 0.2 $g \cdot dL^{-1} \cdot day^{-1}$) compared to ≥ 6 w (n = 6 studies; 0.2 ± 0.1 $g \cdot dL^{-1} \cdot day^{-1}$; p = 0.03). The difference in HDH rate suggests that processes involved in reversing the early stages of hypoxic acclimatisation occur at a faster rate than those involved in reversing the true polycythaemia that accompanies longer hypoxic exposure. These processes are explored further in SECTION 2.3.2.

In 15 studies, serial measurements of [Hb] or Hct were made during and after exposure to a constant level of hypoxia. These data are plotted in FIGURE 2.2 to allow a visual comparison of the relative time course of HAH and HDH for each data set. Although several panels demonstrate an eventual plateau during the hypoxic exposure, HAH rate was calculated from the linear component of each acclimatisation curve. The rates of HAH and HDH were significantly correlated (FIGURE 2.3; r = 0.62, n = 14, p = 0.01) but HDH tended to occur more quickly than HAH (2.0 ± 0.4 vs. 1.5 ± 0.3 % change$\cdot day^{-1}$; p = 0.06).

Not surprisingly, it appears that longer, more severe hypoxic exposures that elicit larger increases in [Hb] and Hct will generally require longer periods of DA before haematological variables are returned to pre-exposure levels. However, these patterns are far from universal and many exceptions are presented in TABLE 2.2. In four studies where humans were exposed for 7 d to altitudes of 3500-4500 m, complete HDH occurred after 1 d (Gunga et al 1996), 3 d (Robach et al 2004), 7 d (Ward 1908) or 10 d (Astrand 1954). In two rat studies where Hct increased 40% during HAH, complete HDH occurred after 14 d (Poiani et al 1990) or 25 d (Pichiule and LaManna 2002). Similarly, in human studies where HAH increased [Hb] by 4 $g \cdot dL^{-1}$, HDH was completed after 1 d (Dill et al 1969), 16 d (Ponchia et al 1995) or 25 d (Pace et al 1956). Even when comparing studies with extremely similar characteristics, the associated HDH times clearly remain unpredictable.

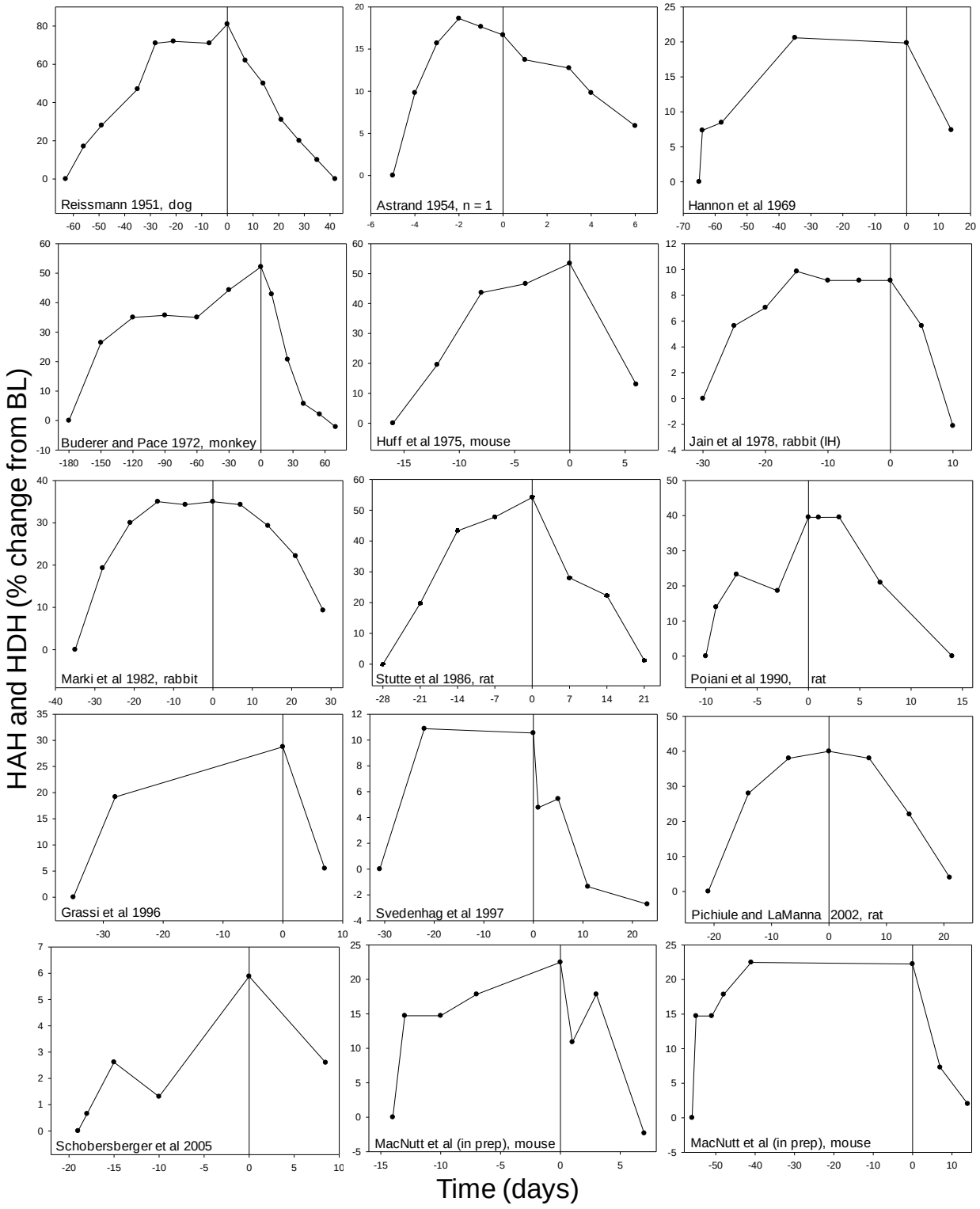


FIGURE 2.2 Comparison of the time course of haematological acclimatisation to hypoxia (HAH) and de-acclimatisation from hypoxia (HDH) in studies that included exposures to a constant severity of hypoxia. Data are presented as relative change in [Hb] or Hct from baseline (BL). Vertical line on each plot represents the cessation of hypoxia. Error bars were omitted for simplification. Details of each hypoxic exposure can be found in TABLE 2.2. Note the scales of both axes are different for each panel.

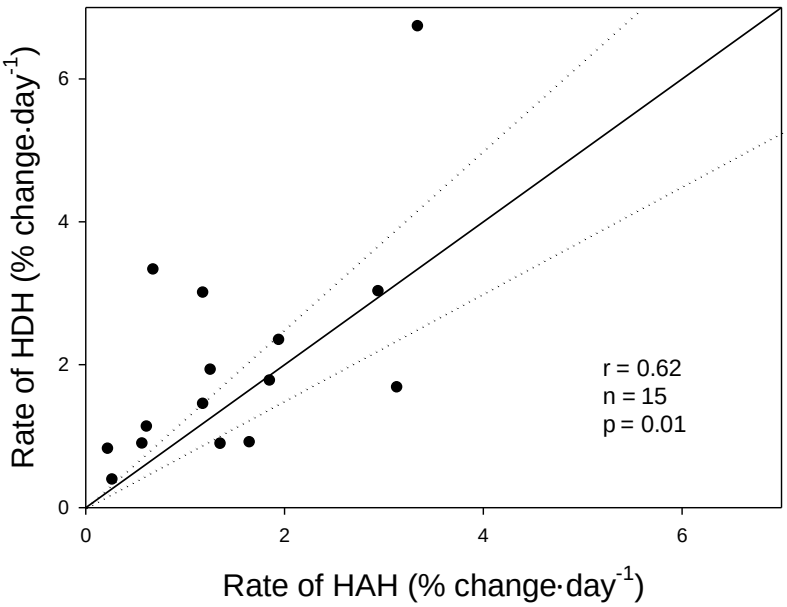


FIGURE 2.3 Rate of change of [Hb] or Hct during hypoxic acclimatisation (HAH) and de-acclimatisation (HDH). Data were calculated from studies presented in FIGURE 2.3. Details of hypoxic exposures can be found in TABLE 2.2. Solid and dotted lines represent unity ± 25%.

2.3.1.2 Plasma volume

The reversal of the hypoxia-induced haemoconcentration response upon RSL seems to depend upon the point during the initial exposure at which normoxia is re-established. The reduction in PV is a relatively acute response to hypoxia (Grover and Bärtsch 1996) and PV can be restored over an extended stay at a constant altitude (Buderer and Pace 1972 - monkey). With shorter stays at a given altitude or extended exposure to progressive altitude, PV generally remains reduced throughout the exposure and is not restored until the hypoxic stimulus is removed. Several studies report that PV had returned to normal by the first post-hypoxic measurement, whether that be after 4-5 w (Merino 1950), 2 w (Hannon et al 1969b), or 4 d (Krzywicki et al 1969). Following 7 d at 4350 m, PV remained elevated above BL at 1d RSL but was normal by 2 d RSL (Robach et al 2002). Others have demonstrated a persistent reduction with PV ~10% below BL after 3-5d RSL (Green et al 2000) and 20% below BL after 6 d RSL (Lyons et al 1995). Two other cases report that plasma re-expansion upon RSL increased PV beyond BL values at 1-3 d (Robach et al 2000) and 10-11 d RSL (Boning et al 1997). In one of the few accounts where serial measurements were made throughout IA and DA, Reissmann (1951) demonstrated in the dog that PV recovered gradually, returning to BL after 2-6 w in individual animals. It is notable that this report of comparatively slow normalisation of PV occurred after a lengthy exposure (10-12 w) to extreme altitude

(6000 m). However, even after 4-6 mo at 3500 m, PV returned to normal within 2 d RSL (Singh et al 1990). Thus, there is little evidence of a consistent effect of the duration or severity of initial exposure on the DA rate for PV.

2.3.1.3 Red cell volume

Direct measurement of RCV would eliminate a great deal of speculation in determining whether changes in [Hb] during HAH and HDH are relative or absolute. However, methodologies for determining RCV are complex and individual responses to hypoxia are notoriously variable (reviewed in Grover and Bärtsch 1996). As a result, only a few studies have examined changes in blood volume and RCV during HDH. At the time of first post-hypoxia measurement, RCV was no different from BL after 4-5 w (Merino 1950; 18-21 d at 4540 m) or 10 d RSL (Jain et al 1978; rabbit, 30 d of 6 $h \cdot d^{-1}$ at ~6100 m). However, Robach and colleagues (2000) demonstrated that RCV remained elevated at 1-3 d RSL following a simulated ascent of Mt Everest (~8848 m). After exposing pig-tailed monkeys to 3800 m for 6 mo, Buderer and Pace (1972) calculated RCV from serial measurements of PV and Hct and demonstrated a linear decrease in RCV for 40 d followed by a plateau of RCV at almost 30% above BL. Given the length of these experiments, this may have represented a new age-appropriate "normal" but since no control animals were included this remains speculative. Reissmann (1951) measured RCV directly in dogs exposed to ~6000 m for 12 w. In this case RCV also showed a linear decrease over time, reaching BL values after about 50 d RSL.

2.3.1.4 [Erythropoietin]

The time required to return [EPO] to BL following exposure to hypoxia also depends on the duration of initial exposure. In humans, [EPO] remained elevated above BL at the end of a 7-d exposure to high altitude but returned to BL after 12-24 h (Gunga et al 1996, Robach et al 2004). However with longer exposure (22 d), [EPO] had fallen back to BL by the end of the expedition (Milledge and Cotes 1985). Though no change was measureable within the first 2 h, [EPO] had fallen significantly below BL by 8 h RSL. After other expeditions, [EPO] remained significantly below BL for at least 6 d (Risso et al 2007; 53 d $\geq$ 4500 m) or 9 d RSL (Savourey et al 2004; 3 w up to 6768 m). Following an earlier expedition to

Mt. Everest (8848 m), Savourey and colleagues (1996) monitored [EPO] for a more extended period and demonstrated that [EPO] remained below BL at 30 d RSL but had normalised by 60 d RSL. In mice exposed to ~4700 m for 2 w, [EPO] remained significantly below BL at 1, 3 and 7 d RSL but had increased to normal by 14 d RSL. In mice exposed to same simulated altitude for 8 w, [EPO] remained below BL at 14 d RSL and was not re-measured at a later time point (see CHAPTER 5). Although, mice exposed for 2 and 8 w experienced similar hypoxia-induced increases in [Hb], the variable returned to BL more slowly following the longer exposure (14 vs. 7 d). In fact, for each of the above studies, the time required for complete HDH of [Hb] (or Hct) and [EPO] are nearly identical. These data suggest that [EPO] will return to BL at a rate that is dependent on the normalisation of [Hb]; [EPO] stays low to halt erythropoiesis while erythrocytes are still in excess, then returns to BL to stimulate erythropoiesis at a rate that will maintain [Hb] at a sea level-appropriate value.

2.3.1.5 Reticulocytes

As with [EPO], exposure to constant hypoxia elicits a relatively transient increase in reticulocyte count (RC), with RC dropping back towards BL within 2 w (Mylrea and Abbrecht 1970, Huff et al 1975). In cases where RC had already partially or completely returned to BL before the end of hypoxic exposure, values remain low upon RSL. In rabbits, RC was measured equivalent to pre-exposure values after 10 d RSL (Jain et al 1978) and, in mice, RC fell significantly below BL at 8 d RSL (Huff et al 1975). With extended exposures to progressive hypoxia that occur during mountaineering expeditions, RC might remain elevated throughout the exposure. Even so, RC had returned to or below BL by 5-10 d (Savourey et al 1996) or 9 d RSL (Savourey et al 2004). Conversely, following 7 d at 3600 m, RC remained elevated at the end of exposure and was still well above BL at 1 d RSL (Gunga et al 1996). Since all of these studies involved only a single observation of RC upon RSL, limited information can be gleaned about the time course of RC during HDH. Although animals were made polycythaemic by transfusion of erythrocytes rather than exposure to hypoxia, the reticulocyte response measured in an experiment by Gurney and colleagues (1961) is more informative. Post-transfusion, reticulocytes disappeared from the circulation and did not re-appear for 18 d, suggesting that erythropoiesis was completely suppressed until Hct (which declined at a steady rate) had fallen to near normal values (from 80 to 50 vs. 44%). Similarly,

serial measurements of RC in mice demonstrated that, even though RC was still elevated at the end of exposure, it dropped immediately to BL (within 1 d RSL), then continued to fall to significantly below BL at 3 and 7 d RSL before returning back to pre-exposure values at 14 d RSL (see CHAPTER 5). Again, this pattern paralleled the drop in [Hb] and Hct, which both reached BL values around 7 d RSL. Presumably, a normal level of erythropoiesis was re-instated at that point to prevent further declines in RBC and [Hb].

2.3.2 Mechanisms of haematological de-acclimatisation

As with the persistent hyperventilation of VDH, elevated [Hb] provides no physiologic advantage upon return to a normoxic environment. Rather, the persistence of polycythaemia and increased blood viscosity upon RSL increases risk of thrombosis, ischemic stroke and pulmonary hypertension (Prchal and Beutler 2006). As a result, it is beneficial to restore [Hb] to a SL-appropriate value as quickly as possible.

There are likely three primary components to the process of HDH: 1) re-expanding PV to reverse the haemoconcentration; 2) down-regulating erythropoiesis to prevent recruitment of new erythrocytes; and 3) destruction of excess erythrocytes and recycling of Hb and other constituents. The relative contribution of these components in restoring haematological status likely depends on the involvement of each process at the point when normoxia is restored. A fourth potential contributor to HDH, re-uptake of erythrocytes by the spleen, is also discussed below.

2.3.2.1 Re-expansion of plasma volume

Mechanisms of the hypoxia-induced reduction in PV are disputable, with conflicting results reported even within the same research group. As a result, the relative contribution of fluid shift from the intra- to extravascular compartment versus frank diuresis in effecting an acute haemoconcentration is unclear (Robach et al 2000, Robach et al 2002). Both studies, however, attributed the re-expansion of PV upon RSL to an antidiuresis, measurable as decreased urine output despite identical water intake at 1 and 2 d RSL following 7 d at 4350 m (Robach et al 2002). Although this water retention was not correlated with changes in renin, aldosterone, atrial natriuretic peptide or arginine vasopressin, Robach and colleagues (2000) did measure an increase in plasma [renin] and [aldosterone] at 1-3 d RSL following a simulated

ascent of Mt. Everest (~8848 m). These hormones were also elevated immediately upon RSL after a real ascent of Mt. Everest, though not significantly so (Savourey et al 1998). Regardless, the potential role of peripheral chemoreceptor sensitivity in driving the antidiuresis during HDH should also be considered (Robach et al 2002).

2.3.2.2 Re-uptake of red cells by spleen

Rapid increases in [Hb] within minutes of exposure to acute hypoxia have been attributed to splenic contraction and ejection of stored erythrocytes into the circulation. In both dogs (Kramer and Luft 1951) and humans (Richardson et al 2008), [Hb] decreased nearly as rapidly when normoxia was restored, concomitant with an increase in spleen weight or volume to pre-exposure values. The spleen has also been implicated in longer term haematological acclimatisation. In humans, spleen volume (assessed by sonography) decreased after 3 and 6 mo at 1750 m (Sonmez et al 2007). In mice exposed to ~4600 m for 1-2 mo, there was a significantly smaller increase in [Hb] (19 vs. 32%) in splenectomised vs. non-splenectomised animals (Cook and Alafi 1956). Although there was evidence of splenic contraction in intact animals, it is likely that the spleen also contributed to HAH as an erythropoietic organ as has been previously demonstrated in the rat (Ou et al 1980, Kam et al 1999). Regardless, Cook and Alafi (1956) showed that with return to normoxia, [Hb] was reduced to pre-exposure values at similar rates in splenectomised and non-splenectomised animals, suggesting the spleen does not play a critical role in HDH.

2.3.2.3 Down-regulation of erythropoiesis

The process by which circulating [EPO] falls back to or below BL is regulated by the availability of its transcription factor, hypoxia-inducible factor (HIF-1α). In cultured HeLaS3 cells, HIF-1α accumulates within 2 min of hypoxic exposure and, in the presence of O_2 is degraded nearly as quickly, with significant reductions within 4 min and near disappearance within 16 min of re-oxygenation (Jewell et al 2001). As a result, DNA-binding is lost almost immediately and upregulation of target genes, including erythropoietin (*Epo)*, ceases. Schuster and colleagues (1987) demonstrated in the rat that changes in plasma [EPO] follow changes in kidney [EPO], which in turn parallel changes in kidney *Epo* mRNA with

a short time lag of less than 1 h. This occurs with both the onset and offset of hypoxic exposure. Following a 4-h hypoxic exposure, kidney [EPO] started declining after 1h RSL, but plasma [EPO] continued to rise until 2 h RSL, likely reflecting secretion of pre-formed EPO from the kidney into the plasma (Fried and Baronevarelas 1984). As a result of this cascade of events, the drop in [EPO] upon RSL triggers apoptosis of erythroid progenitors in the bone marrow (Mide et al 2001, Rice and Alfrey 2005). Although this prevents recruitment of new erythrocytes and can account for the gradual reduction of reticulocytes during HDH (see SECTION 2.3.1.5), committed erythroid precursors are unaffected by a drop in [EPO] and will continue maturing into erythrocytes. Robach et al (2004) reported increased sTfR at 1 and 2 d RSL, despite the return of [EPO] to BL, suggesting that iron transport was required to support continued Hb synthesis in previously stimulated erythroid progenitors. Considering a normal life span of 120 d (Handelman and Levin 2010), complete cessation of erythropoiesis could only account for a decline in [Hb] of 0.8% per day, and not the rate of nearly 4% per day reported in SECTION 2.3.1.1. Clearly another process is also involved in the elimination of excess erythrocytes and [Hb] upon RSL.

2.3.2.4 Neocytolysis

It has been known for decades that excess red cells are removed from the circulation on descent from high altitude (Merino 1950, Pace et al 1956). However, the specific form of erythrolysis that regulates red cell volume in times of plethora has only been described relatively recently (Alfrey et al 1997). Neocytolysis – the selective destruction of the youngest erythrocytes in circulation – occurs when astronauts experience microgravity (Alfrey et al 1996) and during DA from high altitude (Rice et al 2001). It has been implicated as a contributing factor of the anaemia of renal failure (Rice et al 1999) and diabetes mellitus (Wittmann et al 2007), and likely plays a key role in transitioning the polycythaemic newborn to its new normoxic environment (Trial and Rice 2004). Finally, neocytolysis has been demonstrated following withdrawal of recombinant human EPO (rHuEPO) treatment for therapeutic purposes (Kaufman 1998, Besarab et al 2002), and as a prohibited means of blood doping (Chang et al 2009).

Under normal conditions, damaged or aging erythrocytes are removed by splenic macrophages and the components are either recycled or excreted (Beutler 2006). The haeme portion of Hb is converted to

bilirubin and shuttled to the liver for excretion, while globins are broken down into constituent amino acids and later used in protein synthesis. Iron (bound to transferrin) is transported primarily to the liver for storage as ferritin, which can be mobilised to the bone marrow as needed for subsequent erythropoiesis. Thus, elevated serum [bilirubin], increased excretion rates of bilirubin derivatives (i.e. urobilinogen), and increased serum [ferritin] signal an increase in Hb catabolism. In neocytolysis the process is similar, except that young and middle-aged cells are preferentially targeted for phagocytosis instead of senescent or damaged cells (Rice et al 2001, Risso et al 2007). The mechanisms by which younger cells are preferentially targeted are still under investigation but two likely explanations have emerged:

1) Macrophages are altered to preferentially engulf cells expressing markers indicative of their young age.

Human splenic endothelial cells express EPO receptors and respond to a drop in [EPO] by increasing permeability and stimulating adjacent macrophages to increase phagocytic activity, particularly of young erythrocytes (Trial et al 2001). Inflammation (i.e. cytokine release from endothelial cells) may play a role in altering the interaction between adhesion molecules on macrophages and adhesion molecules that are unique to young erythrocytes (Trial and Rice 2004). This process requires further clarification.

2) Young erythrocytes are altered to express surface markers more typical of a senescent cell.

On descent from high altitude, expression of erythrocyte membrane proteins was altered such that cells of all ages took on a senescent phenotype with reduced expression of CD55 (previously known as decay accelerating factor) and CD59 (Risso et al 2007). Both proteins also decrease with cessation of rHuEPO therapy in haemodialysis patients (Ohi et al 2003). Post-expedition, erythrocytes of all ages also had significantly more phoshphatidylserine (PS) exposed on the cell surface compared to pre-altitude exposure. PS is a phospholipid compound normally found on the interior surface of the plasma membrane but that is increasingly exposed to the exterior with normal cell aging; recognition of PS is one of the primary mechanisms by which macrophages typically target older erythrocytes for phagocytosis (Trial and Rice 2004). Although the above changes suggest increased global erythrophagocytosis, they do not account for the selective targeting of younger cells by macrophages. However, on descent from altitude,

surface expression of CD47 (which normally protects cells from phagocytosis) was disproportionately decreased in young and middle-aged cells, suggesting a key role of the protein in identifying cell targets during neocytolysis (Risso et al 2007). Other membrane proteins (CD35, CD44 and CD71) might also be involved but their role has not yet been elucidated (Chang et al 2009).

These two possibilities are not mutually exclusive and evidence exists to support both concepts. Regardless of the precise mechanisms, all evidence suggests that reduced [EPO] is required for neocytolysis to occur. Following an expedition at high altitude, the drop in RCV and increase in serum [ferritin] characteristic of neocytolysis were prevented by administration of rHuEPO (Rice et al 2001). Trial and colleagues (2001) demonstrated that neocytolysis is not triggered by low [EPO] per se, but by an acute drop in [EPO]. As discussed earlier, the increase in [EPO] that occurs with hypoxic exposure is transient and [EPO] falls back towards BL after 1-2 w. Although not previously investigated, it is unlikely that neocytolysis occurs in response to such a decline in [EPO], suggesting that EPO withdrawal is not the only factor regulating neocytolysis and that low absolute [EPO] may also be required. It is also possible that an oxygen-sensing mechanism is involved in overriding the stimulation of neocytolysis at a time when erythropoiesis is a more appropriate response. If so, the observation that neocytolysis still occurs with severe anaemia (Rice et al 1999) suggests that the stimulus is somehow sensed as P_{O_2} rather than C_aO_2.

Data suggest that the process of neocytolysis is initiated immediately upon withdrawal of EPO, which occurs within hours of return to normoxia (see SECTION 2.3.2.3). Risso and colleagues (2007) demonstrated a shift in the age distribution of the erythrocyte population at 6 d RSL and there is indirect evidence that neocytolysis is active even sooner. Increased interaction of macrophages with young erythrocytes was evident at 48 h, but not 24 h, after EPO withdrawal (Trial et al 2001). An increase in serum [bilirubin] was observed within 24 h RSL (Merino 1950). Serum [iron] (bound to transferrin) was elevated at the time of the first measurement (10 d RSL) and remained elevated at 40 d, but not 70 d RSL following a 50-d expedition to 8848 m (Savourey et al 1998). Serum [transferrin] was also elevated at 9 d RSL following a 3-w expedition to 6768 m (Savourey et al 2004). There was no indication of increased serum [ferritin] at 12 or 24 h RSL (Robach et al 2004) but [ferritin] did begin to rise after 6 d RSL (Rice

et al 2001) and 4 d after cessation of rHuEPO treatment (Chang et al 2009). Clearly, neocytolysis begins to contribute to HDH by removing excess erythrocytes from the circulation within a few days of removal from the hypoxic environment.

There is some evidence that the phagocytosis of neocytolysis is also accompanied by intravascular haemolysis upon descent from altitude. Haptoglobin (Hp) transports free Hb to the spleen for metabolism, and its concentration in the circulation is decreased when this function is required. Risso and colleagues (2007) reported a tendency for lower [Hp] at 6 d RSL compared to before the expedition. Following a 50-d expedition to 8848 m, [Hp] was not different from BL at 10, 40 or 70 d RSL (Savourey et al 1998), and [Hp] was significantly higher than BL at 9 d RSL following a 3-w expedition to 6768 m (Savourey et al 2004). Without a repeatable finding, it is difficult to assess the potential significance of intravascular haemolysis as a supplementary mechanism of HDH.

2.3.3 Implications for haematological re-acclimatisation to hypoxia

Evidence has been presented that the early haemoconcentration of HAH is altered during re-acclimatisation (RA) as compared to during an initial acclimatisation (IA). Studying rabbits exposed to IH (6 $h \cdot d^{-1}$ at 6000 m for 30 d), Jain and colleagues (1978) demonstrated a larger [Hb] response that occurred more rapidly during RA than IA when the two exposures were separated by 30 d normoxic DA. This was attributed to an increased haemoconcentration response during RA that was severe enough to cause the death of 4 animals, apparently from pulmonary haemorrhage. Humans exposed for 30 h to hypoxia before and 8 d after acclimatisation at 4300 m for 16 d, also showed an increased haemoconcentration response during the second acute exposure (Lyons et al 1995). However, this was considered an improved response as it was accompanied by a reduction in the severity of AMS, which is known to correlate with fluid retention during hypoxic exposure (Loeppky et al 2005). PV was also reduced in individuals re-exposed to high altitude compared to those exposed for the first time (Singh et al 1990). Conversely, mountaineers exhibited a lesser haemoconcentration during acute hypoxic exposure 9 d after a 3-w expedition to 6768 m as compared to before acclimatisation (Savourey et al 2004). Interestingly, this too was considered indicative of an improved response because it suggested a reduction

in blood viscosity during re-exposure. Finally, Singh and colleagues (1988) reported fluid retention in soldiers re-exposed to high altitude compared to those exposed for the first time (Singh et al 1986), also suggesting that re-exposure puts individuals at greater risk for developing altitude illness. Interpretations of these limited and conflicting data are ambiguous; accordingly, the effect of prior acclimatisation on the haemoconcentration response to hypoxia remains unclear.

There is also theoretical and empirical substantiation that the erythropoietic component of HAH can be both improved and impaired during RA. Okunewick and Fulton (1970) postulated that prolonged hypoxic exposure might alter the stem cell population in such a way that it will respond more efficiently to future calls for erythropoiesis. This could be accomplished by increasing the pool of stem cells and/or by shortening the cell cycle to allow more cells to be recruited and differentiation to occur more quickly. On the other hand, apoptosis of erythropoietic stem cells caused by EPO withdrawal during HDH depletes the pool of erythroid progenitors available for recruitment (Mide et al 2001, Rice and Alfrey 2005).

The early erythropoietic response to acute hypoxic exposure has also been examined before and after extended exposure to high altitude. Savourey and colleagues (1996) reported a hypoxia-induced increase in [EPO] before an extended expedition to Mt. Everest (8848 m) and a decrease in [EPO] afterward. However, blood samples were collected only 50 min after initiation of hypoxia, and previous evidence indicates that observable changes in [EPO] are not measureable for at least 90 (Eckardt et al 1989) to 150 min (Knaupp et al 1992). More credible is a later finding by the same researchers that, compared to pre-expedition, the [EPO] response to a 4-h hypoxic exposure was significantly reduced at 9 d RSL following a 3-w expedition to 6768 m (Savourey et al 2004). Reduced [EPO] was accompanied by increased arterial oxygenation suggesting a reduced hypoxaemic stimulus for EPO production; however, [EPO] was still reduced for a given C_aO_2, indicating a true blunting of the response.

Although evidence of a blunted [EPO] response during hypoxic re-exposure suggests an impaired erythropoietic response, it appears to be offset by increased sensitivity of erythroid progenitors to EPO. Savourey and colleagues (2004) report an increased reticulocyte response in spite of decreased [EPO] as evidence of increased sensitivity of erythroid precursors to EPO. However, the slight but significant

increase in RC is reported after only 3-4 h in hypoxia, whereas a reticulocyte response is typically not measureable for at least 24 h after hypoxia onset (Schobersberger et al 2005), raising questions about the validity of this result. Support, however, comes from earlier work by Okunewick and colleagues. Erythropoiesis (measured by ^{59}Fe uptake) was blunted in polycythaemic mice compared to control animals (1970), but the erythropoietic response to exogenous EPO was greater in mice made polycythaemic by hypoxic exposure versus transfusion (1969, 1970). This suggests that, although erythropoiesis was expectedly inhibited by the existing polycythaemia (Misago et al 1986), recent hypoxic acclimatisation increased sensitivity to EPO.

There is abundant evidence that HAH can be altered by previous acclimatisation to hypoxia. However, the majority of works have examined only the early erythropoietic responses hypoxic re-exposure (Lyons et al 1995, Savourey et al 1996, Savourey et al 2004), and the ultimate impact of reported changes in haemoconcentration, EPO production and EPO sensitivity on the [Hb] response over days and weeks in hypoxia are unknown. With more extended exposures, Jain and colleagues (1978) suggested that altered responses during RA can have devastating consequences for the organism. However, the IH protocol employed in this work is not representative of any naturally occurring condition and evidence from other paradigms of ISH suggest that HAH is not impaired by repeated acclimatisation and DA. South American miners commonly work 7-20-d shifts at altitudes of 3600–4500 m separated by 3-7 d at lower altitudes or SL (Richalet et al 2002a). Even after alternating between high and low altitudes for up to 22 y, mine workers exhibit qualitatively normal changes in [EPO] and RC with each exposure cycle (Gunga et al 1996, Richalet et al 2002b, Heinicke et al 2003). However, the magnitude of some responses does seem to change over time, with cumulative increases in Hct over 19 mo in individuals alternating every 7 d between 3600-4800 m and SL (Richalet et al 2002a). High-altitude values of Hct parallel the pre-exposure values measured at SL, suggesting that gradual increases in Hct are more a result of incomplete HDH rather than augmented HAH responses over time. [EPO] data indicate a decline in pre-exposure values over time, and while [EPO] measured at high altitude also tended to decrease over time, the result was not significant (Richalet et al 2002b). In a slightly different paradigm, an interesting comparison of early erythropoietic responses was made between soldiers who had alternated between 11 d at 3500 m

and 3 d at SL for 6 months and officers who had alternated between altitudes every 3.5 d for 22 y (Heinicke et al 2003). Similar to the work by Richalet and colleagues (Richalet et al 2002b), both soldiers and officers demonstrated elevated Hct and [Hb] at altitude that did not normalise during the brief DA period between high-altitude shifts. In both groups, return to high altitude elicited a similar spike in [EPO], accompanied by an increase in soluble transferrin receptor (sTfR) in soldiers but not officers. Rather, the officers demonstrated significantly elevated [ferritin] throughout the study. The authors suggest this is reflective of enhanced iron storage in officers as a long term response to 22 y of ISH, but [ferritin] only reflects total iron stores in steady state conditions. Therefore, it is more feasible in this case, that serum ferritin reflects the transport of iron liberated during a recent bout of neocytolysis. Although these studies have contributed much towards understanding the process of hypoxic RA, the relatively brief (7-11 d) re-exposures are still of insufficient duration to examine the longer term effects of previous acclimatisation and DA on erythrocyte maturation and Hb synthesis.

2.4 Summary and conclusions

DA is a key component of the process of hypoxic acclimatisation and an improved understanding of DA could benefit those who alternate between high and low altitude for work, recreation, or athletic training. In addition, DA has relevance to a number of clinical populations and an increased awareness of its time course and mechanism could inform treatment for cardiovascular, respiratory and renal disease.

2.4.1 Ventilatory de-acclimatisation and re-acclimatisation

VDH occurs relatively quickly and, with only a few exceptions, normoxic P_aCO_2 is normalised within 1 w RSL, regardless of the duration and severity of initial exposure. $\dot{V}_E$ during exercise and in hypoxia may remain elevated for longer, possibly contributing to the persistence of hypoxia tolerance upon RSL. The rate of VDH does not parallel the gradual reduction of O_2 and CO_2 sensitivity, and alterations in neither peripheral nor central chemoreceptors alone can account for VDH. Rather, it appears that sensitised CNS integration of chemoreceptor input is responsible for VDH. Thus, although VDH occurs at a similar rate to VAH, different mechanisms seem to be responsible for the two processes. Data from a model of IH exposure suggest that VAH might occur more quickly following previous exposure to hypoxia, but this

has yet to be demonstrated with exposure and re-exposure to sustained hypoxia. A more rapid VAH during hypoxic re-exposure could also contribute to the persistence of hypoxia tolerance reported in studies that made assessments during re-exposures of 8-30 h (Lyons et al 1995, Usaj and Burnik 2009) but not likely in those that used re-exposures of only 1-4 h (Savourey et al 1996, Beidleman et al 1997, Boning et al 2001).

2.4.2 Haematological de-acclimatisation and re-acclimatisation

HDH occurs more slowly than VDH and at a similar, if not slightly faster, rate that HAH. Although the time required for complete HDH correlates with the exposure "dose" and initial magnitude of HAH it does not seem possible to predict HDH time based on these measures. HDH is initiated by a re-expansion of PV, and following relatively short hypoxic exposures this mechanism may account almost entirely for normalising [Hb]. However, if HAH is of sufficient duration to induce a true polycythaemia then the process of neocytolysis is primarily responsible for reducing RCV upon return to normoxia. Mechanisms of increased phagocytosis during neocytolysis are well-described but the exact manner in which young erythrocytes are preferentially targeted by macrophages is not entirely clear. To date, the best explanation is reduced surface expression of CD47 by young cells. Erythropoiesis is also inhibited during HDH with persistent blunting of the [EPO] response to hypoxia but increased sensitivity of erythroid progenitors to stimulation by EPO.

Persistently elevated [Hb] would be beneficial during hypoxic re-exposure and likely contributes to improved hypoxia tolerance assessed in the days and weeks following RSL. Ample evidence indicates that hypoxic acclimatisation followed by DA can influence subsequent HAH in a number of ways - via changes in PV, the erythroid stem cell population, O_2-sensitive mechanisms that control EPO production, and sensitivity of erythroid progenitors to the action of EPO. However the effects on haematological RA cannot be consistently described as either advantageous or disadvantageous. Regardless, HAH remains tightly regulated even after years of alternating between high and low altitudes. Numerous questions remain about whether alterations in early erythropoietic responses during hypoxic re-exposure will have longer term implications in terms of the resultant increases in RCV, [Hb] and O_2 carrying capacity.

CHAPTER 3. DESTINATION GOKYO: ACCLIMATISATION IN TREKKERS WITH AND WITHOUT RECENT EXPOSURE TO HIGH ALTITUDE

3.1 Introduction

Acclimatisation to the hypoxia of high altitude has been studied rigorously in humans and other animals with immense clinical and academic interest in the process (Pugh 1962, Dempsey et al 1974, Schoene et al 1984, Cogo et al 1997, Houston 1997, Peacock and Jones 1997, Richalet et al 1999, Hupperets et al 2004). The vast majority of these studies have examined individuals with no prior or recent hypoxic experience, but in reality many people alternate between extended periods at higher and lower altitudes for work and recreation (Gunga et al 1996, Vargas et al 2001, Richalet et al 2002a, Heinicke et al 2003, Farias et al 2006, Brito et al 2007, Prommer et al 2007). For trekkers, mountaineers, military personnel and high-altitude labourers, extended periods of altitude exposure are often interrupted by days, weeks or months in normoxic or less hypoxic conditions. The existing body of literature may not accurately reflect the acclimatisation process in altitude-experienced individuals.

Ideas about how previous hypoxic exposure might affect future acclimatisation and altitude tolerance vary. It has been claimed that previous exposure to high altitude might impair subsequent acclimatisation and pre-dispose re-acclimatisers to illnesses like high-altitude pulmonary oedema (Menon 1965, Singh et al 1988). However, it has been more widely speculated that individuals previously exposed to altitude will acclimatise faster during subsequent exposures than individuals without prior experience (Milledge et al 1983, Mani 1990) with postulated benefits of previous exposures lasting up to several months (Hultgren 1997, Ward et al 2000c). For this reason, several research groups have championed the concept of pre-acclimation to improve safety and climbing performance during subsequent expeditions (Benoit et al 1992, Richalet et al 1992, Savourey et al 1994, Savourey et al 1996, Beidleman et al 2004, Fulco et al 2011).

The effects of altitude pre-exposure are no longer purely speculative, as empirical demonstrations of benefits have recently been reported. Pre-exposure was shown to improve overall function in climbers: those who had recently been to altitude were more likely to reach the summit of Mont Blanc and climbed faster than individuals without recent altitude exposure (Tsianos et al 2006). However, the effect of pre-exposure on clinical outcomes is not entirely clear, since pre-exposure reduced the prevalence of acute mountain sickness (AMS) in some studies (Bircher et al 1994, Schneider et al 2002, Ziaee et al 2003, Pesce et al 2005) but not others (O'Connor et al 2004). In these survey studies, the dose of recent altitude exposure is vague. It is unclear whether all or some pre-exposed individuals spent enough time at altitude to acclimatise or whether recent experience represented brief forays that may have otherwise physically or psychologically prepared them for strenuous exercise in the mountains.

The goal of this field study was to compare the acclimatisation process in individuals with and without recent sustained exposure to altitude. A secondary goal was to determine whether improvements in acclimatisation response would demonstrate a dose response for the severity and duration of altitude pre-exposure. Two novel approaches to address the effect of pre-exposure on the acclimatisation process are offered: 1) trekkers were monitored throughout their gradual ascent to very high altitude; and 2) physiological markers of acclimatisation were assessed at the highest point of the trek.

3.2 Methods

All experimental protocols were approved by the University of British Columbia Clinical Research Ethics Board and the Nepal Health Research Council. Written informed consent was obtained from all participants before commencing data collection.

Individuals completing the Gokyo Valley trek in the Solu Khumbu region of Nepal were invited to participate in a field study of acclimatisation to high altitude. Healthy, low altitude natives who flew from Kathmandu to Lukla and had not been above 3000 m in the previous week were eligible to participate. Non-English-speaking trekkers could participate if a translator was available. Participants were recruited at the trailhead in Lukla (2840 m), in the village of Gokyo (4750 m) or on the summit of Gokyo Ri (5360 m). The ascent profile of the most common Gokyo Valley trekking itinerary is shown in FIGURE 3.1. All

participants completed a physical activity and altitude history questionnaire. Non-exercise maximal oxygen consumption ($\dot{V}O_{2\text{-max}}$) was estimated from sex, age, body mass index, perceived functional ability and current physical activity as described by Bradshaw et al (2005). This method is described in APPENDIX V.1. Two different types of data were collected and participants took part in one or both of the study components.

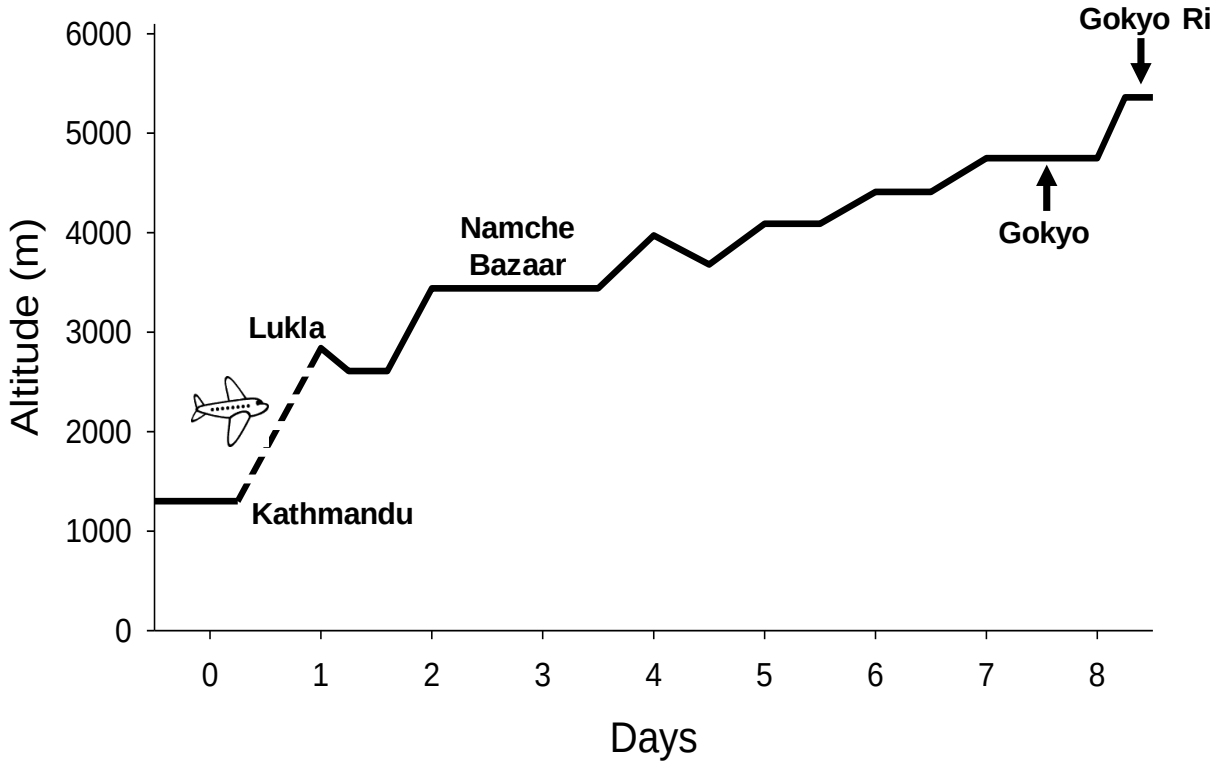


FIGURE 3.1 Ascent profile of a typical itinerary for the Gokyo Valley trek. Trekkers fly from Kathmandu (1350 m) to Lukla (2840 m) and walk between two and eight hours each day, reaching the village of Gokyo (4750 m) on Day 7 and ascending to the viewpoint of Gokyo Ri (5360 m) on Day 8.

3.2.1 Trekking logs

Individuals who were recruited at the trailhead kept a log throughout the trek from Lukla to Gokyo. Self-reported data included a morning and evening assessment of AMS by Lake Louise Questionnaire (LLQ; Hackett and Oelz 1992), as well as daily itinerary, trekking time and medication used. The LLQ can be seen in APPENDIX V.2. The rating of perceived trekking exertion (RPE) was determined each day using the Borg CR10 scale (Borg 1982). This "session RPE" has been previously validated to evaluate the overall intensity of an extended endurance effort (Seiler and Kjerland 2006).

3.2.2 Assessment on Gokyo Ri

Atop the summit of Gokyo Ri, participants completed an LLQ and reported trekking time and exertion for the hike up from the village of Gokyo. After resting on the summit for at least 30 min, resting physiological function was also assessed. Participants were fitted with an automated blood pressure cuff (A&D Medical, San Jose, CA, USA), a Polar RS800sd heart rate transmitter (Polar Electro Oy, Kempele, Finland) and a reflectance pulse oximeter sensor on the forehead (Model 8000R, Nonin Medical Inc., Minneapolis, MN, USA). Air temperature during data collection ranged from +10 to -15 °C therefore participants wore a toque, were gently wrapped in blankets and lay supine on thermal insulating mats to stay as warm as possible during the assessment. Heart rate (f_H) and oxyhaemoglobin saturation (S_pO_2) were recorded continuously for 10 min at 1 kHz and 10 Hz, respectively. Blood pressure was measured after 8 and 10 min of rest.

3.2.3 Data analysis

Heart rate data were extracted from the Polar monitors using Polar ProTrainer 5 (Version 5.30.154, Polar Electro Oy, Kempele, Finland) and pulse oximetry data were extracted using nVision software (Version 5.1e, Nonin Medical Inc., Minneapolis, MN, USA). f_H and S_pO_2 were averaged over the final 5 min of rest. Mean values for systolic (SBP) and diastolic (DBP) blood pressures were used to calculate mean arterial pressure (MAP) as:

$$MAP = 2/3*DBP + 1/3*SBP$$

Participants were separated post hoc into two groups based on their self-reported altitude history. Recent altitude exposure was defined as having spent two or more nights above 3000 m elevation in the 30 d before starting the current trek. Those without recent altitude exposure were considered initial acclimatisers (IA) and those with recent exposure were considered re-acclimatisers (RA). Independent t-tests were used to examine differences between IA and RA. Statistical significance was assumed when $p < 0.05$.

3.3 Results

A total of 93 volunteers were recruited into the study but 21 trekking logs were never recovered and other

problems with missing or incomplete information further reduced the volume of usable data. Ultimately, data sets from 62 participants (45% female) were used in analyses. These individuals spanned a wide age range (20 – 74 years) and represented eighteen nationalities. None reported a serious respiratory, cardiovascular, or blood disorder. Five participants reported smoking behaviour during normal life at home (ranging from five cigarettes per month to 20 per day) which ceased or was greatly reduced during the trek (maximum two cigarettes per day). RA trekkers reported a variety of recent altitude experience (see TABLE 3.1) but had been at low altitude for 14 ± 6 (range 7 – 30) d before starting the Gokyo Valley trek.

TABLE 3.1 Description of the most recent altitude exposure for re-acclimatising participants. Participants 1–20 completed trekking logs and 1-12 were assessed on Gokyo Ri. ALT_{max} is maximum altitude recently attained, $T_{>3000m}$ is number of nights spent above 3000 m and $T_{<3000m}$ is the number of nights below 3000 m before starting Gokyo Valley trek.

Participant	**Most recent altitude exposure**	**ALT_{max} (m)**	**$T_{>3000m}$ (# nights)**	**$T_{<3000m}$ (# nights)**
1	Annapurna Circuit	5416	8	21
2	Annapurna Circuit (incomplete)	4250	8	7
3	Langtang	5500	10	6
4	Gokyo Ri	5360	9	12
5	Gokyo Ri	5360	9	12
6	Tibet	5200	44	12
7	Annapurna Circuit	5416	8	21
8	Alps	3500	2	10
9	Gokyo Ri	5360	9	12
10	Gokyo Ri	5360	9	12
11	Gokyo Ri	5360	9	12
12	Gokyo Ri	5360	9	12
13	Pakistan	5643	7	30
14	Inca Trail	4200	12	12
15	Inca Trail	4200	12	12
16	Annapurna Circuit	5416	8	8
17	Annapurna Base Camp	4131	3	9
18	Annapurna Base Camp	4131	3	9
19	Annapurna	3600	5	21
20	Annapurna	5000	5	21

3.3.1 Trekking logs

Complete trekking logs were collected from 50 individuals and the IA (n = 30) and RA (n = 20) groups are described in TABLE 3.2. Trekkers followed either a 7 or 8-d itinerary from Lukla with no difference between IA and RA in number of days to reach Gokyo. RA trekkers were significantly younger than IA trekkers ($p = 0.03$) but otherwise the two groups were homogeneous.

TABLE 3.2 Description of study participants who completed trekking logs during a trek from Lukla to Gokyo, Nepal. Values represent group mean ± standard deviation (range). Statistical differences between participants on initial acclimatisation (IA) and re-acclimatisation (RA) treks are marked as * ($p < 0.1$) or ** ($p \leq 0.05$).

	IA	**RA**
N	30	20
Sex (% female)	47	40
Age (years)	37 ± 12 (24 - 63)	31 ± 10** (20 – 54)
BMI ($kg \cdot m^{-2}$)	22.7 ± 2.7 (17.1 – 28.2)	22.8 ± 2.3 (18.0 – 26.2)
$\dot{V}O_{2\text{-max}}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	47 ± 7 (30 – 59)	47 ± 7 (32 – 57)
Trekking days to Gokyo	7.5 ± 0.7 (7 - 9)	7.3 ± 0.6 (6 – 8)

Data from trekking logs is summarised in TABLE 3.3. RA trekkers walked approximately 20% faster than IA trekkers ($p < 0.01$) but there was no difference between groups in total perceived trekking exertion. There was no difference in acetazolamide consumption between groups but RA trekkers reported fewer symptoms of AMS ($p = 0.02$) and took less headache medication than IA trekkers ($p = 0.046$).

3.3.2 Assessment on Gokyo Ri

Twenty-five individuals (IA: n = 13; RA: n = 12) were assessed who completed the traditional Gokyo Valley trek, summiting Gokyo Ri 8 or 9 d after flying into Lukla from Kathmandu. IA and RA trekkers are described in TABLE 3.4. On average, RA trekkers were 12 y younger than IA trekkers ($p = 0.01$) and

tended to have a higher predicted $\dot{V}O_{2\text{-max}}$ ($p = 0.06$). Data were also collected for an additional group of individuals ($n = 5$) who had spent several additional days above 4000 m (including three or four day trips over 5000 m) before being assessed on Gokyo Ri. None of these individuals had recent altitude exposure but were assumed to be better acclimatised than IA individuals due to their extended exposure to very high altitude. This group (IA+) summitted Gokyo Ri 13–21 d after arriving in Lukla and was used to determine whether the assessment on Gokyo Ri could detect differences in acclimatisation status by comparison with IA. No descriptive data is available for IA+.

TABLE 3.3 Results from trekking logs completed throughout an initial acclimatisation (IA) or re-acclimatisation (RA) trek from Lukla to Gokyo, Nepal. Values represent group mean ± standard deviation (range). Statistical differences between IA ($n = 30$) and RA ($n = 20$) are marked as ** ($p \leq 0.05$).

	IA	**RA**
Total trekking time (h)	21.4 ± 3.1 (14.3 – 26.8)	17.1 ± 3.3** (12.7 – 22.5)
Total perceived exertion (∑ session RPE)	19 ± 5 (11 - 30)	19 ± 5 (13 – 36)
Total AMS score	15 ± 13 (0 – 52)	9 ± 8** (0 – 26)
Acetazolamide consumed (total mg)	258 ± 510 (0 – 2000)	168 ± 316 (0 – 1000)
Analgesics consumed (total treatments)	1.2 ± 2.1 (0 – 8)	0.3 ± 0.7** (0 - 2)

Results of the assessments on Gokyo Ri are given in TABLE 3.5. The RA group completed the hike from Gokyo to Gokyo Ri faster than the IA group ($p = 0.04$) but there was no difference in perceived trekking exertion. There were no differences between IA and RA in AMS score or in resting f_H, DBP or MAP. However, S_pO_2 was significantly elevated ($p = 0.01$) and SBP tended to be lower ($p = 0.06$) in RA. There were no differences in AMS score between IA and IA+ but resting f_H was lower ($p = 0.03$) and S_pO_2 was higher ($p = 0.01$) in IA+. Comparisons of blood pressure between IA and IA+ show opposite results to the comparisons between IA and RA. SBP was not different between IA and IA+ but DBP ($p = 0.01$) and MAP ($p = 0.05$) were higher in individuals who had spent more time at high altitude.

TABLE 3.4 Description of study participants who trekked from Lukla to Gokyo, Nepal and were assessed on the summit of Gokyo Ri (5360 m). Values represent group mean ± standard deviation (range). Statistical differences between participants on initial acclimatisation (IA) and re-acclimatisation (RA) treks are marked as * ($p < 0.1$) or ** ($p \leq 0.05$).

	IA	**RA**
N	13	12
Sex (% female)	38	50
Age	43.1 ± 15.6	31.0 ± 8.9**
(years)	(24.9 - 74.5)	(20.4 – 47.9)
BMI	22.7 ± 2.4	22.3 ± 1.8
($kg \cdot m^{-2}$)	(18.6 - 24.7)	(18.6–25.2)
$\dot{V}O_{2\text{-max}}$	43 ± 6	47 ± 8*
($mL \cdot kg^{-1} \cdot min^{-1}$)	(34 – 56)	(34 – 57)

TABLE 3.5 A comparison of study participants on Gokyo Ri who have completed an initial acclimatisation (IA, n=13), re-acclimatisation (RA, n = 12) or acclimatisation-plus (IA+, n = 5) trek. Values represent group mean ± standard deviation (range). Statistical differences from IA are marked as * ($p < 0.1$) or ** ($p \leq 0.05$).

	IA	**RA**	**IA+**
Trekking time	2.1 ± 0.6	1.6 ± 0.7**	
(h)	(1.3 – 3.1)	(0.9 – 3.0)	
Trekking exertion	5 ± 2	6 ± 2	
(session RPE)	(1 – 8)	(4 – 8)	
AMS score	1.3 ± 1.7	0.8 ± 1.3	0.8 ± 0.4
	(0 – 5)	(0 – 3)	(0 – 1)
f_H	87 ± 15	88 ± 13	71 ± 17**
($beats \cdot min^{-1}$)	(60 – 106)	(74 – 113)	(57 – 90)
S_pO_2	78 ± 6	85 ± 6**	86 ± 4**
(%)	(68 – 86)	(77 – 96)	(81 – 91)
SBP	123 ± 10	116 ± 9*	124 ± 7
(mmHg)	(110-144)	(105 – 138)	(116 – 132)
DBP	85 ± 6	83 ± 5	94 ± 5**
(mmHg)	(75 – 95)	(73 – 92)	(90 – 102)
MAP	98 ± 7	94 ± 6	104 ± 5**
(mmHg)	(86 – 111)	(86 – 108)	(100-112)

3.4 Discussion

3.4.1 Trekking logs

A comparison of data from trekking logs indicates that the trekking experience was different for individuals who undertook the Gokyo Valley trek with versus without recent exposure to high altitude. Re-acclimatisers were able to trek faster without any increase in perceived trekking exertion and experienced milder symptoms of AMS relative to initial acclimatisers. The principle component of AMS, headache, was also markedly reduced in RA trekkers ($p = 0.02$), who required fewer analgesic treatments to manage pain. These data suggest that sustained high-altitude exposure within 30 d prior to commencing a trek to over 5000 m contributes to better overall altitude tolerance.

An age-difference existed between groups with RA trekkers being an average 6 y younger than IA trekkers. This age-discrepancy could account for some of the improved function seen in RA trekkers and therefore cannot be ignored. Indeed, linear regression using pooled IA and RA data shows that total trekking time increases with age ($p = 0.01$). To determine whether recent altitude exposure influenced trek time independent of age, groups were age-matched by removing the three youngest and oldest individuals from IA and RA, respectively. Re-analysis of IA ($n = 27$, 34 ± 9 years) versus RA ($n = 17$, 33 ± 10 years) showed that RA trekkers still completed the Gokyo Valley trek an average of 4 h, or 20%, faster than IA trekkers (21 ± 3 versus 17 ± 3 h, $p<0.01$). Age was not a significant predictor of AMS score or analgesic treatment and therefore the differences seen in these variables between IA and RA cannot be attributed to the age-difference between IA and RA.

3.4.2 Assessment on Gokyo Ri

Data collected atop Gokyo Ri provided an assessment of how adequately the acclimatisation process prepared trekkers to function and perform at the highest, and therefore most physiologically challenging, point of their trek. Improvements in exercise performance in trekkers with recent altitude exposure continued as the trek climbed steeply over 5000 m, with RA trekkers completing the final climb from Gokyo to Gokyo Ri 24% faster than IA trekkers.

Inconsistent with the trekking log result of improved clinical outcomes in RA trekkers, AMS scores recorded on the summit were not different between IA and RA. This may not be surprising given the high intra-group variability in AMS reported on Gokyo Ri. Also, the ability of AMS scores obtained immediately after arriving at the summit to discern acclimatisation status is questionable since there is no difference in AMS between IA and IA+. In fact, only 6 of 13 IA trekkers report any AMS symptoms on Gokyo Ri and only one meets the criteria for diagnosis with AMS (score ≥ 2, including headache, Roach et al 1993), making it difficult for AMS to be further reduced in either RA or IA+. It is possible that the trekking itinerary was conservative enough to allow adequate acclimatisation in virtually all trekkers to prevent altitude illness on the summit of Gokyo Ri. Alternatively, since AMS symptoms do not generally appear for at least 6-10 h after arriving at a given altitude (Hackett and Roach 2001), the brief (~60 min) exposure to 5360 m may have been too acute to elicit a pathophysiological response to that altitude. Assessing AMS after an extended stay on the summit (or even several hours after descending back to Gokyo) may have provided a better evaluation of the clinical aspects of tolerance to 5460 m.

It is well known that tachycardia occurs with acute exposure to hypoxia but f_H decreases back towards normal with acclimatisation (reviewed in Mirrakhimov and Winslow 1996). Low f_H at altitude may confer some advantage in that higher pulse rates are associated with reduced altitude tolerance and higher AMS scores (O'Connor et al 2004). f_H was significantly lower in IA+ than IA, further advocating the utility of f_H on Gokyo Ri as a marker of acclimatisation status. However, no difference in f_H between IA and RA is reported, suggesting that at least this aspect of cardiovascular acclimatisation is no more complete in trekkers with recent altitude exposure than those without.

Although this study was not designed to determine where in the oxygen cascade the advantage lies, it is evident that RA trekkers are better able to compensate for the reduced P_IO_2 on Gokyo Ri and maintain higher S_pO_2 than IA trekkers (85 ± 6 versus 78 ± 6%). It has been repeatedly demonstrated that S_pO_2 at a given P_IO_2 increases with improved acclimatisation (Bender et al 1989, Calbet et al 2003, Hupperets et al 2004) and acclimation (Richalet et al 1999, Beidleman et al 2004). The significant improvement in S_pO_2 seen in IA+ compared to IA provides further evidence that S_pO_2 on Gokyo Ri is an appropriate measure of acclimatisation status. Given that S_pO_2 values in the range of 78 to 85% reflect O_2-binding on the steep

part of the oxyhaemoglobin dissociation curve, the mean S_pO_2 difference between IA and RA may not reflect a large discrepancy in P_aO_2 between groups. However, even small changes in arterial oxygen tension have substantial biological impact in these trekkers. Assuming equal cardiac output, haemoglobin (Hb) concentration and Hb-O_2 affinity between groups, RA trekkers continuously deliver 9% more O_2 to tissues. The consequence of this disparity is magnified during exercise when improved O_2 delivery effects increases in $\dot{V}O_{2\text{-max}}$ and the capacity to perform higher intensities of metabolically demanding work. Thus, the pulse oximetry data provide some of the most compelling evidence that RA trekkers are better acclimatised than IA trekkers when they reach the summit of Gokyo Ri.

Differences between IA and RA that are unrelated to recent altitude exposure must also be considered when interpreting these results. Of the participants assessed on Gokyo Ri, RA trekkers were younger and tended to have higher predicted aerobic fitness than IA trekkers. Age was not a significant predictor of trek time, AMS score or f_H, but S_pO_2 decreased significantly with age ($p = 0.004$). However, as with the trekking log data set, when groups were age-matched (IA: n = 10, 35 ± 6 years versus RA: n = 9, 34 ± 8 years) re-analysis demonstrated that S_pO_2 was still better compensated in RA (n = 9, 86 ± 7%) than IA (n = 10, 80 ± 5%; $p = 0.03$). Trek time and S_pO_2 were not related to predicted $\dot{V}O_{2\text{-max}}$ but AMS score ($p = 0.09$) and f_H ($p = 0.06$) tended to decrease with improved predicted fitness. Thus, fitness-matching the groups would only reduce the likelihood of demonstrating reduced AMS scores and f_H in RA trekkers. Therefore, the reported differences in trek time and S_pO_2 between IA and RA trekkers on Gokyo Ri reflect grouping by recent altitude exposure and not differences in age or predicted $\dot{V}O_{2\text{-max}}$. Conversely, the strong positive relationship between age and blood pressure ($p < 0.01$) does seem to account entirely for the reduced SBP in RA versus IA. When groups are age-matched, the difference disappears (119 ± 7 versus 118 ± 9) indicating that recent altitude exposure has no effect on systolic blood pressure. DBP and MAP are significantly elevated in IA+ compared to IA and RA (both $p < 0.01$) but this likely reflects the progressive increases in sympathetic outflow, circulating catecholamines and vascular tone that accompany chronic exposure to hypoxia (Rostrup 1998, Fischetti et al 2000, Hansen and Sander 2003) and may not represent a “beneficial” aspect of the acclimatisation process per se.

3.4.3 General discussion

Data from both components of this study indicate that recent altitude exposure leads to improved clinical outcomes and exercise performance during subsequent sojourns to high altitude. Although this provides support for the hypothesis of a carry-over effect of recent altitude experience, a few pressing questions remain.

1. Were RA trekkers still acclimatised from previous exposure?

Since no data were collected from participants before undertaking the Gokyo Valley trek, it is unclear whether RA trekkers had fully de-acclimatised after their respective recent exposures to altitude. If not, improved altitude tolerance in RA may simply reflect partial acclimatisation in Lukla and a head start relative to IA trekkers. Although most IA and RA trekkers reported no AMS symptoms after the first night at altitude (Phakding, 2610 m), average AMS scores were higher in IA than RA trekkers at this point (0.5 ± 0.8 vs. 0.1 ± 0.3, $p = 0.01$). Although this suggests that RA trekkers started the trek in a more acclimatised state than IA trekkers it could also reflect improvements in the early acclimatisation process.

Although a surprising amount of data exists about the various physiological and anatomical aspects of acclimatisation and de-acclimatisation (DA, reviewed in CHAPTER 2), there is still some controversy about how long individuals retain functional aspects of acclimatisation. Beidleman and colleagues (1997) showed that exercise responses were retained to a large extent upon acute re-exposure to 4300 m after an initial 16 d acclimatisation followed by 8 d DA period. In a similar study, AMS scores were reduced during RA and S_pO_2, [Hb] and haematocrit were all higher in RA than during IA (Lyons et al 1995). Richalet and colleagues (1992) also assumed that mountaineers were still partially acclimated after 4-6 d at low altitude. These pre-acclimated climbers went on to ascend Mt. Everest much more quickly than the typical rate. In addition, when participants were re-exposed to hypoxia within ~9 d of leaving high altitude, P_aO_2, and S_aO_2, were maintained higher than before acclimatisation, perhaps at least partly because minute ventilation was increased, as indicated by lower P_aCO_2 and higher [$HCO3^-$] and pH (Savourey et al 2004). Although these data suggest that individuals remain at least partially acclimatised for up to 9 d DA at SL, many others have that reported that DA is complete within 1 w of return to sea

level (reviewed in CHAPTER 2). It is reasonable to expect that individuals in the RA group had de-acclimatised to different extents over 6 to 30 d at low altitude and ultimately one can only speculate about whether or not they were still partially acclimatised at the onset of the Gokyo Valley trek.

2. Are group differences explainable by superior trek-specific fitness in RA trekkers?

Although changes in body composition are known to follow trekking and climbing at high altitude (Krzywicki et al 1969, Rose et al 1988, Westerterp et al 2000) alterations in aerobic capacity that accompany weeks of strenuous mountain walking have not previously been documented. In a companion study, individuals who completed the trek to Gokyo Ri showed marked improvement in aerobic fitness, assessed indirectly by a decrease from pre- to post-trek in f_H and blood [lactate] for a given exercise intensity (see CHAPTER 4). These changes may not necessarily represent actual improvements in $\dot{V}O_{2\text{-max}}$, but it is clear that economy during trekking-specific stepping exercise increased. Although trekkers are likely de-training as they de-acclimatise from high altitude, some fitness improvements might also carry over to the next trek. Improved trek-specific fitness would contribute to the ability of re-acclimatisers to walk faster than initial acclimatisers. However, trek time is a complex variable that is determined not only by physiological limitations like aerobic capacity but also incorporates volitional behaviour. Therefore the powerful role of motivation – well known to be affected by altitude exposure (Richalet et al 1999, Ward et al 2000c) - in determining trek time must not be ignored.

On the other hand, enhanced fitness cannot explain improved clinical outcomes in RA trekkers. Although it has been postulated that enhanced aerobic fitness both increases (Cymerman et al 1979) and decreases (Hackett and Rennie 1983) the risk of developing AMS, the current consensus is that there is no link between fitness and AMS susceptibility (Milledge et al 1991, Honigman et al 1995, Schneider et al 2002).

3. Does altitude tolerance in RA trekkers show a dose response with pre-exposure?

Although detailed itineraries of participants' recent altitude exposure were not usually available, attempts were made to quantify their severity and duration as well as time elapsed since exposure before RA trekkers started the Gokyo Valley trek (see TABLE 3.1). It was anticipated that RA trekkers who had spent

more time at higher altitudes more recently would demonstrate the greatest degree of re-acclimatisation on the Gokyo Valley trek. However, neither maximum recent altitude (ALT_{max}), duration of recent exposure (number of nights above 3000 m; $T_{>3000m}$) nor time between recent exposure and current exposure (number of nights below 3000 m; $T_{<3000m}$) was correlated with any outcome variable from the trekking logs or Gokyo Ri assessments in RA trekkers. Nor was there an association between any logical mathematical combination of these potential covariates ($ALT_{max} \cdot T_{>3000m}$, $ALT_{max} \cdot T_{>3000m}/T_{<3000m}$ or $T_{>3000m}/T_{<3000m}$) and any outcome measure. It is possible that individual variability masks any relationship between aspects of the recent exposure and performance and function during the Gokyo Valley trek.

4. Are there non-physiological explanations for improved altitude tolerance in RA trekkers?

Although repeatedly validated, both self-reported AMS scores and ratings of perceived trekking exertion are subjective assessments that can be affected by psychological factors. It is known that even elite mountaineers experience anxiety during expeditions to high altitude (Noël-Jorand et al 1995). Fear of illness, of appearing weak in front of others and even of death is likely enhanced in individuals with less altitude experience and greatest in those who are completely altitude-naïve. First-time trekkers are often extremely anxious about their ability to tolerate altitude and can be hyper-aware of sensations thought to be related to altitude illness (discussed in CHAPTER 6). These individuals may have an exaggerated perception of breathlessness, headache and other AMS symptoms and their lack of experience may lead to inflated self-reports of RPE and AMS. In support, 8 IA trekkers who had never before been above 3000 m (altitude-naïve), reported higher AMS scores (27 ± 16) than 22 IA trekkers who had previously experienced the process of acclimatisation (non-naïve, 12 ± 10, $p < 0.01$). In fact, elevated AMS scores in naïve trekkers account for the difference between IA and RA; when comparing RA to non-naïve IA trekkers the difference in AMS score disappears. Interestingly, headache score was not different between naïve and non-naïve IA trekkers, and both headache score and analgesic usage was still lower in RA than IA even after naïve IA trekkers were removed from the group.

It is also possible that RA (and non-naïve IA) trekkers report less AMS than naïve IA trekkers because they represent a group that has self-selected to return to altitude. Individuals with very poor altitude

tolerance are likely reluctant to repeat an unpleasant and potentially dangerous experience, and their absence from the pool of returnees could contribute to reduced AMS scores in non-naïve trekkers. This issue has been largely ignored by other researchers when examining the effects of previous exposure (Koller et al 1991a, Bircher et al 1994, Schneider et al 2002, O'Connor et al 2004, Pesce et al 2005). Only two IA trekkers assessed on Gokyo Ri had no previous altitude experience and therefore no attempt was made to compare altitude naïve and non-naïve participants. Removing naïve individuals from the analysis did not alter the original results, indicating that self-selection for travel to altitude is not the basis of faster trek times and improved S_pO_2 in RA trekkers on Gokyo Ri.

3.5 Conclusion

Altitude tolerance was enhanced in trekkers with recent altitude exposure, as evidenced by improved exercise performance, clinical outcomes and S_pO_2 in RA compared to IA trekkers. However, it is not certain that all these improvements are the result of having recently acclimatised to altitude. Faster trek times might primarily reflect fitness gained during recent treks. Clinical outcomes are improved in all previously exposed participants, which might reflect increased anxiety in altitude-naïve trekkers or the absence of altitude-intolerant individuals from the group of returning trekkers. However, it is difficult to argue that resting S_pO_2 at 5360 m could reflect anything but increased compensation in the early steps of the oxygen transport cascade, with improved O_2 loading and a greater capacity for O_2 delivery. Although RA trekkers were better acclimatised on Gokyo Ri, it remains unclear whether this was due to an increased rate of acclimatisation or because they simply started the trek in a more acclimatised state than IA trekkers. This matter could be clarified by establishing the de-acclimatisation status of participants immediately before they re-acclimatise.

CHAPTER 4. DESTINATION GOKYO: REPEATED ACCLIMATISATION IN HIGH-ALTITUDE TREKKERS

4.1 Introduction

The process of acclimatisation to high altitude has primarily been studied in individuals with no prior or recent exposure to hypoxia. However, trekkers, mountaineers, mine and observatory workers and military personnel commonly alternate between extended periods at high and low altitude (Forster 1984, Gunga et al 1996, Vargas et al 2001, Richalet et al 2002a, Heinicke et al 2003, Farias et al 2006, Brito et al 2007, Prommer et al 2007). Some authors have concluded that prior hypoxic exposure leads to adverse responses during re-exposure. High-altitude pulmonary oedema (HAPE) was initially recognised as a condition that predominantly occurs upon re-entry to high altitude after a period at lower altitude (Hultgren et al 1961, Marticorena et al 1964, Menon 1965). Although it is now clear that HAPE also occurs during first-time ascents to altitude (reviewed in Bärtsch et al 2003), Singh and colleagues (1988) suggested that persistently elevated plasma volume (PV) puts individuals recently exposed to high altitude at increased risk for developing HAPE. Interestingly, work in rabbits suggested that recent exposure to altitude is detrimental for the opposite reason; 25% of animals recently exposed to hypoxia did not survive an identical hypoxic re-exposure, apparently due to a severe reduction in PV that placed excessive stress on the cardiovascular system (Jain et al 1978). Despite these reports of deleterious effects of recent altitude exposure, it is now a more commonly-held impression that the acclimatisation process is facilitated by previous high-altitude experience (Mani 1990, Ward et al 2000c, 2000b, Katayama et al 2005a). Although ample indirect evidence supports the notion of improved hypoxic tolerance during re-acclimatisation (discussed below), a direct comparison of the processes of initial acclimatisation (IA) and re-acclimatisation (RA) to sustained high altitude is not yet available.

Numerous studies demonstrate that prior exposure to hypoxia improves altitude tolerance, clinical outcomes, functional capacity and exercise performance during subsequent re-exposure to acute hypoxia

(Brown 1989, Lyons et al 1995, Savourey et al 1996, Beidleman et al 1997, Savourey et al 2004). Improved clinical and functional outcomes have also been reported during sustained re-exposures in high-altitude trekkers (see CHAPTER 3) and climbers (Schneider et al 2002, Pesce et al 2005, Tsianos et al 2006) with recent altitude experience. In addition, there is some evidence that individuals who are repeatedly exposed to sustained altitude acclimatise faster, more efficiently and more completely than individuals exposed for the first time (Milledge et al 1983, Koller et al 1991a, Farias et al 2006, Wu et al 2009). Taken together, these data suggest that altitude tolerance is partially retained if re-exposure occurs before de-acclimatisation (DA) is complete. Recent exposure likely provides a 'head start' that can save time and possibly increase safety during subsequent acclimatisation to altitude (Richalet et al 1992). The degree of acclimatisation attained may even be progressively augmented with consecutive sustained exposures (Richalet et al 2002a, Heinicke et al 2003, Wu et al 2009).

Although none of the above works provide a direct comparison of acclimatisation responses throughout IA and RA, two additional studies come closer to accomplishing this objective. Using repeated series of intermittent hypoxia exposures, it has been shown that haematological (Jain et al 1978) and ventilatory (Katayama et al 2005a) compensation occur more rapidly during RA than IA in rabbits and humans, respectively. However, there are well-known and important differences between physiological responses to intermittent and continuous hypoxia (reviewed in Sheel and MacNutt 2008) and it cannot be assumed the aforementioned results would be repeatable using a different paradigm of hypoxic exposure. Therefore, the primary objective was to provide the first direct and comprehensive comparison of the time course and magnitude of acclimatisation responses throughout IA and RA to sustained high-altitude exposure.

Important differences have been noted between the processes of acclimation and acclimatisation during simulated and real expeditions to high altitude, likely attributable to differences in energy expenditure, food availability and cold exposure (West 1988). In order to assess IA and RA in a realistic setting, the research questions were addressed using a field study. Altitude tolerance and acclimatisation responses were monitored throughout a popular progressive trek to high altitude in the Everest region of Nepal.

Although many individuals either precede or follow this journey with other similar treks in the Himalaya, the same trek was repeated two times to control for differences in terrain, altitude profile and ascent rate. It was hypothesised that acclimatisation would be facilitated during RA compared to IA, demonstrated by improved cardiorespiratory and haematological responses, clinical outcomes, and exercise capacity. An assessment of the DA status of participants prior to re-exposure would indicate whether improved RA was simply due to the retention of previous acclimatisation status upon re-exposure or due to fundamental improvements in the acclimatisation process.

There is an impression that individual altitude tolerance is generally repeatable across exposures in that prior history of mountaineering success and altitude illness predicts clinical and functional outcomes during future exposures (West 1993, Ward et al 2000b, Schneider et al 2002, Pesce et al 2005). In addition, acute mountain sickness (AMS) scores during brief (1 - 1.5 d) altitude exposures have shown good intra-individual repeatability (Robinson et al 1971, Forster 1984). However, there are no known data on the repeatability of haematological or cardiorespiratory acclimatisation responses during an extended high-altitude exposure. Thus, a secondary objective of this research was to evaluate the intra-individual repeatability of the magnitude and time course of acclimatisation responses.

4.2 Methods

Six young, healthy lowlanders were comprehensively monitored throughout two repeated treks up the Gokyo Valley in the Solu Khumbu region of Nepal (maximum altitude 5360 m). Both the trekking itinerary and timing of data collection were identical between the IA and RA treks, which were separated by 10 d DA in Kathmandu (1300 m). All study procedures were approved by the University of British Columbia Clinical Research Ethics Board the Nepal Health Research Council. Written informed consent was obtained from all participants before commencing data collection.

4.2.1 Trekking itinerary

The ascent profile of the Gokyo Valley trek can be seen in FIGURE 4.1. After at least 2 w in Kathmandu, participants flew to Lukla (2840 m) to begin the trek. Participants walked for 6 d and took either one (Namche Bazaar, 3440 m, n = 2) or two (Namche Bazaar and Macchermo, 4410 m, n = 4) acclimatisation

days en route to Gokyo (4750 m). These represent the two most common schedules for completing this trek and each participant kept the same schedule between IA and RA. Participants ascended Gokyo Ri (5360 m) on their seventh trekking day and returned to Gokyo for a second night before re-tracing their steps to Lukla over 3 d. After a 10-d DA period in Kathmandu, participants flew to Lukla to repeat the entire trek.

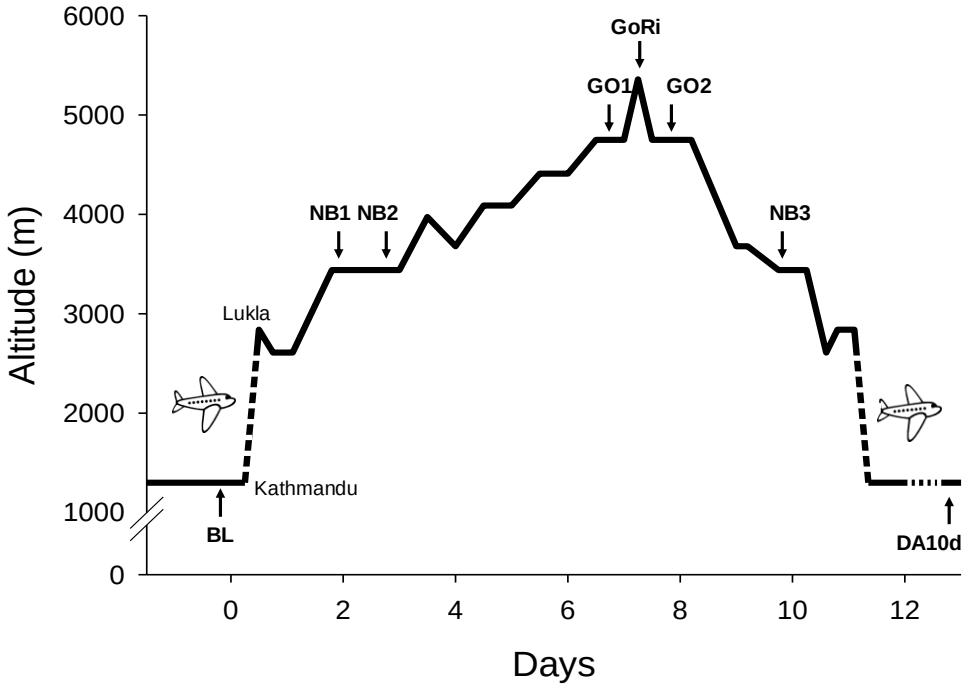


FIGURE 4.1 Ascent profile of the Gokyo Valley trek. Data collection took place in Kathmandu (BL, DA10d), Namche Bazaar (NB1, NB2 and NB3), Gokyo (GO1 and GO2) and atop Gokyo Ri (GoRi). After a 10-d DA period, participants repeated the entire trek with an identical data collection itinerary.

One participant became very ill during the RA trek with gastrointestinal illness and was unable to maintain the trekking schedule and complete data collection. This participant completed a second 10-d DA period in Kathmandu before repeating the trek from Lukla to Gokyo Ri a third time. Data collected on the third trek were used to represent RA for this participant.

4.2.2 Data collection overview and schedule

Each participant maintained a daily trekking log throughout IA and RA. In addition, a battery of tests was repeated a total of 16 times throughout the study period. Data collection included blood sampling, assessment of AMS, and measurement of cardiorespiratory function during rest and during a standardised

exercise challenge. Finally, exercise intensity throughout each day of trekking was continuously recorded during both IA and RA.

The data collection schedule is shown in FIGURE 4.1. Baseline data were collected twice in Kathmandu before departing for the first trek. Data from these two time points were averaged to provide a single baseline reference point (BL_{IA}). Data collection was repeated upon arrival in Namche Bazaar (NB1) and again 24 h after arriving (NB2); upon initial arrival in Gokyo (GO1), on the summit of Gokyo Ri (GoRi, AMS scores and resting data only), after returning to Gokyo from Gokyo Ri (GO2); and upon descent back to Namche Bazaar (NB3). After a day of trekking, participants rested for ≥ 2 h before beginning data collection (≥ 30 min on GoRi). Data collection was repeated at the end of the 10-d DA period, the day before starting the RA trek (DA10d = BL_{RA}). Data collection followed an identical schedule throughout RA, such that pairs of data exist for each testing point (i.e. $NB1_{IA}$ and $NB1_{RA}$).

4.2.3 Procedures

At the beginning of the study, participants completed a physical activity and altitude history questionnaire. Height and weight were measured and non-exercise maximal oxygen consumption (predicted $\dot{V}O_{2\text{-}max}$) was calculated from sex, age, body mass index, perceived functional ability and current physical activity as described by Bradshaw et al (2005).

4.2.3.1 Trekking logs

Participants maintained a daily log throughout the IA and RA treks. Self-reported data included a morning and evening assessment of AMS by Lake Louise Questionnaire (Hackett and Oelz 1992). This questionnaire can be viewed in APPENDIX V.2. Participants reported all medication consumed. A rating of perceived trekking exertion (session RPE, Seiler and Kjerland 2006) was reported each day using the Borg CR10 scale (Borg 1982). Participants also recorded the time required to complete each day's trek, subtracting all rest periods longer than 15 min in duration. AMS scores, medication consumed, trekking time and session RPE were all summed for the complete ascent portion of the trek, from Lukla to Gokyo Ri. One participant suffered from significant gastrointestinal ailments during the first two days of the RA trek. Symptoms started prior to re-exposure and are not attributed to altitude illness. As a result, these

AMS scores were omitted and the corresponding values from the IA trek were also removed from analyses.

4.2.3.2 Haematology

Blood was drawn from an antecubital vein into a 6 mL Vacutainer® EDTA tube (BD Diagnostics – Pre-analytical Systems, Sparks, Maryland, USA) by standard venipuncture procedure. Haematocrit (Hct) was immediately measured in duplicate by centrifuging 100 μL of whole blood in a capillary tube at 4400 g for 5 min in a microhaematocrit centrifuge (ZIPocrit ZPC-04HF-7501, LW Scientific, Lawrenceville GA, USA). Hct values were determined using a Critocaps® microhaematocrit tube reader (McCormick Scientific, Richmond, IL, USA). Haemoglobin concentration ([Hb]) was also measured in duplicate in 10 μL whole blood using an automated Hb analyser (β-Hemoglobin, HemoCue, Ängelholm, Sweden).

Plasma was collected by spinning the remaining blood at 2000 g for 10 min in a microcentrifuge (Galaxy Mini, VWR International LLC, West Chester, PA, USA). In Kathmandu and Namche Bazaar, plasma samples were immediately transferred to -20 °C. In Gokyo, plasma was packed in ice and transferred to a freezer within 3 days. At the end of the study period, plasma samples were transferred (via Kathmandu) to the Genes, RNA, Informatics and Protein Laboratory at the University of British Columbia. There, plasma samples were analysed in duplicate for erythropoietin concentration ([EPO]) using enzyme-linked immunosorbent assay (ELISA, StemCell Technologies Inc., Vancouver, BC, Canada).

4.2.3.3 Rest

Resting cardiorespiratory function was assessed while participants lay supine for 10 min. Minute ventilation ($\dot{V}_I$) was measured using a portable respiratory inductive plethysmograph (LifeShirt™, VivoMetrics, Ventura, CA, USA). The LifeShirt™ consists of a lightweight nylon vest that is worn underneath the clothes. Displacement of the ribcage and abdomen was sensed by pressure transducers embedded in the vest and was calibrated to a fixed tidal volume of 500 mL in standing, seated and supine positions. Heart rate (f_H) was measured with EKG and pulse oximetry (S_pO_2) measured with a reflectance probe at the forehead (Model 8000R, Nonin Medical Inc., Minneapolis, MN, USA). Both signals were integrated with the LifeShirt™ device. All data were recorded continuously at a minimum of 10 Hz and

analysed offline. Mean arterial pressure (MAP) was measured using an automated blood pressure cuff (A&D Medical, San Jose, CA, USA). Air temperature during data collection ranged from +10 to –15 °C so participants were often wrapped gently in blankets to remain as comfortable as possible.

4.2.3.4 Graded exercise

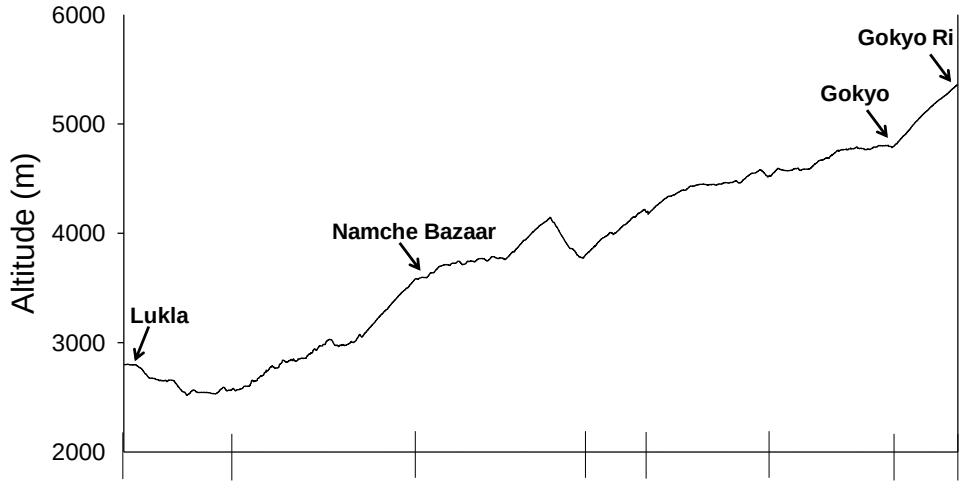
Participants completed a modified Canadian Aerobic Fitness Test (mCAFT; Weller et al 1992) while wearing the LifeShirt™. The test consisted of 3-min stages of stepping exercise separated by 1 min of seated rest. Participants stepped up and down a 20.3 or 40.6 cm step in time with a metronome to elicit the desired workload. Following a 5-min stage of seated rest, exercise began at 0.5 $W \cdot kg^{-1}$, with increments of 0.25 $W \cdot kg^{-1}$ for each subsequent stage. Cardiorespiratory data were collected continuously throughout the test. During the last 30 s of each stage, participants were asked to report separate ratings of perceived exertion for respiratory (RPE_{resp}) and leg effort (RPE_{legs}) using the 15-point Borg scale (Borg 1970). At the end of each stage, subjects promptly sat to allow an immediate measurement of MAP. Blood lactate concentration ([La]) was also measured by finger prick immediately following each exercise stage (LactatePro, KDK Ltd., Kyoto, Japan). The test continued until volitional exhaustion at low altitude or until f_H reached 75% of age-predicted maximum (220 – age) at altitude. When f_H data were not available in real time, exercise was stopped when participants reached an RPE_{resp} of 15. After the final exercise stage, cardiorespiratory data were recorded throughout 3 min of seated recovery.

4.2.3.5 Trekking intensity

Participants wore calibrated altimeters (Polar RS8000sd, Polar Electro Oy, Kempele, Finland) during the seven trekking days from Lukla to Gokyo Ri. One individual walked and ran the entire return route from Lukla to Gokyo twice wearing a calibrated foot pod with the Polar RS8000sd system. Trekking distances were calculated as the average of four measurements on different days. The distance from Gokyo to Gokyo Ri was not measured. An altitude profile and description of each day`s trek is shown in FIGURE 4.2.

Throughout each day of trekking, f_H was continuously measured with either the LifeShirt™ or a Polar RS800sd heart rate transmitter (Polar Electro Oy, Kempele, Finland). Mean values of f_H were calculated

across each day's trek after removing data corresponding to rest periods longer than 15 min in duration ($f_{H_{mean}}$). Maximum values of f_H were also reported for each day, based on a 5-s average (trekking $f_{H_{max}}$). The proportion of trekking time spent in three f_H zones (< 65%, 65-80% and > 80% age-predicted $f_{H_{max}}$) were also calculated based on a 5-s average.



DAY	1	2	3	4	5	6	7	ENTIRE TREK
Average Altitude (m)	2640	2923	3655	3863	4259	4602	5147	**3668**
Gross Ascent (m)	188	1079	627	475	438	424	562	**3793**
Distance (km)	6.1	9.8	8.0	3.6	6.5	8.4	na	**>42.4**

FIGURE 4.2 Ascent profile of Gokyo Valley trek and description of each of seven trekking days from Lukla to Gokyo Ri.

4.2.4 Data analysis

4.2.4.1 Data extraction

All LifeShirt™ data were analysed using VivoLogic software (Version 2.7.2, VivoMetrics, Ventura, CA, USA). f_H data were extracted from the Polar monitors using Polar ProTrainer 5 (Version 5.30.154, Polar Electro Oy, Kempele, Finland). All data were examined visually and aberrant values (those that differed from adjacent values by ≥10 units) were removed. Mean values of $\dot{V}_I$, f_H and S_pO_2 were calculated for the final 5 min of rest, final 30 s of each exercise stage and final 15 s of each recovery stage. MAP (calculated as ⅓SBP + ⅔DBP) was reported as the mean of values recorded at 8 and 10 min.

For exercise data, equations for lines of best fit were established for both exercise (linear) and recovery (linear or quadratic) measures versus workload. A global recovery index (RI) was calculated to quantify each variable's return towards resting levels following each workload (see FIGURE 4.3). The areas bounded by each curve and a horizontal line at the resting value for each measure were calculated and designated as A_E (exercise curve) and A_R (recovery curve). RI was calculated as:

$$RI = 100 \times [(A_E - A_R) / A_E]$$

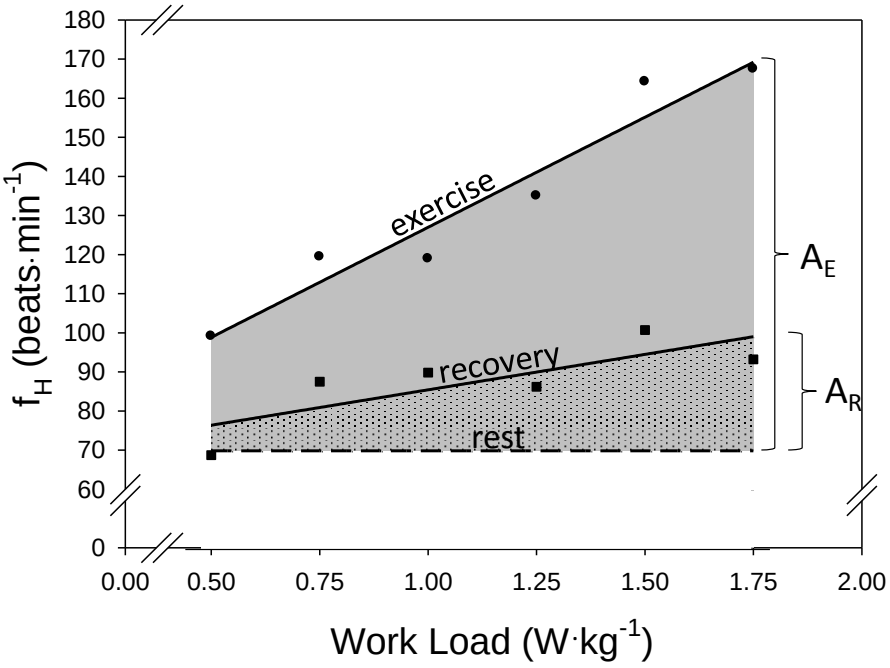


FIGURE 4.3 Schematic demonstrating the calculation of heart rate (f_H) recovery index using data collected for one participant at $NB1_{IA}$. See text for further explanation.

For all cardiorespiratory data, an overall altitude response was calculated as the mean response across all altitude testing points (i.e. $fH_{altitude}$). For each variable, relative change from BL_{IA} was calculated at each time point. $\Delta_{altitude}$ was calculated from the mean altitude responses across IA and RA.

For each variable, coefficients of variation (CV) were calculated for IA-RA pairs of data to determine the repeatability of individual acclimatisation responses throughout consecutive treks. CVs were calculated individually for each subject at every testing point and workload (exercise measures only) and are reported as global means for each variable.

4.2.4.2 Statistical analyses

Data were analysed using statistical software (SPSS 15.0, IBM SPSS Statistics, Chicago, USA). All decisions about data analyses were made in consultation with a professional statistician. Given the small sample size, in order to reduce the likelihood of making a Type II error, the level of statistical significance for all analyses was set at $p < 0.1$ (as suggested in Curran-Everett and Benos 2004).

Trekking log data were analysed with paired t tests. For haematology and cardiorespiratory data, pre-planned comparisons between BL_{IA} and BL_{RA} were made for each variable using paired t tests. Data were then analysed using repeated measures analysis of variance (RM ANOVA) to determine the within-subject effects of testing point and trek. With n = 6, the 2 x 8 (rest) and 2 x 7 (haematology, exercise) RM ANOVAs were underpowered. Therefore, regardless of ANOVA results, paired t tests were used for pair-wise comparisons between IA and RA at each testing point and were Bonferroni-adjusted to correct for multiple comparisons. Mean altitude responses and $\Delta_{altitude}$ were also compared between IA and RA using paired t tests.

4.3 Results

4.3.1 Participants

Two female and four male low altitude residents participated in this study. All participants were young (27 ± 2 y) and healthy, with no reported history of cardiovascular, respiratory or haematological conditions. Further description of participants can be found in TABLE 4.1.

TABLE 4.1 Characteristics of study participants. Individual data are presented with group mean ± standard error.

ID	Sex	Age (y)	BMI ($kg \cdot m^{-2}$)	Predicted $\dot{V}O_{2\text{-max}}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Previous altitude experience
1	F	28	23	50	> 6000 m, 3 y previous
2	M	36	24	56	never > 2500 m
3	M	26	25	43	> 3000m, 2 y previous
4	M	22	19	49	never > 2500 m
5	M	25	21	43	never > 2500 m
6	F	23	22	34	never > 2500 m
mean ± SE		**27 ± 2**	**22 ± 1**	**46 ± 3**	

Participants lost between 0.5 and 5 kg (1 – 7 % of body mass) during the IA trek. After 10 d DA, participants had re-gained between 61 and 170 % of mass lost, and body mass was not significantly different between BL_{IA} and BL_{RA}. The pattern and magnitude of mass lost was similar between the IA and RA treks (see FIGURE 4.4).

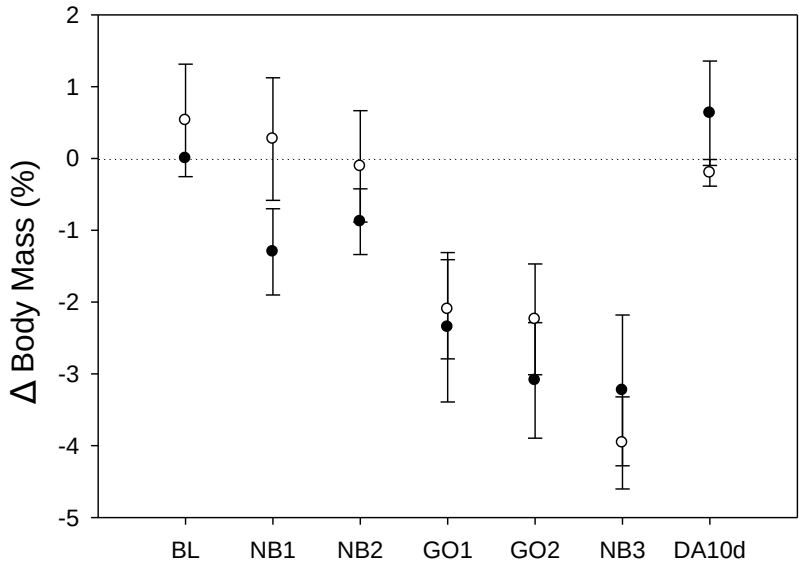


FIGURE 4.4 Body mass of participants across IA (●) and RA (○) treks. Data are expressed relative to baseline (BL_{IA}) and presented as group mean ± standard error. There were no significant pair-wise differences between IA and RA at any testing point.

4.3.2 Trekking logs

TABLE 4.2 summarises data collected from daily trekking logs. Every participant completed the RA trek faster than the IA trek, with an average improvement of 2.9 h (range 1.7 to 5.0 h), or 12%. The total session RPE decreased from IA to RA in every subject with a 15% mean reduction in perceived effort. Participants reported fewer and milder AMS symptoms throughout RA than IA, with a 35% reduction in AMS score.

Participants used analgesics to treat headache on fewer occasions during RA than IA. Five participants self-medicated on a total of eight occasions during IA compared to two subjects on a total of three occasions during RA. Participants also used their own discretion to use acetazolamide to treat AMS. One participant did not use acetazolamide in either IA or RA. Of the other five, four reduced acetazolamide use 50-100% from IA to RA and one increased use by 100%. Although total acetazolamide used dropped

by 64% on average, this difference was not statistically significant (p = 0.11).

TABLE 4.2 Self-reported data for trekking time and intensity, AMS scores, and medication consumed during an initial acclimatisation (IA) and re-acclimatisation (RA) trek. The change in each measure from IA to RA is given in both absolute and relative terms. Data are presented as group mean ± standard error and p values are the result of paired t tests.

Variable	IA	RA	Δ	p value
Trekking time (h)	23.8 ± 2.6	21.0 ± 2.2	-2.9 ± 0.5 -12 ± 1%	<0.01
Σ RPE	26 ± 4	21 ± 1	-5 ± 2 -15 ± 6%	0.07
Σ AMS	11 ± 2	7 ± 2	-4 ± 1 -35 ± 11%	0.02
Analgesics (# times consumed)	1.3 ± 0.4	0.5 ± 0.3	-0.8 ± 0.5 -60 ± 20%	0.07
Acetezolamide (total mg consumed)	290 ± 100	100 ± 80	-190 ± 140 -52 ± 32%	0.11

4.3.3 Haematology

Due to the reduced barometric pressure at testing sites from 1300 to 4750 m, the 6 mL Vacutainer® tubes actually drew between 3.0 and 5.5 mL of blood. The reduced draw volume of evacuated blood collection tubes at altitude is an important consideration for researchers and clinicians (MacNutt and Sheel 2008). This work can be viewed in APPENDIX III.

[Hb] and Hct increased significantly (both $p < 0.001$) throughout the IA trek, reaching peak values in GO1 or GO2 (see FIGURE 4.5). After 10 d DA, Hct had returned to BL but [Hb] remained slightly (0.3 ± 0.2 $g \cdot dL^{-1}$), but significantly ($p < 0.05$), above BL_{IA}. There was no significant effect of trek on either of these variables, and no differences between IA and RA at any testing point. However, the magnitude of Δ[Hb] from BL to peak doubled from 12% in IA to 24% in RA (see FIGURE 4.6). There was no difference in the magnitude of ΔHct between IA and RA. There was also a significant effect of testing point on [EPO] ($p < 0.001$) but no effect of trek and no pair-wise differences between IA and RA at any time point (see FIGURE 4.5). However, there was a significant 31% decrease from IA to RA in the magnitude of Δ[EPO] from BL to peak (FIGURE 4.6, p = 0.05

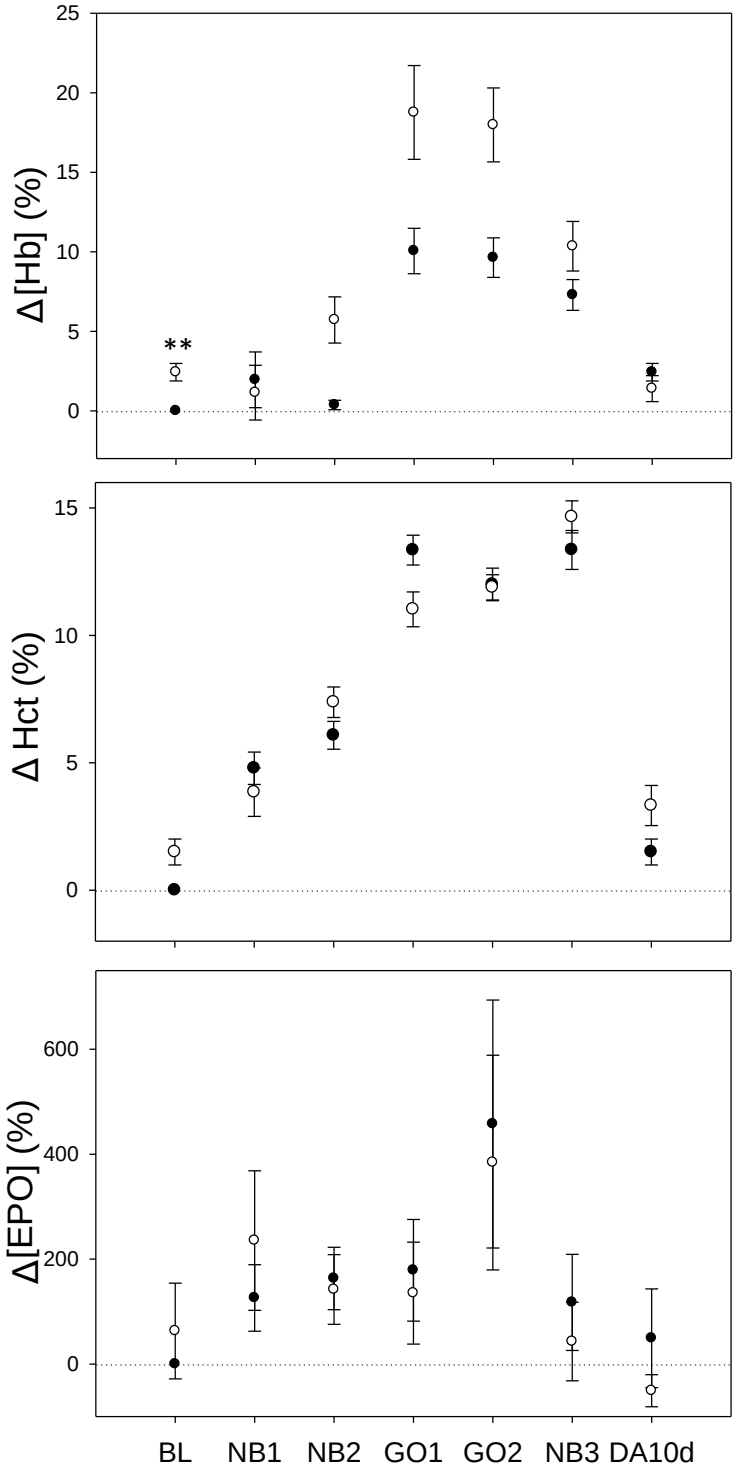


FIGURE 4.5 Haemoglobin concentration ([Hb]), haematocrit (Hct) and plasma erythropoietin concentration ([EPO]) across testing points during IA (●) and RA (○) treks. Data are expressed as percent change from baseline (BL_{IA}) and are presented as group mean ± standard error. Pair-wise comparisons (t tests) were made between IA and RA at each testing point. Significant differences are indicated with ** ($p < 0.05$).

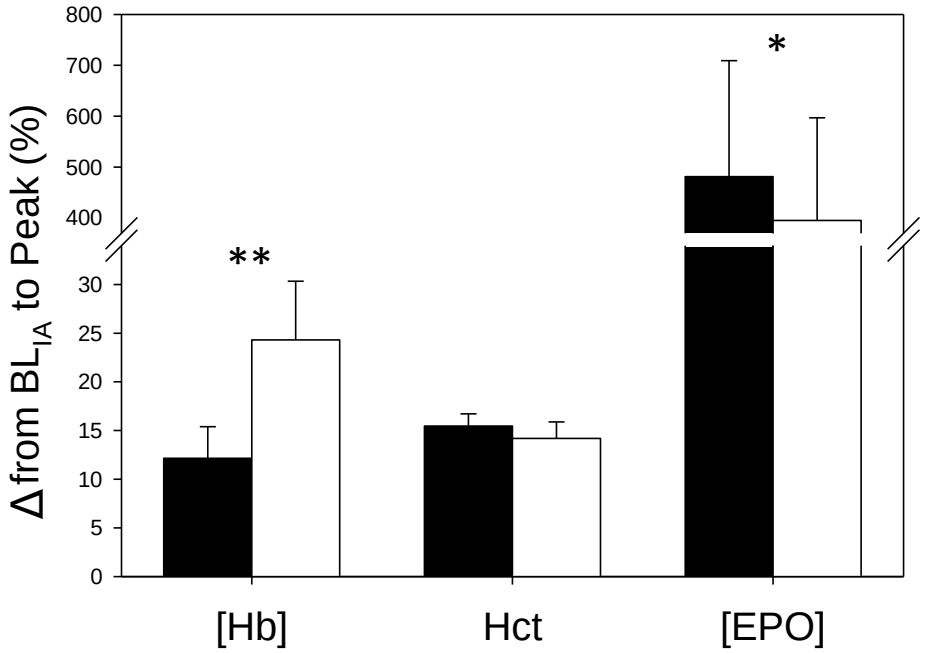


FIGURE 4.6 Percent change in haemoglobin concentration ([Hb]), haematocrit (Hct) and erythropoietin concentration ([EPO]) from BL_{IA} to peak values measured during IA (black bars) and RA (white bars). Data are presented as group mean ± standard error. Significant differences between IA and RA for each variable are indicated with * ($p < 0.1$) and ** ($p < 0.05$).

4.3.4 Rest

Resting measures of cardiorespiratory function throughout IA and RA are shown in FIGURE 4.7. For f_H, MAP and S_pO_2, the effect of testing point was significant (all $p < 0.001$) with a significant $\Delta_{altitude}$ for f_H (↑ at altitude, $p = 0.002$), MAP (↑ at altitude, $p = 0.02$) and S_pO_2 (↓ at altitude, $p = 0.002$). Although $\dot{V}_I$ tended to increase at altitude, there was no effect of testing point ($p = 0.13$) and no significant $\Delta_{altitude}$ ($p = 0.13$). After 10 d DA, S_pO_2 and MAP had returned to normal but f_H and $\dot{V}_I$ remained significantly elevated above BL (f_H: +6%, $p = 0.008$, $\dot{V}_I$: +23%, $p = 0.03$).

There was no effect of trek on any measure of cardiorespiratory function during rest. Nor were there any significant pair-wise differences between IA and RA for any variable at any testing point. However, the mean value for f_H across testing points at altitude ($f_{H_{altitude}}$) was 4 beats·min^{-1} lower ($p = 0.09$), $MAP_{altitude}$ was 3 mmHg higher ($p = 0.08$) and $S_pO_{2\text{-altitude}}$ was 3 percentage points lower ($p = 0.09$) in RA than IA (see FIGURE 4.8). There was no difference in $\dot{V}_{I_{altitude}}$ between IA and RA ($p = 0.48$).

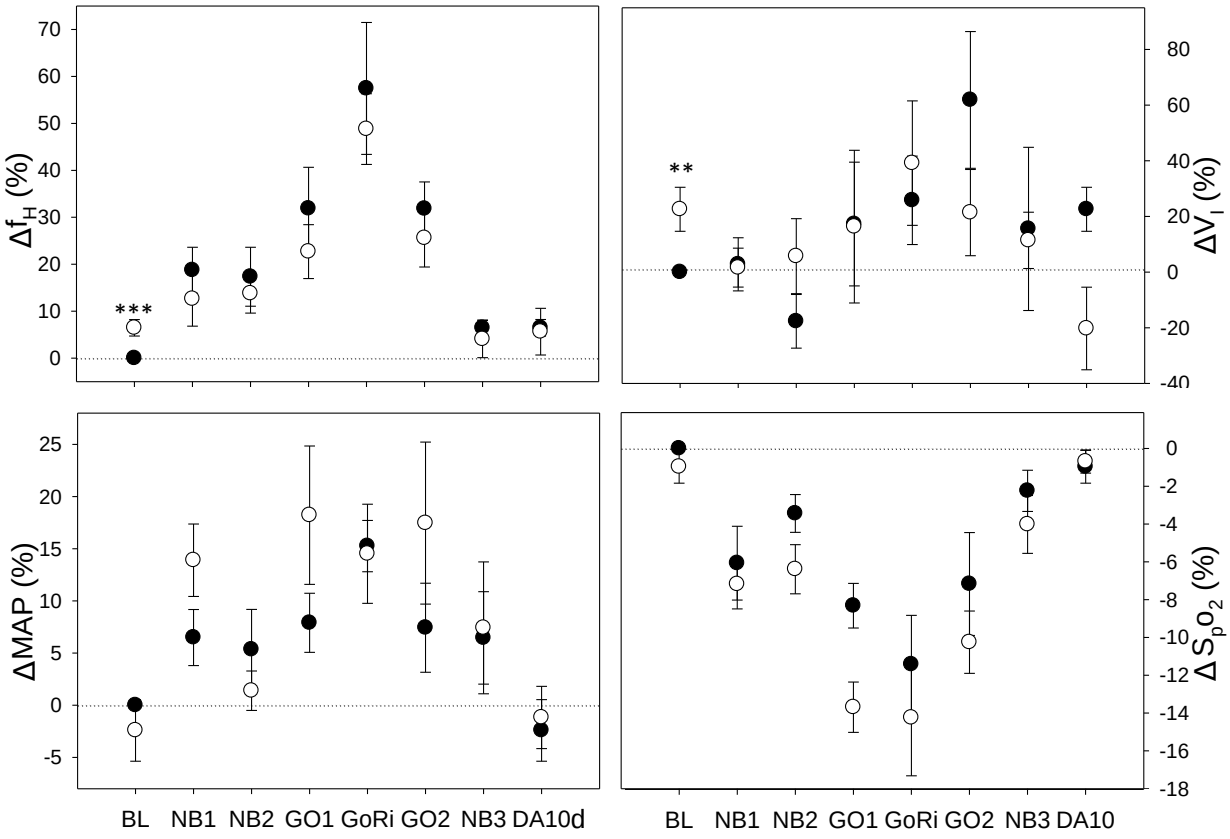


FIGURE 4.7 Resting heart rate (f_H), minute ventilation ($\dot{V}_I$), mean arterial pressure (MAP) and oxyhaemoglobin saturation (S_pO_2) at testing points during IA (●) and RA (○) treks. Data are expressed as percent change from baseline (BL_{IA}) and are presented as group mean ± standard error. Pair-wise comparisons (t tests) were made between IA and RA at each testing point. Significant differences are indicated with ** ($p < 0.05$) and *** ($p < 0.01$).

4.3.5 Graded exercise

4.3.5.1 Exercise stages completed

There was a significant effect of testing point on the number of exercise stages completed ($p < 0.001$, see FIGURE 4.9). Participants completed fewer stages at altitude compared to BL_{IA} ($p < 0.001$), although this indicates only that participants reached the f_H and RPE cut-offs at lower workloads and not necessarily that participants were exhausted sooner at higher altitudes. By BL_{RA}, participants were able to complete the same number of stages as in BL_{IA}. Although one participant consistently completed more exercise stages throughout RA than IA, the final stage completed was consistent between treks for every other participant at all testing points. Thus, there was no significant effect of trek on exercise stage completed ($p = 0.36$), no significant differences between IA and RA at any testing point, and no difference in $\Delta_{altitude}$ between IA and RA.

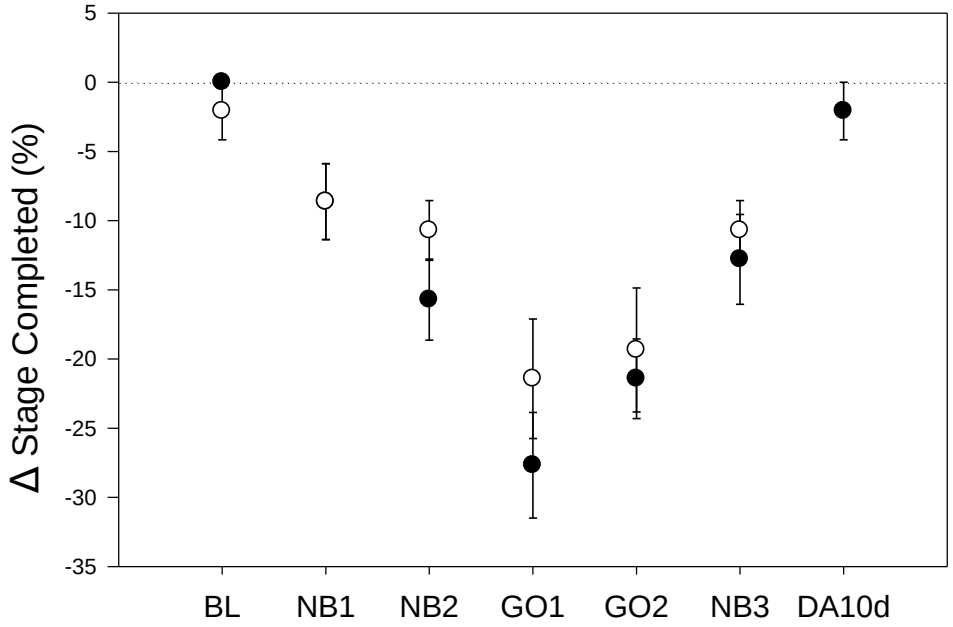


FIGURE 4.8 Mean resting heart rate (f_H), minute ventilation ($\dot{V}_I$), mean arterial pressure (MAP) and oxyhaemoglobin saturation (S_pO_2) throughout IA (solid bars) and RA (open bars) treks. Data are expressed as percent change in mean altitude resposne from baseline (BL_{IA}) and are presented as group mean ± standard error. For each variable, significant differences between IA and RA (paired t-tests) are indicated with * ($p < 0.10$).

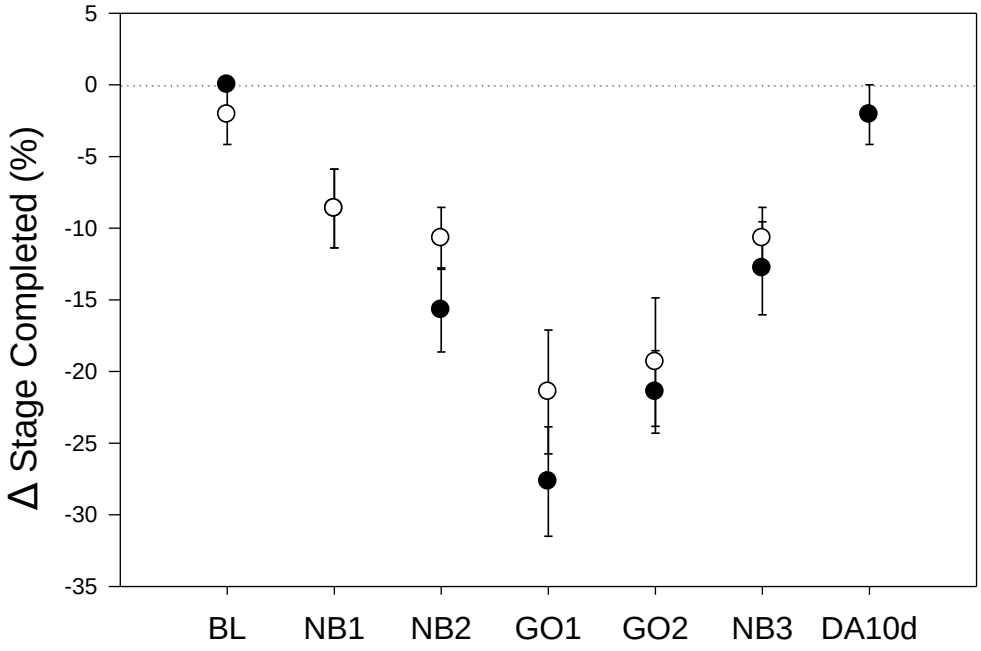


FIGURE 4.9 Final work stage completed during the graded exercise challenge at each testing point throughout IA (●) and RA (○) treks. Data are expressed as percent change from baseline (BL_{IA}) and are presented as group mean ± standard error. Pair-wise comparisons (t tests) were made between IA and RA at each testing point but no significant differences were detected. Note that the symbols representing IA and RA are perfectly overlapped at NB1.

4.3.5.2 Cardiorespiratory and metabolic responses to exercise

FIGURE 4.10 presents a summary of cardiorespiratory and metabolic responses to three intensities of exercise across testing points in IA and RA. Since participants frequently did not complete the hardest workload at altitude, data were individually modelled to calculate exercise responses at 2.0 $W \cdot kg^{-1}$. As such, FIGURE 4.10 includes a combination of measured and modelled data. Positive linear relationships for f_H, $\dot{V}_I$, RPE_{resp} and RPE_{legs} versus workload were very strong across all individuals and testing points with global means for R^2 of 0.98, 0.94, 0.96 and 0.95 respectively. MAP also consistently increased with exercise but the relationship was less robust ($R^2 = 0.75$). S_pO_2 did not change across workloads at low altitude, but did linearly decrease with increasing exercise intensity at altitude ($R^2 = 0.75$). Therefore, S_pO_2 at 2.0 $W \cdot kg^{-1}$ could only be predicted at NB and GO. [La] versus workload was best modelled with a quadratic function, with a resulting mean R^2 value of 0.83.

All variables responded predictably to exercise and were significantly affected by work load (all $p < 0.01$). Testing point had a significant effect on exercise f_H, S_pO_2, and RPE_{resp} (all $p < 0.001$). Although there was no effect of testing point on other variables, examining $\Delta_{altitude}$ indicates that all exercise variables except for MAP ($p = 0.23$) were significantly affected by altitude. The $\Delta_{altitude}$ for each variable is presented in FIGURE 4.11 as the mean of all three exercise intensities. $\Delta_{altitude}$ was significantly positive for f_H ($p = 0.02$), $\dot{V}_I$ ($p = 0.09$), RPE_{resp} ($p = 0.01$) and RPE_{legs} ($p = 0.01$) and negative for S_pO_2 ($p < 0.001$) and [La] ($p = 0.02$).

f_H was the only exercise measure to be significantly affected by trek ($p = 0.09$). Pair-wise comparisons indicated that f_H was lower in RA than IA at BL in moderate ($p = 0.06$) and heavy exercise ($p = 0.04$) and at GO2 in moderate exercise ($p = 0.003$). There were no pair-wise differences in $\dot{V}_I$, MAP, S_pO_2, RPE_{resp} or RPE_{legs} between IA and RA at any testing point or exercise intensity. In heavy exercise, [La] was lower in RA than IA at BL ($p = 0.03$) and NB3 ($p < 0.01$) but this pattern was not consistent across testing points or exercise intensities. Consistent with the ANOVA results, exercise $f_{H_{altitude}}$ was 3 $beats \cdot min^{-1}$ lower in RA than IA ($p = 0.04$). There was no difference in mean altitude response between IA and RA for any other exercise measure.

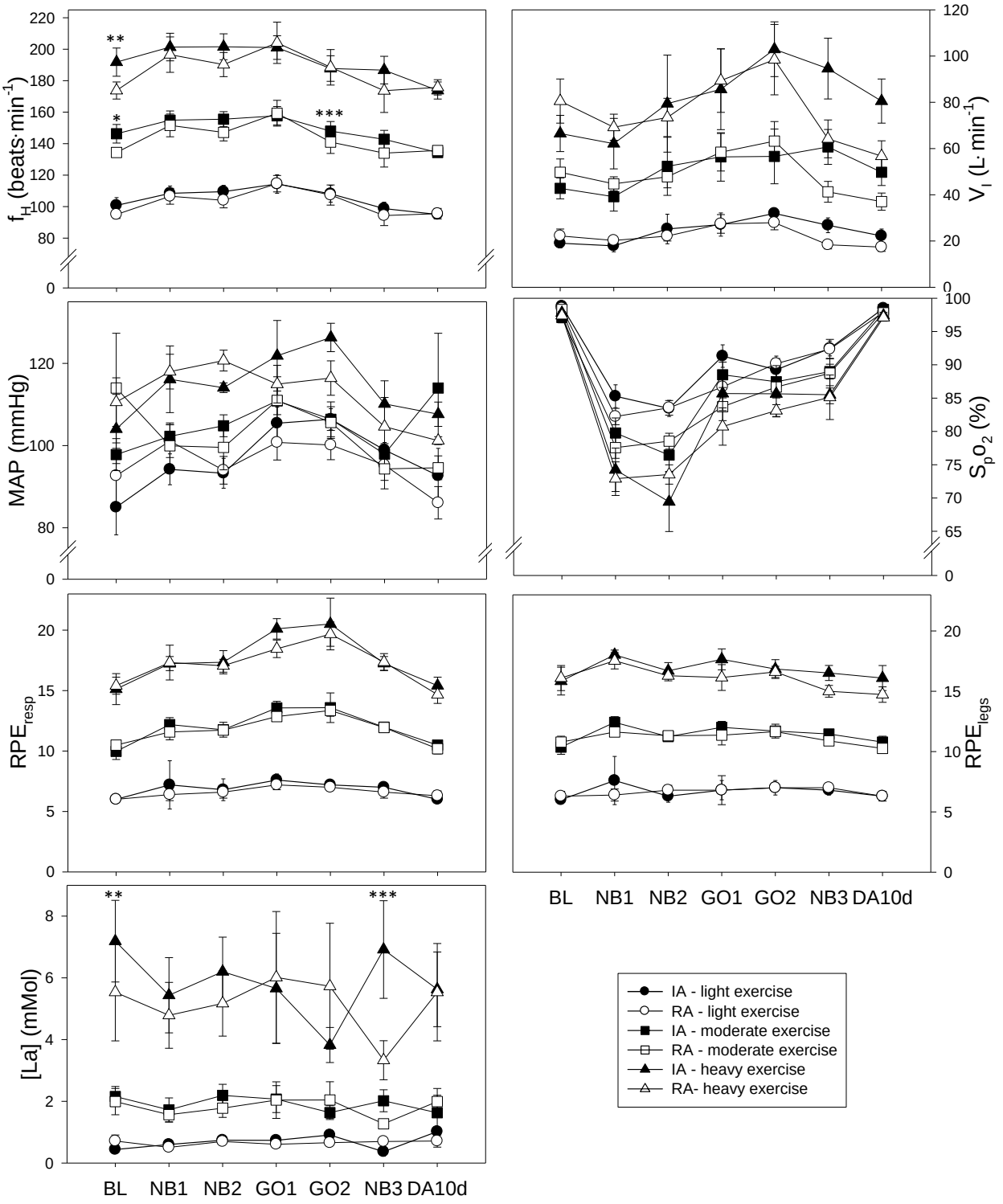


FIGURE 4.10 Heart rate (f_H), minute ventilation ($\dot{V}_I$), mean arterial pressure (MAP), oxyhaemoglobin saturation (S_pO_2), ratings of perceived exertion (RPE_{resp} and RPE_{legs}) and blood lactate concentration ([La]) at three exercise intensities at testing points throughout IA and RA. Data represent values measured or modelled during light (0.5 W·kg^{-1}), moderate (1.25 W·kg^{-1}) and heavy (2.0 W·kg^{-1}) stepping exercise and are presented as group mean ± standard error. Pair-wise comparisons (t tests) were made between IA and RA at each testing point. Significant differences are indicated with ** ($p < 0.05$) and *** ($p < 0.01$).

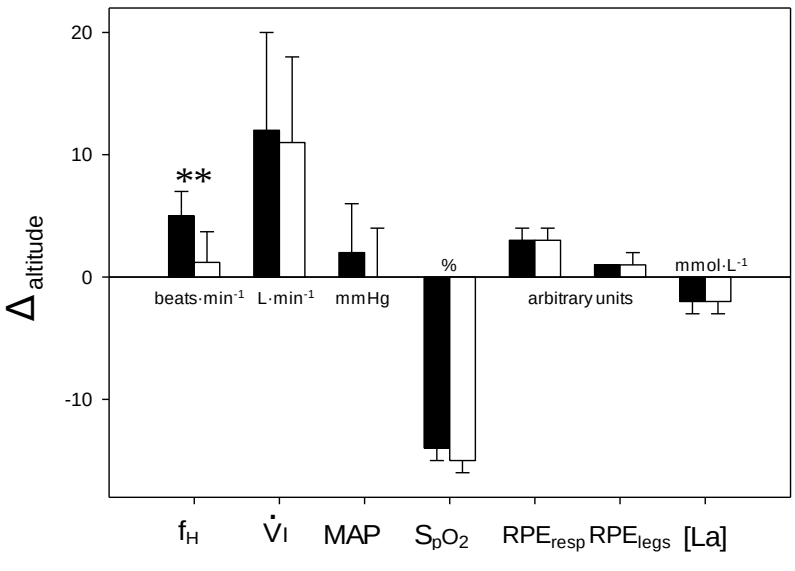


FIGURE 4.11 Mean change in cardiorespiratory and metabolic responses to exercise from BL_{IA} to the mean altitude response in IA (solid bars) and RA (open bars). Change in heart rate (f_H), minute ventilation ($\dot{V}_I$), mean arterial pressure (MAP), oxyhaemoglobin saturation (S_pO_2), rating of perceived exertion for respiratory (RPE_{resp}) and leg sensation (RPE_{legs}) and blood lactate concentration ([La]) were avereaged across three exercise intensities and are presented as group mean ± standard error. Respective units are given for each variable. Significant pair-wise differences between IA and RA are marked with **($p < 0.05$).

4.3.5.3 Recovery from exercise

Recovery indices across testing points and treks are shown in FIGURE 4.12. Time point did not have a significant effect on RI for any variable and no RI was affected by trek. MAP RI was significantly higher in RA at GO1 ($p = 0.006$), but no systematic pair-wise differences between IA and RA were seen in RI for any variable. Throughout IA, the mean f_H RI was 8% higher at altitude than at BL_{IA} ($p = 0.08$) but there was no $\Delta_{altitude}$ for $\dot{V}_I$ RI or MAP RI. Since S_pO_2 did not change throughout exercise at low altitude, S_pO_2 RI was only calculated at NB and GO. Therefore, $\Delta_{altitude}$ for S_pO_2 RI could not be assessed.

4.3.6 Trekking intensity

Trekking f_H data are presented in FIGURE 4.13. A large number of missing data points precluded using RM ANOVA to examine the effects of trek on daily mean and maximum trekking f_H. In both IA and RA, fH_{mean} and fH_{max} were lowest on Day 1, with a notably greater proportion of trekking time spent in the lowest f_H zone. Trekking f_H remained fairly constant throughout the remainder of the trek and neither fH_{mean} nor fH_{max} was correlated with mean trekking altitude. There were no pair-wise differences between

IA and RA in trekking fH_{mean} or fH_{max} on any trekking day. Nor were there any pair-wise differences between IA and RA in the distribution of time spent in each fH zone on any trekking day.

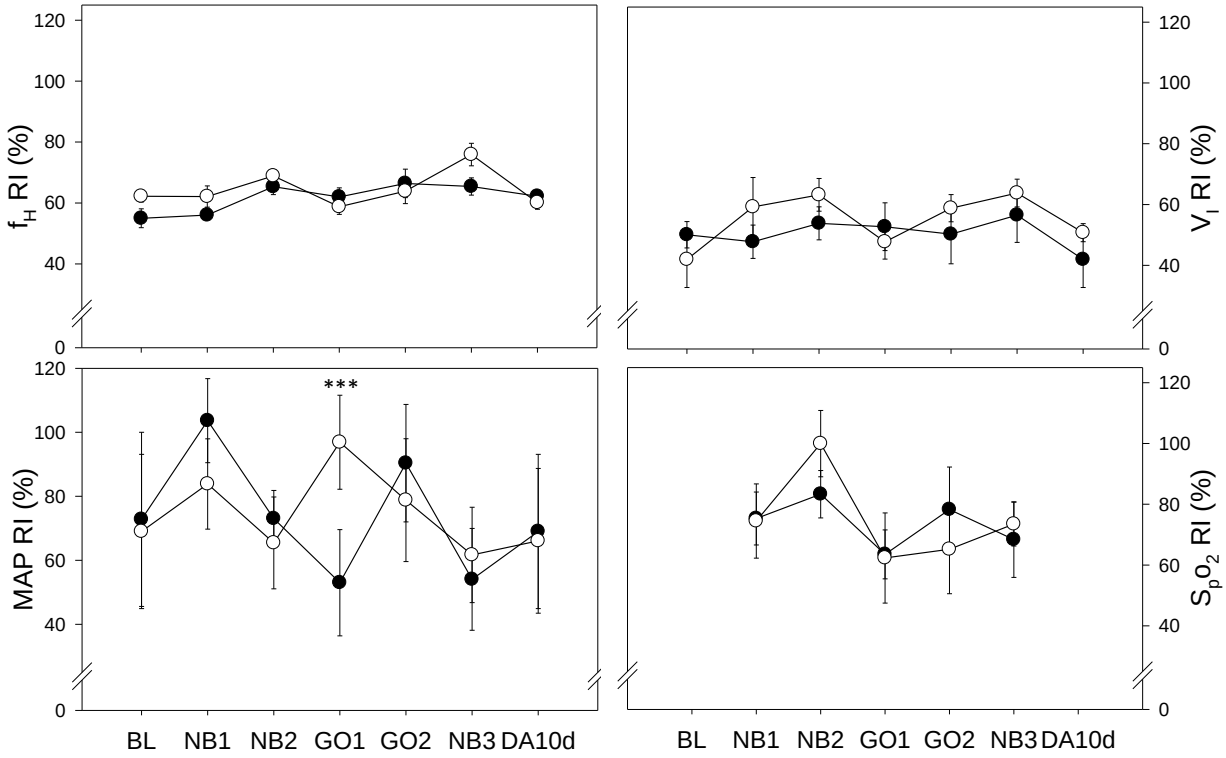


FIGURE 4.12 Recovery index (RI) for heart rate (fH), minute ventilation ($\dot{V}_I$), mean arterial pressure (MAP) and oxyhaemoglobin saturation (S_pO_2) during graded exercise tests throughout IA (●) and RA (○) treks. Calculation of RI is explained in the text (SECTION 4.2.4.1). Data are presented as group mean ± standard error. Pair-wise comparisons (t tests) were made between IA and RA at each testing point. Significant differences are indicated with *** ($p < 0.01$).

4.3.7 Repeatability of acclimatisation responses

CVs for IA-RA pairs of data are presented in TABLE 4.3. [Hb], Hct, resting S_pO_2, exercise fH and S_pO_2, and trekking fH_{mean} and fH_{max} were highly repeatable from IA to RA, with CVs ≤ 5%. CVs for resting fH, resting and exercise MAP, RPE_{resp} and RPE_{legs} were also within 10%. Although there were no patterns in change from IA to RA, acclimatisation responses of [EPO] (CV = 35%), resting and exercise $\dot{V}_I$ (both CVs = 18%) and blood [La] (CV = 23%) were not as consistent between consecutive treks.

4.4 Discussion

Participants responded to altitude exposure as expected during IA and acclimatory responses were

generally reversed at the end of the DA period. Participants suffered less from AMS, trekked faster and perceived the trekking as easier in RA compared to IA. The magnitude of haematological acclimatisation was greater and resting and exercise fH were reduced during the second trek. These main findings are discussed in more detail below.

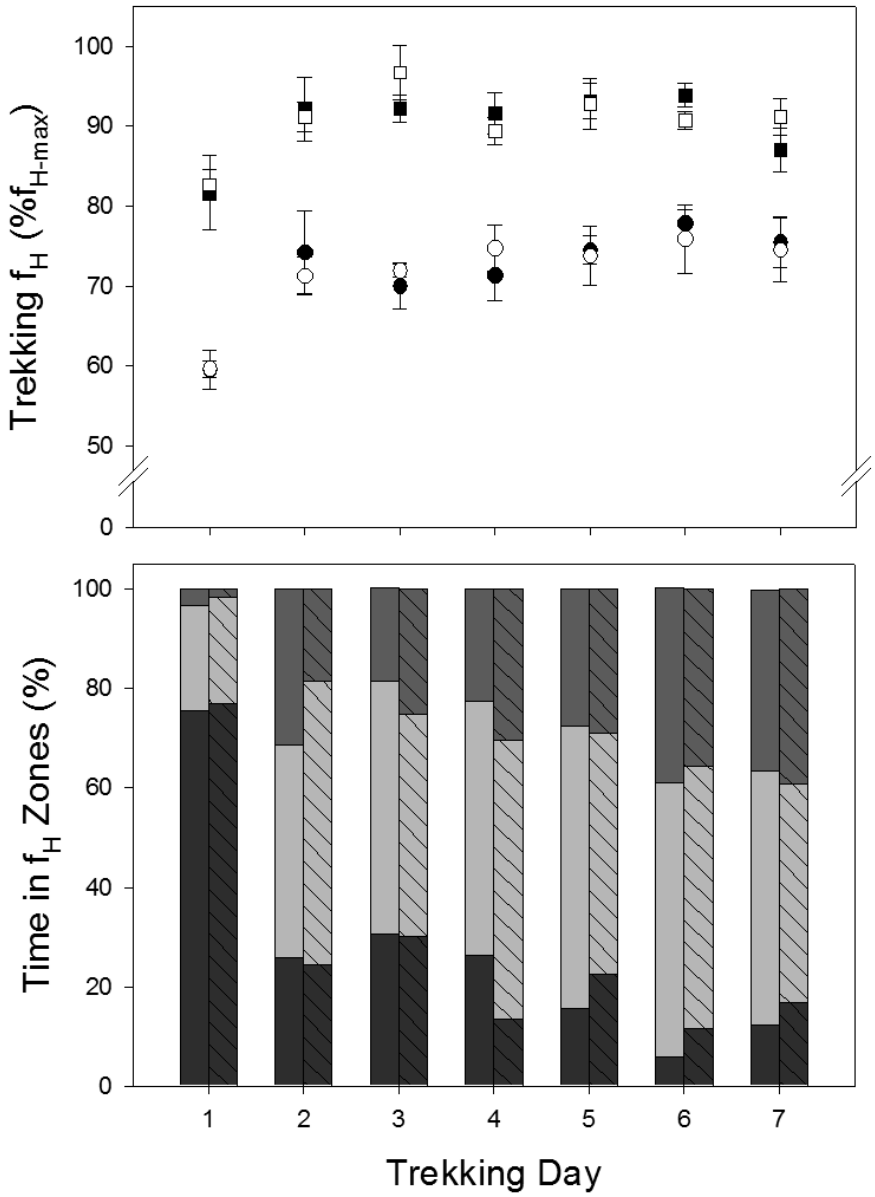


FIGURE 4.13 Heart rate (fH) measured throughout seven days of trekking from Lukla to Gokyo Ri during initial acclimatisation (IA) and re-acclimatisation (RA) treks. Each day's trek is described in FIGURE 4.2. Top panel shows daily mean (circles) and maximum (squares) fH during IA (closed symbols) and RA (open symbols). Data are presented as group mean ± standard error. Bottom panel shows the percent of daily trekking time spent in each of three fH zones: Zone 1 (< 65% fH_{max}, black), Zone 2 (65-80% fH_{max}, light grey) and Zone 3 (> 80% fH_{max}, dark grey). Data are presented as group means for IA (solid bars) and RA (hatched bars). Pair-wise comparisons (t tests) were made between IA and RA for each trekking day but no significant differences were detected.

TABLE 4.3 Coefficients of variation (CV, %) for pairs of data collected throughout IA and RA treks. CVs were calculated for each participant at every testing point and workload (where applicable) and are reported as global mean ± standard error for each variable.

Haematology	**[Hb]**	**Hct**	**[EPO]**				
	4 ± 1	2 ± 0	35 ± 5				
Resting measures	**f_H**	**$\dot{V}_I$**	**MAP**	**S_pO_2**			
	8 ± 1	18 ± 3	6 ± 1	3 ± 1			
Exercise measures	**f_H**	**$\dot{V}_I$**	**MAP**	**S_pO_2**	**RPE_{resp}**	**RPE_{legs}**	**[La]**
	4 ± 0	18 ± 2	7 ± 1	5 ± 0	8 ± 1	8 ± 1	23 ± 2
Recovery index	**f_H RI**	**$\dot{V}_I$ RI**	**MAP RI**	**S_pO_2 RI**			
	14 ± 2	22 ± 4	87 ± 29	23 ± 4			
Trekking intensity	**$f_{H_{mean}}$**	**$f_{H\,max}$**					
	5 ± 1	4 ± 1					

4.4.1 Initial acclimatisation to high altitude

Throughout IA, anticipated responses to high-altitude exposure were observed in all measured variables. All participants experienced at least mild symptoms of AMS (TABLE 4.1) and [Hb], Hct and [EPO] increased over time (FIGURE 4.5). Across testing points at altitude, resting f_H, $\dot{V}_I$ and MAP were elevated and resting S_pO_2 decreased compared to BL_{IA} (FIGURES 4.7 and 4.8). Participants completed fewer exercise stages at altitude (FIGURE 4.9) and in exercise, f_H, $\dot{V}_I$, RPE_{resp} and RPE_{legs} were elevated and S_pO_2 and [La] were reduced compared to low altitude (FIGURES 4.10 and 4.11).

The majority of studies describe the process of acclimatisation to a constant altitude (see Dempsey et al 1972, Grassi et al 1996, Garcia et al 2000, Cornolo et al 2004 for examples). In the present study, participants were exposed to progressively increasing, then decreasing altitude throughout a 10-d trek. As such, this data set represents a combination of acute and partially acclimatised responses to changing altitudes and the timing of responses presented in FIGURES 4.5, 4.7, 4.10 and 4.12 do not demonstrate the classic acclimatisation responses reported in the literature. For example, [EPO] generally peaks after 48 h exposure to a constant altitude before declining back to below pre-exposure values (Ratcliffe et al 1996). However, in both treks, [EPO] is consistently elevated with a secondary spike that followed a brief (1-4 h) exposure to 5360 m on Gokyo Ri (FIGURE 4.5). Clearly, EPO production was continuously stimulated by exposure to increasingly hypoxic conditions. Nevertheless, appropriate acclimatisation responses

become evident when comparing data collected in NB after acute (NB1) and sustained (NB3) exposure to high altitude. For example, resting f_H acutely increased 11 beats·min^{-1} from BL_{IA} to $NB1_{IA}$ ($p < 0.01$). Although f_H continued to increase across testing points as trekkers ascended to higher altitudes, f_H fell to only 4 ± 1 beats·min^{-1} above BL_{IA} by $NB3_{IA}$ ($p = 0.01$), demonstrating the expected acclimatisation response (Wolfel et al 1994). Likewise, S_pO_2 dropped from ~98% at BL_{IA} to 93 ± 2% during rest ($p = 0.01$) and 80 ± 2% during exercise ($p < 0.001$) at $NB1_{IA}$. However, as expected, the degree of desaturation decreased with acclimatisation (Bender et al 1989), rising to 96 ± 2% during rest ($p = 0.01$) and 89 ± 1% during exercise ($p = 0.003$) at $NB3_{IA}$.

It is well known that $\dot{V}_I$ acutely increases upon exposure to hypoxia and progressively increases with acclimatisation in both rest and exercise (Bisgard and Forster 1996). However, a significant increase in $\dot{V}_I$ from BL_{IA} to NB1 occurred only during rest in RA ($p = 0.04$) and not during exercise. Increases in $\dot{V}_I$ from NB1 to NB3 occurred during exercise in both IA and RA ($p = 0.05$), but not during rest in either trek. Due to high inter- and intra-individual variability in measures of $\dot{V}_I$ throughout the study, the ability to detect small changes in $\dot{V}_I$ is questionable. The LifeShirt™ has been previously been validated for measuring $\dot{V}_I$ during rest and exercise (Witt et al 2006, Kent et al 2009). Although there was excellent agreement between $\dot{V}_I$ measured by LifeShirt™ and by pneumotach across a range of workloads ($R^2 = 0.91$), the 95% confidence intervals reported by Witt and colleagues (2006) represent ± 20 L·min^{-1} with individual discrepancies sometimes as large as 40 L·min^{-1}. It is apparent that, although the LifeShirt™ is adept at detecting changes in ventilatory pattern within a recording session, it may not be suitable for use in comparing absolute values of $\dot{V}_I$ between sessions. Thus, despite careful calibration, reported measures of $\dot{V}_I$ do not seem reliable.

Acute increases in both resting and exercise MAP have been reported at altitude with further increases in MAP with acclimatisation (Huang et al 1991). Here, resting MAP was elevated at $NB1_{IA}$ ($p = 0.03$) but no further increases occurred by $NB3_{IA}$. Although mean exercise MAP at altitude was elevated above BL_{IA} there were no significant increases at either $NB1_{IA}$ or $NB3_{IA}$.

Despite a decrease in power output in acute hypoxia, the perception of effort during cycle exercise

remained constant, suggesting that RPE at a given exercise intensity should increase at altitude (Beidleman et al 2009). With acclimatisation, Young and colleagues (1982) reported decreases in local RPE and increases in central RPE. Here, both RPE_{legs} ($p = 0.02$) and RPE_{resp} ($p = 0.002$) increased from BL_{IA} to $NB1_{IA}$, but neither RPE score changed with acclimatisation.

It has previously been shown that f_H and S_pO_2 (Katayama et al 2010) and $\dot{V}_I$ and $\dot{V}_{O_2}$ (Banchero et al 1966) recover more slowly following exercise at high altitude than at sea level but that recovery rates improve over time with acclimatisation. However, no impairment of post-exercise recovery or subsequent improvement in recovery rate with acclimatisation was evident in the present data set (FIGURE 4.12). One possible source of discrepant results is the timing of recovery measurements. While the current novel method of assessing recovery index across a range of workloads examined the degree of recovery only 1 min post-exercise, the above works assessed recovery at ~15 min post-exercise. The recovery of f_H (Darr et al 1988), $\dot{V}_{O_2}$ (Hagberg et al 1980) and $\dot{V}_I$ (Dempsey et al 1995) all have an initial fast (exponential) phase followed by a slow (linear) phase as cardiorespiratory measures return towards pre-exercise values. The works of Katayama and Banchero and colleagues do not distinguish between the fast and slow phases and it is possible that their results reflect changes only in the slow phase of recovery. Indeed, the current results suggest that the early, fast phase is not impaired by hypoxia. There is prior evidence that this is the case, at least for the recovery of $\dot{V}_I$. Dejours and colleagues (1963) independently assessed the fast and slow phases of the recovery of $\dot{V}_I$ at SL and at altitude. Although the slow component of $\dot{V}_I$ recovery was always impaired by hypoxia, the fast component was not impaired in every subject. It is unclear whether this might also be the case for recovery of other cardiorespiratory measures following dynamic exercise. Regardless, although it was anticipated that this novel method of assessing recovery index would prove a sensitive marker of acclimatisation status, this does not appear to be the case.

4.4.2 De-acclimatisation from high altitude

It is well known that all aspects of acclimatisation to hypoxia are reversible upon return to normoxia. The mechanisms and timelines of the DA process are discussed in depth in CHAPTER 2.

BL data were collected immediately prior to the IA trek (BL_{IA}) and, after 10 d DA in Kathmandu (1300

m), immediately before the RA trek (BL_{RA}). For each variable, BL_{IA} and BL_{RA} were compared to determine if DA was complete before re-exposure to altitude. The 10-d DA period was sufficient for participants to regain body mass and for Hct and [EPO] to return to pre-exposure values. [Hb] remained significantly elevated at BL_{RA} compared to BL_{IA} but biological significance of the mean difference of 0.3 $g \cdot dL^{-1}$ is questionable.

After 10 d DA, resting f_H was still a mean 4 beats·min^{-1} (range 0-9 beats·min^{-1}) higher than at BL_{IA}. Although numerically small, this represents an increase of up to 13% from BL_{IA}. Some authors have shown a rapid return of resting f_H back to or below BL (Vogel et al 1967, Hannon et al 1969b), even after extended exposures to extreme altitude (Malconian et al 1990). Others have also reported a persistent elevation in resting f_H following return to sea level (RSL) for at least 3-5 d (Green et al 2000), 7 d (Fischetti et al 2000) or 10-14 d (Ponchia et al 1995). Elevated f_H post-altitude exposure has been attributed to either increased sympathetic or decreased parasympathetic drive (Fischetti et al 2000). Either mechanism would also result in a persistent elevation in MAP, but there is a slight, though not significant decrease in MAP at BL_{RA} compared to BL_{IA}. Although most accounts are that MAP returns to normal following RSL (Ponchia et al 1994, Fischetti et al 2000), others have also reported a decrease in MAP below BL (Vogel et al 1967, Scognamiglio et al 1991). An explanation consistent with an elevated f_H and depressed MAP could be a reduction in blood volume at BL_{RA}. Following 12 d at 4300 m Krzywicki and colleagues (1969) reported that blood volume had normalised within 4 d RSL, but after 16 d at 4300 m plasma volume was reduced for at least 6 d RSL (Lyons et al 1995). With no available data, a persistent reduction in blood volume after 10 d DA cannot be precluded.

Mean resting $\dot{V}_I$ was 1.2 L·min^{-1} higher at BL_{RA} compared to BL_{IA}. Although at least ten authors report that $\dot{V}_I$ decreases rapidly to normal upon RSL (see CHAPTER 2 for review), there are two accounts of persistently elevated $\dot{V}_I$, where arterial P_{CO_2} remained below BL for at least 10 d (Boning et al 1997) or even 35-40 d post altitude exposure (Masuyama et al 1986). However, given the variability of $\dot{V}_I$ measures throughout the study, this result should be considered carefully. Also, although the responses of f_H and MAP were consistent between $DA10d_{IA}$ and $DA10d_{RA}$, the persistent elevation in $\dot{V}_I$ at DA10d was not, further reducing the credibility of the $\dot{V}_I$ data.

The 10-d DA period was sufficient to allow most cardiorespiratory and metabolic responses to exercise to return to BL. $\dot{V}_I$, MAP, S_pO_2, RPE_{resp} and RPE_{legs} were not different at any exercise intensity between BL_{IA} and BL_{RA}. However, in BL_{RA} compared to BL_{IA}, f_H was lower in both moderate and heavy exercise and [La] was lower in heavy exercise. Both responses are consistent with improved fitness (Blomqvist and Saltin 1983) and training adaptations to endurance exercise are measureable after only three 45-min sessions at 70% $\dot{V}_{O2\text{-}max}$ (Ziemba et al 2003). The training stimulus of completing the trek from Lukla to Gokyo Ri was considerably greater, consisting of a total of ~24 h at a mean intensity of 65% $f_{H_{max}}$ over a 10-d period. Thus, completing the trek undoubtedly elicited cardiovascular and metabolic adaptations and improvements in trekking-related fitness. Participants were completely sedentary during the 10-d DA period in Kathmandu. Physiological detraining occurs rapidly, with measureable decreases in central and peripheral adaptations and $\dot{V}_{O2\text{-}max}$, within days to weeks of training cessation (see Mujika and Padilla 2001, Olivier et al 2008 for reviews). However, training adaptations are not completely reversed for several weeks after training cessation and it is likely that some cardiorespiratory and metabolic adaptations would persist after 10 d DA and de-training. On the other hand, improved cardiorespiratory fitness should have also presented itself with a reduced resting f_H (Blomqvist and Saltin 1983) and faster post-exercise recovery (Hagberg et al 1980), neither of which are seen in BL_{RA} compared to BL_{IA}. Thus, reduced f_H and blood [La] at a given exercise intensity may actually represent improved stepping economy, rather than improved cardiorespiratory and metabolic fitness. Exercise economy can be improved with training, though a long term training program is generally needed to see results. However, individuals who are less fit and more naïve to the activity might demonstrate improved economy with less training (reviewed in Jones and Carter 2000). Four participants were sedentary before the IA trek, with no regular participation in any form of physical activity. Although the other two were moderately- to highly-trained, neither had recently completed any training that resembled trekking or stepping exercise. Exercise economy may also be improved following exposure to continuous (MacDonald et al 2001) or intermittent hypoxia (Katayama et al 2004) although this result is not universal (Lundby et al 2007). It is unclear how long improved exercise economy might persist following cessation of training or hypoxic exposure.

4.4.3 Re-acclimatisation to high altitude

Apart from a potentially reduced blood volume and improved aerobic fitness and/or stepping economy at BL_{RA}, it appears that 10 d DA was sufficient to return participants to their pre-IA physiological status. Several authors have shown some retention of altitude tolerance after at least 8 d DA (Lyons et al 1995, Savourey et al 1996, Beidleman et al 1997, Savourey et al 2004), evidenced by decreased AMS scores and increased resting and exercise S_pO_2 during acute hypoxia. However, others have reported a rapid loss of acquired hypoxia tolerance with no difference in resting $P_{ET}CO_2$, acid base status or S_pO_2 during acute hypoxic exposure before, and as little as 7 d after, a 37 to 41-d expedition up to 7600 m (Boning et al 2001). There was no significant reduction in AMS scores in the first 24 h at altitude and no improvement in S_pO_2 at NB1 in RA compared to IA. Thus, participants showed no indication early in RA that they remained partially acclimatised from the IA trek. There were, however, several indications that participants experienced improved altitude tolerance throughout RA compared to IA.

4.4.3.1 Clinical and functional outcomes in RA

Participants demonstrated improved clinical outcomes in RA compared to IA (see TABLE 2). Total AMS scores were decreased in RA and participants reduced the use of analgesics to treat headache pain. In addition, a decrease in the use of acetazolamide to treat AMS from IA to RA approached statistical significance ($p = 0.11$). Exercise tolerance was also improved in RA compared to IA. Ambulatory f_H recordings indicated that exercise intensity during trekking was the same in IA and RA (FIGURE 4.13). However, participants completed the RA trek 11% faster and perceived the overall trekking intensity as 19% easier in RA compared to IA (see TABLE 2). Self-reported trekking times were cross-referenced with trekking f_H data and proved accurate within 5% in all cases. In addition, session RPE has been previously validated for use in evaluating the overall intensity of an extended endurance effort (Seiler and Kjerland 2006).

AMS was also reduced during RA in railroad workers who alternated between 7 mo at 4252 m and 5 mo at SL (Wu et al 2009). Both the incidence and severity of AMS were reduced in repeat acclimatisers compared to first-timers and both measures progressively decreased over 5 y of repeated re-exposure.

These authors acknowledged that AMS scores might be underreported in individuals who had experienced the symptoms during a previous altitude exposure. However, fH and BP tended to be lower and S_pO_2 was significantly higher in re-acclimatisers compared to first-timers, suggesting that AMS scores truly reflect improved hypoxia tolerance during RA, even after DA for 5 mo at SL.

4.4.3.2 Haematological responses in RA

Evidence of improved haematological acclimatisation in RA compared to IA is also reported; the altitude-induced Δ[Hb] doubled from IA to RA (see FIGURE 4.6). Early increases in [Hb] and Hct are primarily caused by a hypoxia-induced haemoconcentration (Grover and Bärtsch 1996). However, erythropoiesis is rapidly initiated upon exposure to high altitude and increased [EPO] is measurable within hours (Knaupp et al 1992). As early as 24 h, reticulocytes begin to be released into the circulation (Schobersberger et al 2005) where they mature into erythrocytes within 4 -5 d (Skadberg et al 2003). Although the initial increases in [Hb] and Hct measured at NB1 were solely due to haemoconcentration, it is likely that reticulocytes and young erythrocytes were contributing to [Hb] and Hct by testing points in GO. The increased Δ[Hb] in RA could therefore be due to an augmented haemoconcentration and/or increased de novo synthesis of erythrocytes.

The magnitude of ΔHb was greater in RA despite a reduction in ΔEPO, which intuitively suggests that erythropoiesis is not likely responsible for the increased ΔHb. In another study, 9 d DA following an expedition to high altitude, re-exposure to acute hypoxia also elicited a reduced EPO response (Savourey et al 1996, Savourey et al 2004). However, the resultant reticulocyte response was augmented, suggesting that erythroid stem cells are more sensitive to EPO stimulation following recent altitude exposure (Okunewick and Fulton 1970, Savourey et al 2004). Thus, the finding of a reduced ΔEPO during RA might not preclude an augmented erythropoietic response during re-exposure.

In rabbits acclimated to two series of intermittent hypoxia ($6h \cdot d^{-1}$ at 6000 m for 30 d, separated by 25 d normoxic DA), a more rapid increase in [Hb] and Hct in RA was attributed to a greater haemoconcentration during the second series of exposures (Jain et al 1978). In fact, an excessive contraction of PV was believed to be responsible for the death of 25% of animals during RA. No deaths

had occurred during IA, leading the authors to conclude that re-exposure to hypoxia "imposes a more severe stress than experienced during acclimatisation [sic]". No direct measures relating to PV or fluid balance are available from the current study to indicate whether a more severe haemoconcentration occurred during RA to continuous hypoxia. However, if the increased Δ[Hb] was due to an altered haemoconcentration response in RA then one would expect a parallel increase in ΔHct. [Hb] and Hct are generally well correlated (Hillman and Finch 1996) and increased similarly (12 and 15%, respectively) during IA. However, the disconnect between Δ[Hb] (24%) and ΔHct (14%) in RA suggests that haemoconcentration was not primarily responsible for improved haematological re-acclimatisation in this study. Rather, [Hb] is elevated disproportionately to Hct when erythrocyte size (MCV) is reduced or when [Hb] per cell (MCH) is increased (Hillman and Finch 1996); the result is improved O_2 delivery without the associated dangerous increases in blood viscosity. Erythrocyte and reticulocyte counts were not determined in this study due to the time-prohibitive method of manual evaluation in field conditions; therefore, MCV and MCH values cannot be calculated. It has previously been reported that MCV increases with hypoxic exposure (Huff et al 1975, Jain et al 1978, Tsantes et al 2004), possibly due to the presence of large reticulocytes. Although microcytosis has been reported in high-altitude populations of humans (Arnaud et al 1985), and other animals (Reynafarje et al 1968, Ruiz et al 1989), a decrease in MCV with short term altitude exposure has not been reported. Conversely, MCH has been shown to increase at altitude (Schobersberger et al 2005), leading us to speculate that an increased MCH was responsible for the increased ΔHb in RA compared to IA.

4.4.3.3 Cardiorespiratory and metabolic responses to rest and exercise in RA

Although there were no clear differences in resting cardiorespiratory function at any given time point between IA and RA, $\Delta_{altitude}$ calculations indicate that, when averaged across all testing points at altitude, f_H was 4 beats·min^{-1} lower, MAP was 3 mmHg higher and S_pO_2 was 3 percentage points lower in RA (FIGURE 4.8). Reductions in f_H are commonly used as an indication of improved altitude tolerance (Wolfel et al 1994) and lower f_H is associated with reduced AMS (O'Connor et al 2004). Conversely an increase in MAP and reduction in S_pO_2 cannot be considered as evidence of improved RA. MAP likely reflects an increase in sympathetic activation which is generally considered a pathophysiological side

effect of hypoxic exposure rather than compensatory mechanism. However, both positive and negative associations between increased blood pressure (BP) and altitude tolerance have been reported. Poor acclimatisation was associated with a drop in diastolic BP and MAP at high altitude (Koller et al 1991a) but diastolic BP was positively correlated with AMS score in another study (Koehle et al 2010). Currently there is no clear indication that MAP can be used as a marker of acclimatisation status. On the other hand, increased S_pO_2 at high altitude is universally acknowledged as an indication of improved hypoxia tolerance, with reduced S_pO_2 suggesting inferior acclimatisation in RA. However, lower S_pO_2 may be at least partially attributable to a clinically important and nearly statistically significant reduction in acetazolamide use in RA (Burtscher 2008). It is unclear how S_pO_2 in RA would differ had acetazolamide use been held constant between the two treks. Acetazolamide improves ventilatory acclimatisation to high altitude but with increased acid-base disruption and greater metabolic costs of breathing (Smith et al 2001). No differences in resting $\dot{V}_I$ between IA and RA are reported.

Assessing cardiorespiratory responses to exercise at high altitude may reveal subtle changes in acclimatisation status that are not apparent when examining acclimatising individuals at rest (Astrand 1954, Robach et al 2000). However, few differences in cardiorespiratory and metabolic responses to exercise are reported between IA and RA. MAP and S_pO_2 responded similarly to exercise throughout both treks despite measurable differences between IA and RA in these variables at rest. There were also no differences in exercise $\dot{V}_I$, RPE_{resp}, or RPE_{legs} between IA and RA and no difference in recovery index of any variable between treks. However, exercise f_H was reduced by an average of 4 beats·min^{-1} across testing points and exercise intensities in RA. Although the pattern was not as universal, [La] was also reduced from 6.0 to 3.3 mmol·L^{-1} during heavy exercise at NB3 in RA compared to IA. Although these findings might represent enhanced exercise tolerance as a result of improved RA, it is also plausible they represent a carryover in improved cardiorespiratory fitness or stepping efficiency brought about by the training stimulus of completing the first trek. It is also possible that repeatedly performing the graded exercise test in IA and RA also served as a training stimulus. However, in a previous study, there was no change in cardiorespiratory responses to exercise in participants who performed a cycling challenge of similar intensity and duration five times at a simulated altitude of 3800 m (see APPENDIX IV).

4.4.3.4 Explanations for improved altitude tolerance during RA

Participants felt better, trekked faster and perceived the trekking as easier during the second trek to high altitude. Resting and exercise f_H were also consistently lower throughout RA than IA. The simplest explanation for these observations is improved oxygen delivery during RA as a result of enhanced hypoxic compensation somewhere in the O_2 transport pathway. There was a greater haematological response with re-acclimatisation, evidenced by a higher peak [Hb] during RA. However, [Hb] was not elevated enough throughout RA to offset the mean 3% decrease in S_pO_2 and, assuming no change in oxyhaemoglobin affinity, there was likely no improvement in resting C_aO_2 from IA to RA. Although exercise S_pO_2 was not significantly reduced in RA, there was still no improvement in exercise C_aO_2 from IA to RA. It is theoretically possible that enhanced peripheral adjustments in RA were able to compensate for what is assumed to be a reduced P_aO_2, increasing the O_2 supply to and/or decreasing the O_2 demand by mitochondria in the brain and muscle. However, there was no assessment of oxygen transport pathways downstream from the blood so there are no data to support this speculation.

Thus, there is little evidence of improved physiological acclimatisation during RA. However, two alternative explanations for improved clinical and functional outcomes during the second trek are suggested: 1) improved cardiorespiratory fitness and/or exercise economy during RA; and 2) improved psychological tolerance of altitude after an initial exposure.

4.4.3.4.1 Improved cardiorespiratory fitness and/or exercise economy in RA

First, reductions in submaximal f_H and blood [La] during the standard exercise challenge suggest that cardiorespiratory fitness and/or exercise economy were improved throughout IA and persisted after 10 d DA. These changes could certainly explain the participants' ability to trek faster throughout RA while maintaining the same trekking f_H as in IA. Although session RPE has been validated as a measure of global intensity of an exercise session (Seiler and Kjerland 2006) it is likely that duration of the exercise bout also contributes to an individual's perception of the effort. Thus, although trekking intensity (in terms of % age-predicted $f_{H_{max}}$) did not change from IA to RA, the decrease in trek duration may have contributed to reduced RPE ratings in RA.

Although it is generally accepted that improved aerobic fitness conveys no protection against the development of AMS (Hansen et al 1967, Milledge et al 1991), there is one report of reduced AMS in individuals who underwent 8 w training prior to altitude exposure, compared to a control group with no training (Gupta et al 1978). In addition, it is understood that exercise at altitude contributes to the development and exacerbation of AMS (Roach et al 2000). Although relative trekking intensities were the same in IA and RA, the overall physiological strain would have been reduced if participants were fitter during the second trek. In addition, the reduced trekking duration in RA decreased the exercise dose and allowed more time for rest and recovery, possibly contributing to reduced AMS scores during the second trek. Perhaps if participants had followed an exercise program during DA to better maintain acquired improvements in fitness, the clinical and functional outcomes would have been further improved in RA.

4.4.3.4.2 Improved psychological tolerance of altitude in RA

Many of the measures that show improvement from IA to RA are subjective in nature or involve volitional behaviour. To date, self report is the only validated means for diagnosing AMS (Koehle et al 2010) and the assessment of AMS severity is based on an individual`s perception of his or her own symptoms. RPE is, by definition, a subjective assessment of effort. Walking speed and medication use are also volitional behaviours that undoubtedly involve complex conscious and subconscious decision-making.

Travel to high altitude is associated with significant anxiety (Noël-Jorand et al 1995, Pollard and Murdoch 2003). The first experience with high-altitude dyspnoea, lethargy, headache and other AMS symptoms can be particularly disconcerting and is accompanied by stress and uncertainty about potential illness, failure to meet objectives, etc. (Pollard and Murdoch 2003). State anxiety was measurably increased upon exposure to 3500 m and decreased during 2 w acclimatisation (Selvamurthy et al 1986). In addition, susceptibility to AMS is associated with anxiety (Missoum et al 1992). Although state anxiety was not assessed in the current study, it is plausible that, following successful completion of IA, participants had increased confidence about their ability to repeat the trek and reduced anxiety about an uncertain environment and physically challenging task. This may have conferred a psychological

advantage in RA which modulated self-reported trekking RPE, AMS scores and the perceived need for analgesics and acetazolamide. Two reports support this supposition. Wilson and colleagues (1993) demonstrated that, following altitude exposure, RPE_{resp} at a given $\dot{V}_I$ was reduced, suggesting that prior experience with dyspnoea may modulate the perception of breathlessness in the future. Second, although the authors claim that AMS scores were highly repeatable in men who were exposed to ~4200 m for 36 h on two occasions separated by 2 w, re-analysis of published data indicates that mean AMS scores were actually significantly lower during the second exposure ($p = 0.04$; Robinson et al 1971). Given the time frame of the experiment it is unlikely that participants retained physiological altitude tolerance through to the re-exposure. Rather, previous experience with the sensation of AMS may have reduced the perception of symptom severity during the second exposure.

The discomforts associated with travel to high altitude are many, including performing strenuous physical activity in the cold, hypoxic environment, impaired quality and quantity of sleep, limited access to desirable foods, and boredom. Improved psychological tolerance of these discomforts could play a major role in enhancing an individual`s perception of his or her experience at high altitude. This concept is discussed further in CHAPTER 6. It is unclear whether the psychological benefits of previous acclimatisation would last indefinitely or also be gradually lost over time.

4.4.4 Repeatability of acclimatisation responses

Existing data indicate that the physiological responses to acute hypoxia are fairly repeatable for a given individual. CVs have been previously reported for resting cardiorespiratory function during acute exposure to ~3800 m that range from 2% for S_pO_2 to 15% for $\dot{V}_I$. In exercise, CVs were similar, ranging from 3.5% for S_pO_2 and f_H to 9% for $\dot{V}_I$ (see APPENDIX IV). There are two reports that AMS scores during brief (24-36 h) exposure to high altitude are also repeatable. Although not included in the original publications, intra-individual CVs of 25% (Robinson et al 1971) and 33% (Forster 1984) were re-calculated from reported data. However, the only available information on the repeatability of acclimatisation responses during extended exposure to high altitude is anecdotal. Individuals who have previously acclimatised well tend to acclimatise well in the future and the best predictor of success on an

expedition is whether a climber has tolerated altitude well on a previous expedition (West 1993). The data presented in TABLE 4.3 represents the first empirical evidence that haematological and cardiorespiratory responses to extended hypoxic exposure are remarkably repeatable during consecutive treks to high altitude. CVs were higher for [EPO] (35%), [La] (23%), and $\dot{V}_I$ (18%), but CVs for the remaining variables were < 10% (resting f_H, MAP, RPE_{resp} and RPE_{legs}) or even ≤ 5% ([Hb], Hct, resting and exercise S_pO_2, exercise f_H, and trekking fH_{mean} and fH_{max}).

4.5 Conclusion

Participants exhibited normal responses throughout an initial acclimatisation to progressive altitude. Most aspects of the hypoxic acclimatisation response reversed during the 10-d DA period such that participants started both treks with a similar physiologic status. A reduced blood volume may have accounted for an increased f_H and a decreased MAP at rest at BL_{RA} compared to BL_{IA}. Decreased submaximal f_H and blood [La] may have resulted from improved aerobic fitness or improved exercise economy.

Evidence is presented that several clinical and functional features of altitude tolerance were improved in RA compared to IA. Throughout RA, participants trekked faster, perceived the trekking as easier, experienced fewer symptoms of AMS and took less headache medication compared to IA. Haematological acclimatisation was also improved in RA, evidenced by an increase in Δ[Hb] that occurred despite a decrease in Δ[EPO]. However, there was little indication that cardiorespiratory acclimatisation was improved between IA and RA. Although f_H tended to be lower during rest and exercise throughout RA, this may have been a consequence of improved cardiorespiratory fitness due to the training stimulus of completing the IA trek. The implications of an increase in resting MAP throughout RA are unclear. Contrary to what would be expected with facilitated re-acclimatisation, resting S_pO_2 was modestly reduced in RA, possibly as result of a nearly significant decrease in the amount of acetazolamide used during the second trek. The additional desaturation offset the increased [Hb] in RA, with the result that resting and exercise C_aO_2 were not different between treks. Improved hypoxic compensation in the periphery may provide a physiological explanation for these results. However it is possible that reduced anxiety and improved psychological altitude tolerance could account for much of the improved functional and clinical outcomes in RA.

CHAPTER 5. HAEMATOLOGICAL ACCLIMATION AND RE-ACCLIMATION TO HYPOXIA IN THE MOUSE (*MUS MUSCULUS*)

5.1 Introduction

The haematological response to hypoxia represents one of the most important features of the acclimation process (Grover and Bärtsch 1996). Haematological acclimation to hypoxia (HAH) leads to progressive increases in erythrocyte count (RBC), haematocrit (Hct) and, most importantly, haemoglobin concentration ([Hb]). Consequently, along with increases in P_aO_2 brought about by increased alveolar ventilation, perfusion and diffusion capacity, C_aO_2 is maintained as closely as possible to normoxic levels and O_2 delivery to the tissues is protected (Grover and Bärtsch 1996).

As with many components of the acclimation response, the mechanisms underlying HAH during a single sustained exposure to hypoxia have been clearly elucidated. With hypoxia, a rapid increase in [Hb] is brought about by an immediate haemoconcentration: a reduction in plasma volume (PV) and blood volume caused by a shift of fluid from the extracellular to intracellular space (Hannon et al 1969a) and/or a frank diuresis (reviewed in Hoyt and Honig 1996). The process of erythropoiesis is also initiated almost immediately, as demonstrated by a rise in serum erythropoietin concentration ([EPO]) within 6 h of hypoxia onset (Knaupp et al 1992) and an increase in circulating reticulocyte count (RC) in as little as 24 h (Schobersberger et al 2005). Reticulocytes mature over the following weeks, leading to relatively linear increases in Hct and [Hb]. When the hypoxic stimulus is removed, haematological variables gradually return to normal. The process of haematological de-acclimation from hypoxia (HDH) is discussed at length in CHAPTER 2 and is mediated by neocytolysis, whereby the most recently formed erythrocytes are targeted for destruction and phagocytised by macrophages in the spleen (Rice et al 2001).

There is some evidence that both the haemoconcentration (Lyons et al 1995) and erythropoietic responses (Savourey et al 1996, Savourey et al 2004) to acute hypoxia are altered by previous hypoxic acclimation

or acclimatisation. However, the process of haematological acclimation to sustained re-exposure has received very little attention. In trekkers who were recently monitored throughout two identical treks to high altitude separated by 10 d at low altitude, a significantly greater [Hb] response was observed during the second exposure (see CHAPTER 4). Given the number of potentially confounding factors in such field studies that cannot be disregarded (i.e. diet, exercise, temperature, psychological state, etc.), it was desirable to determine whether or not these results would be repeatable in a controlled laboratory setting using an animal model. In fact, using a paradigm of intermittent hypoxic (IH) exposure in rabbits (6 $h \cdot d^{-1}$ at ~6100 m for 30 d), Jain and colleagues (1978) had previously demonstrated a greater and more rapid haematological response during re-acclimation (RA) compared to an initial acclimation (IA). However, vital differences between physiological consequences of intermittent versus continuous hypoxia are well known (Sheel and MacNutt 2008) and the results from Jain and colleagues cannot necessarily be extrapolated. Therefore, the first objective was to use a model of sustained exposure and re-exposure to continuous hypoxia in a controlled laboratory environment to re-address the hypothesis that the HAH would occur more rapidly and/or to a greater magnitude during RA compared to IA.

The second objective was to examine the effects of manipulating the duration of IA and DA periods on the time course and magnitude of haematological responses during RA. Starting with a paradigm that closely resembled the repeated altitude exposures in trekkers (see CHAPTER 4), it was hypothesized that extending IA or abbreviating DA would lead to further facilitation of haematological acclimation during RA.

Rudimentary assessments of haematological status were employed by Jain et al (1978) as well as in previous field work with trekkers, offering little insight into physiological mechanisms underlying the results reported in both studies. Thus, the third objective was to explore the process of erythropoiesis and test the hypothesis that alterations in erythropoietic control would account for increased haematological responses reported in RA.

5.2 Methods

Experimental animals were exposed to sustained normobaric hypoxia (IA), allowed to de-acclimate in

normoxia (DA), and were then re-exposed to hypoxia (RA). Blood and tissue samples were collected by terminally sampling groups of animals at several time points throughout IA, DA and RA. All protocols were approved by the University of British Columbia Animal Care Committee.

5.2.1 Animals

Adult female C57BL/6NCrl mice (*Mus musculus*, Charles River Laboratories, Pointe-Claire, QC) were housed five per cage, with each cage representing an experimental group. C57Bl/6 mice have a very similar haematological response to hypoxia as two of the other most commonly-studied inbred strains (Balb/c and 129/Sv; Ward et al 2007) and were chosen for their resistance to disease and general robustness (Hedrich 2004). Animals experienced a 12:12 light:dark cycle and were fed commercial mouse chow ad libitum throughout the experiment. Animals were aged 10 – 24 w and weighed 18 – 24g at time of sacrifice.

5.2.2 Experimental treatments

5.2.2.1 Control animals

Three groups of control animals (no hypoxic exposure) were sampled at age 10 w, 12.5 w and 20 w to test the effect of age and body mass on all outcome variables. Since no clear patterns were seen across this age range for any variables of interest, five animals were randomly selected from the three age groups to represent the control group. Data from the control group represent baseline (BL) values for each variable.

5.2.2.2 Initial acclimation to hypoxia

Animals with no prior hypoxic exposure were sampled throughout a 4-w IA period after 0 (control group), 1, 3, 7, 14 and 28 d of hypoxic exposure.

5.2.2.3 Re-acclimation to hypoxia

Animals that had been initially acclimated to hypoxia and de-acclimated in normoxia were sampled after 0, 1, 3, 7, 14 and 28 d of hypoxic re-exposure. The time domains of IA and DA were manipulated to compare three paradigms of haematological RA to IA.

"RA" = RA after 14 d IA and 14 d DA

"$RA_{\downarrow DA}$" = RA after 14 d IA and an abbreviated (7-d) DA phase

"$RA_{\uparrow IA}$" = RA after an extended (56-d) IA phase and 14 d DA

Exposure paradigms for the four experimental groups are illustrated in FIGURE 5.1. Data was collected from five animals at each of the time points indicated throughout IA, RA, $RA_{\downarrow DA}$ and $RA_{\uparrow IA}$.

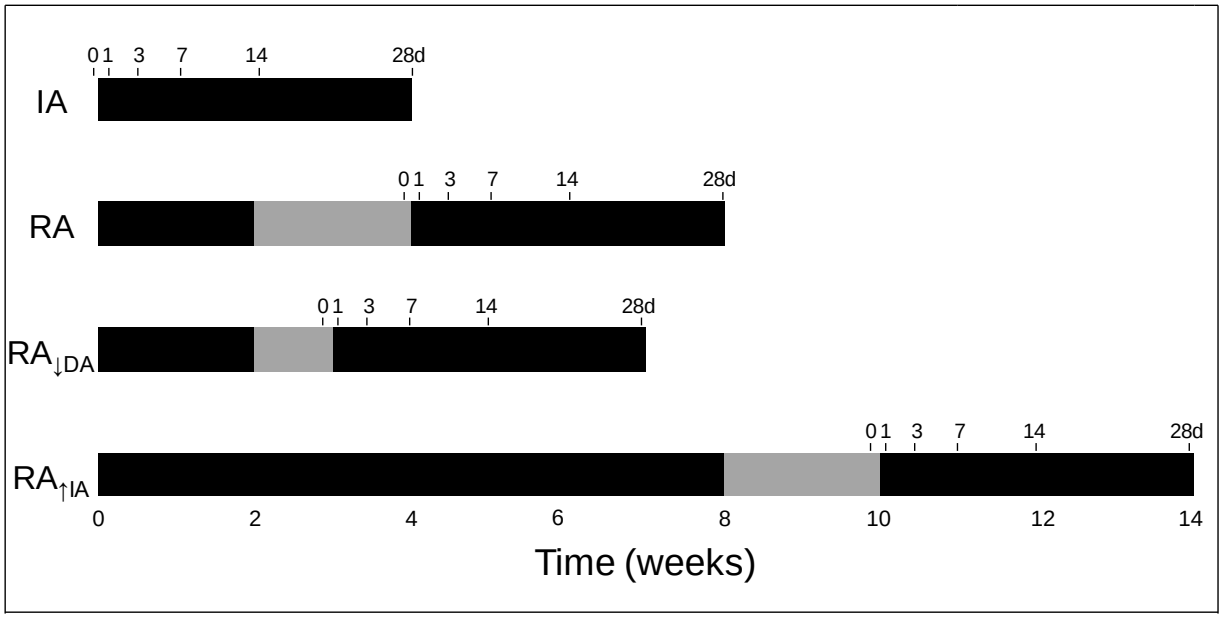


FIGURE 5.1 Schematic of exposure schedule for initial exposure (IA) and three paradigms of re-exposure (RA, $RA_{\downarrow DA}$ and $RA_{\uparrow IA}$) to hypoxia. Black shading denotes periods of hypoxic exposure and grey shading denotes periods of normoxic de-acclimation (DA). Animals in each treatment group were sampled as indicated after 0, 1, 3, 7, 14 and 28 d of hypoxic exposure or re-exposure.

5.2.3 Hypoxic exposures

Up to eight cages were placed in an airtight plexiglas chamber measuring 51 x 71 x 36 cm. Hypoxic gas was produced using a commercially available oxygen extractor (Mountain Air Generator MAG-7, Higher Peak LLC, Winchester, MA, USA) and flushed through the chamber at ~30 $L \cdot min^{-1}$ to maintain a constant hypoxic environment with F_IO_2 = 0.12 (equivalent of ~440 mmHg or ~4700 m terrestrial elevation) with negligible CO_2 accumulation. During DA, cages were placed on a rack beside the hypoxic chamber. Ambient conditions outside the chamber were monitored regularly and F_IO_2 never dropped below 0.20.

5.2.4 Data collection

Animals were sacrificed with an overdose of inhaled isoflurane (AErrane®, Baxter Corporation, Mississauga, ON, Canada). When all breathing movements had ceased, death was verified by cervical dislocation. All animals were sacrificed after 20 ± 3 min (max 45 min) of being removed from the hypoxic chamber.

5.2.4.1 Tissue sampling

5.2.4.1.1 Blood

Immediately post mortem, as much blood as possible was drained by cardiac puncture using a 25 G needle and 1 mL syringe. All blood samples were collected within 1 min of death. 400 μL of whole blood were transferred to an EDTA microtube. Remaining blood (0 – 500 μL) was transferred to an SST microtube and allowed to clot at room temperature for 30 min. Samples were then spun at 2000 g for 10 min in a microcentrifuge. Serum was transferred to a clean microtube and immediately frozen at -80 °C for later assay of [EPO].

5.2.4.1.2 Kidneys

Expression of the erythropoietin gene (Epo) was indirectly examined in the kidney. Within 3 min of death, both kidneys were excised, blotted dry and immersed in 1 mL of RNA stabilising solution (RNAlater®, Applied Biosystems, Streetsville, ON, Canada). Samples were held at 4 °C for 24 h, allowing solution to thoroughly permeate the tissue, and were then moved to -80 °C for storage and quantification of Epo mRNA.

5.2.4.2 Tissue analyses

5.2.4.2.1 Complete blood count

Complete blood count was obtained from fresh, whole EDTA blood using an automated low volume veterinary haematology analyser (Hemavet® 950, Drew Scientific Inc. Dallas, TX, USA). The instrument was calibrated daily to a manufacturer-validated standard (MULTI-TROL mouse, Drew Scientific Inc., Dallas, TX, USA) and re-calibrated after every 25 analyses. Samples were assayed in duplicate and all

blood counts were completed within 6 h of sample collection.

RBC, [Hb] and mean corpuscular volume (MCV) were measured directly by the instrument and several other variables were derived as follows:

$$Hct = (RBC \times MCV) / 10$$

$$\text{mean corpuscular Hb (MCH)} = 10 \times ([Hb] / RBC)$$

$$\text{red cell distribution width (RDW)} = 100 \times (\text{standard deviation of MCV} / \text{mean MCV})$$

One instrument was used to analyse samples from control, IA and RA groups. Samples from two remaining treatment groups ($RA_{\downarrow DA}$ and $RA_{\uparrow IA}$) were analysed using a second instrument (same model) in a different laboratory. Blood from additional control animals was analysed using the second instrument to ensure consistency between instruments. Unfortunately, MCV measurements were significantly different between the first and second instruments so MCV and its derived variables (Hct and RDW) are unavailable for $RA_{\downarrow DA}$ and $RA_{\uparrow IA}$ groups. All other variables of interest were found to be comparable (within 2.5%) between instruments.

5.2.4.2.2 Reticulocyte count

Reticulocytes were stained with Thiazole orange and enumerated using a flow cytometric method (Van Hove et al 1990). Briefly, 5 µL of fresh, whole EDTA blood was mixed with 1 mL of a commercially-available stain (BD RETIC-COUNT™, BD Biosciences, Mississauga, ON, Canada). Another 5 µL was mixed with 1 mL of 1X PBS with 0.1 % sodium azide solution to serve as an unstained negative control. Both samples were incubated in the dark at room temperature for at least 30 min.

Stained samples and unstained controls were analysed by fluorescence-activated cell sorting using a flow cytometer equipped with a 488 nm blue argon laser (FACScan™, BD Biosciences, Mississauga, ON, Canada). Data were collected using CellQuest™ software (V3.3, BD Biosciences, Mississauga, ON, Canada) and thresholds were adjusted manually to exclude noise and debris. 50000 events were acquired for each run and data were analysed using FlowJo software (V7.6, Tree Star Inc., Ashland, OR, USA).

Gating was adjusted around the erythrocyte population to exclude platelets from analyses. Control data were displayed using a fluorescence histogram and a marker was fixed to the right edge of the fluorescence peak. Data from the corresponding stained sample were then displayed in the same manner, with all events to the right of the marker representing stained reticulocytes. As such, RC was calculated as:

$$RC = \#\text{ events to right of marker} / \text{total }\#\text{ gated events}$$

This method of obtaining RC is illustrated in FIGURE 5.2. Reticulocyte enumeration was completed within 6 h of blood collection.

5.2.4.2.3 Serum [EPO]

A commercially-available enzyme-linked immunosorbent assay kit (Quantikine®, R&D Systems, Minneapolis, MN, USA) was used to quantify serum [EPO]. After a total of 4.5 h incubation, plates were read at 450 nm with a correction wavelength of 540 nm (BioTek uQuant Universal Microplate Spectrophotometer, Winooski, VT, USA). The mean absorbance of three blank wells was subtracted from all sample readings and optical densities (ODs) were converted to $pg \cdot mL^{-1}$ using a plate-specific standard curve. Serum samples were assayed in triplicate and mean values reported. Serum [EPO] was determined within 18 mo of blood collection.

5.2.4.2.4 Quantification of kidney *Epo* mRNA

5.2.4.2.4.1 PREPARATION OF CDNA

One kidney from each animal was thawed and homogenised in 1 mL Ambion TRI Reagent® (Applied Biosystems, Streetsville, ON, Canada) on ice. The homogenate was incubated at room temperature for 5 min and then returned to an ice bath. RNA was isolated using a commercially-available kit (Ambion RiboPure™, Applied Biosystems, Streetsville, ON, Canada) and isolated samples were DNAse treated with Ambion® TURBO DNA-free (Applied Biosystems, Streetsville, ON, Canada). The OD of each sample was determined at 260 nm (BioTek uQuant Universal Microplate Spectrophotometer, Winooski, VT, USA) and RNA concentration was quantified as:

$$[RNA] = OD_{260} * \text{dilution factor} * 40\ \mu g \cdot mL^{-1} \cdot OD\ unit^{-1}$$

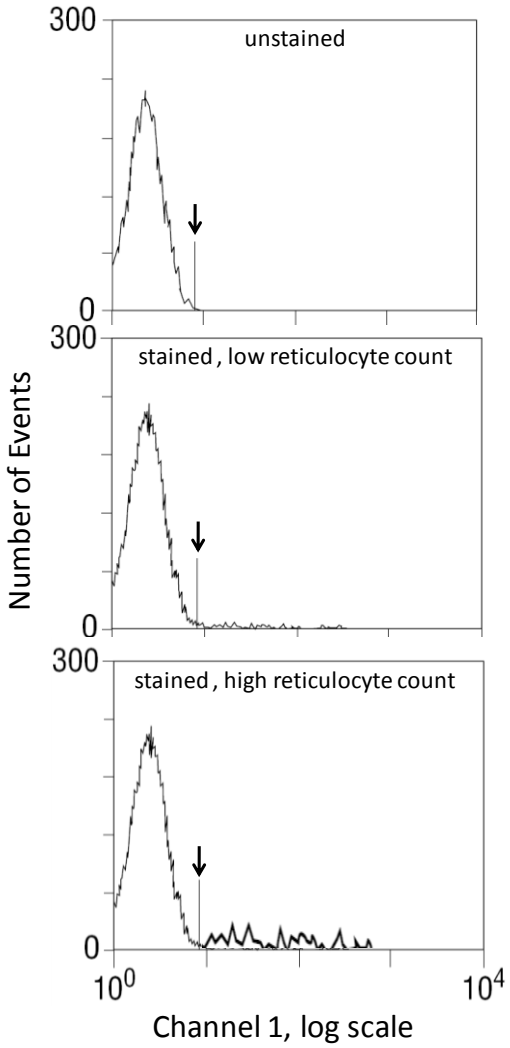


FIGURE 5.2 Schematic of reticulocyte enumeration using the Thiazole orange flow cytometric method. Arrows indicate where the marker was manually set at the edge of the flouresecence peak generated for the unstained control.

1 - 2 µg of RNA was reverse transcribed in a 10 µL solution using a High Capacity cDNA Reverse Transcription (RT) kit (Applied Biosystems, Streetsville, ON, Canada). Briefly, total RNA was combined with an RT master mix containing random primers and MultiScribe™ reverse transcriptase. The reaction was incubated at 25 °C for 10 min, 37 °C for 120 min and 85 °C for 5 s (MJ Mini™ thermal cycler, Bio-Rad Laboratories, Mississauga, ON, Canada). Negative RT (-RT) controls were prepared for each sample in an identical manner except the –RT master mix contained water in place of reverse transcriptase.

5.2.4.2.4.2 QUANTITATIVE POLYMERASE CHAIN REACTION (QPCR)

mRNA for the erythropoietin gene (*Epo*) was quantified using a Stratagene Mx3000P qPCR system (Agilent Technologies, Mississauga, ON, Canada) and normalized to β-actin (*Actb*), a commonly-used

internal control (Li et al 2005, Chan et al 2010). *Epo* primers were designed with PrimerQuest^{SM} software (Integrated DNA Technologies, Whitehead Institute for Biomedical Research, 2002) using a published mouse *Epo* mRNA sequence (Bult et al 2008). Mouse *Actb* primers had previously been used with successful amplification in our laboratory. Sequences of primer pairs for both genes are shown in TABLE 5.1.

TABLE 5.1 Sequences of forward (F) and reverse (R) primers used for amplification of erythropoietin (*Epo*) and β-actin (*Actb*).

Gene	Primer Sequence (5'- 3')
Epo	F: GCT CAG AAG GAA TTG ATG TCG CCT
	R: ACC CGG AAG AGC TTG CAG AAA GTA
Actb	F: CTG GCT CCT AGC ACC ATG AAG ATC
	R: TGC TGA TCC ACA TCT GCT GG

Transcripts were amplified in a 25 μL reaction containing 2 μL of prepared cDNA solution or –RT control, 400 nmol each of forward and reverse primers, 1.25 units of Maxima® Hot Start Taq DNA polymerase (Fermentas Life Sciences, Burlington, ON, Canada) and 1.25 μL EvaGreen^{TM} dye (Biotium, Hayward, CA, USA). A six-point standard curve was included on each plate by preparing fresh dilutions of the same standard (a mixture of cDNA from several individual mice predicted to have high *Epo* mRNA based on their exposure). A no-template control (containing 2 μL water in place of cDNA) was also included on each plate to detect any contamination of the qPCR master mix. All reactions were run in triplicate and amplified by cycling reactions through the following protocol: 10 min at 94°C, 50 cycles of 15 s at 94 °C, 30 s at 58 °C (*Epo*) or 59 °C (*Actb*) and 45 s at 72 °C. Upon completion of the reaction, a melting curve analysis was performed on each qPCR product to confirm the presence of a single amplification product. No *Epo* or *Actb* product was detected in any –RT control, indicating a lack of genomic DNA contamination in cDNA samples.

Critical amplification thresholds were determined for each reaction using MxPro software (Mx 3000P version 4.10, ©2007 Stratagene, Agilent Technologies, Mississauga, ON). For each sample, the quantity of initial transcript was calculated relative to the plate-specific standard curve and normalised to *Actb*

mRNA to account for inter-sample differences in cDNA content. Kidney *Epo* mRNA was quantified within 24 mo of sample collection and RNA stabilisation.

5.2.5 Data analysis

All analyses were completed using a statistical software package (SPSS 15.0, IBM SPSS Statistics, Chicago, USA) and the significance level of all tests was set at $\alpha = 0.05$. Data are presented as group mean ± standard error.

Haematological status immediately prior to re-exposure was compared to BL using unpaired t tests with data collected from mice sampled at IA14-DA14, IA14-DA7 and IA56-DA14. A two-way analysis of variance (ANOVA) was used to determine the effect of time in hypoxia (0 to 28 d) and treatment (IA, RA, $RA_{\downarrow DA}$ and $RA_{\uparrow IA}$) on each outcome variable. If the F statistic for treatment reached significance, the three RA treatments were compared to IA using a Dunnett's test. In cases where data did not meet the assumption of homogeneity of variances (as determined by Levene's test), the Dunnet's T3 test was used for post hoc comparison of each RA treatment to IA. The Tukey's HSD statistic was used to examine post hoc differences between IA and the three RA treatments at a given time point.

Serum was available for two to four animals per group. Groups means were used to replace missing values so analyses for serum [EPO] could be completed as above with n = 4.

5.3 Results

5.3.1 Environmental exposure

Although every attempt was made to maintain a constant environment inside the hypoxic chamber a minimal amount of day-to-day variability in F_IO_2 was unavoidable. After regular monitoring of the chamber environment, the mean F_IO_2 during initial and re-acclimation was calculated for each experimental group and ranged from 0.11 to 0.13 (~5390 to 4060 m). However, when all time points in each treatment were considered, there were no statistical differences between the mean exposure F_IO_2 for IA (0.115 ± 0.003) and RA (0.115 ± 0.001), $RA_{\downarrow DA}$ (0.111 ± 0.002) or $RA_{\uparrow IA}$ (0.121 ± 0.002).

5.3.2 [EPO] and *Epo* mRNA

[EPO] was significantly affected by both time in hypoxia and treatment group (shown in FIGURE 5.3). In IA, [EPO] increased rapidly and peaked after 3 d at more than double its initial value before decreasing back towards, and below, BL. [EPO] was still below BL immediately before all three re-exposure paradigms, though the difference from BL was significant only for $RA_{\downarrow DA}$ and $RA_{\uparrow IA}$. The pattern of [EPO] response was similar across all treatments, but the magnitude was notably reduced in all three RA groups compared to IA.

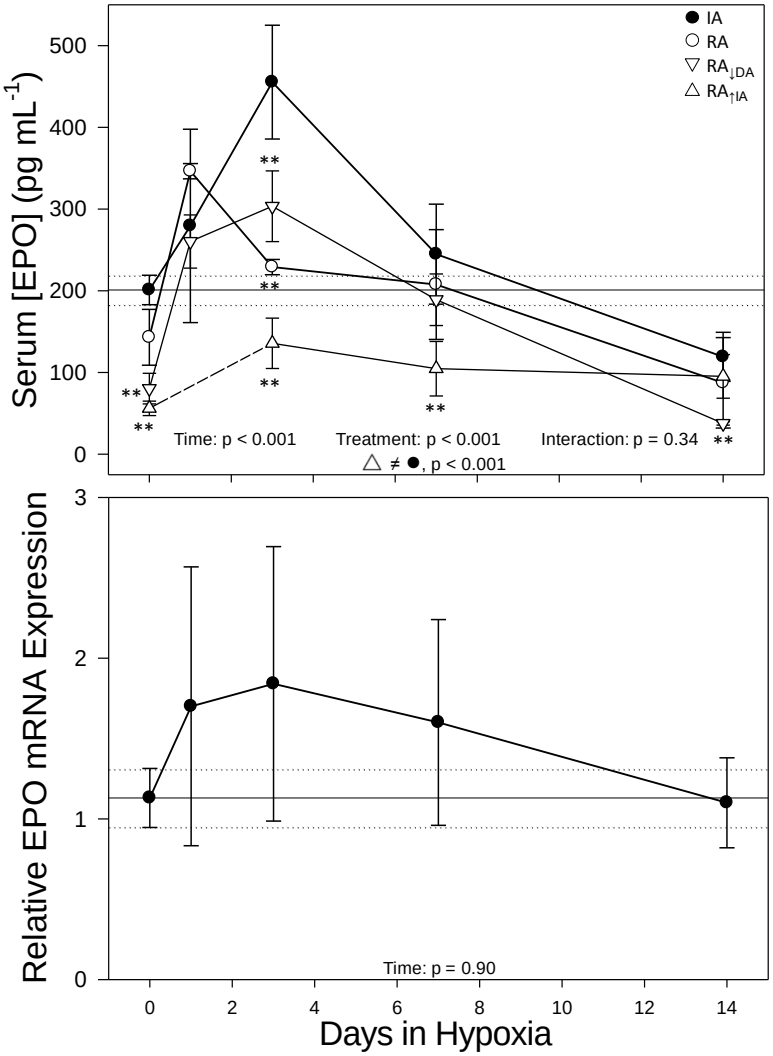


FIGURE 5.3 Serum erythropoietin concentration ([EPO]) and kidney *Epo* mRNA levels (normalized to *Actb*) throughout initial acclimation to hypoxia and three paradigms of re-acclimation (RA) to hypoxia ([EPO] only). Symbols represent group mean ± standard error. Horizontal lines represent the mean ± standard error for control animals with no hypoxic exposure. Results of two-way ANOVA and significant differences between mean IA and RA responses (Dunnett's test) are shown at the bottom of the panel. Significant differences between IA and RA at each time point (Tukey's HSD) are marked with ** ($p < 0.01$).

In RA, peak [EPO] was reached after 1 d, instead of 3 d in hypoxia and although the peak was more than 100 pg $\cdot mL^{-1}$ lower than in IA this did not represent a significant reduction. Conversely, peak [EPO] was significantly lower in both $RA_{\downarrow DA}$ and $RA_{\uparrow IA}$ than in IA. The overall [EPO] response was most notably blunted during $RA_{\uparrow IA}$ and this is the only group determined by Dunnett's test to be significantly different from IA.

Due to a large degree of within-group variability, no effect of time in hypoxia on *Epo* mRNA levels could be detected. However, among the 17 animals for which both types of data were available, there existed a strong correlation ($r = 0.78$, $p < 0.001$) between individual *Epo* mRNA and EPO protein (see FIGURE 5.4), suggesting that the intra-group variability in the quantity of *Epo* mRNA reflects physiological differences and not methodological or random error.

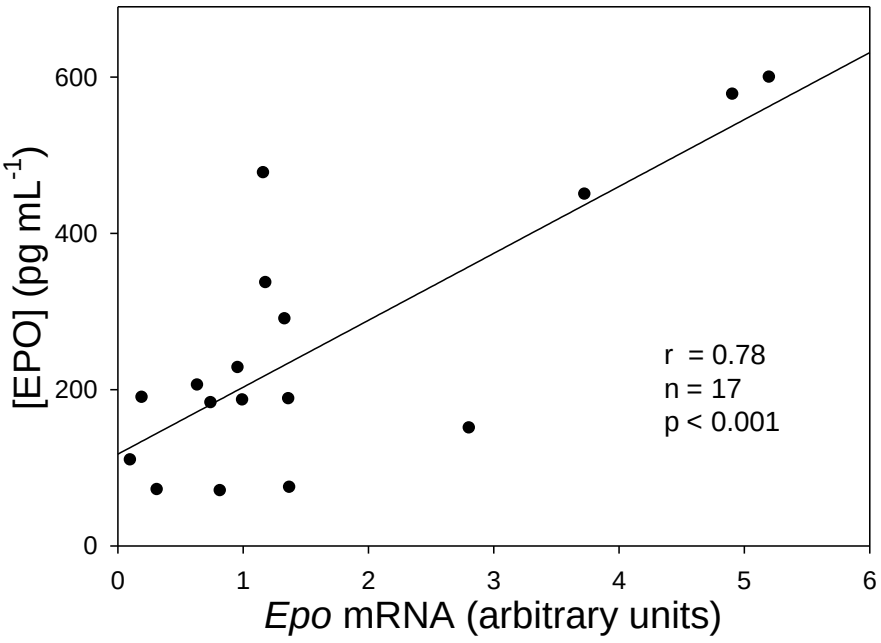


FIGURE 5.4 Scatterplot of serum [EPO] versus normalised *Epo* mRNA for 17 mice with available data. The Pearson product moment correlation and corresponding p value are given.

5.3.3 Reticulocyte count

RC was significantly affected by time in hypoxia and treatment group, but there was also a significant interaction between the main effects (all $p < 0.001$, see FIGURE 5.5). In IA, RC increased after 1 and 3 d in hypoxia then plateaued until measurements stopped at 14 d. After 7 d DA, RC had dropped below BL levels but was back to normal after 14 d DA. Post hoc tests revealed that all three RA conditions were

significantly different from IA (all $p < 0.001$). Although peak RC was consistent across treatment groups at 7-8% after 7 d, RC was lower in all RA groups than IA after 3 d and 14 d in hypoxia.

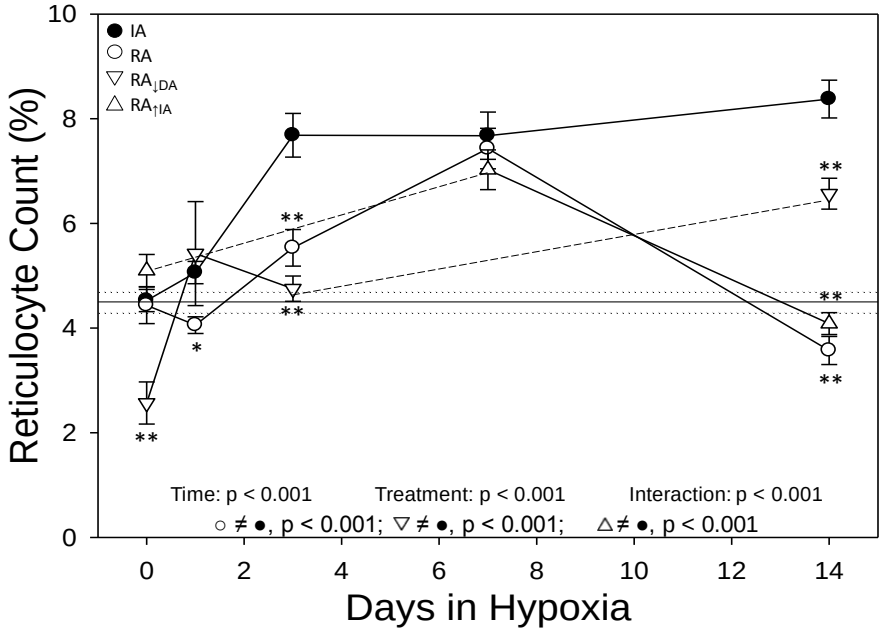


FIGURE 5.5 Reticulocyte count during an initial acclimation (IA) and three paradigms of re-acclimation (RA) to hypoxia. Horizontal lines represent the mean ± standard error for control animals with no hypoxic exposure. Dashed lines connect non-adjacent time points when data are missing. Results of two-way ANOVA and significant differences between mean IA and RA responses (Dunnett's test) are shown at the bottom of the panel. Significant differences between IA and RA at each time point are marked with * ($p < 0.05$) or ** ($p < 0.01$).

5.3.4 RBC, [Hb] and MCH

RBC, [Hb] and MCH were significantly affected by time in hypoxia (all $p < 0.001$). Although [Hb] and MCH were also significantly affected by treatment group (both $p < 0.001$), the treatment effect only approached statistical significance for RBC ($p = 0.09$). However, for all three variables there was a significant interaction between time and treatment, indicating a need for closer examination of response patterns for each variable (see FIGURE 5.6).

The RBC response was consistent between treatment groups (particularly between IA and RA) and Dunnett's test found no significant difference between IA and any RA treatment. Although not significantly different, RBC had not completely returned to BL before re-exposure in $RA_{\uparrow IA}$ and RBC in this group tended to be higher throughout the first week of exposure before plateauing at a similar level to IA and RA after 14 and 28 d in hypoxia. Conversely, although de-acclimation was complete before re-

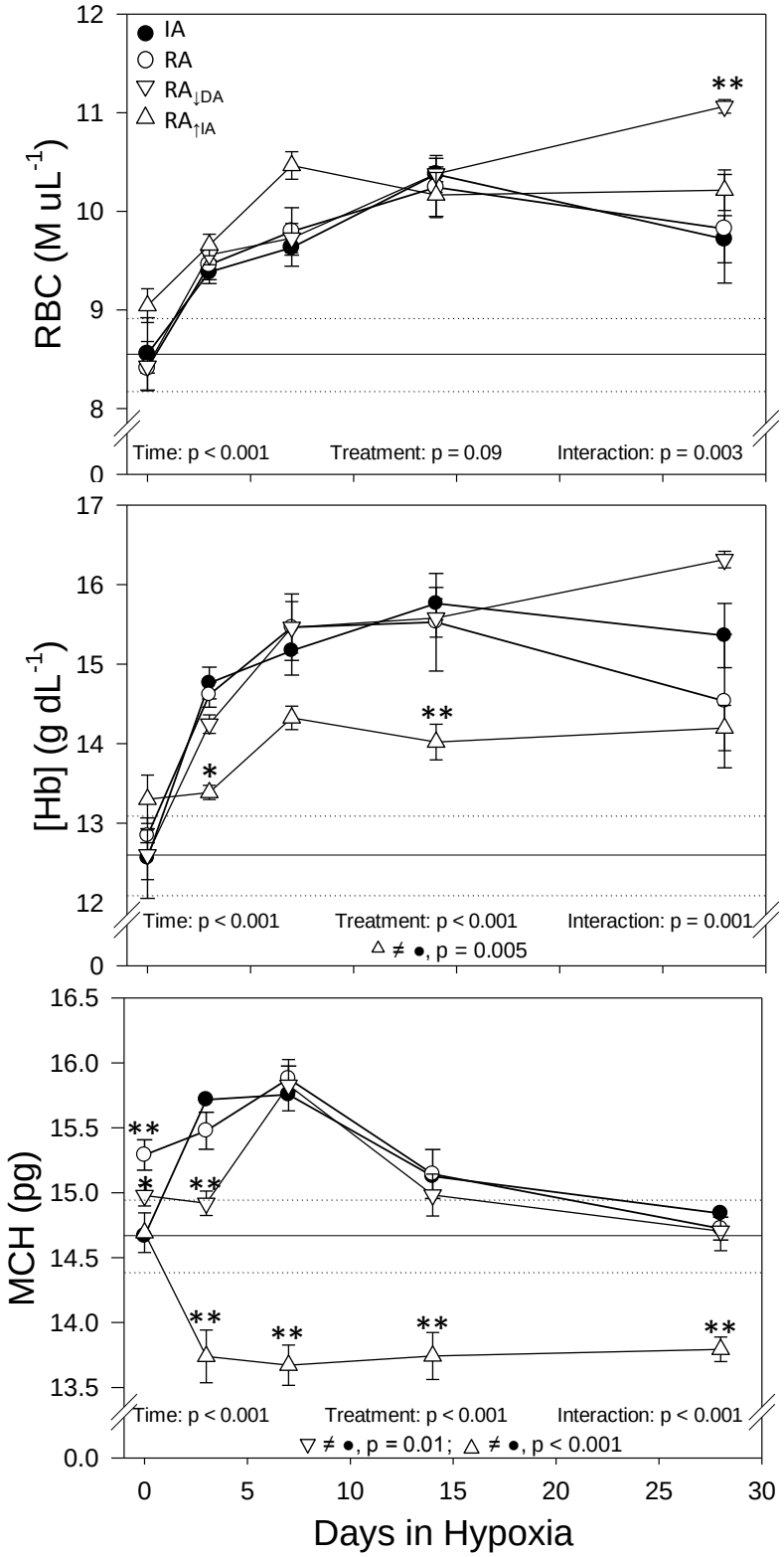


FIGURE 5.6 Erythrocyte count (RBC), haemoglobin concentration ([Hb]) and mean corpuscular haemoglobin (MCH) during an initial acclimation (IA) and three paradigms of re-acclimation (RA) to hypoxia. Symbols represent group mean ± standard error. Horizontal lines represent the mean ± standard error for control animals with no hypoxic exposure. Results of two-way ANOVA and significant differences between mean IA and RA responses (Dunnett's test) are shown at the bottom of each panel. Significant differences between IA and RA at each time point (Tukey's HSD) are marked with * ($p < 0.05$) or ** ($p < 0.01$).

exposure and the early response was identical to IA and RA, RBC reached a significantly higher peak after 28 d in hypoxia in $RA_{\downarrow DA}$ than in IA.

Although [Hb] was highly variable at the end of 28 d in hypoxia, the [Hb] response was consistent throughout IA, RA and $RA_{\downarrow DA}$. In $RA_{\uparrow IA}$, [Hb] remained slightly, but not significantly, above BL immediately before re-exposure. However, [Hb] increased to a lesser degree throughout $RA_{\uparrow IA}$ as compared to the other treatment groups. Dunnett's test indicated a significant difference between IA and $RA_{\uparrow IA}$, with [Hb] significantly lower in $RA_{\uparrow IA}$ than IA at 3 d and 14 d.

MCH was significantly elevated before re-exposure in RA and $RA_{\downarrow DA}$, but the overall MCH response was not different between IA and RA. Dunnett's test indicated a significant difference between $RA_{\downarrow DA}$ and IA, though this was driven largely by differences in the early MCH response. Following an extended IA period, MCH returned to BL after 2 w DA. However, MCH was significantly lower in $RA_{\uparrow IA}$ than IA with MCH consistently below BL throughout the exposure.

5.3.5 MCV, Hct and RDW

MCV, Hct and RDW data were available only for IA and RA treatments (see FIGURE 5.7). There was a significant effect of time and treatment for all three variables, as well as a significant interaction between the main effects (all $p < 0.001$).

MCV increased throughout IA and returned to BL before re-exposure. MCV also increased throughout RA, but to a lesser extent than in IA. Mean cell size was significantly smaller in RA than IA after 7, 14 and 28 d in hypoxia. Hct followed a similar pattern, increasing throughout IA and de-acclimating completely to BL before re-exposure. Again, Hct increased throughout RA but to a lesser degree than in IA. However, there were no significant differences in Hct between IA and RA at any time point. RDW increased sharply during IA, peaking at 7 d then gradually returning towards BL. However, de-acclimation was not complete after 14 d in normoxia and RDW remained significantly greater than in IA at 0 and 3 d in RA. As in IA, RDW peaked at 7 d in RA then steadily decreased towards BL over the remaining weeks in hypoxia.

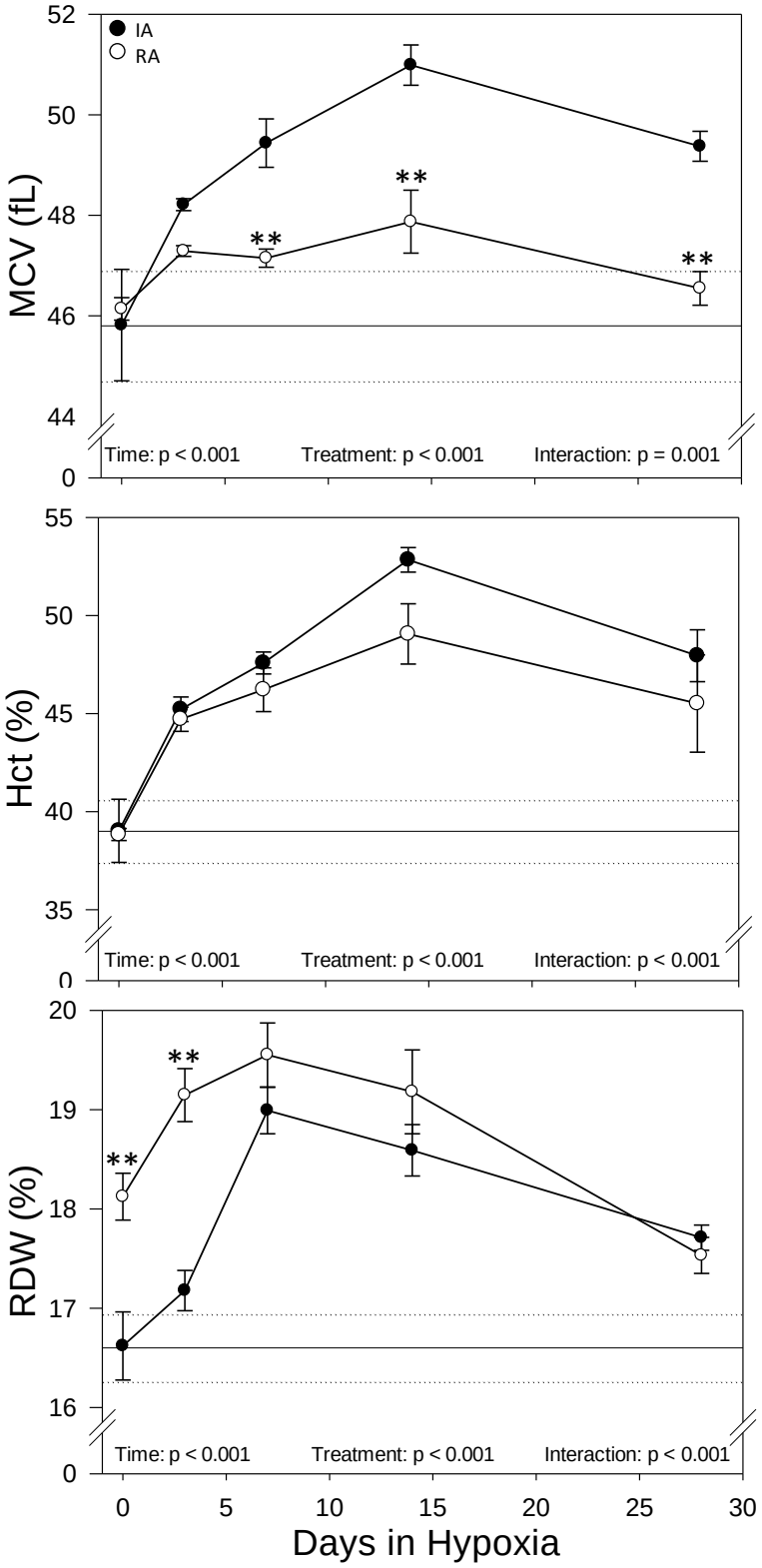


FIGURE 5.7 Mean corpuscular volume (MCV), haematocrit (Hct) and red cell distribution width (RDW) during an initial acclimation (IA) and three paradigms of re-acclimation (RA) to hypoxia. Symbols represent group mean ± standard error. Horizontal lines represent the mean ± standard error for control animals with no hypoxic exposure. Results of two-way ANOVA are shown at the bottom of each panel. Significant differences between IA and RA at each time point (Tukey's HSD) are marked with ** ($p < 0.01$).

5.4 Discussion

It was hypothesised that RBC, Hct, [Hb], and therefore O_2 carrying capacity, would increase faster and to a greater magnitude during hypoxic re-exposure compared to an initial exposure. In addition, it was hypothesised that RA could be further facilitated by increasing the duration of the IA period and/or decreasing the duration of the DA period. In order to explore the mechanisms of facilitated haematological re-acclimation to hypoxia, the early signs of erythropoiesis were also assessed during IA and three paradigms of RA. Few data are presented to support either of these hypotheses.

For most variables, the general pattern of acclimation response was consistent with the literature and repeatable between IA and RA treatments. However, there were several notable exceptions. The most important observations and potential underlying mechanisms and implications are discussed in detail in later sections of the discussion. Ultimately, an argument is presented that previous exposure to hypoxia can actually have a detrimental, rather than facilitative, impact on haematological re-acclimation to hypoxia. Repeated cycles of erythropoiesis and cell destruction can deplete essential nutrients, leading to impaired Hb synthesis and/or erythrocyte development if erythropoietic stimulation continues.

These findings also contribute to a greater understanding of the process of erythropoiesis during the onset and offset of hypoxic exposure. Building on earlier findings by Savourey and colleagues (2004), the current work demonstrates that erythropoietic control is altered following hypoxic exposure and that sensitisation can persist for several weeks. During hypoxic re-exposure, erythropoiesis proceeds normally despite a significantly blunted [EPO] response.

5.4.1 Haematological acclimation during IA

5.4.1.1 Early signs of erythropoiesis

As anticipated, the initial acclimation to hypoxia resulted in a stimulation of erythropoiesis, evidenced by a rapid increase in serum [EPO] (see FIGURE 5.3) followed by an elevated RC (see FIGURE 5.5). As has been reported from human studies (Abbrecht and Littell 1972, Milledge and Cotes 1985), serum [EPO] did not remain elevated throughout the exposure but dropped back towards, and even below, BL by 14 d

in hypoxia. Although RC generally follows this same pattern (Mylrea and Abbrecht 1970, Huff et al 1975), in IA the proportion of reticulocytes remained elevated through 14 d hypoxia, rather than falling to BL as expected.

Increased [EPO] following hypoxic exposure presumably was preceded by increased *Epo* gene expression at the kidney (Schuster et al 1987) and although the expected pattern of kidney *Epo* mRNA was demonstrated, a large degree of inter-individual variability prohibited the detection of a significant effect of time in hypoxia. Given that experimental groups were age- and sex-matched cage mates from an inbred mouse strain, the degree of within-group variability was unexpected. Variability in gene expression between genetically identical cage mates has been attributed to differences in immune status of individual mice (Pritchard et al 2001) and could also reflect differences in maternal environment or hierarchical structures within the cage. Pritchard and colleagues (2001) have also reported an order effect with differential gene expression between the first and last cage mates to be sacrificed. Although the short time frame between sacrifices likely precludes an effect of time of day on gene expression, it is possible that some rapid changes in gene expression occur in response to the removal and sacrifice of cage mates. Schuster and colleagues (1987) reported a decrease in *Epo* mRNA within 1 h of the cessation of hypoxia. In the current study, the time between removing animals from the hypoxic chamber and RNA-stabilizing the excised kidneys ranged from about 5 – 45 min but there was no observable pattern of decreasing *Epo* expression with animals sacrificed after longer periods of time out of the chamber.

Alternatively, increased *Epo* mRNA expression may not be required to elicit an increase in [EPO] since Chikuma et al (2000) suggest that an elongated half life of *Epo* mRNA may also contribute to increased EPO production. Furthermore, although the kidney is the predominant source of *Epo* expression and EPO synthesis, other tissues including the liver, brain, lung, and reproductive organs have been discovered as sites of EPO production in adult mammals and may account for up to 10% of circulating EPO (reviewed in Weidemann and Johnson 2009). Although the latter sources generally contribute insignificantly to circulating EPO, perhaps the contribution of liver *Epo* mRNA should not have been ignored.

5.4.1.2 Complete blood count data

Classic patterns of haematological acclimation were seen in IA for RBC, [Hb] and Hct (FIGURES 5.6 and 5.7) with a rapid initial increase in each variable followed by a more gradual rise to a plateau (see Grover and Bärtsch 1996 for review). MCV, RDW and MCH increased transiently, followed by a gradual decrease after 1-2 w back towards BL (FIGURES 5.6 and 5.7).

There is no known report of an increase in mean corpuscular volume (MCV) in healthy humans following real or simulated high-altitude exposure. However, chronic obstructive pulmonary disease patients (Tsantes et al 2004) and habitual smokers (Cembrowski and Fairbanks 1995) commonly exhibit macrocytosis, possibly due to the episodic nature of their hypoxaemia. Tsantes and colleagues (2004) postulated that repeated stimulation of erythropoiesis by intermittent hypoxia leads to frequent release of large, immature red cells from the marrow. In support, Jain et al (1978) report a 6% increase in MCV in rabbits intermittently exposed (6 $h \cdot day^{-1}$) to ~6100 m for 30 d. Furthermore, an increase in MCV has also been reported in mice (Mylrea and Abbrecht 1970, Huff et al 1975), rats (Germack et al 2002), rabbits (Marki et al 1982) and ground squirrels (McLaughlin and Meints 1972) exposed to continuous hypoxia as well as in mice made polycythaemic with exogenous EPO therapy (Gurney et al 1961). Marki and colleagues (1982) reported that an initial increase in MCV was accompanied by an increased RDW, with both variables returning towards BL over time. Clearly demonstrated in the present data as well (FIGURE 5.7), this pattern suggests the increased MCV is due to the hypoxia-induced reticulocytosis that occurs during the early stages of hypoxic exposure. The presence of large reticulocytes inflates MCV and also increases the variability of cell size within the red cell population (Turgeon 1999). The newly-released RBCs get smaller as they mature, leading to a decrease in both MCV and RDW. However, reported increases in MCV of 4-10 fL cannot be mathematically explained by the observed reticulocytosis alone. In addition, other studies in mice (Mylrea and Abbrecht 1970, McLaughlin and Meints 1972, Huff et al 1975) report that the increased MCV persists throughout the hypoxic exposure, even after RC has returned to normal (Mylrea and Abbrecht 1970). Taken together, these facts suggest that other factors besides reticulocytisis contribute to the increase in MCV seen with hypoxic exposure. Vitamin B_{12} and folate deficiencies during cell development result in the formation of macrocytic red cells and it is

possible that differing patterns of MCV with hypoxic exposure reflect differences in nutritional status between species, or even experimental groups. Although not previously discussed in the literature, hypoxia-induced increases in MCV might also result from general swelling of erythrocytes, possibly due to an increased osmotic gradient between the plasma and red cell.

The finding of a transient increase in MCH in IA (FIGURE 5.6) has been previously observed in rabbits (Marki et al 1982) and humans (Schobersberger et al 2005), and this phenomenon is also likely driven by the hypoxia-induced reticulocytosis. Early reticulocytes continue to synthesise Hb with a steady increase in Hb content from early to late stage, followed by a drop in MCH as reticulocytes develop into erythrocytes (Skadberg et al 2003). This decline in MCH has been explained by the extrusion of Hb-containing vesicles during the final stages of reticulocyte maturation (Colella et al 1991). The pattern and timing of these developmental changes fit with the increase in RC seen in FIGURE 5.5 and could account for the transient increase in MCH reported here and in previous studies.

5.4.2 Haematological re-acclimation during RA, $RA_{\downarrow DA}$ and $RA_{\uparrow IA}$

5.4.2.1 Reduced [EPO] response in RA treatments compared to IA

In all three RA treatments, [EPO] followed the same general pattern as in IA but the magnitude of the response was blunted: peak [EPO] was reduced 25% in RA (NS) and 33% in $RA_{\downarrow DA}$ ($p = 0.05$, see FIGURE 5.3). In $RA_{\uparrow IA}$, [EPO] again followed the same pattern as in IA but remained well below BL throughout the re-exposure. It is possible that missing data concealed an early peak in [EPO] after 1 d in hypoxia (as in RA) but if the shape of the [EPO] response was consistent across treatments it is unlikely that this missing value would have approached the peak [EPO] seen in IA.

Although this is the first study to compare the early stages of erythropoiesis throughout an initial and re-acclimation to sustained hypoxia, other work has compared the EPO response to acute hypoxia before and 1 w after a 3-w expedition to 6768 m. As in the present work, Savourey and colleagues (2004) reported that, in humans, [EPO] was reduced following DA and the EPO response to acute re-exposure was diminished. In previous work (see CHAPTER 4), a reduction in [EPO] also occurred during the early stages

of DA that was restored after 10 d at low altitude. In addition, during an extended re-exposure to high altitude there was a slightly blunted [EPO] response compared to IA. Unlike the present work, Savourey and colleagues (2004) also reported that RBC, [Hb] and Hct had not fully de-acclimatised before re-exposure and that P_aCO_2 was lower and P_aO_2, S_aO_2 and C_aO_2 were higher during acute hypoxia after the expedition as compared to before. They concluded that individuals were still partially acclimatised when they were re-exposed to hypoxia, and with better protected arterial oxygenation higher C_aO_2 at the kidney resulted in reduced erythropoietic stimulation and a blunted EPO response. This might also have been the case in the previous field work in humans, since [Hb] was slightly elevated upon re-introduction to high altitude (see CHAPTER 4). The same was true of mine workers exposed to intermittent sustained hypoxia (7 d at 4200 m, 7 d at SL) for 2.5 y (Richalet et al 2002b). [Hb] and Hct progressively increased at SL and altitude while the EPO response to altitude re-exposure tended to fall over time. In addition, [EPO] was assessed at a single time point after 6 d at altitude, which is not likely to reflect the peak response. Thus, this finding should be interpreted with caution. In the present study, haematological variables had returned to BL before re-exposure but it is possible that improvements in aspects of acclimation that were not measured here (i.e. minute ventilation, blood flow distribution, oxyhaemoglobin affinity) also resulted in higher C_aO_2 and therefore a reduced erythropoietic stimulus at the kidney during RA compared to IA.

However, Savourey et al (2004) still reported a reduced [EPO] response for a given C_aO_2, suggesting that the blunted [EPO] responses reflect decreased sensitivity of renal peritubular cells to hypoxia. This could also account for the common finding that [EPO] drops towards or below BL after 1-2 w of sustained hypoxic exposure (Abbrecht and Littell 1972, Milledge and Cotes 1985, present data set). It is possible that hypoxic sensitivity remains altered for some time following return to normoxia and that this sensitivity can persist into a re-exposure. Given that the [EPO] response was significantly more blunted in $RA_{\uparrow IA}$ than $RA_{\downarrow DA}$, despite a similar starting point immediately pre-exposure suggests that desensitization was greater and/or more persistent in animals that experienced a longer initial acclimation period.

5.4.2.2 Similar reticulocyte response despite blunted [EPO] in RA treatments compared to IA

Despite the blunted [EPO] response during re-exposure, RC peaked at the same value (7-8%) in RA and

$RA_{\uparrow IA}$ as in IA. Unfortunately, data for $RA_{\downarrow DA}$ were unavailable for the 7 d time point so it is uncertain whether the reticulocyte response for this treatment was consistent with the other groups. Although their time frame was much shorter, Savourey et al (2004) actually reported an increased reticulocyte response in spite of reduced [EPO] during re-exposure. One explanation offered was that increased sensitivity of EPO receptors on erythroid progenitor cells resulted in enhanced differentiation for a given EPO stimulus (Savourey et al 2004). In support, Okunewick and colleagues (1970) demonstrated that the erythropoietic response to exogenous EPO (measured by ^{59}Fe uptake) was greater in mice recently made similarly polycythaemic by hypoxic exposure versus transfusion. The previous finding of a greater [Hb] response in RA despite a similar [EPO] response as in IA also bolsters the argument (see CHAPTER 4). The current data set provides evidence that erythropoiesis might be regulated by relative, rather than absolute changes in [EPO]. Despite large differences in absolute values, the relative increases in [EPO] from pre-exposure to peak values were remarkably similar between treatment groups that exhibited a consistent reticulocyte response (130, 140, and 140% increase for IA, RA and $RA_{\uparrow IA}$, respectively). These data suggest that EPO receptors were sensitised to reduced circulating levels of [EPO] during DA and responded to the relative increase in [EPO] that occurred in each RA.

5.4.2.3 Increased peak RBC in $RA_{\downarrow DA}$ compared to IA

Although the RBC response was consistent across treatments, after 28 d in hypoxia RBC reached a significantly higher peak in $RA_{\downarrow DA}$ than in IA (FIGURE 5.6). [Hb] was also higher at this time point, though the difference from IA was not significant. Review of environmental conditions in the exposure chamber indicates that $RA_{\downarrow DA}$ mice were exposed to lower than average F_IO_2 from 14 – 28 d in hypoxia (0.108 compared to 0.115). Although this only represents a reduction in P_IO_2 from ~87 to 81 mmHg, the resulting P_aO_2 would fall on the steep part of the mouse oxyhaemoglobin dissociation curve (Johansen et al 1976) and the small difference likely represents a large change in C_aO_2 and physiological stimulus. Thus, the increased RBC is likely a result of increased haemoconcentration, splenic contraction and/or erythropoiesis caused by an increase in the hypoxic stimulus, rather than a physiological difference caused by the re-acclimation paradigm per se.

5.4.2.4 Decreased [Hb] and MCH throughout $RA_{\uparrow IA}$ compared to IA

As shown in IA and in previous studies (Marki et al 1982, Schobersberger et al 2005), MCH showed a transient increase in the early stages of RA and $RA_{\downarrow DA}$ before decreasing back to BL (FIGURE 5.6). In contrast, the [Hb] response was significantly reduced by ~9% across time points in $RA_{\uparrow IA}$ compared to IA with a persistent drop in MCH below BL, despite a similar RBC response. Thus, contrary to the original hypothesis, O_2 carrying capacity was reduced, rather than increased in $RA_{\uparrow IA}$ compared to IA. This is not the first report that previous hypoxic exposure can have apparently adverse, rather than beneficial, effects on re-acclimation responses. Using a model of intermittent hypoxic exposure in rabbits, Jain and colleagues (1978) observed a faster haematological response with a larger increase in [Hb] in RA compared to IA. However, there was no evidence of increased erythropoiesis during RA and the increased [Hb] response was driven by four animals who exhibited a stronger than average haemoconcentration during RA. In fact, hypovolaemia in those individuals was so severe that they died during RA, apparently from pulmonary haemorrhage. An increased haemoconcentration during acute hypoxic re-exposure has also been demonstrated in humans following continuous initial exposure to HA; however, this was associated with the positive outcome of reducing severity of acute mountain sickness during RA (Lyons et al 1995). Conversely, data from Savourey and colleagues (2004) suggest a reduced haemoconcentration response during acute re-exposure following a 3-w sojourn to high altitude. No measures of PV or fluid balance are reported here. However, based on the repeatability of early changes in RBC, Hct and [Hb] across treatments, there is no evidence of altered haemoconcentration during RA groups compared to IA.

The reasons for decreased [Hb] and MCH throughout $RA_{\uparrow IA}$ are not entirely clear but a transient decrease in MCH has also been reported in the pig-tailed monkey (Buderer and Pace 1972) and in the mouse (Mylrea and Abbrecht 1970). Interestingly, in the latter study this pattern only occurred in animals exposed to more severe hypoxia (~6200 m) and not during milder hypoxic exposures (~4600 and ~3400 m). The greater hypoxic stimulus elicited a larger reticulocyte response, including relatively more "shift" reticulocytes than following less hypoxic exposures. Shift reticulocytes are prematurely released from the bone marrow during times of particular erythropoietic stress (Turgeon 1999) and contain less Hb than

normal reticulocytes (Skadberg et al 2003). As these shift reticulocytes mature they continue to synthesize Hb, bringing MCH back towards BL. Given the identical environmental exposure, the reduced [EPO] response, and the similar reticulocyte and RBC response between $RA_{\uparrow IA}$ and the other treatments, there is no evidence to suggest that hypoxic stimulus was more severe in $RA_{\uparrow IA}$. However, the extended initial exposure to hypoxia may have depleted the pool of mature reticulocytes, leading to the premature release of more shift reticulocytes during $RA_{\uparrow IA}$.

Alternatively, the drop in MCH might be the result of reduced Hb content of normal-age reticulocytes released in $RA_{\uparrow IA}$. This could reflect impaired Hb synthesis in the erythrocyte precursors, perhaps as a result of reduced availability of iron, transferrin or other required elements such as folate, Vitamin B_{12}, succinyl coenzyme A, and amino acids (Turgeon 1999). Given that MCH did not increase back towards BL in $RA_{\uparrow IA}$ as it did in the above-mentioned studies (Mylrea and Abbrecht 1970, Buderer and Pace 1972) and that the [Hb] response was reduced throughout the re-exposure suggests that impairment of Hb synthesis persisted in the circulating reticulocytes.

Still, it is not simply the quantity of circulating Hb that must be considered when assessing compensatory mechanisms to improve gas exchange during acclimation to hypoxia. Upon initial assessment, the finding of similar or decreased [Hb] response in RA treatments compared to IA indicates that there is no improvement in O_2 carrying capacity with re-acclimation. However, evidence suggests that repeated stimulation of erythropoiesis (Tsantes et al 2004) and apoptosis triggered by the drop in [EPO] that occurs during and after extended hypoxic exposure (Mide et al 2001) can both deplete the normal pool of erythroid precursors (colony forming units, CFU-E) in the bone marrow and spleen of mice. When this occurs, new cells will be recruited into the erythrocyte population from the more primitive pool of burst forming units (BFU-E). Differentiated BFU-E cells retain the ability to synthesize foetal Hb (HbF) and their recruitment has been shown to cause considerable increases in the proportion of circulating HbF in humans (Kidoguchi et al 1978, Clarke et al 1979). The increased O_2 affinity of HbF relative to normal adult Hb (HbA) facilitates O_2-loading, which is arguably advantageous for protecting gas exchange in moderate hypoxia compared to the decrease in O_2 affinity that typically occurs with hypoxic acclimation and favours O_2-unloading (Eaton et al 1974, Bencowitz et al 1982). In theory, extended and repeated

erythropoietic stimulation is more likely to deplete the CFU-E pool and ultimately increase the ratio of HbF:HbA than a single initial exposure to hypoxia. Since data are not available to compare either P_{50} or S_ao_2 between IA and RA treatments, the potential beneficial impact of HbF synthesis during re-acclimation to hypoxia is purely speculative.

5.4.2.5 Reduced MCV and Hct but increased RDW in RA compared to IA

The response patterns of MCV, Hct and RDW were similar between IA and RA but the magnitude of responses differed between initial exposure and re-exposure. Hypoxia-induced increases in MCV were significantly reduced in RA compared to IA and, since the RBC response was the same for both treatments, the outcome was a slightly reduced Hct response in RA (FIGURE 5.7). Since the reticulocyte response was also similar between IA and RA, additional increases in MCV throughout IA must be attributed to other factors that affect mean cell size besides the sheer number of reticulocytes. Given that the mechanistic basis for the increase in MCV during IA is unclear (see SECTION 5.4.1.2), it is difficult to speculate on explanations for a reduced MCV response during RA. In fact, there are several reasons one might expect the MCV response to be greater during RA than IA. First, nutrient deficits are likely to be augmented by the recent cycle of haematopoiesis and neocytolysis, resulting in a tendency for increased macrocytosis during RA (Turgeon 1999). Second, the CFU-E pool is also more likely to be depleted followed an extended period of erythropoietic stimulation. Erythrocytes derived from BFU-Es are even larger than shift reticulocytes and can be present in sufficient numbers to increase both RDW and MCV (Tsantes et al 2004).

Regardless of the physiologic explanation for the reduced MCV and Hct throughout RA, this result can be interpreted as a preferred acclimation response to hypoxia. Typically, the trade off with increasing [Hb] in response to hypoxia is the concomitant increase in Hct and blood viscosity, adding strain to the cardiovascular system, increasing pulmonary vascular resistance and increasing the risk of myocardial and cerebral infarct (Turgeon 1999). However, a reduced MCV response in RA resulted in the same [Hb] response with a slight reduction in Hct (and therefore viscosity) as compared to IA. Several species of HA-adapted camelids employ a similar strategy to keep [Hb] elevated while maintaining low blood

viscosity (Reynafarje et al 1968).

The significant difference in RDW between IA and RA is driven by the persistent elevation of RDW immediately before and during the early stages of hypoxic re-exposure. During DA, the process of neocytolysis causes the selective destruction of the most recently formed erythrocytes (Rice et al 2001), contributing to the return of RBC, [Hb] and Hct to BL. With the removal of these younger, larger cells, MCV and RDW should also return to BL. Although MCV did return to normal, the finding of persistently elevated RDW after 2 w DA was surprising, indicating the presence of both very large and very small erythrocytes within the population. Although cell fragments from the recent spate of haemolysis could be erroneously counted as small cells, it is more difficult to explain the presence of larger cells. While there is no known reason to expect increased cell agglutination following DA, clumps of cells can be mistakenly evaluated as single macrocytic cells, contributing to an artefactual increase in RDW. Visual examination of blood smears would better inform the cause of the increased anisocytosis following DA but unfortunately no blood films were prepared during this study.

5.4.3 Methodological considerations

5.4.3.1 Absolute values of haematological measures

Following 4 weeks in hypoxia, peak values of [Hb], Hct and RBC were lower than have previously been reported for mice following a similar hypoxic exposure (~4600 m for 35 d: [Hb] 19.4 $g \cdot dL^{-1}$; Hct 61% and RBC 12.0 $M \cdot \mu L^{-1}$; Mylrea and Abbrecht 1970 – no strain given). However, the present control values were also lower than those published by these authors, as well as those provided by the supplier of experimental animals (see TABLE 5.2). Whether due to instrument or other methodological error, it is apparent that the current haematology data represent a significant reduction from the true absolute values for RBC, [Hb], Hct, MCV, MCH and RDW. However, data seem to be affected consistently across all treatment groups, as evidenced by similar relative changes in [Hb] (27 vs. 25%), Hct (28 vs. 20%) and RBC (22 vs. 20%) reported here and by Mylrea and Abbrecht (1970). Thus, I am confident that comparisons made within the present data set are sound and that all reported similarities and differences between IA and RA treatments are real.

5.4.3.2 *Actb* as housekeeping gene

Actb is commonly used as an endogenous reference gene because of its known stability during hypoxic exposure (To and Huang 2005, Chan et al 2010, Arvidsson et al 2011). However, a recent study challenged the stability of *Actb* expression in human chondrocytes during exposure to severe (F_IO_2 = 0.01) hypoxia (Foldager et al 2009). Although the stability of *Actb* in mouse peritubular cells following exposure to more moderate hypoxia (F_IO_2 = 0.12) has not been explicitly refuted, the use of an alternative, potentially more stable, reference gene such as 28S rRNA (Zhong and Simons 1999) or RNA polymerase II (Radonic et al 2004) might have substantially altered the results. Selecting a different endogenous reference might have reduced inter-individual variability and improved the ability to detect an effect of time in hypoxia on *Epo* mRNA expression.

TABLE 5.2. Haematological data provided by Charles River Laboratories for female C57BL/6NCrl mice compared to data for control animals measured in the present study. Data are presented as mean ± standard error.

Variable	Charles River Laboratories	Control Animals
RBC ($M \cdot \mu L^{-1}$)	9.8 ± 0.3	8.6 ± 0.4
[Hb] ($g \cdot dL^{-1}$)	15.8 ± 0.7	12.6 ± 0.5
Hct (%)	56.4 ± 2.7	39.0 ± 1.6
MCV (fL)	57.3 ± 1.5	45.8 ± 1.1
MCH (pg)	16.0 ± 0.5	14.7 ± 0.3
RDW (%)	14.2 ± 0.8	16.7 ± 0.3

5.4.3.3 Reduced physical activity in caged animals

Although a consistent factor across most animal studies, the lack of vigorous physical activity in experimental animals deserves mention. This is in stark contrast to previous field work studying humans who participated in high volumes of exercise each day throughout initial and re-exposures to high altitude (see CHAPTER 4). Apart from obvious species differences, it is possible that relatively un-investigated effects of exercise on erythropoietic processes could be responsible for the inability to reproduce the finding of an increased haematological response during RA. Even when considering only human studies, others have noted differences between responses during studies of acclimation and acclimatisation that are likely attributable to factors other than the manner of simulating high altitude (West 1988, 2004),

including physical activity, nutrition and exposure to cold. As such, comparisons between studies using exercising versus sedentary research subjects should always be undertaken with caution.

5.5 Conclusions

(1) Based on previous work in humans (see CHAPTER 4), it was hypothesised that haematological acclimation to hypoxia would be facilitated by recent exposure to the same stimulus. Using a similar time frame as the recent field study (see CHAPTER 4), the finding of increased [Hb] in RA compared to IA could not be confirmed in controlled laboratory experiments. Nor could the hypotheses that decreasing the duration of DA and/or increasing the duration of IA would further improve haematological acclimation during RA be confirmed. Instead, evidence is presented that haematological re-acclimation can actually be impaired relative to an initial acclimation, as demonstrated by a reduction in [Hb] and MCH throughout $RA_{\uparrow IA}$ compared to IA. This corresponded with a normal reticulocyte and RBC response, suggesting that impaired Hb synthesis in early erythrocytes was responsible. Although not the case with an initial 14-d acclimation period, extended erythropoietic stimulation for 56-d likely diminished stores of elements required for Hb synthesis. Although iron status before and during travel to high altitude has been a common concern (Hannon et al 1969b, Richalet et al 1994, Jean et al 2005), sojourners should also ensure adequate stores and sufficient replenishment of folate and Vitamin B_{12}, not only to allow adequate Hb synthesis but to prevent an undesirable macrocytosis that can occur when these nutrients are deficient. Since repeated cycles of haematopoiesis/neocytolysis are likely to further stress nutritional reserves, supplementation with iron, folate and Vitamin B_{12} may be of particular importance during repeated travel between high and low altitude. In order to ensure that haematological responses are unaffected by potential nutrient deficiency, researchers are also encouraged to consider supplementation with all three nutrients when studying humans and other animals during extended or repeated exposure to hypoxia.

(2) A normal erythropoietic response during hypoxic re-acclimation is reported despite a blunting of the [EPO] response to hypoxic re-exposure. This finding represents a substantial expansion on work by Savourey and colleagues (2004), in four key ways. First, while previous work showed a blunted [EPO]

response following acute (4-h) hypoxic re-exposure, the current work demonstrates that the [EPO] response remains blunted throughout an extended re-exposure of days to weeks. Second, it is demonstrated that EPO receptor sensitisation also persists for days to weeks beyond what was previously reported by Savourey et al (2004). Third, the recruited reticulocytes seem to develop normally into mature reticulocytes and increases in RBC, Hct and [Hb] are also evident despite the blunted [EPO] signal. Finally, present data suggest that the degree to which the [EPO] response is blunted is dependent on the nature of the previous hypoxic exposure and may depend on the duration of both the IA and DA phases prior to re-exposure.

The blunted [EPO] response during re-exposure seems to reflect a fine-tuning of erythropoietic control as a result of the recent exposure to sustained hypoxia. The time limits of altered erythropoietic control have yet to be explored, and it is currently unknown whether sensitization of EPO receptors also occurs following exposure to chronic intermittent hypoxia, intermittent sustained hypoxia or treatment with exogenous EPO or prolyl hydroxylase inhibitors. Further expanding the current understanding of the on- and off-responses of erythropoiesis could advance therapies for anaemia, allow better management of chronic obstructive pulmonary disease and sleep apnoea, and better inform individuals repeatedly exposed to high altitude for work or recreation. A more thorough understanding of erythropoietic control could also be exploited by athletes who seek to improve O_2 carrying capacity and endurance performance through both legitimate and prohibited means.

CHAPTER 6: NON-PHYSIOLOGICAL ASPECTS OF ACCLIMATISATION AND RE-ACCLIMATISATION: INSIGHT FROM HIGH-ALTITUDE-EXPERIENCED INDIVIDUALS

6.1 Introduction

Increasing numbers of people now alternate between extended periods at high and low altitude for recreation (Milledge et al 1983, Oelz et al 1986, Koller et al 1991a), employment (Richalet et al 2002a, Heinicke et al 2003, Farias et al 2006, Prommer et al 2007, Wu et al 2009), and pursuit of athletic excellence (Robach et al 2006, Gore et al 2007, Stray-Gundersen and Levine 2008). As a result, there has been an increased interest in understanding the physiological effects of repeated exposure to high altitude. Potential differences in the physiological aspects of acclimatisation and re-acclimatisation to high altitude have been postulated. There are numerous reports of improved hypoxia tolerance during re-exposure (Koller et al 1991a, Lyons et al 1995, Beidleman et al 1997, Wu et al 2009) but physiological acclimatisation seems to proceed normally even after years of exposure and re-exposure to high altitude (Richalet et al 2002b, Heinicke et al 2003). RA in high-altitude trekkers resulted in improved subjective hypoxia tolerance: reduced perception of effort despite trekking faster and reduced self-reported symptoms of acute mountain sickness (AMS; see CHAPTERS 3 and 4). However there was little evidence of improved physiological re-acclimatisation (CHAPTER 4), suggesting that non-physiological factors might play a large role in determining altitude tolerance. This has been previously suggested by Oelz and colleagues (1986), who reported that physiological characteristics cannot account for the exceptional ability of relatively few individuals to exercise at extreme altitudes without supplementary oxygen. Nonetheless, non-physiological aspects of acclimatisation and hypoxia tolerance have received little attention in the field of high-altitude medicine and biology.

In order to gain insight about acclimatisation and re-acclimatisation from a non-physiological perspective, a group of high-altitude-experienced individuals with no formal knowledge of high-altitude medicine and

biology were interviewed. This research was not hypothesis-driven but was intended to explore general perceptions about the consistency and repeatability of the acclimatisation process. Of particular interest were attitudes about potential differences in the acclimatisation process between altitude-experienced and altitude-naïve individuals and the role of non-physiological factors in determining high-altitude tolerance.

6.2 Methods

All experimental protocols were approved by the University of British Columbia Behavioural Research Ethics Board. Written informed consent was obtained from all participants before commencing data collection.

During a trek in the Solu Khumbu region of Nepal, mountaineers and mountain guides were invited to participate in a study about perceptions of the acclimatisation process in altitude-experienced individuals. Seven participants completed 1-2 h semi-structured interviews that were guided by the interview schedule included in APPENDIX V.3. Participants were asked to primarily discuss their own experiences with repeated acclimatisation but were encouraged to draw on their experience witnessing the acclimatisation process in colleagues and clients. Five interviews were one-on-one and one interview was conducted simultaneously with two altitude-experienced individuals (ID 5 and 6; married climbing partners). Interviews were audio-recorded and transcribed verbatim. Transcripts were checked for accuracy by an objective second party.

Transcripts were reviewed several times and statements related to perceived determinants of acclimatisation success were assigned to a number of categories. In the following section, a discussion of each category is built around a number of direct quotes from the interviews. In direct quotes, pauses are indicated with ellipses (…) and omitted text is indicated by two consecutive ellipses separated by a space (… …). Unspoken clarifications and equivalent altitudes in metres have been inserted for clarity and are contained within square parentheses.

6.3 Results and discussion

A general description of study participants can be found in TABLE 6.1. Participants had repeatedly

travelled between high and low altitudes for several years, for both professional and recreational purposes. Exposures to high altitude ranged from days to months and periods of de-acclimatisation between trips ranged from zero to five months. Four individuals considered themselves to be "average" in terms of how easily they acclimatise to high altitude. Two considered themselves to be better than average and one self-identified as relatively poor at acclimatising. All reported having had positive experiences at altitude, characterised by feeling well-acclimatised and energetic, meeting objectives, and being in good spirits. Everyone also reported previous negative experiences at altitude, typified by experiencing symptoms of AMS (headache, impaired sleep quality, reduced appetite, lassitude, vomiting), altered mental status, severely limited exercise capacity and failure to meet objectives.

TABLE 6.1 Description of interviewees with self-reported estimates of previous mountain experience.

ID	Sex	Nationality	Reason for repeated acclimatisation	Years of mountain experience	Number of times acclimatised to HA
1	Male	Spanish	professional trekking guide	8	80x >3000 m 30x >5000 m
2	Male	Swiss	part-time mountain guide	7	15 >3000 m 7x >5000 m
3	Male	American	professional mountain guide	13	50 >3000 m 22x >5000 m
4	Male	American	professional mountain guide	7	20x >5000 m
5	Male	American	recreational mountaineer	16	50x >3000 m 20x >5000 m
6	Female	American	recreational mountaineer	10	20x >3000 m 12x >5000 m
7	Male	Canadian	professional mountain guide	7	20x >3000 m 14x >5000 m

In addition to developing their own ideas about acclimatisation directly from their personal experiences, participants undoubtedly had been influenced by external sources. One participant acknowledged a number of information sources that influenced his opinions.

> …altitude docs, books…reference books on altitude medicine, quite a few different sources.

Another participant expressed some frustration about the mixed messages available from such resources.

> Some of them tell you one thing and some tell you another thing…it depends on the book you read. So I rely on my experience.

Regardless of outside influence, participants clearly had extensive personal experience with acclimatisation, having experienced the process an estimated 15-80 times each. One participant had climbed a single peak more than seventy times (though often many times in a row with little or no de-acclimatisation in between). In addition, each participant had a great deal of experience observing other individuals acclimatise, with both positive and negative outcomes. Observations of hundreds of clients and companions also undoubtedly shaped participants' opinions about the acclimatisation process in altitude-experienced and -inexperienced individuals.

6.3.1 Perceived determinants of successful acclimatisation

Participants were asked to speak about factors that have brought about positive experiences at high altitude. Acclimatisation success was generally discussed in terms of preventing altitude illness and maintaining exercise capacity and motivation at altitude. Allowing ample time for acclimatisation was universally described as resulting in the best outcomes.

> Every time I climb Denali I always feel amazingly well. But that's after acclimatising for 5 or 6 weeks. I've usually been climbing other peaks.

> You know I'd been up here for six full weeks and I just ran up it.

Recent exposure to high altitude on other trips was also repeatedly described as leading to success on subsequent expeditions.

> We know from experience from the other guides that if they do multiple trips one after the other then it gets really easy.

Some participants specifically credited recent altitude experience as the explanation for their most successful acclimatisation experiences.

> Going to Bolivia about a month after being on Denali.

> …those times when coming from another trek.

> Because I had been to altitude before. For sure.

Participants frequently described an awareness that some degree of acclimatisation is retained following descent to lower altitudes, improving performance and well-being on subsequent climbs.

> I really think you keep some kind of acclimatisation after some days.
>
> It feels like it holds, like it really holds over. I've been surprised.
>
> You're up there for like three or four months and then you take a month off. I always have felt like I could pretty much go right back up.
>
> I've also had clients who were on Everest and then went pretty much directly to Denali. And I took them up Denali in six days. Which is very rapid for Denali because it's long distances as well. And they were doing great… …They did way better [with enthusiasm] than your average un-acclimatised client…way, way, way, way better.
>
> …because I know I retain a lot 'cause I come back, and come in and go out a lot.
>
> There is something… you keep something for some days. I do not know how many days.

However, recent exposure to high altitude and adequate acclimatisation do not always ensure good performance and well-being at altitude. Two participants described occasions of negative experiences despite being well-acclimatised.

> Yeah we were six weeks acclimatised. We had already climbed five peaks. Something happened that day or the night before. I got really cold. I was doing a lot of work for the group because they were struggling a little bit and so I put myself in a compromised situation and then on summit day I just had a terrible, terrible time…
>
> I had a very bad experience coming down from Kala Pattar. I was perfectly…actually I had done Annapurna's trek then I spent like five, six days in Kathmandu. I came here to Khumbu, I climbed up to Kala Pattar perfectly, no symptoms, everything ok and then on the way down from Kala Pattar to Lobuche I got again a very bad headache. So when we returned to Lobuche I had to rest for a while. My friends had to - I was with friends and not clients - they had to take my rucksack and we went down to Pheriche... …That was actually the time I felt worst in the mountains. And I don't know what happened it was just one time.

6.3.2 Perceived determinants of unsuccessful acclimatisation

Negative experiences at high altitude were most commonly attributed to rapid ascent rates and inadequate acclimatisation.

> [after only 1-2 days at 12000 ft (~3700 m)] …we went up to the glacier to work on some skills with clients at 15000 ft [~4600 m]. Spent the bulk of the afternoon there and on the drive back down to the hacienda we were staying at around 12000 ft I just had a splitting headache. I felt like my head was going to explode. I was really nauseous and super bad headache…Yeah I mean I had been to 14000 ft

> [~4300 m] before in the States on Mt. Rainier but that was my new high point at that point in time. It was my first taste of what altitude sickness symptoms can feel like. It was miserable.
>
> So we… um … went from sea level to 13000 ft (~4000 m) …in a day… …And you just feel exhausted. You just, you can't … you feel like lead. We had one member of our party puking. It was really, really bad. And the next day we were forced to go over a 16800 ft pass which is 4800, 4900m. You just feel like you've got lead legs. You're just… yeah…
>
> I have been dizzy. I have had headaches but usually it's on rapid ascents.
>
> My worst experience was the highest I've been on Aconcagua. We summitted in [only] ten days from sea level. So it was… I have to take back what I said before. It was the only trip when we were kind of on a time constraint. Kind of, kind of not. We were fighting weather windows and we had the chance and there it was, so we went for it. And we camped one night at 21000 ft [6400 m] on a nice night. It was pretty rough. The summit day was just one of the most exhausting, weirdest days of my life. I could take maybe ten steps before I would just collapse. And then take ten more steps….

However, it was acknowledged that many factors unrelated to acclimatisation status can have a substantial impact on success and well-being in the mountains.

> ..freeze-dried food… doesn't agree with my body. Low altitude, high altitude it doesn't really matter. So, I've had some bad experiences with that…like at high altitude… camping above 5000 m and eating freeze-dried foods.
>
> Maybe I have [a] cold or diarrhoea or anything that will make it more difficult.
>
> …the more physical activity there is the more difficult it is to acclimatise. I think also that hot weather is also not so good. Maybe if you go over maybe 35 degrees centigrade and you are already high and in the mountains like in Africa, then it gets quite difficult.
>
> I think a lot of times when people get altitude headaches it's not necessarily the altitude, it's just dehydration.
>
> My worst-feeling experiences at altitude have all been on Denali. And they've all been being stuck in a tent for days at a time… … not being able to get my blood moving too much…just laying there and my head starts to feel really heavy and I'm really lazy [laughs] and I have to get really motivated just to sit up and get water. But it's usually all head and energy related.

Clearly, altitude-experienced individuals perceive that determinants of acclimatisation success are multi-factorial.

6.3.3 The role of experience in acclimatisation

There are numerous claims in the scientific literature that the acclimatisation process improves with successive high-altitude exposures over many years (Hultgren 1997, Ward et al 2000b). Only one

individual expressed an opinion that the acclimatisation process has not changed across his climbing career.

> …still the same…sometimes good, sometimes bad. Some days I just feel … really tired some days you know and other days I feel really energized…

Others described a general improvement in the acclimatisation process with years of experience.

> Well let me first say I am not very good at acclimatising. But I think because of the experience I am doing better and better each time.

> It seems like I'm doing better each time that I go up.

> I think my body now knows when I… like maybe it's a mental thing… my body knows what to do and knows when to like, ok here we go….ok switch. And it starts doing certain things… …it feels like my body is starting to maybe adapt to it or to be much more comfortable at these elevations.

One participant acknowledged that his perception of such an improvement was heavily influenced by the opinion of others.

> I think when I first started climbing bigger peaks I was told by colleagues, coworkers that the body develops… you know there is some evidence that the body develops a memory for the process that it gets easier each time. So maybe that's a bit of a psychological factor for me – I've been to altitude so many times, and someone told me that… so I just assume it's going to get easier each time.

6.3.3.1 Role of experience: altered behaviour at high altitude

Regardless of whether or not they perceived a systematic improvement of acclimatisation across the years, all participants acknowledged that they had become "smarter" in their behaviour and decision-making at high altitude.

> I think that the two main things are walking slow and drinking a lot a lot a lot. And with time, I have done this more. I have insist[ed] on that deeper and deeper with the clients. So they complain, just joking, that I am giving them tea, tea, tea the whole day and I force them to walk very slow. The other thing is about using Diamox. Years ago I used to be much more conservative about using it. And now, as soon as I see clients feeling sick I tell them to start on Diamox.

> I think the key learning is really to go up slowly and slowly means weeks not days.

> Just common sense sort of things I guess… I think staying well hydrated is a big part of it. I intentionally force myself to drink more to try to avoid any dehydration….And I mean all the new inventions every year the new high tech gear getting lighter and better I've trimmed 10-20% off my kit in the last five years. Just because there's better gear available. So that makes a big difference too – carrying less.

> I don't know if that's because I've learned things and I'm much more… I'm cautious. I just make sure that I don't overextend myself and just try to be patient as I can because it'll come.
>
> I mean, if I am feeling some headache or whatever, I know when to take an aspirin.
>
> But I always try to go slow and I like taking as much time as possible between 3000 and 4000 [m] because I think at that altitude is where your body is really working for acclimatisation. So I like that, slowly between 3000 and 4000m.

Participants consistently reported becoming more conservative with ascent profiles and an increased focus on ensuring adequate hydration and avoiding over-exertion. These factors are known to play a critical role in the development of altitude illness and adopting these "smarter" behaviours undoubtedly leads to improved clinical outcomes at altitude (Basnyat et al 2000, Hackett and Roach 2001, West 2004).

6.3.3.2 Role of experience: reduced anxiety at high altitude

Participants expressed increased physical self-awareness after repeatedly experiencing the effects of high-altitude exposure and the acclimatisation process.

> … I think I'm getting more aware, with my body.
>
> And experience in knowing how your body reacts and taking care of it. You know, just listening.
>
> I think the only thing that I could say that I do differently or know differently is, uh, that I… I know the signs of my body, how it's feeling… it's like an old friend. I know… ok here we go… that lack of oxygen ….that, you know… …Ok just relax. It'll come. And give it time.

Some participants have repeated certain trekking or climbing itineraries so frequently that they know exactly when to expect certain sensations and symptoms.

> Because I know very well how I have to feel in Dingboche normally.
>
> I have some trips where I know exactly where I am going to get a headache.

Participants described learning to recognise the physical sensations associated with exposure to high altitude and, with experience, gaining an understanding that they are both normal and temporary.

> For example, I know that if I get a headache it is probably going to be gone in one day.

> The first time I go up to 15 [thousand ft (~4600 m)] I can expect to feel a little more winded if I'm coming up from sea level. Maybe a little headachy and I just kind of anticipate that ahead of time so it doesn't give me any kind of shock value. I'm not surprised by it… When I know it's coming it doesn't really bother me too much.
>
> I think it has something to do with experience. It's …you know your body … maybe you are coming here quite calm because you know you will be ok… …I don't know but I feel more and more comfortable when I come here and maybe that helps to acclimatise.

Conversely, participants spoke about witnessing altitude-naïve clients experience stress and anxiety as a result of unfamiliar physical sensations and uncertainty about their ability to acclimatise successfully, meet objectives, and keep up with the group.

> We came up with a whole group of [inexperienced] trekkers with our company up to Everest Base Camp. And their experience is that they were stressed a lot of the time because these new things are happening in their bodies. Should I take Diamox? How much should I take? When should I take it during the day? What about these strange dreams I've been having? They're so strong. And is my tummy altitude? So many things…
>
> Yes. I think that if someone hasn't been in…so high before they always get quite stressful about it. And they have a lot of questions and they feel like maybe at 2000 m they already have problems, which is funny… and they don't know what will be coming next.
>
> Whereas I think people who are experiencing it for the first time… I think get into a downward spiral when their body is experiencing something new and they think 'oh I don't feel right' and then the anxiety just compounds the whole problem.

Participants suggested that the anxiety experienced by altitude-naïve individuals generally did not affect them because they knew what to expect. There is clear evidence that a variety of anxiety-inducing experiences can become less stressful with repeated experience. In mice, prior experience in a testing apparatus had a similar effect in reducing anxious behaviour as pre-treatment with diazepam (Rodgers and Shepherd 1993). In humans, repeated virtual reality exposure therapy is commonly used to reduce anxiety in situations that initially induce fear and stress (reviewed in Parsons and Rizzo 2008). And prior to delivery, anxiety is substantially reduced in women who have previously given birth compared to first time mothers (McCall Sellers 2007). Repeated experience at altitude also appears to reduce its anxiogenic effects.

6.3.3.2.1. Anxiety at altitude: effects on self-assessment

Clearly psychological factors can have a considerable impact on perceived well-being at altitude.

Participants acknowledged that stress can influence their own perceptions of acclimatisation, even after years of experience.

> For me I think that it's all mental….if I'm mentally stressed, then I don't acclimatise as well as if I'm really relaxed and if I'm just able to focus on my body.

For that reason, one participant described feeling his best at altitude on personal trips that lacked the additional stress of being responsible for clients.

> So I'm not taking care of them. I'm only thinking about myself. In those situations, I have excelled the most at altitude, technically, mentally, physically, with my climbing…

Another participant acknowledged that the stress of leading a new trip in a different country can also impact the experience at altitude.

> Well my trip in Ecuador this year, back in July I went into it feeling like, ok I've been here enough times this is going to be smooth and easy for me, and, and it was… …after eight times in Ecuador it's very familiar to me. And here, first time in Nepal, like I said I walked in Namche yesterday and just didn't really feel that well. And I'm thinking, wow this is only 11000 ft [~3400 m], why do I not feel better than this?... …I felt great [in Ecuador]. And again I think a lot of that may have been psychological. Familiarity with the trip and knowing the places and the people and just having a good routine after eight trips. I don't know.

In high-altitude medicine and biology, the most clinically important condition (AMS) is evaluated primarily by self-report of symptoms (Hackett and Oelz 1992). AMS score is used to diagnosis the condition, assess its severity and determine treatment. AMS score is also a common metric of hypoxia tolerance and acclimatisation status used in research studies. Historically, clinicians and physiologists have applied a biomedical model when interpreting results of self-reported data, assuming a direct, causal relationship between a sensory stimulus and perception of its sensation (reviewed in Schwartz 1982). According to the biomedical model, AMS score is directly related to the underlying pathology such that an increased score indicates physiological deterioration and a reduced score means a resolution of the physical illness. Although authors commonly acknowledge the subjective nature of the measure (Hackett and Roach 2001, Schoene 2008), AMS scores are generally interpreted in the context of the biomedical model. However, it is almost certainly more appropriate to interpret AMS scores using the cognitive-perceptual model of symptom assessment. According to this model, self-evaluation involves complex

interpretation of somatic sensation, and symptom assessment is influenced by an individual's experience, surroundings and beliefs about the meaning and significance of the sensations (Cioffi 1991). One participant eloquently described how elements of the cognitive-perceptual model are relevant to self-assessment in individuals with and without high-altitude experience.

> I think that having [experience]…you will feel…you will evaluate not so tough or not so bad in your second or third or fourth visit....because on the first time if everything is new... …Even if I have a headache, I know it will be gone. I know I am going to get to Kala Pattar. Normally I will get Kala Pattar even if I have a headache in Dingboche. So it makes you evaluate your own condition in a… let's say a more optimistic way.

It was suggested that guides do not necessarily feel any physically better than their altitude-naïve clients, but that sensations and symptoms associated with exposure to high altitude are more familiar and therefore less disconcerting for altitude-experienced individuals. In this application of the cognitive-perceptual model, increased AMS scores might reflect an exaggerated perception of novel sensations that is intensified by the uncertainty of their seriousness, duration, and impact on climbing objectives or holiday plans. In support, during a simulated ascent of Mt. Everest (8848 m) increases in the cerebral symptoms of AMS paralleled changes in state anxiety (reviewed in Richalet 2010). Viewed another way, reduced AMS scores could reflect a blunted perception of unpleasant sensations that are familiar and believed to be temporary. This is the sentiment postulated by Wu and colleagues (2009) as a potential explanation for reduced AMS scores in railroad workers who were exposed year after year to high altitude. The same reasoning might account for the progressive reduction in AMS scores with increasing altitude experience (Ziaee et al 2003) and could explain the reported differences in behaviour exhibited by individuals with and without previous altitude experience during a brief exposure to simulated altitude (Koller et al 1991b). Altitude-naïve participants were noticeably anxious, inattentive and fatigued; two individuals were sufficiently distressed to require early termination of the exposure. The effect of experience and reduced anxiety on perception of symptoms could account for reduced AMS scores in altitude-experienced trekkers reported in CHAPTERS 3 and 4. However, previous experience at altitude might also have the opposite effect on anxiety and perception of well-being. In a model of intermittent sustained hypoxia (4 d at 3550 m, 3 d at SL), mine workers reported significant increases in headache and

sleep disturbances during the last night at SL and these anticipatory symptoms were increased in those who experienced headache at high altitude (Brito et al 2007).

Clearly, inter- and intra-individual differences in AMS scores cannot be assumed to solely reflect physiological changes; psychological factors that could impact the perception of AMS symptoms must be considered. However, the complexity of the issue is illustrated by a report that both trait and state anxiety are increased in AMS-susceptible individuals (Missoum et al 1992). Although authors conclude that anxiety is a good predictor of AMS, at least two other interpretations of their finding are plausible. As discussed above, increased AMS in high-anxious individuals might actually reflect a heightened perception of AMS symptoms rather than any increase in underlying pathophysiology. Second, the causal relationship between anxiety and AMS might be opposite to that suggested by the authors. As such, the prevalence of anxiety at altitude might be higher among individuals who tend to feel unwell, and not vice versa.

Other types of self-reported data can also be influenced by psychological state. Ratings of perceived exertion (RPE) and of dyspnoea are commonly used in both research and clinical practice as a means to assess the perception of physiological responses. RPE is affected by mood state, with anxiety causing an uncoupling between RPE and physiological work (Morgan 1973). The powerful effect of psychology on perception of effort was demonstrated in an experiment by Williamson and colleagues (2001). In a crossover study design, participants cycled at a fixed workload but were given the hypnotic suggestion that they were either cycling downhill, uphill or on a level grade; RPE was significantly higher when participants believed they were cycling uphill. Dyspnoea is also affected by mood state and is over-perceived (relative to respiratory work) in anxious compared to non-anxious asthmatics (reviewed in Rietveld and van Beest 2007). Dyspnoea itself is anxiogenic and there is evidence that prior experience with dyspnoea at high altitude may modulate the perception of breathlessness in the future (Wilson et al 1993). Thus, altitude experience and psychological state must also be considered when interpreting changes in RPE and dyspnoea ratings.

6.3.3.2.2 Anxiety at altitude: effects on physiology

Anxiety is associated with sleep disturbances and poor appetite (Yager 2009) and, as such, could inflate AMS scores by mimicking its characteristic symptoms. Psychological factors can also directly affect physiological function. Anxiety is characterised by heightened sympathetic activation which manifests as elevated heart rate (f_H), blood pressure (BP) and ventilation rate (Yager 2009). f_H is the best known physiological correlate of AMS (O'Connor et al 2004) and is known to decrease with acclimatisation (Mirrakhimov and Winslow 1996); thus the tachycardic effect of anxiety could also be interpreted as physiological evidence of illness or poor acclimatisation status. It is also feasible that the combination of anxiety-induced hyperventilation and tachycardia could result in impaired gas exchange via increased dead space ventilation and reduced transit time of erythrocytes through the pulmonary capillaries. As such, anxiety could theoretically lead to reductions in oxyhaemoglobin saturation (S_pO_2). S_pO_2 is the most common measure of physiological hypoxia tolerance and is used to assess clinical condition (Koehle et al 2010) and physiological acclimatisation status at altitude. Although it is not clear which is the causative factor, anxiety is also associated with increases in vasopressin, a powerful antidiuretic hormone (reviewed in Frank and Landgraf 2008). Antidiuresis and fluid retention is a known contributor to altitude illness (Loeppky et al 2005) and the importance of blood volume regulation in determining haemoglobin concentration is well known (reviewed in Grover and Bärtsch 1996). Thus, the physiological effects of anxiety mediated by vasopressin release might also be substantial. The magnitude of anxiety required to elicit these physiological effects might indeed be large and therefore the clinical and functional implications to the typical altitude sojourner are unknown. However, in the research setting, small differences in outcome measures can be interpreted as meaningful and the possible impact of mood state on physiological function should be considered.

There are cases where the potential psychological influences on physiological markers of acclimatisation have been overlooked. For example, Koller and colleagues (1991a) interpreted increases in f_H and BP during a brief hypoxic exposure as impaired physiological acclimatisation in altitude-naïve compared to altitude-experienced individuals. However, anxiety in these same altitude-naïve individuals was evidenced by dilated pupils, increased BP and hyperventilation even before the hypoxic exposure started

(1991b), suggesting that at least some of the observed physiological differences between the two groups can be attributed to psychological factors. Similarly, reduced f_H in altitude-experienced railroad workers (Wu et al 2009) might also reflect reduced anxiety rather than improved acclimatisation. Even in controlled laboratory experiments, reduced AMS scores and improved physiological function might be partially attributable to reduced anxiety in individuals who have previously experienced sensations associated with hypoxic exposure (Milledge et al 1983, Koller et al 1991a, Lyons et al 1995).

6.4 Implications and conclusions

The first mild symptoms of AMS serve as a warning to the altitude sojourner, reminding of the physiological stress imposed by the environment. It could be argued that "paranoia" about AMS symptoms and conservatively estimating physical capacity could be protective in altitude-naïve individuals. Not everyone is capable of reaching high-altitude objectives using a typical acclimatisation schedule and first-time trekkers and climbers will not know if they are susceptible to serious altitude illness like high-altitude pulmonary or cerebral oedema. Exaggerated perception of symptoms and mild anxiety could prevent an inexperienced individual from pushing past his or her limits into potentially dangerous territory. In the same sense, a blunted perception of the seriousness of AMS symptoms could put altitude-experienced individuals at risk because of the expectation that acclimatisation will be successful. Although the best predictor of performance at high altitude is previous performance (Ward et al 2000c), successful acclimatisation in the past does not preclude the development of serious altitude illness in the future. Perhaps with the stress of novel circumstances, including unfamiliar climbs or ascents to progressively higher altitudes, altitude-experienced individuals revert to a more heightened perception of symptoms and sensations. This could also be protective by encouraging conservative decision-making in uncertain situations.

The influence of psychology on self-reported subjective measures and physiological function has widespread relevance throughout the health and biological sciences. In high-altitude medicine and biology, the impact of mood state on perceived exertion, dyspnoea, and particularly AMS scores, must not be ignored. The effects of anxiety on physiological acclimatisation should also be considered.

Exposure to simulated altitude in confined chambers might elicit at least as much anxiety as exposure to harsh conditions at natural altitude and even natural altitude-experienced individuals might be stressed by hypoxic exposures in confined spaces. Researchers might consider high trait anxiety as an exclusion criterion for study participants. To aid with interpretation of data, altitude experience of study participants should be disclosed with study results and psychological assessment throughout field and laboratory exposures is encouraged. A non-hypoxic control group should be included wherever possible. However, this improvement in study design does not eliminate the confounding influence of psychological factors on outcome measures since an interaction between physiological effects of hypoxia and state anxiety is likely.

The observation of anxiety and "paranoid" self-assessment in first-time altitude sojourners has prompted a somewhat speculative examination of the potential implications for both physiological and psychological tolerance of hypoxia. Improved psychological tolerance might explain the exceptional performance of a few individuals at extreme altitude (Oelz et al 1986), yet it is rarely considered as an important contributor to outcome measures in field and laboratory investigations of high-altitude physiology. The role of anxiety and other psychological factors in determining altitude tolerance merits further investigation.

CHAPTER 7. CONCLUSIONS AND FUTURE DIRECTIONS

7.1 Review of thesis objectives

This body of work set out to address the following questions:

- Is hypoxic acclimatisation facilitated by previous exposure to the stimulus?
- If so, is improved re-acclimatisation (RA) mediated by the retention of previously-acquired hypoxia tolerance?
- Or, is the process of acclimatisation fundamentally altered by previous exposure hypoxia?

The primary research objective was to explore these questions by conducting the first direct comparison of the processes of initial acclimatisation (IA) and RA to sustained hypoxia. This was accomplished in a field study by monitoring clinical and functional outcomes as well as physiological markers of cardiorespiratory and haematological acclimatisation throughout repeated high-altitude treks in Nepal. In a separate set of experiments, a more detailed examination of haematological IA and RA to normobaric hypoxia was conducted under controlled laboratory conditions using a mouse model.

Additional approaches used to address each of the secondary research questions are described below.

- How long is hypoxia tolerance retained following acclimatisation to high altitude?

Ventilatory and haematological de-acclimatisation were examined in trekkers returning to low altitude. Haematological de-acclimation (DA) was examined in mice following acclimation to normobaric hypoxia. In mice, the IA period was manipulated to determine the effects on DA. These data were assembled with published data from 1908 to present to create the only known synthesis of the timelines and mechanisms of ventilatory and haematological de-acclimatisation from hypoxia.

- Is the acclimatisation process altered by previous hypoxic exposure, even after all aspects of the acclimatised phenotype have been completely reversed?

The originally-proposed research project aimed to examine early hypoxic re-acclimation responses following de-acclimatisation periods of up to 1 y. Unfortunately this project was not completed due to logistical restraints. In a field study, trekkers demonstrated little evidence of their previous acclimatisation immediately before re-exposure to high altitude; there was a clinically insignificant elevation in [Hb], and a more substantial increase in resting f_H that could both be attributable to a theoretical reduction in plasma volume. However, these might also be indications that 10 d at low altitude was insufficient to allow complete DA. In the investigation of haematological re-acclimatisation in mice, the duration of DA was manipulated to examine the effects on the re-acclimatisation process. Following the longest DA period (8 w), RC and [Hb] remained slightly, though not significantly, above and [EPO] remained significantly below control values. As such, I am not confident that the process of RA has been examined in humans or mice that were completely de-acclimatised. Thus, no data are presented with which to address this research question.

- Are there non-physiological explanations for improved altitude tolerance following previous acclimatisation to hypoxia?

The influence of non-physiological factors on real and perceived acclimatisation status were examined through interviews with individuals with extensive experience at high altitude. Although empirical data are limited, the theoretical effects of psychological factors on self-assessment and physiology were thoroughly explored.

7.2 Review of major research findings

Research findings are placed in the context of the literature and discussed at great length within each chapter. Here, the main research findings are summarised as they relate to the thesis objectives.

7.2.1 Improved functional and physiological tolerance in RA

A new field study of IA and RA contributes to the increasing body of work that supports the beneficial

effects of recent altitude exposure on clinical and functional outcomes during re-exposure (CHAPTER 3). Previous cross-sectional studies have only evaluated AMS at a single time point (Schneider et al 2002, Pesce et al 2005) or climbing performance as binary measure (summitted vs. did not summit - Pesce et al 2005, Tsianos et al 2006). In contrast, this new work (CHAPTER 3) monitored functional hypoxia tolerance throughout the acclimatisation process. In addition, this study provides the first evidence of improved physiological acclimatisation (increased S_pO_2) upon extended re-exposure to high altitude. This is in contrast to earlier work that showed no effect of recent altitude exposure on S_pO_2 (O'Connor et al 2004). RA trekkers were better acclimatised from the beginning of the exposure, suggesting that improved hypoxia tolerance throughout the trek was the result of incomplete DA and a head start on the acclimatisation process.

7.2.2 Improved functional tolerance in RA but no evidence of improved hypoxic compensation

The longitudinal study presented in CHAPTER 4 also provides evidence of improved functional hypoxia tolerance throughout repeated exposures to progressive altitude. However, there was no indication of improved physiological compensation for hypoxia during RA compared to IA, indicating that other mechanisms are likely responsible for superior functional outcomes. There was evidence the initial trek elicited a training response and that improved cardiorespiratory fitness and/or stepping economy persisted throughout 10 d DA and de-training. Improved fitness during RA could certainly account for reduced trekking times and decreased trekking RPE, as well as decreased f_H during the graded exercise challenge, and could indirectly contribute to reduced AMS scores via mechanisms outlined in CHAPTER 4.

7.2.3 Improved psychological tolerance in RA?

It was also suggested that reduced anxiety and improved psychological tolerance of altitude could account for much of the improved functional and clinical outcomes in RA. Further exploration of this concept was initiated by conversations with mountaineers and mountain guides with extensive high-altitude experience (see CHAPTER 6). It was a common perception among these individuals that altitude exposure evokes palpable anxiety in altitude-naïve individuals, commonly making them "paranoid" about their ability to acclimatise and hyper-aware of negative sensations associated with hypoxic exposure. Given the

potential impact of anxiety on self-assessment, improved psychological tolerance of high altitude could have a substantial influence on ratings of perceived exertion, dyspnoea, and particularly AMS scores. In both experimental and clinical use, these data should be interpreted in the context of the cognitive-perceptual model not the outdated biomedical model.

7.2.4 Haematological acclimation is not facilitated by recent hypoxic exposure

Mice exposed and re-exposed to normobaric hypoxia demonstrated remarkably similar increases in [Hb] during IA and RA. The response was similar following an abbreviated DA period but an extended IA period led to impaired haematological acclimatisation during $RA_{\uparrow IA}$ with reduced [Hb] and MCH, despite a similar RBC response. It was suggested that extended erythropoietic stimulation depleted stores of iron, folate, and/or Vitamin B_{12} leading to impaired Hb synthesis in reticulocytes during $RA_{\uparrow IA}$. This surprising finding indicates that the effects of previous exposure on haematological re-acclimation are dependent on characteristics of the initial exposure. It also highlights the importance of ensuring adequate nutrient stores in individuals who are repeatedly exposed to high altitude.

7.2.5 Erythropoietic regulation is altered by previous hypoxic exposure

As part of the DA process, [EPO] is blunted upon return to sea level (RSL) and erythropoiesis down-regulated until [Hb], Hct and C_aO_2 have decreased to normal values. After the first trek, [EPO] normalised in humans after 10 d DA but the [EPO] response in RA was slightly blunted compared to IA (CHAPTER 4). In mice, [EPO] remained below BL immediately before re-exposure in RA, $RA_{\downarrow DA}$ and $RA_{\uparrow IA}$ and the EPO response was blunted in all three re-exposures compared to IA (CHAPTER 5). These findings build on earlier work showing a blunted EPO response to acute hypoxic re-exposure (Savourey et al 1996, Savourey et al 2004). Hypoxia-induced EPO secretion was also slightly blunted in recently (48 h prior) hypoxic mice compared to control animals (Martinez et al 2010). However, in this case, [Hb] was elevated ~80% in recently hypoxic mice and the retention of any other aspects of the previous acclimatisation would only serve to further increase C_aO_2 compared to control animals. Thus, the ~25% reduction in [EPO] actually represents an increased response for a given hypoxic stimulus. This is the only known report of increased sensitivity in the EPO response to hypoxic re-exposure; however the

results are quite convincing. A dose-response relationship existed between severity of the previous hypoxic exposure and the increased sensitivity of the EPO response to re-exposure. Furthermore, increased sensitivity of the EPO response did not occur in similarly polycythaemic mice that had been recently exposed to exogenous or endogenous EPO (stimulated by exposure to carbon monoxide or phenylhydrazine plus transfusion). Given that neocytolysis appears to occur similarly to reduce plethora brought about by any means, it is not likely that mechanisms of DA are responsible for the increased EPO response during hypoxic re-exposure. This suggests that mechanisms responsible for increasing sensitivity of renal peritubular cells are unique to hypoxic pre-exposure and not caused by the recently upregulated erythropoiesis.

In high-altitude trekkers, there was a greater [Hb] response despite the blunted EPO response in RA (CHAPTER 4). In mice, the reduced EPO responses during hypoxic re-exposure did not preclude a normal reticulocyte response in all three RA paradigms (CHAPTER 5). These data indicate that erythropoiesis occurs normally in RA, despite reductions in the hormone regulating the process. An increased sensitivity of erythropoietic stem cells to EPO has been previously suggested (Okunewick and Fulton 1970, Savourey et al 2004) and could explain these new results in humans and mice. The present data set also indicates that recruited reticulocytes seem to develop normally into mature reticulocytes and increases in RBC, Hct and [Hb] are also evident despite the blunted [EPO] signal. Thus, the blunted [EPO] response is offset by an increased EPO-sensitivity and these modifications might represent a fine-tuning of erythropoietic control during repeated exposure to hypoxia.

7.3 Critique of methodologies

In addressing a common set of research questions, complementary methodologies were applied, including human and animal experiments, cross sectional and longitudinal study designs, laboratory- and field-based experiments, and both quantitative and qualitative approaches. Physiological acclimatisation was evaluated from the molecular to the whole organismal level and the influence of brain and behaviour were considered. The overall strength of my Doctoral research lies in the diversity of investigative approaches that were employed. However, this work demonstrates the unavoidable trade off between breadth and

depth and could be criticised for its lack of focus on any single concept, mechanisms or study system. The scope of my work has also presented a substantial challenge in acquiring sufficient expertise to appropriately interpret my diverse data sets within the context of other work. However, I believe it is appropriate to tackle relatively unexplored concepts with broad strokes. In so doing, my work has exposed several areas that deserve additional attention and has laid a foundation for future work by me or others. The strengths and weaknesses of specific research methodologies are discussed below.

7.3.1 Review of ventilatory and haematological de-acclimatisation

This review represents the first known synthesis of available data to discuss the timelines and mechanisms of hypoxic de-acclimatisation. Given the inconsistency of terms used to describe the DA process, many of these data cannot be located with a simple literature search. In addition, re-analysis and re-presentation of unearthed data have allowed the first systematic comparison of processes of DA following a variety of exposure paradigms. This assemblage of works will serve as a convenient resource for other scientists.

Reviewing studies on physiological responses to sustained hypoxic exposure is challenging for a number of reasons. Although generally considered to be physiologically equivalent, there is some evidence of differential responses to hypo- and normobaric hypoxia (Roach et al 1996, Sheedy et al 1996, Savourey et al 2003). Acclimatisation also appears to occur differently in a hypobaric chamber compared to at natural altitude (West 2004). Even direct comparisons among field studies are problematic because of differences in pre-exposure status, ascent rates, maximum altitudes attained and durations of exposures. Comparisons become even more complex when you consider differences in data collection methodologies and age, sex, training status and altitude experience of study subjects. Data from different species should obviously be interpreted together with caution, and strain-differences within a species must also be considered (Hill et al 1987, Ou et al 1992, Ward et al 2007). Nonetheless, valuable information can be gleaned from cautiously comparing and contrasting available data. It is certainly possible that the heterogeneity of study designs and study subjects can account for substantial variability in the reported rates of ventilatory and haematological de-acclimatisation. However, although less clearly

defined for VDH, the mechanisms of HDH appear to be consistent across a diversity of examined paradigms.

7.3.2 Comparison of acclimatisation in trekkers with and without recent altitude exposure

This study involved much smaller numbers of participants (n = 62) than similar cross-sectional studies (n = 169 - 919; Schneider et al 2002, Ziaee et al 2003, O'Connor et al 2004, Pesce et al 2005, Tsianos et al 2006). However, the current sample size was sufficiently powered to detect improvements in trekking time and S_pO_2 and reductions in analgesic use. Effect sizes were large enough that differences between IA and RA persisted even when the samples sizes were reduced to age-match the groups.

As with previous studies, acclimatisation status of study participants was not determined immediately before re-exposure to high altitude. Although one participant had been cycling on the Tibetan plateau for 44 d before trekking to Gokyo Ri, the remainder of recent exposures were relatively brief (5-12 d). Considering data discussed in CHAPTER 2, it is feasible that DA periods of 6-30 d (mean 14 ± 1 d) were sufficient to allow complete reversal of ventilatory and haematological acclimatisation in most participants. However, assessment of AMS scores throughout the trek allowed us to compare the severity of symptoms reported early in the exposure. After the first night at altitude, RA trekkers reported fewer AMS symptoms than IA trekkers, suggesting that they had retained hypoxia tolerance from previous exposures. However, early acclimatisation responses in ventilatory control or fluid regulation could also account for discrepancies between IA and RA. Thus the de-acclimatisation status of RA trekkers immediate before re-exposure to high altitude remains speculative.

7.3.3 Longitudinal study of repeated acclimatisation in high-altitude trekkers

The group of trekkers studied throughout repeated treks to high altitude was somewhat heterogeneous in terms of age and fitness level, but each participant served as his or her own control when comparing acclimatisation throughout IA and RA treks. A small sample size limited statistical power, but this problem was mitigated in a number of ways. First, the level of significance was set at $p < 0.1$ to reduce the likelihood of overlooking an important difference between IA and RA. In several cases, pooled, averaged data were analysed to increase power in order to examine global differences between IA and

RA. Despite limited power, several significant differences were reported between IA and RA and, because of the small sample size, I am confident that reported statistical differences are real.

In undertaking this study, the goal was to examine the concept of facilitated re-acclimatisation to high altitude in a realistic, field setting so a popular Himalayan trekking route was selected as the study system. Although every attempt was made to keep the acclimatisation stimulus the same, potentially important variables such as food and water intake were not controlled. Participants completed the same trek with an identical itinerary in both IA and RA, carrying the same load on both treks. However, it cannot be irrefutably stated that the physiological stimulus for acclimatisation was identical during both treks. Participants tended to use less acetazolamide during RA compared to IA which may be partially responsible for the finding of lower mean S_pO_2 at altitude during RA. S_pO_2 is commonly evaluated as a simple assessment of hypoxic compensation (Lyons et al 1995, Savourey et al 1996, Beidleman et al 1997, Savourey et al 2004). However, S_pO_2 (along with oxyhaemoglobin affinity) reflects P_aO_2, which drives cardiorespiratory acclimatisation via stimulation of peripheral and central chemoreceptors. S_pO_2 (along with [Hb]) also reflects C_aO_2, which is sensed at the kidney to drive erythropoietic acclimatisation (Ratcliffe et al 1996). Clearly, the determination of acclimatisation status becomes increasingly complex when you consider that some variables considered to be markers of acclimatisation directly feed back to affect the acclimatisation process.

Although the acclimatisation process was assessed as comprehensively as possible within the logistical restraints, it was not possible to measure every variable of interest. Information about the regulation of plasma volume would have been particularly informative. In addition, the data cannot speak to possible changes in oxyhaemoglobin affinity or blood flow distribution that potentially contributed to clinical and functional improvements in RA.

While spending time at high altitude, individuals are primarily occupied by three activities: resting, exercising or sleeping. It is desirable to evaluate physiological responses during each of these activities in order to obtain a complete view of how effective the body has compensated for the hypoxic environmental conditions. For example, although enhanced sensitivity in the ventilatory control system is

known to increase ventilation during wakefulness, this is not always paralleled during sleep, resulting in further reductions in S_pO_2 (Anholm et al 1992, Smith et al 2001, West 2004). Similarly, hyperventilation and blood flow redistribution during exercise may be inadequate to meet additional metabolic requirements, further depriving the brain and other vital organs of sufficient oxygen (Wagner et al 1986, Reeves et al 1987, Roach and Kayser 2001, Imray et al 2005). Acclimatory changes in ventilatory and cardiovascular control and haematology may be evident when measuring function at rest but their implications to function during sleep and exercise are often overlooked. Taken together, the physiological responses to high altitude during wakefulness, exercise and sleep provide better resolution as to which individuals are "better" or "worse" at acclimatizing. For these reasons, the intent was to collect 24-h ambulatory $\dot{V}_I$, f_H and S_pO_2 data throughout IA and RA using the LifeShirt™ system. Unfortunately, these efforts were thwarted by the effects of cold temperature on battery life and cloudy skies on the ability to re-charge with solar power. Although continuous data were collected in a few individuals throughout IA and DA, this aspect of the research plan was aborted in RA. It was a great disappointment to have lost the opportunity to compare the processes of IA and RA in this novel way.

7.3.4 Haematological acclimation and re-acclimation in mice

Mice were selected as the experimental animal for this study for two main reasons. First, large numbers of mice could be simultaneously exposed to hypoxia in the available chamber. Second, terminal sampling at each time point allowed the collection of kidney tissue for analysis of *Epo* mRNA expression and this work was made feasible by the readily available sequence and map of the mouse genome. A considerable disadvantage of the study design was the sampling of different individuals at each time point. The alternative was a repeated measures design using a larger animal such as the rat so that individual animals could be repeatedly sampled throughout IA, DA and RA. Although this would have precluded the study of kidney *Epo* mRNA expression it would have allowed comparisons of haematological measures between IA and RA to occur in the same individual, eliminating a great deal of inter-individual variability and allowing the use of more robust repeated measures analyses. I am now quite certain that using a rat model would have been a better option.

An obvious methodological problem was the use of two automated analysers to acquire complete blood counts. This occurred because the study was originally designed to compare HAH between IA and RA. After analysing these data it was decided to run additional experiments using $RA_{\downarrow DA}$ and $RA_{\uparrow IA}$, and at this point the original instrument was no longer available. Although this precluded comparisons of MCV, Hct and RDW between IA, $RA_{\downarrow DA}$ and $RA_{\uparrow IA}$, I am confident that other variables were comparable between the two instruments.

Fluid balance was not addressed in these experiments and the repeatability of plasma volume shifts with repeated hypoxic exposure is unknown. The haemoconcentration response plays an important role in determining early haematological outcomes, and information about plasma volume throughout IA, DA and RA would allow a more complete interpretation of the role of previous hypoxic exposure on the process of haematological re-acclimatisation.

Although these experiments focussed on haematological IA, DA and RA, it is actually impossible to isolate haematological responses from other acclimatory, de-acclimatory and re-acclimatory processes. The primary erythropoietic stimulus (C_aO_2 at the kidney) is influenced by both [Hb] and S_aO_2. Without measurement of C_aO_2 or any of its determinants, there is no way to determine whether the hypoxaemic severity was actually constant between IA and RA exposures.

7.3.5 Examining the role of psychological altitude tolerance

The comparative strengths and weaknesses of quantitative and qualitative research approaches are ardently debated. Qualitative approaches allow open-minded, non-hypothesis-driven exploration of a topic and are aptly suited to the study of complex questions that do not have simple answers. Limitations include the unavoidability of researcher subjectivity and the possibility that reported findings will be poorly accepted as "science" by quantitative researchers. In CHAPTER 6, conversations with altitude-experienced individuals were used to generate ideas and possible explanations for the widespread belief that acclimatisation becomes easier across a lifetime of repeated exposures. The plausibility and applicability of generated ideas were examined in the context of my and others' work. While admittedly speculative, the discussion was intended to encourage consideration of the possible psychologically-

mediated effects of previous experience on self-evaluation, perceived well-being and even physiological responses during hypoxic re-exposure. Given the nature of the method, the arguments are infused with my own opinions and biases. As with other qualitative work, it is the responsibility of the reader to determine the transferability or generalisability of the concepts.

7.4 Unanswered questions and future directions

A large number of major questions remain about the effects of previous hypoxic exposure on subsequent RA to high altitude. The duration of IA and DA could be manipulated to investigate the process of RA across an infinite number of time scales. Altering the altitude of initial exposure relative to re-exposure adds another element of complexity to be examined. In addition to surveying the time domains over which hypoxia tolerance in RA is influenced by previous acclimatisation, the mechanisms driving an improved RA merit investigation. Given the infinite combinations of duration and hypoxic severity of IA, DA and RA, and the number of mechanisms to be explored, there will remain more questions than answers for quite some time.

A handful of areas of that warrant attention are described below. This list is by no means exhaustive and reflects particular areas of personal interest within the gaps in the literature.

7.4.1 Direct comprehensive comparisons of IA and RA

The experiments described in APPENDICES I and II remain timely and would still constitute a substantial contribution towards understanding the processes of IA, DA and RA. Such studies would reduce the number of uncontrollable factors that influenced work presented in CHAPTER 4 and would provide a more comprehensive assessment of the timelines and mechanisms of hypoxia tolerance and hypoxic compensation than work presented in CHAPTER 5. The evaluation of RA after various extended periods of DA (as proposed in APPENDIX I) would be particularly informative.

7.4.2 Manipulating the time course of DA

Much work remains in determining the time course and mechanisms involved in ventilatory and haematological DA following various paradigms of hypoxic exposure. One unexplored area of

tremendous practical application is whether or not the time course of DA can be manipulated. One research group has suggested that blood donation upon RSL is an effective way to speed HDH, avoid the negative consequences of elevated Hct and blood viscosity at SL, and save energy by circumventing the need for neocytolysis (Zubieta-Calleja et al 2007). Alternatively, it might be desirable to prolong the persistence of general hypoxia tolerance until a future re-exposure to hypoxia. Similarly, athletes would undoubtedly like to extend the benefits of live high – train low exposures for as long as possible into the competitive season. Muza and colleagues (2004) suggested that DA can be delayed by occasional brief re-exposure to hypoxia but no data are yet available to indicate whether hypoxia tolerance can be retained in this way. If successfully demonstrated, the use of IH to prolong acclimatisation status could become as popular a concept as pre-acclimatisation to improve safety, well-being and exercise capacity on subsequent exposure to high altitude.

7.4.3 Using various models of polycythaemia to study erythropoietic control during DA and RA

Much work is required to better understand the on/off responses of erythropoiesis and its underlying regulation. Suppression of erythropoiesis and neocytolysis seem to be involved in reducing red cell mass no matter what the initial cause of plethora. As a result, polycythaemia induced by erythrocyte transfusion or exogenous EPO administration present excellent models with which to study processes involved in haematological DA. The effects of altered EPO kinetics and EPO sensitivity on haematological RA might also be investigated using a model whereby the initial "acclimatisation" is to exogenous EPO instead of hypoxia. Perhaps more than other aspects of hypoxic acclimation, the study of haematological acclimatisation to hypoxia (HAH) attracts a great deal of interest beyond the field of high-altitude medicine and biology. Insights about erythropoietic control during IA, DA and RA could inform management of clinical conditions characterised by suppression or overstimulation of erythropoiesis. In addition, endurance athletes are keen to understand the process of HAH so as to best manipulate it and reap the gains in performance. Such experiments would also be of great interest to those with vested interests in developing blood doping and anti-doping technologies.

7.4.4 Exploring the importance of psychological altitude tolerance

The prevalence of anxiety in first time sojourners to altitude should be better quantified and the effects of age, trait anxiety, and level of education about altitude illness examined. Furthermore, the potential effects of anxiety on self-reported AMS scores, the perception of effort and dyspnoea, and physiological markers of high-altitude acclimatisation warrant further investigation.

7.4.5 Assessing the health consequences of long term exposure to intermittent sustained hypoxia

Exposure to the paradigm of chronic intermittent hypoxia that characterises sleep-disordered breathing elicits a myriad of ill effects ranging from cardiovascular disease to metabolic disorders and neuropsychiatric consequences (see Chiang 2006, Dempsey et al 2010 for reviews). There is also evidence that paradigms of intermittent sustained hypoxia (ISH) have negative health including issues of hyperviscosity (Jain et al 1978), impacts on reproductive health (Cikutovic et al 2009), pulmonary hypertension, right ventricular hypertrophy and elevated circulating triglycerides (Brito et al 2007) and progressive reductions in exercise capacity at sea level (Richalet et al 2002a). Given the large number of individuals who commute between high and low altitude for work it is imperative to better understand the short and long-term health consequences of repeated prolonged exposure to high altitude. Further monitoring studies exploring comprehensive assessment of health across years of ISH exposure are required.

7.5 Recommendations

- It is clear that altitude tolerance persists for some time and recent exposure is associated with improved altitude tolerance during re-exposure. However, we do not yet know enough DA and RA to make predictions about acclimatisation status upon re-exposure. In order to maximise the carryover from previous acclimatisation, it is recommended that the DA period be as short as possible. Nonetheless, it is prudent to assume that DA is complete and the previous exposure will confer no advantage to re-acclimatisation. Conservative ascent profiles, adequate hydration and minimal exertion are recommended for re-exposure as for initial exposure. If exposure and re-exposure involve substantial exercise at altitude, sojourners should also consider endurance training at sea level during DA to maintain the added

benefit of fitness gained from the initial exposure to altitude. In addition, it is possible that intermittent exposure to hypoxia during the DA period might extend the beneficial effects of pre-acclimatisation. However, this possibility requires further study.

- Anyone alternating between high and low altitude should consider supplementation with iron, folate and Vitamin B_{12} to ensure that erythropoiesis and Hb synthesis are not impaired by nutrient deficiency. This might be most critical for individuals spending extended periods at altitude but again, this matter requires further study.

- Fluid retention is associated with the development of AMS (Loeppky et al 2005). Since there have been mixed reports of either increased (Jain et al 1978, Singh et al 1990, Lyons et al 1995) or decreased (Singh et al 1988, Savourey et al 2004) diuresis during RA, it is recommended that re-acclimatisers monitor urine output during re-exposure. If fluid retention is suspected, descent or treatment with acetazolamide might be considered to prevent the development or progression of AMS.

- Given the numerous health risks associated with frequently alternating between high and low altitude, it is recommended that workers be fully informed of the potential, though not universally reported (Sarybaev et al 2003), health consequences of long term ISH. Obviously numerous social and economic factors are involved but it should be the responsibility of the employer to ensure that workers are sufficiently educated to make informed decisions about employment opportunities.

7.6 Conclusions

Clinical and functional outcomes during RA are reported here, but with little evidence of improved hypoxic compensation. The potential role of psychological tolerance in determining clinical and function outcomes at high altitude should not be undervalued.

There is clear evidence that processes involved in haematological IA and DA alter the internal milieu sufficiently that hypoxic re-stimulation of erythropoiesis might elicit drastically different responses than an initial exposure. However, we provide no evidence that the process of HAH is facilitated by previous

exposure, and in fact we present evidence that it can be impaired. Despite conflicting results from newly presented data and the published literature, it remains that normal erythropoietic pathways are instigated with each hypoxic re-exposure, even after many years of exposure to ISH. Thus, apart from an apparent effect of the "head-start" phenomenon, there remains no evidence that the process of HAH is functionally altered by recent exposure. This does not mean that the effects of hypoxic exposure in erythropoietic regulation could not be harnessed for clinical and other purposes. These processes deserve much more attention.

To date, most reports of improved hypoxia tolerance during re-exposure can be attributed to the partial retention of previous acclimatisation status, with no clear evidence that the process of acclimatisation is altered by previous exposure. However, it was recently reported in railroad workers that both clinical outcomes and physiological compensation were improved during early RA to high altitude after 5 mo at SL (Wu et al 2009). Furthermore, both AMS scores and S_pO_2 at altitude were progressively increased over 5 y of ISH (7 mo at 4500 m, 5 mo at SL). These findings are similar to those reported in other paradigms of ISH where individuals alternate between high and low altitudes much more frequently (Brown 1989, Richalet et al 2002a, Farias et al 2006). Does the finding by Wu and colleagues suggest that some degree of altitude tolerance is retained even after 5 mo at SL? Or does this represent the first documented case of physiological memory in the acclimatisation process leading to faster acclimatisation with each subsequent re-exposure? It is possible that reduced AMS scores could be attributable to improved psychological altitude tolerance and unfortunately few other measures of physiological function were presented to explain the mechanisms of improved S_pO_2 with each exposure. However, this result prompts further exploration of the possibility that previous exposure to hypoxia does fundamentally alter the process of acclimatisation. Much about this fascinating and complex topic remains to be discovered.

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APPENDIX I. HYPOXIC ACCLIMATISATION AND RE-ACCLIMATISATION: THE ROLE OF DE-ACCLIMATISATION DURATION (PROPOSED RESEARCH)

I.1 Introduction

The initial approach to addressing the research questions involved the design of two inter-related studies to assess re-acclimatisation following short and longer-term de-acclimatisation (DA) from hypoxia.

The first study was to investigate whether the acute and sustained responses to hypoxia differ between two identical altitude exposures separated by a relatively brief DA period. In this scenario, subjects would likely retain some of the acclimatised phenotype upon re-exposure to altitude and the effect of having a "head start" on the re-acclimatisation (RA) process could be evaluated.

In order to consider the acclimatisation process is fundamentally changed by previous hypoxic exposure, subjects were to de-acclimatise for more extended periods to allow acclimatory changes to be completely reversed. At various time points throughout the DA period, subjects were to be re-challenged in a hypoxic tent to determine whether hypoxic compensation is improved compared to before acclimatisation. Ideally the acclimatisation process could be re-examined at these time points but due to logistical issues, only relatively acute responses were to be examined.

Both study designs are outlined below with detailed methodologies.

I.2 Study 1. Hypoxic acclimatisation, de-acclimatisation and re-acclimatisation

I.2.1 Study design

In order to address the above questions, the time course of compensatory changes in several variables of interest were to be monitored throughout a initial period of hypoxic acclimatisation (IA), normoxic DA and hypoxic RA. Baseline (BL) testing in normoxia and hypoxia (F_IO_2 = 13%) was to occur at sea level

(SL) in Vancouver prior to travel to the White Mountain Research Station near Bishop, CA. Serial measurements of all variables were to be made throughout the study and each subject was to serve as his own control. During the IA and RA periods, each variable was to be compared to BL values. During the DA period, each variable was to be compared to BL and end-acclimatisation values. For each variable, the acute response to hypoxia and the rate of change was to be compared between IA and RA.

I.2.2 Time course of exposures

Subjects were to be acclimatised to 3810 m (Barcroft Station), de-acclimatised at the Owens Valley Lab (OVL, 1250 m) and re-acclimatised 3810 m. Ideally, the hypoxic exposures would allow sufficient time for "complete" acclimatisation to occur but given that haematological variables are still increasing after eight weeks at 3800 m (Garcia et al 2000), this was an unrealistic proposition. A 2-w altitude exposure was deemed sufficient for hypoxic ventilatory response (Garcia et al 2000), ventilation and blood gases (Hupperets et al 2004) to stabilise at acclimatised levels and also represented a more logical time scale for this study. The DA period was chosen to represent a realistic time frame that individuals might spend at sea level (SL) or low altitude between sojourns to altitude. Two studies have shown that after 8 d at SL, subjects were still acclimatised to some degree such that the incidence and severity of acute mountain sickness (AMS) was less on re-exposure than during the initial exposure to hypoxia (Lyons et al 1995) and that exercise responses associated with acclimatisation were retained to a large degree (Beidleman et al 1997). It was therefore desirable to extend the duration of the de-acclimatisation period beyond this 8-d period to 2 w.

I.2.3 Subjects

Ten young (19-35 y), healthy (no asthma, anaemia, cardiovascular or respiratory disease or sleep apnoea) male subjects from the Vancouver area were to participate. The sample size was limited by the capacity of the research team to collect sufficient data each day. Potential participants must not have been to altitude (sleeping over 3000 m) in the 12 months preceding the study and needed to have a minimum level of fitness ($\dot{V}O_{2\text{-max}} > 50$ ml $O_2 \cdot kg^{-1} \cdot min^{-1}$) such that repeated submaximal exercise testing would not serve as a training stimulus. Highly trained subjects ($\dot{V}O_{2\text{-max}} > 65$ ml $O_2 \cdot kg^{-1} \cdot min^{-1}$) were also to be

excluded to avoid de-training during extended periods living at the field station. Physical activity level of subjects was to be monitored throughout the study.

I.2.4 Variables

The variables of interest could be subdivided into "mechanistic" and "functional" variables. The mechanistic variables included measures of chemosensitivity and haematology, which represent the fundamental changes that allow the body to compensate oxygen uptake and transport in a hypoxic environment. Changes in mechanistic variables underlie adjustments in the more integrative functional variables. This group of functional variables included measures such as oxy-haemoglobin saturation (S_pO_2) during exercise and symptoms of altitude illness that are perhaps of greater practical application. It is uncertain whether changes in the mechanistic and functional variables remain coupled throughout the processes of IA, DA and RA.

The following variables were to be measured twice at BL (UBC) and 1 d, 3 d, 1 w and 2 w into the IA, DA and RA phases of the experiment.

- Body mass
- Symptoms of AMS by Lake Louise scores (Hackett and Oelz 1992) and Environmental Symptoms Questionnaire III (Sampson et al 1983)
- Complete blood count (including haemoglobin and haematocrit), erythropoietin concentration ([EPO]), reticulocyte count, soluble transferrin receptor (sTfR), plasma osmolality (a metric of haemoconcentration and used to normalise other measures) by venipuncture. Blood counts were to be measured on site at WMRS and plasma was to be frozen and transported to Vancouver for all other assays.
- Ventilatory and cerebrovascular sensitivity to steady state exposures to hypoxia, hypercapnia and hypocapnia.
- Cardioventilatory and metabolic variables (heart rate, blood pressure, minute ventilation, S_pO_2, partial pressure of end-tidal CO_2, $\dot{V}O_2$, $\dot{V}CO_2$, ventilatory equivalents for O_2 and CO_2, blood lactate concentration) measured at rest and during submaximal exercise. These variables were to

be measured under ambient conditions throughout the exposures with additional measurements made in hypoxic conditions during BL testing and immediately before the hypoxic re-exposure. Pilot work was conducted to design an exercise challenge and determine any potential training effect of repeated hypoxic exercise testing (see APPENDIX IV).

- Blood gases at rest and during exercise would be ideal but not possible. Repeated arterial catheterisation carries considerable risk and single arterial samples are not representative of true rest (anxious subjects) or true exercise (subjects have recovered by the time sample is taken).

I.2.5 Outcomes

- A thorough assessment of the compensatory changes in mechanistic and functional variables with sustained exposure to 3800 m was to be obtained and compared to previous profiles by Garcia et al (2000) and Hupperets et al (2004).
- A thorough time course of the reversal of the above compensatory changes was to be obtained during the 2-w DA period.
- Acute responses upon reintroduction to hypoxia were to be obtained following a "brief" DA period and compared to initial acute responses.
- The time course of compensatory changes in mechanistic and functional variables were to be obtained during RA and compared to the IA profile.
- The relationship between compensation in mechanistic and functional variables was to be determined throughout the processes of IA, DA and RA.

I.3 Study 2. Hypobaric chamber exposures before and after hypoxic acclimatisation

Although the first study would have provided a great deal of insight into the compensation of functional aspects of repeated acclimatisation and their underlying mechanisms, its weakness was that it was to examine only one of countless permutations of the time courses of IA, DA and RA. A positive result showing improved compensation during RA would have been exciting but would only have provided information about re-acclimatisation following a specific DA period. Considering the financial and temporal involvement of acclimatising subjects (twice) it was desirable to examine responses upon re-introduction to hypoxia following DA periods of different lengths.

I.3.1 Study design

Although several exposures to sustained hypoxia were not feasible due to the time commitment, it was possible to briefly re-expose subjects to simulated altitude (F_IO_2 = 0.134, ~3800 m) in a hypoxic tent at UBC. Participants were to undergo the first tent exposure prior to acclimatisation at WMRS. Measurements made on subsequent exposures (1, 3, 6 and 12 months following acclimatisation at WMRS) were to be compared to this initial chamber exposure to determine whether subjects are better able to compensate for hypoxia at various time points following an acclimatisation period.

I.3.2 Exposure duration

Ideally subjects would remain in the chamber for ~72 hours in order to monitor the initial stages of re-acclimatisation. Aside from the logistical impracticalities of an extended exposure, it would be undesirable for these hypoxic exposures to act as an acclimatisation stimulus. In order to evaluate AMS symptoms, exposures should last at least 4-12 h and include an overnight stay (Beidleman et al 2004). Increases in [EPO] have been seen after as little as 4 h with more severe hypoxic exposures (~5800 m, Knaupp et al 1992) and after 15 h with less severe hypoxia (~2900 m, Abbrecht and Littell 1972). Therefore, an overnight tent exposure of 12 h was to be used as the hypoxic stimulus.

I.3.3 Testing schedule

Measurements were to be made before, during and after the tent exposure as in Study 1. In addition, arterial and venous blood gases were to be evaluated at rest and during exercise via indwelling catheters. Prior to each tent exposure, haematology, normoxic and hypoxic resting cardioventilatory function, and ventilatory and cerebrovascular chemosensitivity were to be measured to determine DA status. These measures were to be repeated immediately after each hypoxic exposure. Throughout the exposure, f_H and S_pO_2 were to be measured continuously using electrocardiogram and pulse oximetry. $\dot{V}_I$ and $P_{ET}CO_2$ were to be monitored continuously via nasal prongs. Arterial and venous blood would be sampled after 1, 2, 6 and 12 h for blood gas analysis. AMS scores would also be measured before and after 1 and 12 h in the tent. Participants would give an additional venous blood sample 24 h after entering the tent in order to measure the peak circulating [EPO].

I.3.4 Outcomes

- The DA of mechanistic variables was to be monitored for an extended period of time.
- Responses to acute hypoxia were to be compared before and at various time points after IA to 3800 m.
- The functionality of the status of mechanistic variables was to be tested at various time points following IA with repeated re-exposures to hypoxia.

I.4 Project collapse

After more than one year of fine-tuning the study design, securing ethical approval, ordering supplies, testing equipment, piloting methods, recruiting study participants and sorting out the logistics of a seven-week international research endeavour, data collection commenced in May 2007. A team of twelve researchers and physicians had been assembled to tackle this comprehensive project. Unfortunately, several volunteer participants backed out during the following month and the project was cancelled only a few weeks before travelling to California.

A significant amount of time and money was lost as a result of the untimely end of this study.

APPENDIX II. A COMPREHENSIVE ASSESSMENT OF ACCLIMATION, DE-ACCLIMATION AND RE-ACCLIMATION TO HYPOXIA IN THE RAT (PROPOSED RESEARCH)

II.1 Introduction

Following the completion of field work in Nepal it was clear that several questions about re-acclimatisation to hypoxia remained unanswered. It was desirable to complete a controlled laboratory-based study that would allow both a reductionist and integrative approach to studying initial acclimation (IA), de-acclimation (DA) and re-acclimation (RA) to hypoxia. The thesis committee agreed on an ambitious proposal that would incorporate three areas of research into one comprehensive study. The primary objectives of the research project were to:

1) compare physiological hypoxia tolerance and hypoxic compensation throughout IA and RA.
2) compare functional hypoxic tolerance throughout IA and RA.
3) compare underlying mechanistic changes that could potentially account for differences in whole animal responses between IA and RA.

The rat was selected as the preferable animal model because body size is small enough to allow housing the large numbers of animals required by the study design but large enough to make surgical procedures feasible. The rat's docile nature makes it ideal for use in studies where repeated handling is required. Finally, the rat has been previously used in numerous studies of acclimation to hypoxia (Olson and Dempsey 1978, Hill et al 1987, Ou et al 1992, Takahashi et al 1993, Harik et al 1996, Gonzalez et al 1998, Pichiule and LaManna 2003, Kusakabe et al 2004, Matsuda et al 2006, Ripamonti et al 2006, Panisello et al 2008), providing ample literature for comparison.

II.2 Proposed methods

An enthusiastic collaborator was identified at the University of Northern British Columbia (UNBC). This

work was to be completed at the Animal Care Facility in the Northern Health Sciences Research Facility on the UNBC campus.

II.2.1 Animals

A total of 110 male Sprague Dawley rats were to be used in these experiments. All animals were to be aged 8 w at the beginning of experiments and familiarised with handling, experimental equipment and procedures before starting data collection.

II.2.2 Familiarisation

During the week preceding surgical intervention, animals were to be handled daily to accustom them to scientist interaction. At least three times during the week all animals were to be placed in a dark restraint tube for ten minutes (simulating resting data collection) and complete the 15-min exercise protocol. This process was intended to reduce stress associated with data collection procedures and minimise any learning artefact.

II.2.3 Hypoxic exposures

Animals were to be exposed to normobaric hypoxia for 10 d (IA) and then returned to normal sea level (SL) conditions (normoxia) for an additional 10 d (DA) before a second 10-d re-exposure to hypoxia (RA).

For IA and RA phases, cages were to be placed inside a large plexiglass enclosure and the oxygen concentration reduced to 12.0% using an oxygen extractor (Mountain Air Generator™) to simulate an altitude of ~4700 m. There was to be sufficient air flow through the enclosure to prevent the accumulation of carbon dioxide (CO_2). Ambient CO_2 level was to be monitored continuously to ensure this was the case. For the DA phase, cages were to be placed next to the hypoxic chamber.

II.2.4 Experimental protocols

Three groups of animals were to undergo initial and re-exposure to hypoxia in order to collect three types of data: 1) blood gas and cardiovascular measurements, 2) volitional running behaviour and 3) tissue

sampling.

II.2.4.1. Blood gas and cardiovascular measurements (18 animals)

II.2.4.1.1 Surgical procedures

Under general anaesthesia (pentobarbital 25 $mg \cdot kg^{-1}$), experimental animals were to have a cannula (PE10) surgically implanted into the carotid artery and another into the jugular vein. These lines were each to be attached to PE-50 tubing threaded under the skin, exteriorised at the back of the neck and secured with an adhesive tape collar. A small (2.2 x 14 mm), sterile and biocompatible temperature transponder was also to be implanted subcutaneously on the dorsum, between the shoulder blades, using a manufacturer-provided injector device.

Hypoxic exposures were to begin 5 days after surgery and cannulae were to stay in place for the 30-d duration of the experiments. The cannulae were to be initially flushed and filled with a heparinised saline solution and lines later flushed and re-filled following every blood sample. Lines were to be checked for patency and incision sites checked for infection daily.

II.2.4.1.2 Data collection

Blood gases, blood pressure and heart rate were to be measured throughout the initial and re-exposures to hypoxia while the animal was at rest and during exercise. At ten time points (PRE, 1 h, 6 h, 12 h, 24 h, 2 d, 3 d, 5 d, 7 d and 10 d) throughout the IA and RA periods, data was be collected at rest and during exercise. At each time point, body temperature was to be determined using the Bio Medic Data Systems pocket scanner (BMDS Inc., Seaford, DE, USA). This requires that the scanner be held 5 cm away from the implanted transponder and does not disturb the animal. Each data collection period was to last about 30 min.

II.2.4.1.2.1 REST

After a measurement of body mass, the animal was to be placed in a dark restraining tube that would prevent animal movement but allow access to the cannulae without disturbing the animal. The arterial line was to be connected to an external fluid-filled pressure transducer for continuous measurement of mean

arterial pressure and heart rate. This data was to be collected on a personal computer via an analog to digital converter and averaged over a 3-min period after the animal had been quiet for 10 min. Next, a sample of arterial and venous blood (150 µL each) was to be collected for immediate analysis using the iSTAT® portable blood gas analyzer (Abbott, Mississauga, ON, CA). All blood gas measurements were to be made in duplicate and corrected for body temperature.

II.2.4.1.2.2 EXERCISE

Animals were to run on a speed-controlled wheel for 5 min at 10 $m \cdot s^{-1}$ (warm-up) followed by 10 min at 25 $m \cdot s^{-1}$ (challenge). Heart rate and blood pressure were to be averaged over minutes 5 to 8 of the exercise challenge. Arterial (150 µL) and venous blood (200 µL) were to be collected during the final minute of the exercise challenge to measure blood gases and venous lactate concentrations (using the LactatePro portable analyzer, KDK Ltd., Kyoto, Japan). Again, all blood measures were to be made in duplicate and blood gas measurements corrected for body temperature.

Nine animals were to undergo surgery, hypoxic exposures and data collection procedures as described above. An additional nine control animals were to undergo surgery and an identical schedule of data collection but remain normoxic for the duration of the experiment. These animals were to be housed adjacent to experimental animals and experience the same environmental conditions apart from the hypoxia.

II.2.4.2 Volitional running behaviour (20 animals)

Animals were to be individually housed in cages with attached running wheels. Animals were to have 24-h access to the wheels to exercise ad libitum at any self-selected pace. Speed and distance were to be recorded continuously using a computer-interfaced magnetic sensor on each wheel. Body mass was to be measured every two days.

Volitional running behaviour was to be monitored in 10 experimental animals that experienced the initial and re-exposures to hypoxia. An additional ten control animals were to remain normoxic throughout the monitoring period.

II.2.4.3 Tissue Sampling (96 animals)

After measuring body mass, animals were to be overdosed with anaesthetic (100 $mg \cdot kg^{-1}$ pentobarbital) followed by cervical dislocation. As much blood as possible was to be immediately collected from the saphenous vein. The brain, carotid bodies, heart, diaphragm and vastus lateralis were to be harvested. Collected tissues were to be used in analyses of haematology, gene expression, capillary and mitochondrial density, and muscle fibre type.

II.2.4.3.1 Haematology

Blood was to be analysed using an automated haematology system to determine haematocrit and haemoglobin concentration. Serum was to be collected by centrifugation and stored at -80 ºC for later analysis of erythropoietin, soluble transferrin receptor, angiotensin converting enzyme, vascular endothelial growth factor and C-reactive protein by enzyme-linked immunosorbent assay.

II.2.4.3.2 Gene expression

Small tissue samples from the brain, kidney, carotid bodies, heart, diaphragm and vastus lateralis were to be stored in RNA stabilization solution for later analyses of gene expression using quantitative PCR.

II.2.4.3.3 Capillary density

Samples of brain, carotid bodies, heart, diaphragm and vastus lateralis muscle were to be mounted in an imbedding matrix (OCT) and frozen in isopentane in liquid nitrogen. A cryostat was to be used to cut 12 μm slices of each tissue to be fixed, treated with a 1% amylase solution, and stained with periodic acid-Schiff reagent to visualize capillaries. Under magnification, the number of capillaries per known area was to be counted to calculate capillary density per mm^2 and per muscle fibre.

II.2.4.3.4 Muscle fibre type

Additional slices of diaphragm and vastus lateralis were to be stained for myosin ATPase activity. Fibres were to be classified as Type I, Type IIa or Type IIb and each type expressed as a percentage of total fibres.

II.2.4.3.5 Mitochondrial enzyme activity

Additional samples of brain, heart, diaphragm and vastus lateralis were to be immediately frozen in liquid nitrogen, powdered and stored at -80ºC. The activity of mitochondria-specific enzymes (citrate synthase and cyochrome c-oxidase) were to be measured by addition to excess substrate and spectophotometric analysis of the quantity of product produced in a set time period.

Terminal sampling of eight experimental animals was to occur immediately before and after 5 d and 10 d of IA and RA. Eight control animals were also to be terminally sampled at the same time points. Control animals were to remain in normoxic conditions at all times.

II.3 Project collapse

After several months carefully designing all scientific aspects of the research project, an application was submitted to the Animal Care Committee at UBC in June 2008 (A08-0421). Logistical arrangements continued for the next month, including a site visit to the Northern Health Sciences Research Facility. Preparations were made to re-locate to Prince George for six months to complete the study, starting in July. Three days before moving, the collaborator notified us of several insurmountable issues with equipment incompatibility. With the stability of the proposed research environment and commitment of the collaborator suddenly very much in question, the project was immediately suspended. Appropriate facilities were not available at the UBC campus and a willing collaborator could not be located. Thus, the project was cancelled and a new study was designed that could be successfully completed with in-house resources and expertise.

APPENDIX III. PERFORMANCE OF EVACUATED BLOOD COLLECTION TUBES AT HIGH ALTITUDE[1]

III.1 Introduction

Since their development in the 1940's, evacuated blood collection tubes have offered a convenient alternative to traditional syringe techniques and have become the most widely used method of blood collection both in the clinical setting and in research (Turgeon 1993). They offer the advantage of automatically drawing a pre-determined blood volume and when multiple samples are needed, evacuated tubes can be switched much more easily than syringes. Blood is drawn directly into tubes containing an appropriate volume of desired additive, minimising phlebotomist exposure to blood and reducing the risk for needle stick injury during blood transfer (Little et al 2007).

Evacuated tubes draw pre-determined volumes of blood based on a precisely calculated negative pressure established within the tubes by the manufacturer (McCall and Tankersley 2003). However it is not the absolute magnitude of the internal vacuum that determines draw volume but the pressure differential from outside to inside the tube. Using an evacuated tube in a hyperbaric chamber, where both ambient pressure and the pressure differential are increased would result in a larger than expected draw volume. Alternatively, if ambient pressure (and environment to tube pressure gradient) were decreased in a hypobaric chamber or by ascent to high altitude, evacuated tubes would be expected to draw reduced volumes. Indeed, online product literature from a major manufacturer of evacuated blood collection systems (Becton, Dickson and Company: (BD Technical Services Department, 2007) states that "the quantity of blood drawn can vary with altitude". However, details on the magnitude to which draw volumes are affected by moderate to high altitudes are not available. Considering that an estimated 400

[1] A version of this chapter has been previously published. MacNutt, M.J. and Sheel, A.W. (2008) Performance of evacuated blood collection tubes at high altitude. High Altitude Medicine and Biology 9 (3): 235-37. Reprinted with permission from Mary Ann Liebert, Inc.

million people live above 1500 m (Cohen and Small 1998) and that countless scientific endeavours occur at altitudes and barometric pressures simulating upwards of 9000 m, information on tube performance should be available to clinicians and researchers. Therefore we conducted field tests to determine the draw volumes of four sizes of evacuated blood collection tubes at altitudes ranging from sea level (SL) to 5341 m.

III.2 Methods

We tested the draw volume of BD Vacutainer® tubes (BD Diagnostics – Pre-analytical Systems, Sparks, Maryland, 2007) at eight altitudes ranging from SL to 5341 m. At each altitude, three tubes of each manufacturer-specified volume (2, 4, 6 and 10 mL) were punctured with a BD Eclipse™ 21G needle attached to a standard needle holder (BD Medical – Medical Surgical Systems, Franklin Lakes, New Jersey, 2007) and filled from a beaker containing 100 mL of body temperature water. Needle tips were submerged at least 2 cm below the water's surface for 1 min to allow a complete draw then tubes were removed and inverted several times to mix water with the additive. Draw volume was recorded to the nearest 0.05 mL using a 10-mL glass graduated cylinder. Draw volumes were determined at SL in Vancouver, Canada, at 1350 m in Kathmandu, Nepal and at various locations along the trekking route up the Gokyo Valley in the Solu Khumbu region of Nepal (Lukla: 2860 m, Namche Bazaar: 3440 m, Mong La: 3973 m, Dole: 4230 m, Gokyo Village: 4750 m, and Gokyo Ri: 5341 m).

Least squares linear regression (SigmaPlot 8.02, SPSS Inc., Chicago, Illinois, 2002) was used to determine the relationship between draw volume and altitude for each tube size. For each data set, the regression was forced to intercept the y axis at the manufacturer-specified draw.

III.3 Results

There was a progressive decrease in evacuated tube performance as terrestrial elevation increased (FIGURE III.1). Very strong linear relationships existed between altitude and draw volume within each tube size (adjusted R^2s from 0.95 to 0.98, all $p < 0.0001$). The slope of each regression equation indicates that for each 1000-m gain in altitude, draw volume decreased by 0.5, 0.3, 0.6 and 0.5 mL in 2, 4, 6 and 10 mL tubes respectively. No water was drawn into the 2-mL tubes at 3973 m, and above this altitude, gas

bubbles were visibly ejected into the water from these small tubes. Percentage reductions in predicted draw volumes were calculated based on regressions for each tube size and extrapolated to 9000 m (TABLE 1).

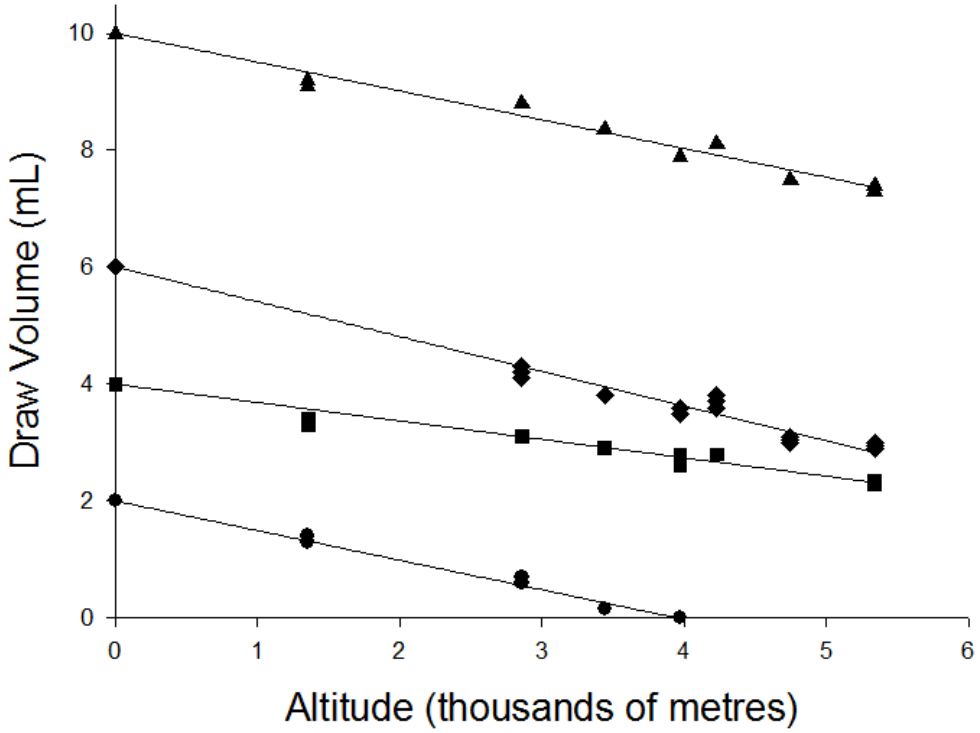

FIGURE III.1 Draw volumes of four sizes of Vacutainer® tubes at altitudes ranging from sea level to 5341 m. Circles represent manufacturer-specified 2-mL tubes: $y = 2.0 – 0.5071x$, $R^2 = 0.98$; squares represent 4-mL tubes: $y = 4.0 – 0.3152x$, $R^2 = 0.95$; diamonds represent 6-mL tubes: $y = 6.0 – 0.5935x$, $R^2 = 0.97$; and triangles represent 10-mL tubes: $y = 10.0 – 0.4926x$, $R^2 = 0.97$.

TABLE III.1 Percent predicted reduction in draw volume at altitudes up to 9000 m based on regression equations developed using collected data.

Tube size	**Reduction in draw volume (%)**								
	1000m	2000m	3000m	4000m	5000m	6000m	7000m	8000m	9000m
2 mL	25	51	76						
4 mL	8	16	24	32	39	47	55	63	71
6 mL	10	20	30	40	49	59	69	79	89
10 mL	5	10	15	20	25	30	34	39	44

III.4 Discussion

As anticipated, Vacutainer® performance was progressively impaired at high altitude with decrements in

draw volume ranging from 0.3 to 0.6 mL per thousand metres altitude gain. The strong linear relationships between draw volume and altitude reflects the linear decrease in barometric pressure (West 1996). TABLE 1 extrapolates our results to altitudes as high as 9000 m, approximating the highest point on earth and the site of remarkable feats of physiological data collection in the field (West et al 1983, Grocott et al 2009) and in simulated conditions (Houston et al 1987, Richalet et al 1999). Although absolute reductions in draw volume are fairly consistent across tube sizes, the relative reduction is obviously greater in smaller tubes for a given altitude. For example, draw volume is reduced by approximately 25% in 2 mL tubes at only 1000 m but 10 mL tubes aren't affected to this relative degree until 5000 m. However, substantial reductions in draw volume of 10% were observed in these larger tubes at altitudes as low as 2000 m.

Reduced draw volumes in evacuated tubes are of concern for several reasons. First, an insufficient blood volume may be available to complete all desired analyses. In the clinical setting this could result in the inconvenience of having to recall a patient to provide an additional sample. In research, this would likely result in missing data and the need to prioritize some tests over others. Second, and of greater impact, is the effect an inappropriate blood-to-additive ratio in under-filled tubes might have on test results. This is of greatest concern with sodium citrate tubes that are used in coagulation studies (Chuang et al 2004) and of particular relevance due to the interest in changes in the clotting properties of blood that occur at high altitude (Grover and Bartsch, 1996). Haematological measures are also extremely common at high altitude in assessing health status (Vargas and Spielvogel 2006), responses to high-altitude athletic training (Wehrlin et al 2006) and aspects of the acclimatisation process (Savourey et al 1996). An excess of the most common additive used for these tests, ethylenediaminetetraacetic acid (EDTA) is also known to adversely affect test results by shrinking erythrocytes and causing an under-prediction of red cell counts and haematocrit (McCall and Tankersley 2003). Third, we demonstrated that, as expected, the direction of flow reverses when the environmental pressure falls below the pressure established within the tube. In this case gas can be injected into a blood vessel, potentially resulting in a gas embolism. This can occur as low as 4000 m with a 2 mL tube and the volume of gas injected will increase at higher altitudes. Currently, the volume of introduced gas that can lead to a clinically significant (potentially fatal) venous

gas embolism is unknown (Chilvers 1999).

Researchers and clinicians should be aware that the performance of evacuated blood collection systems such as Vacutainer® tubes is impaired at high altitude. In our study conducted in the Himalaya, draw volumes were reduced by approximately 0.5 mL for every 1000 m gain in terrestrial elevation. These performance reductions may not be of great functional significance when using large tubes at relatively low altitudes but the impact grows as tubes get smaller and altitudes get higher. Barometric pressure was not measured but all tests were completed during clear, stable weather. An important point to consider is that, for a given terrestrial elevation, barometric pressure is higher in the Himalaya than in other high-altitude regions of the world because of the proximity to the equator where the troposphere is the thickest (West 1996). Therefore, our data actually slightly underestimate the decrement in evacuated tube performance that would be seen at latitudes greater than 30º.

III.5 Recommendations

If a slight excess of additive will not affect the results of intended assays then researchers can use a larger than normal tube to ensure an appropriate blood volume is collected. For example, at 4000 m, a 6 mL tube can be used to draw 4 mL of blood. Appropriate tube sizes can be estimated using the equation:

$$\text{tube size} = \text{required volume} + 0.5 \times \text{altitude (in km)}$$

Liquid additives should be avoided as the dilution of blood samples will cause an underestimate of haematocrit, [haemoglobin] or other protein concentrations.

The required blood volume can be collected by syringe and transferred to an evacuated tube containing an appropriate additive. A blood transfer device should always be used because transfer from syringe to evacuated tube through either a used or new needle is associated with increased rates of haemolysis and increased risk for needle stick injury (McCall and Tankersley 2003). This technique requires additional equipment (and generates additional waste) and is therefore somewhat less desirable, particularly when working in field conditions. However, it offers the best alternative for ensuring appropriate blood to additive ratios and securing accurate results.

APPENDIX IV. DAY TO DAY VARIABILITY IN CARDIORESPIRATORY RESPONSES TO HYPOXIC CYCLE EXERCISE

IV.1 Introduction

The effects of hypoxia on cardiorespiratory responses to maximal and submaximal exercise are of great interest to healthy individuals travelling to high altitude, diseased individuals coping with chronic hypoxaemia and researchers exploring the limits of human performance. Hypoxic exercise testing is commonly used to address fundamental questions in physiology: to explore the minutiae of metabolic pathways (Hochachka et al 2002), to test mechanisms of oxygen transport (Calbet et al 2003), to investigate the effects of training status on performance decrements in hypoxia (Mollard et al 2007), and to examine cardiovascular and respiratory plasticity associated with the processes of acclimation and acclimatisation to sustained (Boning et al 2001) and intermittent (Beidleman et al 2008) hypoxic exposure. Hypoxic exercise testing has also been used to investigate the pathophysiology of high-altitude illness (Eldridge et al 2006) and to assess potential therapies for improving exercise capacity in healthy (Ghofrani et al 2004) and clinical populations (Fischler et al 2009). Finally, hypoxic exercise testing has been used to examine hypoxic adaptation in high-altitude natives (Hochachka et al 1991) and to explore the genetic bases of extreme altitude tolerance (Patel et al 2003). Although researchers regularly interpret study results based on cardiorespiratory responses to hypoxic exercise, little has been reported about the reliability and repeatability of these measures.

In addition to numerous studies using pre- and post-hypoxia measurements, responses to maximal and submaximal exercise are commonly assessed throughout the process of acclimation or acclimatisation to sustained (Pronk et al 2003) or intermittent hypoxia (Townsend et al 2005). In these time course studies, participants repeatedly perform a standardized exercise challenge such that physiological responses to the same stimulus can be tracked over days, weeks or months. Over time, decreases in heart rate (f_H), blood lactate concentration ([La]) and ratings of perceived exertion (RPE) and increases in minute ventilation

($\dot{V}_I$) and oxyhaemoglobin saturation (S_pO_2) at a given workload are evidence of improved exercise tolerance and acclimatization status, as is faster post-exercise recovery of these cardiorespiratory measures (Bender et al 1989, Beidleman et al 1997). However, many cardiorespiratory adjustments that occur to improve hypoxia tolerance are similar to those that occur with endurance training (Hochachka et al 1998) and several of the above changes would also occur over time with increasing aerobic fitness (Bassett and Howley 2000). Depending on the intensity and duration of the exercise challenge, the frequency at which it is performed, and the initial fitness level of study participants, it is feasible that the repeatedly administered test could elicit a training response and confound the interpretation of results (Wenger and Bell 1986). Thus, a repeated exercise bout used as a monitoring tool has the potential to become a co-intervention and its potential influence on study results must be evaluated.

A hypoxic exercise challenge to be repeated throughout a field study of acclimatisation, de-acclimatisation and re-acclimatisation to high altitude was carefully designed. It was desirable to develop a test that would include a range of workloads, from low intensities that would represent activities of daily living through to the highest intensities tolerable during acute hypoxia. This would provide data on the repeatability of cardiorespiratory responses to exercise at a number of workloads that might be used by me or other researchers in future studies. It was important that the test be as short as possible and still give meaningful, repeatable results. A brief test would be of particular utility for comprehensive field-based studies that are often conducted in extremely challenging conditions. More importantly, the exercise dose associated with a brief test would less likely be of sufficient intensity and duration to elicit a cardiorespiratory or neuromuscular training response with repeated testing (Wenger and Bell 1986).

In testing the suitability of the exercise protocol, the goals of this study were two-fold: 1) to ensure that cardiorespiratory responses to hypoxic exercise did not change across five repeated testing sessions; and 2) to assess the day to day variability of commonly-used measures of cardiorespiratory responses to hypoxic exercise. Both objectives were met and the described exercise challenge proved a suitable protocol for use in repeated hypoxic exercise testing. Although this exercise challenge was designed for use in a specific investigation, it provides a valuable reference protocol for use by other researchers.

IV.2 Methods

All methods were approved by the University of British Columbia's Clinical Research Ethics Board. Eight healthy male volunteers gave written informed consent before participation. Volunteers were excluded if they had a history of cardiovascular disease, asthma, or other respiratory disease, were smokers, or were not able to participate in exercise for any reason. Volunteers were also ineligible if they participated in > 4 h of vigorous aerobic exercise each week or had travelled to altitudes > 3000 m within the previous year. Enrolled participants visited the Health and Integrative Physiology Lab at the University of British Columbia on six occasions and were instructed to avoid alcohol for 12 h and food, caffeine and exercise for 4 h prior to each visit. Cycle ergometer seat height was adjusted for each participant at the initial visit and kept consistent throughout all testing sessions.

During the initial visit, height and weight were measured and basic lung function was evaluated using a portable spirometer (Spirolab II, Medical International Research, Rome, Italy). Forced vital capacity and maximum voluntary ventilation were measured in triplicate according to standards of the American Thoracic Society (1995). Next, participants performed a normoxic maximal exercise ($\dot{V}O_{2\text{-max}}$) test on a programmable cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) using a 30 watt·min^{-1} ramped protocol. Participants were instructed to continue pedalling until volitional exhaustion and the test was terminated when cycling cadence fell below 60 rpm. Peak power (P_{max}) was recorded immediately upon termination of the test. After the $\dot{V}O_{2\text{-max}}$ test, participants breathed a humidified gas mixture of 13% O_2, balance N_2 during seated rest to become familiar with the hypoxia. This level of hypoxia simulates approximately 3800 m, the elevation of exposure during the proposed acclimatization study.

Participants returned to the lab on five non-consecutive days (H1 to H5) over a two-week period to repeatedly perform the hypoxic exercise test. Each hypoxic exposure started with 5 min seated rest and continued throughout exercise. Participants completed 3-min exercise stages at 20%, 40% and 60% normoxic P_{max} and recovered for an additional 3 min at 10% normoxic $P_{max.}$ The original exercise protocol included a 3-min stage at 80% normoxic $P_{max.}$ However, all four pilot subjects struggled to complete this work load in acute hypoxia and all desaturated below the safety cut-off of 75% that is

regularly used in our laboratory and imposed by the UBC Clinical Research Ethics Board. Therefore this workload was not included in the testing protocol. Cycling cadence was self-selected between 60 - 120 rpm during the first testing session and was held constant throughout the remaining sessions.

Throughout maximal and submaximal exercise tests, participants wore a heart rate transmitter (Polar FS1, Polar Electro Oy, Kempele, Finland) and pulse oximeters at the ear and finger (Model 504, Criticare Systems, Milwaukee, WI). Heart rate (f_H) and oxyhaemoglobin saturation (S_pO_2) were recorded every 30 s. To avoid overestimating the degree of desaturation, if there was a discrepancy between the two oximeter readings, the higher S_pO_2 value was recorded. A rating of perceived exertion was determined separately for sensation in the legs (RPE_{legs}) and respiratory muscles (RPE_{resp}) using the 15-point Borg scale (Borg 1970). Both RPE measures were recorded every minute during the $\dot{V}O_{2\text{-max}}$ test and at the end of each workload during the submaximal test. During the last 30 s of each workload 100 μL of blood was collected by finger prick for measurement of blood lactate ([La]; YSI 1500 SPORT^{TM} lactate analyser, YSI Life Sciences, Yellow Springs, OH). Participants wore a nose clip and mouthpiece and breathed through a two-way non-rebreathing valve (Model 2700B, Hans Rudolph, Kansas City, MO). Inspired flow was measured using a pneumotachograph (Model 3818, Hans Rudolph, Kansas City, MO) and mixed expired gases were measured using O_2 (Model S/3A-I) and CO_2 (Model CD-3A) sensors and analyzers (AEI Technologies, Pittsburgh, PA). Instruments were interfaced with an analog to digital convertor (PowerLab, ADInstruments, Colorado Springs, CO) and data were recorded at 1000 Hz on a personal computer. Files were analyzed offline (LabChart software, version 6.1.3, ADInstruments, Colorado Springs, CO) to determine inspired minute ventilation ($\dot{V}_I$), and rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). Values were averaged during the last 30 s of each submaximal exercise stage and during the final 15 s of the $\dot{V}O_{2\text{-max}}$ test.

Data are presented as mean ± standard deviation. Cardiorespiratory responses to hypoxic exercise were evaluated using RM ANOVA. Analyses were completed with statistical software (SPSS Statistics 17.0, SPSS Inc. Chicago, IL). $\dot{V}_I$, $\dot{V}O_2$, $\dot{V}CO_2$ and R were not measured during normoxic rest. Due to numerous missing data points, [La] data is included only in repeatability analyses.

IV.3 Results

Study participants were young (22.5 ± 2.4 years), moderately trained ($\dot{V}O_{2\text{-max}}$ = 50.7 ± 4.7 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$), healthy males of 175 ± 9 cm and 72.6 ± 12.5 kg. All participants had normal pulmonary function, with FVC and $FEV_{1.0}/FVC \geq 80\%$ of predicted value. Peak power on the normoxic $\dot{V}O_{2\text{-max}}$ test was 304 ± 52 (range 238 to 398) W.

Across hypoxic testing sessions, participants exhibited normal responses to graded exercise with significant (all $p < 0.001$) increases in $\dot{V}_I$, $\dot{V}O_2$, $\dot{V}CO_2$, R, f_H, RPE_{legs} and RPE_{resp} and a decrease in S_pO_2 with increasing workload (FIGURE IV.1). FIGURE IV.1 illustrates the tight clustering of cardiorespiratory responses to hypoxic exercise across testing sessions. Indeed, there were no statistical differences in any variable across testing days. To increase the likelihood of detecting change across testing sessions, a 2 (H1 vs. H5) x 5 (workload) RM ANOVA was used to compare cardiorespiratory responses between the first and last testing session. Again, testing session was not a significant factor in any analysis.

Coefficients of variability (CVs) were calculated to determine the intra-individual day to day variability for each cardiorespiratory response (TABLE IV1). Most measures were highly repeatable across testing sessions with inter-day variability averaging ≤ 10% of the mean value in all variables except $\dot{V}O_2$ (CV = 17%), $\dot{V}CO_2$ (CV = 11%) and [La] (CV = 17%). For f_H and S_pO_2, CV was < 5%. Work load had a significant effect on CV for all variables except [La]. The effect of exercise intensity on CV approached significance for S_pO_2 ($p = 0.05$) and R ($p = 0.08$). However, there was no universal pattern in CV across exercise stages for other variables. $\dot{V}_I$, $\dot{V}O_2$, $\dot{V}CO_2$, R and f_H were most variable at rest, with CV dropping throughout exercise and increasing again during recovery. Conversely, both RPE measures exhibited increasing variability from rest to exercise to recovery.

IV.4 Discussion

IV.4.1 Effect of hypoxia on cardiorespiratory responses

Acute exposure to moderate hypoxia is well known to educe marked changes from normoxia in cardiorespiratory responses to both rest and exercise (reviewed in Ward et al 2000d). Cardiorespiratory

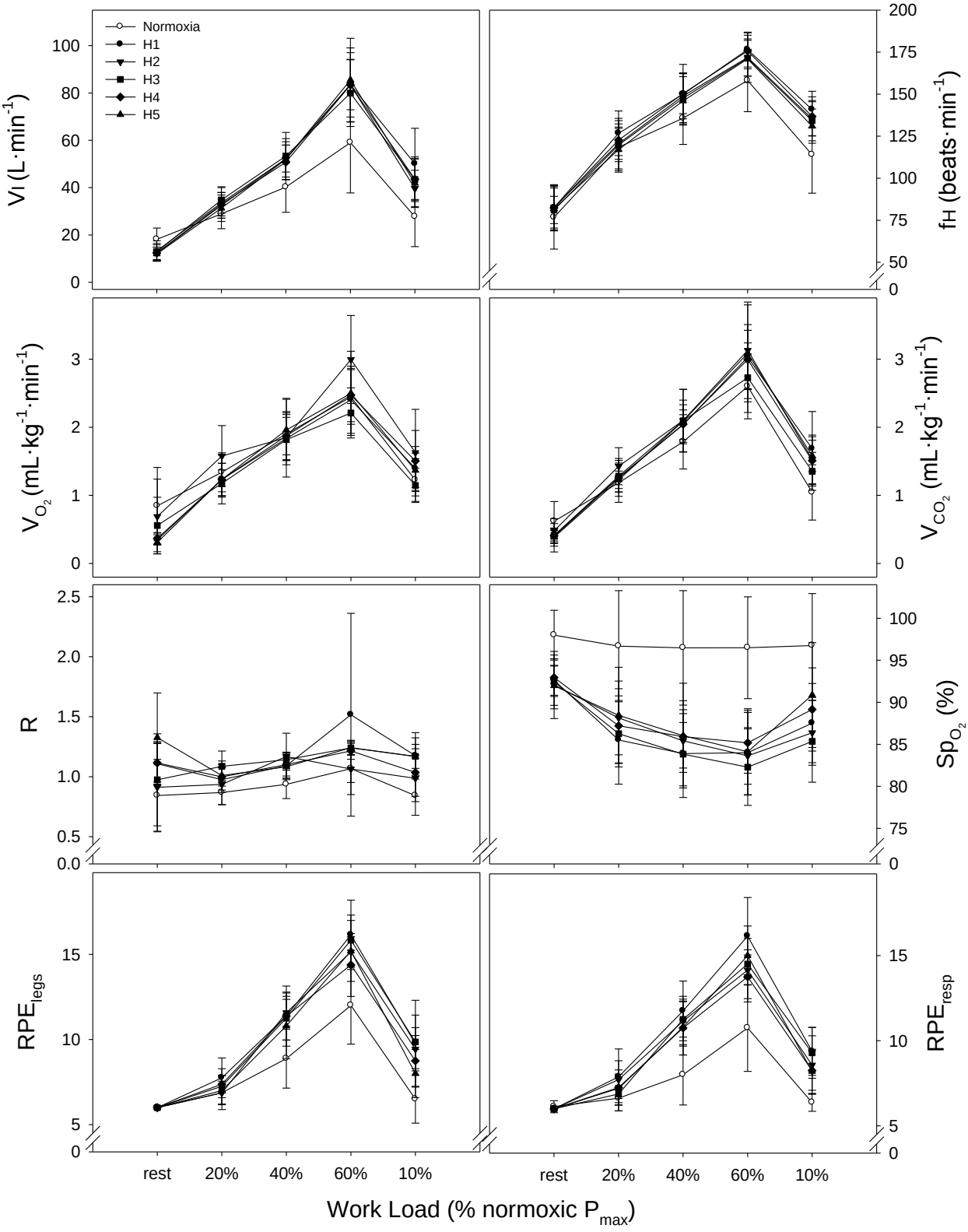

FIGURE IV.1 Exercise responses of minute ventilation ($\dot{V}_I$), heart rate (f_H), O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$), respiratory exchange ratio (R), oxyhaemoglobin saturation (S_pO_2), and rating of perceived exertion for leg (RPE_{legs}) and respiratory sensation (RPE_{resp}) in normoxia and repeated five times in hypoxia (H1 to H5; $F_IO_2 = 0.13$). Data are presented as mean ± standard deviation for all eight participants at each testing session.

responses to normoxic exercise are shown in FIGURE 1 along with data from the repeated hypoxic tests. However, normoxic data represent 15-s averages at each desired power output during the initial $\dot{V}O_{2\text{-max}}$ test as compared to 30-s averages taken at the end of each 3-min hypoxic exercise stage. Although direct comparisons between normoxic and hypoxic cardiorespiratory responses are inappropriate in this case, it seems that hypoxia substantially increased $\dot{V}I$, f_H, RPE_{legs} and RPE_{resp} and decreased S_pO_2 but had little effect on pulmonary gas exchange. These apparent changes reflect the expected effects of acute hypoxia on cardiorespiratory responses to submaximal exercise (Wagner et al 1986).

TABLE IV.1 Day to day variability of cardiorespiratory variables in rest and exercise responses. Intra-individual coefficients of variation (CVs) for minute ventilation ($\dot{V}I$), heart rate (f_H), O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$), respiratory exchange ratio (R), oxyhaemoglobin saturation (S_pO_2), rate of perceived exertion for legs (RPE_{legs}) and breathing (RPE_{resp}) and blood lactate concentration [La] are presented as group mean ± standard deviation (range) for each workload and as a global mean across all workloads. P values indicate effect of workload on CV (RM ANOVA).

	Coefficient of Variation (%)						
	rest	**20% P_{max}**	**40% P_{max}**	**60% P_{max}**	**10% P_{max}**	**mean**	**p value**
$\dot{V}I$	15 ± 5 (8-20)	6 ± 3 (2-10)	8 ± 2 (6-12)	9 ± 5 (3-17)	12 ± 7 (4-23)	10 ± 3	0.01
$\dot{V}O_2$	30 ± 22 (5-69)	15 ± 10 (4-35)	9 ± 4 (4-16)	12 ± 7 (5-28)	18 ± 12 (7-40)	17 ± 8	0.02
$\dot{V}CO_2$	22 ± 15 (5-54)	8 ± 4 (3-14)	7 ± 3 (1-10)	8 ± 4 (3-14)	11 ± 8 (4-28)	11 ± 6	0.04
R	17 ± 18 (5-61)	8 ± 6 (3-17)	6 ± 5 (2-16)	6 ± 7 (1-21)	12 ± 9 (4-28)	10 ± 5	0.08
f_H	7 ± 3 (2-13)	5 ± 2 (3-8)	4 ± 1 (1-4)	2 ± 1 (1-4)	3 ± 2 (1-5)	4 ± 2	0.02
S_pO_2	2 ± 1 (1-4)	3 ± 2 (1-7)	3 ± 2 (1-6)	3 ± 2 (2-6)	5 ± 2 (2-6)	3 ± 1	0.05
RPE_{legs}	0 ± 0 (0-0)	9 ± 6 (0-16)	9 ± 4 (5-15)	10 ± 6 (5-22)	13 ± 5 (6-18)	8 ± 5	< 0.001
RPE_{resp}	0 ± 0 (0-0)	14 ± 4 (8-20)	11 ± 4 (7-16)	12 ± 5 (6-23)	13 ± 5 (6-22)	10 ± 6	<0.001
[La]	21 ± 14 (8-40)	22 ± 12 (5-34)	21 ± 13 (10-41)	8 ± 6 (2-13)	16 ± 16 (7-39)	17 ± 6	0.19

IV.4.2 Cardiorespiratory responses across testing sessions

Repeated measures designs are statistically robust because error due to irrelevant variables is reduced (Myers and Well 1991). However, repeatedly performing any test can lead to either performance improvements (via practice or training) or decrements (via fatigue or deterioration at HA) that can confound results. These effects can be minimized by randomizing testing order but randomization is constrained in time course studies and order effects cannot be disregarded. Hypoxic exercise testing has two important components (hypoxia and exercise) with potential to elicit training responses and cause systematic changes in the highly plastic cardiorespiratory responses to exercise.

Exposure to intermittent hypoxia (IH) has been repeatedly shown to increase hypoxic ventilatory response (HVR) (Katayama et al 2005b), resulting in higher $\dot{V}_I$ and S_pO_2 during hypoxia. HVR has increased following as little as 30 min · day^{-1} at 12% O_2 for 10 d (Foster et al 2005) and 20 min · day^{-1} at 10% O_2 for 14 consecutive days (Mahamed and Duffin 2001). However, as expected, there was no change in resting or exercise $\dot{V}_I$ from H1 to H5, indicating that the brief hypoxic exposure (17 min · day^{-1} at 13% O_2 for 5 non-consecutive days) was insufficient in severity, duration and/or frequency to elicit similar IH-induced changes in ventilatory control. Consequently, there was no improvement of S_pO_2 across hypoxic testing sessions to indicate improved hypoxia tolerance.

Aerobic training elicits several notable cardiovascular and metabolic adaptations, some of which are evident after only 1 w and 3-4 sessions of moderate exercise (Ziemba et al 2003). Resting and submaximal f_H both decrease with aerobic training (reviewed in Blomqvist and Saltin 1983) and there is a shift in substrate utilization from carbohydrate to lipid at submaximal workload (Friedlander et al 1998). The reduced respiratory quotient is detectable as a reduced R during standard metabolic testing and is accompanied by a lower RPE at a given workload (Ekblom and Goldbarg 1971). Moreover, endurance training is known to speed post-exercise recovery rates of f_H and $\dot{V}_{O_2}$ (Short and Sedlock 1997). No downward shift in the relationship between f_H, R, or RPE and workload was evident (FIGURE 1) and there was no difference in the degree to which f_H ($p > 0.10$) or $\dot{V}_{O_2}$ ($p = 0.20$) had recovered towards BL after 3 min active recovery at 10% $P_{max.}$ These results confirm that, by design, the exercise challenge was of

insufficient duration and/or intensity to elicit even the earliest signs of an aerobic training response after five testing sessions. These results are specific to the moderately-trained participant population. Fitter individuals would be even less likely (while those of lower fitness may be more likely) to exhibit a training response to such a nominal dose of exercise (Wenger and Bell 1986).

IV.4.3 Repeatability of cardiorespiratory measures

Intra-individual repeatability of cardiorespiratory responses to hypoxic rest and exercise was generally very good, with average CVs across workloads as low as 3 and 4% for S_pO_2 and f_H, respectively. Measurement of respiratory gas exchange was less repeatable and is reflected in the higher CVs for $\dot{V}O_2$,$\dot{V}CO_2$ and R.

IV.4.3.1 Rest

For most cardiorespiratory responses measured here, variability was markedly higher during rest and recovery than during exercise, possibly reflecting behavioural, rather than physiological differences from day to day. Anxiety or restlessness can lead to alterations in cardiorespiratory responses and their impacts are masked by physiological responses as exercise intensity increases (discussed in Wasserman et al 2005). If rest and recovery stages are excluded, exercise CVs for most variables are substantially reduced, and the result is most notable for $\dot{V}O_2$ (CV drops from 17 → 12%), $\dot{V}CO_2$ (11 → 8%) and R (10 → 7%).

IV.4.3.2 Exercise

Day to day variability of cardiorespiratory responses to hypoxic exercise was similar to values previously reported from a normoxic exercise test – re-test study on a much larger sample population (Wilmore et al 1998). In agreement with these findings, Wilmore and colleagues reported that variability in f_H, $\dot{V}I$, $\dot{V}O_2$, $\dot{V}CO_2$ and R decreased in heavier exercise. In the present data set, the variability of S_pO_2 also decreases with increasing work. However, both RPE CVs were lowest at rest and tended to increase throughout exercise and recovery. The same pattern has been previously reported for RPE CVs in normoxic exercise (Lamb et al 1999).

It should be acknowledged that CV expresses variability relative to the mean. Therefore, any measure-

ment error that is consistent across workloads will always result in the highest CVs when mean values are the lowest. All cardiorespiratory responses (except S_pO_2) increased with exercise intensity; thus the general trend for accompanying decreases in variability may partially reflect an artefact in calculating CV. This does not exclude a physiological explanation, particularly since the mean and CV for S_pO_2 both decreased with increasing work.

IV.5 Conclusion

An exercise challenge was developed that was appropriate for use in repeated testing of the cardiorespiratory responses to hypoxic exercise. The hypoxic stimulus (FIO_2 =0.13, ~3800 m) is known to alter cardiorespiratory responses in rest and exercise, but the frequency and duration of hypoxic exposure was insufficient to elicit measurable IH-induced changes in ventilation. The cycle exercise protocol (3-min stages at 20%, 40%, 60% and 10% of normoxic P_{max}) was not of sufficient intensity, duration or frequency to elicit a training response when repeated five times during a two-week period. The day to day variability of cardiorespiratory responses to hypoxic exercise was low across a range of workloads, indicating the exercise challenge elicited repeatable responses. Therefore, this protocol allows detection of small changes in cardiorespiratory responses to hypoxic exercise that might occur during exposure to chronic or intermittent hypoxia, or in response to therapeutic interventions for pathological hypoxaemia or high-altitude illness.

Information is provided so the protocol can be modified to address other research questions. For example, it might be desirable to customize the exercise challenge to maximize statistical power; using TABLE 1, investigators can select an exercise intensity that will minimize the intra-individual variability for a cardiorespiratory variable of interest. For those who wish to relate these findings to exercise studies that have been (or will be) conducted at work loads described relative to maximum performance in hypoxia, estimates are offered to convert exercise intensities from % normoxic to % hypoxic P_{max}. According to two accounts, acute exposure to 13% O_2 causes a 15% (Lawler et al 1988) or 15-20% (Martin and O'Kroy 1993) reduction of $\dot{V}O_{2\text{-max}}$ in individuals of the same fitness levels as the current study participants. Bebout and colleagues (1989) surmise a 25% reduction of normoxic $\dot{V}O_{2\text{-max}}$ with the same severity of

hypoxia. Therefore, assuming that maximal performance is reduced 15-25% with acute exposure to 3800 m, workloads of 20%, 40%, 60% and 10% of normoxic P_{max} represent approximately 24-27%, 47-53%, 71-80% and 12-13% of hypoxic P_{max}.

APPENDIX V. RESEARCH MATERIALS

V.1 Calculating non-exercise $\dot{V}O_{2\text{-max}}$

As described by Bradshaw et al (2005), $\dot{V}O_{2\text{-max}}$ was estimated from the following formula:

$$\dot{V}O_{2\text{-max}} = 48.0730 + 6.1779a - 0.2463b - 0.6186c + 0.7115d + 0.6709e$$

where:

a = sex [0 for female, 1 for male]

b = age in years

c = body mass index (BMI) = [mass in kg] / [height in m]^2

d = perceived functional ability (see below)

e = current physical activity (see below)

Perceived functional ability is the sum of responses from the following questionnaire (from George et al 1997):

Suppose you were going to exercise continuously on an indoor track for 1 mile (1.6km). Which exercise pace is just right for you – not too easy and not too hard?

1 Walking at a *slow* pace (18 minutes per mile or more)
2
3 Walking at a *medium* pace (16 minutes per mile)
4
5 Walking at a *fast* pace (14 minutes per mile)
6
7 Jogging at a *slow* pace (12 minutes per mile)
8
9 Jogging at a *medium* pace (10 minutes per mile)
10
11 Jogging at a *fast* pace (8 minutes per mile)
12
13 Running at a *fast* pace (7 minutes per mile or less)

How fast could you cover a distance of 3 miles (4.8km) and NOT become breathless or overly fatigued? Be realistic.

1 I could walk the entire distance at a *slow* pace (18 minutes per mile or more)
2
3 I could walk the entire distance at a *medium* pace (16 minutes per mile)
4
5 I could walk the entire distance at a *fast* pace (14 minutes per mile)
6
7 I could jog the entire distance at a *slow* pace (12 minutes per mile)
8
9 I could jog the entire distance at a *medium* pace (10 minutes per mile)
10
11 I could jog the entire distance at a *fast* pace (8 minutes per mile)
12
13 I could run the entire distance at a *fast* pace (7 minutes per mile or less)

Current physical activity is the response from the following questionnaire (modified from Jackson et al 1990):

Select the number that best describes you overall level of activity for the previous six months:

0 = avoid walking or exertion: e.g. always use elevator, drive when possible instead of walking

1 = *light activity*: walk for pleasure, routinely use stairs, occasionally exercise sufficiently to cause heavy breathing or perspiration

2 = *moderate activity*: 10 to 60 minutes per week of moderate activity such as golf, horseback riding, calisthenics, table tennis, bowling, weight lifting, yard work, cleaning house, walking for exercise

3 = *moderate activity*: over 1 hour per week of moderate activity as described above

4 = *vigorous activity*: run less than 1 mile (1.6km) per week or spend less than 30 minutes per week in comparable activity such as running/jogging, lap swimming, cycling, rowing, aerobics, skipping rope, or engaging in vigorous aerobic-type activity such as soccer, basketball, tennis, racquetball or handball

5 = *vigorous activity*: run 1 mile (1.6km) to less than 5 miles (8km) per week or spend 30 to less than 60 minutes per week in comparable physical activity as described above

6 = *vigorous activity*: run 5 miles (8km) to less than 10 miles (16km) per week or spend 1 hour to less than 3 hours per week in comparable physical activity as described above

7 = *vigorous activity*: run 10 miles (16km) to less than 15 miles (24km) per week or spend 3 hours to less than 6 hours per week in comparable physical activity as described above

8 = *vigorous activity*: run 15 miles (24km) to less than 20 miles (32km) per week or spend 6 hours to less than 7 hours per week in comparable physical activity as described above

9 = *vigorous activity*: run 20 miles (32km) to less than 25 miles (40km) per week or spend 7 hours to less than 8 hours per week in comparable physical activity as described above

10 = ***vigorous activity***: run over 25 miles (40km) per week or spend over 8 hours per week in comparable physical activity as described above

V.2 Lake Louise Questionnaire

Acute mountain sickness (AMS) was evaluated using the self-report section of the Lake Louise Questionnaire (Hackett and Oelz 1992). AMS score was calculated as the sum of responses from the following questionnaire.

For each category, circle the number that best describes your current symptoms.

Headache	
No headache	0
Mild headache	1
Moderate headache	2
Severe, incapacitating	3
Gastrointestinal (GI)	
No GI symptoms	0
Poor appetite or nausea	1
Moderate nausea or vomiting	2
Severe N&V incapacitating	3
Fatigure/weakness	
Not tired or weak	0
Mild fatigue/weakness	1
Moderate fatigue/weakness	2
Severe F/W, incapacitating	3
Dizzy/lightheaded	
Not dizzy	0
Mild dizziness	1
Moderate dizziness	2
Severe, incapacitating	3
Difficulty sleeping	
Slept well as usual	0
Did not sleep as well as usual	1
Woke many times	2
Could not sleep at all	3

V.3 Perceptions of acclimatisation: interview guide

The following interview guide represents an overarching agenda for interviews with high-altitude-experienced individuals. Probes were pursued flexibly and sometimes altered or added to as different themes and patterns emerged in the interview.

- Tell me a bit about yourself.
- Tell me about some of your experiences at high altitude.
- Describe the ascent/descent profiles of some of your travels to high altitude.
- How do you choose your ascent profiles?
- On a typical expedition, describe how you feel when you first arrive at high altitude?
- As you spend time at altitude, do you feel your body changing in any way? If so, how?
- How would you describe your ability to acclimatize to high altitude (relative to others around you)?
- Can you think about a particular time when you did not feel physically well at high altitude? If so, can you describe how you felt?
- Can you describe a time when you have acclimatized very easily to high altitude? A time when you felt unable to acclimatize properly?
- To what extent do you respond in a similar (or different) way each time you travel to high altitude?
- Can you describe any aspects of the acclimatization process that seem to be consistent for you across different exposures? Are there any aspects that have not been consistent across different exposures?
- Are you aware of any factors that seem to affect whether you acclimatize well or poorly?
- How do you feel when you return to sea level after an extended period at high altitude?
- Could you tell me (a) how much time do you typically spend at low altitude between climbs, and (b) what do you do between climbs?
- What have been your experiences during re-exposure to high altitude after spending time at low altitude?