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One week of daily voluntary apnoea training does not alter acute hypoxic ventilatory response or erythropoietin concentration in healthy males

by

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Abstract

Exposure to intermittent hypoxia (IH) increases ventilatory chemosensitivity and various haematological parameters. It is unknown whether voluntary apnoea training can be used as a model of IH to produce similar physiological effects. It was hypothesized that seven days of voluntary apnoea training would increase the acute hypoxic ventilatory response (HVR), erythropoietin concentration ([EPO]), haemoglobin (Hb), haematocrit (Hct) and VO_2max. No significant ($P > 0.05$) differences were found in HVR (0.59 ± 0.24 vs. 0.54 ± 0.27 $L.min^{-1}.\%^{-1}$), VO_2max (48.4 ± 7.8 vs. 48.5 ± 6.8 $ml.kg^{-1}.min^{-1}$) or [EPO], Hct, and Hb were not different across all time points (EPO: 7.5 ± 2.6 vs. 6.5 ± 2.5 $mIU.mL^{-1}$, Hct: 45.0 ± 2.3 vs. $45.7 \pm 3.4\%$, Hb: 14.9 ± 2.46 vs. 14.2 ± 3.37 $g.100mL^{-1}$) for first and last measures, respectively. These findings indicate that seven days of daily voluntary apnoea training, does not alter HVR, [EPO], [Hb] or Hct.

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BP – blood pressure

CO_2 – carbon dioxi3de

CV – coefficient of variation

DBP – diastolic blood pressure

EPO – erythropoietin

[EPO] – erythropoietin concentration

F_IO_2 – fraction of inspired oxygen

Hb - haemoglobin

[Hb] – haemoglobin concentration

Hct – haematocrit

HR – heart rate

HIF-1 – hypoxia-inducible factor-1

HVR – hypoxic ventilatory response

IH – intermittent hypoxia

kp - kilopond

LDIH – long duration intermittent hypoxic exposure

MAP – mean arterial pressure

PCO_2 – partial pressure of carbon dioxide

PO_2 – partial pressure of oxygen

PR – pulse rate

PaO_2 – arterial partial pressure of oxygen

$P_{ET}CO_2$ – end-tidal partial pressure of carbon dioxide

$P_{ET}O_2$ – end-tidal partial pressure of oxygen

P_IO_2 – inspired partial pressure of oxygen

Q_c – cardiac output

RBC – red blood cell

RER – respiratory exchange ratio

rpm – revolutions per minute

SaO_2 – arterial oxygen saturation

SBP – systolic blood pressure

SDIH – short duration intermittent hypoxic exposure

SV – stroke volume

V_I – inspired ventilation

V_E – pulmonary ventilation

VO_2 – oxygen consumption

VO_2max – maximal oxygen consumption

W – watts

CHAPTER 1: INTRODUCTION

1.1 OVERVIEW

1.1.1 HYPOXIA

Hypoxia is defined as a decrease in oxygen (O_2) availability and commonly occurs with ascent to altitude. Exposure to hypoxia presents substantial challenges to human physiology, including the cardiovascular and respiratory systems. The primary physiological challenge associated with ascent to altitude is the decrease in ambient partial pressure of oxygen (PO_2). The decline in inspired PO_2 (P_IO_2) reduces the driving pressure for the transfer of O_2 from the atmosphere to the lung and throughout the O_2 cascade from the lung to the muscle mitochondria. Atmospheric air is composed of ~ 20.93% O_2 and barometric pressure at sea level (0 m) averages ~ 760 mm Hg resulting in a P_IO_2 of approximately 160 mm Hg at sea level. Barometric pressure progressively declines with altitude to approximately 674, 526, 462, 405 and 267 mm Hg at 1,000, 3,000, 4,000, 5,000 and 8,000 m, respectively. This results in a decrease in P_IO_2 to approximately 141, 110, 97, 85 and 56 mm Hg at the latter mentioned elevations, respectively. Consequently, as the elevation increases so too does the severity of the hypoxic stimulus. Arterial hypoxaemia resulting from reduced P_IO_2 elicits immediate physiological adjustments that maintain physiological function in the face of diminished O_2 availability and initiate the process of altitude acclimatization. Altitude acclimatization occurs when people ascend to high altitude for extended periods (23) resulting in both central (cardiopulmonary) and

peripheral (muscular) adaptations that improve O_2 carrying capacity/delivery and muscle O_2 utilization (32), which counteract the reduced O_2 availability. Immediate physiological adjustments involve the respiratory and cardiovascular systems followed by more gradual haematological adjustments that augment the O_2 carrying capacity of the blood (4). Hypoxia is sensed by peripheral chemoreceptors which initiate an increase in pulmonary ventilation (V_E) in an attempt to maintain arterial oxyhaemoglobin saturation (SaO_2) near normal levels (4). These adjustments attenuate the effects of a decreased P_IO_2 and help restore the O_2 content of the blood and improve tissue oxygenation thereby aiding in the process of altitude acclimatization (19). However, this compensation is often inadequate, particularly at higher altitudes, and the arterial partial pressure of oxygen (PaO_2) is reduced below sea-level values, which leads to a reduction in the amount of O_2 delivered to the working muscles during exercise. As a result of this reduced O_2 delivery, aerobic performance at altitude tends to decline.

1.1.2 AEROBIC POWER

Endurance exercise performance is largely dependent upon an individual's maximal oxygen consumption (VO_2max) (5), which reflects the ability to maximally uptake, transport and utilize O_2. Given that VO_2max is the product of the volume of blood being pumped by the heart (cardiac output) and the amount of O_2 utilized for metabolic work (arterial-venous oxygen content difference), any measurable increase in either of these variables results in an improvement in VO_2max and endurance performance. Cardiac output (Q_c) may be improved through an increase in heart rate (HR) or cardiac stroke volume (SV), while

arterial-venous oxygen content difference may be increased either through an improved arterial O_2 content or improved utilization of O_2 in working muscles. Increased arterial O_2 content occurs through an increase in the number of haemoglobin (Hb) molecules, which bind and transport O_2, facilitating an increase in O_2 delivery at the same Q_c, assuming no change in arterial O_2 saturation (SaO_2). Exposure to hypoxia has been proposed as a potential method for inducing haematological adaptations associated with O_2 transport; however results are equivocal and largely dependent upon the hypoxia protocol (4). Moreover, there appears to be large inter-individual variability in the haematological response to hypoxia (4, 9, 14, 15, 19, 29, 39).

1.1.3 HYPOXIA, ALTITUDE AND HAEMATOLOGICAL ADJUSTMENTS

Ascent to altitude represents a substantial physiological challenge as O_2 availability is reduced (e.g. decrease in P_IO_2). Exposure to hypoxia results in important haematological adjustments related to the O_2 carrying capacity of the blood. Erythropoietin concentration ([EPO]) increases following both acute and chronic exposure to hypoxia (decreased P_IO_2) (14, 15, 19, 25, 29). The heterodimer transcription factor hypoxia-inducible factor-1α (HIF-1α) is responsible for the increased synthesis of EPO in response to hypoxia (42). During hypoxic conditions the degradation of HIF-1α is blocked causing HIF-1α subunits to accumulate eliciting an increase in EPO production, primarily in the adult kidney (21). If the production of EPO in response to hypoxia is of sufficient magnitude and/or sustained duration an increase in erythrocyte production may occur and lead to an increase in circulating reticulocytes, haemoglobin

concentration ([Hb]), haematocrit (Hct) and red blood cell (RBC) mass (4, 15, 25). Previous research has demonstrated that as little as 84 minutes of exposure to 4,000 m or 114 minutes of exposure to 3,000 m of simulated altitude significantly increases EPO levels in healthy humans (14). Eckardt and colleagues demonstrated a continuous rise in EPO for approximately 90 minutes post-exposure and then a decline approximately three hours post-exposure (14). These results indicate the responsiveness of EPO to acute hypoxia is almost immediate and appears to be dependent upon both the intensity and duration of the hypoxic stimulus (14, 19). In comparison, increases in RBC mass are more gradual and tend to require longer duration (4, 22) or a more severe hypoxic stimulus (4).

In healthy humans, acute hypoxic exposure elicits an immediate increase in V_E as a result of changes in the peripheral chemoreflex, specifically in the carotid body (13). The carotid body is considered a principle component of the O_2 sensing system that is required to activate the brainstem respiratory centre to elicit hyperventilation during periods of acute hypoxaemia (e.g. altitude exposure) (33). Carotid body glomus cells are considered the chemoreceptive elements responsible for changes in chemoreceptor responsiveness during periods of hypoxia (33). Hypoxic conditions lead to an inhibition of potassium currents and channel activity which in turn leads to an increase in external calcium dependent cytosolic calcium, catecholamine and adenosine triphosphate release from hypoxic glomus cells (33). The increase in V_E observed during acute hypoxic conditions is thus a result of the cellular responses occurring in the carotid body (33). The responsiveness of peripheral chemoreceptors is commonly measured

using an acute hypoxic challenge test where the acute isocapnic hypoxic ventilatory response (HVR) is measured (13). The HVR measurement involves a progressive decrease in the fraction of inspired O_2 (F_IO_2) to elicit a concurrent decrease in SaO_2 and an increase in V_E (41). The acute HVR is quantified by determining the magnitude of the increase in inspired ventilation (V_I) for a given decrease in SaO_2 (44) and is typically expressed in $L.min^{-1}.\%^{-1}$. Consequently, any change in the acute HVR may be considered the result of altered carotid body chemosensitivity, processing of carotid body input within the central nervous system or ventilatory efferent output (41). Longer-term ventilatory adjustments to altitude also occur with chronic hypoxia. The increase in V_E in response to hypoxia in an attempt to increase alveolar PO_2 produces a considerable decrease in the body's carbon dioxide (CO_2) levels and results in a physiologic disequilibrium. A decrease in CO_2 associated with hyperventilation results in an increase in pH and as a result bodily fluids become more alkaline (4). To mitigate the ventilatory induced alkalosis the kidneys excrete bicarbonate which restores pH to normal and allows for an even greater increase in V_E in response to hypoxia (4). When compared, poikliocapnic hypoxia (allowing arterial partial pressure of CO_2 to fall during hypoxic hyperventilation) attenuates an increase in V_E as compared to isocapnic hypoxia (allowing arterial partial pressure of CO_2 to remain constant) (41).

1.1.4 ACUTE HYPOXIA AND THE CARDIOVASCULAR SYSTEM

Hypoxic exposure also places an added stress on the cardiovascular system (35). Immediately following exposure to hypoxia, resting HR increases

while SV remains unchanged, resulting in an overall increase in Q_c (35). At moderate altitudes, the magnitude of the increase in Q_c is sufficient to offset the decrease in arterial O_2 content, such that, tissue O_2 delivery remains similar to sea-level values under basal conditions (35).

With acclimatization to high altitude, alterations in the autonomic control of the cardiovascular system occur, evidenced by a marked reduction in maximal HR (4, 7). Enhanced parasympathetic nerve activity has been postulated as one of the mechanisms responsible for the reduced maximal HR with chronic exposure to altitude (4, 7). Consistent with this postulate, Boushel and colleagues demonstrated a pronounced increase in HR at rest and during exercise following the administration of a parasympathetic nerve blockade (glycoporolate) in subjects who had resided at an altitude of 5,260 m for nine weeks (7). Despite significant variations in HR following the administration of glycopyrrolate, no change in Q_c or O_2 transport were found suggesting that SV was reciprocally adjusted to maintain the same Q_c (7).

Chronic and acute hypoxia initiate a complex set of physiological responses that regulate vasomotor tone (6). In the pulmonary arteries, hypoxia causes vasoconstriction thereby increasing pulmonary vascular resistance, while in the systemic arteries hypoxia causes vasodilation (3). In response to alveolar hypoxia, pulmonary arteries constrict in an attempt to help improve gas exchange efficiency by diverting blood flow away from poorly ventilated lung regions, towards areas with better oxygenation (43). However, at high altitudes, global alveolar hypoxia leads to chronic pulmonary arterial hypertension, which can

trigger vascular remodeling. Hypoxic pulmonary vasoconstriction is biphasic as there is an acute phase (initial transient constriction which does not require an intact endothelium) and a chronic phase (slow sustained constriction which does require an intact endothelium) (43). The acute phase occurs within seconds of hypoxic exposure, causes calcium release from intracellular stores, thereby causing the opening of voltage dependent and independent calcium channels. As a result, there is an increase in cytosolic calcium which activates pulmonary artery smooth muscle cell contraction. The chronic phase occurs within minutes of hypoxic exposure and persists if hypoxia is maintained and affects the myofilament sensitivity to calcium (43). Although the responses differ between the systemic and pulmonary arteries, the mitochondria act as the main O_2 sensor in both (43). During hypoxic conditions, the mitochondria detects and translates O_2 availability into a signal that triggers the above mentioned adaptive responses (43). The systemic vasodilation and pulmonary vasoconstriction that occur in response to hypoxia are modulated by endothelium-derived nitric oxide (6). Specifically, in the pulmonary circulation, endothelium-derived nitric oxide attenuates pulmonary vasoconstriction, whereas in the systemic circulation it contributes to the vasodilation that occurs in response to hypoxia (6).

There is a graded increase in muscle sympathetic nerve activity in response to hypoxia (24). This increase in muscle sympathetic nerve activity is attributable to an increase in baroreceptor unloading caused by a hypoxia induced fall in blood pressure (BP). However, it appears that the increase in muscle sympathetic nerve activity in response to hypoxia is generally blunted by dilator

influences during systemic hypoxia (24). To this extent, it has been shown that femoral vascular resistance decreases in a graded manner in response to graded increases in hypoxia (two minutes at an F_IO_2 of 12%, 10% and 8%), indicating peripheral vasodilation (24). As a result of peripheral vasodilation there is a reduction in total peripheral resistance indicating that an increase in BP during hypoxic conditions would most likely be the result of an increase in Q_c associated with hypoxia and not an increase in total peripheral resistance.

1.1.5 INTERMITTENT HYPOXIC EXPOSURE

Exposure to multiple brief bouts of hypoxia over a period of time is known as intermittent hypoxia (IH). Intermittent hypoxia protocols are often classified as either short duration (SDIH) or long duration (LDIH) according to the duration and frequency of the hypoxic exposure. SDIH includes multiple brief bouts of hypoxia separated by bouts of normoxia over one day or a number of days (e.g. five minutes hypoxia interspersed with five minutes normoxia for one hour per day over seven days) whereas LDIH typically involves longer bouts of hypoxia for a continuous period of time over a number of days (e.g. one exposure of 60 minutes per day of sustained hypoxia for seven days).

1.1.6 INERMITTENT HYPOXIA AND VENTILATORY CHEMOSENSITIVITY

Similar to altitude exposure, IH leads to significant cardiovascular and ventilatory adjustments. Intermittent hypoxia affects ventilatory chemosensitivity as observed by an increase in the acute HVR, which is demonstrated by an increase in the slope of the regression line relating V_I to SaO_2 (26, 27, 30, 34, 41).

Ventilatory acclimatization to altitude may be facilitated more effectively through a greater increase in V_I for a given drop in SaO_2 (a brisk HVR), than a smaller increase or lack of change (a blunted HVR) (13). Both SDIH and LDIH exposure elicit an increase in ventilatory chemosensitivity with the acute HVR (1, 16, 18, 27, 28, 31) increasing by 34-49% (16, 30, 31, 34) and 67% (30, 31), respectively. Additional studies demonstrated a 200% and 103% increase in HVR following 12 consecutive days of LDIH, two hours per day at a simulated altitude of 3,800 m and 10 consecutive days of IH, one hour per day at an SaO_2 of 80%, respectively (18, 34). However, the increase in HVR following IH appears to be transient and returns to baseline within five days post-exposure (1, 16, 18, 30, 31).

1.1.7 INTERMITTENT HYPOXIA AND HAEMATOLOGICAL ADJUSTMENTS

Similarly, prolonged and acute exposure to IH at simulated altitudes between 3,550 - 5,000 m elicit significant increases in plasma and serum [EPO] (20, 23). Repeated hypoxic stimuli for a total of 60 hours (three hours per day, five days per week, four weeks) between 4,000 - 5,500 m of simulated altitude elicits a significant increase of 136% in serum [EPO] three hours post exposure (20). It has also recently been established that four days of IH, six hours of continuous cycles of two minutes hypoxia (end tidal partial pressure of O_2 ($P_{ET}O_2$) = 45.0 mm Hg) followed by two minutes of normoxia ($P_{ET}O_2$ = 88.0 mm Hg) is sufficient to significantly increase [EPO] by 36% from baseline (8). In the same study, Brugniaux and colleagues strengthened the evidence for the acute response of [EPO] following IH as they found a significant increase in [EPO] of 50% from baseline on day two. In contrast to [EPO], it remains unclear whether IH exposure

consistently causes significant changes in RBC volume, [Hb] and Hct (20, 23, 39). The confounding results on the effects of IH on various haematological parameters suggest that the IH paradigm plays an important role in the haematological response. More importantly, there appears to be large inter-individual variability in the haematological response to IH and a sensitivity to the specificity of the IH protocol (18, 20, 23, 39).

1.1.8 INTERMITTENT HYPOXIA AND CARDIOVASCULAR ADJUSTMENTS

Exposure to IH elicits various changes in cardiovascular function, most notably an increase in mean arterial pressure (MAP) (16). Foster and colleagues measured MAP during daily SDIH exposures (10 exposures of one hour, cycling between five minutes of hypoxia ($F_IO_2 = 0.12$) followed by five minutes of normoxia) and found a significant increase in MAP (~ 5 mm Hg) from the first five minutes of hypoxia to the last five minutes of hypoxia (16). It appears that repeated states of deoxygenation and reoxygenation experienced during IH exposure are an important stimulus for increasing BP (16). There were small and non-significant increases (approximately 1 beat.min^{-1}) in HR during IH exposure (16), suggesting that the increase in BP may have been mediated by alterations in peripheral vascular resistance, not an increase in Q_c. The sympathetic nervous system plays an important role in the hemodynamic response to hypoxia; however local metabolic factors may be primarily responsible for the hypoxic vasodilation (12). As such, it is probable that the rise in MAP occurs as a result of a lack of this hypoxia-mediated vasodilation (16). Repeated exposure to IH (60 minutes per day, cycling between five minutes hypoxia ($F_IO_2 = 12\%$) followed by five

minutes of normoxia for 10 days) tends to increase the BP sensitivity to hypoxia, however not significantly (16). This possible increase is the result of a unique contribution of the carotid body, which leads to an increase in the amount of sympathetic stimulation resulting in down-stream cardiovascular consequences, namely an increase in BP (36). Therefore, the increase in resting BP associated with IH exposure is mediated by carotid body afferents and their sympathetic innervations (36). Ultimately, hypoxia leads to an increase in sympathetic nervous system activity, which elicits a peripheral vasoconstrictor response thereby increasing BP.

1.1.9 VOLUNTARY APNOEAS AS A MODEL OF INTERMITTENT HYPOXIA

In humans, voluntary apnoeas occur in a variety of settings including some sports (e.g. breath-hold diving and synchronized swimming) and other periods of controlled apnoea training. The human diving response is characterized by bradycardia, reduced Q_c, peripheral vasoconstriction and increased BP (2) and is considered an acute physiological adaptation that permits humans to endure the lack of O_2 experienced during apnoeas (17). Splenic contraction during apnoeas may play an important role in O_2 availability by increasing [Hb] and Hct leading to enhanced O_2 transport/capacity throughout the body (17, 37, 38). In some animals, like Weddell seals, splenic contraction contributes to prolonged diving (10); however in humans splenic contraction plays a relatively minor role in augmenting the O_2 carrying capacity of the blood. Moreover in humans, the increase in Hct and [Hb] associated with splenic contraction is an acute response and does not enhance the O_2 carrying capacity of the blood in the long-term.

Although voluntary apnoeas can alter haematological parameters through splenic contraction (17, 37, 38, 40), the effects of voluntary apnoea training on total circulating EPO concentration and the subsequent erythropoiesis are not known. In addition, literature is lacking on the changes in ventilatory chemosensitivity and cardiovascular adjustments to acute hypoxia following voluntary apnoea training. Although the various physiological changes that occur as a result of laboratory controlled IH exposure have been examined, it is not known whether voluntary apnoea training has similar effects and can be used as a model of IH exposure.

Recently, de Bruijn and colleagues demonstrated a significant increase in circulating serum [EPO] following an acute bout of voluntary apnoeas consisting of three series of five maximal duration voluntary apnoeas (11). Following only one bout of 15 maximal voluntary apnoeas, mean serum [EPO] increased by an average individual maximum of 24% from baseline, with the peak occurring three hours post-apnoea exposure. However, this protocol only elicited transient increases in serum [EPO]; [EPO] levels returned to baseline five hours post-exposure. Only one acute bout of voluntary apnoeas was examined. These findings suggest that one session of 15 voluntary apnoeas is a sufficient stimulus to induce acute increases in serum [EPO]. Data do not, however, address the effects of repeated voluntary apnoeas on other haematological variables associated with the O_2 carrying capacity of the blood, ventilatory chemosensitivity (as measured by the acute HVR) or cardiovascular function (as measured by resting MAP).

1.2 STATEMENT OF THE PROBLEM

Although acute exposure to maximal duration voluntary apnoeas significantly increases serum [EPO], the chronic effects of voluntary apnoea training on plasma [EPO] are not known. Therefore, it is unclear whether voluntary apnoea training over a number of days can evoke similar increases in [EPO] as previously observed following a single bout. The effects of voluntary apnoea training on other haematological parameters associated with the O_2 carrying capacity of the blood and on ventilatory chemosensitivity are also not well established. Therefore, it is unknown whether voluntary apnoea training over a number of days acts as a SDIH stimulus similar to that previously seen in other laboratory controlled SDIH protocols.

1.3 PURPOSE

The purpose of this study was to investigate the haematological, ventilatory and cardiovascular responses to seven days of voluntary apnoea training. More specifically, the current study examined the effects of seven days of voluntary apnoea training on plasma [EPO], Hct, [Hb], the acute HVR and resting MAP. In addition, this study investigated the effects of this type of training on VO_2max.

1.4 HYPOTHESES

It was hypothesized that voluntary apnoea training would increase plasma [EPO] within three hours of the initial-apnoea training session and that this

increase would be maintained throughout the seven days of training. It was also hypothesized that seven days of voluntary apnoea training would elicit an increase in Hct and [Hb]. Given that these haematological variables are associated with an increase in the O_2 carrying capacity of the blood, it was hypothesized that VO_2max would be increased following the seven days of apnoea training. The IH exposure in the present study is similar to previous poikliocapnic IH exposures, consequently it was hypothesized that seven days of repeated voluntary apnoeas would elicit an increase in ventilatory chemosensitivity (measured as an increase in the acute HVR) and resting cardiovascular activity (as evidenced by a increase in resting MAP).

1.5 SIGNIFICANCE OF THE STUDY

Previous research involving [EPO] and voluntary apnoea exposure has been limited to a single exposure. Furthermore, EPO has been the only haematological variable studied following this type of hypoxic stimulus. Therefore, it is unknown whether repeated voluntary apnoea exposure over a number of days can further enhance [EPO] and increase other haematological parameters associated with the O_2 carrying capacity of the blood, namely [Hb] and Hct. In addition, literature is currently lacking on the effects of voluntary apnoea exposure over a number of days on ventilatory chemosensitivity and cardiovascular variables. Therefore, this study will determine in healthy humans the effects of repeated voluntary apnoeas on ventilatory chemosensitivity to acute hypoxia and resting BP.

If shown to be effective at increasing the haematological variables associated with the O_2 carrying capacity of the blood, voluntary apnoea training could provide a more practical and cost effective model of IH than those seen previously in other laboratory controlled IH protocols. As a result, voluntary apnoea training has the potential to be used as a model of IH in replacement of ascent to altitude or the use of a hypobaric or hypoxic chamber. Ultimately, this type of training provides a unique model of poikliocapnic IH that would allow for the examination of repeated apnoea exposure on haematological variables and measures of ventilatory chemosensitivity to acute hypoxia and resting cardiovascular variables in the absence of pre-existing diseases.

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CHAPTER 2: One week of daily voluntary apnoea training does not alter acute hypoxic ventilatory response or erythropoietin concentration in healthy males

2.1 INTRODUCTION

Repeated exposure to IH at simulated altitudes between 3,550 - 5,000 m (equivalent to a P_IO_2 of between approximately 110 mm Hg and 85 mm Hg) elicits a significant increase in plasma and serum [EPO] (9, 10). Recently, it was established that serum [EPO] was significantly increased by 50% (day two) and 36% (day four) from baseline following four days of IH (six hours continuous cycles of two minutes hypoxia, $P_{ET}O_2$ = 45.0 mm Hg, followed by two minutes of normoxia, $P_{ET}O_2$ = 88.0 mm Hg), respectively (2). These results demonstrate that a relatively short bout of IH exposure was sufficient to increase [EPO] which may be important to attenuate the negative physiological effects associated with decreased O_2 availability.

However, the effects of IH on RBC volume, [Hb] and Hct (9, 10, 20) are equivocal. These inconsistent results suggest that the length and severity of the IH play key roles in the haematological response to IH. More importantly, there appears to be large inter-individual variability in the haematological response to IH and a sensitivity to the specificity of the IH protocol (8-10, 20).

IH also affects ventilatory chemosensitivity, measured as a change in the acute HVR (8, 11, 12, 14, 16, 22). Previous research has demonstrated an increase in HVR of 34 - 49% following SDIH (7, 14, 15). LDIH produces greater increases in the acute HVR with a 200% and 103% increase following 12 and 10 consecutive days of IH (two hours per day at a simulated altitude of 3,800 m

(F_IO_2 = 0.13 and one hour per day at an SaO_2 = 80%, respectively) (8, 16). However, this increase in HVR appears to be transient as it returns to baseline within five days of exposure (1, 7, 8, 14, 15).

Recently, de Bruijn and colleagues demonstrated a significant increase in serum [EPO] following a single bout of 15 maximal duration voluntary apnoeas (5). Specifically, they found an individual maximum increase in serum [EPO] of 24%, with the peak occurring three hours post-exposure. However, the increase in serum [EPO] was transient as values returned to baseline five hours post exposure. These results suggest that voluntary apnoeas act as a model of IH, but do not address the effects of repeated voluntary apnoea exposure on [EPO] or other haematological parameters associated with improved O_2 carrying capacity of the blood. Further, data do not address the effects of this type of IH exposure on ventilatory chemosensitivity as measured by the acute HVR or cardiovascular function as measured by resting MAP.

Therefore the purpose of this study was to investigate whether seven days of voluntary apnoea training would alter plasma [EPO], Hct, [Hb] and consequently VO_2max, as well as the acute HVR and resting MAP. It was hypothesized that seven days of voluntary apnoea training would increase plasma [EPO], Hct, [Hb], VO_2max, the acute HVR and resting MAP.

2.2 METHODS

2.2.1 SUBJECTS

Twelve healthy male subjects (24.6 ± 3.4 yrs; 180.4 ± 6.4 cm; 79.8 ± 9.4 kg; 24.6 ± 3.0 $kg.m^2$) volunteered for the present study. Subjects were all apparently free from cardiovascular, haematological, and respiratory diseases (e.g. heart disease, anaemia, asthma) and had no history of sleep apnoea as established by the sleep apnoea criteria from the American Academy of Sleep Medicine. Participants were all non-smokers. Subjects had no significant previous apnoea experience (e.g. synchronized swimmers or free divers) and were all Edmonton area residents who had not been exposed to significant altitude (≥ 3,000 m for more than one week) for at least six months prior to participation in the study. Competitive endurance athletes (VO_2max > 60 $ml.kg^{-1}.min^{-1}$) were excluded. This study was approved by the Physical Education and Recreation, Agricultural, Life and Environmental Sciences and Native Studies Research Ethics Board at the University of Alberta. The subjects were informed of the experimental procedures and possible risks involved in the present study, and their written informed consent was obtained.

2.2.2 EXPERIMENTAL PROTOCOL

The time course of the experimental procedures in this study is presented in Figure 1-1. Subjects reported to the lab on eight consecutive days. Prior to the first day and on day eight subjects underwent a VO_2max test. On days one through seven subjects engaged in one daily supervised apnoea training session during

which SaO_2, end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) and length of apnoea were monitored. On days one, three, and seven subjects were required to provide a pre- and three hour post-training blood sample. An additional post-apnoea blood sample was obtained on day eight. On these days subjects reported to the lab at the same time each morning to standardize the time at which blood was drawn to account for diurnal variations in [EPO] (4). Subjects were instructed to refrain from caffeine intake on the mornings of days one, three, seven and eight. During the three-hour break subjects were instructed to engage in their normal daily activities, including exercise. [EPO] was measured from all blood samples while measures of [Hb] and Hct were performed on the pre-training blood samples on days one and three and on the post-training sample on day eight. On days one and eight subjects completed a pre and post-training acute hypoxic challenge test, during which the acute HVR was measured. Resting MAP was measured prior to the start of both acute hypoxic challenge tests. The MAP response to acute hypoxia was also measured throughout the acute hypoxic challenge test.

2.2.3 APNOEA PROTOCOL

The voluntary apnoea training consisted of a total of 15 apnoeas per session. The training session was typically divided into three series of five apnoeas; however throughout data collection the grouping of the 15 apnoeas was adjusted slightly to fit the abilities of the individual subjects. Throughout each apnoea training session subjects rested in a semi-recumbent position. Individual apnoeas were separated by two minutes and subjects were given a maximum of

two five minute breaks. Subjects hyperventilated for one minute, by increasing both tidal volume and breathing frequency, prior to each apnoea, such that their $P_{ET}CO_2$ was approximately half that of rest. Throughout each apnoea SaO_2 was monitored using a digital finger pulse oximeter (Nonin Avant™ 9600; Nonin Medical Inc., Plymouth, MN, USA). Subjects were instructed to maintain the apnoea as long as possible, with the goal of reducing SaO_2 below 85%. However, for safety reasons, if SaO_2 dropped below 80% subjects were instructed to resume breathing. Throughout the apnoea training session, subjects were provided feedback on the duration of each apnoea and/or their SaO_2 values.

2.2.4 VO_2MAX MEASURE

Following 5 - 10 minutes at a self-selected intensity, subjects performed an incremental exercise test on a cycle ergometer (Ergomedic 828E, Monarch Exercise, Sweden). Subjects were instructed to maintain a pedal cadence of approximately 75 revolutions per minute (rpm) throughout the test. Resistance was initially set at 0.5 kiloponds (kp) and increased 0.5 kp per minute until volitional exhaustion. Expired gases were collected and analyzed and ventilation was measured (True Max 2400 Metabolic Measurement System; Parvo Medics, Salt Lake City, Utah, USA) and averaged every 15 s. Heart rate was measured by telemetry (Polar RS800; Kemple, Finland) and averaged every 15 s. For the purposes of the present study, VO_2max was measured as the highest 15 s valued reached on the cycle ergometer. The following criteria were used following the test to determine if VO_2max was achieved: a respiratory exchange ratio (RER) > 1.10; achieved age predicted maximal heart rate (220-age); less than 100 $mL.min^{-}$

[1] change in VO_2 (over two consecutive minutes) to indicate VO_2max and volitional fatigue as observed by the tester.

2.2.5 BLOOD MEASUREMENTS

Erythropoietin concentration was assessed from a small venous blood sample (approximately four mL) obtained from a superficial vein in the forearm via venipuncture. Blood was collected using a vacutainer lined with the anticoagulant EDTA. Within 30 minutes of collection the blood was centrifuged for 15 minutes at 1891 rpm. The plasma was then drawn off, placed in a microcentrifuge tube, and stored at ≤ -10 degrees Celsius in a non-self-defrosting freezer. Frozen plasma was subsequently analyzed by immunoassay (Quantikine In Vitro Diagnostic Human EPO Immunoassay; R&D Systems Inc., Minneapolis, MN, USA). EPO values were measured in $mIU.mL^{-1}$. The intra-assay coefficient of variation (CV) for the duplicate samples was 12.7%. Haematocrit was measured using approximately 25 µl of the venous blood previously drawn. Blood was collected in a micro-capillary plastic haematocrit tube lined with heparin (Fisherbrand Plasticrit Micro-Hematocrit Capillary Tubes, Fisher Scientific, Pittsburgh, PA, USA) and was then centrifuged for five minutes (International Micro-Capillary Centrifuge Model MB, International Equipment Company, Massachusetts, USA). An Hct scale (International Micro-Capillary Reader; International Equipment Company, Massachusetts, USA) was subsequently used to determine the total %Hct of the blood sample. The CV for the Hct measures was 1.6%. Using approximately 20 µl of the blood previously drawn [Hb] was determined using the cyanmethemoglobin assay. The absorption of the assay was

read at 540 nm using a spectrophotometer. The absorption was compared to a standard curve and values were provided in $g.100mL^{-1}$. All blood measures were completed in duplicate to ensure accuracy and account for potential inter-assay variability. Duplicate values were then averaged and the mean of the two samples was then used for all subsequent data analysis.

2.2.6 HYPOXIC VENTILATORY RESPONSE AND MEAN ARTERIAL PRESSURE

Prior to each test, the gas analyzer was calibrated using a two-point calibration. High and low values of both O_2 and CO_2 were sampled from a standard gas calibration tank to form two-point conversion. Following this, the pneumotach was calibrated using a syringe of known volume (3 L) of air. The volume of air pushed through the syringe was then integrated to produce a flow measure. During the acute hypoxic challenge test subjects breathed through a respiratory mask (Hans Rudolph R8932, Hans Rudolph Inc., Shawnee, KS, USA) that was sized to fit their face (small, medium or large) that was attached to a one-way non-rebreathing valve (Hans Rudolph R2700, Hans Rudolph Inc., Shawnee, KS, USA) and heated pneumotach (Hans Rudolph Linear Pneumotachometer R3813, Hans Rudolph Inc., Shawnee, KS, USA). Ventilatory (tidal volume, breathing frequency and V_I) and gas concentration (O_2 and CO_2) values were sampled at the mouth, analyzed (ADInstruments ML206, ADInstruments, Colorado Springs, CO, USA) and displayed in real time during the testing (PowerLab, ADInstruments, Colorado Springs, CO, USA). Inspiratory flow was measured by the pneumotach and displayed in real time throughout the test. The pneumotach was connected to a 15 L mixing chamber on the inspired side of the

apparatus to allow mixing of the inspirate. $P_{ET}CO_2$ was calculated using the peak expired CO_2 values and was displayed in real time throughout the testing. All data were recorded at 200 Hz (ADInstruments PowerLab 16/30, ADInstruments, Colorado Springs, CO, USA).

The subjects rested in a semi-supine position in a dimly lit room while breathing room air for five minutes prior to the test. Throughout the duration of the test subjects listened through earphones to pre-selected music in an attempt to minimize external stimuli that may affect V_E and to properly blind the subjects to the initiation of the test. The pre-selected music was identical for both the pre- and post- hypoxic challenge tests. Following the five-minute rest period the average $P_{ET}CO_2$ was calculated. The subjects then rested for an additional three minutes before the start of the test. The test started when 100% nitrogen (N_2) was introduced into the inspired gas mixture. F_IO_2 was manually reduced by progressively increasing the flow of 100% N_2 into the inspirate. The flow of N_2 began at 2 $L.min^{-1}$ and was increased at a rate of 1 $L.min^{-1}$ every 30 seconds. This protocol evoked a gradual drop in SaO_2 to 75% over approximately five minutes. To maintain isocapnia, $P_{ET}CO_2$ was monitored in real time throughout the hypoxic challenge test and trace amounts of CO_2 were added to the inspirate approximately 15 cm upstream of the mouth via a 23 gauge needle using a manually controlled regulator. Isocapnia was maintained within ± 1 - 2 mmHg of the averaged resting $P_{ET}CO_2$. Once SaO_2 reached 75% the hypoxic challenge test was terminated and the subjects breathed 100% O_2 and were encouraged to hyperventilate mildly to increase SaO_2 back to normal values. One data point was

exported every second from the HVR data file. V_I was subsequently plotted against SaO_2, and HVR was calculated as the slope of the regression line ($L.min^{-1}.\%^{-1}$). Figure 2-1 demonstrates how the HVR was calculated and represents a typical HVR, with the slope of the regression line representing the value of the HVR. The *x*-axis is plotted from right-to-left by convention to display a positive slope.

Beat-by-beat resting MAP was measured throughout the three minute resting period and the hypoxic challenge test non-invasively via finger pulse photoplethysmography (Finometer™; Finapres Medical Systems BV, Amsterdam, Netherlands) placed on the mid-phalanx of the middle digit of the left hand. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded allowing for the calculation of MAP in mm Hg. Resting pulse rate (PR) was recorded via finger pulse oximeter. Resting SBP, DBP, MAP and PR were averaged over the final minute of the three minute resting period. The average resting values were measured and recorded during the pre- and post-test. The change in PR, SBP, DBP and MAP relative to the change in SaO_2 throughout the acute hypoxic challenge test was measured and recorded during the pre- and post-test.

2.3 STATISTICAL ANALYSIS

The acute HVR, resting BP and PR, VO_2max and resting ventilatory data were analyzed using a separate paired t-test. All haematological data were analyzed using a one-way repeated measures ANOVA. When significant omnibus F ratios were uncovered Tukey's post-hoc analysis was used to locate significant

pair-wise differences. Significance was accepted at $P < 0.05$. Statistics were performed using the analytical software SigmaPlot (SigmaPlot Version 11.0, Systat Software Inc., Germany). A trend was defined as a statistical analysis with a P value between 0.06 and 0.10. All values are reported as mean ± standard error of the mean. Two out of 84 blood samples were not included in the statistical analysis of [EPO]. Two subjects had incomplete haematological testing (day one post-training for both subjects). Only the final nine subjects were included in the Hb analysis, as there were technical difficulties receiving the proper chemicals to perform the Hb assay.

2.4 RESULTS

2.4.1 HYPOXIC STIMULUS

Figure 2-2 depicts the typical SaO_2, $V_{I,}$ $P_{ET}CO_2$ and PR response during a sample apnoea training session for an individual subject. Voluntary apnoea duration was 139 ± 40 s (mean ± S.E) (range of a single apnoea was 15 s to 4 min 9 s) and total apnoea training time per day was 74.2 ± 9.6 min (range 57.6 min to 88.4 min). Pre-training resting SaO_2 was 95.9 ± 1.0% and SaO_2 was not altered by apnoea training (post-training SaO_2 = 96.1 ± 1.1%). Subjects achieved an SaO_2 below 85% on 998 of 1,260 apnoeas with the mean nadir of SaO_2 across all apnoeas being 79.9 ± 4.8%. On average subjects spent 7.8 ± 4.2 min per day of training below 85% SaO_2 (range 2.2 min to 12.1 min) and a total of 55.1 ± 1.1 min below 85% SaO_2 over the course of the seven day protocol.

2.4.2 ERYTHROPOIETIN

There was no significant difference ($P > 0.05$) in plasma [EPO] across all time points. Figure 2-3 illustrates the mean plasma [EPO] across all seven blood measures. Although there was no significant increase, there was a trend ($P = 0.095$) for plasma [EPO] to increase. Specifically, plasma [EPO] increased by 17.2%, 9.8%, 21.8%, 20.1% and 24.7% from baseline at day one post-training, day three pre-training, day three post-training, day seven pre-training and day seven post-training, respectively. Figure 2-4 illustrates the individual EPO response for all subjects. Baseline [EPO] ranged from 4.8 to 11.8 $mIU.mL^{-1}$. On day one post-training, day three pre- and post-training, day seven pre- and post-training and on day eight plasma [EPO] values ranged from 3.5 to 11.9 $mIU.mL^{-1}$; 2.5 to 19.7 $mIU.mL^{-1}$; 3.3 to 13.5 $mIU.mL^{-1}$; 5.2 to 13.1 $mIU.mL^{-1}$; 3.4 to 14.9 $mIU.mL^{-1}$ and 4.0 to 10.0 $mIU.mL^{-1}$, respectively.

2.4.3 HAEMOGLOBIN

[Hb] was not significantly different ($P > 0.05$) on days one and three post-training and on day eight (Figure 2-5). Figure 2-6 represents the individual [Hb] responses to voluntary apnoea training across the three blood samples. On day one, three and eight [Hb] values ranged from 11.8 to 19.7 $g.100mL^{-1}$; 11.0 to 17.6 $g.100mL^{-1}$ and 10.9 to 21.0 $g.100mL^{-1}$, respectively. All [Hb] were within the normal range (approximately 15.0 $g.100mL^{-1}$) across all time points.

2.4.4 HAEMATOCRIT

Hct was not significantly different ($P > 0.05$) on days one and three post-training and on day eight (Figure 2-7). Figure 2-8 represents the individual responses in Hct across all three time points. On day one, three and eight Hct ranged from 41.0 to 50.5%; 41.0 to 51.0% and 40.0 to 52.5%, respectively.

2.4.5 VO_2MAX

Relative VO_2max was not significantly different ($P > 0.05$) from pre- to post-training (Figure 2-9). Figure 2-10 depicts the individual relative VO_2max values for all subjects from pre- to post-training. Pre-training relative VO_2max values ranged from 35.4 to 61.9 $mL.kg^{-1}.min^{-1}$. Post-training relative VO_2max values ranged from 37.7 to 58.8 $mL.kg^{-1}.min^{-1}$. There was also no significant change in absolute VO_2max values from pre- to post-training ($P > 0.05$).

2.4.6 HYPOXIC VENTILATORY RESPONSE

Figure 2-11 depicts an acute hypoxic challenge test in a representative subject. Throughout the test $P_{ET}CO_2$ was maintained constant and did not differ from resting values. Resting $P_{ET}CO_2$ decreased from pre- to post- training ($P < 0.05$). Mean resting $P_{ET}CO_2$ pre-training was 43.0 ± 2.0 mm Hg. This value significantly decreased post-training to 42.0 ± 1.6 mm Hg. There was no significant difference in resting V_I from pre- to post-training ($P > 0.05$). Mean pre- and post- training resting V_I were 9.7 ± 1.3 $L.min^{-1}$ and 10.2 ± 2.5 $L.min^{-1}$, respectively. HVR was not significantly different ($P > 0.05$) from pre- to post-

training (Figure 2-12). Figure 2-13 depicts the individual HVR responses to seven days of voluntary apnoea training. On day one and eight HVR ranged from 0.1 to 1.0 $L.min^{-1}.\%^{-1}$ and 0.2 $L.min^{-1}.\%^{-1}$ to 1.2 $L.min^{-1}.\%^{-1}$, respectively. Inter- and intra-individual variation was present in these values, but there was no obvious trend in HVR. Four subjects were brought back to re-test HVR on two separate occasions. The calculated mean CV for HVR for these subjects was 13.9%. Test-retest correlations were calculated for four subjects between three different days (Day 1, Day 2 and Pre-test). The test-retest correlations for day 1 to 2, day 1 to pre-test and day 2 to pre-test were $r = 0.94$, $r = 0.96$ and $r = 0.85$, respectively. All three test-retest correlations for the measure of HVR were strong and positive.

2.4.7 RESTING MEAN ARTERIAL PRESSURE AND PULSE RATE

Resting PR was not significantly different from pre- to post-training ($P > 0.05$). Mean resting PR pre-training was 65.2 ± 11.6 $beats.min^{-1}$. Mean resting PR was similar post-training at 64.1 ± 9.7 $beats.min^{-1}$. Resting MAP was not significantly different ($P > 0.05$) from pre- to post-training (Figure 2-14). Individual resting MAP values from pre- to post- training are presented in Figure 2-15. Resting MAP values ranged from 68.8 to 106.8 mm Hg and 60.9 to 100.8 mm Hg on days one and eight, respectively. There was no significant difference in resting SBP from pre- to post-training ($P > 0.05$). Mean resting SBP was 127.4 ± 12.7 mm Hg at pre-training and 124.3 ± 13.9 mm Hg at post-training. Resting SBP ranged from 110.4 to 145.6 mm Hg and 107.6 to 149.2 mm Hg at pre- and post-training, respectively. There was also no significant difference in resting

DBP from pre- to post-training ($P > 0.05$). Mean resting DBP was 64.8 ± 10.4 mm Hg at pre-training and 61.3 ± 12.5 mm Hg at post-training. Resting DBP ranged from 48.0 to 87.5 mm Hg and 37.5 to 77.6 mm Hg at pre- and post-training, respectively. The mean SBP, DBP and MAP throughout the pre- (HVR 1) and post-training (HVR 2) HVRs are presented in Figure 2-16. The mean change in SBP relative to the mean change in SaO_2 ($\Delta SBP/\Delta SaO_2$) was significantly reduced from pre- to post-training ($P < 0.05$). $\Delta SBP/\Delta SaO_2$ was 1.0 mm $Hg.\%^{-1}$ at pre-training and 0.6 mm $Hg.\%^{-1}$ at post-training. The mean changes in DBP, MAP and PR relative to the change in SaO_2 ($\Delta DBP/\Delta SaO_2$, $\Delta MAP/\Delta SaO_2$ and $\Delta PR/\Delta SaO_2$, respectively) were not significantly different during the first and second HVR ($P > 0.05$, respectively). $\Delta DBP/\Delta SaO_2$ was 0.3 mm $Hg.\%^{-1}$ and 0.2 mm $Hg.\%^{-1}$ during the first and second HVR, respectively. $\Delta MAP/\Delta SaO_2$ was 0.5 mm $Hg.\%^{-1}$ and decreased slightly, but not significantly to, 0.3 mm $Hg.\%^{-1}$ from pre- to post-training, respectively. Similarly, the $\Delta PR/\Delta SaO_2$ was 1.2 $beats.min^{-1}.\%^{-1}$ at pre-training and was similar at post-training at 1.0 $beat.min^{-1}.\%^{-1}$.

2.5 DISCUSSION

2.5.1 APNOEAS AND HAEMATOLOGICAL PARAMETERS

The purpose of the present study was to investigate whether seven days of voluntary apnoea training would alter haematological parameters associated with the O_2 carrying capacity of the blood, namely, [EPO], [Hb] and Hct and thus result in a change in VO_2max, and whether this type of hypoxic exposure would alter ventilatory chemosensitivity and resting cardiovascular parameters as

evidenced by a change in the acute HVR and resting MAP, respectively. There are two main findings to the present study. First, there was no significant increase in mean [EPO], [Hb] and Hct across all time points and no change in VO_2max. Second, there was no significant change in the acute HVR or resting MAP. Together these results suggest that seven days of voluntary apnoea training did not alter the O_2 carrying capacity of the blood, ventilatory chemosensitivity or VO_2max.

Previous research involving the effects of IH on [EPO] have produced equivocal results (2, 8, 9). EPO is a glycoprotein hormone that is produced primarily in the adult kidney in response to reduced blood O_2 availability. It operates on the bone marrow to regulate feedback changes in erythropoiesis (18). Indeed, EPO is considered the principle regulator of RBC production in healthy adults (19, 24). Given that increases in circulating [EPO] initiate changes in other haematological parameters (e.g. Hct, [Hb], reticulocyte count and RBC mass), stimulating and maintaining an elevated [EPO] may be the key determinant to increasing the O_2 carrying capacity of the blood. However, the optimal IH protocol for stimulating [EPO] has yet to be established. Most recently, Brugniaux and colleagues demonstrated that relatively short duration IH (four days, six hours per day cycling between two minutes of hypoxia ($P_{ET}O_2$ = 45 mm Hg) and two minutes of normoxia ($P_{ET}O_2$ = 88 mm Hg) was sufficient to significantly increase serum [EPO] (2). Following this protocol, significant increases of 50% and 36% from baseline were found on day two and four, respectively. These results suggest that as little as six hours of SDIH over two days was a sufficient hypoxic stimulus

to elicit adaptations in [EPO]. Similarly, Gore and colleagues exposed subjects to three hours of hypoxia (simulated altitude between 4,000 m and 5,500 m), five days a week for four weeks and found a significant increase in [EPO] of 136% and 103% three hours post-exposure at week two and four, respectively (9). In contrast, Garcia and colleagues demonstrated that exposure to IH for two hours per day for 12 consecutive days at a simulated altitude of 3,800 m ($F_IO_2 = 0.13$) was not sufficient to elicit a significant increase in plasma [EPO] (8). However, these results must be interpreted with caution as plasma [EPO] was sampled immediately following the IH protocol while previous research has determined that [EPO] typically reaches peak values three hours following both acute hypoxia and IH (5, 9).

Recently, de Bruijn and colleagues demonstrated that one bout of 15 maximal duration voluntary apnoeas was a sufficient IH stimulus to induce significant increases in [EPO] (5). Specifically, they showed an average individual maximum increase in serum [EPO] of 24% following voluntary apnoea exposure. However, the results of the present study are not in agreement with those previously published, as there was no significant increase in plasma [EPO] across all time points. Interestingly, three hours following the first apnoea training session plasma [EPO] in the present study had increased by 17.2% from baseline compared to 16% found by de Bruijn and colleagues. Despite a 1.2% greater increase in plasma [EPO] in the present study than that found previously, the lack of significant change may be explained by large inter-individual variability in baseline plasma [EPO]. In comparison to the results previously published,

subjects in the present study spent less time below 85% (average seven minutes and 48 seconds vs.12 minutes and 25 seconds in the present study compared to those previously published, respectively). In addition, the average apnoeic duration in the present study was 139 seconds compared to 206 seconds previously reported. The average SaO_2 nadir in the present study was approximately 7% higher than that previously reported (79.9% vs. 72.7%). The discrepancy between apnoeic duration, average SaO_2 nadir and time spent below 85% SaO_2, was likely the result of a difference in the SaO_2 safety cut-off used. In the present study subjects were instructed to resume breathing once SaO_2 reached 80%, which was 20% higher than that previously reported by de Bruijn and colleagues. Consequently, the results of the present study when compared with those of laboratory controlled and previous voluntary apnoea IH studies, suggest that the hypoxic stimulus may not have been severe enough to induce an increase in plasma [EPO]. When combined, the results of the present study and those previously reported, on both laboratory controlled IH and voluntary apnoea exposure, suggest a large inter-individual variability in the [EPO] response to IH. It appears that the IH stimulus must either be longer in duration and/or higher in intensity in order to elicit significant increases in [EPO]. Additionally, these results suggest that [EPO] sampling is time sensitive and as a result significant changes in the present study may have gone undetected.

Additional haematological parameters associated with the O_2 carrying capacity of the blood have been shown to be effected by IH; however similar to [EPO] the results are equivocal (9, 20). Rodriquez and colleagues exposed

subjects to 90 minutes of hypoxia at simulated altitudes between 4,000 – 5,500 m (greater than the present study) three times per week for four weeks and found significant increases in [Hb] and Hct (20). Specifically, significant increases in Hct were observed at the end of the second week (42.5%) and reached peak values at the end of the third week (44.8%). Similarly, [Hb] increased by 4.9% following the first week of exposure (from 14.3 to 15.0 $g.dl^{-1}$ at baseline and end of week one, respectively). By the end of the third week [Hb] had further increased by 8% to 16.2 $g.dl^{-1}$ and was maintained at similar values for the subsequent two weeks. Contrary to these results Gore and colleagues found no significant increase in [Hb] or Hct following four weeks of IH at simulated altitudes between 4,000 – 5,500 m, three hours per day, five days per week (9). The overall mean change in [Hb] was 1%. Across the IH protocol mean [Hb] values ranged from 14.3 to 14.7 $g.dl^{-1}$ across all four weeks of exposure. Similarly, there was no significant change in Hct throughout the duration of the protocol, as mean Hct ranged from 43.1% to 44.1% across all four weeks of exposure. The effects of repeated exposure to voluntary apnoeas on both [Hb] and Hct have not previously been studied. However, similar to the results produced by Gore and colleagues there was no significant change in either [Hb] or Hct throughout the duration of the present study. Given that [EPO] plays an important role in stimulating increases in other haematological variables associated with the O_2 carrying capacity of the blood, it stands to reason that a lack of significant increase in plasma [EPO] would correspond with no change in Hct or [Hb]. The confounding results demonstrated by previous research on the effects of IH

exposure on Hct and [Hb] suggest a large inter-individual variability in the haematological response to IH and a sensitivity to the specificity of the IH protocol. More importantly, when combined, the results of the present study and of previous research demonstrate the need to determine the relationship between the nature of the IH protocol (duration and intensity) and the degree of haematological change.

2.5.2 APNOEAS AND VO_2MAX

Given that the present study did not expose subjects to an exercise training stimulus, any increase in VO_2max would be the result of an increase in the O_2 being transported to working muscles. However, the present study did not elicit any such increases in haematological variables, indicating that there was no physiological rationale for an increase in VO_2max. Consistent with this notion, there was no significant increase in either mean absolute or relative VO_2max in the present study. Specifically, mean absolute VO_2max was 3.8 $L.min^{-1}$ at pre-training and 3.8 $L.min^{-1}$ at post-training while mean relative VO_2max was 48.4 $mL.kg^{-1}.min^{-1}$ and 48.5 $mL.kg^{-1}.min^{-1}$ at pre- and post-training, respectively. These results are similar to those presented by Rodriguez and colleagues who demonstrated no significant difference in VO_2max (53.8 $mL.kg^{-1}.min^{-1}$ vs. 54.2 $mL.kg^{-1}.min^{-1}$) following three weeks of IH, three times per week for 90 minutes at simulated altitudes between 4,000 – 5,500 m (20). Contrary to these results, Brugniaux and colleagues demonstrated a significant increase in both absolute and relative VO_2max scores following 18 days of hypoxic exposure for 14 hours per day (six nights at 2,500 m and 12 nights at 3,000 m) (3). In addition to this

hypoxic exposure, subjects completed on average, 68 minutes of training each day (54%, 27%, 9% and 7% of training time spent below 75%, between 75 – 85%, between 85 – 87% and between 87 – 100% of maximal HR, respectively). VO_2max was measured at 1,200 m above sea level and increased significantly from 63.3 to 69.4 $ml.kg^{-1}.min^{-1}$. These results suggest that higher intensity and longer duration IH exposure would be needed to elicit any significant increases in VO_2max. Moreover, the results from Brugniaux and colleagues suggest that in order to be effective, IH exposure, either laboratory controlled or voluntary apnoea exposure may need to be combined with a regular exercise regime in order to elicit a significant change in VO_2max.

2.5.3 APNOEAS AND VENTILATORY CHEMOSENSITIVITY

Unlike the confounding results previously reported regarding the effects of IH on various haematological parameters, the effects of IH on ventilatory chemosensitivity appear to be uniform based on previous research (8, 11, 12, 14-17). However, the results of the present study are not in agreement with this latter research. Previous research has demonstrated that IH protocols differing in length and severity produce different increases in the acute HVR (8, 11, 12, 14-17). Katayama and colleagues exposed subjects to an IH protocol similar in duration (seven days) to the present study. Specifically, subjects were exposed to one hour per day of IH at an average F_IO_2 of 12.3% (11). Following this protocol, HVR increased significantly and remained elevated one week after the cessation of IH exposure. In a similar study, HVR was significantly increased following exposure to IH for three hours per day, for one week, with an F_IO_2 of 12.3% (12). Koehle

and colleagues also demonstrated a significant increase of approximately 49% (0.47 to 0.79 $L.min^{-1}.\%^{-1}$) in HVR following seven consecutive days of 115 minutes (12 cycles of five minutes hypoxia with an F_IO_2 = 12% and five minutes normoxia) of IH (15). Using the same protocol (12 cycles of five minutes hypoxia at an F_IO_2 = 12% followed by five minutes normoxia for seven days), Koehle and colleagues demonstrated a significant increase in HVR of 65%, which remained significantly elevated for one week following the cessation of the IH protocol (14). Other studies examining the effects of IH on ventilatory chemosensitivity have produced greater increases in the acute HVR. Following 12 consecutive days of IH (two hours per day at an F_IO_2 = 12.3%), HVR significantly increased, reaching peak values on day five at 200% from baseline (8). Similarly, Lusina and colleagues demonstrated a 103% increase in HVR following 10 days of IH for one hour per day at an SaO_2 = 80% (16). More recently, Pialoux and colleagues demonstrated that relatively short duration IH exposure is sufficient to induce a significant increase in the acute HVR (17). Subjects were exposed to four days of IH consisting of six hours per day (cycling between two minutes of hypoxia, $P_{ET}O_2$ = 45 mm Hg and two minutes of normoxia, $P_{ET}O_2$ = 88 mm Hg). Following this protocol, HVR significantly increased progressively on days one and two but reached peak values on day four at 83% greater than baseline values. However, similar to previous studies the increases in HVR were transient, as they had returned to baseline values following four days of recovery (day eight) (17).

Contrary to results previously published, one week of voluntary apnoea training in the present study did not elicit a significant increase in the acute HVR.

Potential reasons for the lack of increase in the acute HVR in the present study are two fold. First, it is possible that the length of voluntary apnoea training was not adequate to elicit a significant change in the acute HVR. Second and more importantly, the IH stimulus produced by voluntary apnoea training appears to not have been severe enough, compared to previous IH protocols, to elicit a change in the acute HVR. In comparison to the results produced by Pialoux and colleagues, the results of the present study may be different due to the time course for measuring the acute HVR. In the present study, the acute HVR was measured pre- and post-training and no IH stimulus was given prior to each test. In contrast, Pialoux and colleagues measured the acute HVR within 30 minutes after the end of the exposure to IH. As a result, it is possible that the measurement of the acute HVR was affected by the same day exposure to IH, which did not occur in the present study. Previous research examining the effects of laboratory controlled IH on the acute HVR have exposed subjects to different IH protocols varying in length and duration. These IH protocols have varied in exposure length from four days to two weeks and exposure duration from one hour to three hours (8, 11, 12, 14-17). Similar to previous research, the present study exposed subjects to seven days of IH for an average of 74.2 minutes per day. However, the severity of the IH protocol in the present study was vastly different than seen previously in laboratory controlled IH protocols. Subjects in the present study only experienced hypoxia ($SaO_2 < 85\%$) for an average of 7.8 minutes per day for seven days (approximately 55 minutes in total) compared to between one and three hours per day in previous laboratory controlled IH studies. As a result, it appears that

changes in ventilatory chemosensitivity following IH exposure, whether laboratory controlled or in the form of apnoea training, are dependent upon the duration of the training protocol, the length of the exposure time and the severity of the IH stimulus. Interestingly, it appears that the changes in ventilatory chemosensitivity associated with IH are dependent on the severity and/or duration of the IH protocol rather than the amount of time spent cycling between hypoxia and normoxia.

2.5.4 APNOEAS AND BLOOD PRESSURE

Exposure to IH elicits changes in cardiovascular variables caused by a chemoreflex-mediated increase in sympathetic nerve activity (21). The increase in sympathetic nerve activity results in an increase in systemic arterial pressure and contributes to a change in HR, which is dependent upon the degree of activation of the chemoreflex (21). However, the results from previous research are confounding as to the magnitude of the change in resting MAP, SBP, DBP and HR following exposure to IH (7, 13, 16). Foster and colleagues exposed subjects to 10 days of SDIH consisting of alternating cycles of five minutes hypoxia (F_IO_2 = 12%) followed by five minutes of normoxia for 60 minutes per day and found no significant change in any resting cardiovascular variables throughout or following the training period (7). Lusina and colleagues exposed subjects to a similar protocol (10 days, 60 minutes per day, SaO_2 = 80%) and found no significant change in resting cardiovascular variables (16). Similar results were reported by Katayama and colleagues, who demonstrated no significant change in resting SBP, DBP or HR following seven days of IH for 60 minutes per day at a

simulated altitude of 4,500 m (13). These results are similar to the results in the present study as there was no significant change in resting SBP, DBP, MAP or PR following seven days of voluntary apnoea training.

However, in contrast to the present study and others previously described, exposure to IH has also been shown to significantly alter resting cardiovascular variables (6, 17). Foster and colleagues exposed subjects to six hours of continuous cycles of two minutes hypoxia ($P_{ET}O_2$ = 45 mm Hg) followed by two minutes of normoxia ($P_{ET}O_2$ = 88 mm Hg) for four consecutive days and found a significant increase in resting DBP and MAP; however the increases were transient as values had returned to normal by day eight (6). In addition, this IH protocol tended to increase resting SBP; however not significantly. As previously described, Pialoux and colleagues exposed subjects to a relatively SDIH stimulus (four days, six hours per day, two minutes hypoxia $P_{ET}O_2$ = 45 mm Hg and two minutes of normoxia, $P_{ET}O_2$ = 88 mm Hg) and found a significant increase in resting MAP (17). Resting MAP increased after exposure to this protocol of IH from 79.5 mm Hg at baseline to 80.6 mm Hg on day one, 82.6 mm Hg on day two and 83.4 mm Hg on day four (17).

The discrepancy between the findings of the present study and those presented by Pialoux and colleagues may be the result of differences in the protocol of measurement rather than differences in the IH protocol. Specifically, in the present study resting MAP was measured on day one prior to the subject being exposed to any IH stimulus and on day eight, when no IH stimulus was given. As a result, in the present study, resting MAP was never measured

following IH exposure. This is contrary to the protocol performed by Pialoux and colleagues where resting MAP was taken within 30 minutes after the end of exposure to the IH protocol. Therefore, it is possible that same day exposure to IH may have influenced the measurement of resting MAP, which may not have been accounted of for in the results and which was not present in the current study. In addition, the method of assessing resting MAP between the two studies is vastly different. In the present study, resting MAP was measured beat-by-beat via finger pulse photoplethysmography. In contrast, Pialoux and colleagues assessed resting MAP as the mean of three values taken every three minutes from a sphygmomanometer. Consequently, it is possible that the values in the present study contain greater accuracy as they provided a continuous recording of resting MAP. The lack of change in resting BP and PR in the present study suggests that, baseline autonomic control of the heart and peripheral vasculature does not appear to be affected by seven days of voluntary apnoea training. In addition, the results demonstrate that subjects in the present study were in a comparable basal state prior to the commencement of both acute HVR tests.

In addition to examining changes in resting cardiovascular variables, previous research has also examined the effects of IH on cardiovascular sensitivity to hypoxia. More specifically, previous research has examined the change in various cardiovascular variables, including SBP, DBP and HR, relative to the change in SaO_2 throughout the acute HVR test. Foster and colleagues examined the change in cardiovascular sensitivity to hypoxia following exposure to 10 episodes of IH, consisting of continuous cycles of five minutes normoxia

followed by five minutes of hypoxia (F_IO_2 = 12%) for 60 minutes (7). Following this protocol, it was found that, the $\Delta SBP/\Delta SaO_2$ and the $\Delta DBP/\Delta SaO_2$ appeared to increase, however the increase was not statistically significant, while the $\Delta HR/\Delta SaO_2$ did not change. In comparison, Katayama and colleagues demonstrated a significant increase of 48% and 127% in the $\Delta SBP/\Delta SaO_2$ and $\Delta DBP/\Delta SaO_2$ relationships, respectively, but no change in the $\Delta HR/\Delta SaO_2$ relationship, following seven days of exposure to IH (60 minutes per day at a simulated altitude of 4,500 m) (13). These results suggest that short term IH leads to an increase in the BP response to hypoxic stimuli in humans.

However, in contrast to previous results, the results from the present study indicate that the $\Delta SBP/\Delta SaO_2$ significantly decreased from pre- to post-training from 1.0 to 0.6 mm Hg.$\%^{-1}$, respectively. In addition, the $\Delta DBP/\Delta SaO_2$ and the $\Delta PR/\Delta SaO_2$ tended to decrease following seven days of voluntary apnoea training; however, the decrease was not significant. Therefore, it appears that the IH stimulus induced by one week of voluntary apnoea training in the present study, tended to induce the opposite BP adaptations to acute hypoxia as those previously found following other laboratory controlled IH protocols; however further research is warranted. As such, these results would indicate that, seven days of voluntary apnoea training in the present study did not alter cardiovascular sensitivity to acute hypoxia. It is important to note that, Foster and colleagues reported that the trend of the $\Delta SBP/\Delta SaO_2$ and $\Delta DBP/\Delta SaO_2$ to increase following exposure to IH was not statistically significant due to large inter-individual variability in the blood pressure response to acute hypoxia which is

comparable to the results of the present study, as there was large inter-individual variation in the blood pressure response to acute hypoxia during the hypoxic challenge test. Although the $\Delta SBP/\Delta SaO_2$ was found to significantly decrease following voluntary apnoea training, the results are lacking in power demonstrating the need to include a greater amount of subjects in subsequent research.

2.6 CONCLUSION

In summary, the purpose of the present study was to investigate whether seven days of voluntary apnoea training would elicit increases in haematological variables associated with the O_2 carrying capacity of the blood, VO_2max, ventilatory chemosensitivity and resting BP. The results of the present study demonstrate that the IH stimulus produced by this voluntary apnoea training protocol was not sufficient to elicit significant changes in the haematological variables associated with the O_2 carrying capacity of the blood or VO_2max. It was also not sufficient to elicit a change in ventilatory chemosensitivity and resting cardiovascular variables as evidenced by no significant change in the acute HVR and resting MAP, SBP, DBP and PR, respectively. Therefore, it appears that voluntary apnoea training may not provide the same haematological and performance benefits as those commonly observed with both ascent to altitude and other laboratory controlled IH exposures (e.g. use of a hypoxic chamber). These results also suggest that voluntary apnoea training may not aid in the process of ventilatory acclimatization to altitude as was originally hypothesized. Ultimately, it appears that to achieve haematological and ventilatory benefits

associated with improved performance and an improved capacity to withstand hypoxia, respectively, other models of IH should be used rather than seven days of voluntary apnoea training.

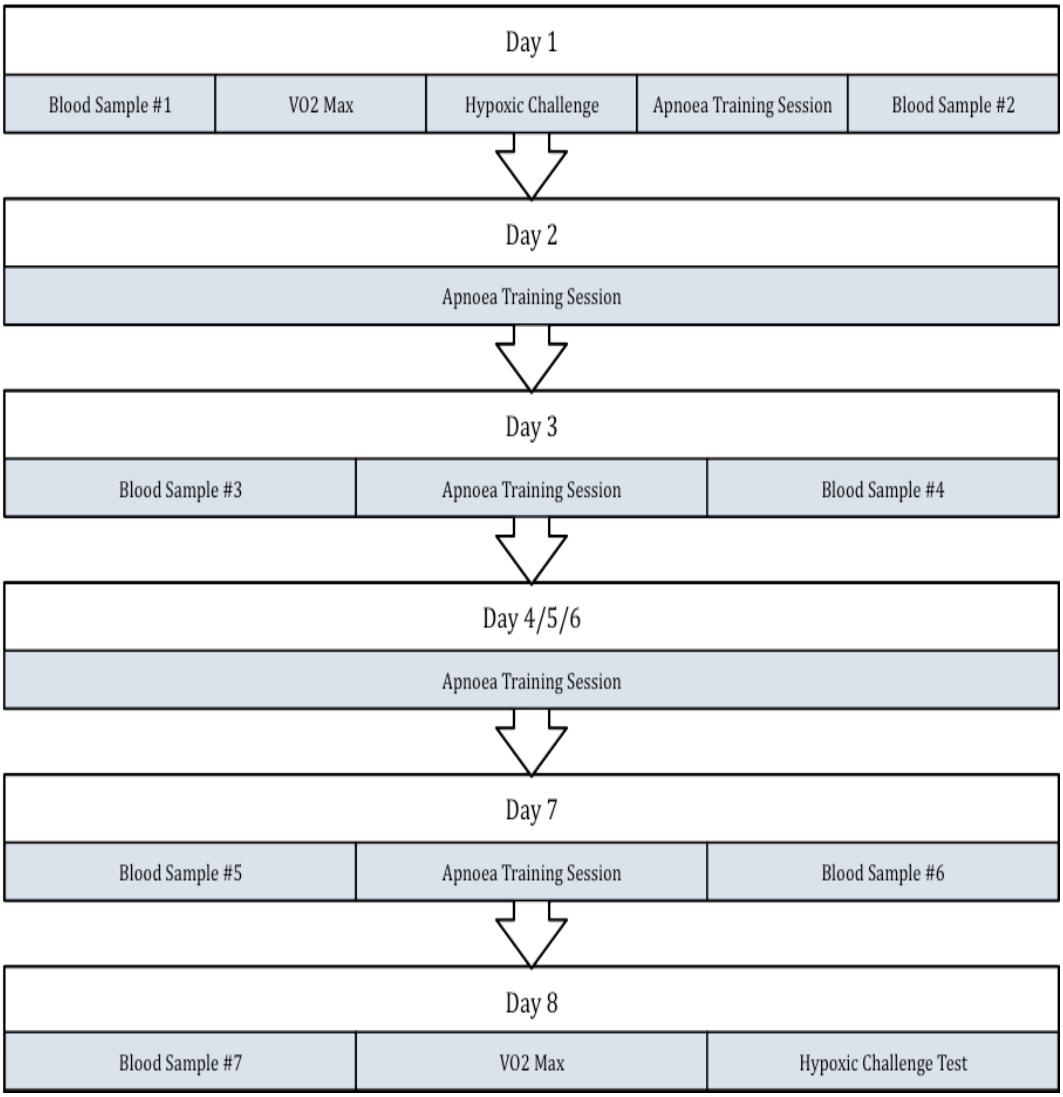

Figure 1-1: Experimental Protocol.

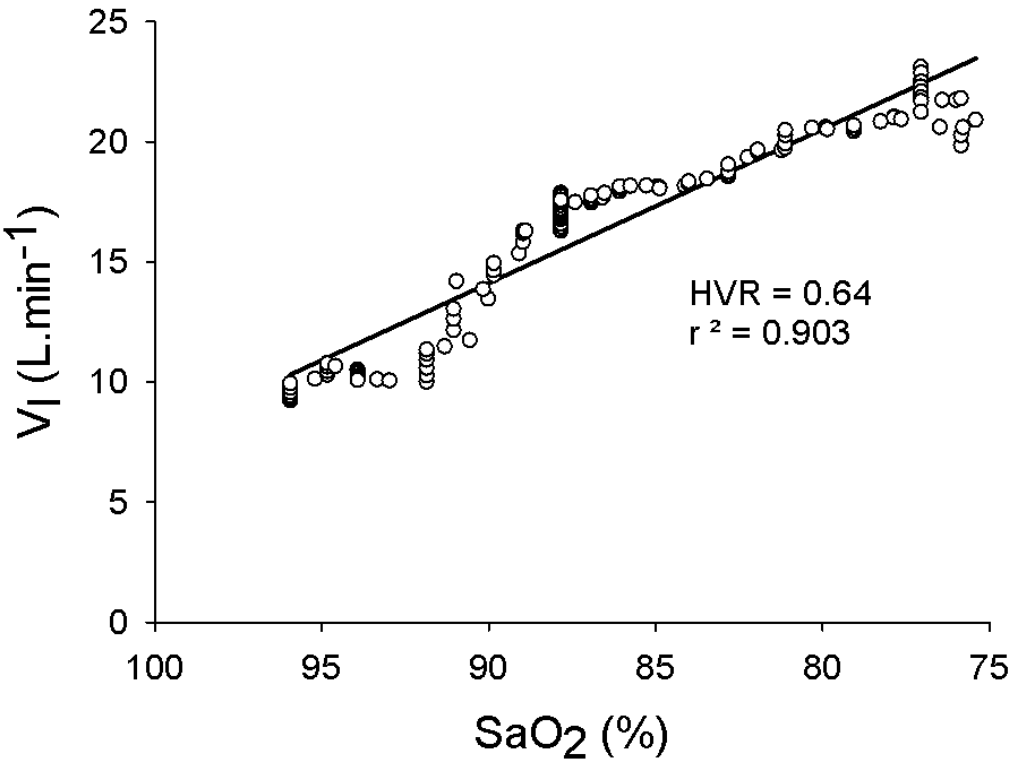

Figure 2-1: Hypoxic ventilatory response plot for one subject. The *x*-axis is plotted from right-to-left by convention to display a positive slope. V_I, inspired ventilation; SaO_2, arterial oxygen saturation

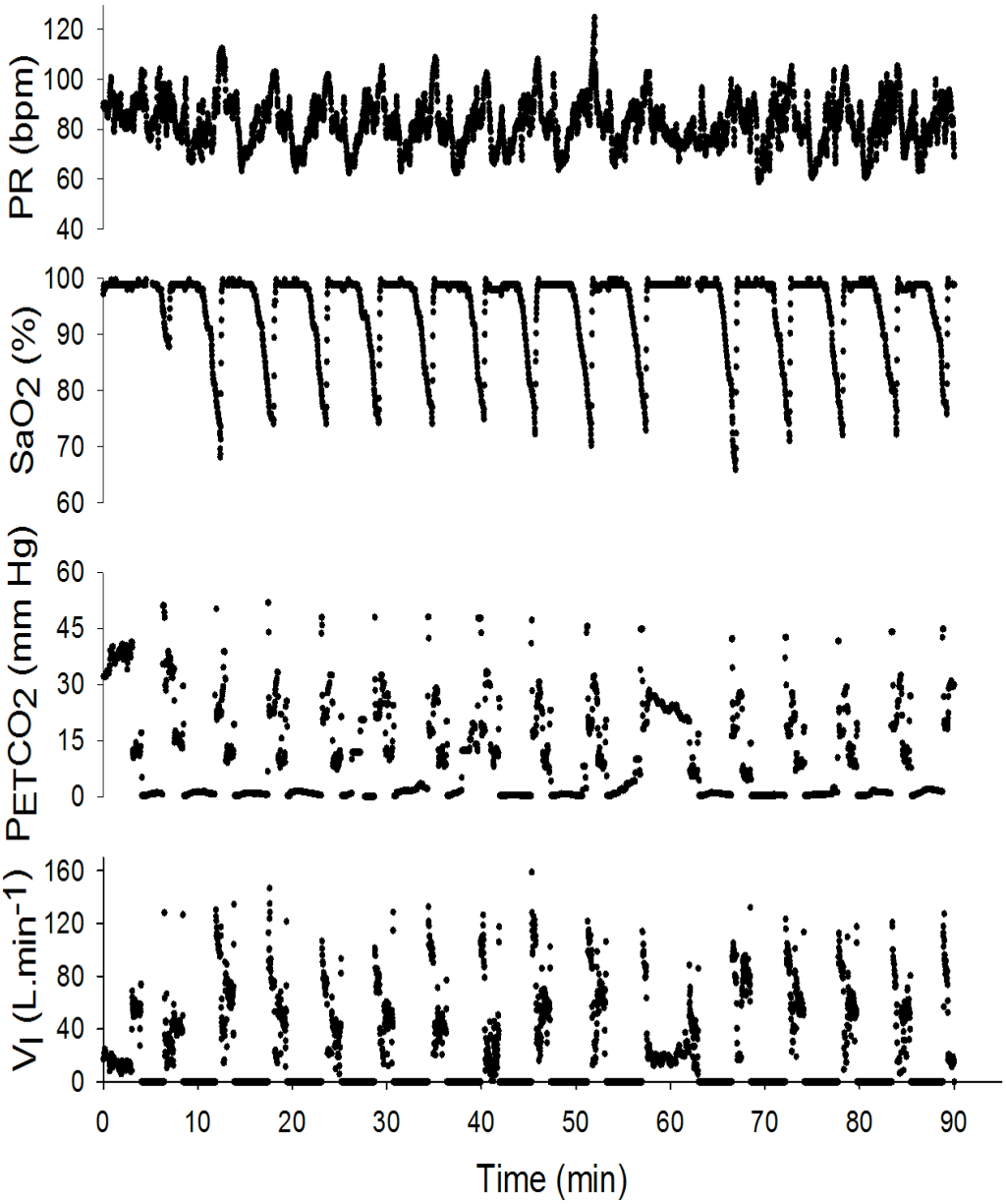

Figure 2-2: Sample data from a single representative complete apnoea training session for one subject. V_I, inspired ventilation; $P_{ET}CO_2$, end-tidal partial pressure of CO_2; SaO_2, arterial oxygen saturation; PR, pulse rate.

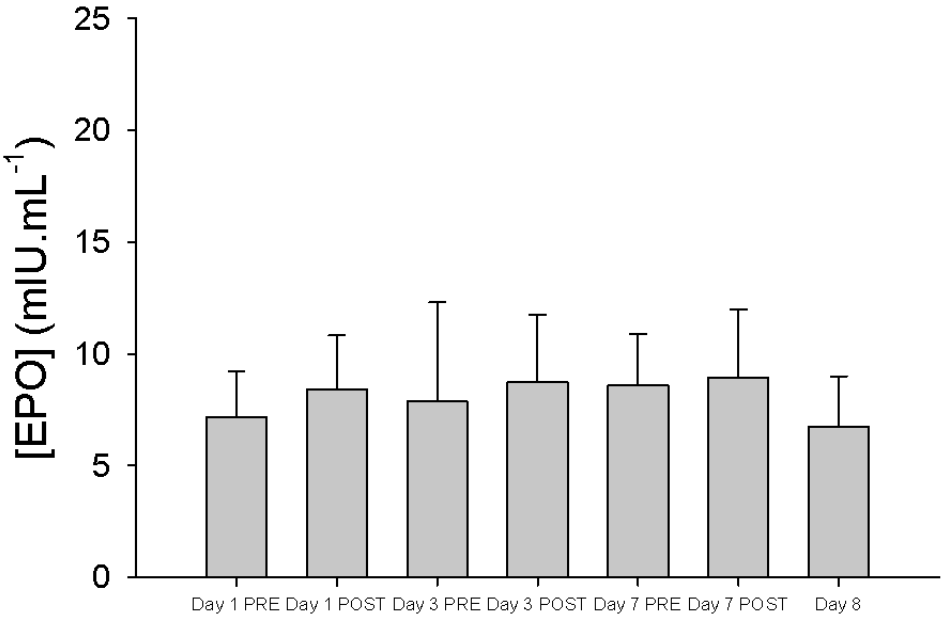

Figure 2-3: Mean plasma [EPO] during seven days of voluntary apnoea training. On Day 1 POST, Day 3 POST and Day 7 POST blood sampling was completed 3 hours after apnoea training. Day 1 POST value represents n = 10.

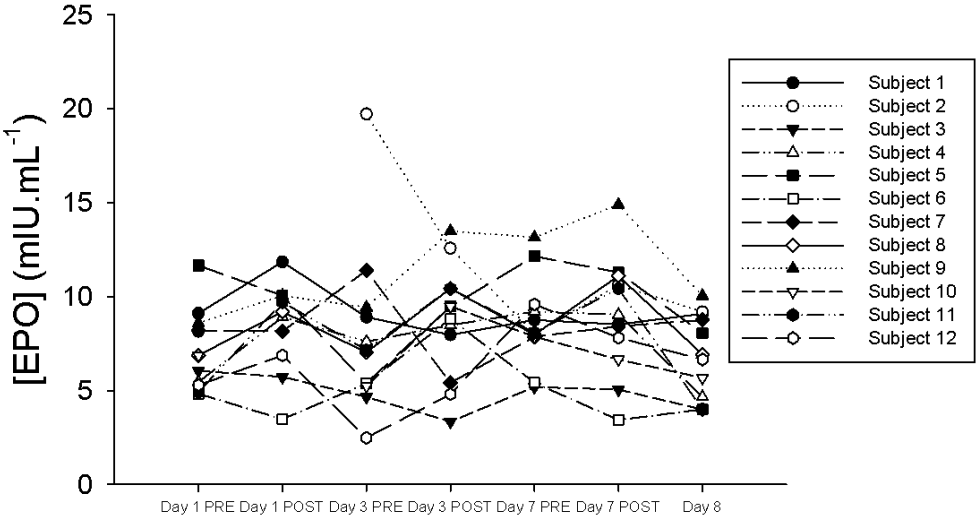

Figure 2-4: Individual plasma [EPO] during seven days of voluntary apnoea training. On Day 1 POST, Day 3 POST and Day 7 POST blood sampling was completed 3 hours after apnoea training. Missing values for subject 2 and subject 10 for day 1 POST sample.

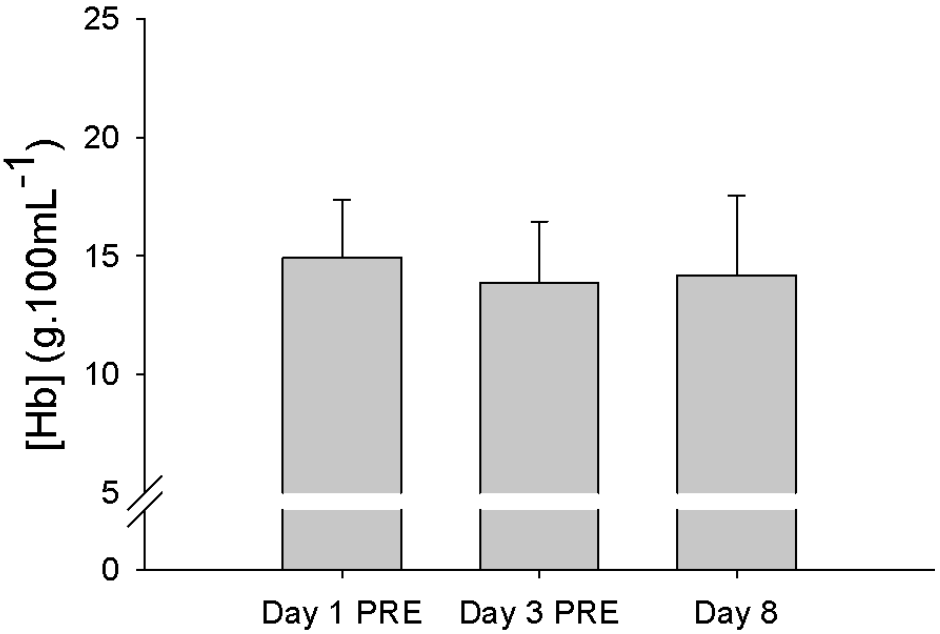

Figure 2-5: Mean [Hb] during seven days of voluntary apnoea training. Mean [Hb] represents n = 9.

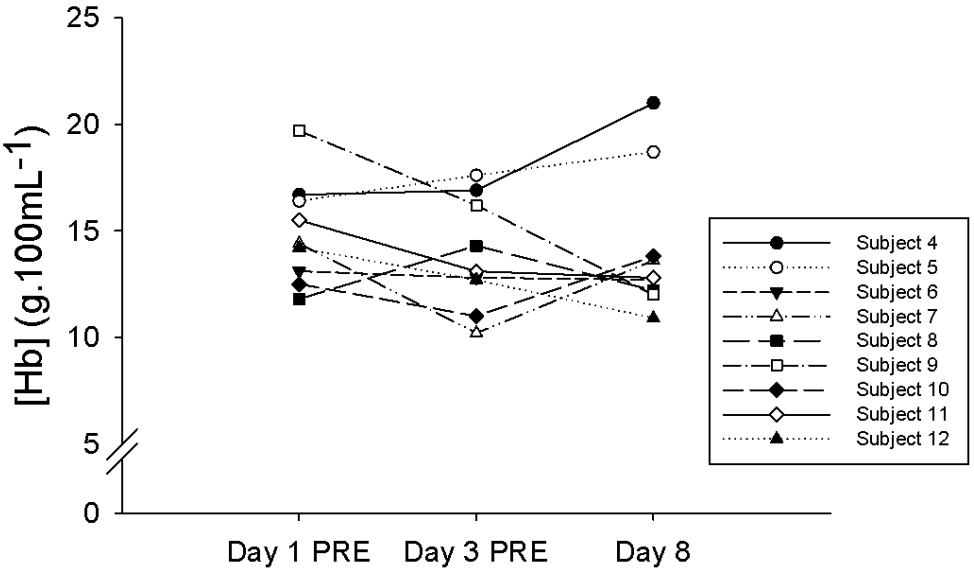

Figure 2-6: Individual [Hb] during seven days of voluntary apnoea training. Subjects 1 through 3 were omitted as not data were available for the [Hb] measure.

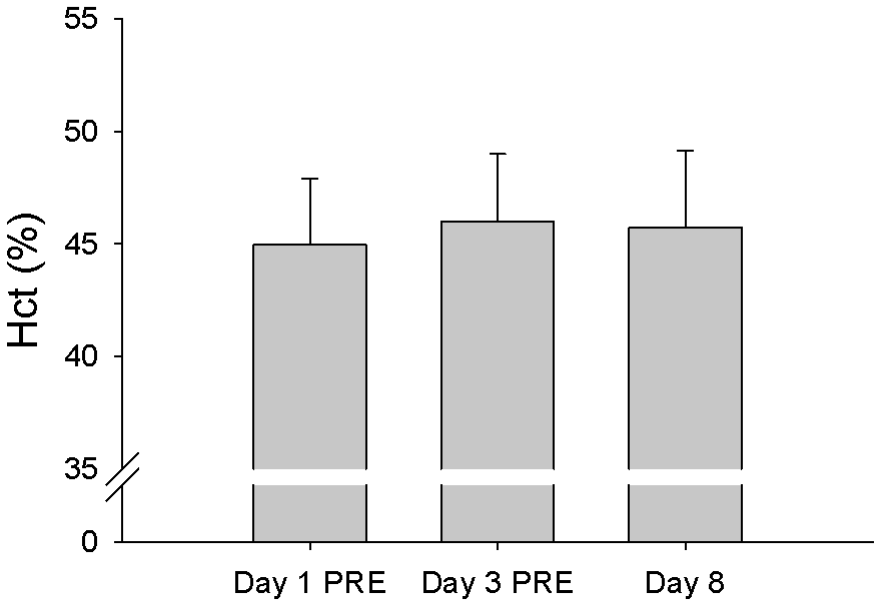

Figure 2-7: Mean Hct during seven days of voluntary apnoea training.

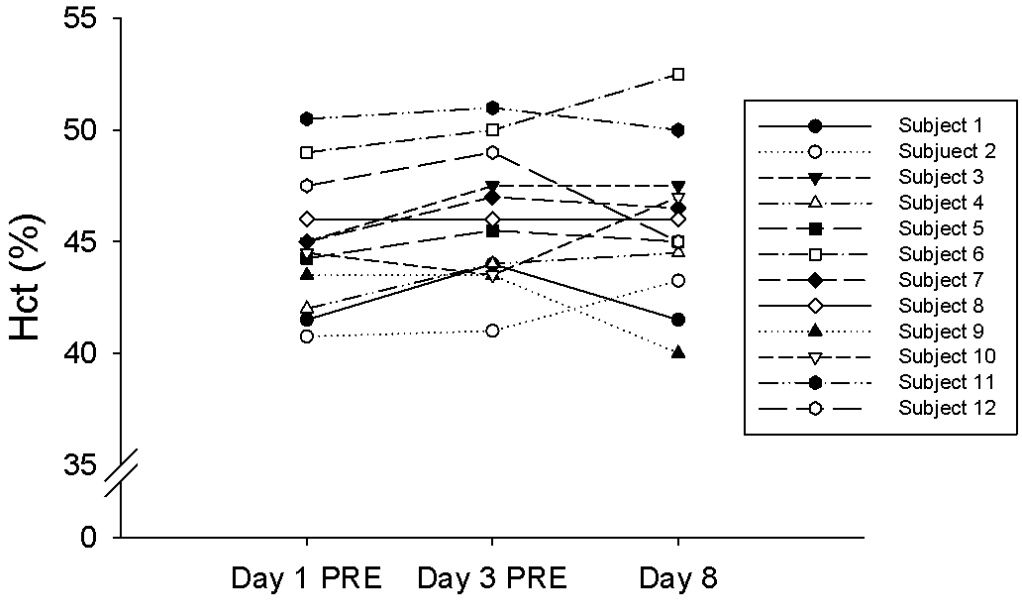

Figure 2-8: Individual Hct during seven days of voluntary apnoea training.

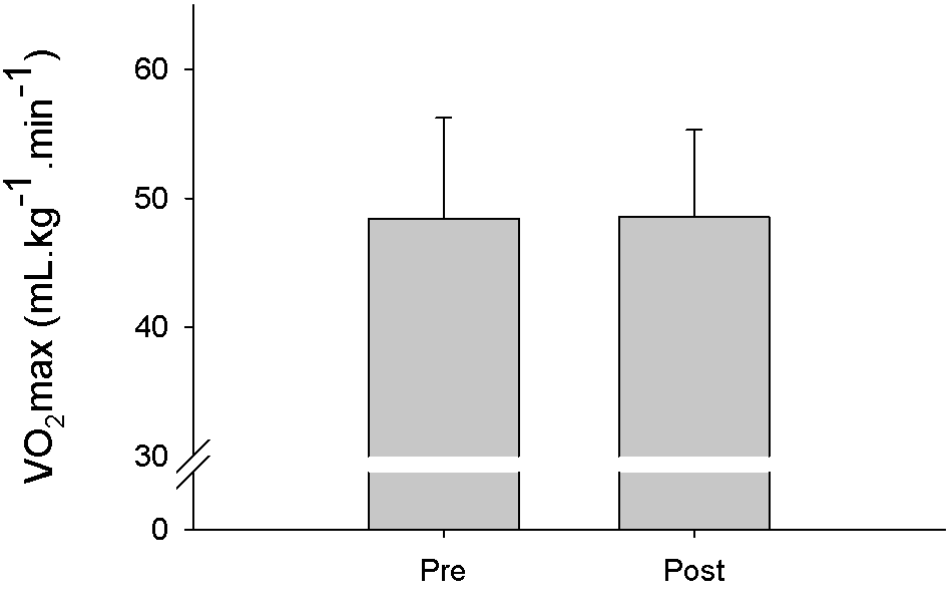

Figure 2-9: Mean relative VO_2max scores from before and after seven days of voluntary apnoea training.

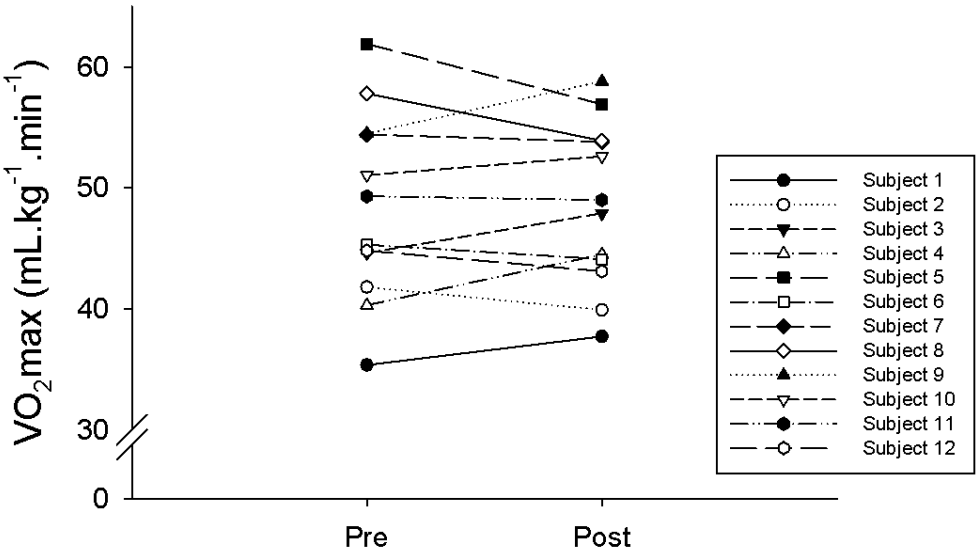

Figure 2-10: Individual relative VO_2max scores from before and after seven days of voluntary apnoea training.

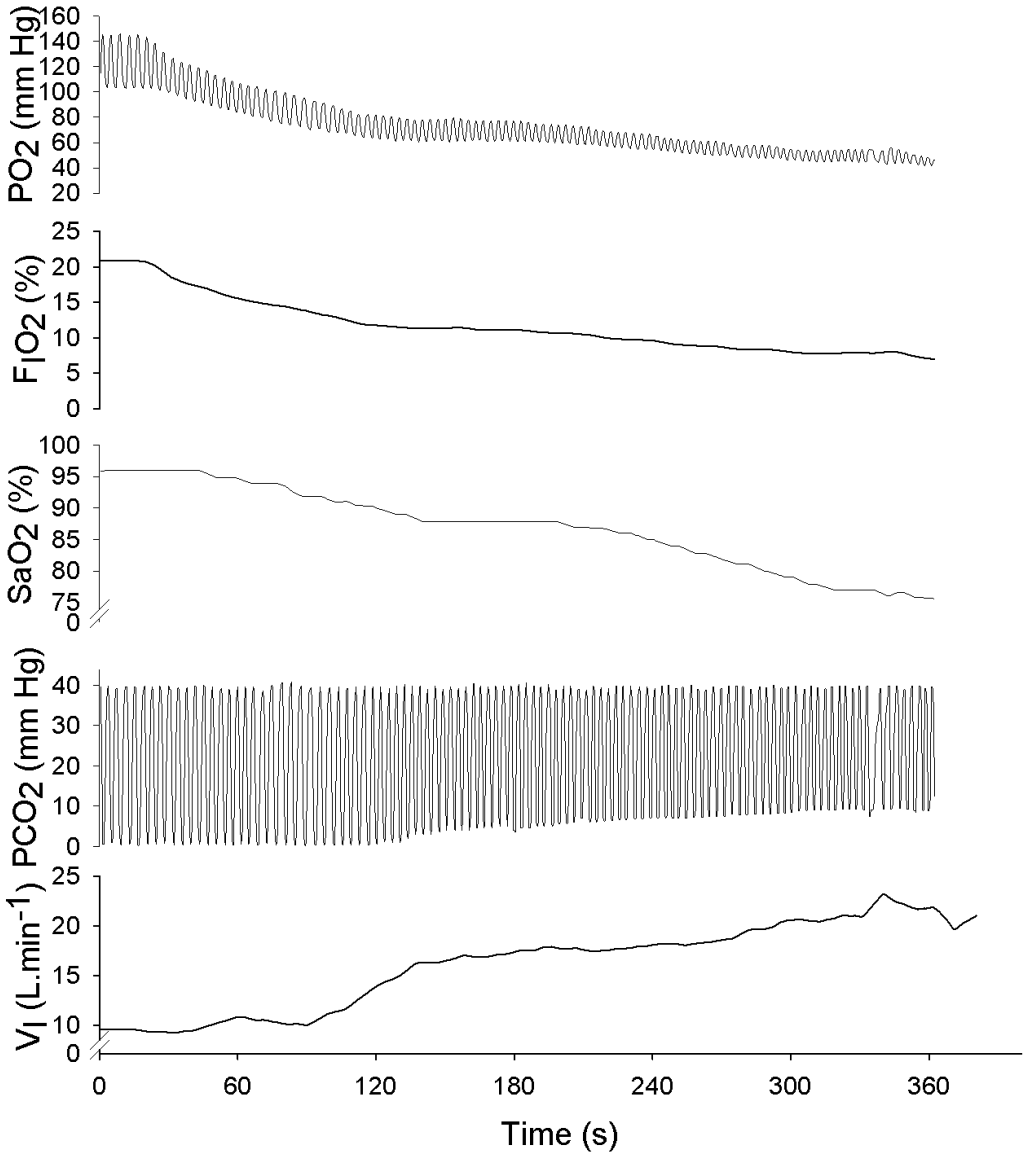

Figure 2-11: Sample data from a single representative acute hypoxic challenge test on one subject. V_I, inspired ventilation; PCO_2, partial pressure of carbon dioxide; SaO_2, arterial oxygen saturation; F_IO_2, fraction of inspired oxygen; PO_2, partial pressure of oxygen.

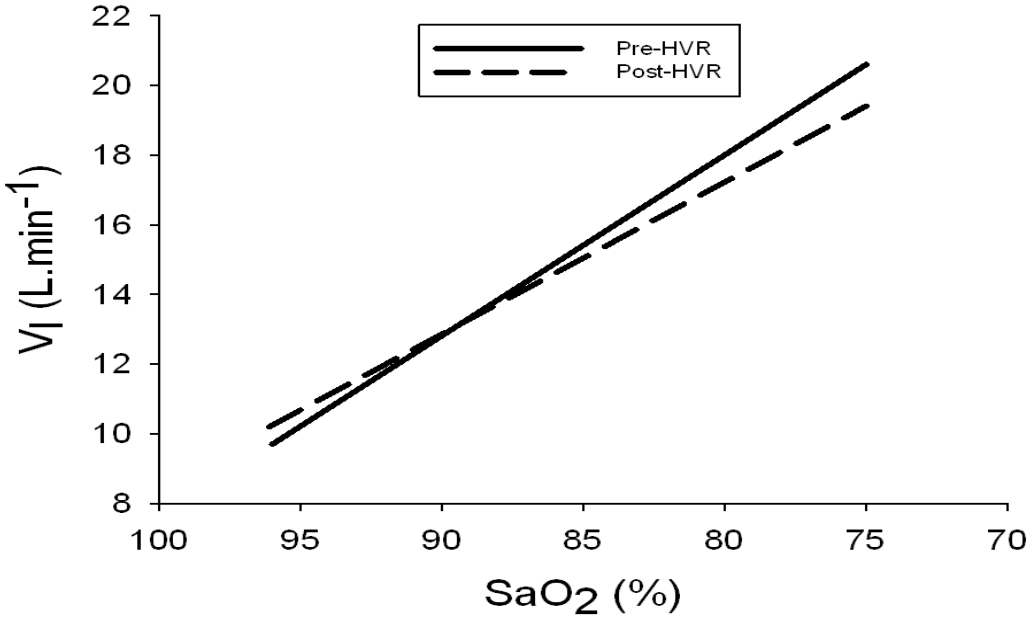

Figure 2-12: Mean HVR values from before and after seven days of voluntary apnoea training for all subjects.

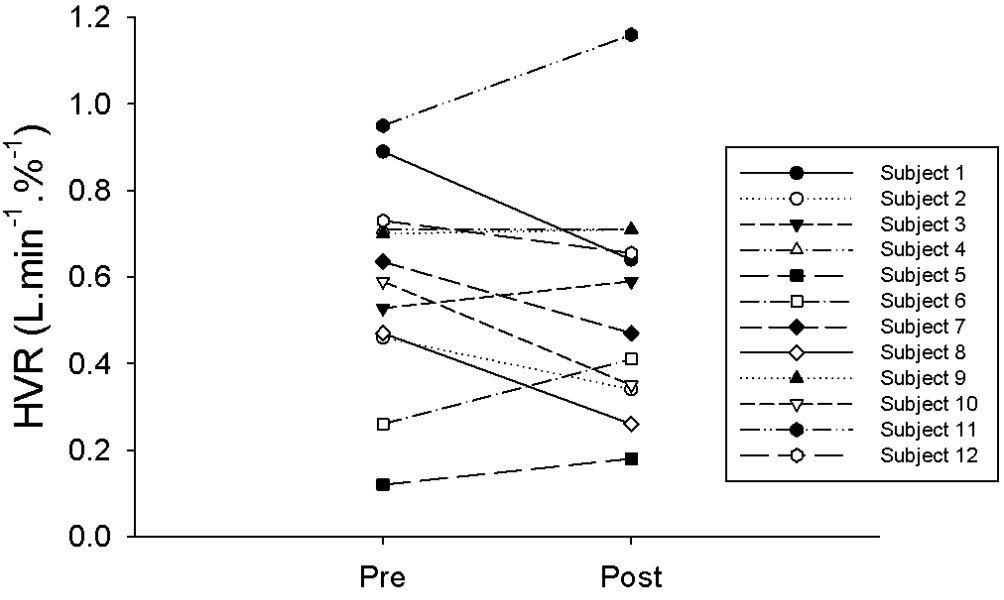

Figure 2-13: Individual HVR values from before and after seven days of voluntary apnoea training.

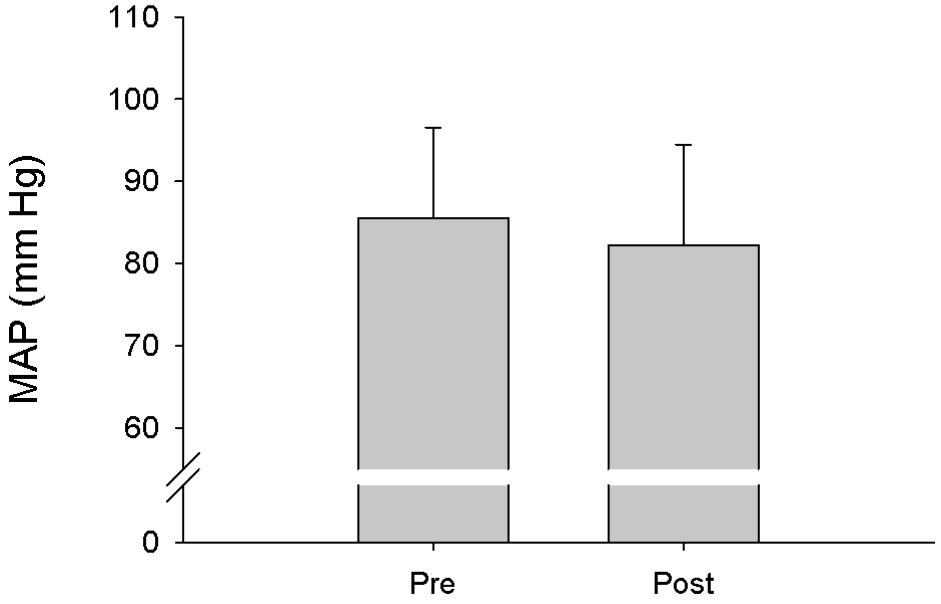

Figure 2-14: Mean resting MAP prior to and following seven days of voluntary apnoea training.

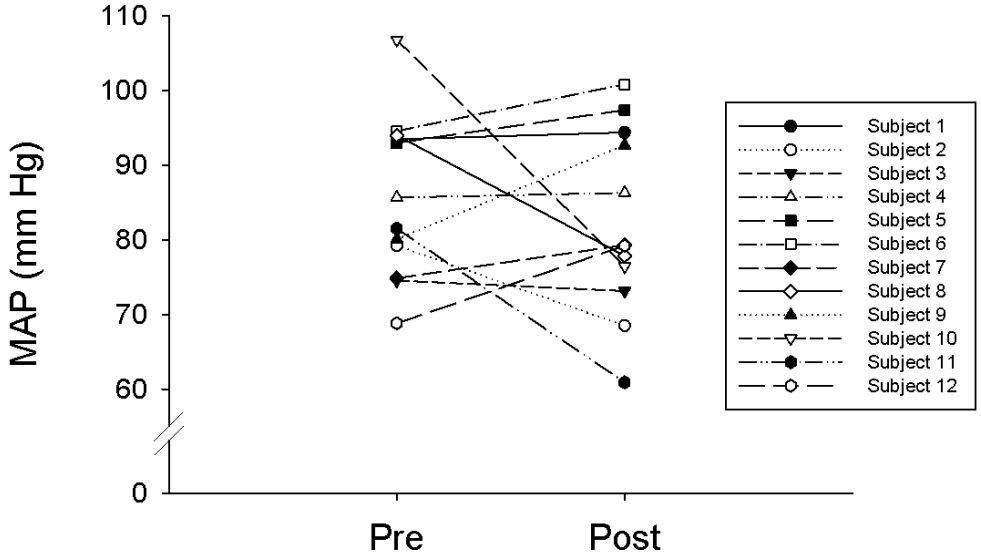

Figure 2-15: Individual resting MAP before and after seven days of voluntary apnoea training.

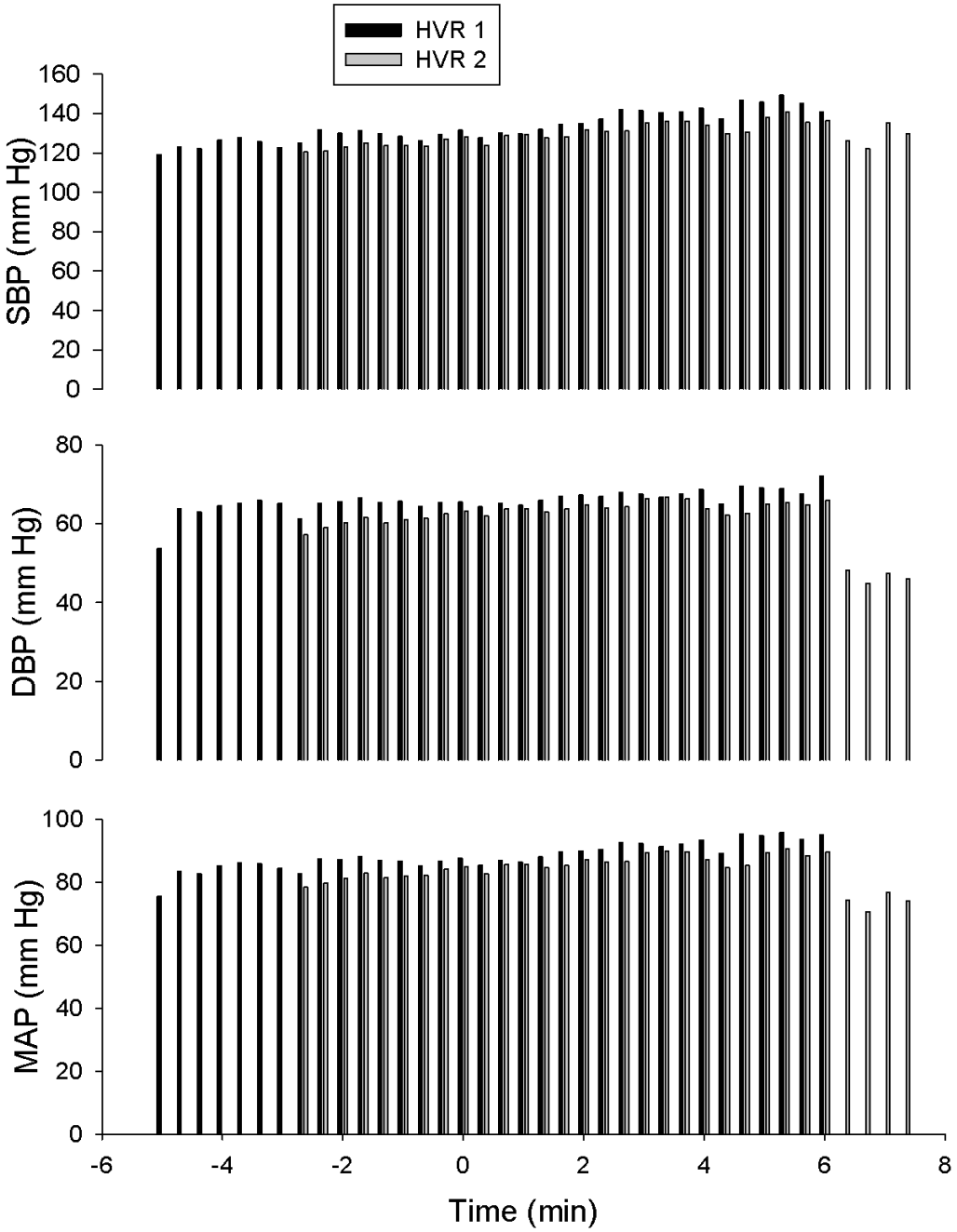

Figure 2-16: Mean SBP, DBP and MAP responses throughout rest and the HVR test prior to and following seven days of voluntary apnoea training. Black bars represent values during the pre-training HVR. Individual apnoeas varied in length therefore the number of subjects represented by each bar is the following. Black bars number 1 (n = 1), 2-7 (n = 2), 8-10 (n = 10), 11-13 (n = 11), 14-24 (n =12), 25-26 (n = 11), 27-28 (n = 9), 29 (n = 7), 30 (n = 6), 31-32 (n = 5), 33 (n = 4) and 34 (n = 2). Grey bars represent values during the post-training HVR. Grey bars number 1 (n = 9), 2-3 (n = 10), 4-18 (n = 12), 19-20 (n = 11), 21 (n = 7), 22 (n = 6), 23 (n = 5), 24-25 (n = 4), 26-27 (n = 3), 28-31 (n = 1). Time point 0 represents the start of the HVR test. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

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CHAPTER 3: GENERAL DISCUSSION

3.1 MAIN FINDINGS

This study produced several novel findings. First, seven days of voluntary apnoea training did not significantly increase plasma [EPO], [Hb] and Hct. Second, there was no change in VO_2max following apnoea training, which appears consistent with the lack of effect on haematological parameters. Third, there was no significant change in the acute HVR or in resting MAP following voluntary apnoea training. Collectively, these results demonstrate that seven days of voluntary apnoea training was not a sufficient stimulus to alter ventilatory chemosensitivity or haematological variables associated with the O_2 carrying capacity of the blood or maximal aerobic power.

3.2 EXPERIMENTAL CONSIDERATIONS

This is the first study to examine the effects of repeated voluntary apnoea training on haematological parameters associated with the O_2 carrying capacity of the blood ([EPO], [Hb] and Hct), VO_2max, the acute HVR and resting MAP. Seven days of voluntary apnoea training did not significantly increase [EPO] and other haematological parameters associated with the O_2 carrying capacity of the blood, namely [Hb] and Hct. It was previously demonstrated by de Bruijn and colleagues that a single bout of 15 maximal duration voluntary apnoeas, that were terminated when SaO_2 reached 60%, was sufficient to induce significant increases in serum [EPO] (1). In the present study, each apnoea was terminated at an SaO_2 of 80%. Thus, the subjects in the present study experienced a less severe hypoxic

stimulus during each training session and it is conceivable that, a severe enough hypoxic stimulus to induce increases in [EPO] and various haematological parameters associated with the O_2 carrying capacity of the blood ([Hb] and Hct), was not achieved in the present study. The average voluntary apnoea training duration was 139 s, with an average total training time per day of 74.2 minutes, in the present study. Subjects on average were exposed to hypoxia ($SaO_2 < 85\%$) for 7.8 minutes per day compared to approximately 12 minutes in previous research by de Bruijn and colleagues. If this protocol were to be repeated, voluntary apnoeas should be allowed to continue to a lower SaO_2 to achieve a greater hypoxic stimulus. On a number of occasions throughout the apnoea training, subjects were instructed to resume breathing before they felt the need to take a breath. If apnoeas were terminated at a lower SaO_2, the subjects could potentially hold their breath until a true minimum SaO_2 was achieved, rather than being instructed to resume breathing prior to achievement of a maximal apnoea. However, a lowering of the SaO_2 termination criteria would introduce the risk of physiological complications associated with hypoxia, such as loss of consciousness. A major advantage of the present approach is that no subjects experienced hypoxia related problems suggesting this type of training could potentially be carried out with minimum supervision in a non-laboratory setting. In contrast, the lack of hypoxia related problems does suggest that subjects may have had the capacity to withstand a lowered SaO_2.

In addition to lowering the SaO_2 at which apnoeas were terminated and increasing the severity of the IH stimulus on a given day, increasing the length of

the training protocol should also be considered. Given that seven days of exposure to voluntary apnoea training did not significantly alter haematological variables associated with the O_2 carrying capacity of the blood or ventilatory chemosensitivity, it is possible that a more prolonged exposure to this type of IH may be required to augment haematological variables associated with the O_2 carrying capacity of the blood and ventilatory chemosensitivity. By increasing both the severity and length of the voluntary apnoea training protocol, subjects would experience a greater IH stimulus which may lead to adaptations in [EPO], [Hb], Hct, the acute HVR and VO_2max.

The measurement of [EPO] only occurred on days one, three, seven and eight in the present study. However, the time course for changes in [EPO] following this type of IH exposure have yet to be confirmed; therefore if repeated, it would have been beneficial to take EPO measurements on all days. This would allow for an in depth examination of the time course of changes in [EPO] following voluntary apnoea training in healthy individuals. In addition, [Hb] and Hct were only measured on days one and three pre-training and on day eight. The low number of measures for these two variables does not allow for an adequate interpretation of the [Hb] and Hct responses to voluntary apnoea exposure. Further, this may have led to significant changes being undetected. As such, future studies should include both pre- and post-training measures of [Hb] and Hct on all training days (days one through seven), in an attempt to determine the time course of changes in these haematological variables following voluntary apnoea training. An increase in the number of measures for these variables in

combination with an increase in the number of EPO measures would provide an improved understanding of the haematological adaptations associated with voluntary apnoea training. Upon examination of [EPO], there was a significant increase from day one (baseline) to day seven post-training ($P < 0.05$). Specifically, at baseline average [EPO] was 7.2 ± 2.5 $mIU.mL^{-1}$ and increased to 8.9 ± 3.1 $mIU.mL^{-1}$ by day seven post-training. However, the statistical power for this measurement was lacking (power = 0.5). Therefore, future studies examining the effects of voluntary apnoea training should include a greater number of subjects to increase statistical power and detection of time dependent changes in [EPO], [Hb] and Hct associated with voluntary apnoea training.

An increase in the severity and duration of IH stimulus during future voluntary apnoea training protocols may also alter ventilatory chemosensitivity. Previous research has demonstrated that longer duration and more severe IH protocols can produce greater increases in the acute HVR. Garcia and colleagues demonstrated a 200% increase in HVR following 12 consecutive days of LDIH (two hours per day at a simulated altitude of 3,800 m), while Lusina and colleagues demonstrated a 103% increase in the acute HVR following 10 consecutive days of IH (one hour per day at an $SaO_2 = 80\%$) (2, 3). An increase in the protocol duration and/or severity would strengthen the argument that voluntary apnoea training could be used as a model of IH to aid in ventilatory acclimatization to altitude, similar to other laboratory controlled IH protocols. As such, this type of training would have the potential to be used in replacement of ascent to altitude or the use of a hypoxic or hypobaric chamber.

In the present study, the acute HVR was measured on day one and eight. In future studies, an acute hypoxic challenge familiarization trial should also be considered. Given that many individuals have never experienced the lowered O_2 associated with this type of test, it would be beneficial if subjects were familiarized with the acute hypoxic challenge test prior to the commencement of the study. Thus, if the present study were repeated, it would be recommended that subjects report to the lab on a separate day prior to day one for a familiarization session with the acute hypoxic challenge test. A familiarization day would ensure a more accurate pre-measurement of the acute HVR. Despite the lack of familiarization, our laboratory has demonstrated good reproducibility of the HVR measure, with an HVR CV of 13.9%. In addition, test-retest correlations were calculated between three different days (Day 1, Day 2 and Pre-test). Strong positive test-retest correlations of $r = 0.94$, $r = 0.96$ and $r = 0.85$ were found between day one and day two, day one and pre-test and day two and pre-test, respectively.

Due to technical difficulties related to improper input of subject characteristics, the change in Q_c in response to acute hypoxia and resting Q_c following seven days of voluntary apnoea training was not measured. As a result, the effects of repeated exposure to voluntary apnoea training on Q_c remain unclear. Subsequent research should therefore aim to determine the effects of this type of IH on Q_c as this may be a better indicator of changes in cardiovascular function than resting MAP.

3.3 FUTURE RESEARCH

Given that seven days of voluntary apnoea training did not elicit significant changes in the variables studied, future research involving voluntary apnoea training should first seek to determine the minimum training length required to elicit significant increases in various haematological parameters associated with the O_2 carrying capacity of the blood, ventilatory chemosensitivity and maximal aerobic power. Also, considering de Bruijn and colleagues demonstrated a significant increase in serum [EPO] using an SaO_2 cut-off of 60%, the severity of hypoxia needed to elicit these changes should also be investigated. Specifically, it should be determined what level of SaO_2 is safe yet effective for increasing [EPO], [Hb], Hct, the acute HVR and VO_2max. As a result, future research should examine differences in the length and severity of different voluntary apnoea training protocols.

In the present study, reticulocyte count was not measured; however future research should investigate the effects of voluntary apnoea exposure on reticulocyte count as this is may be a better indicator of the effectiveness of voluntary apnoea training on increasing the O_2 carrying capacity of the blood rather than both [Hb] and Hct. Given that reticulocytes mature into RBC's, an increase in this parameter may signify acute changes in the blood that may otherwise go undetected. Moreover, given the short duration of the training, this measure may detect earlier changes in haematological parameters that suggest an early effect on the O_2 carrying capacity of the blood, which may not be possible when only measuring [Hb] and Hct.

3.4 CONCLUSION

In summary, the present study investigated the effects of seven days of voluntary apnoea training on haematological variables associated with the O_2 carrying capacity of the blood, VO_2max, ventilatory chemosensitivity and resting BP. The results demonstrate that the IH stimulus produced by the present voluntary apnoea training protocol was not sufficient to elicit significant changes in the haematological variables examined or VO_2max. It was also not sufficient to elicit a change in ventilatory chemosensitivity or resting BP. Therefore, further research is needed to determine the optimal voluntary apnoea training protocol for eliciting similar changes in haematological variables associated with the O_2 carrying capacity of the blood and ventilatory chemosensitivity benefits as previously established with other models of IH. Until future determinations can be made, it currently appears that other models of IH should be used rather than seven days of voluntary apnoea training.

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