INTERMITTENT HYPOXIA AND THE CHEMOREFLEX CONTROL OF BREATHING

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Human Kinetics)

THE UNIVERSITY OF BRITISH COLUMBIA

August 2006

ABSTRACT

Rationale: Intermittent Hypoxia (IH) consists of bouts of hypoxic exposure interspersed with normoxic intervals. In animals, there is some evidence that multiple brief short duration exposures to intermittent hypoxia (SDIH) provoke more profound changes in chemosensitivity than longer duration bouts of intermittent hypoxia (LDIH). The purpose of this study was to test the hypothesis that SDIH would have differential effects from LDIH on chemosensitivity during rest and exercise in humans.

Methods: Ten males underwent two intermittent hypoxic protocols of 7 days duration/each. The LDIH protocol consisted of daily 60-minute exposures to normobaric 12% O_2. The SDIH protocol consisted of twelve 5-minute bouts of normobaric 12% O_2, separated by 5-minute bouts of normoxia. Measured resting variables included the hypoxic ventilatory response (HVR), hypercapnic ventilatory response (HCVR), CO_2 threshold and CO_2 sensitivity. Submaximal exercise variables included minute ventilation, oxygen saturation, hyperoxic and hypercapnic ventilatory response in both hypoxia and normoxia. Peak exercise variables included power and oxygen consumption in hypoxia. Measurements were made immediately prior to intermittent hypoxic training and on the first day following IH. Resting measures were repeated 7 days following IH.

Results: For both protocols, the HVR was significantly ($p< 0.05$) increased after IH. One week post IH, the HVR was not different from pre-IH. The HCVR was increased and remained elevated at 7 days post-IH ($p<0.01$). The CO_2 sensitivity was unchanged by either intervention. In hypoxia and hyperoxia, the CO_2 threshold was significantly reduced following IH ($p<0.05$). The submaximal minute ventilation, hyperoxic and hypercapnic responses in normoxia and hypoxia were unchanged by IH. Submaximal oxygen saturation and peak power were both increased ($p<0.05$), while maximal ventilation and oxygen consumption were unaltered. There were no significant differences between the two IH protocols for any of the above measures.

Conclusions : A 7-day IH protocol causes increases in the HVR and HCVR at rest and a left-shift in the CO_2 threshold and an improvement in oxygen saturation during submaximal hypoxic exercise. SDIH is no more efficacious than LDIH at effecting these changes in respiratory control.

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CO-AUTHORSHIP STATEMENT

A version of Chapter 2 has been previously published as:
Koehle MS, Foster GE, McKenzie DC and Sheel AW (2005) Repeated measurement of hypoxic ventilatory response as an intermittent hypoxic stimulus **Resp Physiol Neurobiol** 145(1): 33-39.

MS Koehle was the primary author and played the principal role in identification and design of the research programme, performance of research, data analysis and manuscript preparation.

GE Foster assisted in the performance of research.

DC McKenzie assisted in identification and design of the research programme and manuscript preparation.

AW Sheel assisted in identification and design of the research programme, data analysis and manuscript preparation.

CHAPTER 1: INTRODUCTION

The term Intermittent Hypoxia (IH) refers to an exposure to multiple brief bouts of hypoxia over a period of time. This length of time can be as short as a few minutes or as long as several weeks. IH has been shown to increase red cell mass[1], blood pressure[2] and cerebrovascular and sympathetic responses to hypoxia[3 4]. IH can cause alterations in the chemoreflex control of breathing in humans that may have potential clinical or ergogenic applications. There is some evidence that it may protect the heart from ischaemia and have the potential to improve ventilation in spinal cord transection patients[6]. For the respiratory system, IH can increase an individual's ventilatory response to hypoxia at rest[7-11]. There is some evidence that IH can also increase exercise ventilation during exercise tests at simulated altitude (4500 metres)[12]. This increased ventilation is associated with improved arterial oxygen saturation during exercise. IH does not appear to increase exercise ventilation at sea level[13]. However, although no research has examined exercise ventilation at sea level and simulated altitude following the same IH exposure.

Proposed Mechanism of Intermittent Hypoxia

Due to its invasive nature, the majority of the research into the mechanism of IH has been performed in animal models. These concepts are applied to human physiology with appropriate caution. Acute hypoxic exposure leads to a series of alterations in neural control of respiration; these are the short-term hypoxic phrenic response, the post-hypoxia frequency decline and phrenic Long-term Facilitation (LTF). The short-term hypoxic phrenic response is an increase

in integrated phrenic amplitude during the hypoxic exposure. This modulation does not persist beyond the hypoxic exposure, but it can be augmented by intermittent hypoxia through a serotonin-mediated mechanism[14]. Following the hypoxic exposure, a transient decrease in phrenic motorneuron frequency occurs that returns to baseline after several minutes.[14] Subsequent to the hypoxic exposure, there is a persistent increase in phrenic motorneuron output amplitude lasting minutes to hours, termed the phrenic LTF[15]. LTF following an acute hypoxic exposure is a form of neural "plasticity". Plasticity is defined as "a persistent change in the neural control system based on prior experience"[16]. With repeated exposures to hypoxia (intermittent hypoxia) this increased phrenic motorneuron output (LTF) is further augmented[14]. This amplification of the LTF is believed to be a form of "metaplasticity" whereby a prior exposure (to hypoxia) modifies one's ability to express plasticity[16]. This metaplasticity can be blocked using the serotonin blocker methysergide[14], indicating that the mechanism is at least partially serotonin-mediated. Other mechanisms may also play a role in the generation of LTF, such as the nitric oxide pathway[15]. Following intermittent hypoxia, the augmented LTF can be induced with either hypoxia or electrical carotid sinus nerve stimulation, indicating that this plasticity occurs (at least partially) through central facilitation of chemoreceptor afferents as opposed to occurring entirely at the carotid peripheral chemoreceptors themselves[14]. LTF can occur in response to hypoxia in carotid-denervated cats, indicating that there may be a possible role for the direct effect of hypoxia on the CNS neurons themselves.[17]

Duration of Intermittent Hypoxia

Many different IH protocols have been utilized in previous studies in humans (see Table 1.1). Exposures have ranged from twenty minutes to two hours in length, and have typically been repeated between 7 and 14 times. The optimal IH intervention for increasing hypoxic ventilatory response is unknown. One way to better characterize the minimum duration of IH on ventilation would be to measure hypoxic ventilatory response (HVR) daily during the administration of an IH regime. When working with hypoxia and hypoxic sensitivity, one must be careful to ensure that the measurements used in a study are not interventions themselves. During a single HVR test, a subject receives a short exposure to significant hypoxia (lasting approximately 5 minutes) where F_IO_2 (fraction of inspired oxygen) can reach as low as 5%. An HVR test therefore represents a shorter but more severe hypoxic exposure than the typical bout of hypoxia used during IH. It is possible that the brief, more profound exposures of an HVR test, if repeated daily, could present an intermittent hypoxic stimulus and therefore affect ventilation during subsequent hypoxic exposures. Before incorporating daily assessment of HVR into a study, the significance of this potential co-intervention would need to be assessed.

Paper	N	Intervention	Testing	HVR	HCVR	*HCVR sb*	*Modified Read Rebreathe*	VO_2max	Exercise Ventilation	Haem.	Comments
Foster et al. 2006[18]	9♂SDIH 9♂LDIH	***Normobaric*** *12 % O_2* 10 of 12 days SDIH/LDIH	-VO2max, min Ve, Fb at various %max	N/A	N/A	N/A	N/A	-ØΔ (at sea level)	-ØΔ (at sea level)	N/A	-isocapnic
Townsend et al.2005[8]	12♂A 11♂B 10♂C	***Normobaric*** *16.3 % O_2* A=20d X 8-10h B=4 X (5d X 8-10h) C=Control	-HVR (pre-, Post-) -submaximal exercise Ve (Pre-, after 4, 10 19 nights)	-increased in both IH groups	N/A	N/A	N/A	N/A	-increased after 4 nights of hypoxia for both IH groups	N/A	-exercise ventilation measured in normoxia -change in exercise ventilation correlated to change in HVR
Foster et al. 2005[7]	9♂SDIH 9♂LDIH	***Normobaric*** *12% O_2* 10 of 12 days SDIH/LDIH	-HVR days 1,3,5,8,10,12	increased in both groups	-ØΔ	N/A	N/A	N/A	N/A	N/A	-isocapnic
Katayama et al. 2005[19]	7♂A-E 7♂A-C 8♂B-E 7♂B-C	***Normobaric*** *12.3% O_2* A = 3h X 7d B =3h X 14d	-HVR, pre-,post-, 1-2-wks post- -HCVR, pre-,post-, 2-wks post-	-increased in both groups, back to N w/I 2/52	-incr. only after 2/52 of IH	N/A	N/A	N/A	N/A	N/A	N/A
Katayama et al. 2004[20]	8♂E 7♂C	***Normobaric*** *12.3% O2* 3h X 14d	-maximal, submaximal X 2, TT -pre- and post -haematology	N/A	N/A	N/A	N/As	-ØΔ in VO2max	-↓ ***submax VO_2*** (efficiency?)	-ØΔ in Hb, Hct, RBC, Rct	-trend to improved TT performance
Ainslie et al. 2003[21]	12♂	***Normobaric*** 4300mX8-9hX5d	-HVR, HCVR pre-test X 2, post, and 5d-post	-increased by 1.6L/min/% Sa02	-increase in slope -left shift in intercept	N/A	N/A	N/A	N/A	N/A	-HVR used PETO2
Hendrikse n and Meeuwsen 2003[22]	12♂	***Hypobaric*** 2hX2500mX10d -2h cycling 65%HRR -crossover control	-VO2max, Wingate, Hb, Hct -pre-, 9d post-	N/A	N/A	N/A	N/A	-ØΔ in VO_2max ↑anaerobic power	N/A	-↑Hb, Hct	-triathletes

Table 1.1 Summary of Selected Research on intermittent hypoxia and ventilatory control on humans

Paper	N	Intervention	Testing	HVR	HCVR	*HCVR sb*	*Modified Read Rebreathe*	VO2max	Exercise Ventilation	Haem.	Comments
Mateika et al. 2003[23]	7♂ 4♀	***Normobaric*** 4'X 8%O_2 X 8	-MRR test pre- and 1h post- -PO2=50 and 140	N/A	N/A	N/A	-increase in slope -ØΔ threshold	N/A	N/A	N/A	
Katayama et al. 2003[13]	6♂C 6♂E	**Hypobaric** *(4500m)* -90' X 9 (over 3/52)	-3k run time, run to exhaustion, VO2max, haematology -pre-, post, 3/52 post-	N/A	N/A	N/A	N/A	-ØΔ in VO_2max	-ØΔ (at sea level) -↓ ***submax VO_2*** (efficiency?)	-ØΔ in Hb, Hct, RBC, Rct, Epo, Ferritin	-↓3k times, ***submax VO2*** -↑ run time to exhaustion **-trained runners**
Fahlman et al. 2002[24]	5♂ 7♀	**Normobaric** -novel repeat HVR circuit -2'X8.3%O_2 X 4, alternating w/ 21%O2	-repeated HVR measurements following square 4m waveform	-'steady' response -variable response	N/A	N/A	N/A	N/A	N/A	N/A	**-CV** was 70%
Katayama et al. 2002[9]	8♂E 6♂C	**Hypobaric** *(4500m)* -60' X 7d	-HVR, HCVRsb, pre- post- -Ve @ 40, 70, 100%max pre-,post	-↑HVR at rest - ØΔ in C	N/A	-non-signifi cant- ↑ post-	N/A	N/A	- ØΔ at any exercise level	N/A	-exercise at sea level
Katayama et al. 2001[12]	6♂	**Hypobaric** *(4500m)* -60' X 7d	-HVR, HCVR, HCVRsb, max and submax Ve, VO_2 -pre-, post-, 7d post-	-↑HVR post- and 7d post- -incr by ~90%	- ØΔ	-↑ post- - ØΔ 7d post-	N/A	- ØΔ in VO_2max	-↑ Ve/VO_2 and SaO_2 @ rest, 40%, 70% of max -↑ SaO_2 @ max	N/A	-maximal and submaximal tests ***at 4500m***
Katayama et al. 2001[2]	14♂	**Hypobaric** *(4500m)* -60' X 7d	-ventilatory and CV responses to hypoxia -pre-, post-	N/A	N/A	N/A	N/A	N/A	- ØΔ in resting Ve	N/A	-enhanced arterial BP responsiveness to hypoxia
Gore et al. 2001[25]	6♂E 7♂C	**Normobaric** *15.48% O_2* -23 X 9.5h	-VO_2 during maximal and submaximal exercise	N/A	N/A	N/A	N/A	N/A	-↓VO_2 during submaximal exercise		-normoxic exercise

Table 1.1 (continued) Summary of Selected Research on intermittent hypoxia and ventilatory control on humans

Paper	N	Intervention	Testing	HVR	HCVR	*HCVR sb*	*Modified Read Rebreathe*	VO2max	Exercise Ventilation	Haem.	Comments
Mahamed & Duffin 2001[26]	5♂ 2♀	**Normobaric** 20'X*10%O_2*X14d	-MRR pre-,post- each exposure	-↑ Ve during resting hypoxia after intervention	N/A	N/A	-left shift in threshold (pre-hypoxia), right-shift post- - ØΔ in sensitivity	N/A	N/A	N/A	-increase in resting ventilation during hypoxia and HVD became more apparent
Casas et al. 2000[27]	5♂ 1♀	**Hypobaric** -3-5hX*4000-5500M*x17d	-haematology	N/A	N/A	N/A	N/A	-La curve shifted to right	-↑ Ve @ max and La threshold	-↑ PCV, RBC, Hb	**-climbers**
Garcia et al. 2000[10]	9♂	**Hypobaric** *(3800m)* -2h X 12d	-HVR pre- and post- -haematology pre-, post-	-↑HVR, peaked on Day 5 then ↓	N/A	N/A	N/A	N/A	- ØΔ in resting Ve in normoxia -↑ Ve in hypoxia	N/A	-ØΔ in hb, Hct -↑in Rct by Day 5
Garcia et al. 2000[11]	4♂	**Hypobaric** *(3800m)* -2d CH vs. -2h X 2d	-HVR pre- and post-	-↑HVR, greater in IH group	N/A	N/A	N/A	N/A	N/A	N/A	
Rodriguez et al. 1999[28]	8♂C 2♀C 6♂E 1♀E	**Hypobaric** -3-5h X *4000-5500m* X 9d -low intensity exercise in E	-VO2max, La threshold, haematology -pre-, post-	N/A	N/A	N/A	N/A	-La curve shifted to R -ØΔ in VO2max	-↑ Ve @ max at sea level	-↑ PCV, RBC, Hb, Rct	- ØΔ b/w C and E groups **-climbers**
Katayama et al. 1998[29]	7♂ 6♂	**Hypobaric** *(4500m)* -exercise:2 X 15' @40%VO_2max X 6 -both groups 60'X6	-HVR, HCVR, VO2max -pre-, post-, 6d post-	-↑ in control group	-ØΔ in either group	N/A	N/A	-↑ in training group	N/A	N/A	-HVR used finger SaO2 -↑ resting SaO_2 in control group

Table 1.1: Summary of selected research on intermittent hypoxic training and ventilatory control in humans.
MRR= Modified Read Rebreathe; '= minutes; h = hours; HVR = hypoxic ventilatory response; HCVR = hypercapnic ventilatory response; HCVRsb = hypercapnic ventilatory response (single-breath); ØΔ = no change; Haem = haematology; Hb = Haemoglobin concentration; Hct = Haematocrit; RBC = erythrocyte count; Rct = reticulocyte count; Epo = erythropoietin count; E = Experimental; C = Control; SDIH = Short-duration intermittent hypoxia; LDIH = Long-duration intermittent hypoxia; CH = Continuous Hypoxia; Ve = ventilation; VO_2 = oxygen consumption; La = Lactate; TT = Time Trial

Hypobaric vs. Normobaric Intermittent Hypoxia

Hypoxia can be hypobaric or normobaric. In hypobaric hypoxia, the fractional concentration of oxygen remains unchanged, while the absolute pressure is decreased. Hypobaric hypoxia requires altitude exposure or a hypobaric chamber. In contrast, normobaric hypoxia involves maintenance of the ambient pressure, but decreasing the inspired oxygen concentration. Most of the research examining the relationship between IH and ventilatory control has been conducted using hypobaric hypoxia, although there have been a few recent studies using normobaric hypoxia [11 23 26 30]. Similar changes in HVR have been demonstrated using both protocols. No research has compared the two types of hypoxia in the same study population.

Poikilocapnia vs. Isocapnia

IH protocols can be either poikilocapnic or isocapnic. The vast majority of studies do not control end-tidal CO_2 levels, therefore, during the hypoxic bouts of IH; end-tidal CO_2 is decreased, leading to a respiratory alkalosis. Only one study has looked at the effect of isocapnic IH on HVR[7 26]. Foster et al. showed similar increases in HVR following isocapnic IH to those observed in poikilocapnic studies. Presumably, in isocapnic IH, the respiratory alkalosis would be mitigated, and any changes in respiratory control would be secondary to the hypoxia alone.

Hypoxia Duty Cycle

Another possible variable that may be important in intermittent hypoxic training is the duty cycle. This term refers to the duration and frequency of each hypoxic exposure. Most published studies in humans have involved a continuous dose of hypoxia each day over several days. This protocol is called continuous intermittent hypoxia or long duration intermittent hypoxia (LDIH). It is not known whether repeated shorter doses of hypoxia each day might provide a more or less profound effect. This type of protocol is termed intermittent-intermittent hypoxia or short-duration intermittent hypoxia (SDIH). Peng and Prabhakar[31] examined carotid body hypoxic response in rats following two different protocols of (poikilocapnic) intermittent hypoxia. The SDIH procedure consisted of 15 seconds of normobaric hypoxia (F_IO_2=5%) followed by 5 min of normoxia for 9 episodes per hour, eight hours per day for ten days. The long-duration protocol (LDIH) consisted of 4 hours of hypobaric hypoxia (0.4 atmospheres) per day for ten days. Hypoxic response was significantly enhanced in the SDIH animals but not in the LDIH group. Only one published study has compared the two protocols in humans. Foster et al. 2005[7], examined chemoreceptor responses in humans exposed to either an SDIH or LDIH protocol but were unable to demonstrate a difference between the two protocols. The study had a few weaknesses: the hypoxic exposures were short (only 30 minutes total daily hypoxia exposure) and occurred as ten exposures over 12 days and subjects were not their own controls. These factors may have

increased the variability in response to IH, and thus made it more difficult to demonstrate a difference between SDIH and LDIH.

Response to Carbon Dioxide and Intermittent Hypoxia

Recently, three separate groups have studied the effects of different IH protocols on the carbon dioxide control of breathing. Interestingly, the results have varied significantly between the three laboratories. Duffin's group[26 32] uses a modified rebreathing protocol which incorporates prior hyperventilation to reduce end-tidal carbon dioxide levels (to approximately 20mmHg) before testing. With this technique, one can assess both the CO_2 threshold (below which ventilation does not respond to a rise in CO_2) and the ventilatory sensitivity to CO_2. The experimental paradigm keeps the subject iso-oxic while the carbon dioxide gradually rises as a result of rebreathing. Both hypoxic and hyperoxic conditions are assessed (end-expiratory oxygen pressure of 50 mmHg or 150 mmHg). The hyperoxic trial is intended to reduce the output from the peripheral chemoreceptors[33]This assertion is controversial, in that Dahan et al.[34] examined the response to CO_2 in normoxia and hyperoxia. They divided the response into a fast and a slow component, attributing the fast component to the peripheral chemoreceptors. This fast component was reduced but not completely attenuated by hyperoxia. In interpreting the results, therefore, one must consider that there may remain a small residual effect from the peripheral chemoreceptors contributing to the hyperoxic trial.

By comparing a hypoxic test where the peripheral chemoreceptors are contributing to respiratory drive to such a hyperoxic test, the investigators can approximate the role of the peripheral chemoreceptors. Using an IH protocol consisting of daily *isocapnic* twenty-minute exposures to an F_IO_2 of 10%, Mahamed and Duffin examined carbon dioxide response before and after each exposure[26]. Following the 14-day IH intervention, the subjects demonstrated a leftward shift in the carbon dioxide/ventilation curve. This shift represented a lowering of the carbon dioxide threshold, but no change in carbon dioxide sensitivity. Mateika et al.[23] used the same paradigm to examine respiratory response to a shorter IH protocol. Subjects completed eight four-minute bouts at an F_IO_2 of 8% in one session. There was no ensuing change in carbon dioxide threshold, but the sensitivity was increased. These findings are clearly different from Duffin's study, but the IH protocol was also dramatically different. Ainslie et al.[4 21] also found an increase in CO_2 sensitivity following five nights of hypoxia (4300m). There were some methodological differences between Duffin's protocol (modified Read rebreathing method) and Ainslie's protocol (hyperoxic acute hypercapnic ventilatory response), which make it difficult to make a direct comparison. However, it is interesting that Mateika's study used a shorter IH protocol than Duffin's, and Ainslie's protocol was longer, but they both found an increase in CO_2 sensitivity that Duffin did not. Perhaps the fact that Duffin's exposure was the least hypoxic of the three may play a role. Clearly the changes in carbon dioxide sensitivity with IH remain ambiguous. Moreover, no study has looked at both HVR and the modified Read rebreathing method in the same

subjects. There may be a possible interaction between changes in CO_2 response and changes in HVR. For example, if carbon dioxide threshold is lowered during an iso-oxic test (as a result of IH), ventilation would be increased for a given oxygen concentration. When an HVR test is then performed, this change in CO_2 threshold could manifest as an increased HVR. It would be therefore be worthwhile to measure CO_2 response both before and after an IH protocol using Duffin's rebreathing method and compare it to the changes that occur in HVR.

Respiratory Drive during Exercise

Thus far, the ventilatory parameters that have been tracked following IH have been resting values such as HVR and carbon dioxide response. The effect on exercise ventilation is not well understood. Katayama has demonstrated that IH induces an increase in ventilation during hypoxic exercise[12 35] (at 40% and 70% of VO_2peak at altitude) but not during sea level (normoxic) exercise[13]. Conversely, other studies by Casas et al.[27 28] and Rodriguez[27 28] et al. have demonstrated an increase in ventilation during maximal exercise tests under normoxic conditions following intermittent hypoxia. The IH protocols that the subjects undertook were similar in all three studies except for the fact that Katayama's IH protocol was slightly shorter at seven days (instead of nine or twelve days for the other two studies). Rodriguez' study also had the subjects exercising during their hypoxic exposures, unlike the others which involved passive exposure to hypoxia. Thus, although there is much less work examining

ventilatory response to exercise following IH, it appears that ventilation may be increased during hypoxic exercise. During normoxia, there is considerable disagreement. A study examining exercise ventilation under both normoxic and hypoxic conditions is necessary to help resolve this issue.

The control of breathing during exercise is multifactorial, while the relationship between the various inputs remains unclear. Arterial blood gases are well-maintained during steady-state below the ventilatory threshold[36]. Typically, the hyperpnoea of subthreshold exercise follows three phases. At the initial onset of exercise there is a rapid increase in ventilation within one gait cycle. This 'fast component' is likely neurally controlled, either by a central or peripheral stimulus (or a combination of the two)[37 38]. The second phase of exercise hyperpnoea involves a slower increase in ventilation (the slow component), which is possibly mediated by peripheral chemoreceptors in humans[39 40]. Eventually, ventilation reaches a steady state, which has been classically described as a summation of the neural (fast) and chemoreceptor (slow) drives to exercise ventilation[41]. An increase in exercise ventilation resulting from IH would putatively affect the slow component through a similar central neural facilitation mechanism as that proposed for the upregulation of HVR. No studies have examined chemoreceptor response during exercise in humans following an IH protocol. Instead, only resting measures (such as HVR) have been assessed.

The process of measuring hypoxic and hypercapnic ventilatory response during exercise is much different than at rest[42 43]. To measure the hypoxic

response, a subject exercises at steady state and then the inspired gas is switched from air to 100% oxygen for three breaths. The ratio of hyperoxic ventilation to normoxic ventilation is used to indicate the chemoreflex drive to oxygen. A similar mechanism is used to assess hypercapnic response. Instead of oxygen, subjects are given a three-breath stimulus of hypercapnic gas while exercising at steady state. The proposed study would assess both resting and exercising indicators of chemoreflex drive to gain a better understanding of the effect of IH on exercising ventilation.

Summary

In summary, IH has many effects on the respiratory system that may lead to ergogenic or clinical applications. This study aims to comprehensively assess the resting and exercising control of ventilation while comparing two different types of IH (SDIH and LDIH). The overall aim is to gain a better understanding of the most effective IH protocol and how it affects oxygen and carbon dioxide control of breathing during rest and exercise.

RESEARCH QUESTIONS:

The proposed study attempts to address the following questions:

1) Is daily measurement of hypoxic ventilatory response a form of intermittent hypoxia?
2) What is the effect of an intermittent hypoxia protocol on *resting* ventilatory response to hypoxia and hypercapnia?
3) Do two types of intermittent hypoxia protocols (SDIH vs. LDIH) affect the above changes differently?
4) What is the time course of the change in resting ventilatory response to hypoxia as a result of intermittent hypoxia?
5) What is the relationship between changes in hypoxic ventilatory response and changes in both carbon dioxide threshold *and* sensitivity with intermittent hypoxia?
6) What is the relationship between changes in hypercapnic ventilatory response as measured by the traditional method as compared with the modified Read rebreathing method?
7) Does an intermittent hypoxia protocol affect *exercising* ventilatory response to hyperoxia and hypercapnia?
8) Does an intermittent hypoxia protocol change submaximal and maximal exercising ventilation under *normoxic* conditions?

9) Does an intermittent hypoxia protocol change submaximal and maximal exercising ventilation under *hypoxic* conditions?

10) Does an intermittent hypoxia protocol affect exercise capacity in hypoxia?

HYPOTHESES:

1) Daily measurement of hypoxic ventilatory response would act as an intermittent hypoxic stimulus, thus altering hypoxic ventilatory response.
2) An intermittent hypoxic protocol would result in a co-ordinated increase in oxygen and carbon dioxide chemosensitivity at rest. An intermittent-intermittent hypoxia protocol would lead to larger changes in resting hypoxic ventilatory response and hypercapnic response than the continuous intermittent hypoxia protocol. These changes would occur after fewer days of intermittent hypoxia following the intermittent-intermittent hypoxic protocol, than after the continuous intermittent protocol.
3) An intermittent hypoxic protocol would result in an increase in oxygen and carbon dioxide chemosensitivity during exercise. These augmented responses would lead to increased exercise ventilation under normoxic and hypoxic conditions, improving the arterial saturation and performance during submaximal and maximal hypoxic exercise.

PURPOSE:

The purpose of the study was to compare the effects of two different intermittent hypoxia protocols on respiratory chemoresponse and to examine the relationship between carbon dioxide and oxygen sensitivity during rest and exercise.

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CHAPTER 2: REPEATED MEASUREMENTS OF HYPOXIC VENTILATORY RESPONSE AS AN INTERMITTENT HYPOXIC STIMULUS[⊕]

[⊕] A version of this chapter has been published as:
Koehle MS, Foster GE, McKenzie DC and Sheel AW (2005) Repeated measurement of hypoxic ventilatory response as an intermittent hypoxic stimulus **Resp Physiol Neurobiol** 145(1): 33-39.

RESEARCH QUESTION:

1) Is daily measurement of hypoxic ventilatory response a form of intermittent hypoxia?

METHODS:

The study was approved by the University of British Columbia's Clinical Research Ethics Board. Nine male subjects were recruited. Subjects were screened by a physician for a history of respiratory disease, cardiovascular disease or smoking. They were not taking any medication during the study period. Mean subject age was 26.7 ±6.2 years (mean ±SD).

Subjects visited the lab on a total of six occasions over a ten-day period. The visits took place on days 1, 6, 7, 8, 9 and 10. Subjects were asked to refrain from caffeine and ethanol intake and moderate exercise in the six hours prior to the laboratory tests. On the first visit, subjects were informed of the details and protocol for the study and informed consent was obtained. Resting spirometry was then performed according to the standards of the American Thoracic Society[1] to exclude occult respiratory disease by using a portable spirometer (Spirolab II, Medical International Research, Rome, Italy). Following spirometry, subjects underwent a resting isocapnic HVR test. This initial HVR test acted as the baseline test to which the repeated tests would later be compared. On subsequent test days, subjects came to the lab and relaxed quietly for a minimum of ten minutes before HVR testing.

The HVR testing protocol was based on an earlier method[2-6]. Subjects breathed through a respiratory mask (Hans-Rudolph 8980, Kansas City, MO, USA) attached to a one-way non-rebreathing valve (Hans-Rudolph 2700, Kansas City, MO, USA). Inspiratory flow was measured using a heated pneumotach (Hans-Rudolph HR800, Kansas City, MO, USA). Arterial O_2 saturation was measured using pulse oximetry at the finger (Model 503, CSI Criticare Systems Inc., Waukesha, WI, USA). End-tidal P_{CO_2} was sensed at the mouth using a CO_2 sensor (Model CD-3A, Applied Electrochemistry, Pittsburgh, PA, USA). Inspired O_2 concentration was sampled upstream of the non-rebreathing mask using an O_2 sensor (Model S-3-A/I, Applied Electrochemistry, Pittsburgh, PA, USA). A 13.5-litre mixing chamber was located upstream of the pneumotach. The manual addition of varying flows of 100% N_2 to the mixing chamber allowed control of F_IO_2. Ventilatory and gas values (flow, tidal volume, frequency, SaO_2, P_{CO_2}, F_IO_2) were displayed in real time during testing (PowerLab, ADI Instruments, Colorado Springs, CO, USA). Inspired volume was calculated using the integrated flow signal and the frequency of breathing. Data were sampled at 400 Hz.

The subjects rested in a supine position and breathed room air for five minutes in a darkened room while listening to ambient music to reduce external stimuli that could affect respiration. The test period started when 100% N_2 was introduced into the inspired gas mixture. The flow of N_2 was started at 2 litres per minute and increased at a rate of one litre per minute every 30 seconds. This protocol gradually lowered inspired O_2 concentration to approximately 5% over a period of approximately five minutes. To maintain isocapnia, CO_2 was added to

the inspired mixture using a manually controlled valve. The investigator monitored the displayed end-tidal P_{CO_2} value and adjusted the flow of CO_2 added to the inspired gas mixture, just proximal to the non-rebreathing mask. The test was terminated once the arterial saturation fell below 80%.

Data and Statistical Analysis

Ventilatory data were acquired on a breath-by-breath basis. The last minute of rest was used to calculate the resting ventilatory values. Ventilation was then plotted against saturation. Using the trendline function, a best-fit slope was plotted by computer (Microsoft Excel, Redmond, WA, U.S.A.). The absolute value of the slope was taken as the hypoxic ventilatory response. Using statistical software (STATISTICA 6.1, Stat Soft Inc., Tulsa OK, USA), resting tidal volume, frequency, minute ventilation and HVR were analysed using repeated measures analysis of variance (ANOVA) procedures, with time as the independent variable. Linear regression was used to find a trend in hypoxic ventilatory response over the five sequential test days. A p-value of 0.05 was used to determine statistical significance.

RESULTS:

Anthropometric data and spirometry are presented in Table 2.1. Mean resting ventilatory parameters (minute ventilation, frequency, tidal volume and end-tidal P_{CO_2}) are included in Table 2.2. No significant differences were noted

between the sample days for any of the resting parameters. (p-values were 0.75, 0.81, 0.94 and 0.83 for minute ventilation, frequency, tidal volume and end-tidal P_{CO_2}, respectively). The mean intra-individual coefficient of variation for resting end-tidal P_{CO_2} was 4%.

	Mean ±SD	% Predicted
Age (years)	26.8 ±6.1	
Height (centimetres)	181.0 ±5.8	
Mass (kilograms)	76.3 ±6.1	
Forced Vital Capacity (FVC) (litres)	5.60 ±0.97	103 ±13
Forced Expired Volume ($FEV_{1.0}$) (litres)	4.64 ±0.92	100 ±16
FEV/FVC (%)	82.6 ±4.0	99 ±6

Table 2.1: Anthropometric and spirometry data for all subjects. Data are expressed as means ±SD.

Day	Frequency (breaths·min^{-1})	Tidal Volume (litres)	Minute Ventilation (litres·min^{-1})	End-tidal P_{CO_2} (mmHg)	Hypoxic Ventilatory Response (litres·min^{-1}%·SaO_2^{-1})
1	12.9 ±4.3	0.92 ±0.31	10.56 ±2.42	41.6 ±2.1	0.70 ±0.58 (0.12 - 2.04)
6	13.2 ±3.1	0.90 ±0.20	11.04 ±2.00	43.1 ±2.1	0.70 ±0.40 (0.36 - 1.58)
7	13.6 ±3.9	0.92 ±0.22	11.60 ±2.46	42.2 ±2.1	0.70 ±0.59 (0.23-2.23)
8	14.2 ±3.2	0.85 ±0.24	11.44 ±1.19	41.9 ±4.0	0.68 ±0.38 (0.36 - 1.56)
9	14.2 ±3.7	0.91 ±0.23	12.36 ±3.53	42.4 ±6.0	0.80 ±0.72 (0.40 - 2.63)
10	13.6 ±3.1	0.86 ±0.17	11.43 ±3.52	42.7 ±2.1	0.66 ±0.43 (0.33 - 1.71)
Mean	13.6 ±3.6	0.89 ±0.23	11.40 ±2.52	42.3 ±3.3	0.71 ±0.51

Table 2.2: Resting respiratory parameters and hypoxic ventilatory response (HVR). Values are expressed in means ±SD. HVR range is shown in parentheses.

Figure 2.1 demonstrates a sample tracing from a single representative HVR test. Two minutes of resting data are demonstrated. The beginning of the test occurs once the F_IO_2 begins to decrease from its baseline value. As the haemoglobin saturation decreases following the F_IO_2, the ventilation gradually increases. In general, the increased minute ventilation was mediated through an

increase in both frequency and tidal volume, but the contribution of tidal volume seemed to be the larger of the two components.

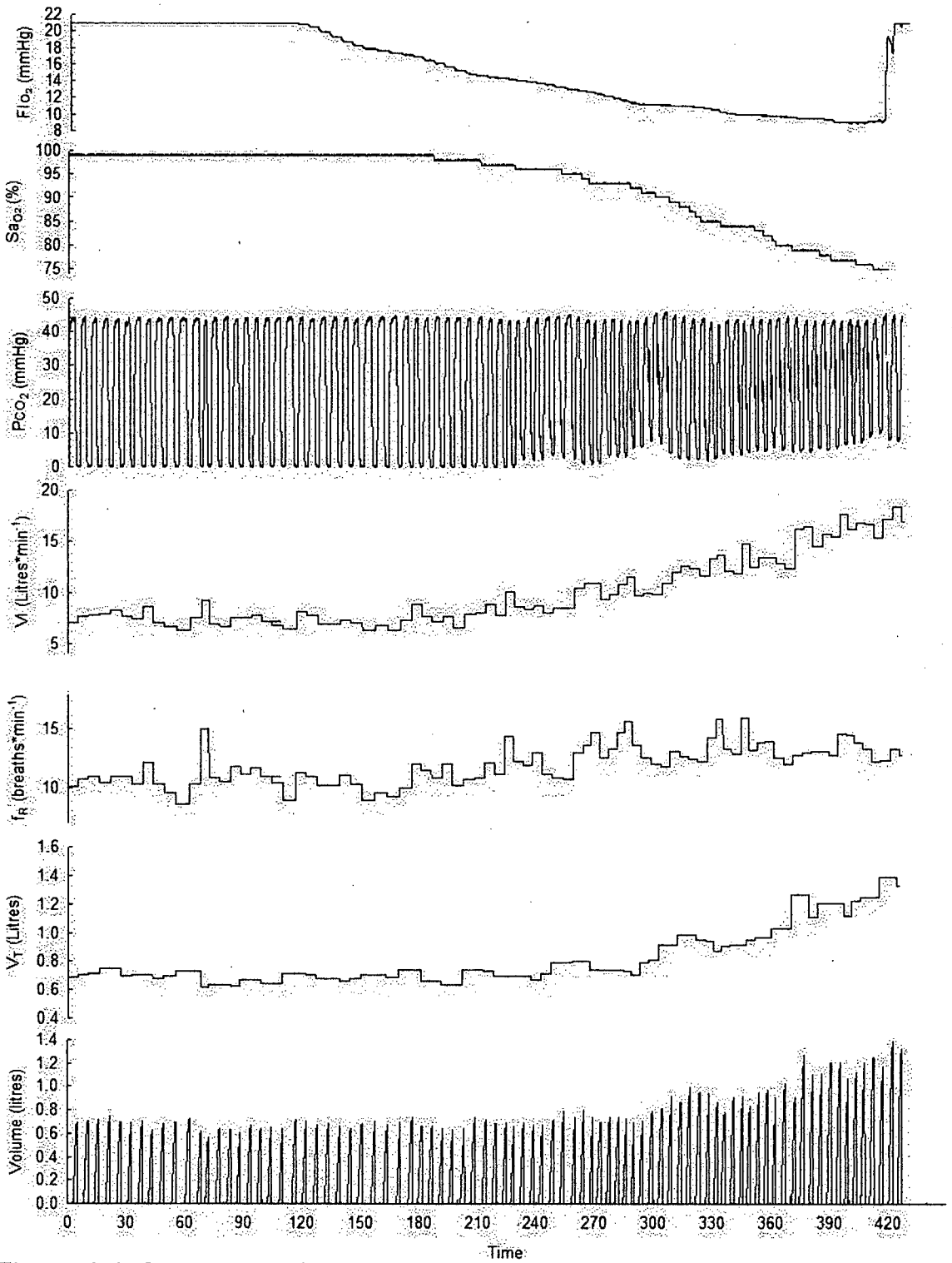

Figure 2.1: Sample data from one HVR test on one subject.

The HVR plot derived from the sample tracing in Figure 2.1 is shown in Figure 2.2. The x-axis (saturation) is plotted from right-to-left (with 100% saturation on the left) by convention to display a positive slope. Figure 2.3 demonstrates the individual HVR values for each of the subjects on each of the test days. Inter- and intra-individual variation was present in these values, but there was no obvious trend in HVR. Mean HVR values (with standard deviations) are presented in Table 2.2. There were no significant differences in HVR between any of the test days ($p=0.86$). Regression failed to show any trend in HVR over the five sequential days ($p=0.97$). The calculated mean coefficient of variation for HVR for each subject was 27%.

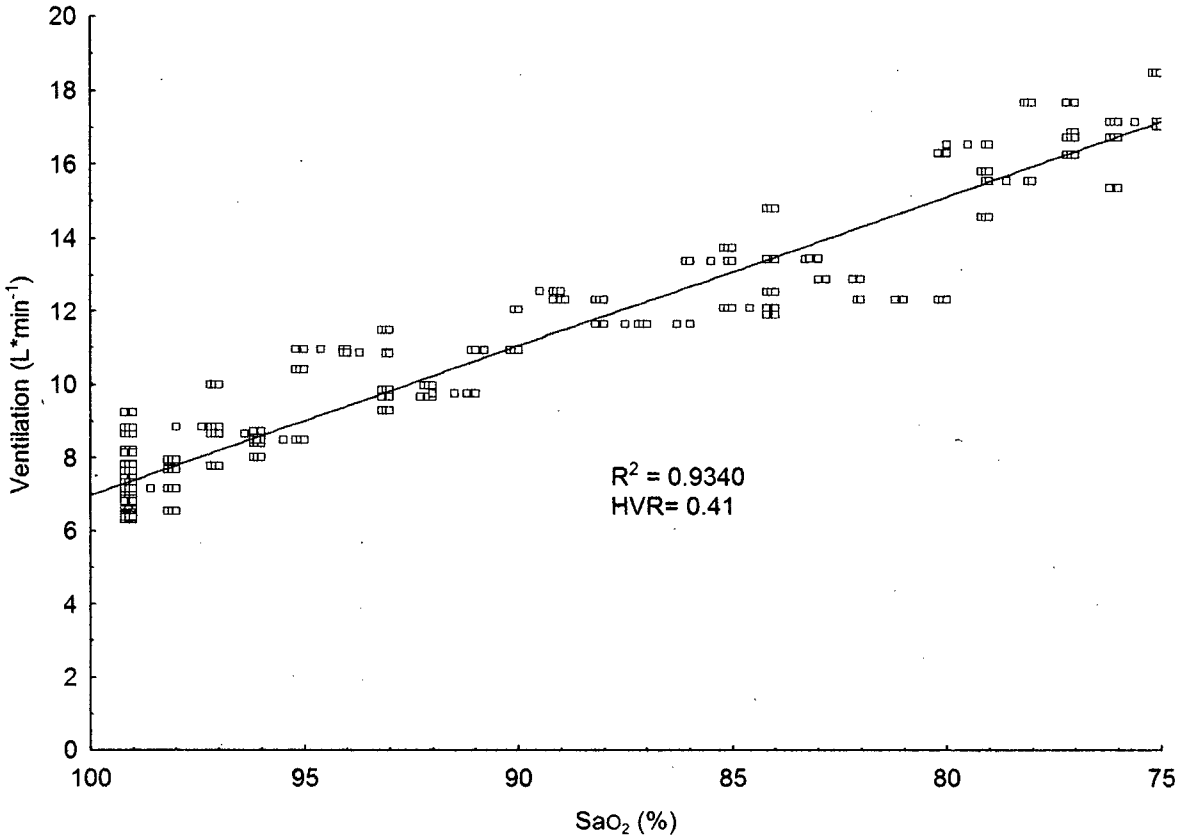

Figure 2.2: HVR plot using the data from Figure 2.1. The x-axis (saturation) is plotted right-to-left by convention.

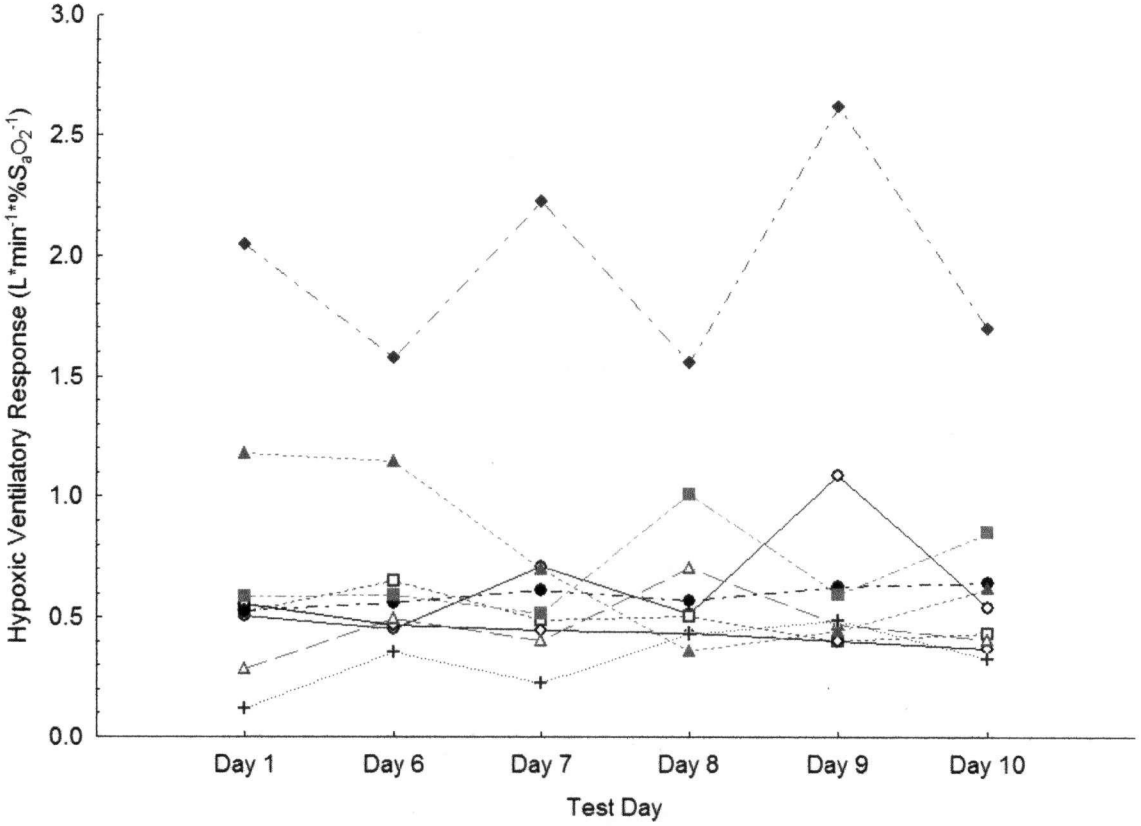

Figure 2.3: Summary plot of all individual HVR results for all subjects.

DISCUSSION:

To determine the optimal duration and frequency of intermittent hypoxia required to achieve the greatest change in HVR, daily measurements of HVR are required. Similarly, to measure the changes in HVR during acclimatisation to continuous hypoxia, daily measurements are desirable. To date, human studies that have involved repeated measurements of HVR during IH or acclimatisation (over days) [7 8] have not included a control group (i.e. subjects not exposed to episodic or intermittent hypoxia, but still had their HVR measured daily). Therefore, the independent effect of repeated measurements of HVR is unknown. This study is the first to examine whether repeated measurement of

HVR does in fact change HVR. The results failed to show a trend over 5 days of repeated HVR measurements, or a difference between the five measurements and a control measurement taken 5 days prior. The short exposure to hypoxia as part of HVR measurement is, therefore, likely not a co-intervention when measured repeatedly (24 hours apart) in physiological studies of acclimatisation and intermittent hypoxic training in humans.

This study also provides information about the repeatability of HVR measurements. HVR is a notoriously variable parameter[9-12]. Sahn et al.[9] compared intra-individual variability in HVR, and demonstrated that intra-day variability was much less than between-day variability. The coefficient of variation (CV) was 19% for intra-day measurements and between-day variability was 1.2-15 times greater than within-day variability. Zhang and Robbins[10] examined between-day variability in HVR (measurements were at least one week apart) and found a coefficient of variation of 26%. Other studies examining multiple HVR measurements in the same subject have shown much more variability,[9 11] with CV's reported as high as 76%. To our knowledge, there have been no published studies that have examined repeatedly measured HVR over several consecutive days (in the absence of other stimuli). Previous reports of between-day HVR variability have been at least a week apart.

The mean coefficient of variation for the present experiment was 27% (±13), comparable to the 26% reported by Zhang and Robbins[10]. Both studies measured HVR on six occasions in each subject. Unique to our study was the measurement of HVR over five consecutive days. Since the CV was comparable

between the two studies, it makes us more confident that repeated measurement of HVR does not lead to an independent augmentation of HVR. Furthermore, the methodology used in the present study represents a repeatable paradigm for assessing HVR which demonstrates a coefficient of variation that is comparable to previously published data [9-11].

Methodological Considerations

Arterial oxygen saturation was measured by pulse oximetry instead of by arterial catheterisation. Pulse oximetry has potential disadvantages that may influence the results. This method was chosen because it is non-invasive, portable and has a negligible complication rate as compared to arterial catheterisation. Pulse oximetry can be influenced by poor peripheral perfusion, as can occur during exercise, cold and Raynaud's phenomenon. In the present study, all these factors were excluded. Additionally, pulse oximetry fails to compensate for changes in pH and temperature which both affect the haemoglobin-oxygen dissociation curve. As subjects were at rest during the present study, there were no changes in temperature during the course of an HVR measurement. Changes in pH were minimised by strict control of end-tidal P_{CO_2} during testing.

The hypoxic exposures in this study involved isocapnic hypoxia. Although many intermittent hypoxic protocols utilise poikilocapnic hypoxia, there have also been studies documenting ventilatory changes following isocapnic intermittent

hypoxia[13-16]. As HVR is most often measured using an isocapnic hypoxic stimulus, we chose isocapnia for this study.

CONCLUSIONS

Repeated measurement of HVR does not lead to a change in HVR itself. Therefore, repeated HVR measurement does not act as a significant co-intervention in short-term acclimatisation and intermittent hypoxia studies. The HVR method used in the present study demonstrates comparable reproducibility to previously published data.

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CHAPTER 3: INTERMITTENT HYPOXIA AND ITS EFFECT ON RESTING MEASURES OF CHEMORESPONSE

RESEARCH QUESTIONS:

1) What is the effect of an intermittent hypoxia protocol on *resting* ventilatory response to hypoxia and hypercapnia?
2) Do two types of intermittent hypoxia protocols (short duration intermittent hypoxia and a long duration intermittent hypoxia) affect the above changes differently?
3) What is the time course of the change in resting ventilatory response to hypoxia as a result of intermittent hypoxia?
4) What is the relationship between changes in hypoxic ventilatory response and changes in both carbon dioxide threshold *and* sensitivity with intermittent hypoxia?
5) What is the relationship between changes in hypercapnic ventilatory response as measured by the traditional method as compared with the modified Read rebreathing method?

METHODS:

The study was approved by the University of British Columbia Clinical Research Ethics Board. A non-blinded randomised crossover study design was used. Subjects were evaluated before, during and following two intermittent hypoxia protocols: a short duration intermittent hypoxia (SDIH) and a long duration intermittent hypoxia (LDIH) protocol. The study paradigm is depicted in Figure 3.1. Ten male subjects were recruited from the University population and the local mountaineering and endurance sports community. The subjects were

healthy, recreationally active and had not travelled to altitude in the preceding 6 months. All subjects were asked to come to the laboratory a total of 19 times, totalling approximately 40 hours each. These visits included one orientation visit, seven visits for short-duration intermittent hypoxic exposure, seven visits for long-duration intermittent hypoxic exposure and four follow-up measurement sessions.

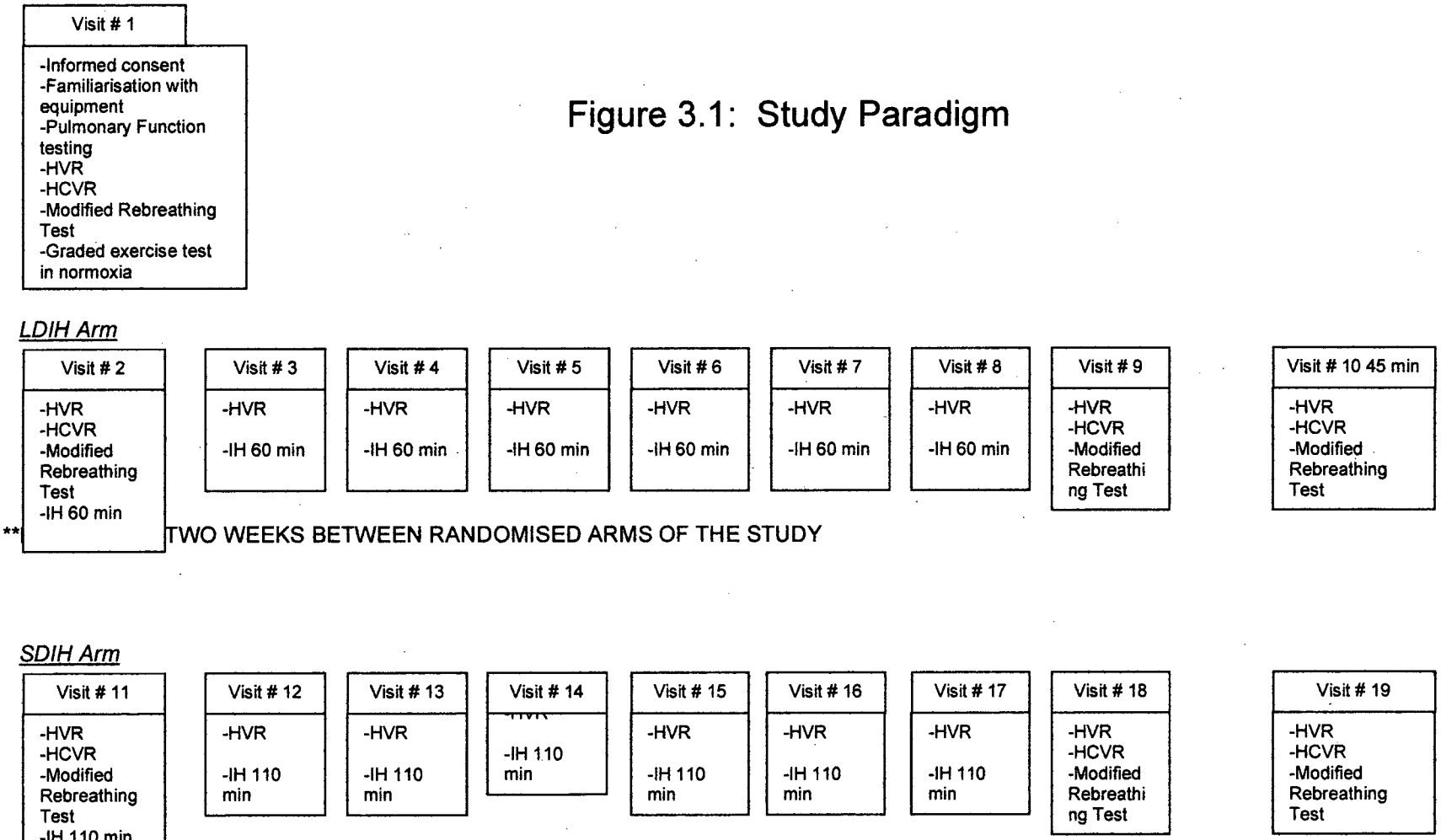

Figure 3.1: Study Paradigm

On the initial visit, informed consent was obtained, followed by baseline spirometry and familiarisation with the equipment. Subjects filled out a physical activity screening questionnaire[1] (PAR-Q, CSEP, www.csep.ca, Canada). At least one HVR test and two modified rebreathing tests were performed to acquaint the subjects with the testing protocol and equipment. Subjects also performed a normoxic maximal oxygen uptake test at this time on an electronically braked cycle ergometer (Lode, Groningen, Netherlands). A ramp protocol was used with wattage starting at 0 W and increasing by 0.5 W/s until volitional fatigue. Inspiratory flow was measured using a heated pneumotach (Hans-Rudolph, Kansas City, MO, USA). Minute ventilation was calculated using the integrated flow signal and the frequency of breathing. Arterial oxygen saturation was measured using pulse oximetry at the finger. Expired gases were collected in a mixing chamber and analysed using a carbon dioxide and an oxygen sensor (Applied Electrochemistry, Pittsburgh, PA, USA). Ventilatory and gas values were displayed in real time during testing (PowerLab, ADI Instruments, Colorado Springs, CO, USA). Respiratory values were averaged every 15s. The highest 4 consecutive values were averaged to determine maximal values. Peak power at the end of exercise was recorded.

Hypoxic Ventilatory Response

Resting ventilatory tests were performed in a quiet environment with distractions minimised. The HVR testing protocol followed the protocol used in

previous studies in the laboratory[2 3] and is based on an earlier method used in other facilities[4]. It is described in detail in Chapter 2 of this dissertation. Briefly, subjects breathed through a respiratory mask attached to a one-way non-rebreathing valve (Hans-Rudolph, Kansas City, MO, USA). Inspiratory flow was measured using a heated pneumotach (Hans-Rudolph, Kansas City, MO, USA). Minute ventilation was calculated using the integrated flow signal and the frequency of breathing. Arterial oxygen saturation was measured using pulse oximetry at the finger. End-tidal carbon dioxide and inspired oxygen concentrations were measured on a breath-by-breath basis using a carbon dioxide and an oxygen sensor (Applied Electrochemistry, Pittsburgh, PA, USA). Ventilatory and gas values were displayed in real time during testing (PowerLab, ADI Instruments, Colorado Springs, CO, USA). During the entire HVR test, subjects listened to quiet, ambient music through headphones. The subjects rested in a supine position while breathing room air for five minutes. The resting end-tidal carbon dioxide was determined from the last minute of this rest period. The test started when 100% nitrogen was introduced into the inspired gas mixture. The flow of nitrogen increased at rate of one litre per minute every 30 seconds. This protocol gradually lowered inspired oxygen concentration from 21% to approximately 5% over a period of five minutes. To maintain isocapnia, carbon dioxide was added to the inspired mixture using a manually controlled valve. The test ended once the arterial saturation reached 80%. Ventilation was then plotted against saturation, with the absolute value of the magnitude of the slope representing the HVR.

Breath-by-breath ventilation was then plotted against saturation. A best-fit slope was plotted by computer using the built-in trendline function in Microsoft Excel (Redmond, WA, U.S.A).

Hypercapnic Ventilatory Response Test

The hypercapnic ventilatory response (HCVR) testing protocol was based on the protocol of Katayama et al.[5] This test involved no prior hyperventilation. Following the HVR testing, subjects rested in a seated position prior to their HCVR test for approximately 5 minutes. Wearing nose clips, subjects breathed room air ad libitum through a three-way rebreathing valve (Hans-Rudolph, Kansas City, MO, USA) that was connected to a rebreathing bag. This bag was filled with 7% N_2, balance O_2. Inspiratory flow was measured using a heated pneumotach (Hans-Rudolph, Kansas City, MO, USA). Inspired volume was calculated using the integrated flow signal and the frequency of breathing. Arterial oxygen saturation was measured using pulse oximetry at the finger. End-tidal carbon dioxide and inspired oxygen concentrations were assessed on a breath-by-breath basis using a carbon dioxide and an oxygen sensor (Applied Electrochemistry, Pittsburgh, PA, USA). Ventilatory and gas values were displayed in real time during testing (LabVIEW 7.0, National Instruments, Austin, TX, USA).

After reaching a steady-state resting ventilation, subjects exhaled completely before they were switched over to the rebreathing bag. They took

three large breaths to equilibrate the gas in their lungs with that in the bag. Subjects were then asked to breathe ad libitum. The test was terminated once $P_{ET}CO_2$ reached 60mmHg, minute ventilation reached 100 litres per minute or in the instance of subject discomfort. HCVR sensitivity was determined as the slope of minute ventilation ($L \bullet min^{-1}$) plotted against end-tidal CO_2 (mmHg).

Modified Rebreathing Test

The modified rebreathing testing protocol was based on the protocol of Read[6] and modified by Duffin[7] [8]. At the start of each test, subjects hyperventilated for five minutes to reduce their end-tidal CO_2 partial pressure to between 19 and 25 mmHg. They were coached during this rebreathing period to maintain this desired end-tidal CO_2 level. Using a rebreathing valve (Hans-Rudolph, Kansas City, MO, USA), subjects were then switched to breathe into a bag that was filled with a mixture of 42mmHg CO_2 and either 50mmHg or 150mmHg of oxygen (for the hypoxic and hyperoxic tests, respectively). The rebreathing bag was maintained iso-oxic using a computer-controlled valve[9] (LabVIEW 7.0, National Instruments, Austin, TX, USA) while end-tidal CO_2 was allowed to progressively rise. The test was performed twice, with O_2 pressures maintained at either 50 mmHg (hypoxic condition) or 150 mmHg (hyperoxic condition). The test was terminated once P_{CO_2} reached 60mmHg, minute ventilation reached 100 litres per minute or in the instance of subject discomfort. Using specifically designed software[10], the data from each test was used to calculate CO_2 threshold and sensitivity. The model parameters are described in

detail in Duffin et al. 2000.[11] Briefly, the software fits a straight line to the CO_2/time relationship and derives a predicted CO_2 for each breath based on this model. Ventilation is then plotted against this predicted CO_2, and the model then fits three line segments to the rebreathing data. The first segment follows the exponential decline to basal ventilation following the cessation of hyperventilation. The second segment begins at the first breakpoint, the slope of which is reported as the sensitivity. If there is a second breakpoint, a third segment is plotted beyond this point. Breakpoints and other parameters are adjusted in an iterative manner to optimise fit via minimisation of the sum of squares (Levenberg–Marquardt algorithm)[12]. Using the technique, one will often find that above the threshold, the CO_2/ventilation relationship can appear to consist of two segments (Figure 3.2), the first one is more gradual and is mainly mediated through increases in tidal volume, whereas the second slope seems to be more frequency-mediated[11]. The sensitivity calculated using this software is for the first (lower) slope. As the second segment was not uniformly present, it was not assessed in the present analysis.

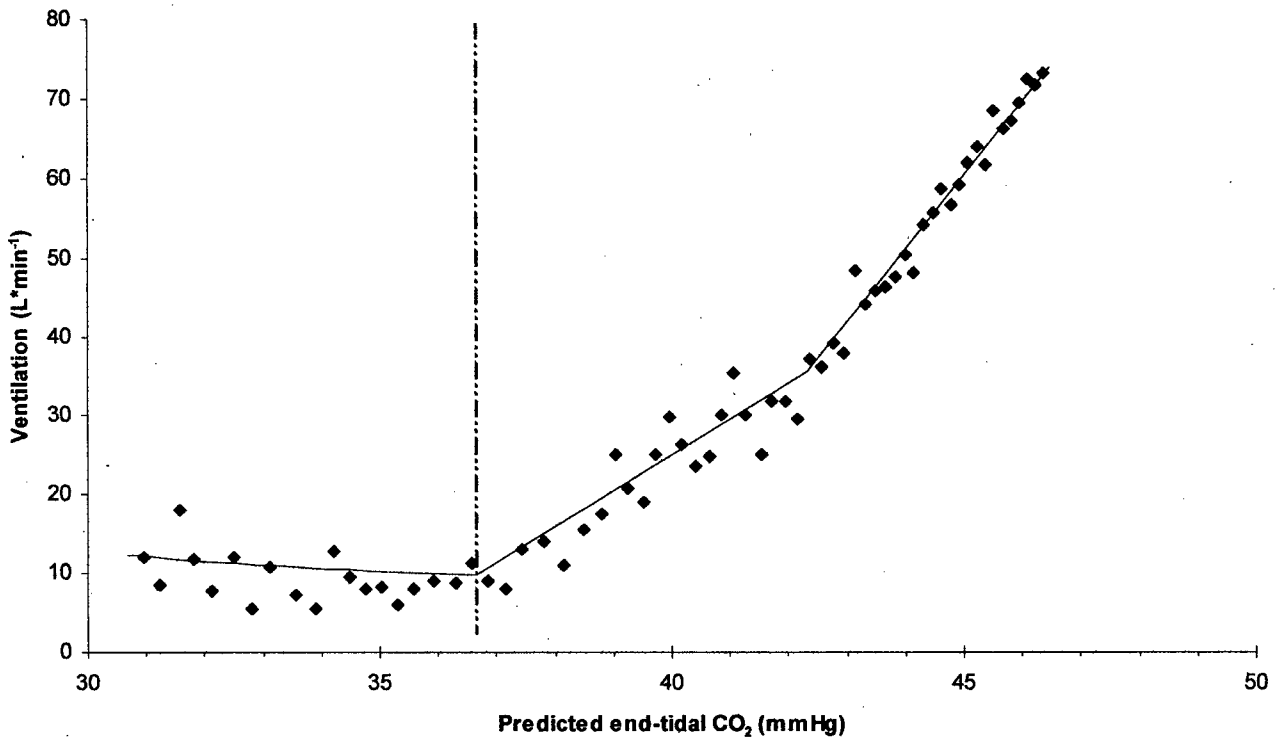

Figure 3.2: Sample data from an individual modified rebreathing test demonstrating the CO_2 threshold (dashed vertical line) and the two subsequent slopes in the CO_2/ventilation relationship. For the purpose of this study, the first slope was assessed to determine CO_2 sensitivity.

Intermittent Hypoxic Training

The final task for the subjects on their second visit day was their intermittent hypoxic training. Normobaric hypoxic gas (12% O_2, balance N_2) was provided by mask. For the LDIH protocol, subjects breathed the hypoxic gas for 60 minutes daily for seven days. This protocol was chosen because it was the normobaric equivalent of that used in Katayama's study that showed the increases in exercise ventilation during hypoxia[13]. For the SDIH protocol, the subjects spent 115 minutes breathing from the mask each day. They alternated

through 12 cycles of 5 minutes of hypoxia (simulated 4400 metres) followed by 5 minutes of normoxia.

Subjects were then required to return to the lab for 6 additional IH sessions. These were identical to the session on the first day. Each IH exposure was preceded by measurement of isocapnic HVR.

Follow-up

The first day following the final IH protocol the subjects returned for Post-IH testing. This testing was exactly the same as the pre-testing. It consisted of an isocapnic HVR test, an HCVR test and two modified rebreathing tests (hypoxic and hyperoxic).

One week following the completion of the first round of IH, subjects returned to the laboratory for 7-days Post-IH testing. This session included HVR testing, an HCVR test and two modified rebreathing tests.

Subjects were given at least two weeks washout between each arm of the study (range 14-94 days). Two weeks was chosen because previous studies had shown that HVR remained somewhat elevated at one week after intermittent hypoxia[13], but not two weeks post-IH[14]. Pre- and Post- testing was the same in both arms of the study; the only difference was the nature of the intermittent hypoxic training (SDIH or LDIH). The entire visit paradigm is demonstrated in Figure 3.1.

Statistics

Each of these resting tests (HVR, HCVR, Modified Rebreathing) were compared at three time points (Pre-, Post- and 7 days Post-IH) using ANOVA with repeated measures over time and IH protocol has an independent factor. Where the null hypothesis was rejected, Tukey's HSD was calculated to determine the significant differences. Data are presented as means (±SD). Linear correlations were also performed between HVR and CO_2 threshold (Pre-, Post- and Delta). Statistical analysis was performed using computer software (SPSS Inc., Chicago, IL, U.S.A.); an alpha of 0.05 was used to determine statistical significance.

Using previously reported data, using a very similar intermittent hypoxic protocol,[13] with a mean post-protocol HVR of 0.71 ±0.15 $L \cdot min^{-1} \cdot \%Sa_{O_2}^{-1}$, to detect a 25% difference in HVR (SDIH vs. LDIH, $\alpha = 0.05$ and $\beta = 0.80$) a sample size of 9.586 was required.

RESULTS:

All ten subjects completed all parts of the study. Anthropometric data, respiratory and exercise data are presented in Table 3.1.

Parameter	Mean	Standard Deviation
Age (years)	26.0	6.7
Height (cm)	177.2	8.1
Mass (kg)	72.8	13.9
Forced Vital Capacity - (FVC)	5.84	0.70
% Predicted FVC	112.9	9.8
Forced Expired Volume (1-second) ($FEV_{1.0}$)	5.01	0.61
% Predicted ($FEV_{1.0}$)	114.2	9.1
FEV/FVC (%)	85.9	3.8
% Predicted FEV/FVC (%)	101.5	4.0
Maximal Oxygen Consumption (Absolute) ($L \cdot min^{-1}$)	4.23	0.82
Maximal Oxygen Consumption (Relative) ($mL \cdot kg^{-1} \cdot min^{-1}$)	58.2	3.9
Maximal Power (Watts)	335	67

Table 3.1: Anthropometric, Respiratory and Exercise Baseline Data. FVC = forced vital capacity, $FEV_{1.0}$ = forced expired volume in one litre.

Hypoxic Ventilatory Response

HVR results are presented in Figure 3.3. Mean baseline HVR for both protocols was 0.47 (±0.23) $L \cdot min^{-1} \cdot \%SaO_2^{-1}$. After the 7-day IH protocols, HVR was increased by 93 (±120%) and 65% (±74%) (for LDIH and SDIH, respectively). This difference was significant ($p<0.01$ and $p<0.05$, for LDIH and SDIH respectively). The difference between the two protocols was not significant. One week post-IH, HVR remained non-significantly elevated with

both protocols. Individual data are presented in Figures 3.4 and 3.5. When protocol order was examined (to assess for potential learning effect) there was no difference in the change in HVR between the protocol that the subjects performed chronologically first and that which they performed second (Figure 3.6).

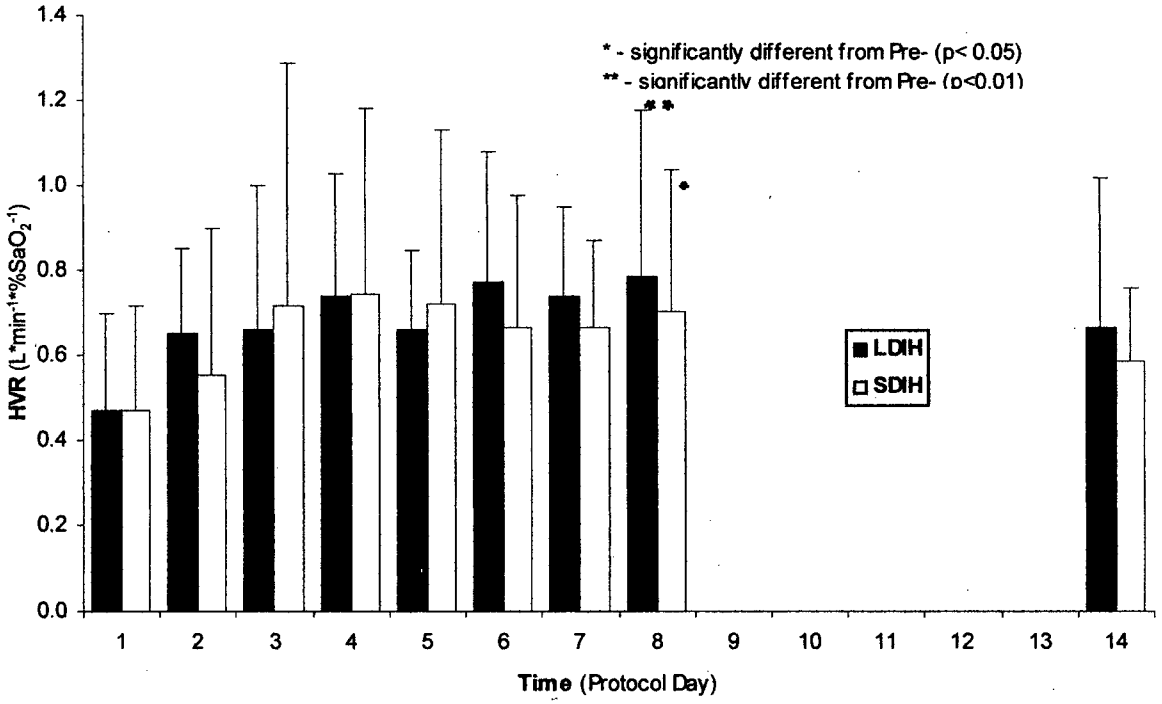

Figure 3.3: Mean (±SD) Hypoxic Ventilatory Response (HVR) vs. Time. The measurement on Day 1 occurs prior to the first hypoxic exposure. The measurement on Day 8 occurred the first day following intermittent hypoxia.

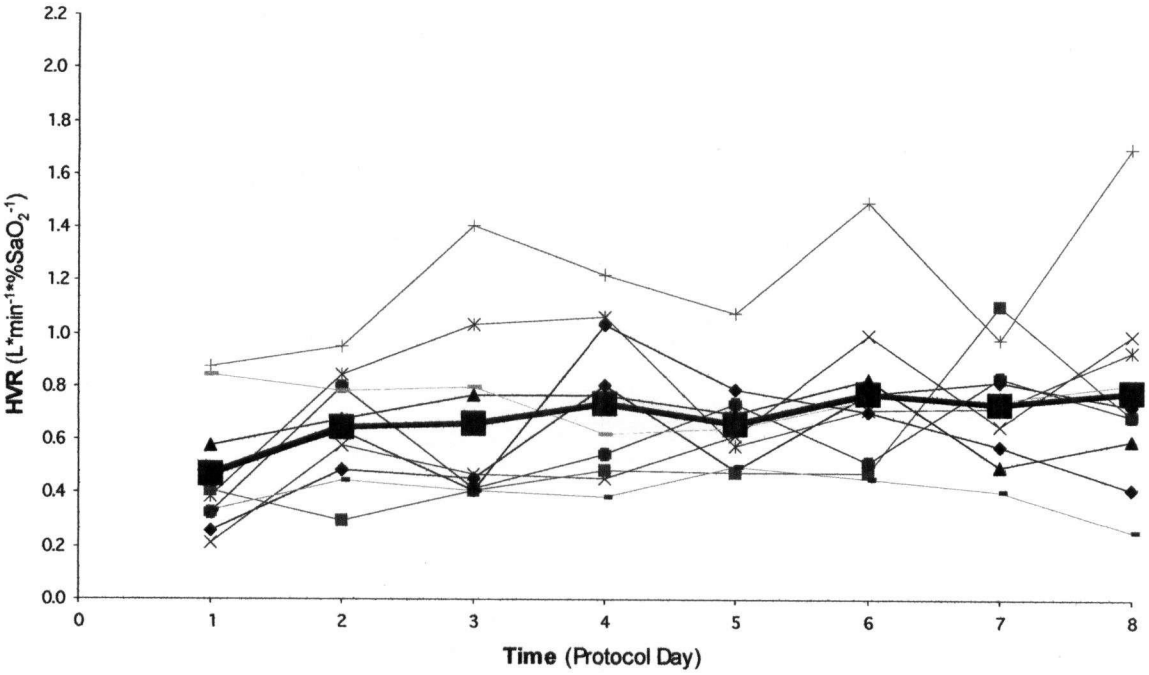

Figure 3.4: Individual Hypoxic Ventilatory Response (HVR) vs. Time during the LDIH Protocol. Thick black line denotes mean response. The measurement on Day 1 occurs prior to the first hypoxic exposure. The measurement on Day 8 occurred the first day following intermittent hypoxia.

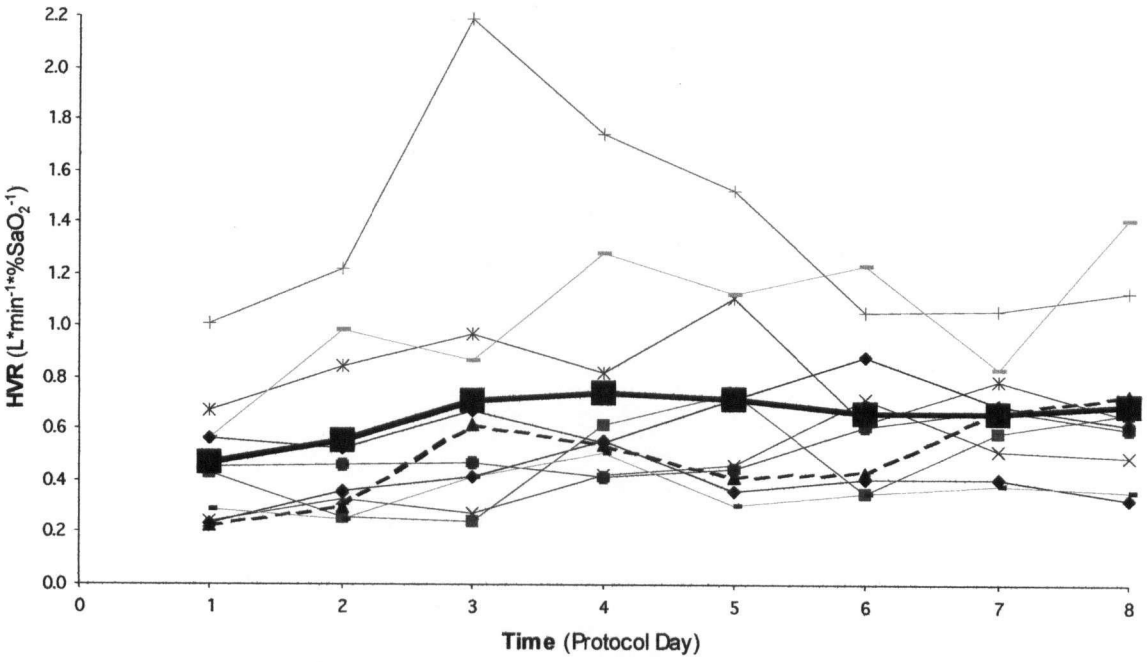

Figure 3.5: Individual Hypoxic Ventilatory Response (HVR) vs. Time during the SDIH Protocol. Thick black line denotes mean response. The measurement on Day 1 occurs prior to the first hypoxic exposure. The measurement on Day 8 occurred the first day following intermittent hypoxia.

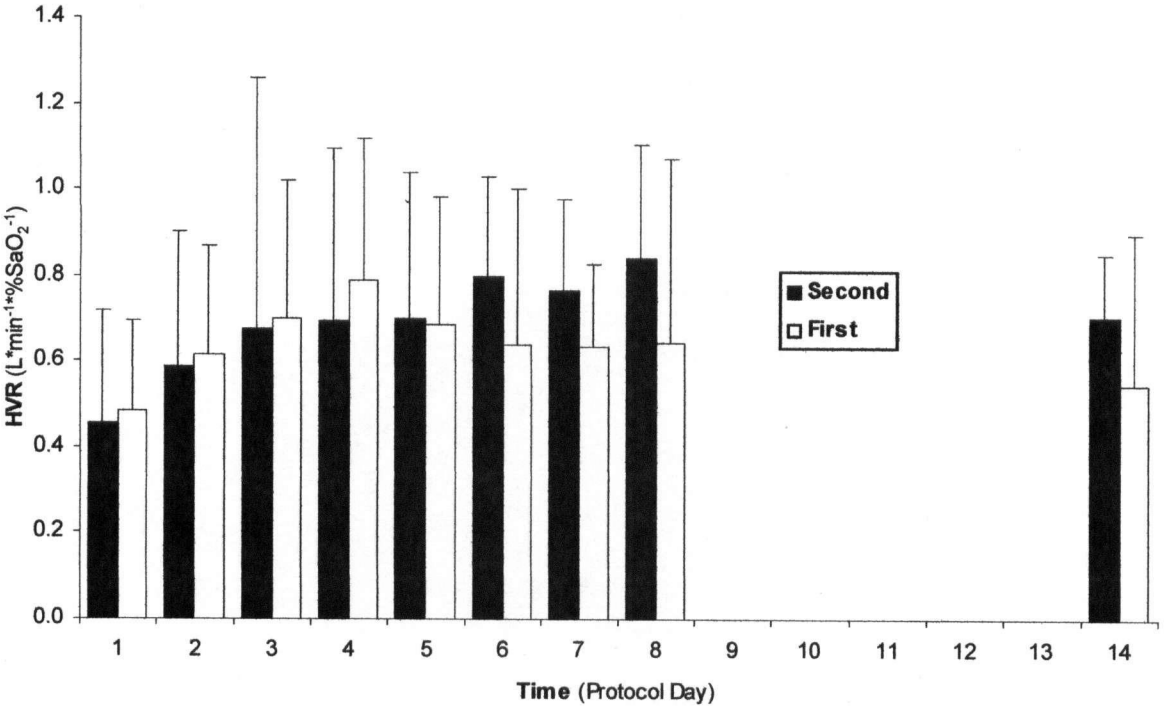

Figure 3.6: Mean (±SD) Hypoxic Ventilatory Response (HVR) vs. Time by Protocol Order. The measurement on Day 1 occurs prior to the first hypoxic

exposure. The measurement on Day 8 occurred the first day following intermittent hypoxia.

Both protocols caused non-significant increases in resting ventilation with the increase from LDIH (12.7 ±19.9%, p=0.074), more pronounced than that following SDIH (8.4 ±15.9%, p=0.13). There was no significant difference between the two protocols.

Modified Rebreathing

In both the hyperoxic and hypoxic modified rebreathing tests, the CO_2 sensitivity was unchanged by either protocol of IH. In hypoxia, the CO_2 threshold was significantly reduced following both protocols. LDIH reduced the threshold by 1.60 (±0.98) mmHg, whereas following SDIH it was reduced by 1.98 (±2.60) mmHg. Under hyperoxic conditions, LDIH reduced the CO_2 threshold by 2.06 (±2.33) mmHg, and SDIH caused a reduction of 2.53 (±1.36) mmHg. There were no significant differences between the two protocols. At 7 days following the IH, these threshold values were still lower than baseline (but not significantly so). These results are displayed in Figures 3.7 and 3.8.

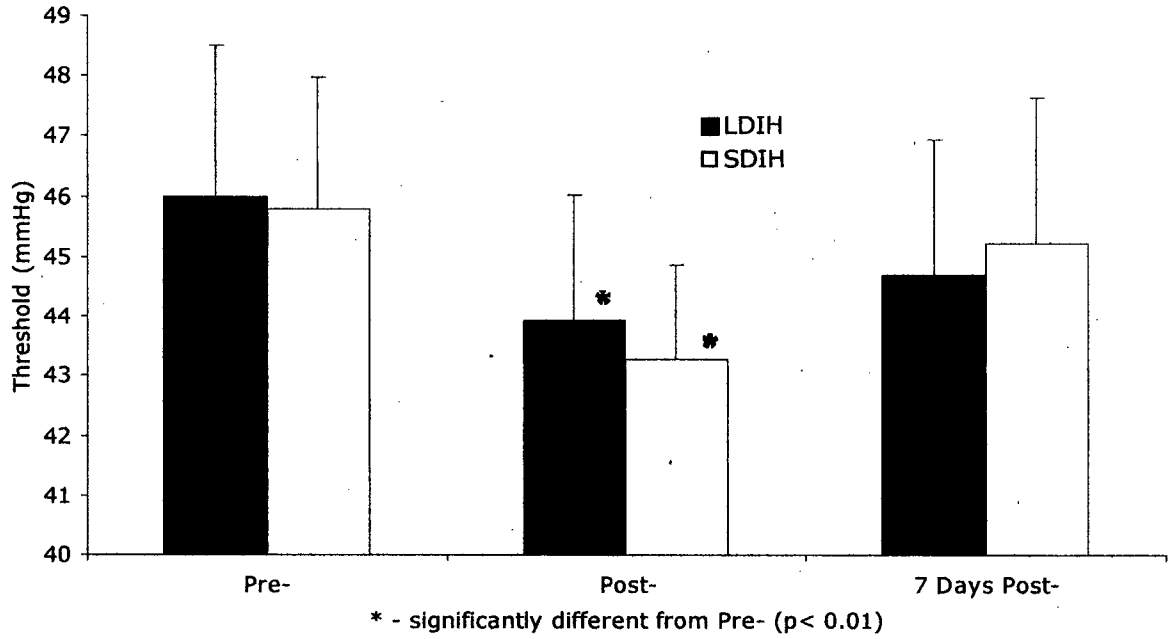

Figure 3.7: Mean (±SD) Carbon dioxide Threshold in Hyperoxia vs. Time (P_{O_2} = 150 mmHg).

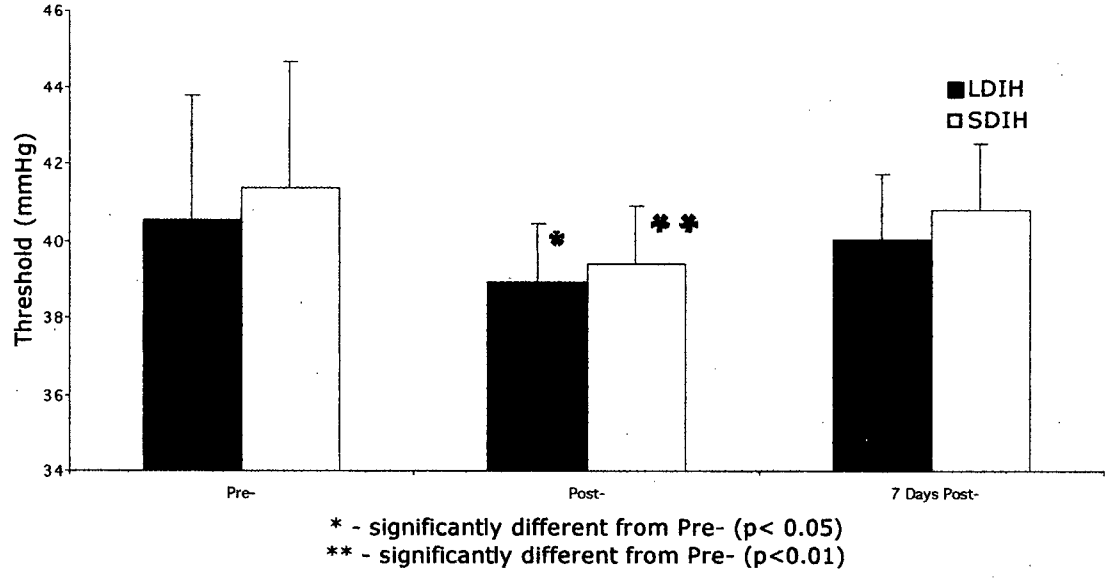

Figure 3.8: Mean (±SD) Carbon dioxide Threshold in Hypoxia vs. Time (P_{O_2} = 50 mmHg).

Hypercapnic Ventilatory Response

The Hypercapnic Ventilatory Response (HCVR) was significantly increased by IH by 42.9 (±63.4)% ($p<0.01$). This value remained elevated by 38.1 (±70.9)% at 7 days following IH ($p<0.01$). When analysed by protocol, HCVR was increased significantly by the LDIH protocol by 56.1 (±71.6)% ($p<0.01$) and remained elevated by 54.4 (±94.0)% at 7 days post ($p<0.01$). The changes following the SDIH protocol were smaller at 29.7 (±54.6)% and 21.9 (±34.4)%, at 1 and 7 days post IH, respectively. The increases following SDIH were not significant, nor were the differences between the two protocols. These data are presented in Figure 3.9.

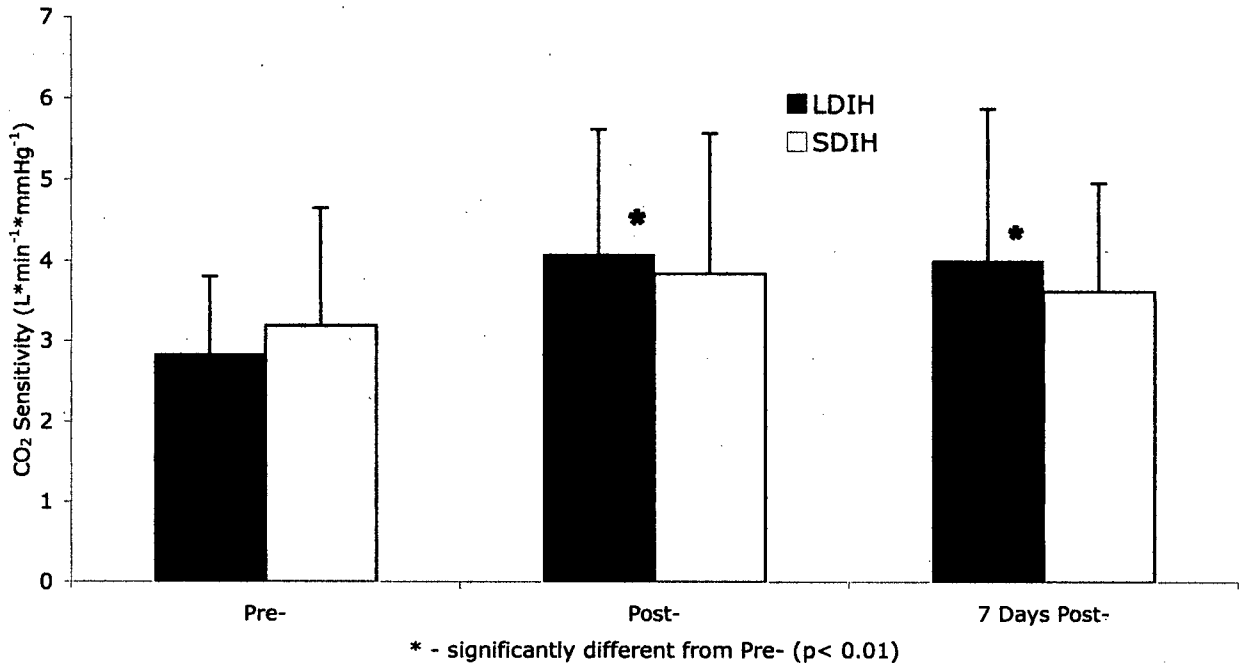

Figure 3.9: Mean (±SD) Hypercapnic Ventilatory Response (HCVR) vs. Time.

Associations between Chemosensitivity Measures

When HVR, measured prior to the first performed protocol was compared with the values from the Duffin technique, no significant correlations were found for CO_2 threshold (r= -0.499 and -0.625, for hypoxic and hyperoxic conditions, respectively) or sensitivity (r= 0.137 and 0.233). Secondly, when the change in HVR was compared with the change in CO_2 threshold by the Duffin technique, no significant correlations were found (r= -0.058 and 0.091).

When HCVR was compared with CO_2 sensitivity by the Duffin method (in hyperoxia), there was no significant relationship (p=0.532).

DISCUSSION:

Ten male subjects, acting as their own controls, underwent two consecutive different IH protocols in a random order.. Resting measurements of HVR, HCVR, CO_2 threshold and CO_2 sensitivity were performed prior to, immediately following, and one week following the IH. HVR measurements were also performed daily during the protocol. Intermittent hypoxia increased HVR by approximately 50% while decreasing the threshold to CO_2 in hypoxia and hyperoxia. HCVR was also increased following IH.

All the volunteers were healthy and recreationally active. The mean relative Vo_2max was 58.2 $mL \cdot kg^{-1} \cdot min^{-1}$, with a standard deviation of only 3.9. Thus in terms of fitness, these subjects represent a relatively homogeneous group with above average fitness for a recreationally active cohort.

Hypoxic Ventilatory Response

The observed increases in HVR are consistent with previous work that showed comparable changes in HVR with similar IH protocols[15 16]. This is the first study to measure the HVR daily over 7 consecutive days of IH. From these measurements it appears that majority of the augmentation of resting HVR following these protocols occurs in the first 4 days. The HVRs from the fourth and the eighth days were not statistically different. This finding may indicate that shorter IH protocols may be adequate if the goal of IH is augmentation of HVR.

Hypercapnic Ventilatory Response

The relationship between IH and augmentation of HCVR has been much less consistent. In earlier work, Katayama et al.[13] showed no change in HCVR after 7 days of poikilocapnic IH (60 minutes per day), but more recently they demonstrated an increase after a 14 day protocol (3 hours per day)[17]. Ainslie et al.[18] were also able to demonstrate an increase in the slope of HCVR following 5 nights of 8-9 hours of poikilocapnic IH. Using isocapnic IH (with only 30 minutes per day of hypoxia), Foster[15] showed no difference in HCVR. It appears that the studies that incorporate longer durations of poikilocapnic hypoxia tend to affect HCVR whereas those that maintain isocapnia or employ shorter bouts of hypoxia do not augment HCVR. Longer and poikilocapnic exposures may cause a more profound, prolonged hypocapnic stimulus to increase the sensitivity to CO_2.

Modified Rebreathing

Using the modified Read rebreathing tests, we were able to examine both the CO_2 threshold and the CO_2 sensitivity. The test was performed under both hypoxic and hyperoxic conditions. In theory, the hyperoxic trial attenuates the contribution from the peripheral chemoreceptors to preferentially target the central chemoreceptors as discussed previously. We found that CO_2 threshold was reduced following both IH protocols in hypoxia and hyperoxia. In the only other study to examine IH and CO_2 threshold[19], subjects were exposed to 14 consecutive daily exposures to 20 minutes of isocapnic hypoxia. Mahamed and Duffin found a decrease in threshold only under the hypoxic condition and not the hyperoxic

hyperoxic condition, attributing this alteration to the effects of intermittent hypoxia on the peripheral chemoreceptor in the absence of any change in CO_2. The current study differs in that the exposures were longer and were poikilocapnic; no study had previously looked at the effects of poikilocapnic IH on CO_2 response by the Duffin technique. Mahamed et al.[12] showed that the repeated hypoxic *hypercapnic* exposures of obstructive sleep apnoea caused an overnight increase in sensitivity to CO_2 (in the hyperoxic test) but no change in threshold. They found no changes in the hypoxic rebreathe test. In summary, it appears that intermittent hypoxia has variable effects on the CO_2 sensitivity and threshold in hypoxia and hyperoxia that depend on the level of CO_2 (poikilo-, iso- or hypercapnic) and the duration and severity of the hypoxia. A study that compares the CO_2 responses to intermittent exposures to a given dose of hypoxia but under poikilocapnic, isocapnic and hypercapnic conditions is required to clarify the role of CO_2 level on the effect of IH on CO_2 response.

In the current study, sensitivity to hypercapnia was increased following IH in the HCVR test, but not the hyperoxic modified rebreathing test. Furthermore, there was no correlation between HCVR and CO_2 sensitivity by the Duffin technique. This study is the first to compare HCVR and the Duffin technique following the same intervention, but is not the first time that discordant results have been obtained with the two measurements. Fuse et al.[20] and Mahamed et al.[12] both looked at overnight changes in response to hypoxic hypercapnia in patients with obstructive sleep apnoea using the HCVR and the Duffin method, respectively. Fuse found no change in HCVR overnight, while in a later study,

Mahamed found an increase in CO_2 sensitivity. Mahamed attributed this discrepancy to the fact that the Duffin method measures CO_2 sensitivity over a different range of end-tidal CO_2 than the HCVR method. Because the hyperventilation (used in Duffin's technique) reduces end-tidal CO_2 to a subthreshold level, the slope of the CO_2 sensitivity was measured from a lower point (by about 4 to 8 mmHg) than in the HCVR. This lower starting point becomes even lower following IH. Such a difference becomes important if the CO_2/ventilation relationship is not truly linear. In the tests where more than one sensitivity slope was evident, the lower slope (starting at the threshold) is assessed by the analysis software. As HCVR slope assessment occurs at higher CO_2 levels, these two assessments may not overlap as much as one would initially expect. Thus, the HCVR may be assessing response at higher partial pressures of CO_2 than the Duffin technique, leading to the different outcomes that were observed.

This is the not the first study to compare two measurements of CO_2 sensitivity and find differing results. Pandit et al.[21] compared CO_2 sensitivity by the steady-state and the Read rebreathing methods (without prior hyperventilation), and found that the sensitivity response was steeper in the rebreathing method. They also found differing effects on cerebral blood flow sensitivity to carbon dioxide between the two methods. Variations in cerebral blood flow may play a role in the differing responses to CO_2 observed in the present study. Hyperventilation reduces cerebral blood flow through its effect on

cerebral vasodilatation. This reduced cerebral blood flow could alter the tissue P_{CO_2}, and thus the response of the central chemoreceptors.

The 5 minutes of hyperventilation in the Duffin technique may cause other inputs to the control of breathing which are not present in HCVR measurement. For example, in some individuals, the 5 minutes of voluntary hyperventilation can induce a short-term potentiation of ventilation[22]. Furthermore, there may be behavioural inputs to ventilation following hyperventilation that may affect the result. Datta et al.[23] showed that ventilation following a period of hyperventilation to induce hypocapnia is affected by wakefulness. Subjects that were asleep showed a longer, more consistent apnoea following hyperventilation than while awake. The authors concluded that other behavioural drives affect ventilation during this period. Thus, cerebral blood flow, STP or behavioural drives to breathing may act as further modulators of ventilation, diluting the effect of an alteration in CO_2 sensitivity from IH. As the HCVR technique does not involve hyperventilation, it would not be subject to these other influences.

In summary, although both the HCVR and the modified rebreathing method each assess a form of CO_2 sensitivity, the results are not equivalent.

This discordance between HCVR and the modified Read rebreathing method may also relate to the CO_2 levels at which the sensitivity is assessed (higher with HCVR), or different inputs to ventilation brought about by the 5 minutes of prior hyperventilation. One way to evaluate the role of the prior hyperventilation would be to use a eucapnic voluntary hyperpnoea protocol, whereby the subject increases their ventilation but by inspiring a mixed gas

containing CO_2, they do not become hypocapnic. The behavioural and STP components of the hyperventilation would be maintained but CO_2 sensitivity would be measured in the range of the HCVR.

The advantage of the HCVR is that it measures central chemoresponsiveness without the confounding effects of the 5-minute prior rebreathe. The modified rebreathing technique is able to determine the CO_2 threshold under both hypoxic and hyperoxic conditions. Thus if threshold or peripheral chemoresponsiveness is the most important outcome, the modified rebreathing technique is most appropriate, while if central chemoreceptor response to CO_2 is the variable of interest, HCVR would be more appropriate.

SDIH vs. LDIH

There was no difference between SDIH and LDIH for any of the measured variables. As with the other studies of IH in humans, there is a large amount of inter- and intra-individual variation in the chemosensitivity measures[2]. This factor makes it more difficult to notice subtle differences between protocols. We therefore may not be able to rule out a small difference in efficacy between SDIH and LDIH, but a large (and arguably physiologically significant) difference between them is unlikely. Foster et al.[15] had similar findings when comparing SDIH and LDIH. In their study, the subjects did not act as their own controls, increasing the potential for variation. Furthermore, the IH protocol was 5 days on, two days off, 5 days on. This led to a somewhat irregular protocol causing a more uneven profile of HVR augmentation. The doses of IH in the current study

were also approximately double (in daily duration) that of Foster et al. Finally, in the study of animals that demonstrated the increased efficacy of SDIH over LDIH, Peng and Prabhakar[24] used poikilocapnic hypoxia, unlike Foster's group (who used isocapnic hypoxia). The current study used poikilocapnic hypoxia, which better replicates the animal work. These four factors should make the current study design more sensitive to a difference between SDIH and LDIH. Consequently, the absence of a difference in the current study should make one more confident of a lack of benefit of SDIH over LDIH in affecting resting chemosensitivity in humans.

SDIH and LDIH differ in the number of hypoxic on-transients and off-transients. The duration of SDIH (5-minute bouts) chosen in the present study was chosen to mitigate the effects of hypoxic ventilatory decline (HVD) on the stimulus to ventilation during the intermittent hypoxia. Theoretically, each bout was short enough to end before HVD could become a significant factor. Conversely, the LDIH protocol would expose the subjects to HVD each day. The finding that SDIH was not more efficacious than LDIH lends support to the concept that in humans, the number of transitions from hypoxia to normoxia may not be as important as the total exposure time to hypoxia. Furthermore, avoidance of any HVD effect may not be instrumental in stimulating augmentation of chemoresponse following IH.

Limitations

Oxygen saturation was estimated by pulse oximetry at the finger for all tests. The concordance between oxygen saturation at rest by pulse oximetry and by arterial blood gas measures is reasonable at saturations above 85%.[25] At saturations below this value, the accuracy of these devices deteriorates. The HVR protocol used in the current study required monitoring the subjects to an oxygen saturation of 80%. Presumably, the accuracy during the final portion of the HVR would be reduced, increasing the error. Unfortunately, because breath-by-breath monitoring of oxygen saturation is required, pulse oximetry is necessary (as direct measures could not provide the breath-by-breath values in real time). This factor has the potential to increase the variability in the HVR measurements and reduces the power of the study.

Another potential criticism is that the washout period may have been inadequate. Recent work from Katayama[17], suggests that if two IH protocols are done consecutively, the HVR might increase sooner in the second than in the first protocol (indicating a form of metaplasticity). To assess whether the length of the washout period was adequate, we compared the daily HVRs from the first and second protocols (chronologically) and found no significant differences. In Katayama's study, the daily IH exposures were 3 times as long as in the present study, which may mean that a more prolonged daily dose is required to cause this potentiation of HVR response to IH.

A sample size of 10 was chosen to detect a 25% difference between SDIH and LDIH post-protocol. Thus, a difference between the two protocols of less

than 25% would not have been detected. Thus there could have been a small difference between the two protocols that was undetected by the current study.

CONCLUSIONS:

Following two different 7-day IH protocols administered to subjects in a crossover fashion, there were increases in HVR and HCVR, along with a left shift in the CO_2 threshold in both hypoxia and hyperoxia. The majority of the augmentation in HVR occurred by the fourth day of the IH. No differences occurred between the SDIH and LDIH protocols in terms of respiratory response at rest. The poikilocapnic IH protocol appeared to cause more potentiation of the central chemoreceptors (as measured by HCVR and hyperoxic rebreathing methods) than in previous studies using shorter doses of isocapnic IH.

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CHAPTER 4: INTERMITTENT HYPOXIA AND ITS EFFECT ON EXERCISE CHEMOSENSITIVITY

RESEARCH QUESTIONS:

1) Does an intermittent hypoxia protocol affect exercise performance in hypoxia?
2) Does an intermittent hypoxia protocol affect *exercising* ventilatory response to hyperoxia and hypercapnia?
3) Does an intermittent hypoxia protocol change submaximal and maximal exercising ventilation under *normoxic* conditions?
4) Does an intermittent hypoxia protocol change submaximal and maximal exercising ventilation under *hypoxic* conditions?
5) Do two types of intermittent hypoxia protocols (short duration intermittent hypoxia and a long duration intermittent hypoxia) affect the above changes differently?

METHODS

This study was approved by the University of British Columbia Research Ethics Board. It was conducted in conjunction with the experiment described in Chapter 3 of this dissertation. The same crossover study design, intermittent hypoxia protocol and subjects were used for both studies. Using exercise testing, subjects were evaluated prior to and following two intermittent hypoxia protocols: a short duration intermittent hypoxia (SDIH) and a long duration intermittent hypoxia (LDIH) protocol. Ten male subjects were recruited from the University population and the local mountaineering and endurance sports community. All subjects were asked to come to the laboratory a total of 17 times. These visits

included one orientation visit, seven visits for short-duration intermittent hypoxia and seven visits for long-duration intermittent hypoxia and two follow-up measurement sessions.

On the initial visit, informed consent was obtained, followed by baseline spirometry and familiarisation with the equipment. Subjects filled out a physical activity screening questionnaire[1 2] (PAR-Q, CSEP, Canada). All exercise testing was performed on an electronically braked cycle ergometer (Lode, Groningen, Netherlands). Subjects also performed a normoxic maximal oxygen uptake test at this time. The initial normoxic graded exercise test was used to determine the subsequent submaximal work rates (the protocol for this test is previously described in Chapter 3 of this thesis). For the submaximal exercise test, work rates corresponding to 35% and 60% of the normoxic maximal work rate were used. (These values are expected to correspond to approximately 40% and 70% of maximal work rate breathing 15% O_2, based on previous research conducted in our laboratory.)[3]

Exercise Test

The exercise test consisted of three components: a normoxic submaximal test, a hypoxic submaximal test and a hypoxic graded exercise test. The normoxic and hypoxic components were separated by ten minutes of rest. Two work rates were used for each submaximal test. Low intensity corresponded to 35% of the normoxic maximal work rate, while moderate intensity corresponded to 60% of the normoxic maximal work rate. The exercise test protocol is depicted

in Figure 4.1.

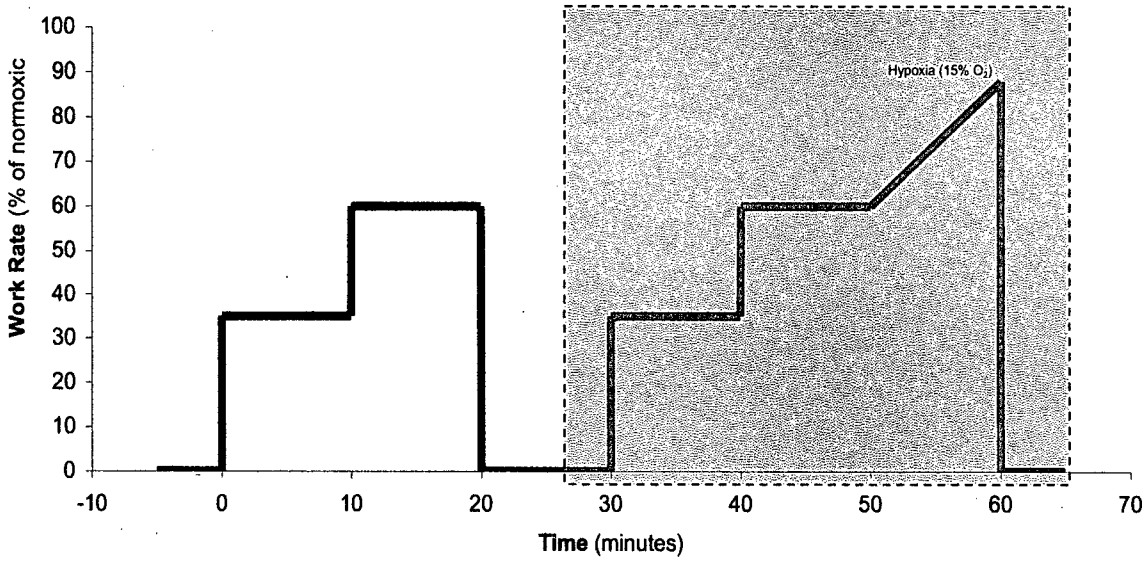

Figure 4.1: Exercise Testing Protocol – shaded area indicates hypoxic condition

The protocol used to assess the chemosensitivity during exercise was based on method previously used in this laboratory[4] and others[5]. After a self-selected warm-up, subjects commenced with the normoxic submaximal exercise test. They pedalled at 35% of normoxic maximal work rate for 10 minutes. During the final seven minutes of this exercise bout they received two hyperoxic and two hypercapnic stimuli. Each of these stimuli was separated by approximately two minutes (ventilation had always returned to the pre-stimulus level by approximately one minute post-stimulus). Steady-state ventilation was determined from the mean ventilation for each 30-second period prior to each chemoreceptor stimulus. The hyperoxic test consisted of abruptly switching the inspired gas from room air to 100% oxygen for 3 breaths before reverting to room air. To estimate the hyperoxic chemosensitivity during exercise, the mean

minute ventilation during the 30 seconds prior to the stimulus was compared to the nadir of the three-second moving average of the breath-by-breath minute ventilation post-stimulus. For the hypercapnic test, the inspired mixture was switched to 10% carbon dioxide (21% oxygen, balance nitrogen) for one single breath. To estimate the hypercapnic chemosensitivity during exercise the mean minute ventilation during the 30 seconds prior to the stimulus was compared to the peak of the three-second moving average of the breath-by-breath minute ventilation post-stimulus. The order of the hyperoxic and hypercapnic stimuli was randomised. The subjects were blinded to this order. Following ten minutes and two bouts of both hyperoxia and hypercapnia the low-intensity submaximal test was complete. Without stopping exercise, work rate was then increased to 60% of normoxic maximal work rate. Subjects then performed the same protocol a second time at this new work rate.

At the conclusion of the normoxic submaximal work rate subjects rested for approximately ten minutes before starting the hypoxic exercise test. The hypoxic mixture consisted of 15% oxygen (balance nitrogen) provided by mask at atmospheric pressure. This oxygen concentration was chosen because it was the lowest concentration at which subjects can consistently finish a maximal exercise test without reaching an arterial oxygen saturation below 70% by pulse oximetry[3]. Before starting to exercise in hypoxia, subjects rested for approximately 2 to 3 minutes while breathing the hypoxic mixture. The hypoxic submaximal exercise test followed the same protocol as the normoxic submaximal test. For the hypercapnic chemosensitivity test, subjects breathed

one breath of 10% carbon dioxide with 15% oxygen and balance nitrogen in order to maintain the F_IO_2 constant while only altering the F_ICO_2. The hyperoxic stimulus was unchanged from normoxic exercise (at 100% O_2 for three breaths).

Immediately following the hypoxic submaximal test, subjects transitioned to a graded maximal oxygen uptake test (still in hypoxia). At this point, the gas sample line was transferred from the mouthpiece (for breath-by-breath analysis) to a mixing chamber to allow for determination of oxygen consumption.

Intermittent Hypoxia Protocol

The final task for the subjects on their second visit day was their intermittent hypoxic exposure. Normobaric hypoxic gas (12% O_2, balance N_2) was provided by mask. For the LDIH protocol, subjects breathed the hypoxic gas for 60 minutes daily for seven days. For the SDIH protocol, the subjects spent 115 minutes breathing from the mask each day. They were exposed to 12 cycles of 5 minutes of hypoxia (simulated 4400 metres) followed by 5 minutes of normoxia. The order of the SDIH and LDIH arms of the study was randomised but not blinded. Subjects were required to return to the lab for 6 subsequent IH sessions.

Follow-up

The first day following the final IH protocol the subjects returned for follow-up exercise testing. This testing protocol was the same as in the pre-test.

Following a washout period of at least 2 weeks, subjects entered the second arm of the study, undergoing the IH protocol that they had not already completed.

Data Analysis

All exercise variables were assessed at two time points, on the first day of IH (Pre-IH) and on the first day following the completion of the IH protocol (Post-IH). Variables were compared using analysis of variance with repeated measures over time. Significance was set at $p<0.05$. Data are presented as means (±SD).

RESULTS

All subjects attended all laboratory sessions and performed all tests (to the best of their ability). No subjects left the study for any reason.

Submaximal Exercise Test

Minute ventilation was unchanged during both normoxic and hypoxic submaximal exercise following IH. These data are presented in Table 4.1.

Condition	**Intensity**	**LDIH**		**SDIH**	
		Pre-IH	**Post-IH**	**Pre-IH**	**Post-IH**
Normoxia	Low	40.4 ±8.5	41.2 ±7.6	41.4 ±8.5	41.4 ±7.8
	Moderate	68.6 ±12.1	70.1 ±11.0	72.1 ±15.6	71.6 ±11.4
Hypoxia	Low	46.3 ±9.9	47.3 ±8.7	48.8 ±9.4	48.9 ±10.3
	Moderate	91.6 ±19.4	89.4 ±14.1	93.2 ±17.4	92.2 ±17.3

Table 4.1: Exercise Minute Ventilation Pre- and Post-Intermittent Hypoxia (IH). Values are expressed in $L \cdot min^{-1}$ (±SD).

The hyperoxic trials caused a transient nadir in minute ventilation following 3 breaths of 100% oxygen. The decrease in minute ventilation following hyperoxic challenge was significantly higher in moderate intensity exercise than in low intensity exercise ($p<0.01$). The hyperoxic test also caused larger changes in ventilation in hypoxia than in normoxia ($p<0.01$). The effect of the hyperoxic tests on minute ventilation increased in magnitude following IH ($p< 0.05$) from 14.4 (±9.5) to 15.3 (±9.9). $L \cdot min^{-1}$. There was no difference between the two protocols. Data for each condition and protocol are presented in Table 4.2.

Condition	**Intensity**	**LDIH**		**SDIH**	
		Pre-IH	**Post-IH**	**Pre-IH**	**Post-IH**
Normoxia	Low	7.0 ±4.1	7.1 ±4.4	6.2 ±4.1	6.2 ±4.6
	Moderate	10.7 ±5.9	11.6 ±6.5	11.0 ±5.1	12.5 ±4.5
Hypoxia	Low	13.7 ±6.5	15.2 ±5.9	16.2 ±6.5	17.3 ±6.9
	Moderate	24.5 ±7.6	27.1 ±10.7	26.1 ±10.7	27.3 ±7.3
Mean		**14.0 ±8.9**	**14.7 ±10.1**	**14.9 ±10.1**	**15.6 ±9.6**

Table 4.2: Mean decreases in Minute Ventilation following 3 breaths of hyperoxia (Pre- and Post-IH). Values are expressed in % (±SD).

Results of the hypercapnic challenge were similar to those of the hyperoxic challenge. In contrast to the hyperoxic results, there were no differences in the decrease in the ventilation following the hypercapnic challenge as a result of the intermittent hypoxia ($p<0.01$ for both). Hypercapnia had more

of an effect in moderate than low intensity exercise and a larger effect in hypoxia than in normoxia ($p<0.01$ for both). Data for each condition and protocol are presented in Table 4.3.

Condition	Intensity	LDIH		SDIH	
		Pre-IH	Post-IH	Pre-IH	Post-IH
Normoxia	Low	8.8 ±4.1	12.5 ±7.1	8.6 ±4.1	13.6 ±5.4
	Moderate	10.7 ±5.9	11.6 ±6.5	13.6 ±5.4	13.9 ±6.8
Hypoxia	Low	12.6 ±6.1	11.5 ±6.7	12.8 ±6.8	13.3 ±7.8
	Moderate	17.3 ±5.9	15.3 ±6.9	13.6 ±6.6	17.0 ±7.9
Mean		**12.8 ±6.5**	**12.2 ±6.7**	**12.1 ±6.1**	**13.7 ±7.2**

Table 4.3: Mean increases in Minute Ventilation following 1 breath of hypercapnia (Pre- and Post-IH). Values are expressed in % (±SD).

Pulse oximetry results were unchanged during submaximal *normoxic* exercise following an IH protocol. In *hypoxia*, there was a significant increase ($p<0.01$) in mean arterial oxygen saturation following IH (both protocols combined). This increase was highly significant following the SDIH protocol, but not significant following the LDIH protocol. (Figure 4.2) The difference between the two protocols was not statistically significant.

To test the relationship between resting HVR and exercising ventilation in hypoxia, a correlation was performed between HVR and the change in minute ventilation between normoxic submaximal exercise and hypoxic submaximal exercise. No relationship was evident ($r=0.024$ and $p=0.947$).

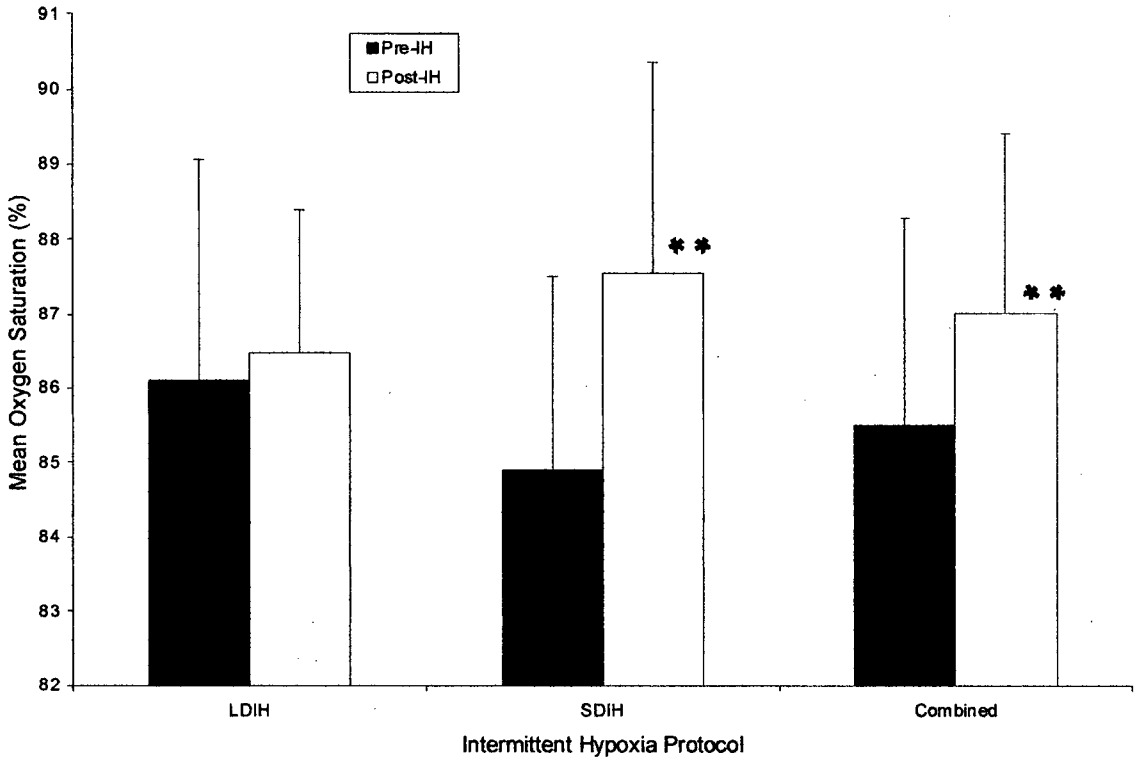

Figure 4.2: Mean (±SD) Oxygen Saturation (%) during Submaximal Hypoxic Exercise vs. Intermittent Hypoxia Protocol. **-denotes statistically significant ($p < 0.01$) from Pre- value.

Hypoxic Graded Exercise Test

Of the 10 subjects, 8 were able to complete the exercise protocol each time, including some portion of the hypoxic graded exercise test at the conclusion of the hypoxic submaximal exercise test. The two subjects who were unable to complete the entire exercise protocol stopped due to volitional exhaustion. For tests where the subjects were unable to complete the entire submaximal portion of the test, their highest submaximal wattage was taken as the peak wattage for that particular exercise test. Following IH, there were no changes in peak wattage, peak ventilation or peak oxygen consumption. These

data are presented in Figure 4.3. There were no significant differences between the two protocols.

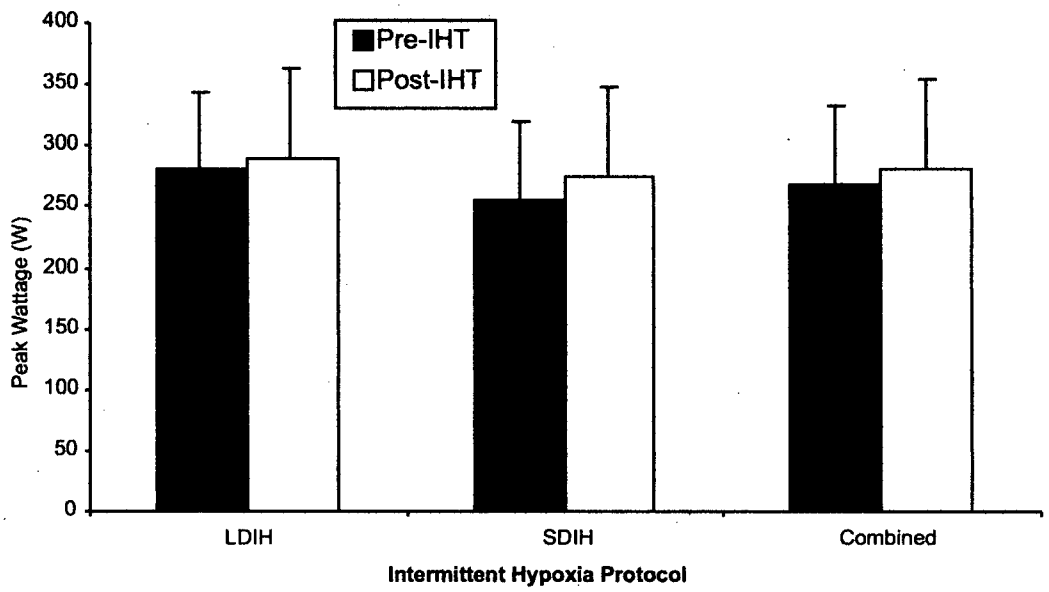

Figure 4.3: Mean (±SD) Peak Wattage vs. Intermittent Hypoxia Protocol. Combined refers to the collective data for both SDIH and LDIH protocols.

Of the 8 subjects who completed the entire exercise test, there were only complete oxygen consumption data on 5 of those subjects (due to difficulties switching the gas sampling from breath-by-breath to mixed gases mid-test). Following IH, peak oxygen consumption in hypoxia was 3.7 (±9.7)% higher, but this difference was not significant (p=0.12). This increase was similar with both protocols (displayed in Figure 4.4). Peak ventilation was unchanged following the LDIH protocol, but increased by 10.9 (±14.7) % after the SDIH protocol. Peak ventilation data are presented in Figure 4.5.

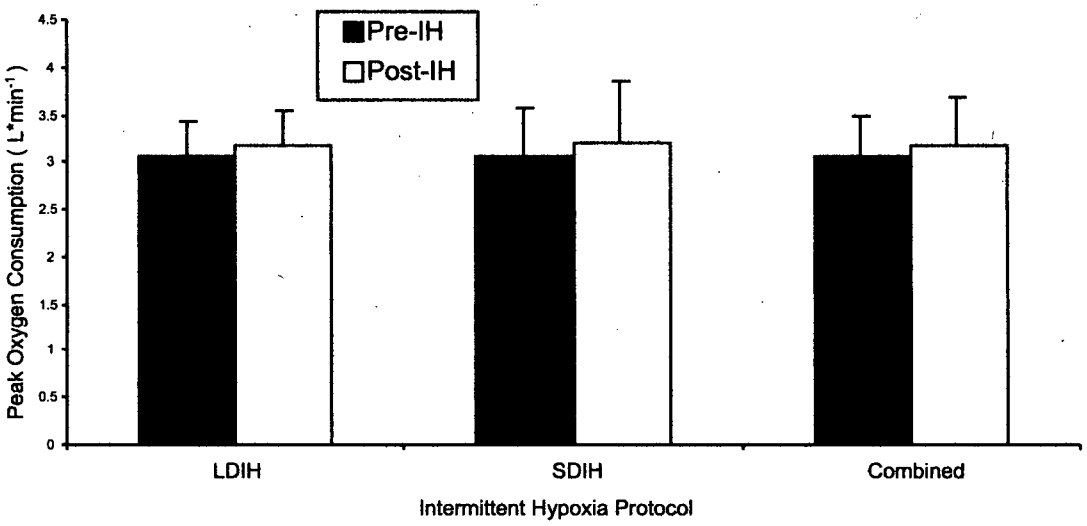

Figure 4.4: Mean (±SD) Peak Oxygen Consumption ($L \cdot min^{-1}$) vs. Intermittent Hypoxia Protocol. Combined refers to the collective data for both SDIH and LDIH protocols.

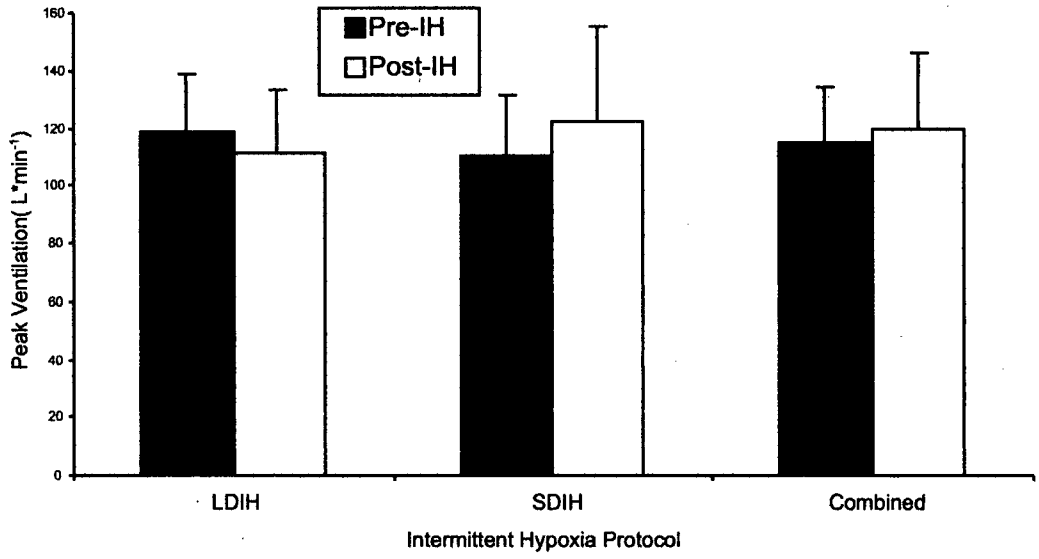

Figure 4.5: Mean (±SD) Peak Exercise Ventilation ($L \cdot min^{-1}$) vs. Intermittent Hypoxia Protocol. Combined refers to the collective data for both SDIH and LDIH protocols.

DISCUSSION:

Ten male subjects completed two different IH protocols in a random order, acting as their own controls. Submaximal exercise measurements of ventilation, saturation and response to hypercapnic and hyperoxic stimuli were assessed in normoxia and hyperoxia. A modified graded exercise test in hypoxia was also performed. All measurements were performed prior to and following IH. Following IH, small increases in arterial oxygen saturation during submaximal exercise were observed. IH did not affect minute ventilation, peak power or oxygen consumption during peak exercise. The responses to hyperoxic or hypercapnic stimuli during submaximal exercise were not significantly altered by IH, although the increase in response to the hyperoxic stimulus approached significance.

Minute Ventilation During Normoxic Exercise

Submaximal minute ventilation was unchanged following IH in both the normoxic and hypoxic conditions. Under normoxic conditions, many previous studies have shown no increase in submaximal exercise ventilation [6-9]. Using a similar IH protocol, the current study corroborates these findings. Conversely, two studies employing longer IH protocols[10 11] found an increase in submaximal minute ventilation during normoxic exercise following IH. The protocols involved 23 and 19 nights of 8-10 hours of hypoxic exposures that were slightly hypobaric (F_{IO_2}= 15.48% and 16.3% with P_{atm} ~710 mmHg). Townsend[11] found that the augmentation in ventilation was already apparent after the first four nights of IH

exposure. In these "live-high, train-low" protocols, subjects were sleeping in hypoxic enclosures (for 8-10 hours) as opposed to breathing from a mask while awake for only one hour each day. Two possible explanations exist for the difference between these two types of studies. One possibility would be that the longer durations of hypoxia might provide a more substantial stimulus to exercise ventilation that was only apparent in the overnight studies. Secondly, sleeping during hypoxia may have a different effect on ventilation than remaining awake during hypoxia. While alert, the subjects would have more ventilatory stimuli and would have a higher arterial oxygen saturation for a given level of hypoxia. Periodic breathing occurs in hypoxia[12], but would be more common in sleeping subjects than in awake subjects. An increase in periodic breathing could also lead to more profound hypoxaemia (and a stronger IH stimulus) in the sleeping subjects than if they were awake. Thus, these "live-high, train-low" studies might provide a greater hypoxic stimulus than those of Katayama and the current study leading comparatively larger increases in HVR[11]. As exercise ventilation is controlled by a larger number of factors than resting ventilation, the effect of augmented HVR on exercising ventilation could be obscured by these other factors. Consequently, a larger increase in HVR (by longer exposures during sleep) may be required to cause measurable changes in submaximal exercise ventilation. More research is needed to compare awake to sleeping IH and to assess the effects of longer daily doses of hypoxia.

Minute Ventilation During Hypoxic Exercise

In the one previous study to look at submaximal exercise under hypoxic conditions, Katayama showed a significant increase in submaximal exercise ventilation under *hypobaric hypoxic* conditions[13]. In that study, subjects exercised at 432 mmHg (approximately equivalent to an F_{IO_2} of 11.9%). In contrast, our subjects exercised in normobaria at an F_{IO_2} of 15%. The exercise type (cycle ergometer) and duration in both studies was 10 minutes each at low and moderate intensities, respectively. Even the exercise intensities were comparable; in Katayama's study, low and moderate intensity corresponded to 40 and 70% of *hypoxic* V_{O_2peak}, while in the current study, low and moderate intensity corresponded to 35 and 60% of *normoxic* V_{O_2peak}. The IH protocols (60 minutes/day for 7 days) were very close, except that in the Katayama study the exposures were hypobaric.

There are two potential explanations for the discrepancy between the two studies: the effects of hypobaria, and the timing of the hypoxic exposures with the onset of exercise. The differential effects of normobaric hypoxia and hypobaric hypoxia are unclear. Two studies in humans have shown that resting ventilation in normobaric hypoxia is higher than in an equivalent level of hypobaric hypoxia[14 15]. If an equivalent augmentation of *exercising* ventilation occurred in normobaric hypoxia, over hypobaric hypoxia, it could possible explain the discrepancy. That is to say, higher baseline ventilation occurring during normobaric hypoxic exercise could mask an effect of IH on exercise ventilation.

In a hypobaric chamber, to bring subjects to the desired simulated altitude requires approximately 30 minutes; subjects therefore would have a short period of progressive hypoxia prior to the onset of exercise. In the current (normobaric) study, subjects breathed hypoxic gas at rest for only 2-3 minutes prior to the start of exercise. Thus, in the two studies, subjects' submaximal exercise ventilations were tested at different time points during an acute exposure to hypoxia. Hypoxic Ventilatory Decline (HVD) refers to the decrease in the acute ventilatory response to hypoxia that occurs within 5 to 30 minutes of exposure to hypoxia[16]. Presumably, in the normobaric hypoxic study, subjects would have started exercising prior to the onset of HVD, while in the hypobaric study; the measurements would not have commenced until after this decline had already occurred. HVD has been described only during rest, but this attenuation of ventilatory response could also occur during exercise. If IH acted to mitigate the effects of HVD on exercising ventilation, its effect would only be evident if the exercise took place following the onset of HVD (as in Katayama's study) and not in the present study.

In summary, IH does not appear to augment exercising ventilation in normobaric hypoxia as it does during hypobaric hypoxia, possibly because the effects of hypobaria on ventilation or the presence of an HVD phenomenon in the hypobaric study.

Hyperoxic Test

The 3-breath doses of 100% oxygen caused transient decreases in ventilation that were more pronounced during the hypoxic condition and the moderate exercise intensity. IH significantly enhanced these changes. The purpose of this hyperoxic challenge test was to remove the hypoxic drive to ventilation from the peripheral chemoreceptors. The difference between the nadir in post-hyperoxic stimulus ventilation and the mean pre-stimulus ventilation was ascribed to the hypoxic drive from the peripheral chemoreceptors during exercise. These findings could indicate that augmentation of hypoxic ventilatory response at rest leads to a concomitant increase in the magnitude of the hypoxic drive during exercise. The findings do not improve our understanding of the location of the modulation in hypoxic respiratory control. Evidence indicates that either the carotid bodies themselves, or central facilitation of chemoreceptor afferents are modulated through a metaplastic process.[17 18]. Either mechanism (or a combination of both) could potentially augment the chemoreceptor afferent input to exercise ventilation.

One would expect that if resting hypoxic ventilatory response played a significant role in the control of the hyperpnoea of exercise, that those with a higher resting HVR would have a larger augmentation in ventilation when transitioned from normoxic to hypoxic exercise. This situation did not occur, in the present study. Furthermore, the lack of effect on submaximal minute ventilation would indicate that although this hypoxic drive to breathe may have been increased, that it's effect on total ventilation during exercise is modest.

Work by Sheel et al.[19] compared resting HVR to maximal ventilation during a graded exercise test. No relationship was found, indicating that resting HVR is not a major determinant of peak exercise ventilation.

There are two potential reasons for the unchanged minute ventilation in the face of augmented hypoxic drive to exercise. The augmented hypoxic drive could alter the pattern of ventilation without changing the minute ventilation. For example, when HVR is increased by chronic hypoxic exposure to altitude, there is an augmentation of tidal volume[20] preferentially over breathing frequency. In the current study, although minute ventilation was unchanged, there could have been an increased in tidal volume that was not apparent when minute ventilation data was compared. This change would have the effect of enhancing alveolar ventilation without increasing minute ventilation.

Alternatively, the increased hypoxic drive to ventilation during exercise is of too small a magnitude to increase minute ventilation. The size of the ventilatory transients following hyperoxic challenge was only increased by about 6%. Considering the magnitude of other neural and humoral inputs to exercise hyperpnoea, this altered chemoresponse could be just to small to lead to a perceptible effect. Further analysis of the pattern of breathing during exercise is warranted to understand the interaction between enhanced hypoxic drive and minute ventilation.

Hypercapnic Test

To test the effect of IH on CO_2-mediated control of breathing during exercise, subjects were given single breath doses of 10% CO_2 at several points during their submaximal exercise tests. These hypercapnic breaths caused a transient increase in ventilation that was unaffected by IH. At rest, IH caused a left shift in the CO_2 threshold that would increase the ventilation at a given end-tidal CO_2 pressure. This study is the first to modulate response to CO_2 during rest, and then to test its effect during exercise. There were no differences in the effect of the hypercapnic tests following IH, suggesting that the augmentation of resting CO_2 response following IH did not lead to significant changes in CO_2 control of exercise ventilation.

The changes in ventilation following the hyperoxic and hypercapnic tests were larger in moderate exercise than in low intensity exercise. The load on the ergometer was independent of cadence. Because subjects were encouraged to maintain their cadence constant throughout the exercise test, workload would have limited the effect of mechanically sensitive peripheral limb afferents on exercise hyperpnoea. The modulation of exercise hyperpnoea would therefore have been mediated by a variety of central, humoral, behavioural and factors. At the moderate intensity, the magnitude of the hypoxic drive was increased, indicating that its effect on exercise hyperpnoea was undiminished as compared to low intensity exercise. These changes in ventilation were also more pronounced in hypoxia than in normoxia. Presumably in hypoxic exercise, the

contribution of the hypoxic drive to exercise minute ventilation would be more important. Removing this drive with hyperoxia would then cause a larger drop in ventilation.

Oximetry during Submaximal Exercise

Arterial oxygen saturation was obtained using pulse oximetry, which has limitations during exercise[22], and must be interpreted cautiously. There was no effect of IH on normoxic oxygen saturation during exercise, but IH caused a significant increase in saturation during hypoxic exercise. This increase in saturation was not associated with a concomitant increase in minute ventilation, so the reasons for this finding are unclear. Two potential explanations (decreased oxygen consumption, and improved oxygen delivery) are presented.

Several studies[7 10 23] have shown a decreased oxygen cost during submaximal running following IH with no change in exercise minute ventilation. This decreased oxygen consumption for a given workload has been attributed to improved efficiency. Potential mechanisms for enhanced efficiency include a decreased cost of breathing, greater carbohydrate utilisation for oxidative phosphorylation, or reduced consumption of adenosine triphosphate by the muscle[10 23]. A similar increase in exercise efficiency in the present study could explain the increased arterial oxygen saturation.

Secondly, if the pattern of ventilation had changed, such that tidal volume was preferentially increased, improving alveolar ventilation, saturation would be increased without an overall increase in minute ventilation. The increased

hypoxic drive to ventilation could have led to a preferential increase in tidal volume thus reducing the physiological dead space and enhancing oxygen saturation. IH could also have improved arterial oxygenation with no change in ventilation through beneficial effects on diffusion capacity, lung ventilation/perfusion or a decrease in shunt (although these changes following IH have not been studied). In summary, several possible mechanisms could explain all or part of the improved saturation following the SDIH protocol; a more in-depth assessment of oxygen consumption, respiratory parameters and arterial blood gases would be required to better characterise this finding.

Maximal Exercise Test

When the data for both protocols was assessed, there were no significant differences in peak wattage, peak ventilation or peak oxygen consumption during a normoxic graded exercise test. From previous work, the effect of IH on maximal performance is unclear. Studies that have looked at maximal exercise performance in *normoxia* following IH have failed to show a significant increase in peak power output[9 13 24 25], except with concurrent exercise training[26]. This study is the first to examine the effect of IH on peak exercise performance in *hypoxia*. Conceivably, IH could have improved oxygen delivery during hypoxic exercise, either through alterations in mitochondrial activity, muscle buffering capacity or oxygen delivery, but the current study provided no indication of such an improvement.

Unfortunately, due to technical constraints, the sample size for peak V_{O_2} determination was only 5. No significant changes in V_{O_2} were evident. A larger sample size would be necessary to confidently determine the effects of IH on peak oxygen consumption during exercise.

SDIH vs. LDIH

There were no significant differences among any of the measured variables between the two IH protocols in the current study. We had hypothesised that the SDIH protocol, which seemed to more profoundly augment carotid sinus nerve activity in animals[27], may have had a similar effect in humans (observable as an increase in ventilatory response to hypoxia). Intermittent hypoxia appeared to enhance the hypoxic drive to breathe during submaximal exercise, but there was no differential benefit of SDIH over LDIH. This lack of benefit of SDIH over LDIH parallels the results found in the resting measures discussed in Chapter 3. Although the current study had no effect on submaximal minute ventilation, one could argue that if there had been a more profound augmentation of hypoxic ventilatory response as in the "live-high, train low" protocols, a difference between the two protocols might have become be evident. Unfortunately, the protocols that seem to have some effect on exercising ventilatory parameters (such as that of Townsend et al.[11]) are not conducive to SDIH, because tents and chambers need such a long time to establish hypoxia (30 minutes to 2 hours).

Limitations

Due to subject fatigue and technical difficulties, the sample size was very limited for the final part of the graded exercise test. With such a low sample size (n = 5), a very large difference (or low variability) in the measured values would need to be present to be detectable. A rest period before the graded exercise test would give the subjects a short recovery period prior to attempting the graded exercise test. The disadvantage of this modification is that the exercise testing sessions for the subjects were already extremely long and this modification would lengthen it by approximately 20 minutes.

With the current setup, breath-by-breath data was collected during the submaximal exercise bouts, and mixed gases were collected for the graded exercise testing. Using a parallel setup, it would be possible to collect both types of data throughout the entire test. Such a paradigm would provide valuable information about submaximal exercise efficiency.

CONCLUSIONS:

Following two different 7-day IH protocols administered to subjects in a crossover fashion there was a slight increase submaximal oxygen saturation during hypoxic exercise. There were no changes in submaximal exercise ventilation in hypoxia or normoxia. Peak exercise ventilatory parameters and peak power were unchanged by IH. The response to hyperoxia during exercise was augmented by IH whereas the response to hypercapnia was unchanged by IH. No differences occurred between the SDIH and LDIH protocols in terms of respiratory response during exercise. Thus, although an augmentation of response to hyperoxia occurred following IH, it was not translated into an increase in minute ventilation during normoxic or hypoxic exercise.

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CONCLUSIONS:

Intermittent hypoxic exposures increase the ventilatory response to hypoxia at rest. IH has the potential for applied and clinical benefits, including improving exercise performance[1], reducing cardiac ischaemia[2] and it may have a role in improving ventilation in spinal cord transection patients[3]. Many different protocol intensities, durations and duty cycles have been studied, but as yet the optimal IH protocol for augmenting HVR in humans is not yet known.

The purpose of the study was to compare the effects of two different types of intermittent hypoxia protocols on respiratory chemoresponse and to examine the relationship between carbon dioxide and oxygen sensitivity during rest and exercise. Furthermore, HVR was assessed daily to follow the pattern of HVR augmentation during the course of IH. To determine whether repeated measurement of HVR caused a confounding co-intervention, repeatability study was performed. This study demonstrated: 1) acceptable values for coefficient of variation for HVR and 2) no apparent IH stimulus from daily measurements of HVR. Two types of intermittent hypoxia: short-duration intermittent hypoxia (SDIH) and long duration intermittent hypoxia (LDIH) were assessed in the second phase of the project. Ten male subjects underwent two seven-day intermittent hypoxic training protocols while being assessed for a battery of measurements of resting and exercise ventilatory control.

As anticipated, following the IH, HVR was augmented and CO_2 threshold was decreased (in hypoxia and hyperoxia). HCVR was also increased by IH. These resting alterations in chemoreflex control remained different from baseline

at 7 days post-IH. Intermittent hypoxia had no effect on exercise minute ventilation, but increased the response to hyperoxic challenge. Also during submaximal exercise, there was a slight increase in submaximal O_2 saturation (by oximetry). Peak exercise values were unchanged by intermittent hypoxia. It was initially hypothesised that SDIH would cause more profound changes in the measured variables than LDIH. However, the effects of SDIH and LDIH were similar throughout, with no significant differences for any of the measurements.

Significance

This research comprehensively compares SDIH and LDIH, demonstrating no significant differences between the two protocols. It is the first research to examine HVR, HCVR and the modified Read rebreathing techniques in the same subjects following the same intervention. It confirms that IH both augments HVR and lowers the CO_2 threshold, but that there were no correlations between these two parameters. It also highlights (and discusses potential mechanisms for) the discrepancy between CO_2 sensitivity as measured by HCVR and by the modified rebreathing method.

Furthermore, this work comprises the most comprehensive assessment of resting *and* exercising chemosensitivity in subjects undergoing IH. Previous work examining the relationship between resting chemosensitivity and exercise control of breathing have not employed specific chemosensitivity tests or have focussed on peak exercise ventilation. For the IH protocols tested, augmentation of resting hypoxic ventilatory response resulted in an increase in

exercising chemosensitivity. This alteration did not affect the minute ventilation or the change in exercise ventilation between normoxia or hypoxia, indicating that its effect on the control of breathing during exercise was modest at best. Further analysis is required to assess the changes in respiratory pattern (such as tidal volume and frequency of breathing) that may potentially occur following IH. An increased tidal volume during submaximal ventilation could potentially explain the enhanced saturation during hypoxic exercise without an increase in minute ventilation.

The current experimental design with it's systematic assessment of resting and exercising chemoresponse in a crossover controlled fashion is the most sophisticated comparison of SDIH and LDIH on the control of breathing in humans. The results fail to support any benefit of SDIH over LDIH on the augmentation of chemoresponse in humans. This information lends credence to the concept that the normoxic-hypoxic transients that are more plentiful in SDIH are not instrumental to the augmentation of ventilation in humans.

Limitations & Future Research

Ventilatory measures during wakefulness have a high degree of variation. The repeatability study demonstrated that the technique for measurement of HVR used in the lab shows a sizeable coefficient of variation (27%), but one which is comparable to the lowest previously reported values. Although the other testing methods used in this research have not been assessed in terms of their repeatability, they likely have significant coefficients of variation as well. It is

therefore possible that changes occurred in the chemosensitivity to hypoxia or hypercapnia that were not detected due to this variation. Larger alterations in resting chemoreceptor control may be needed to induce detectable changes in the ventilatory control of breathing during exercise. A similarly extensive assessment of resting and exercising chemoresponse, but with a "live-high, train-low" protocol would provide valuable insight not only into the effects of IH, but also on the control of breathing during exercise in humans.

Potential mechanisms for the increased saturation during submaximal exercise following IH include an increase in alveolar ventilation or a decrease in oxygen consumption (improved efficiency). One could derive approximate values for oxygen consumption by integrating the breath-by-breath data collected during this study. Ultimately, a more detailed assessment of gas exchange, using arterial blood gases and a combination of both breath-by-breath and mixing chamber analysis during submaximal exercise would provide more definitive answers regarding the effects of IH on exercise efficiency.

The differences between normobaric and hypobaric IH remain unclear. It is tempting to equate the hypoxia from a reduced F_IO_2 in normobaria with a constant F_IO_2 in hypobaria. The two conditions are not identical and may have differential effects on fluid status, baroreceptor tone and other physiological processes that affect the response to hypoxia. A head-to-head trial comparing a hypobaric IH protocol with its normobaric equivalent, although technically challenging, would provide valuable insight into these differences.

The importance of sleep during IH is also not understood. IH protocols that involve sleeping in hypoxia[4] cause greater increases in HVR than when the subjects are awake[5] [6]. It is unclear whether this due to the duration of the hypoxic exposure or the state of wakefulness. A comparison of two IH protocols with a consistent exposure duration, and F_IO_2, but with subjects either awake or asleep would provide interesting insight.

Summary

IH has many potential applied and clinical applications. A thorough understanding of its mechanism of action and optimal dosages would be beneficial to properly tailor IH for its intended use. From this work it appears that a 7-day course of poikilocapnic IH causes 1) an increase in HVR that occurs rapidly in the first four days of exposure, 2) a left-shift in the CO_2 threshold in both hypoxic and hyperoxic conditions, 3) a concomitant increase in the ventilatory response to hyperoxic challenge during exercise 4) all changes are essentially equivalent following an SDIH or an LDIH protocol. This work raises many important questions that provide opportunity for further study.

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APPENDIX I:

Certificates of Ethical Review

The University of British Columbia
Office of Research Services and Administration
Clinical Research Ethics Board

Certificate of Expedited Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	[redacted]
Sheel, W.	Human Kinetics	C03-0466

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT

UBC Campus

CO-INVESTIGATORS:

Guenette, Jordan, ; Koehle, Michael, Human Kinetics; McKenzie, Donald, Human Kinetics; Sporer, Benjamin, Human Kinetics

SPONSORING AGENCIES

Unfunded Research

[redacted]

APPROVAL DATE	TERM (YEARS)	DOCUMENTS INCLUDED IN THIS APPROVAL:
OCT 01 2003	1	Consent form version 1.1 dd 4 September 2003; posters, protocol

CERTIFICATION:

In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of the this Research Ethics Board have been documented in writing.

The documentation included for the above-named project has been reviewed by the Chair of the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

The CREB approval for this study expires one year from the approval date.

Approval of the Clinical Research Ethics Board by one of:
Dr. P. Loewen, Chair
Dr. A. Gagnon, Associate Chair
Dr. J. McCormack, Associate Chair

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V6Z 1L8

Certificate of Expedited Approval

Clinical Research Ethics Board Official Notification

PRINCIPAL INVESTIGATOR	DEPARTMENT	NUMBER
McKenzie, D.C.	Family Practice	C04-0402

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
UBC Campus

CO-INVESTIGATORS
Guenette, Jordan. ; Hughes, Bevan. ; Koehle, Michael, Human Kinetics; Lusina, Sarah, Human Kinetics; Milsom, William, Zoology; Sheel, William, Human Kinetics

SPONSORING AGENCIES
Natural Science Engineering Research Council

TITLE
Intermittent Hypoxia and the Chemoreflex Control of Ventilation

APPROVAL DATE	TERM (YEARS)	DOCUMENTS INCLUDED IN THIS APPROVAL
11 Aug 2004	1	Protocol: Consent version 1.1 dated 27 July 2004; Advertisement: PAR-Q Questionnaire

CERTIFICATION
In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of the this Research Ethics Board have been documented in writing

The documentation included for the above-named project has been reviewed by the Chair of the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

The CREB approval for this study expires one year from the approval date.

Approval of the Clinical Research Ethics Board by one of:
Dr. P. Loewen, Chair
Dr. A. Gagnon, Associate Chair
Dr. J. McCormack, Associate Chair

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V6Z 1L8

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V6Z 1L8

Certificate of Expedited Approval: Renewal

Clinical Research Ethics Board Official Notification

PRINCIPAL INVESTIGATOR	DEPARTMENT	NUMBER
McKenzie, D.C.		C04-0402

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
UBC Campus

CO-INVESTIGATORS
Guenette, Jordan. : Hughes, Bevan. : Koehle, Michael, Human Kinetics; Lusina, Sarah, Human Kinetics; Milsom, William, Zoology; Sheel, William, Human Kinetics

SPONSORING AGENCIES
Natural Science Engineering Research Council

TITLE
Intermittent Hypoxia and the Chemoreflex Control of Ventilation

APPROVAL RENEWAL DATE	TERM (YEARS)	AMENDMENT	AMENDMENT APPROVED
19 July 2005	1		

CERTIFICATION
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The Chair of the UBC Clinical Research Ethics Board has reviewed the documentation for the above named project. The research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved for renewal by the UBC Clinical Research Ethics Board.

The CREB approval for renewal of this study expires one year from the date of renewal.

Approval of the Clinical Research Ethics Board by one of:
Dr. Gail Bellward, Chair
Dr. James McCormack, Associate Chair

APPENDIX II:

Informed Consent Forms

capable of inducing haematological changes, such as increases in the ability of the blood to carry oxygen. The ideal protocol is not yet known. In animals, multiple short bouts (less than 5 minutes) each day has been shown superior to single long daily bouts. This has not been examined in humans. Likewise the ideal length of an IHT protocol is also unknown. This study will attempt to answer these questions.

Purpose:
The purpose of this study is to investigate the breathing response to hypoxia over seven daily hypoxic ventilatory response tests.

Procedures:
All subjects recruited for the study will be normal healthy male volunteers, between 18-40 years of age. All subjects will be non-smoking, have normal pulmonary function and free of any history or symptoms of cardiopulmonary disease including exercise-induced asthma. Subjects will not have had any significant exposure to altitude or hypoxia in the preceding four weeks. Each subject will undergo a standardized screening history (Physical Activity Readiness Questionnaire; PAR-Q).

If you consent to become a subject in this study you will be asked to participate in nineteen data collection test days. The session will take place at the Health and Integrated Physiology Laboratory at the Osborne Centre (Unit 2, Room 202) on the University of British Columbia campus. The study will require approximately thirty-four (34) hours of your time. We will schedule your testing sessions to be most convenient for you.

On the first day, your height and weight will be measured. You will then undergo a simple, non-invasive breathing test to ensure that you do not have any obstructive lung disease (i.e., asthma). This requires you to breathe deeply and exhale quickly through a mouthpiece.

You will then be required to lie comfortably on a bed in which you will breath through a two-way valve so that expired gases and flow can be monitored. A small plastic clip will slip onto your fingertip. This will permit us to measure the amount of oxygen in your blood. After 10 minutes of breathing normal air, experimenters will slowly and progressively add nitrogen gas to the air you are breathing. We will measure the amount that your breathing (rate and depth) increases in response to this. The test will stop once your blood oxygen saturation level reaches 80%. This experiment will simulate high altitude exposure and will take approximately 15 minutes. This is the hypoxic ventilatory response (HVR) test.

You will then perform a similar test where you breathe from a large bag while resting. We will control the concentration of gases in the bag. Gradually the carbon dioxide in the bag will accumulate, and you will breathe more and more. We will stop the test once it has become too uncomfortable or the amount of carbon dioxide in the bag reaches a pre-determined amount (60 mmHg). This is the hypercapnic ventilatory response test (HCVR).

The next test is the maximal oxygen uptake test. This is a test where you ride a stationary bicycle while wearing a mask to collect the gas that you breath out. The resistance on the bicycle gets higher and higher until you can no longer continue. This test determines aerobic fitness.

On your second visit, you will repeat the HVR test and the HCVR test. You will also complete the multi-stage exercise test. During this test, you will be exercising on a bicycle at two relatively low resistances. You will first exercise breathing room air, and then secondly while breathing hypoxic air. You will finish the exercise test by performing another maximal oxygen uptake test (while breathing the hypoxic gas).

All your pre-testing is complete. You will then start your intermittent hypoxic training (IHT). This will last for 7 days. You will do two different protocols, and they will each last seven days. Each day you will come to the lab, and breath a gas mixture while relaxing, watching movies, reading or working quietly. Each time you come in you will also do an HVR test. At the end of each 7-day IHT session we will repeat the HVR, HCVR and multi-stage exercise test. You will do each IHT programme at least 2 weeks apart. One week after each IHT programme, you will return to the lab and HVR and HCVR will be re-measured.

Risks:
There are no significant risks associated with a short exposure to simulated altitude (approximately 20,000 feet). A physician (Dr. Koehle or Dr. Hughes) will be present at all testing sessions, if you feel any discomfort, or have any concerns, you will be attended to immediately. Some people find it feels a little uncomfortable when they are breathing hypoxic air. The maximal oxygen uptake test has a small chance of adverse effects, such as vomiting (5%), abnormal blood pressure (less than 1%), fainting (less than 1%), disorders of the heartbeat (less than 0.1%), and very rare instances of heart attack (less than 0.001%). All procedures used in this study have been previously performed in our laboratory without incident.

Benefits:
By participating in the study, the subjects will enhance the understanding of the effects of hypoxia on the control of breathing; this knowledge will be used to further our understanding of the safety of diving in the asthmatic population. Furthermore, you will receive a maximal oxygen uptake test (VO2max test) and two courses of intermittent hypoxic training at no charge. After completion of the study, you will receive an honorarium of one hundred dollars.

Confidentiality:
Your rights to privacy are protected by the Freedom of Information and Protection of Privacy Act of British Columbia. This Act lays down rules for the collection, protection, and retention of your personal information by public bodies, such as the University of British Columbia and its affiliated teaching hospitals. Further details about this Act are available upon request. Your confidentiality will be respected. No information that

discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices. You are encouraged to ask for an explanation or clarification of any of the procedures or other aspects of this study before signing this consent from or at any time during your participation in the study.

YOU MAY DECLINE TO ENTER THIS STUDY OR WITHDRAW FROM THE EXPERIMENT AT ANY TIME.

If you have any concerns or questions about your rights or experience as a research subject, you may contact the Research Subject Information Line in the UBC Office of Research Services at (604) 822-8598.

Consent:
In signing this form you are consenting to participate in this research project and acknowledge receipt of a copy of this form. Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

_____ _____
Signature of Participant Date

_____
Printed Name of Participant

_____ _____
Signature of Witness Date

_____
Printed Name of Witness

_____ _____
Signature of Investigator Date

_____
Printed Name of Investigator

(less than 5 minutes) each day has been shown superior to single long daily bouts. This has not been examined in humans. Likewise the ideal length of an IHT protocol is also unknown. This study will attempt to answer these questions.

Purpose:
The purpose of this study is to investigate the breathing response to hypoxia over seven daily hypoxic ventilatory response tests.

Procedures:
All subjects recruited for the study will be normal healthy male volunteers, between 18-40 years of age. All subjects will be non-smoking, have normal pulmonary function and free of any history or symptoms of cardiopulmonary disease including exercise-induced asthma. Subjects will not have had any significant exposure to altitude or hypoxia in the preceding four weeks. Each subject will undergo a standardized screening history (Physical Activity Readiness Questionnaire; PAR-Q).

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The next test is the maximal oxygen uptake test. This is a test where you ride a stationary bicycle while wearing a mask to collect the gas that you breath out. The resistance on the bicycle gets higher and higher until you can no longer continue. This test determines aerobic fitness.

On your second visit, you will repeat the HVR test and the HCVR test. You will also complete the multi-stage exercise test. During this test, you will be exercising on a bicycle at two relatively low resistances. You will first exercise breathing room air, and then secondly while breathing hypoxic air. You will finish the exercise test by performing another maximal oxygen uptake test (while breathing the hypoxic gas).

All your pre-testing is complete. You will then start your intermittent hypoxic training (IHT). This will last for 7 days. You will do two different protocols, and they will each last seven days. Each day you will come to the lab, and breath a gas mixture while relaxing, watching movies, reading or working quietly. Each time you come in you will also do an HVR test. At the end of each 7-day IHT session we will repeat the HVR, HCVR and multi-stage exercise test. You will do each IHT programme at least 2 weeks apart. One week after each IHT programme, you will return to the lab and HVR and HCVR will be re-measured.

Risks:
There are no significant risks associated with brief mild hypoxia exposure (approximately 20,000 feet). A physician (Dr. Koehle or Dr. Hughes) will be present at all testing sessions, if you feel any discomfort, or have any concerns, you will be attended to immediately. Some people find it feels a little uncomfortable when they are breathing hypoxic air. The maximal oxygen uptake test has a small chance of adverse effects, such as vomiting (5%), abnormal blood pressure (<1%), fainting (<1%), disorders of the heartbeat (<0.1%), and very rare instances of heart attack (<0.001%). All procedures used in this study have been previously performed in our laboratory without incident.

Benefits:
By participating in the study, the subjects will enhance the understanding of the effects of hypoxia on the control of breathing; this knowledge will be used to further our understanding of the safety of diving in the asthmatic population. Furthermore, you will receive a maximal oxygen uptake test (VO2max test) and two courses of intermittent hypoxic training at no charge. After completion of the study, you will receive an honorarium of one hundred dollars.

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inspected in the presence of the Investigator or his or her designate by representatives of the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices. You are encouraged to ask for an explanation or clarification of any of the procedures or other aspects of this study before signing this consent from or at any time during your participation in the study.

YOU MAY DECLINE TO ENTER THIS STUDY OR WITHDRAW FROM THE EXPERIMENT AT ANY TIME.

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Consent:
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_____ _____
Signature of Participant Date

_____
Printed Name of Participant

_____ _____
Signature of Witness Date

_____
Printed Name of Witness

_____ _____
Signature of Investigator Date

_____
Printed Name of Investigator

APPENDIX III:

Physical Activity Readiness Questionnaire

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

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APPENDIX IV:

Data Tables

	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 14	
Subject	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**
1	0.24	0.32	0.36	0.66	0.41	0.43	0.56	0.63	0.36	0.77	0.41	0.51	0.41	0.61	0.33	0.49	0.39	0.59
2	0.43	0.83	0.26	0.65	0.25	0.67	0.62	0.88	0.75	0.83	0.35	0.69	0.59	0.89	0.66	0.84	0.71	0.48
3	0.22	0.35	0.30	0.45	0.62	0.64	0.54	0.58	0.41	0.57	0.43	0.48	0.68	0.59	0.73	0.58	0.56	0.50
4	0.24	0.41	0.33	0.57	0.28	0.40	0.42	0.66	0.46	0.43	0.72	0.91	0.52	0.74	0.49	0.71	0.47	0.78
5	0.67	0.59	0.84	0.63	0.97	0.32	0.82	0.81	1.11	0.82	0.63	0.67	0.79	0.63	0.65	0.75	0.82	0.69
6	0.46	0.35	0.46	0.33	0.47	0.66	0.41	0.38	0.45	0.60	0.61	0.61	0.68	0.69	0.60	0.64	0.58	0.55
7	1.01	0.82	1.22	0.78	2.20	0.90	1.75	0.92	1.53	0.87	1.06	0.93	1.06	0.94	1.14	0.81	0.58	0.87
8	0.29	0.72	0.25	0.74	0.42	0.64	0.51	0.54	0.30	0.82	0.35	0.55	0.39	0.46	0.36	0.55	0.35	0.74
9	0.57	0.37	0.98	0.69	0.87	0.58	1.28	0.73	1.13	0.65	1.24	0.63	0.84	0.81	1.41	0.79	0.87	0.84
10	0.56	0.80	0.53	0.79	0.67	0.82	0.54	0.86	0.72	0.83	0.89	0.80	0.70	0.67	0.62	0.90	0.54	0.79
Mean	**0.47**	**0.55**	**0.55**	**0.63**	**0.71**	**0.61**	**0.75**	**0.70**	**0.72**	**0.72**	**0.67**	**0.68**	**0.66**	**0.70**	**0.70**	**0.71**	**0.59**	**0.68**
SD	**0.25**	**0.22**	**0.34**	**0.15**	**0.57**	**0.18**	**0.43**	**0.17**	**0.41**	**0.15**	**0.31**	**0.16**	**0.20**	**0.15**	**0.34**	**0.13**	**0.17**	**0.14**

Table 6.1: HVR results during the SDIH protocol. SD- standard deviation.
HVR values are expressed in litres·min^{-1}%·SaO_2^{-1}

	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 14	
Subject	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**	**HVR**	**R^2**
1	0.261	0.461	0.484	0.634	0.455	0.508	0.807	0.545	0.488	0.703	0.778	0.749	0.824	0.592	0.734	0.661	0.651	0.473
2	0.414	0.748	0.298	0.513	0.408	0.716	0.485	0.697	0.477	0.814	0.477	0.793	1.111	0.637	0.694	0.884	0.580	0.861
3	0.576	0.774	0.674	0.615	0.768	0.785	0.771	0.471	0.704	0.448	0.826	0.539	0.504	0.784	0.599	0.745	0.652	0.536
4	0.212	0.504	0.579	0.884	0.469	0.668	0.456	0.342	0.619	0.785	0.996	0.817	0.656	0.634	1.001	0.823	0.541	0.678
5	0.391	0.508	0.846	0.463	1.036	0.644	1.065	0.669	0.577	0.350	0.719	0.578	0.722	0.649	0.936	0.658	0.778	0.566
6	0.327	0.839	0.796	0.398	0.417	0.470	0.551	0.577	0.740	0.471	0.520	0.660	0.835	0.603	0.696	0.632	0.650	0.532
7	0.875	0.633	0.954	0.842	1.409	0.873	1.222	0.807	1.081	0.948	1.500	0.856	0.981	0.775	1.704	0.851	1.594	0.826
8	0.334	0.452	0.450	0.519	0.414	0.571	0.387	0.496	0.499	0.504	0.457	0.553	0.414	0.665	0.261	0.623	0.295	0.628
9	0.844	0.883	0.782	0.737	0.802	0.784	0.622	0.839	0.649	0.694	0.750	0.704	0.754	0.650	0.814	0.767	0.500	0.658
10	0.470	0.821	0.634	0.803	0.414	0.750	1.039	0.751	0.791	0.796	0.708	0.928	0.578	0.854	0.416	0.766	0.391	0.744
Mean	**0.470**	**0.662**	**0.650**	**0.641**	**0.659**	**0.677**	**0.741**	**0.619**	**0.662**	**0.651**	**0.773**	**0.718**	**0.738**	**0.684**	**0.785**	**0.741**	**0.663**	**0.650**
SD	**0.229**	**0.170**	**0.202**	**0.169**	**0.343**	**0.130**	**0.289**	**0.160**	**0.183**	**0.196**	**0.306**	**0.134**	**0.213**	**0.088**	**0.391**	**0.094**	**0.356**	**0.129**

Table 6.2: HVR results during the LDIH protocol. SD- standard deviation.
HVR values are expressed in litres·min^{-1}%·SaO_2^{-1}

SDIH

Subject	Pre-	Post-	7 days Post-
1	1.57	2.63	2.64
2	3.26	3.27	2.41
3	2.49	2.59	2.77
4	4.34	7.52	5.78
5	2.67	3.06	3.78
6	1.40	1.28	1.80
7	5.86	3.97	5.08
8	4.93	4.59	5.22
9	3.27	4.32	3.00
10	2.07	5.23	3.68
Mean	**3.19**	**3.85**	**3.62**
Standard Deviation	**1.47**	**1.73**	**1.34**

LDIH

Subject	Pre-	Post-	7 days Post-
1	2.46	2.70	1.47
2	2.18	4.79	2.70
3	2.86	2.57	3.21
4	1.05	3.34	4.12
5	3.95	6.29	6.17
6	2.86	2.63	2.36
7	3.37	5.77	4.73
8	4.43	4.77	5.94
9	2.07	2.32	2.52
10	3.13	5.69	6.92
Mean	**2.84**	**4.09**	**4.01**
Standard Deviation	**0.97**	**1.54**	**1.86**

Table 6.3: HCVR results during the LDIH and SDIH protocols. HCVR values are expressed in litres·min^{-1}%·mmHg^{-1}

SDIH	Threshold			Sensitivity		
Subject	Pre-	Post-	7 days Post-	Pre-	Post-	7 days Post-
1	44.12	42.20	42.66	2.48	1.56	3.49
2	45.23	42.59	42.72	3.06	4.17	3.30
3	44.22	41.53	47.16	2.61	1.73	2.32
4	47.30	42.43	43.15	4.68	4.10	4.40
5	45.11	41.93	44.86	3.71	2.91	3.38
6	48.92	45.78	48.89	1.72	1.64	2.31
7	45.76	45.22	48.56	2.82	3.14	4.54
8	43.44	42.24	43.66	5.21	4.25	4.82
9	44.26	43.13	44.03	2.49	2.78	1.91
10	49.61	45.61	46.56	4.29	3.43	2.69
Mean	**45.80**	**43.27**	**45.23**	**3.31**	**2.97**	**3.32**
Standard Deviation	**2.12**	**1.63**	**2.39**	**1.12**	**1.05**	**1.02**

LDIH	Threshold			Sensitivity		
Subject	Pre-	Post-	7 days Post-	Pre-	Post-	7 days Post-
1	47.91	41.84	43.02	2.31	2.48	3.02
2	47.15	41.64	43.35	4.28	6.59	4.85
3	44.46	43.05	43.05	2.05	1.78	2.37
4	49.07	47.55	48.45	3.84	3.29	4.86
5	44.11	43.06	44.05	3.50	4.34	4.09
6	46.81	46.70	47.60	3.38	2.81	2.68
7	45.21	43.37	45.98	4.27	2.12	2.81
8	42.06	42.58	42.97	5.75	3.67	3.91
9	43.75	44.02	42.33	1.98	2.16	1.94
10	49.31	45.41	46.22	4.15	3.07	5.10
Mean	**45.98**	**43.92**	**44.70**	**3.55**	**3.23**	**3.56**
Standard Deviation	**2.43**	**2.01**	**2.18**	**1.18**	**1.41**	**1.14**

Table 6.4: Hypoxic modified rebreathing method results during the LDIH and SDIH protocols. Threshold units mmHg. Sensitivity values are expressed in litres·min^{-1}%·mmHg^{-1}

SDIH	Threshold			Sensitivity		
Subject	Pre-	Post-	7 days Post-	Pre-	Post-	7 days Post-
1	39.73	37.86	38.75	4.70	5.01	4.54
2	40.00	37.96	41.35	4.37	5.82	6.40
3	40.54	40.94	42.25	4.94	5.19	5.68
4	44.16	38.02	41.40	5.00	4.80	5.75
5	37.75	39.33	39.54	4.17	4.60	4.85
6	46.58	41.77	41.77	2.60	2.80	3.90
7	44.35	40.30	42.93	4.40	6.55	7.46
8	36.29	37.85	37.64	7.56	7.51	6.11
9	40.71	38.93	39.83	5.40	5.48	3.80
10	43.82	41.15	42.34	4.93	3.29	8.98
Mean	**41.39**	**39.41**	**40.78**	**4.81**	**5.11**	**5.75**
Standard Deviation	**3.24**	**1.52**	**1.74**	**1.23**	**1.39**	**1.61**

LDIH	Threshold			Sensitivity		
Subject	Pre-	Post-	7 days Post-	Pre-	Post-	7 days Post-
1	39.86	38.32	39.15	3.80	5.72	4.45
2	39.63	39.64	39.39	10.71	7.58	7.37
3	40.69	38.48	38.97	3.89	3.13	4.48
4	43.15	40.98	40.31	4.43	4.12	4.44
5	38.61	36.59	37.66	6.58	4.69	4.16
6	40.43	38.61	43.15	4.48	4.24	5.43
7	42.86	39.93	42.73	7.98	6.34	5.57
8	36.87	36.06	37.45	6.58	5.23	9.24
9	39.64	39.52	39.39	3.35	3.61	3.35
10	43.64	41.27	41.97	4.44	5.51	3.96
Mean	**40.54**	**38.94**	**40.02**	**5.62**	**5.02**	**5.25**
Standard Deviation	**2.14**	**1.70**	**2.00**	**2.33**	**1.34**	**1.79**

Table 6.5: Hyperoxic modified rebreathing method results during the LDIH and SDIH protocols. Threshold units mmHg. Sensitivity values are expressed in litres·min^{-1}%·mmHg^{-1}

	Mean Minute Ventilation Pre-IH				Mean Minute Ventilation Post-IH			
SDIH	**Normoxic**		**Hypoxic**		**Normoxic**		**Hypoxic**	
Subject	**Low**	**Moderate**	**Low**	**Moderate**	**Low**	**Moderate**	**Low**	**Moderate**
1	53.1	77.8	58.0	116.4	48.5	79.5	57.1	96.4
2	39.5	64.4	48.3		41.8	69.4	45.1	
3	39.2	70.1	47.2	96.7	41.4	66.7	49.6	90.1
4	37.2	60.7	44.8	83.0	37.4	69.2	46.1	89.7
5	42.1	72.0	46.0	89.2	40.9	75.6	49.5	95.0
6	23.8	48.5	28.3	59.3	23.8	44.6	23.0	50.6
7	40.8	77.9	46.4	90.0	39.7	78.3	48.5	100.3
8	47.3	103.1	57.7	113.5	45.8	85.9	56.6	114.0
9	52.7	87.5	62.4	104.9	53.4	80.0	63.7	100.9
10	38.0	59.0	49.3	86.0	41.2	67.2	50.2	92.6
Mean	**41.4**	**72.1**	**48.8**	**93.2**	**41.4**	**71.6**	**48.9**	**92.2**
Standard Deviation	**8.5**	**15.6**	**9.4**	**17.4**	**7.8**	**11.4**	**10.7**	**17.3**

Table 6.6: Submaximal minute ventilation values during exercise before and after SDIH. Values are expressed in litres·min^{-1}

	Mean Minute Ventilation Pre-IH				Mean Minute Ventilation Post-IH			
LDIH	**Normoxic**		**Hypoxic**		**Normoxic**		**Hypoxic**	
Subject	**Low**	**Moderate**	**Low**	**Moderate**	**Low**	**Moderate**	**Low**	**Moderate**
1	54.1	78.8	60.0	119.5	44.5	78.8	51.9	98.8
2	35.1	60.7	42.9		44.3	66.9	47.1	
3	36.8	59.0	38.2	83.9	41.8	65.1	45.2	90.8
4	42.5	70.0	51.3	106.6	36.9	64.1	47.9	81.9
5	38.7	70.2	41.5	76.2	41.1	66.6	45.8	84.4
6	22.9	42.6	24.9	52.6	22.0	45.6	25.0	56.6
7	38.6	77.8	47.5	100.2	43.6	82.2	49.4	103.0
8	46.9	80.5	50.8	100.5	47.5	81.2	52.1	99.3
9	48.2	80.5	54.7	95.8	49.5	77.9	57.8	96.7
10	39.9	65.8	51.4	89.3	40.4	72.8	50.8	93.1
Mean	**40.4**	**68.6**	**46.3**	**91.6**	**41.2**	**70.1**	**47.3**	**89.4**
Standard Deviation	**8.5**	**12.1**	**9.9**	**19.4**	**7.6**	**11.0**	**8.7**	**14.1**

Table 6.7: Submaximal minute ventilation values during exercise before and after LDIH. Values are expressed in litres·min^{-1}

	SDIH		
Subject	**Pre-**	**Post-**	**Delta**
1	83.9	89.0	5.1
2	90.9	92.6	1.7
3	85.0	87.3	2.2
4	85.0	85.6	0.6
5	81.5	86.5	5.0
6	86.2	90.6	4.4
7	86.2	87.7	1.5
8	84.7	87.5	2.8
9	82.4	86.3	3.9
10	83.1	82.4	-0.6
Mean	**84.9**	**87.6**	**2.7**
Standard Deviation	**2.6**	**2.8**	**1.9**

	LDIH		
Subject	**Pre-**	**Post-**	**Delta**
1	86.9	84.5	-2.4
2	90.2	90.2	0.0
3	84.3	86.0	1.7
4	80.4	84.8	4.4
5	86.4	87.7	1.3
6	86.8	86.9	0.1
7	87.8	86.6	-1.2
8	86.4	87.7	1.3
9	89.2	86.8	-2.4
10	82.8	83.6	0.8
Mean	**86.1**	**86.5**	**0.4**
Standard Deviation	**2.9**	**1.9**	**2.0**

Table 6.8: Submaximal saturation values during hypoxic exercise before and after intermittent hypoxia. Values are expressed in %·SaO_2

	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta
Subject	Pre-SDIH											
1	50.0	42.7	7.3	51.6	38.8	12.8	66.7	61.5	5.2	78.2	62.9	15.3
2	39.2	34.6	4.6	40.5	28.7	11.9	62.5	57.9	4.6	72.7	56.5	16.1
3	39.4	30.1	9.4	39.8	32.5	7.2	72.5	54.2	18.3	69.6	58.4	11.2
4	37.0	30.8	6.2	40.0	32.2	7.8	64.8	48.0	16.8	60.3	47.3	13.0
5	42.2	34.9	7.3	41.1	37.8	3.3	65.4	51.6	13.8	77.2	67.7	9.5
6	23.0	22.9	0.1	24.7	23.6	1.0	46.3	41.8	4.4	52.6	45.4	7.2
7	43.1	35.3	7.8	39.9	33.8	6.1	78.5	64.4	14.0	81.1	67.0	14.1
8	37.1	36.6	0.6	50.8	42.9	8.0	91.8	78.0	13.9	111.7	94.2	17.5
9	53.9	40.7	13.3	53.7	44.9	8.9	85.2	77.1	8.1	90.2	79.2	11.0
10	33.4	33.5	-0.1	39.2	38.0	1.2	53.2	49.9	3.3	59.3	57.2	2.1
Mean	**39.8**	**34.2**	**5.6**	**42.1**	**35.3**	**6.8**	**68.7**	**58.4**	**10.2**	**75.3**	**63.6**	**11.7**
SD	**8.6**	**5.6**	**4.4**	**8.4**	**6.4**	**4.0**	**13.9**	**12.0**	**5.7**	**17.1**	**14.6**	**4.6**
	Post-SDIH											
1	52.3	38.4	13.9	47.8	38.8	9.1	78.8	61.6	17.2	83.1	68.0	15.1
2	42.0	34.3	7.7	42.0	40.0	2.0	70.9	60.6	10.3			
3	39.6	34.4	5.2	40.3	38.5	1.8	62.4	51.2	11.2	69.9	59.0	10.9
4	35.5	31.0	4.5	37.8	36.5	1.3	67.7	51.8	15.8	71.6	55.2	16.4
5	42.9	33.9	8.9	42.3	36.0	6.3	79.7	66.4	13.3	77.5	68.4	9.1
6	22.6	20.2	2.4	22.6	20.2	2.4	44.2	32.9	11.3	46.4	41.2	5.2
7	41.3	36.7	4.6	38.1	23.8	14.4	79.0	60.4	18.6	83.0	61.1	21.9
8	42.9	36.2	6.7	46.5	43.0	3.5	79.5	70.2	9.3	87.5	80.0	7.5
9	62.6	45.5	17.2	49.3	42.0	7.3	80.0	62.5	17.5	78.1	72.5	5.6
10	40.9	36.7	4.2	37.4	37.2	0.2	69.4	58.1	11.3	67.7	57.3	10.4
Mean	**42.3**	**34.7**	**7.5**	**40.4**	**35.6**	**4.8**	**71.2**	**57.6**	**13.6**	**73.9**	**62.5**	**11.3**
SD	**10.3**	**6.4**	**4.7**	**7.6**	**7.6**	**4.4**	**11.3**	**10.4**	**3.4**	**12.2**	**11.3**	**5.5**

Table 6.9: Hyperoxic Test ventilation values during normoxic exercise before and after SDIH. 3bMA = Three-breath moving average. Pre = mean ventilation for the 30 seconds prior to the challenge. Values are expressed in litres·min^{-1}

	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta
Subject	Pre-LDIH											
1	55.3	40.3	15.0	52.0	48.0	4.0	74.7	58.1	16.7	83.5	61.7	21.8
2	36.2	26.7	9.5	31.8	28.0	3.8	63.3	52.6	10.7	60.1	53.9	6.3
3	33.2	33.3	0.0	39.0	32.7	6.3	59.4	50.8	8.6	62.5	50.3	12.2
4	41.1	29.1	12.0	44.1	30.4	13.6	68.6	62.6	6.0	75.2	63.4	11.8
5	40.3	35.2	5.1	37.0	35.3	1.7	70.8	53.0	17.8	75.7	55.5	20.3
6	22.6	20.6	2.1	21.5	18.1	3.4	42.9	39.4	3.4	42.1	37.9	4.3
7	37.5	31.5	6.0	40.5	32.1	8.4	68.9	64.5	4.4	84.6	64.8	19.9
8	49.1	38.2	10.9	48.5	42.5	6.0	76.8	68.6	8.2	82.8	73.8	9.0
9	48.0	36.0	12.0	47.1	39.1	8.0	79.7	68.0	11.7	85.2	73.0	12.2
10	41.3	34.0	7.4	41.0	36.0	5.0	65.9	62.1	3.7	66.7	62.3	4.5
Mean	**40.5**	**32.5**	**8.0**	**40.3**	**34.2**	**6.0**	**67.1**	**58.0**	**9.1**	**71.9**	**59.6**	**12.2**
SD	**9.1**	**5.8**	**4.8**	**8.8**	**8.2**	**3.4**	**10.5**	**9.1**	**5.1**	**14.0**	**10.8**	**6.5**
	Post-LDIH											
1	50.4	36.9	13.4	44.7	37.3	7.4	79.4	59.6	19.8	80.1	58.1	21.9
2	43.4	37.7	5.7	41.6	30.4	11.2	65.3	56.9	8.4	70.5	51.1	19.5
3	40.7	35.5	5.2	42.0	40.4	1.6	59.3	56.0	3.3	67.5	60.6	7.0
4	33.4	27.8	5.5	36.0	33.4	2.6	62.4	46.8	15.6	65.2	55.1	10.1
5	42.0	37.7	4.3	39.5	36.0	3.5	74.5	63.0	11.5	73.1	63.8	9.3
6	22.0	17.5	4.5	21.2	18.7	2.5	42.7	38.9	3.8	45.8	42.0	3.8
7	43.0	33.0	10.0	45.0	34.8	10.1	72.1	64.3	7.8	83.8	74.7	9.2
8	52.1	39.8	12.3	47.4	40.5	6.9	76.8	67.5	9.3	83.8	60.8	23.0
9	53.0	35.0	18.0	49.9	44.2	5.7	74.0	66.7	7.3	79.0	73.7	5.3
10	39.6	28.8	10.8	40.9	39.4	1.5	73.2	58.8	14.4	74.7	53.2	21.5
Mean	**41.9**	**33.0**	**9.0**	**40.8**	**35.5**	**5.3**	**68.0**	**57.8**	**10.1**	**72.3**	**59.3**	**13.1**
SD	**9.3**	**6.7**	**4.7**	**8.0**	**7.1**	**3.5**	**11.0**	**9.0**	**5.3**	**11.3**	**10.0**	**7.5**

Table 6.10: Hyperoxic Test ventilation values during normoxic exercise before and after LDIH. 3bMA = Three-breath moving average. Pre = mean ventilation for the 30 seconds prior to the challenge. Values are expressed in litres·min^{-1}

	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta
Subject	Pre-SDIH											
1	55.2	59.3	4.1	55.6	59.5	3.9	81.8	91.8	10.0	84.6	92.1	7.5
2	38.7	56.5	17.8	39.6	55.7	16.1	61.6	76.2	14.6	61.0	80.0	19.0
3	37.9	43.4	5.5	39.7	43.8	4.1	66.5	83.2	16.7	71.6	82.8	11.2
4	34.7	45.3	10.7	37.0	44.8	7.8	57.6	70.5	12.9	60.1	76.0	15.9
5	40.6	53.8	13.2	44.7	54.9	10.2	70.3	80.5	10.2	75.2	91.1	16.0
6	24.0	32.1	8.1	23.8	31.8	8.0	47.1	55.2	8.1	48.0	60.5	12.5
7	39.3	47.7	8.4	40.9	48.9	8.0	73.2	87.4	14.3	78.8	91.3	12.5
8	49.3	59.1	9.8	51.8	65.2	13.4	99.8	116.7	16.9	108.9	122.6	13.6
9	50.5	53.6	3.1	52.4	61.3	8.9	80.3	97.5	17.2	94.4	124.0	29.6
10	39.2	43.4	4.2	40.2	47.3	7.1	60.2	63.6	3.4	63.1	72.2	9.1
Mean	**40.9**	**49.4**	**8.5**	**42.6**	**51.3**	**8.8**	**69.8**	**82.3**	**12.4**	**74.6**	**89.2**	**14.7**
SD	**8.9**	**8.6**	**4.6**	**9.2**	**10.0**	**3.8**	**14.9**	**17.6**	**4.5**	**18.1**	**20.4**	**6.2**
	Post-SDIH											
1	44.9	58.6	13.6	48.8	58.2	9.4	77.9	91.1	13.2	78.2	95.0	16.8
2	42.1	47.8	5.7	41.1	59.8	18.7	67.8	80.1	12.3			
3	42.0	47.2	5.2	43.5	51.6	8.1	66.3	84.1	17.8	68.2	80.2	11.9
4	38.0	53.9	15.8	38.2	53.1	14.9	68.4	88.8	20.4	69.2	93.6	24.4
5	38.0	45.3	7.3	40.4	47.3	6.9	67.5	83.0	15.5	77.6	86.3	8.6
6	26.8	27.9	1.1	23.0	38.6	15.7	42.0	48.2	6.2	46.0	50.7	4.7
7	41.0	53.7	12.7	38.2	54.5	16.3	72.5	93.5	21.0	78.8	108.6	29.8
8	45.9	66.4	20.6	48.0	63.0	15.0	85.0	95.9	10.9	91.5	105.7	14.1
9	53.8	64.1	10.3	47.9	61.3	13.4	76.2	90.0	13.8	85.8	97.3	11.5
10	40.5	45.7	5.1	46.1	51.5	5.4	62.7	69.0	6.3	68.9	72.8	3.9
Mean	**41.3**	**51.1**	**9.8**	**41.5**	**53.9**	**12.4**	**68.6**	**82.4**	**13.7**	**73.8**	**87.8**	**14.0**
SD	**6.9**	**11.1**	**5.9**	**7.7**	**7.3**	**4.5**	**11.4**	**14.3**	**5.1**	**13.1**	**18.0**	**8.6**

Table 6.11: Hypercapnic Test ventilation values during normoxic exercise before and after SDIH. 3bMA = Three-breath moving average. Pre = mean ventilation for the 30 seconds prior to the challenge. Values are expressed in litres·min^{-1}

	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta
Subject	Pre-LDIH											
1	57.2	66.1	8.9	52.0	64.3	12.3	76.2	97.7	21.5	81.0	93.7	12.7
2	35.8	43.6	7.8	36.6	44.0	7.4	58.0	61.8	3.8	61.5	68.1	6.6
3	34.6	46.0	11.4	40.4	51.8	11.3	56.2	66.6	10.4	57.9	72.6	14.7
4	44.0	49.9	5.9	40.7	51.1	10.4	68.7	96.3	27.5	67.5	93.9	26.3
5	39.6	45.4	5.8	37.7	42.3	4.6	64.0	72.3	8.3	70.4	82.8	12.4
6	24.7	34.8	10.1	22.9	27.1	4.2	41.0	50.9	9.9	44.6	58.1	13.5
7	39.1	41.6	2.5	37.1	55.9	18.9	75.6	88.5	12.9	82.2	97.8	15.7
8	44.7	51.5	6.8	45.0	59.3	14.2	79.7	86.7	7.0	82.7	94.9	12.2
9	47.6	58.2	10.6	50.1	62.8	12.7	75.4	82.1	6.7	81.7	103.3	21.6
10	35.5	39.7	4.3	41.9	47.4	5.5	63.5	65.5	2.0	67.0	72.0	5.0
Mean	**40.3**	**47.7**	**7.4**	**40.4**	**50.6**	**10.2**	**65.8**	**76.8**	**11.0**	**69.6**	**83.7**	**14.1**
SD	**8.8**	**9.2**	**2.9**	**8.1**	**11.1**	**4.7**	**11.9**	**15.7**	**7.9**	**12.7**	**15.2**	**6.3**
	Post-LDIH											
1	38.1	53.1	14.9	44.7	57.1	12.3	74.3	84.0	9.7	81.5	96.7	15.2
2	49.4	51.2	1.8	42.9	51.4	8.5	64.9	80.3	15.4	66.7	81.7	14.9
3	41.1	47.9	6.8	43.6	50.6	7.0	64.0	75.9	11.9	69.5	80.7	11.2
4	39.3	46.7	7.5	39.0	46.3	7.3	65.2	80.0	14.8	63.4	84.7	21.3
5	43.8	49.0	5.2	39.1	45.0	5.8	58.4	64.3	5.9	60.3	67.8	7.5
6	21.8	25.7	3.9	22.9	26.8	3.9	46.6	62.3	15.7	47.4	57.7	10.2
7	41.9	54.8	12.9	44.4	58.6	14.2	89.7	116.9	27.2	83.1	114.9	31.8
8	45.3	55.9	10.5	45.0	59.9	14.9	81.5	98.1	16.6	82.7	98.8	16.1
9	45.0	53.7	8.7	50.3	58.3	8.0	70.8	75.7	4.9	88.0	101.2	13.2
10	39.7	45.5	5.8	41.4	47.9	6.5	67.3	81.3	14.0	76.2	76.7	0.5
Mean	**40.5**	**48.3**	**7.8**	**41.3**	**50.2**	**8.8**	**68.3**	**81.9**	**13.6**	**71.9**	**86.1**	**14.2**
SD	**7.4**	**8.7**	**4.0**	**7.2**	**9.9**	**3.7**	**11.9**	**15.9**	**6.3**	**12.7**	**17.0**	**8.3**

Table 6.12: Hypercapnic Test ventilation values during normoxic exercise before and after LDIH. 3bMA = Three-breath moving average. Pre = mean ventilation for the 30 seconds prior to the challenge. Values are expressed in litres·min^{-1}

	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta
Subject	Pre-SDIH											
1	57.5	38.0	19.5	54.9	37.9	17.0	101.7	55.1	46.6	104.1	68.3	35.9
2	47.7	29.4	18.2	47.4	30.7	16.7	50.9	37.0	13.9			
3	44.6	30.5	14.1	48.8	35.5	13.2	104.1	72.9	31.2	107.7	77.2	30.5
4	43.5	24.3	19.2	45.7	26.7	18.9	78.7	50.9	27.9	89.5	55.4	34.1
5	44.7	37.0	7.7	46.6	38.6	8.0	82.5	59.8	22.7	89.8	70.3	19.6
6	28.9	16.8	12.1	28.1	22.2	6.0	57.6	46.4	11.2	60.3	43.4	16.9
7	47.0	32.4	14.6	42.9	35.3	7.6	90.6	65.8	24.8	89.4	66.5	22.9
8	55.5	41.9	13.7	59.0	44.0	15.0	86.5	86.5	0.0	123.8	98.6	25.2
9	58.4	33.4	25.0	62.1	36.6	25.5	105.5	70.8	34.6	106.9	73.3	33.6
10	47.0	25.9	21.1	53.8	22.9	30.9	82.4	48.3	34.1	92.3	61.2	31.1
Mean	**47.5**	**31.0**	**16.5**	**48.9**	**33.0**	**15.9**	**84.0**	**59.4**	**24.7**	**96.0**	**68.2**	**27.7**
SD	**8.6**	**7.4**	**5.0**	**9.6**	**7.2**	**7.9**	**18.4**	**14.8**	**13.5**	**17.7**	**15.3**	**6.9**
	Post-SDIH											
1	58.1	43.9	14.2	56.8	39.9	16.9	98.7	66.9	31.8	99.5	74.4	25.0
2	46.3	21.3	24.9	42.6	31.6	11.0						
3	49.6	26.1	23.6	51.3	39.2	12.1	83.8	55.5	28.3	89.5	63.9	25.6
4	45.1	23.9	21.1	46.6	27.3	19.3	87.3	63.5	23.8	94.6	64.0	30.6
5	50.1	39.8	10.3	51.8	41.3	10.5	97.8	78.9	19.0	99.9	79.2	20.8
6	21.8	15.2	6.6	23.9	18.2	5.8	51.3	34.8	16.4	55.5	37.9	17.6
7	47.9	33.0	14.9	49.2	33.0	16.2	99.1	69.4	29.7	105.0	74.2	30.8
8	55.1	37.9	17.2	58.3	37.7	20.6	102.8	81.2	21.6	120.0	94.1	25.9
9	61.9	32.7	29.2	66.4	36.9	29.5	99.7	58.2	41.5	98.2	69.0	29.2
10	51.4	33.5	17.8	49.4	25.2	24.3	93.0	49.0	44.0	93.1	64.2	28.9
Mean	**48.7**	**30.7**	**18.0**	**49.6**	**33.0**	**16.6**	**90.4**	**61.9**	**28.5**	**95.0**	**69.0**	**26.1**
SD	**10.8**	**9.0**	**6.9**	**11.2**	**7.5**	**7.1**	**15.9**	**14.6**	**9.5**	**17.2**	**15.1**	**4.5**

Table 6.13: Hyperoxic Test ventilation values during hypoxic exercise before and after SDIH. 3bMA = Three-breath moving average. Pre = mean ventilation for the 30 seconds prior to the challenge. Values are expressed in litres·min^{-1}

	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta
Subject	Pre-LDIH											
1	57.5	38.7	18.8	59.5	47.5	12.0	97.3	76.2	21.2	128.3	106.5	21.8
2	43.8	27.7	16.1	42.2	29.7	12.6	73.1	46.4	26.7	72.4	48.2	24.2
3	37.9	26.8	11.2	38.8	31.1	7.7	90.7	55.5	35.2	90.0	65.7	24.3
4	49.0	25.0	24.0	57.0	31.6	25.4	103.4	64.6	38.8	109.5	76.6	32.9
5	40.8	37.0	3.8	42.1	36.3	5.8	74.5	52.9	21.5	78.5	64.6	13.9
6	23.6	14.1	9.5	26.6	20.4	6.2	55.4	36.5	19.0	54.0	40.5	13.5
7	44.8	37.7	7.2	51.6	33.7	17.8	90.5	79.3	11.3	105.0	81.5	23.5
8	50.1	33.5	16.5	50.5	41.1	9.5	92.7	67.4	25.4	107.4	81.0	26.5
9	55.9	37.2	18.8	57.4	33.6	23.7	92.8	64.1	28.7	99.7	80.7	19.0
10	50.1	32.8	17.4	51.4	41.0	10.4	89.4	64.8	24.7	90.1	51.8	38.3
Mean	**45.4**	**31.0**	**14.3**	**47.7**	**34.6**	**13.1**	**86.0**	**60.8**	**25.2**	**93.5**	**69.7**	**23.8**
SD	**9.8**	**7.7**	**6.2**	**10.2**	**7.5**	**7.0**	**14.2**	**13.1**	**7.9**	**21.3**	**19.6**	**7.7**
	Post-LDIH											
1	50.5	38.6	11.9	52.1	37.3	14.8	93.5	70.6	22.9	99.5	74.7	24.8
2	47.4	34.1	13.3	46.2	32.2	14.1						
3	43.4	35.4	8.0	48.7	31.0	17.8	88.3	59.8	28.4	99.8	58.8	41.0
4	47.9	21.2	26.6	46.7	26.1	20.5	84.7	38.1	46.6	81.4	35.8	45.6
5	46.0	37.4	8.5	47.6	38.6	9.0	88.2	62.4	25.8	95.6	78.0	17.5
6	23.6	19.8	3.8	25.0	15.5	9.5	53.7	34.1	19.6	56.4	37.5	18.9
7	51.0	39.2	11.8	48.9	31.9	16.9	88.2	71.5	16.7	104.9	95.6	9.3
8	51.8	35.7	16.1	51.3	32.1	19.2	91.3	70.4	20.9	100.1	78.6	21.4
9	54.4	28.6	25.9	58.2	40.5	17.7	90.6	60.1	30.5	100.1	68.6	31.6
10	50.1	32.5	17.5	50.6	30.7	19.9	98.1	54.9	43.2	104.0	80.8	23.2
Mean	**46.6**	**32.2**	**14.3**	**47.5**	**31.6**	**16.0**	**86.3**	**58.0**	**28.3**	**93.5**	**67.6**	**25.9**
SD	**8.7**	**6.9**	**7.4**	**8.6**	**7.1**	**4.1**	**12.8**	**13.7**	**10.4**	**15.5**	**20.1**	**11.6**

Table 6.14: Hyperoxic Test ventilation values during hypoxic exercise before and after LDIH. 3bMA = Three-breath moving average. Pre = mean ventilation for the 30 seconds prior to the challenge. Values are expressed in litres·min^{-1}

	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta
Subject	Pre-SDIH											
1	59.7	66.3	6.6	60.1	66.1	6.1	128.3	135.0	6.6	131.3	145.8	14.4
2	47.1	74.3	27.2	51.0	73.7	22.7	78.9	97.6	18.7			
3	46.5	57.8	11.3	48.9	64.1	15.3	83.4	103.5	20.1	91.6	108.5	16.9
4	42.3	54.8	12.5	47.8	68.2	20.4	79.3	94.3	15.0	84.5	97.6	13.1
5	44.4	52.3	7.9	48.3	53.7	5.4	86.5	105.1	18.6	98.1	101.0	3.0
6	28.7	37.6	8.9	27.5	41.6	14.1	57.9	61.9	4.0	61.6	68.8	7.2
7	49.3	55.2	5.8	46.3	54.8	8.5	84.7	97.7	13.0	95.2	101.1	5.8
8	58.3	79.3	21.0	57.9	80.1	22.2	123.3	135.7	12.4	120.1	135.3	15.2
9	66.1	83.3	17.2	63.0	71.2	8.2	99.4	121.0	21.6	107.7	134.8	27.1
10	45.7	51.9	6.2	50.7	58.7	7.9	78.9	97.3	18.4	90.6	98.1	7.6
Mean	48.8	61.3	12.5	50.1	63.2	13.1	90.1	104.9	14.8	97.9	110.1	12.3
SD	10.5	14.2	7.2	9.8	11.3	6.8	21.5	21.7	5.9	20.3	24.2	7.3
	Post-SDIH											
1	55.0	67.6	12.6	58.5	76.6	18.2	94.0	118.6	24.6	93.4	109.6	16.2
2	44.8	64.7	19.9	46.8	56.6	9.8						
3	46.6	58.7	12.2	50.8	60.1	9.3	92.0	114.8	22.7	95.1	130.2	35.1
4	44.6	75.1	30.5	48.2	71.2	22.9	85.5	110.3	24.8	91.2	106.6	15.4
5	47.4	54.8	7.4	48.7	53.7	5.0	85.4	104.3	18.9	96.7	103.0	6.2
6	21.9	32.6	10.7	24.6	30.5	5.9	46.7	63.9	17.3	48.7	51.8	3.1
7	47.0	60.2	13.3	49.9	55.4	5.5	91.8	110.6	18.9	105.3	124.1	18.8
8	55.5	84.0	28.5	57.2	78.4	21.2	113.6	131.4	17.8	119.5	140.2	20.6
9	60.8	75.3	14.6	65.6	72.5	6.9	100.7	110.7	10.0	105.0	124.1	19.1
10	51.4	55.8	4.4	48.7	57.0	8.3	92.2	95.6	3.4	92.3	105.9	13.6
Mean	**47.5**	**62.9**	**15.4**	**49.9**	**61.2**	**11.3**	**89.1**	**106.7**	**17.6**	**94.1**	**110.6**	**16.5**
SD	**10.5**	**14.3**	**8.5**	**10.7**	**14.3**	**6.8**	**18.1**	**18.8**	**7.0**	**19.3**	**25.4**	**9.1**

Table 6.15: Hypercapnic Test ventilation values during hypoxic exercise before and after SDIH. 3bMA = Three-breath moving average. Pre = mean ventilation for the 30 seconds prior to the challenge. Values are expressed in litres·min^{-1}

	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta	Pre	3bMA	Delta
Subject	Pre-LDIH											
1	60.1	85.5	25.4	62.9	74.1	11.2	113.0	131.5	18.5	139.4	164.4	24.9
2	42.9	55.0	12.2	42.8	48.8	6.1	65.8	83.1	17.2	70.3	83.8	13.5
3	34.4	51.6	17.2	41.6	52.2	10.6	73.0	98.3	25.2	81.8	102.7	20.9
4	47.6	60.9	13.3	51.7	69.5	17.8	102.1	120.4	18.3	111.3	122.1	10.8
5	40.6	44.2	3.6	42.6	51.1	8.5	68.8	99.3	30.5	83.1	89.5	6.4
6	24.3	38.8	14.5	25.0	29.1	4.1	49.2	66.2	16.9	51.5	69.8	18.3
7	48.1	66.9	18.7	45.5	61.1	15.6	98.8	113.4	14.7	106.4	128.3	21.9
8	51.6	69.8	18.2	51.1	71.2	20.1	98.8	112.7	13.9	103.0	125.0	22.0
9	52.8	59.2	6.4	52.5	63.7	11.2	91.0	107.2	16.2	99.7	110.6	11.0
10	53.9	57.1	3.2	50.0	64.3	14.3	85.4	94.9	9.5	92.1	107.9	15.8
Mean	**45.6**	**58.9**	**13.3**	**46.6**	**58.5**	**11.9**	**84.6**	**102.7**	**18.1**	**93.9**	**110.4**	**16.5**
SD	**10.5**	**13.3**	**7.2**	**9.9**	**13.5**	**5.1**	**19.8**	**18.8**	**5.9**	**24.2**	**26.8**	**6.0**
	Post-LDIH											
1	51.8	56.8	5.0	53.2	58.8	5.6	92.6	110.7	18.1	109.5	122.0	12.5
2	45.1	46.7	1.6	49.7	66.3	16.6						
3	42.9	65.8	22.9	45.8	66.9	21.1	81.5	97.3	15.8	93.6	111.2	17.7
4	47.5	58.8	11.3	49.5	58.1	8.6	78.1	100.6	22.5	83.6	96.9	13.4
5	43.5	53.0	9.5	46.1	51.4	5.3	73.8	89.4	15.6	80.1	90.4	10.3
6	25.0	33.5	8.6	26.3	32.9	6.6	58.4	63.3	4.9	57.8	68.6	10.8
7	52.0	61.7	9.7	45.6	66.9	21.3	112.6	126.0	13.5	106.3	144.8	38.5
8	52.1	73.8	21.7	53.2	70.8	17.6	97.8	111.8	13.9	108.1	122.6	14.5
9	57.0	63.1	6.0	61.7	69.2	7.5	98.4	110.4	12.1	97.6	113.3	15.7
10	51.8	68.8	17.0	50.7	56.9	6.2	81.5	94.2	12.7	89.0	101.7	12.7
Mean	**46.9**	**58.2**	**11.3**	**48.2**	**59.8**	**11.7**	**86.1**	**100.4**	**14.3**	**91.7**	**107.9**	**16.2**
SD	**8.9**	**11.6**	**7.1**	**9.0**	**11.3**	**6.7**	**16.0**	**17.8**	**4.8**	**16.6**	**21.9**	**8.7**

Table 6.16: Hypercapnic Test ventilation values during hypoxic exercise before and after LDIH. 3bMA = Three-breath moving average. Pre = mean ventilation for the 30 seconds prior to the challenge. Values are expressed in litres·min^{-1}

Subject	SDIH Protocol Day 1			SDIH Protocol Day 8		
	Ramp Time (s)	*Peak VO2*	*Peak Ve*	*Ramp Time (s)*	*Peak VO2*	*Peak Ve*
1	9	3.06	122.3	64	2.92	131.9
2	0			0		
3	0		106.5	150		103.3
4	134		83.3	62		96.1
5	205	2.87	103.3	166	2.84	94.4
6	220		84.0	217		95.8
7	50			174		
8	130	2.43	141.4	165	2.65	166.6
9	210	3.81	131.8	330	4.30	174.7
10	160	3.23	111.7	154	3.28	117.8
Mean	**111.8**	**3.1**	**110.5**	**148.2**	**3.2**	**122.6**
Standard Deviation	**89.8**	**0.5**	**20.9**	**91.6**	**0.7**	**32.4**

Table 6.17: Peak Ramp time, oxygen consumption VO2, and ventilation during a graded exercise test in hypoxia before and after SDIH. Ventilation and oxygen consumption values are expressed in litres·min^{-1}

Subject	LDIH Protocol Day 1			LDIH Protocol Day 8		
	Ramp Time (s)	*Peak VO2*	*Peak Ve*	*Ramp Time (S)*	*Peak VO2*	*Peak Ve*
1	60	3.26	132.6	142	3.07	97.2
2	30			0	N/A	
3	142		111.9	150		102.3
4	190		136.6	143		89.9
5	216	2.60	97.3	232	2.61	110.5
6	188		89.2	176		88.5
7	148			155		
8	180	2.86	149.3	225	3.60	154.8
9	244	3.44	116.2	310	3.35	122.0
10	217	3.24	118.3	241	3.29	125.1
Mean	**161.5**	**3.1**	**118.9**	**177.4**	**3.2**	**111.3**
Standard Deviation	**69.0**	**0.3**	**20.1**	**83.0**	**0.4**	**22.2**

Table 6.18: Peak Ramp time, oxygen consumption VO2, and ventilation during a graded exercise test in hypoxia before and after LDIH. Ventilation and oxygen consumption values are expressed in litres·min^{-1}