The Health Effects of Intermittent Hypoxic Exposure in a Sedentary Population

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A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at

Lincoln University

by

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Lincoln University

2013

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

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The use of simulated altitude is frequently used in an attempt to enhance athletic performance. Similar techniques have also recently been used in a clinical population to improve cardiovascular risk. However, the effects of different frequencies of simulated altitude per week, follow up assessments and the effects of passive simulated altitude in addition to exercise (rather than exercising in hypoxia) has not been tested in a sedentary, middle-aged population.

To simulate altitude, an intermittent hypoxic exposure (IHE) protocol was used whereby 5-min of hypoxia was alternated with 5-min of normoxia for 1 hour. Heart rate variability (HRV), i.e. the beat-to-beat fluctuations between R-peaks, cardiovascular fitness ($\dot{V}O_{2peak}$), systolic blood presssure (SBP), highly-sensitive C-Reactive protein (hs-CRP, indicative of systemic inflammation associated with athlersclerosis), high density lipoprotein, total cholesterol, and arterial stiffness were used to assess cardiovascular health during 3 IHE studies.

In the first study, 4 IHE sessions/week for 4 weeks resulted in increased HRV compared to a placebo treatment (71.6 ± 52.5%, mean between-group change from the natural logarithm ± 90% confidence interval), indicating increased parasympathetic activity. The second study assessed the effects of IHE frequency on selected risk factors. Following the 5-week intervention, HRV (during paced breathing) increased in participants given both 2-3 (IHE3) and 5 (IHE5) IHE sessions per week (IHE5: 47.3 ± 41.6%; IHE3: 6.2 ± 6.9%), thereby confirming the changes in HRV reported in Study 1. In addition, haemoglobin concentration (2.7 ± 2.8%), time taken to complete the maximal fitness assessment (34.5 ± 36.8%), and maximal workload (14.4 ± 14.9%) were increased in IHE5 compared to the control group, while changes in hs-CRP (12.7 ± 48.7%) were trivial. None of these changes were seen in IHE3. There was an increased $\dot{V}O_{2peak}$ (12.6 ± 9.3%) in IHE3 compared to the Control, but not IHE5.

Due to the benefical effects on $\dot{V}O_{2peak}$ and HRV, 2-3 IHE sessions/week were used together with 3 exercise sessions/week (IHE3+Ex), and compared to exercise only (Ex) in the third study. Immediately

post intervention, 4- and 8-wk follow up measurements were taken. Both groups showed a tendency to decrease total cholesterol, improve arterial compliance (pulse wave velocity) and increase $\dot{V}O_{2peak}$ and HRV, but these changes were unclear. High density lipoprotein (Post: 8.0 ± 8.0%; 8-wk: 10.0 ± 8.5%), SBP (Post: -3.4 ± 3.4%; -3.5 ± 3.7%) improved in IHE3+Ex more than Ex, and while $\dot{V}O_{2peak}$ was qualitatively unclear at Post it was increased at the 4-wk (9.4 ± 8.0%) and 8-wk (7.9 ± 8.3) follow-up sessions. While differences in systemic arterial stiffness (augmentation index) was unclear between groups at Post, the augmentation index increased at 4- and 8-wk (4-wk: 11.8 ± 18.4%; 8-wk: 24.8 ± 19.7%) in the IHE3+Ex group compared to Ex.

Overall, IHE appears to increase the parasympathetic contribution to the sympathovagal balance of the heart at rest in sedentary middle-aged participants, and may improve SBP and some fitness parameters, particularly with 4–5 IHE sessions/week. While the short-term effects of IHE on arterial health are promising, more research on the long-term effects of IHE treatments is needed before IHE can conclusively be considered 'safe'.

Keywords: Intermittent hypoxic exposure, heart rate variability, exercise, sedentary, health, cardiovascular risk, systolic blood pressure, total cholesterol, high density lipoprotein, highly sensitive C-Reactive protein, arterial stiffness

Acknowledgements

The road to submission has been a long winding one that has traversed steep inclines, unexpected challenges, long dry deserts, and amazing view-points. It has been a journey that could not have been completed, without the help and support of so many along the way.

My supervisor, Associate Professor Mike Hamlin, has been a mentor in the truest sense of the word. Over the last 4 years, I have greatly appreciated his guidance, support, and critical insight. I could not have asked for a better (or more patient) collaborator and advisor. I also owe thanks to my associate supervisor, Dr Gary Steel, for planting the 'why not apply to do a doctorate instead' seed, and all his ongoing support in both thesis and work-related spheres.

For the last 3 years I have received a stipend and contribution to university fees from The National Heart Foundation of New Zealand, for which I am incredibly grateful.

I have been immensely fortunately to have been able to collaborate with researchers outside of Lincoln University, which has greatly contributed to the depth of investigation we were able to achieve. To Dr Lee Stoner, who has provided expert guidance, support and generous use of his equipment in the assessments of arterial stiffness in Study 3 (Appendix G) and Dr Sam Lucas, who so willingly drove up from the University of Otago to drop off NIRS equipment for each of the measurement time points, and for his critical analysis of the subsequent data analysis (Appendix G), I owe you both a great depth of gratitude. Dr Yaso Kathiravel and Dr John Elliott have both gone above and beyond the call of duty in volunteering their very busy time to medically assess the participants prior to inclusion into the study, and to Dr John Hellemans for his expert advice on both hypoxic training and medical matters.

Then, to all the people who have helped with the long hours of exercise testing, specifically Adam Lucero, Meegan Fraser, Apiwan and Nuttaset Manimmanakorn, Darrell Lizamore, Sam Russell, and Helen Marshal. In a post-earthquake space-limited campus, the thinking-out-the-box can-do attitude of Marianne Richards and her team of ground staff at Lincworks was like a cool glass of water on a desert stretch of the road.

Finally, I am indebted to all the participants for each of the studies who have so willingly and cheerfully volunteered their time to the project.

On a personal note, I would like to extend my gratitude to David and Eriko Viviers, and the Metcalf family who have been like family to us since we arrived in New Zealand. To our family, the Rendalls, Lizamores, Simpkins, and Blacklaws, your thoughts, prayers and encouragement have been especially

valuable. Then, to my Grandpa whose love for endurance running and recovery from cardiovascular disease have been two of the biggest sources of inspiration, both academically and personally, thanks.

Finally, to my husband, Darrell Lizamore, who has been up front in the passenger seat and has shared all of the views, excitement and the detours along the way –all while negotiating a PhD of his own. I couldn't have done this without all your encouragement, love and support. You are my sunshine, thank you.

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List of frequently used abbreviations

AIx	Augmentation Index (measure of systemic arterial compliance)
Ex	Group receiving exercise only in Study 3
Hb	Haemoglobin
HF	High frequency (indication of parasympathetic contribution to the autonomic control of the beat-to-beat intervals)
HR_{max}	Maximal heart rate achieved during a fitness assessment
HR_{submax}	Average heart rate taken during submaximal exercise exertion
HRV	Heart rate variability
Hyp	The group receiving 4 intermittent hypoxic exposure sessions / wk in Study 1
IHE	Intermittent hypoxic exposure (5-min hypoxia alternated with 5-min normoxia for 1 h)
IHE3	The group receiving 2 - 3 intermittent hypoxic exposure sessions / wk in Study 2
IHE3 + Ex	The group receiving 2 - 3 intermittent hypoxic exposure sessions / wk in addition to 3 exercise sessions /wk in Study 3
IHE5	The group receiving 5 intermittent hypoxic exposure sessions / wk in Study 2
LF	Low frequency (representing a combination of parasympathetic and sympathetic activity in the control of the beat-to-beat intervals)
PWA	Pulse wave analysis (measure of systemic arterial stiffness)
PWV	Pulse wave velocity (indication of local arterial stiffness)
rMSSD	Root mean square of successive differences between R-peaks, indicates parasympathetic contribution to the autonomic control of the beat-to-beat intervals
RPE	Rating of perceived exertion
SBP	Systolic blood pressure
SDNN	Standard deviation of all normal-to-normal beats, indicates overall heart rate variability
$\dot{V}O_{2peak}$	Maximal oxygen consumption measured over the last 30 s of maximal exertion
$\dot{V}O_{2submax}$	Oxygen consumption measured during submaximal exercise.

Chapter 1

Introduction

"*If exercise could be purchased in a pill, it would be the single most widely prescribed and beneficial medicine in the nation.*"

Robert H. Butler

Atherosclerotic cardiovascular disease is the leading cause of death worldwide (Gersh et al., 2010, Perk et al., 2012), and is responsible for approximately 40% of all deaths in Europe and America (Heidenreich et al., 2011, Perk et al., 2012). New Zealand citizens are no exception to these trends, with cardiovascular disease and cancer listed as the joint leading causes of death nationwide (Ministry of Health, 2013). Aside from premature death, the steady decline in health attributed to cardiovascular disease results in an increase in disability restricted years (Perk et al., 2012), and a substantial economic burden related to health care (Leal et al., 2006). However, as cardiovascular disease is progressive in nature (Dzau et al., 2006), the pathway to full cardiovascular disease can be disrupted by early detection and treatment of identified risk factors (Perk et al., 2012). For example, there are a number of health conditions such as hypertension, diabetes, elevated cholesterol and obesity, or lifestyle influences such as physical inactivity, or cigarette smoking that have been identified as pre-cursors to cardiovascular disease (Dzau et al., 2006, Chobanian et al., 2003).

In terms of health improvement and maintenance, exercise is widely regarded as one of the best and most cost effective means of interrupting the pathway to full cardiovascular disease. Indeed, given the low cost and cardioprotective benefits conferred by regular physical activity, in 1998 New Zealand established "Green Prescription" whereby a doctor can 'prescribe' physical activity to their patient (Ministry of Health, 2012). If the patient chooses to enrol in the Green Prescription programme, they are offered nutritional advice, discuss physical activity goals and receive follow up support (Ministry of Health, 2012). In a similar spirit to the Green Prescription programme, a new initiative, "Exercise is Medicine" has been established in association with the American College of Sports Medicine and the American Medical Association (Sallis, 2009). The purpose of this initiative is to encourage medical practitioners to view physical activity as a prescription that can be given to patients requiring the medical benefit of exercise.

While exercise is the best option for most people, there are many people who cannot exercise or who can only exercise in limited amounts. Such people include those who are confined to bed-rest and those with spinal injuries, patients with chronic diseases such as arthritis, multiple sclerosis and patients undergoing muscular rehabilitative therapy following a sports injury or surgery. For this population, an intervention

that may afford some of the cardiovascular benefit of exercise, but without physical activity, would be very valuable indeed.

Interestingly, research in the Former Soviet Union has long recommended the use of simulated altitude training (as is used in many athletic protocols) to improve the outcome of a number of different diseases such as hypertension, diabetes mellitus and respiratory disease (Serebrovskaya, 2002). However, much of this research is written in Russian and warrants further investigation. In 'Western' countries, the majority of the research regarding altitude training has been focussed on elite athletes and is directed at improving performance (Millet et al., 2010). Still, there are some simulated altitude exposure interventions that have been conducted in a clinical population with the aim of improving exercise tolerance and thereby improving cardiovascular health (Burtscher et al., 2009, Burtsher et al., 2004). While the available literature regarding the use of simulated altitude in a sedentary or clinical population has demonstrated beneficial responses, both with and without concurrent exercise, there are still a number of questions that warrant further investigation.

The purpose of this thesis is to determine the effects of intermittent hypoxic exposure on cardiovascular risk factors in a sedentary population.

1.1 Thesis statement and aims

The purpose of this thesis is to determine the effects of intermittent hypoxic exposure on cardiovascular risk factors in a sedentary population. In order to achieve this, the following aims have been outlined:

1) Identify gaps in the literature regarding the use of simulated altitude for health improvement.

2) Informed by findings in the literature review, determine the effects of simulated altitude (without the use of exercise) on selected risk factors in a sedentary population

3) Informed by findings in the literature review, determine the effects of simulated altitude (with the use of exercise) on selected risk factors in a sedentary population

As this research is based at a university rather than at a medical school, we have elected to use sedentary participants as the target research population, rather than clinical or acutely unwell participants.

To guide the progression of the research, a decision tree was used (see Figure 1).

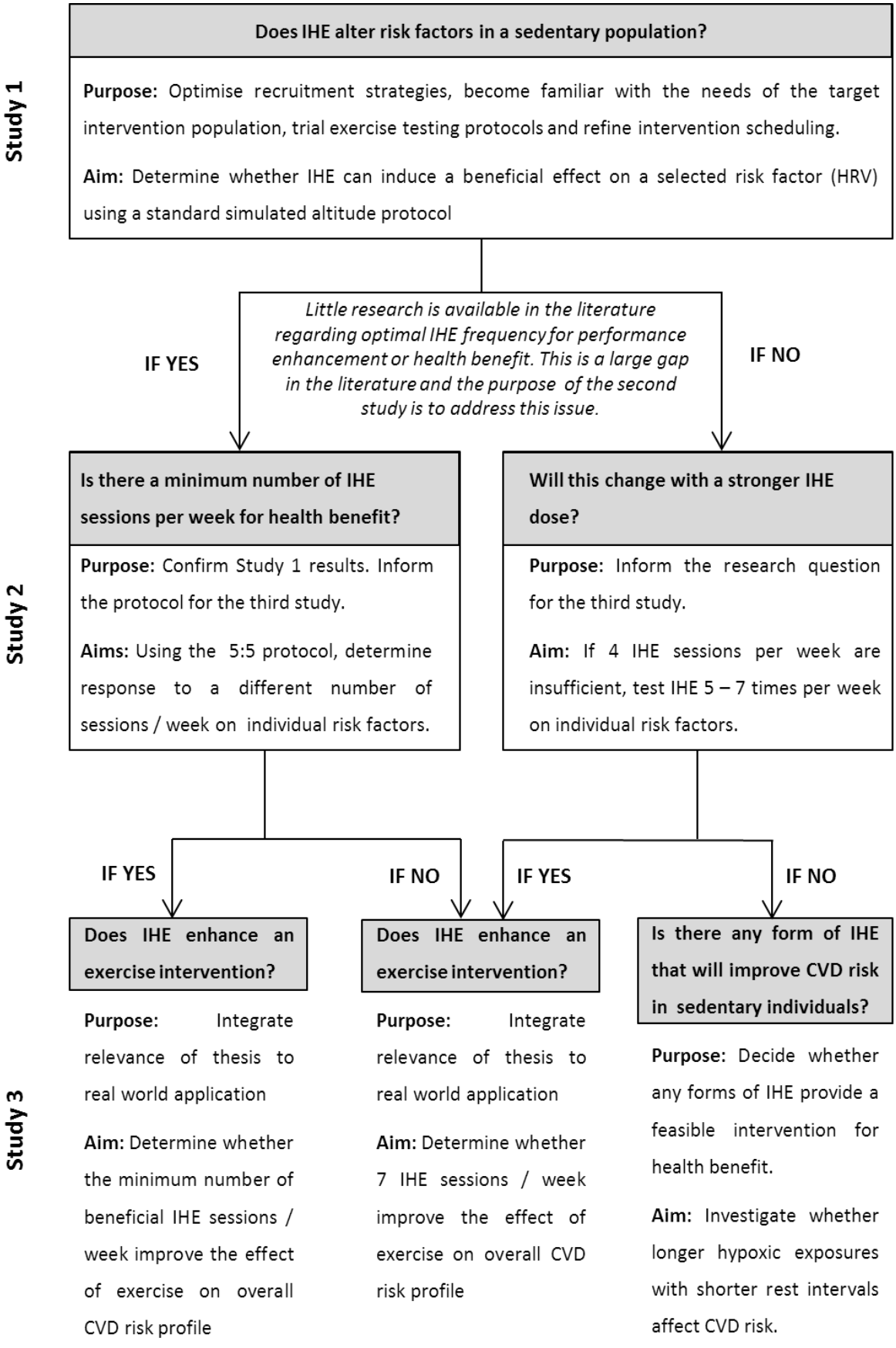


Figure 1: Decision making process for research progression

1.2 Thesis format

The best means of testing these hypotheses was to run successive studies, each developing the learnings from the previous one. Therefore, the nature of this research lends itself to a 'thesis by papers' approach, whereby each chapter of the thesis is written in the format of an academic journal article. It follows then, that each paper is self-contained, including an introduction and background section to the research covered in that chapter, as well as a discussion of the results. Additionally, a brief description of the 'learnings' from the previous study and how they relate to the following study has been included between chapters to improve readability and the flow between chapters. All references are listed in the reference list at the end of the thesis.

Early on in the research planning, we realised that heart rate variability (HRV), which is both a predictor of cardiovascular health, and reflects instantaneous alterations in the autonomic control of the heart (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996) would be a useful tool in all three studies. Accordingly, the assessment of HRV in each of the 3 studies has become a sub-theme that has been threaded through each of the studies. However, while the assessment of HRV is inexpensive, non-invasive, and time-efficient, there are various methodological caveats (such as data capture techniques, how the data are filtered, and the presentation of the final HRV results) that need to be addressed. An introduction to HRV and it's measurement, and a draft paper on the repeatability of HRV are included in the Appendix A and B respectively.

1.3 An overview of the following thesis chapters

1.3.1 Chapter 2: Literature Review on the effects of simulated altitude

To fully understand the research to date a comprehensive literature review was undertaken. While the primary focus of this thesis is on a clinical or sedentary population, the review did include literature on athletic and well trained populations. The decision to include literature focussed on athletic populations was due to the large amount of research that has already been conducted in this population. The findings from these studies could provide insight into any physiological changes observed in an unwell or sedentary population or help to generate ideas for protocol development. The review includes a quantitative assessment of the currently available literature including a profile of the research (sex and ages of participants, types of training programmes used and physical abilities of participants), and an in-depth discussion relating to studies that have investigated the use of simulated altitude exposure in either a clinical or sedentary population. Tables summarising the literature relating to healthy/active, and well trained/athletic populations are also included, but are not extensively discussed. Directions for future research are recommended. The literature review did not include extended hypoxic exposure such as long

sojourns at altitude (including native mountain dwellers, or mountaineering expeditions) and disease-related hypoxic exposure (such as in obstructive sleep apnoea, or ischemic heart disease). Several key areas for future research include:

I. The effects of simulated altitude, particularly an intermittent hypoxic exposure (IHE) protocol which alternates several minutes of severe hypoxia with several minutes of normoxic recovery cycled for an hour, on cardiac autonomic modulation, vascular health, blood lipid profile, and cardiovascular risk profile in addition to conventional cardiovascular fitness assessments is needed.

II. The need to report the participant's subjective response to the simulated altitude treatments.

III. A greater emphasis is needed on research in a clinical or sedentary population. These population groups appear to demonstrate promising improvements to simulated altitude.

IV. Research regarding the effects of different frequencies of simulated altitude sessions per week on health (assessed via modulation of cardiovascular risk factors) is missing.

V. One study has reported beneficial responses of the effect of IHE in conjunction with exercise on cardiovascular risk factors in a sedentary, overweight population of 18 – 20 year old men. More research should be conducted on the effects of passive simulated altitude in conjunction with exercise (versus exercise alone or exercise in hypoxia) in a sedentary, middle-aged group who are likely to demonstrate greater cardiovascular risk factors.

To address these gaps in the literature, three different studies were undertaken.

1.3.2 Chapter 3: Study 1: The effect of intermittent hypoxic exposure on heart rate variability in a sedentary population

While meeting the aim and purpose outlined in Figure 1, this research also sought to determine both the response in the autonomic control of the heart rate during an IHE session and the adaptive response to 4 weeks of 4 IHE sessions per week. As HRV is both an indicator for cardiovascular risk, and provides an instantaneous reflection of parasympathetic and sympathetic activity on the heart rhythm, this was used as the central measure in this study. The results of this study indicated an improvement in parasympathetic activity during rest in response to the 4-week intervention. We also indicate substantive parasympathetic withdrawal and reactivation between hypoxic and rest intervals respectively which was not present in the control group.

1.3.3 Chapter 4: Study 2: The minimum dosage of intermittent hypoxic exposure per week for health improvement in sedentary, middle-aged individuals

The aim of this study was to build on from Study 1's findings and determine whether there is a dose response to the frequency of IHE treatments per week, and if so, what the minimum number of IHE sessions per week is to elicit a health benefit. To achieve this, participants were divided into groups receiving 2 – 3 IHE sessions per week and 5 IHE sessions per week (with one group as a control group) for 5 weeks. By using these frequencies, we have effectively gathered data from the effects of 2, 3, 4 and 5 IHE sessions per week in a sedentary population.

Along with HRV, other independent risk factors for CVD, including highly sensitive C-Reactive protein, cardiorespiratory fitness, and systolic blood pressure (SBP) were included in this study to give a more comprehensive overview of the effect of IHE on CVD risk factors in a sedentary population.

The results of Study 2 indicated that while cardiovascular benefit associated with 5 IHE sessions per week demonstrated enhanced HRV and exercise tolerance, there was some improvement in HRV and possible improvement in $\dot{V}O_{2peak}$ in the group receiving IHE 2 - 3 times per week.

1.3.4 Chapter 5: Study 3: Does intermittent hypoxic exposure enhance the effect of an exercise intervention on overall cardiovascular risk profile?

The aim of the final study was to assess whether IHE has an additive health benefit when used in conjunction with an exercise training programme. To test this, one group received 2 – 3 IHE sessions per week in conjunction with 3 supervised exercise sessions while another attended only exercise training.

This study included independent risk factors and a full cardiovascular risk profile. Independent risk factors included arterial stiffness, HRV and cardiovascular fitness, while the overall cardiovascular risk was assessed using the New Zealand Cardiovascular Risk Chart. This risk profile uses a compilation of risk factors including systolic blood pressure, total cholesterol: high density lipoprotein level, age, smoking and diabetes status to determine an overall 5-year risk of fatal or non-fatal cardiovascular disease (New Zealand Guidelines Group, 2009). See Figure 31 in Appendix E for a copy of the NZ Guidelines risk assessment chart.

While the group receiving the IHE and exercise treatment demonstrated slightly superior results in independent risk markers, the cardiovascular risk profile was unchanged in both groups. When weighed against the time commitment of the three IHE sessions per week for only a marginal improvement in health, it is likely that an individual's time would be better spent exercising one or two addition days. Additionally, more research is needed on the long-term (greater than 2 months) effects of IHE on vascular

health. However, for those who are restricted to a limited amount of exercise, IHE may be worthwhile. In this case 4 or 5 IHE sessions per week should be considered.

1.3.5 Chapter 6: Overall discussion and conclusion

The findings from each of the three studies are analysed collectively, final recommendations for the use of IHE in a sedentary, middle-aged population, and projections for future research are discussed.

1.3.6 Appendices

Supplementary material has been included in the appendices. Appendices A and B include an overview of HRV and how it was interpreted in this thesis. Appendices C and D report the raw data for studies 2 and 3 (raw data for Study 1 are included in Chapter 3). Appendix E contains the chart used to assess overall 5-year cardiovascular risk used in Study 3. Finally, Appendix F explains how the qualitative outcomes are assessed using the spreadsheets designed by Will Hopkins. Appendix G contains a paper that was based on the baseline results of Study 3.

Chapter 2

Literature Review: The use of simulated altitude techniques for beneficial physiological adaptation.

2.1 Introduction

Altitude training has long been used in athletic populations in an attempt to improve competitive performance. However, while there is potential for improved performance following altitude training, it is also expensive, time consuming and disruptive to family and work life. For example, it is recommended that athletes reside at 2000 – 2500 m for greater than 3 - 4 weeks, for more than 12 - 22 hours/day (Wilber et al., 2007, Rusko et al., 2004, Duke et al., 2012). Clark et al. (2009) specify that for improvements in haemoglobin mass (Hb_{mass}) at least 100 hours of hypoxic exposure at between 2200 – 3000 m for at least 12 hours/day are required for every 1% increase in Hb_{mass}. In addition to the long duration required, the currently recommended best practice for altitude training is to use a "live high train low" model (Millet et al., 2010) which involves living at altitude while driving to and from lower altitude for exercise training. The time, inconvenience and expense associated with real altitude training has resulted in the development of several simulated altitude protocols which aim to provide a convenient method of achieving the benefits of altitude training but without the disruption and financial costs associated with real altitude training.

To simulate altitude, researchers (and coaches/athletes) either employ the use of hypobaric chambers or create a normobaric hypoxic environment. The latter is achieved by either diluting the ambient air with nitrogen (Wilber, 2001), using re-breathers which recycle expired air (Sausen et al., 2003), or 'hypoxicators' which extract oxygen from the air through the use of a polymeric membrane air separation process (Serebrovskaya et al., 2003). The relevance of differences between the hypo- and normobaric techniques have been the heart of a recent debate (Millet et al., 2012b), with most agreeing that hypobaric hypoxia provides a different and far more complex network of stresses (Millet et al., 2012a); for example, greater susceptibility of acute mountain sickness (Roach et al., 1996). However, this is also debated by Schommer and Bartsch who point out only a negligible difference in acute mountain sickness (50% in real altitude versus 58% in normobaric hypoxia) following 18 h of exposure (Bailey et al., 2005, Schommer et al., 2011). Taylor, Snyder and Johnson as well as Guenette and Koehle have also suggested that there is a greater risk of pulmonary oedema with hypobaric hypoxia via increased pulmonary fluid flux in lower barometric pressure (Levine et al., 1988, Snyder et al., 2006). Other differences reported were the lower air resistance in hypobaric hypoxia (Girard), quality of sleep (Nespoulet, Wuyam, Tamisier, Verges, and Levy), and greater

respiratory muscle energy cost in normobaric hypoxia (For all points and counterpoints, see Girard et al., 2012).

However, it is the reduction of oxygen that is the trigger for adaptations associated with the oxygen-sensing transcription factor, the α-subunit of Hypoxic Inducible Factor – 1 (HIF-1α). Indeed, the oxygen sensing pathway is the central argument in Mounier and Brugniaux's counter-point to the non-importance of hypobaric pressure in altitude adaptation (Mounier and Brugniaux, 2012a, Mounier and Brugniaux, 2012b).

On arrival in an oxygen reduced environment (in real or simulated altitude) there is a spontaneous increase in ventilation known as the hypoxic ventilatory response. This increase in ventilation includes an increase in tidal volume and respiratory frequency (Powell et al., 1998) and is essential in maintaining adequate tissue oxygenation. The hypoxic ventilatory response is activated primarily via the carotid body peripheral chemoreceptors (Prabhakar and Peng, 2004) and can be up-regulated within one breath of oxygen reduced air (Powell et al., 1998). Other researchers have also observed increases in heart rate, cardiac output and left ventricular ejection fraction (Huez et al., 2009) with hypoxia. The concurrent activation of HIF-1α up-regulates genes responsible for angiogenesis, erythropoiesis (Semenza, 2009) iron homeostasis (observed as an increase in haemoglobin (Clark et al., 2009, Saunders et al., 2009)) and altered glucose metabolism (Semenza, 2001) which are important for long-term adaptation and maintenance of tissue and organ oxygenation in a low oxygen environment. In addition to these adaptations, physiological changes such as increased chemoreflex sensitivity to hypoxia (Bernardi et al., 2001), increased pulmonary arterial pressure (Zhao et al., 2001) and increased subsarcolemmal mitochondrial expression (Schmutz et al., 2010) which is a subgroup of mitochondria which appears to be most responsive to endurance training (Koves et al., 2005) are observed.

It is the influence of these adaptations that are thought responsible for increased maximal oxygen consumption (Robertson et al., 2010c) and improved exercise economy following altitude training. The haematological adaptations (red blood cells and haemoglobin in particular) have typically thought to be the primary adaptation responsible for improved athletic performance (Levine and Stray-Gundersen, 2005). However, a study by Garvican et al. (2011) suggests that other non-haematological adaptation to hypoxia may be responsible for improvements in performance. To isolate the role of Hb in exercise performance, Garvican et al. (2011) allowed one group of sub elite cyclists to adapt normally to a simulated live high train low protocol, and withdrew a proportion of blood in a matched control group to negate any gains in Hb. These researchers found that despite no net increase in Hb_{mass} following blood removal, the control group still improved $\dot{V}O_{2peak}$ similar to that of the test group which suggest that the 'upstream changes' and triggers leading to the increase in Hb mass may also be important in the adaptation to exercise. Some

examples of non-haematological adaptation include improved muscle buffering capacity, lactic acid tolerance, and greater mitochondrial efficiency (Gore et al., 2007).

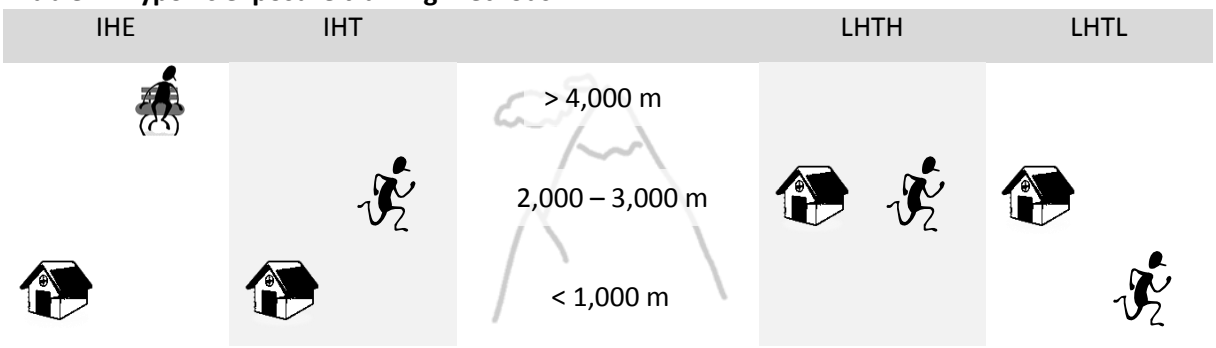
While the physiological adaptation to real or simulated altitude training are promising, in reality, the training response can be quite varied, with some athletes demonstrating considerable improvements (Saunders et al., 2009, Beidleman et al., 2003), and others showing either no change (Taylor et al., 2011, Aulin et al., 1998, Siebenmann et al., 2012) and in 'non-responders' a drop in performance (Chapman et al., 1998) following altitude training. Additionally, even if the individuals indicate HIF-1α -related physiological adaptation, such as increased erythropoeitin and haemoglobin mass, or demonstrate enhanced laboratory assessments of fitness such as improved running economy or $\dot{V}O_{2peak}$, these do not always transfer into improved competitive or time trial performance (Robertson et al., 2010c). However, the chance of beneficial athletic performance has generally out-weighed the risk of either no improvement or a small decline. As such a number of real and simulated altitude training protocols have been suggested.

All altitude and simulated altitude training protocols assume the return of the individual to sea level. As such, the hypoxic exposure is 'intermittent'. However, the 'intermittent' nature of the hypoxic dosage is highly varied and can be broken into several training models such as Live High Train High (LHTH), Live High Train Low (LHTL), intermittent hypoxic training (IHT) and intermittent hypoxic exposure (IHE). Refer to Table 1 for a description of these techniques. Typically, prolonged hypoxic exposure such as the LHTH and LHTL models, or hypoxic exposure during exercise, such as in LHTH and IHT protocols, use lower hypoxic dosages (equivalent to 2,000 – 3,000 m), while shorter hypoxic exposures, as is used in passive IHE models, use a higher hypoxic dosage (equivalent to 4,000 m). At times, a combination of these techniques is used. For example, IHT may be used in conjunction with IHE whereby a participants initially performs 30 – 60 min exercise in hypoxia followed by another 3 h of passive hypoxic exposure. Alternatively, additional IHT may be performed in conjunction with the LHTL model (also referred to as "live high train low and high").

Interestingly, it is not only athletes and coaches who are interested in simulated altitude training devices. The overlap between convenience and potential for physiological improvement and exercise-enhancing adaptations has meant that the use of simulated altitude has also become a point of interest in clinical cohorts (Burtscher et al., 2010). In most cases, patients with cardiovascular disease exhibit poor exercise tolerance which makes any sort of physical activity difficult and short lived. Additionally, sedentary individuals who typically suffer from poor exercise tolerance and fitness levels would also stand to benefit from such treatments. For clinicians and exercise professionals, an intervention that promises to improve the exercise tolerance of their patients and clients prior to, or in conjunction with an exercise programme would be widely beneficial.

The purpose of this review is to determine the extent to which simulated altitude has been investigated in sedentary or clinical populations, and to examine the treatments used and findings from the retrieved papers. Furthermore, a summary of the literature pertaining to well-trained and athletic populations is also provided.

Table 1: Hypoxic exposure training methods



IHE: intermittent hypoxic exposure, IHT: intermittent hypoxic training, LHTL: live high train low, LHTL: Live high train low. **IHE** is usually achieved using hypobaric chambers, altitude tents, or by delivering a lower fraction of inspired oxygen (FiO_2) through a hand held face mask. This method of simulated altitude exposure is distinct from the others in that there is no physical training performed within or alongside the altitude exposure. Hypoxic exposure can range from several repeated doses of a few minutes of hypoxic exposure interrupted by several minutes of ambient air over 40 – 90 min, or a single dose of several hours. **IHT** requires that the individual either travels to real altitude for their training programme or trains within a hypobaric chamber (hypobaric hypoxia) or altitude tent (normobaric hypoxia), or while inspiring a hypoxic gas delivered through a face mask. The individual then returns to sea level following the exercise. During **LHTH**, the individual either resides at and trains in real altitude, in a hypobaric chamber (hypobaric hypoxia), or in an 'altitude apartment' (normobaric hypoxia). The hypoxic exposure is uninterrupted, and can range in time from several days to several weeks. However, with **LHTL** the individual either resides at real altitude, in a hypobaric chamber, in an 'altitude apartment', or in an 'altitude tent', but returns to a normoxic environment for training. The hypoxic exposure usually ranges from 8 h (in the case of altitude tents), to 18 – 20 hours (in altitude apartments, real altitude or hypobaric chambers).

2.2 Search methods

Google Scholar, Science Direct and PubMed (via Medline) were searched using a combination of "include" and "excluding" terms. In addition to these databases, all APS journals and the "High Altitude Medicine and Biology" journals were searched individually. The focus of this literature review is on the adaptive response to short term, simulated altitude in un-acclimatized humans. Therefore, any long-term hypoxic exposure associated with extended exposure to high altitude (such as high altitude residents and mountain tribes or mountaineering expeditions) or as a consequence of disease states (such as obstructive sleep apnoea,

ischemic heart disease or neonatal hypoxia) were also excluded. While a wealth of research conducted on rats, mice and dogs has investigated the role of hypoxia in gene expression and molecular pathways, the focus of this review is on intervention-based research in humans and therefore all animal studies were excluded from this review. Given the focus on the adaptive response to the simulated altitude, only research examining a training response to the simulated altitude was accepted, and therefore studies examining an 'instantaneous' physiological response to a single dose of hypoxia were excluded. Finally, only English-speaking, full text, peer reviewed academic journal articles were accepted in this review.

In an attempt to balance the number of articles between people of different health states, the descriptors "Sedentary" OR "untrained" OR "Inactive" were included in the Google Scholar and Science Direct search terms (anywhere in article). A subsequent Google Scholar search which included the terms, "well trained", "active", "athlete" and "simulated altitude", "interval hypoxia" and "normobaric hypoxia" was also included. After reviewing the first 100 articles in the second Google Scholar search, the remainder of the 2390 search results were restricted to those published after 2009. Refer to Figure 2 for a full description of the inclusion and exclusion terms used, and the number of articles accepted at each filtering stage. Following the searching process, the reference lists of selected review articles (Burtscher et al., 2010, Bärtsch et al., 2008, Muza, 2007, Wilber, 2007) were reviewed and any additional articles were added.

It is important to note that while this is an extensive review of the literature, and is likely to contain the majority of hypoxia-related interventions performed in sedentary individuals, it is likely that research regarding athletic populations published before 2009 has not been included. Additionally, given the specificity of populations in the search terms ("Sedentary" OR "untrained" OR "Inactive" OR "well trained" OR "active" OR "athlete"), it is possible that relevant research that has not specified the training status of their population was not captured in the search process. After all articles were retrieved and documented, they were then sorted into one of 4 participant categories:

- Well-trained / athlete
- Healthy / active
- Sedentary / untrained
- Clinical.

Regarding the grouping of participants, the distinction between healthy/ active groups and sedentary / untrained were, at times, blurred. For example, if participants were described as healthy but not involved in exercise or physical activity, or untrained, they were included in the "sedentary/ untrained". In cases where participants were described as healthy but no indication of level of physical activity was recorded, but included a description akin to "healthy lowlanders, not taking medications and without disease" the study

was included in the "healthy/ active" category. Therefore there is a possibility that some studies have been misclassified. The details of the training protocols and key outcomes of the studies were also noted.

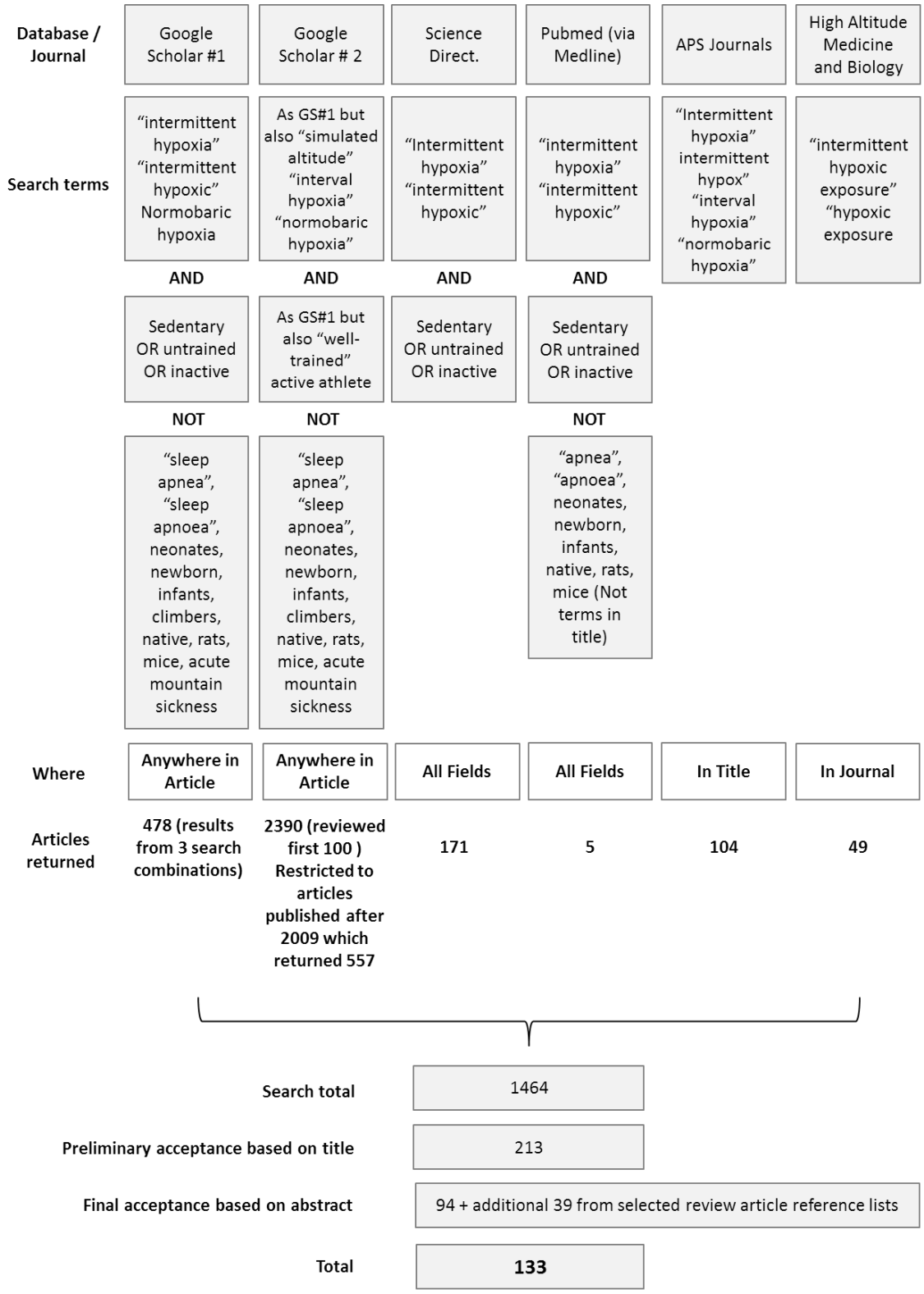


Figure 2: Database and journal search procedure

2.3 Results and discussion

There were 1464 articles returned from the searching process, and 213 were accepted based on their titles. Of these 213 articles, 94 articles were focussed on intervention style studies in humans. In the rejected articles, 16 were focussed on real altitude only with no simulated altitude content, 40 were reviews, commentaries or debates, 57 were 'instantaneous' or non-intervention based and 6 were either duplicates, animal studies or irrelevant. An additional 39 articles were added after reviewing the reference lists of selected articles, all of which were published before 2009 (one was in 2009). Articles ranged in date from 1992 to 2013.

Of the retrieved articles, 85% were focussed on healthy, physically active participants of which 52.6% of the articles were based on "well-trained / athlete" cohorts and 32.4 % were in "healthy / active" participants. In the remaining 15% of the retrieved articles, 12% were in "sedentary / untrained" populations and 3% investigated the response of simulated altitude in a clinical population, see Figure 3.

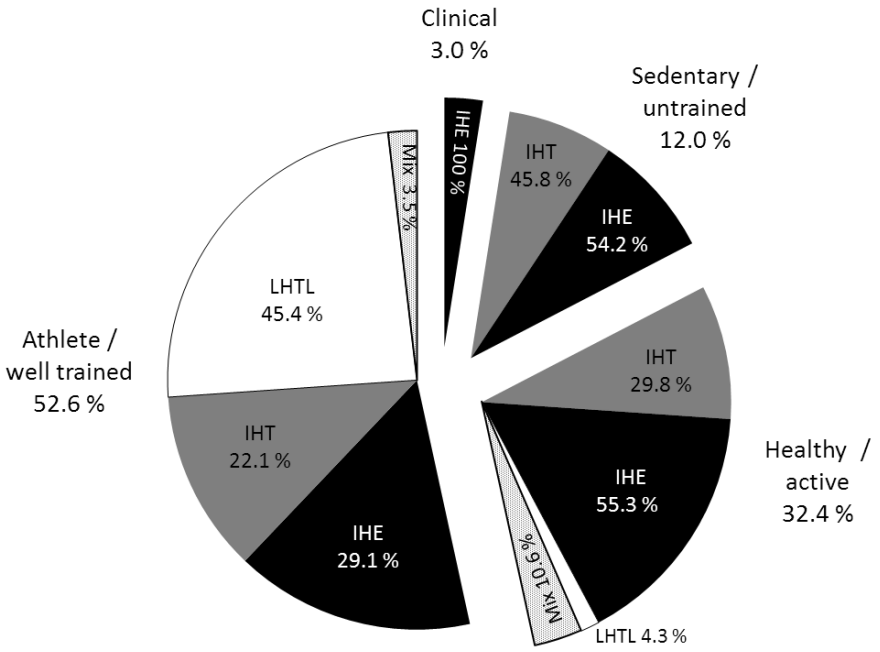


Figure 3: Proportion of studies focussing on different populations, and within those portions, the methods used to simulate altitude

See Table 3 - Table 6 for the studies included in this Pie Diagram.

Figure 4 at the end of this chapter represents the prevalence of the authors cited in this literature review.

Altitude simulation methods were grouped into 3 main training modalities, namely LHTL (n = 41), IHT (n = 44) and IHE (n = 68), refer to Table 1 for a description of these protocols. (If a research article used two or more types of simulated altitude protocol, these were counted separately). Eight articles made use of a

combination of techniques: 7 combined IHE+IHT (5 in the healthy / active population, and 2 in the well trained / athlete population) and 1 combined LHTL with IHE. Twenty one studies used hypobaric hypoxia to simulate altitude: 13 in IHE protocols (2 in Clinical, 2 in sedentary / untrained, 1 in healthy / active, and 8 in well trained / athletes), 6 in IHT (2 in sedentary / untrained, 2 in healthy / active, and 2 in well trained / athlete), and 2 in IHE+IHT combinations (1 in healthy / active, and 1 in well trained / athlete).

Within the IHE protocol, there were two distinct techniques: Those that included one continuous, passive exposure of simulated altitude, usually over 3 – 4 h, and those that included short (several minutes) of severe hypoxic exposure, usually around an FiO_2 of 12%, alternated with a similar duration of ambient air, and repeated for 40 – 90 min. For the sake of clarity, for the remainder of this discussion, repeated, short intervals of hypoxic alternated with short periods of normoxic breathing will be referred to as intermittent hypoxic exposure (IHE), while the sustained periods of passive hypoxic exposure will be referred to as prolonged hypoxic exposure (PHE).

Traditionally, male participants have been recruited more frequently than female participants into simulated altitude research, at ratios of approximately 7:1; 6:1; 17:1 and 5:1 in the clinical; sedentary / untrained; healthy/active; and well-trained / athlete groups respectively. Studies focussing on active participants have typically recruited younger participants; while in the sedentary cohorts, the participants have generally been older (see Table 2). In the sedentary / untrained group however, there seemed to be specific groupings regarding participant age. That is, 18 studies ranged in age from 19 – 28 years, and the participants in the 6 remaining studies were between 42 – 64 years old (which accounts for the much larger standard deviation in the age of this group).

Table 2: Overview of participant profiles in simulated altitude research

Participant group	Number of studies	Male n	Female n	Approximate average age (years ± SD)
Clinical	4	71	11	54.6 ± 8.2
Sedentary / untrained	16	339	55	29.9 ± 14.4
Healthy / active	43	529	32	24.5 ± 3.1
Well trained / athlete	70	1037	201	24.5 ± 4.3

Given the vested interest in athletic performance by the coaches, athletes and even government, and the very small differences in performance between the top elite athletes, it makes sense that most research regarding the use of simulated altitude has been centred on athletic performance. Indeed, numerous recent review articles on the effects of simulated altitude on athletic performance have already been

published (Bärtsch et al., 2008, Bonetti and Hopkins, 2009, de Paula and Niebauer, 2012, Wilber, 2011). However, the smaller branch of the research that has focussed on sedentary participants and those with cardiovascular disease may have more potential for health improvement than for athletic improvement, and has typically received a lot less attention than its athletic counterpart. In response to this, the first part of this discussion will review the available literature on the use of simulated altitude in "clinical" and "sedentary/ untrained" population groups, with a closer look at the effectiveness of each simulated altitude technique in each group. The key outcomes following the examination of this literature will then be compared to findings observed in the wider population. The literature review will conclude with recommendations for future research directions.

2.3.1 Clinical populations

There were only 4 articles examining the effect of IHE in a clinical population, all of which used passive hypoxic exposure (see Table 3). Within the IHE protocols, one article focussed on interval IHE (Haider et al., 2009) and 3 focussed on PHE (del Pilar Valle et al., 2006, Tin'kov and Aksenov, 2002, Saeed et al., 2012).

Intermittent hypoxic exposure

The aim of Haider et al. (2009) was to assess whether short intervals of hypoxic exposure would influence the cardiovascular autonomic dysfunction associated with chronic obstructive pulmonary disease. To assess functional respiratory control, Haider et al. (2009) reported the ventilatory response to normocapnic normoxia (control), normocapnic hypoxia (index of peripheral chemoreflex), and hyperoxic hypercapnia (index of central chemoreflex). Baroreflex sensitivity was estimated using autoregressive power spectral analysis of the interval between R- R peaks in heart rate data, and systolic blood pressure (SBP).

Following the 3-week trial, participants receiving IHE demonstrated improved baroreflex sensitivity and a slight (non-statistically significant) decrease in SBP. The RR interval also increased, suggesting improved vagal activity. However, the increase in RR interval could also have been related to the tendency for participants in the IHE group to breathe slower and more deeply following the intervention. Additionally, Haider et al. (2009) did not report how well the participants tolerated the hypoxic intervals, only that no clinical problems arose. Therefore future research in this population, using this technique should monitor changes in RR interval under controlled breathing, and monitor tolerance to the hypoxic dosage.

Prolonged hypoxic exposure

The reports of improved exercise tolerance (Saeed et al., 2012), myocardial perfusion (del Pilar Valle et al., 2006) and blood lipid profile (Tin'kov and Aksenov, 2002) in a clinical population following PHE are promising. However, the small number of studies in this population and the different focus in each of the

studies makes it difficult to assess the repeatability of the respective findings. There are also limitations in each of these studies that cloud the clarity of the outcomes in these trials.

The major limitation in these studies has been the lack of a control group. For example, despite a relatively large number of participants recruited into the study by Tin'kov and Aksenov (2002), particularly when compared to other studies of this nature (n = 46 in Tin'kov and Aksenov (2002), versus 18, 12 and 6 by Haider et al. (2009), Saeed et al. (2012), and del Pilar Valle et al. (2006) respectively), Tin'kov and Aksenov (2002) did not include a group receiving a normoxic placebo. The absence of a control group makes it difficult to conclusively pinpoint the alteration of the blood lipid profile to the intermittent hypobaric hypoxic exposure. For example, it is possible that the change in the blood lipid profile over the course of the study could have been attributed to the participant's ongoing use of medication over the course of the 3-week intervention period and the 10-month follow up, or indeed a change in season (Ockene et al., 2004) or physical activity levels. Similarly, oral acetazolamide (a carbonic anhydrase inhibitor which acts as a diuretic) was administered to 11 of the 12 participants in the study by Saeed et al. (2012) prior to the PHE treatment to prevent altitude sickness. While all of the participants tolerated the hypoxia well, there is also a small possibility that the ingestion of the acetazolamide could have augmented some of the training responses. As the time course of the changes in the blood profile variables almost perfectly matches the changes in the intervention, particularly immediately following the intervention period, and the 3 month follow up, it is likely that the hypobaric hypoxic treatment was indeed responsible for this change.

The small sample size in del Pilar Valle's study was possibly the largest limitation in their study. However, given the high risk population used in their trial, and the absence in participant motivation required for their measurements, their results are likely reliable. The improvement in myocardial perfusion was attributed to improved blood flow due to an increase in angiogenesis, and an improvement in vasodilation due to improved serum nitric oxide following the PHE protocol (del Pilar Valle et al., 2006).

Overall, 3 – 4 h episodes of PHE at a simulated altitude of 2,700 – 4,200 m for 2 – 3 weeks in patients with stable chronic heart failure or coronary heart disease appears to have beneficial effects on blood lipid profile, myocardial perfusion and exercise capacity. While there appears to be high potential for PHE to improve health outcomes in this population, further placebo-controlled (or randomised crossover), double blind studies are required before conclusive statements can be made regarding the effectiveness of PHE in this population.

Table 3: Studies researching simulated altitude training in a clinical population

Authors	Participant description (cohort, MF, Age	Group treatment	Hypoxia frequency (sessions, per week, weeks)	Hypoxic severity	Main Outcome
Intermittent hypoxic exposure (n=1)					
Haider et al. (2009)	Patients with mild COPD symptoms; 10M8F; 51.5 y	IHE	3-5 min hypoxia: 3 min recovery cycled for 3 - 5 rotations; 5 sessions / wk, 3 wks.	FiO_2: 15, 13, 12 in wk 1, 2, 3 respectively (2600 m, 3750 m, 4400 m)[ɣ]	↑ Baroreflex sensitivity & RR interval, slight ↓ in SBP but NS. ↑ HCVR but not HVR
	As above, 5M4F, 52 y	C: (sham)	As above but with normoxic placebo	FiO_2: 21 in wk 1 – 3 (SL)	Slight ↓ baroreflex sensitivity & RR interval (NS), ⇆ SBP
	Age matched healthy, 5M9F, 47 y	Healthy comparison	Baseline data only	No treatment	
Prolonged hypoxic exposure (n=3)					
del Pilar Valle et al. (2006)	Severe stable CHD, 6M, 66.5 y	PHE*	4 h / session, 1 session / wk, 14 sessions	2 h acclimatizing to target, 1 h at target, 1 h descent. Starting target 2400 m, ↑ by 250 - 300 m each following session until 4200 m	Improved myocardial perfusion, no evidence of impairment
Saeed et al. (2012)	Patients with stable chronic heart failure, 9M3F, 52.5 y	PHE	3-4 h / session, 10 sessions over 22 d	Started at 1500 m, ↑ by 300 m every session until 2700 m.	↑ VO_{2max} (up to 4 wk post), ↑ exercise time, ↓ 6 min walk distance, ↑ skeletal muscle strength. Trend to improved left ventricular ejection fraction
Tin'kov and Aksenov (2002)	Coronary heart disease patients (30 with myocardial infarction, 16 ischemic heart disease); 46M, 48 y	PHE*	3 h / session, 22 sessions in 22 d	3500 m	↓Total cholesterol & low density lipoprotein, ↑ high density lipoprotein (up to 6 months post). ↓ coefficient of atherogenity

*hypobaric hypoxia; PHE: prolonged hypoxic exposure; IHE: intermittent hypoxic exposure; C: control; sham: normoxic placebo; ɣ estimated using altitude conversion chart by www.higherpeak.com; FiO2: fraction of inspired oxygen; ↑ increase; ↓ decrease; ⇆ no change; SL: sea level; COPD: Chronic obstructive pulmonary disease; RR interval: average distance between RR peaks; SBP: systolic blood pressure; HCVR: hypocapnic ventilatory response; HVR: hypoxic ventilatory response; M: male; F: female.

2.3.2 IHT and IHE in sedentary / untrained populations

The results of the studies utilising sedentary / untrained, but otherwise relatively healthy individuals are summarised in Table 4. The research involving this cohort focussed exclusively on IHE and IHT training modalities, with 4 articles using IHE (Balykin et al., 2004, Burtscher et al., 2009, Burtsher et al., 2004, Shatilo et al., 2008), 3 using normobaric, PHE (Wang et al., 2007a, Wang et al., 2007b, Wang et al., 2010), 2 using hypobaric PHE (Katayama et al., 1998, Ricart et al., 2000), 8 using normoxic IHT (Balykin et al., 2004, Friedmann et al., 2003, Geiser et al., 2001, Mao et al., 2011, Vogt et al., 2001, Wang et al., 2010, Wiesner et al., 2010, Pesta et al., 2011), and 2 using hypobaric IHT (Katayama et al., 1998, Nishiwaki et al., 2011). Balykin et al. (2004) and Wang et al. (2010) tested both IHE and IHT groups. Overall, the quality of these studies were better, with more researchers reporting comparisons relative to control groups.

Intermittent hypoxic exposure

The population used in these studies included people with mild COPD (or at risk for COPD), who were overweight, or who were healthy and active but elderly (60 – 74 y). Most studies reported an improvement in exercise tolerance during a submaximal workload (Burtscher et al., 2009, Burtsher et al., 2004, Shatilo et al., 2008), particularly in those with greater disease (Burtsher et al., 2004) or a sedentary nature (Shatilo et al., 2008). Changes in maximal capacity were somewhat harder to detect. Only the elderly men in the study by Burtsher et al. (2004) demonstrated a clear increase in $\dot{V}O_{2peak}$ compared to the control group. While the later study by Burtscher et al. (2009) and the study by Balykin et al. (2004) both noted a small increase in $\dot{V}O_{2peak}$ following IHE, neither increase was statistically significant (despite an increase in time to exhaustion reported by Burtscher et al. (2009)).

There were several conflicting adaptations in response to the IHE protocol. For example, while some studies report an increase in red blood cells (Burtsher et al., 2004) and haemoglobin (Burtscher et al., 2009, Burtsher et al., 2004), others have noticed no meaningful increase (Shatilo et al., 2008). Interestingly, both Shatilo et al. (2008) and Burtscher et al. (2009) reported small, but not statistically significant decreases in total cholesterol which is in line with the improvement in blood lipid profile following PHE in a clinical population (Tin'kov and Aksenov, 2002). However, the decrease in cholesterol in the test group in the study by Burtscher et al. (2009) was similar to the decrease in the control group and therefore these results were equivocal.

The study by Balykin et al. (2004) primarily investigated the effectiveness of different IHE protocols with, and without the use of exercise. The IHE protocols used in conjunction with exercise training (either simultaneously, or sequentially) were more effective regarding exercise capability, and these

results are discussed under the “Intermittent hypoxic training” heading. One particularly interesting outcome of the IHE group was the decrease in sympathetic and increase in parasympathetic activity in the autonomic control of the heart following the hypoxic exposure intervention, resulting in a reduced strain index (regarding cardiac autonomic regulation). This was also reflected in the reduced resting heart rate and systolic blood pressure. Unfortunately neither the frequency nor the duration of the hypoxic stimulus was clearly reported, and therefore such variables warrant further investigation.

Participants in these studies appear to have tolerated the hypoxia well. Prior to the IHE intervention, the elderly participants in the study by Shatilo et al. (2008) underwent a sustained 10 min exposure at an FiO_2 of 12 % to test hypoxic tolerance. Of the 36 participants who participated in the sustained hypoxia, 1 was excluded due to a SBP increase of more than 30% between minutes 3 – 5 of the exposure (and was excluded from the study), and 4 participants voluntarily stopped the sustained exposure due to dizziness, shortness of breath or weakness. These symptoms occurred approximately 7 min into the hypoxic interval. After the first IHE session of the 10 d intervention, 3 participants reported mild chest discomfort, dizziness and tinnitus. No further distress was reported during the IHE intervention periods of 5 min. In Burtscher et al.’s studies, participants (in both the control and in the test groups) reported feeling dizzy or sleepy during the hypoxic exposure (Burtscher et al., 2009, Burtsher et al., 2004)

In general, all of the IHE studies made use of a control group of sorts (while Shatilo et al. (2008) and Balykin et al. (2004) did not use a placebo group, they did examine the intervention in different population groups). Of note is that while some of the haematological parameters are unclear, there were no negative health outcomes attributed to the IHE interventions (such as increased SBP, or a decline in exercise tolerance).

Therefore, for those who are unable to exercise, the possibility of health benefit compared to the seemingly absent risk of harm would make this intervention worthwhile, particularly regarding the positive effects regarding submaximal exercise tolerance. The potential for IHE to reduce sympathetic dominance and improve the strain index of autonomic control of the heart, as reported by Balykin et al. (2004), is also intriguing, and should be explored further in a clinical or sedentary population.

Prolonged hypoxic exposure

The research regarding normobaric PHE has been conducted by one research group who have focussed primarily on the differences in the hypoxic severity (FiO_2 of 12% vs 15%) on haemodynamic

control (Wang et al., 2007a, Wang et al., 2010), eosinophil- and neutrophil- platelet aggregation, cytokine response to strenuous exercise (Wang et al., 2007b), as well as exercise tolerance and ability. The results of these studies indicate a large variation in physiological response depending on both the time and severity of the hypoxic dosage. For example, 4 or 8 weeks of PHE, for 1 h, 5 times/week using an FiO_2 of 15% resulted in improved pulmonary ventilation, and submaximal and maximal exercise performance, as evidenced by an approximately 9% change in $\dot{V}O_{2peak}$ from baseline (Wang et al., 2007a, Wang et al., 2007b), which were not statistically significant following 30 minutes of the same PHE protocol (Wang et al., 2010). Additionally, while the arterial hyperaemic response (an indication of vascular haemodynamic function) following occlusion remained largely unchanged following 4 weeks of 30 min or 60 min PHE, at an FiO_2 of 15%, the hyperaemic response tended to worsen after 4 weeks of 60 min PHE at an FiO_2 of 12% (Wang et al., 2007a). In addition to the reduced hyperaemic response, the group receiving an FiO_2 of 12% demonstrated an increase in lipid peroxidation, decrease in vitamin E levels and a decrease in vascular endothelial function which was attributed to a decrease in antioxidative capacity (Wang et al., 2007a). Contrastingly, 8 weeks of the same PHE protocol at an FiO_2 of 12 or 15% resulted in a reduction in the pro-inflammatory cytokine and thrombo-inflammatory responses to strenuous exercise, but only the group receiving an FiO_2 of 12% demonstrated a substantial increase in circulatory anti-inflammatory cytokines IL-6 and IL-10 during hypoxia, exercise, and at rest (Wang et al., 2007b).

Table 4: Studies relating to simulated altitude in a sedentary / untrained population

Authors	Participant description (cohort, MF, Age)	Group	Frequency (sessions, per week, weeks)	Hypoxic severity	Main Outcome
			Intermittent hypoxic exposure		
Balykin et al. (2004)	Healthy, sedentary, overweight, no disease, 11M, 18 - 20 y	C: Cycling (100W)	30 min/d, 3 d/week, 4 wk	Sea level	Very small changes, only resting $\dot{V}O_2$ & $\dot{V}CO_2$ worthwhile, ↓ strain index of ANS (not significant)
	As above; 9M; 18-20 y	**IHE**	5 min hypoxia alternated with 5 min recovery, 10 sessions, over 22 d	FiO_2: 10	↓ SNS, ↑ PNS, ↓ strain index of ANS, ↑ physical work capacity
	As above; 9M; 18 - 20 y	**IHE** before exercise	As above followed by C exercise. 10 IHE and 10 C exercise sessions	FiO_2: 10	⇆ SNS, ↑ PNS, ↓↓strain index of ANS, ↑ physical work capacity, better than IHE alone
	As above; 10M; 18 - 20 y	IHT: cycling (100 W)	30 min/d, 3d/week, 10 sessions	FiO_2: 10	⇆ ANS changes between IHE + Exercise and IHE only, but greater ↑ in physical work capacity
	As above; 8M; 18 - 20 y	**IHE** alternated with IHT	IHE and IHT protocols alternated, as described above	FiO_2: 10	Improved ANS balance & physical work capacity (less than combination groups). Greater improvement in anthropometric data.
Burtsher et al. (2004)	Normally active, half with & half without prior MI, 8M, 59 y	**IHE**	3 - 5 minutes hypoxia cycled with 3 min normoxia repeated for 3 -5 cycles, 5 d / wk, 3 wk	FiO_2: 14, 12 10 in wk 1, 2, 3	↑RBC & Hb, ↑submaximal efficiency & tolerance. ⇆ peak workload, but ↑ efficiency
	As above, 8M, 61 y	C	As above but normoxic placebo	FiO_2: 21	↓RBC, ⇆ other bloods, small changes in submaximal & maximal exercise
Burtscher et al. (2009)	Normally active (2 h / wk), with chronic obstructive pulmonary symptoms, 5M4F, 51 y	**IHE**	3-5 min hypoxia alternated with 3 min normoxia, cycled for 3-5 repetitions, 5 d/ wk, 3 wk	FiO_2: 15, 13, 12, in wk 1, 2, 3	↓SBP, DBP, ↑forced expiratory volume, Hb, & plasma volume, ↑exercise time compared to C
	As above, 5M4F, 52 y	C:	As above but normoxic placebo	FiO_2: 21	↓ SBP, DBP, arterial O_2 saturation, ↑triglycerides

Authors	Participant description (cohort, MF, Age)	Group	Frequency (sessions, per week, weeks)	Hypoxic severity	Main Outcome
Shatilo et al. (2008)	Healthy active seniors; 14M; 67 y	**IHE**	5 min hypoxia alternated with 5 min normoxia cycled 4 times, for 10 d	FiO_2: 12%	⇆ Haemodynamic indices or work capacity. ↑ skin blood flow during submaximal exercise & hyperaemic recovery time
	Sedentary seniors; 21M; 61 y	**IHE**	As above	As above	↓ exercising blood pressure, ↑ submaximal work, & anaerobic threshold. ↓ HR & BP during submaximal exercise. ↑ skin blood flow. ↑ improvements compared to active group
			Prolonged hypoxic exposure		
Katayama et al. (1998)	Healthy, no regular physical activity, no cardiorespiratory disease or medication; 7M; 19 y	IHT*: 2 X 15 min cycling (5 min recovery between) at 40 % sea level max; remainder as PHE*	2 h/d, 6 sessions in 6 d	30 min up to 4500 m; 1 h at 4500 m, 15 min back to sea level	⇆ HVR or HCVR, ↑ $\dot{V}O_{2peak}$
	As above; 6M; 19.5 y	**PHE*** (rest)	As above	As above	↑ HVR but ⇆HCVR or $\dot{V}O_{2peak}$
Ricart et al. (2000)	4WD members; 5M4F; 45 y	**PHE***	2 h/d, 7 d/wk, 2 wk	5000 m	↑ SaO_2 & expired minute volume (↑ tidal volume). ⇆ Resting variables, packed cell volume or Hb after 2 wks.
Wang et al. (2007b)	healthy, non-smokers, no medications, disease free; 10M; 24 y	**PHE**: Moderate (15%)	1 h/d, 5 d/wk, 8wk	FiO_2: 15%; 2733 m	Lower ↑ in hypoxia & exercise induced eosinophil & neutrophil platelet-aggregation, interleukin-1ß & malondialdehyde levels
	As above; 10M, 23 y	**PHE**: Severe (12%)	As above	FiO_2: 12%	As above, but also ↑ plasma interleukin 6 & interleukin 10
Wang et al. (2007a)	Healthy, non-smokers, no medications, disease free; 10M; 24 y	**PHE**: Moderate (15%)	1 h/d, 5 d/wk, 4 wk	FiO_2: 15%; 2733 m	↑ pulmonary ventilation & oxygen uptake
	As above; 10M, 23 y	**PHE**: severe (12%)	As above	FiO_2: 12%	As above, but also ↑ exercising BP ↑ Plasma malondialdenhyde & nitric oxide, ↓hyperaemic arterial response (haemodynamic function), venous compliance & endothelium-dependant vasodilation. ↓ Plasma antioxidant & Vitamin E
	As above, 10M, 24 y	C	As above	FiO_2: 21%	No change

Authors	Participant description (cohort, MF, Age)	Group	Frequency (sessions, per week, weeks)	Hypoxic severity	Main Outcome
Wang et al. (2010)	Healthy, non-smokers, no medications, disease free; 12M; 23 y	IHT: cycling 50% maximal heart rate reserve	30 min/d, 5 d/wk, 4 wk	FiO_2: 15%; 2733 m	↓ Stroke volume, hyperaemic & re-oxygenation suppression induced by FiO_2: 12%. ↑perfusion & O_2 extraction in FiO_2: 12% exercise
	As above; 12M; 23 y	IHT: 50% max work at FiO2: 15%	As above	FiO_2: 15%; 2733 m	As above but also better improvement in aerobic capacity than normoxic exercise.
	As above; 12M; 23 y	C: sedentary normoxic	As above	Sea level	No worthwhile change
	As above; 12M; 21 y	C: normoxic cycling (50% maximal work rate)	As above	Sea level	No worthwhile change
	As above; 12M; 21 y	**PHE**	As above	FiO_2: 15%; 2733 m	No worthwhile change
			Intermittent hypoxic training		
Balykin et al. (2004)	Healthy, sedentary, overweight, no disease, 11M, 18 - 20 y	C: Cycling (100W)	30 min/d, 3 d/week, 4 wk	Sea level	Very small changes all NS except resting vo2 & VCO2, ↓in strain index of ANS (NS)
	As above; 9M; 18-20 y	IHE	5 min hypoxia alternated with 5 min recovery, 10 sessions, over 22 d	FiO_2: 10	↓ SNS, ↑ PNS, ↓ strain index of ANS, ↑ physical work capacity
	As above; 9M; 18 - 20 y	IHE before exercise	As above followed by C exercise. 10 IHE and 10 C exercise sessions	FiO_2: 10	⇆ SNS, ↑ PNS, ↓↓strain index of ANS, ↑ physical work capacity, better than IHE alone
	As above; 10M; 18 - 20 y	**IHT**: cycling (100 W)	30 min/d, 3d/week, 10 sessions	FiO_2: 10	⇆ANS changes between IHE + Exercise and IHE only, but greater ↑ in physical work capacity
	As above; 8M; 18 - 20 y	IHE alternated with IHT	IHE and IHT protocols alternated, as described above	FiO_2: 10	Improved ANS balance & physical work capacity (less than combination groups). Improvement in anthropometric data.

Authors	Participant description (cohort, MF, Age)	Group	Frequency (sessions, per week, weeks)	Hypoxic severity	Main Outcome
Friedmann et al. (2003)	Recreationally active or untrained, 10M, 25 y	**IHT**: Low resistance, high repetition leg strength training	40 min/d, 3 d/wk, 4 wk	FiO_2: 12 4500 m	As below, but also correlation between hypoxic markers & glycolytic enzyme mRNA linked to hypoxic specific adaptation. No practical benefit of IHT
	As above, 9M, 24 y	C: normoxic low resistance, high repetition leg strength training	As above but normoxic placebo	Sea level	↑ Strength endurance capacity, but ⇆ muscle cross sectional area, fibre type distribution or fibre cross sectional area. High inter-individual variation in mRNA analyses.
Geiser et al. (2001)	Healthy untrained, 10M, 23 y	**IHT**: high intensity (80% $\dot{V}O_{2peak}$) hypoxia	30 min/d, 5 d / wk, 6 wk	3850 m	↑ $\dot{V}O_{2peak}$ & maximal power tested in hypoxia and normoxia. ↑↑ mitochondrial volume density. ↑ knee extensor muscle volume & capillary length density (length of capillary in 1 mm^3)
	As above, 8M, 23 y	**IHT**: low intensity (67% $\dot{V}O_{2peak}$) hypoxia	30 min/d, 5 d/wk, 6 wk	3850 m	↑$\dot{V}O_{2peak}$. ↑ maximal power, but less so than high intensity groups. ↑ mitochondrial volume density
	As above, 8M, 25 y	C: high intensity (80% $\dot{V}O_{2peak}$) in normoxia	As above but normoxic placebo	600 m	↑ $\dot{V}O_{2peak}$. ↑ maximal power (more so than low intensity.) ↑ mitochondrial volume density
	As above, 7M, 29 y	C: low intensity (67% $\dot{V}O_{2peak}$) normoxia	As above but normoxic placebo	600 m	↑$\dot{V}O_{2peak}$. ↑ maximal power, but less so than high intensity groups; ↑ mitochondrial volume density
Katayama et al. (1998)	Healthy, no regular physical activity, no cardiorespiratory disease or medication; 7M; 19 y	**IHT***: 2 X 15 min cycling (5 min recovery between) at 40 % sea level max; remainder PHE*	2 h/d, 6 sessions in 6 d	30 min up to 4500 m; 1 h at 4500 m, 15 min back to sea level	⇆HVR or HCVR, ↑ $\dot{V}O_{2peak}$
	As above; 6M; 19.5 y	PHE*	As above	As above	↑ HVR but ⇆HCVR or $\dot{V}O_{2peak}$
Mao et al. (2011)	Healthy, non-smoker, no medication or history of disease: 12 M; 22 y	**IHT**: cycling at 60% work rate	30 min/d, 5 d/wk, 5 wk	FiO_2: 15%; 2733 m	↓ Erythrocyte health and durability under shear stress. ↑eryptotic response to hydrogen peroxide
	As above; 12M; 22 y	C: cycling at 60% work rate	As above	FiO_2: 21% (sea level)	⇆ Erythrocyte rheological properties under rest or exercise.

Authors	Participant description (cohort, MF, Age)	Group	Frequency (sessions, per week, weeks)	Hypoxic severity	Main Outcome
Nishiwaki et al. (2011)	Postmenopausal women, sedentary/ recreationally active, no hormone replacement therapy; 8F; 56 y	**IHT***: 30 min swimming 50 % $\dot{V}O_{2peak}$, PHE remainder	2 h/d, 4 d/wk, 8 wk	2000 m	↓ Brachial pulse wave velocity; ↑ Flow mediated dilation & peak diameter
	As above; 8F; 56 y	C: 30 min swimming	30 min/d, 4 d/wk, 8 wk	Sea level	No worthwhile changes
Pesta et al. (2011)	Healthy sedentary: 7M; 29 y	**IHT**: endurance training	35-55 min/d, 3 d/wk, 10 wk	FiO_2: 13.5%, 4000 m	↑ Fatty acid oxidation capacity per muscle mass mainly due to qualitative mitochondrial change, & partly due to tissue density.
	As above; 8M; 28 y	C: endurance training, normoxia	As above, normoxic placebo	Sea level	↑ Physiological oxidative phosphorylation & key mitochondrial changes.
	Healthy sedentary; 7M; 24 y	**IHT**: strength training	12 - 17 min/d, 3 d/wk, 10 wk	FiO_2: 13.5%, 4000 m	As above
	As above; 3M; 24y	C: strength training	As above, normoxic placebo	Sea level	As above.
Vogt et al. (2001)	Untrained, 7M, 23 y	**IHT**: high intensity (4 - 6 mmol blood lactate)	30 min/d, 5 d/wk, 6 wk	3850 m	↑ $\dot{V}O_{2peak}$ & maximal power output; ↑HIF-1α & splice variant HIF-$1\alpha^{736}$; ↑ myoglobin & vascular endothelial growth factor mRNA.
	Untrained, 8M, 25 y	C: High intensity as above	As above but normoxic placebo	Sea level	↑ $\dot{V}O_{2peak}$ & maximal power output
	Untrained, 7M, 23 y	**IHT**: low intensity (2 - 3 mmol blood lactate)	30 min/d, 5 d/wk, 6 wk	3850 m	↑ $\dot{V}O_{2peak}$ & maximal power output ↑HIF-1α & splice variant HIF-$1\alpha^{736}$
	Untrained, 8M, 29 y	C: Low intensity as above	As above, normoxic placebo	Sea level	↑ $\dot{V}O_{2peak}$ & maximal power output

Authors	Participant description (cohort, MF, Age)	Group	Frequency (sessions, per week, weeks)	Hypoxic severity	Main Outcome
Wang et al. (2010)	Healthy, non-smokers, no medications, disease free; 12M; 23 y	**IHT**: cycling 50% maximal heart rate reserve	30 min/d, 5 d/wk, 4 wk	FiO_2: 15%; 2733 m	↓ Stroke volume, hyperaemic & reoxygenation suppression induced by FiO_2: 12%. ↑perfusion and O_2 extraction in FiO_2: 12% exercise
	As above; 12M; 23 y?	**IHT**: 50% max work at FiO2: 15%	As above	FiO_2: 15%; 2733 m	As above; also more ↑aerobic capacity than normoxic exercise.
	As above; 12M; 23 y	C: sedentary normoxic	As above	Sea level	No worthwhile change
	As above; 12M; 21 y	C: normoxic cycling (50% maximal work rate)	As above	Sea level	No worthwhile change
	As above; 12M; 21 y	PHE	As above	FiO_2: 15%; 2733 m	No worthwhile change
Wiesner et al. (2010)	Sedentary, overweight, healthy, 10M14F; 42 y	**IHT**: Treadmill (65% maximal HR)	1 h/d, 3 d/wk, 4 wk	2740 m	↓ Workload during training; ↑ $\dot{V}O_{2peak}$ & time to exhaustion; greater improvement in respiratory quotient & lactate at anaerobic threshold & body composition.
	As above, 8M13F, 42 y	C: Treadmill (65% maximal heart rate)	As above, normoxic placebo	Sea level	↑ $\dot{V}O_{2peak}$ and time to exhaustion

*hypobaric hypoxia; IHE: intermittent hypoxic exposure; M: male; F: female; PHE: prolonged hypoxic exposure; IHT: intermittent hypoxic training; C: control; ɣ estimated using altitude conversion chart by www.higherpeak.com; FiO_2: fraction of inspired oxygen; ↑ increase; ↓ decrease; ⇆ no change; VO_2: oxygen uptake; $\dot{V}CO_2$: volume of carbon dioxide; ANS: autonomic nervous system; PNS: parasympathetic nervous system; SNS; Sympathetic nervous system; Hb: haemoglobin; RBC: red blood cells; HR: heart rate; BP: Blood pressure; NS: not significant SBP: systolic blood pressure; HCVR: hypocapnic ventilatory response; HVR: hypoxic ventilatory response; HIF-1α: the alpha subunit of the hypoxic inducible factor transcription factor.

Regarding the studies utilising hypobaric hypoxia in a sedentary population, one was focussed on the acclimatization potential of hypobaric PHE, and was assessed using rest and exercising parameters in hypobaric hypoxia (Ricart et al., 2000). The results of this study demonstrated no change in RBC or Hb (however, given the 2-week duration of this study, this was not unexpected). On the other hand exercising ventilation and SpO_2 both improved while cycling at an estimated 30% of $\dot{V}O_{2peak}$ following 14 PHE sessions in the hypobaric chamber (Ricart et al., 2000). The increase in both SpO_2 and ventilation was interpreted as beneficial altitude acclimatization. Similarly, Katayama et al. (1998) reported an increase in the hypoxic ventilatory response, but after a much shorter intervention (1 h / d for 6 d at 4500 m, compared to 2 h / d for 14 d at 5000 m in the Ricart et al. (2000) study).

Overall, while the effects of hypobaric PHE are likely to improve hypoxic sensitivity and acclimatization through increased ventilation in hypoxia, there is insufficient literature available to comment on its effectiveness as a means of improving exercise tolerance or health in a sedentary population. Conversely, the use of normobaric PHE in this population appears to consistently improve exercise tolerance and capacity following > 4 weeks of 5 PHE sessions/ week for at least 60 min. Given the similarity between the physiological responses to an FiO_2 of 12 and 15% regarding improvements in exercise parameters and a resistance to pro-inflammatory cytokine and thrombotic responses to strenuous exercise, the added potential of PHE at an FiO_2 of 12% to increase circulatory IL-6 and IL-10 (Wang et al., 2007b) seems offset by the possibility of an increase in lipid peroxidation and a decrease in vascular endothelial function (Wang et al., 2007a). Therefore, based on the limited available research, a PHE protocol of >4 weeks for 1 h/ d, 5 d / week at an FiO_2 of 15 % should be recommended in this population.

Intermittent hypoxic training

When researchers have used identical exercise intensity protocols in hypoxic, compared to normoxic conditions, the participants training in hypoxia have demonstrated superior aerobic capacity following as little as 6 – 10 IHT sessions (Katayama et al., 1998, Balykin et al., 2004) or as long as 4 – 5 weeks (Wang et al., 2010, Mao et al., 2011) of IHT (30 min/d, 5 d/ week). However, other researchers have demonstrated no improvement in sea level performance compared to the control group following 6 weeks of high (80% $\dot{V}O_{2peak}$) or low (67% $\dot{V}O_{2peak}$) intensity IHT for 30 min/d, 5d/week (Geiser et al., 2001, Vogt et al., 2001) and no clear advantage following 10 weeks of IHT (Pesta et al., 2011).

Interestingly, even when there is little change in exercise performance, there are likely to be hypoxic-related adaptations at the cellular level (Friedmann et al., 2003, Geiser et al., 2001, Vogt et al., 2001). For example, despite positive correlations between mRNA levels of vascular endothelial

growth factor (VEGF) and myoglobin, and between phosphofructokinase and lactate dehydrogenate following IHT (but not before), there was no additional advantage of strength training in hypoxia compared to normoxia (Friedmann et al., 2003). Other structural alterations reported as a consequence of IHT include significant increases in muscle volume of knee-extensors, capillary length density, which is the capillary length in 1 mm^3 (Tomanek, 2013), and mitochondrial volume density (Geiser et al., 2001, Vogt et al., 2001) and elevated mRNA concentrations of the alpha subunit of hypoxic inducible factor – 1 (HIF-1 α) (Vogt et al., 2001).

None of the studies reported any beneficial erythropoietic responses as a consequence of 4 – 6 weeks of IHT (Wang et al., 2010, Geiser et al., 2001, Mao et al., 2011). Indeed, Mao et al. (2011) demonstrated accelerated aging, decreased size, a reduction in the ability of erythrocytes to deform under stress, and increased eryptotic (or suicidal cell death) response to hydrogen peroxide, following IHT.

However, potentially valuable adaptations for a sedentary population that were reported following IHT include improvements in the cardiac autonomic balance between sympathetic and parasympathetic contributions to the heart period, and increased lipid metabolism as evidenced by a lower RER during exercise (Balykin et al., 2004) which was possibly responsible for the 13% decrease in body fat weight compared to little change following normoxic exercise (Balykin et al., 2004). Additionally, Nishiwaki et al. (2011) reported improvements in vascular health (decreased pulse wave velocity and improved flow-mediated vasodilation) following IHT, but not normoxic exercise following hypobaric IHT. Further research should examine whether these improvements in vascular health can be repeated following normobaric hypoxia.

2.3.3 Simulated altitude in an active cohort

There have been numerous literature reviews that have already been published on the effectiveness of simulated altitude training on an active / athletic population. Therefore, a literature review, per se will not be discussed here and instead, a brief overview of some of the already published literature reviews will be provided. For the reader's interest, Table 5 and Table 6 provide a summary of research investigating the role of simulated altitude in an active / well-trained population.

The reviews have included general overviews (de Paula and Niebauer, 2012, Wilber, 2007, Levine, 2002) or meta-analyses (Bonetti and Hopkins, 2009, Lancaster and Smart, 2012) of simulated altitude techniques. (The meta-analysis by Lancaster and Smart (2012) has been criticised by Duke et al. (2012) for an inappropriate use of meta-analyses as the studies included were too dissimilar to draw generalisations). Others have examined the effectiveness of specific protocols such as IHE and

PHE (Bärtsch et al., 2008, Muza, 2007), IHT (Vogt and Hoppeler, 2010), or LHTL (Wilber, 2007). Physiological mechanisms have also been reviewed (Bailey and Davies, 1997). However, the key drivers for improved performance are still widely debated (See Point: Counter Point debate between Levine and Stray-Gundersen (2005), Gore and Hopkins (2005), and others (Noakes, 2005)). For example, some posit that the central modifier of performance is in adaptation of haemodynamic parameters such as erythropoietin or haemoglobin concentrations which increases $\dot{V}O_{2peak}$ (Levine, 2002, Rusko et al., 2004, Levine and Stray-Gundersen, 2005), while others argue that improved muscle efficiency, mitochondrial function, buffering and lactate tolerance (Gore et al., 2007), or even placebo/nocebo effects (Bonetti and Hopkins, 2009) are more likely to be responsible.

Regardless of which mechanisms are 'most important', one of the conflicting features regarding simulated (or real) altitude training is the lack of performance despite hypoxic-specific haemodynamic or cellular adaptation (Fudge et al., 2012, Vogt and Hoppeler, 2010, Clanton and Klawitter, 2001). Vogt and Hoppeler (2010), argue that the absence of observed improvements in performance despite gene expression changes may simply be due to a lack of sensitivity in $\dot{V}O_{2peak}$ or power output testing. Indeed, the variation in those who benefit from or do not benefit from simulated (or real) altitude training has led to the discussion of the possibility of 'responders' and 'non-responders' to altitude training (Gardner, 2009, Levine, 2002). However, others argue that the notion of responders and non-responders may simply represent a normal distribution of responses and therefore question the success of such interventions (de Paula and Niebauer, 2012).

Overall, the effects of simulated altitude in an athletic population has received mixed outcomes, with some researchers arguing against (Bärtsch et al., 2008, de Paula and Niebauer, 2012), and others supporting the use of simulated altitude (particularly IHE or PHE) for athletic enhancement (Hamlin and Hellemans, 2007, Bonetti et al., 2006, Wood et al., 2006), or for improving acclimatization prior to performance at altitude (Muza, 2007, Vogt and Hoppeler, 2010). For athletes and well trained individuals, the most widely supported model of simulated altitude for improvement in performance is the LHTL (Bonetti and Hopkins, 2009, Levine, 2002, Loffredo and Glazer, 2006, Rusko et al., 2004).

2.3.4 Key areas for future research in a sedentary or clinical population

In the sedentary and clinical populations, IHT does not appear to be particularly more effective than normoxic training in improving exercise capacity (Friedmann et al., 2003, Pesta et al., 2011, Vogt et al., 2001). However, the effects on vascular health are promising (Nishiwaki et al., 2011, Shi et al., 2013). Contrastingly IHE appeared to be effective in inducing beneficial adaptation in this cohort,

particularly regarding aerobic fitness and exercise tolerance (Burtscher et al., 2009, Burtsher et al., 2004, Shatilo et al., 2008), restoring cardiac autonomic balance between parasympathetic and sympathetic activity (Balykin et al., 2004), and blood lipid profiles (Tin'kov and Aksenov, 2002). Further research regarding the hypoxic dosage required for beneficial adaptation in required.

Cardiac autonomic modulation and systolic blood pressure

During hypoxia there is an increase in the sympathetic activity of the autonomic nervous system as evidenced by an increase in systolic blood pressure and heart rate with hypoxia (Rodway et al., 2007). However, while systolic blood pressure may remain elevated following PHE, there was no such increase following 10 IHE sessions (Foster et al., 2005) in an active population. Indeed, a decrease in resting (Shatilo et al., 2008) and exercising (Burtsher et al., 2004) systolic blood pressure has been observed following an IHE protocol at an FiO_2 of 12% (particularly in a sedentary population), but there was an increase in exercising SBP following a similar dose of PHE (Wang et al., 2007a). Therefore, when selecting a mode of treatment for a sedentary cohort an IHE protocol (rather than PHE) may be preferential in a population who may be at risk of high BP.

Interestingly, some authors have reported a shift towards increased parasympathetic activity in cardiac autonomic control following an IHE intervention period (Balykin et al., 2004, Haider et al., 2009), particularly in an unhealthy population (overweight and at risk of or with mild chronic obstructive pulmonary disease). However, while there was reduced parasympathetic withdrawal during the hypoxic exposure, no further changes in resting HRV were found in a healthy population (Bernardi et al., 2001). The greater sympathovagal improvement via increased parasympathetic activity in the autonomic control of the heart in the sedentary population, compared to the fit cohort suggests that there is greater potential for improvement following an IHE intervention in a less healthy population. As a decrease in vagal activity in cardiac control may be linked to numerous risk factors for cardiovascular disease, and indeed even precede the risk factors themselves (Thayer and Lane, 2007), the potential for IHE to improve HRV in a sedentary population and potentially reduce the risk for future disease warrants further investigation.

Blood lipid profile

High density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol are important considerations in the assessment of a person's overall cardiovascular risk (New Zealand Guidelines Group, 2003). Therefore, the reports of decreased total cholesterol and LDL and a corresponding increase in HDL following PHE (Tin'kov and Aksenov, 2002) warrants further investigation. It is important that future research makes use of a control group to cater for seasonal or biological variation in these measures.

Vascular health

Two recent studies have demonstrated an improvement in vascular health following IHT protocols in both postmenopausal women and healthy young men (Nishiwaki et al., 2011, Shi et al., 2013). However, Wang et al. (2007a) has reported a decrease in haemodynamic function following a PHE of 60 min (FiO_2 of 12%) but not following 60 min at an FiO_2 of 15% (Wang et al., 2007a, Wang et al., 2010) which suggests that the severity of the hypoxic stimulus should pass a minimum threshold, but not be excessive. That is, there was no change in haemodynamic function following 30 or 60 min of PHE at 15%. An improvement in flow mediated dilation and arterial compliance was noted following 2 h of IHT+PHE at a simulated altitude of 2000 m (IHT: 30 min of swimming at 50% $\dot{V}O_{2peak}$ +90 min PHE) (Nishiwaki et al., 2011) or 50 min IHT (60% HR_{max}) +30 min PHE at a simulated altitude at approximately 2400 m or an FiO_2 of 15.5% (Shi et al., 2013). However, 60 min of PHE at an FiO_2 of 12% had a detrimental effect on haemodynamic function (Wang et al., 2007a). An IHE protocol may provide a good balance between short intervals of severe hypoxia sufficient to improve arterial function, but regular alternation with ambient air may prevent distress or maladaptation.

Dosage

Katayama and colleagues have investigated various alterations and adjustments between the different hypoxic delivery modalities. In doing so, Katayama's research group have established that, there is no added advantage to using a 3 h exposure compared to 1 h of hypoxia, that 14 d are better than 7 d, and that an FiO_2 of 12.3% is better than 15% (Katayama et al., 2009, Katayama et al., 2007, Katayama et al., 2005). However, most of these studies have been conducted in well trained cohorts, using the PHE model, and were focussed on acclimatization. Further research regarding dosage should be conducted in a sedentary population. Furthermore, no researchers have investigated the differences in the frequency of hypoxic exposures per week. As there are no additional advantages to a longer session, compared to a shorter session (1 h compared 3 h), or IHE to PHE (Koehle et al., 2007), a 60 min IHE session may prove most beneficial for a sedentary population. Additionally, IHE is unlikely to cause any increases in systolic blood pressure (Foster et al., 2005), and may avoid the reduction in haemodynamic function with 60 min of PHE at an FiO_2 of 12%. Further research should investigate the frequency of IHE sessions per week necessary for an improvement in health in a sedentary population.

2.4 Key points and further research from literature review:

* Simulated altitude seems more likely to be beneficial in a health context, but only 15% of the retrieved articles examined the health response to simulated altitude in a sedentary population.

* Based on the current literature, 1 h of IHE to an FiO_2 of 12% for at least 2 weeks is likely to be most worthwhile for health adaptation. However, more research is needed on the frequency of IHE per week for beneficial adaptation.

* The effects of simulated altitude on the sympathovagal balance in cardiac autonomic modulation, vascular health, blood lipid profile as well as cardiovascular fitness should be further investigated.

* While the assessment of specific variables is useful, the effect of simulated altitude on an overall cardiovascular risk profile should be pursued.

* More information regarding the tolerance to hypoxia is desirable.

Figure 4: The size of each author's surname represents their contribution to the overall literature analysed in Tables 3 – 6 (www.wordle.net, accessed on the 17 November 2013)

Table 5: Literature summary of the use of simulated altitude in a healthy / active population (Listed alphabetically)

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall Main Outcome(s)
Ainslie et al. (2003)	Healthy, non-smoker, no medication or history of disease, 12M, 27 y	PHE	9 h / d, 5 sessions in 5 d	4300 m	↑ Hypoxic ventilator response ↑ Hypoxic & hypercapnic ventilatory response
Ainslie et al. (2007)	Healthy, non-smoker, no medication or history of disease, 3M4F, 25 y	IHE then real	5 min hypoxia alternated with 5 min normoxia for 90 min, 10 - 12 sessions in 12 d	SpO_2: 82 - 75%	↑ HVR during hypoxic interval (related to associated ↑ BP & ↓ middle cerebral artery blood flow velocity) following IHE.
	As above, 4M3F, 25 y	C then real	As above, normoxic placebo	SpO_2: >95%	⇆ Response in IHE & 12 d real altitude (1560 m)
Ainslie et al. (2008)	Healthy, non-smoker, no medication or history of disease, 6M5F, 26 y	11 IHE + real altitude;	5 min hypoxia alternated with 5 min normoxia for 90 min, 10 - 12 sessions in 15 d; followed by 12 d real altitude (1560 m)	SaO_2: 82 - 75% during IHE, 1560 m at real altitude	⇆ Change to cerebral auto-regulation in hypoxia, ↑ Muscle oxygenation in hypoxia Real altitude ⇆ IHE
	As above, 4M3F, 26 y	C then real	No treatment followed by 12 d at real altitude	1560 m	
	As above, 6M4F, 26 y	Intermittent hypercapnia	As IHE + real, but with $FiCO_2$: 5%	$FiCO_2$: 5%, then 1560 m real	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall Main Outcome(s)
Aulin et al. (1998)	Cross country skiers, middle and long distance runners, road cyclists, rowers and triathletes, 5M1F, 22 y	LHTL	12 h/d, 10 sessions in 10 d	2000 m	Initial ↓Hb after 2 d but returned to baseline. ↑ EPO in 3 -5 d and ↑ reticulocyte during 7 d LHTL. ⇆ $\dot{V}O_{2submax}$ or $\dot{V}O_{2peak}$, BP (rest or exercise) or mood states.
	As above, 8M1F, 27 y	LHTL	12 h/d, 10 sessions in 10 d	2700 m	
	As above, 4M1F, 32 y	C	no treatment	sea level	
Bailey et al. (2000)	University students, 2 - 4 h exercise/wk, no smoking or medications,18 M, 22 y	IHT: Cycling at 70 - 85% HR maximum for 20 - 30 min	20 min/d in wk 1&2; 30 min/d in wk 3&4, 3d/wk, 4wk	FiO_2: 16%	<u>IHT:</u> ↑ Lean mass & $\dot{V}O_{2peak}$ ↓ SBP & $exercise_{submax}$ lactate concentration. ↓ homocystein
	As above, 14 M, 22 y	C (sham): as above	As above, normoxic placebo	FiO_2: 21%	<u>Both:</u> ↓ nonesterified fatty acids, total cholesterol, HDL & LDL & ↑ power $output_{max}$ <u>C:</u> ↓Apolipoproteins AI & B
Bailey et al. (2001a)	University students, 2 - 4 h exercise/wk, no smoking or medications, 18M, 22 y	IHT: Cycling at 70 - 85% HR maximum for 20 - 30 min	20 min/d in wk 1&2; 30 min/d in wk 3&4, 3d/wk, 4wk	FiO_2: 16%	↓ Peripheral cholecystokinin (CCK) during IHT (correlated to SpO_2) ⇆ CCK at rest or exercise.
	As above, 14 M, 22 y	C (sham): cycling at 70 - 85% HR for 20-30 min	As above, normoxic placebo	FiO_2: 21%	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall Main Outcome(s)
Bailey et al. (2001b)	University students, 2 - 4 h exercise/wk, no smoking or medications, 18M, 22 y	IHT: Cycling at 70 - 85% HR maximum for 20 - 30 min	20 min/d in wk 1&2; 30 min/d in wk 3&4, 3d/wk, 4wk	FiO_2: 16%	In hypoxia: exercise induces ↑ hydroperoxide & malondialdehyde at ↓ $\dot{V}O_{2peak}$; increases correlated with ↓ Hb saturation.
	As above, 14 M, 22 y	C (sham): cycling at 70 - 85% HR maximum for 20 - 30 min	As above, normoxic placebo	FiO_2: 21%	After IHT: Greater ↓ in exercise-induced increase in lipid hydroperoxides & malondialdehyde in IHT (through selective mobilization of α-tocopherol.)
Bakkman et al. (2007)	Used to regular bicycling, no endurance training. Healthy, no smoking, disease or medication, 5M3F, 27 y	IHT*: 1 leg 30 min at 65% of maximal power output	45 min/d, 4d/wk, 4 wk	3000 m	Both: ↑ max power output C: ↑ citrate synthase, ⇆ cytochrome C oxidase
	As above	C: Other leg 30 min at 65% of maximal power output	As above	sea level	
Beidleman et al. (2003)	healthy, active no disease or smoking, 3 participants, 23 y	PHE*	4 h/d, 5d/wk, 3wk	4300 m	⇆ PHE vs IHT+PHE: data pooled ↑ TT performance (22 %) ↑ Adductor pollicis endurance, ↑ Resting SpO_2
	As above, 3 participants, 23 y	IHT*: Cycling 45 - 60 min, 70 - 85% maximal HR; remainder PHE*	4 h/d, 5d/wk, 3wk (exercise during first h PHE*)	4300 m	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall Main Outcome(s)
Beidleman et al. (2004)	healthy, no disease smoking, 1-2h exercise/wk, 3 participants, 23 y (Total participants: 5M1F)	PHE*	4 h/d, 5d/wk, 3wk	4300 m	⇆ PHE vs IHT+IPE: data pooled In 24 h hypoxia: ↓ acute mountain sickness & $PETCO_2$ via ↑ ventilation after training
	As above, 3 participants, 23 y	IHT*: Cycling 45 - 60 min, 70 - 85% maximal HR; remainder PHE*	4 h/d, 5d/wk, 3wk (exercise during first h PHE*)	4300 m	
Beidleman et al. (2008)	Healthy, no disease smoking, 1-2h exercise/wk, 5 participants 26 y (Total participants: 8M2F)	5 PHE*	4h/d, 7 sessions in 9 d	4300 m	Both: ↑ time trial performance & SS work rate. Data pooled: 16 % improvement in time trial performance ↑ SaO_2 , ↓ HR & RPE during steady state exercise
	As above, 5 participants, 26 y	5 IHT*	4h/d, 7 sessions in 9 d, exercise in first h PHE*	4300 m	
Beidleman et al. (2009)	Healthy lowlanders, non-smoking, 11M, 22 y	PHE & IHT: 2 X 25 min cycling at 80% peak HR	2h/day, 7 consecutive sessions	4500 m for PHE, 3000 m for IHT	⇆ Time trial ⇆ HR, SaO_2, or RPE during steady state exercise in either group
	As above, 6M, 20 y	C: 6	As above, normoxic placebo	Normoxia	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall Main Outcome(s)
Berglund et al. (2002)	Healthy, 3M, 20-23 y	PHE&IHT: 10 min cycling at 50 W	24 h/d, 10 consecutive d	SpO_2: 91%, 14 kPa	↑ EPO (especially after 2 d hypoxia, still elevated after 10 d)
	As above, 4M, 20-23y	PHE&IHT: as above but also 45 min cycling at 75 % maximum HR every other d	24 h/d, 10 consecutive d	SpO_2: 91%, 14 kPa	↑ Hb & Hct ↓ Ferritin
Bernardi et al. (2001)	Ukrainian soldiers, 12M, 26 y	IHE	5-7 min rebreathing alternated with 5-7 min normoxia for 1h, 7d /wk, 2wk	$PETO_2$: 35 - 40 mmHg	⇆ Resting respiration, ↑ HVR, ↓ vagal withdrawal in hypoxia, trend to ↑ Hb & RBC, ⇆ resting HRV
	As above, 6M, 27 y	C	As above	Sham	
Debevec et al. (2010)	Moderately active, healthy, 9M, 20 y	IHT: Cycling at HR corresponding to 50% hypoxic peak power output	70 min/d, 5 d/wk, 4 wk	FiO_2: 12%	C: ↑ $\dot{V}O_{2peak}$, peak power output and time to exhaustion IHT: ↑ Time to exhaustion
	As above, 9M, 22 y	C: Cycling as above at 50% normoxic peak power output	70 min/d, 5 d/wk, 4 wk	FiO_2: 21%	⇆hypoxic $\dot{V}O_{2peak}$, pulmonary function or haematological variables
Debevec and Mekjavic (2012)	Active, healthy, non-smoker, 10 M, 23 y	PHE	4h/d, 4 consecutive d	4200 m FiO_2: 12%	In hypoxia: ↑ SpO_2 & Ventilation ⇆ Constant power test or oxygenation (cerebral, vastus lateralis, or serratus anterior).
	As above, 9M, 22 y	C	4h/d, 4 consecutive d	Sham	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall Main Outcome(s)
Faulhaber et al. (2010)	Cyclists, 6M, 40 y	PHE	1h/d, 7 sessions over 9 d	FiO_2: 12.5%	↓ Mean power output in hypoxia PHE ⇆ C.
	As above, 6M, 34 y	C	As above	FiO_2: 21%	⇆ Time trial
Foster et al. (2009)	Healthy, no confounding medical condition, 10M, 21 - 36 y	IHE	4 d sham, 10 d IHE: 2 min hypoxia (or placebo) cycled with 2 min normoxia for 6 h	$PETO_2$: 45 mmHg	↑ MAP ↓ Nitric oxide derivatives In hypoxia: ↑ pressor response ↑ cerebrovascular resistance
Foster et al. (2005)	Recreationally active, healthy, 9M, 26 y	IHE	5 min hypoxia cycled with 5 min normoxia for 1 h, 10 sessions in 12 d	FiO_2: 12%	↑ HVR (baseline after 17 d), ↑ SBP in PHE but not in IHE. In hypoxia: IHE ⇆ PHE regarding ventilatory & cardiovascular response; ↓ cerebral oxygenation.
	As above, 9M, 26 y	PHE	30 min/d, 10 sessions in 12 d	FiO_2: 12%	
Foster et al. (2006)	Recreationally active, healthy, 9M, 26 y	IHE	5 min hypoxia cycled with 5 min normoxia for 1 h, 10 sessions in 12 d	FiO_2: 12%	⇆ Maximal exercise or ventilatory responses during exercise (no relationship).
	As above, 9M, 26 y	PHE	30 min/d, 10 sessions in 12 d	FiO_2: 12%	↑ HVR in IHE and PHE.
Garcia et al. (2000)	Healthy, non-smokers, 9M, 29.3 y	PHE	2h/d, 12 consecutive d	3800 m FiO_2 : 13%	⇆ Hct or Hb, ↑ Ret count to d 5. ↑ HVR in hypoxia to d 5, then ↓ to lower value on d 12, ⇆ Ventilatory data (normoxia or poikilocapnic hypoxia).

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall Main Outcome(s)
Green et al. (1999)	Active, but not endurance trained, healthy, 9M, 19 - 27 y	IHT: 30 min of 1 leg cycling at 75% maximal mechanical power of weaker leg. First 6 wk continuous Last 2 wk interval	30 min/d, 3d/wk, 8 wk	FiO_2: 13.5%	In hypoxia: Mitochondrial potential up-regulated Na^+ -K^+ -ATPase pump expression down-regulated. Normoxic training ↑ both.
	As above	C: as above, but 30 min on other leg in normoxia	As above	FiO_2: 21%	
Holliss et al. (2013)	Physically active, healthy, 9M, 22 y	IHT: single leg high intensity interval training	25 min/d, 5 d/wk, 3wk	FiO_2: 14.5%	⇆ Muscle metabolism between legs after training
	As above	As above, other leg	As above	FiO_2: 21%	
Katayama et al. (1999)	Healthy, no medication, 7M, 21 y	IHT*: cycling at 70% altitude $\dot{V}O_{2peak}$	30 min/d, 5d/wk, 2 wk	4500 m	Both groups ↑ $\dot{V}O_{2peak}$ after training, & ↓ after detraining. ↑ HVR in IHT (not sig), ↓ C. ⇆ HVR after detraining
	As above, 7M, 22 y	C: cycling at 70% sea level $\dot{V}O_{2peak}$	30 min/d, 5d/wk, 2 wk	Sea level	
Katayama et al. (2000)	Healthy, no medication, 7M, 21 y	IHT*: cycling at 70% altitude $\dot{V}O_{2peak}$	30 min/d, 5d/wk, 2 wk	4500 m	SBP changes in parallel with HVR in progressive hypoxia, ⇆ DBP & HR. sig correlation between change in HVR and change in SBP/change in SaO_2
	As above, 7M, 22 y	C: cycling at 70% sea level $\dot{V}O_{2peak}$	30 min/d, 5d/wk, 2 wk	Sea level	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall Main Outcome(s)
Katayama et al. (2001)	Healthy, no cardiovascular disease, 6M, 21 y	PHE*	30 min ascending, 60 min at target/d, 7 consecutive d	4500 m	↑ HVR (rest) & ↑ ventilatory equivalent for O_2, and SaO_2 in submaximal exercise for 7 d.
Koehle et al. (2007)	University students (mountaineering and endurance backgrounds), 10 M, 26 y	PHE	1 h/d, 7 consecutive d	4200 m FiO_2: 12%	↑ HVR (plateau after 3 d). HVR returned to baseline within a week. PHE ⇆ IHE
	As above (crossover)	IHE	5 min of hypoxia alternated with 5 min normoxia, for 2 h; 7 consecutive d	4400 m	
Kolb et al. (2004)	Healthy, no smoking, mediation or disease, 12 M, 26 y	PHE	8 h/d, 5 consecutive d	4300 m FiO_2: 13.8%	↓ $PETCO_2$ & ↑ SaO_2 as night progressed. ↑ AMS on 1st & 2nd night. ↑ Cerebral blood flow sensitivity to change in O_2 & CO_2. Regional cerebral O_2 saturation correlated with SpO_2
Kounalakis et al. (2013)	Healthy, active, 8M, 20 y	PHE	24 h/d, 10 consecutive d	3400 m FiO_2: 15.4%	⇆ C in any measure ⇆ PHE NIRS in cerebral, serratus anterior, $\dot{V}O_{2peak}$ or peak work rate
	As above (crossover)	C	24 h/d, 10 consecutive d	Sham	↑ ventilation after PHE, & lower ↓ in VL Hb dissociation

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall Main Outcome(s)
Levine et al. (1992)	Recreationally active, 7 participants, 27 y	IHT*: cycling at 70% sea level $\dot{V}O_{2peak}$	45 min/d, 5 d/wk, 5 wk	2500 m	C: ↑ $\dot{V}O_{2peak}$ C, ⇆ HVR,
	As above, 7 participants, 27 y	IHT*: cycling at 70% altitude $\dot{V}O_{2peak}$	As above	2500 m	IHT: ↑ $\dot{V}O_{2peak,}$ ↑ HVR, (possibly ↑ chemoreceptor sensitivity)
	As above, 7 participants, 26 y	C: cycling at 70% sea level $\dot{V}O_{2peak}$	As above	sea level	
Lundby et al. (2005)	Healthy, moderate-well trained, 8M, 24 y	PHE*	2 h/d, 7 d/wk, 2 wk	4100 m	⇆ Hb, HCT, Ret, serum transferrin, EPO, or exercise capacity
Lusina et al. (2006)	Healthy, no smoking, mediation or disease, 11 M, 25 y	PHE	1 h/d, 10 consecutive d	SpO_2: 80 %	↑ HVR following IH. ↑ SNS during hypoxia & stayed ↑ after SpO_2 normalised. Correlation: MSNA& HVR (SNS & HVR centrally controlled.)
McLean et al. (2006)	Healthy, non-smokers, 6M, 26.5 y	PHE	8h/d, 3 consecutive d	FiO_2: 12.9	↑ EPO & Hct, Hb & RBC (likely natural variation or dehydration as ⇆ markers for EPO such as ret counts or serum ferritin concentration
	As above, 5M, 26 y	5 C	As above	FiO_2: 20.4%	
Melissa et al. (1997)	Recreational activity, 10 M, 19 - 25 y	IHT: 30 min unilateral cycling at 75% maximal power output of weaker leg for first 6 wk, interval training in last 2 wk	30 min/d, 3d/wk, 8 wk	FiO_2: 13.5%	↑ Both legs: $\dot{V}O_{2peak}$, time to exhaustion, citrate synthase, succinate dehydrogenase & phosphofructokinase. <u>IHT:</u> ↑↑ Citrate synthase
	As above (crossover)	As above , other leg	As above	FiO_2: 21%	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall Main Outcome(s)
Morton and Cable (2005)	Healthy, active in team sports, 8M, 21 y	IHT: moderate - high intensity interval training	30 min/d, 3d/wk, 4 wk	2750 m FiO_2: 15%	Both: ↑ $\dot{V}O_{2peak}$, Maximum work rate, onset of blood lactic accumulation, mean & peak power. IHT ⇆ C ⇆ Hb & Hct
	As above, 8M, 20 y	C: as above	As above	Sea level	
Querido et al. (2008)	Healthy, normal pulmonary function, 8M, 41 y	IHE	5 min hypoxia alternated with 5 min normoxia for 1h, 10 consecutive d	FiO_2: 12%	↓ Cerebrovascular sensitivity to hypoxia, ⇆ Ventilatory, BP or HR sensitivity change
Querido et al. (2009)	Healthy, normal pulmonary function, 9M, 24 y	PHE	1 h/d, 10 consecutive d	SpO_2: 80%	↑ Minute ventilation in hypoxia. ⇆ Exercise ventilation, middle cerebral artery mean velocity & MAP
Querido et al. (2012)	Healthy, normal pulmonary function & no breath holding activities, 9M, 24 y	PHE	1 h/d, 10 consecutive d	SpO_2: 80%	⇆ Cytokines / markers of inflammation
Rodway et al. (2007)	Healthy (not obese, smoking or medicated), not training, 10 M, 20 - 35 y	IHE	10 min hypoxia alternated with 10 min normoxia for 1 h, 3 consecutive d	FiO_2: 13.5%	In hypoxia: ↑ HR, SBP, DBP ⇆ Nitric oxide synthase mRNA correlated to nitric oxide synthase mRNA & average end exposure DBP & MAP during d 1 - 3 in IHE
	As above	PHE	1 h/d, 3 consecutive d	FiO_2: 13.5%	
Shi et al. (2013)	Healthy, no smoking or disease, 8M, 26 y	IHT: Treadmill exercise at HR corresponding to 60% $\dot{V}O_{2peak}$	50 min IHT followed by 30 min PHE, 3 d/wk, 4 wk	FiO_2: 15.4%, 2500 m	Improved brachial PWV & $\dot{V}O_{2peak}$ after IHT, not C
	As above (crossover)	C: As above	As above, normoxic placebo	Fio2: 21%	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall Main Outcome(s)
Shin et al. (2013)	Healthy, 9M, 23 y	IHT: Treadmill, 60% Vo2peak for 40 min (5 min warm up/down) followed by 30 min PHE	80 min/d, 3d/wk, 4 wk	2500 m FiO_2: 15.4%	↑ $\dot{V}O_{2peak}$ & HR after training, "Fairly Light" corresponded to higher loads.
Taylor et al. (2011)	Healthy, recreationally active, 8M, 20 y	PHE	75 min/d, 10 consecutive d	2980 m	⇆ $\dot{V}O_{2peak}$ or time to exhaustion ↑ Monocyte expressed heat shock protein 72 (day 2 - 2 d post-intervention. ↑ Daily elevations in oxidative stress markers & sustained ↑ in EPO.
Tonini et al. (2011)	Active healthy volunteers, 10M2F, 23 y	PHE	30 desaturations/h for 8 h, 7 d/wk, 2 wk	FiO_2: 13%	⇆ Exercise capacity, ↓ $PETCO_2$ & ↑ VE/VCO_2 (hyperventilation during exercise) ↓ HR_{max}, ↑ DBP, after 1 min of submaximal exercise recovery ↓ Lactate & RER < 1 at higher power output

*: Hypobaric hypoxia; ↑ Increased; ↓ Decreased; ⇆ No change, or no difference; AMS: Acute mountain sickness; BP: Blood pressure; C: Control group; CO_2: Carbon dioxide; DBP: diastolic blood pressure; EPO: Erythropoeitin; F: Females; $FiCO_2$: fraction of inspired carbon dioxide; FiO_2: Fraction of inspired oxygen; Hct: Haematocrit; Hb: Haemoglobin; HR: heart rate; HR_{submax}: heart rate measured during submaximal exercise; HRR: heart rate reserve; HDL: High density lipoprotein; HIIT: High intensity interval training; HVR: hypoxic ventilatory response; IHE: passive intermittent hypoxic exposure; IHT: intermittent hypoxic training (exercise in hypoxia); LDL: Low density lipoprotein; LHTL:Live high train low; LMTM: Live moderate train moderate; M: Males; MAP: Mean arterial pressure; MSNA: Muscle sympathetic nervous activity; O_2: Oxygen; $PETCO_2$: Partial pressure of end-tidal carbon dioxide; PHE: Prolonged hypoxic exposure; PWV: Pulse wave velocity; RPE: Rating of perceived exertion; RBC: Red blood cells; Ret: Reticulocytes; RER: Respiratory exchange ratio sEPO: serum erythropoeitin; SaO_2: arterial oxygen saturation; SpO_2: peripheral oxygen saturation; SBP: Systolic blood pressure; SNS: Sympathetic nervous system; TT: Time trial; $\dot{V}O_2$: oxygen uptake; $\dot{V}O_{2peak}$: peak oxygen uptake; $\dot{V}O_{2submax}$: submaximal exercising oxygen uptake; VE: expired volume; VCO2: volume of carbon dioxide.

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Table 6: Summary of the literature examining simulated altitude exposure in a well-trained / athletic cohort

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Abellan et al. (2005)	Triathlete, 8M	PHE*	3h/d, 5d/wk, 4 wk	4000 – 5500 m	↑ Serum erythropoietin, ⇆ Reticulocytes or RBC.
	As above, 8M	C	As above, normoxic placebo	Sea level	
Ashenden et al. (1999a)	Triathletes, cyclists, cross country skiers, 6M, 25 y	LHTL	8 - 10 h/d, 23 consecutive d	3000 m FiO_2: 15.5%	⇆ Hb, Reticulocyte parameters.
	As above, 7M, 25 y	C	Slept at home	Sea level	
Ashenden et al. (1999b)	Road cyclists, 5F, 26 y	LHTL	8-10 h/d, 12 consecutive d	2650 m FiO_2: 16%	⇆ Hb, Reticulocytes or any reticulocyte related variables.
	As above, 6F, 26 y	C	Slept in dormitories	Sea level	
Ashenden et al. (2000)	Middle distance runners, 6 athletes, 22 y	LHTL	8 - 11h, 5 d LHTL, 3 d recovery for 24 d	2650 m FiO_2: 16%	↑ EPO (after 5 d), but not after 2nd & 3rd 'block' of 5 d. ⇆ Reticulocyte production.
	As above, 6 athletes, 22 y	C	Slept in dormitories	Sea level	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Aughey et al. (2005)	Triathletes, cross country skiers, road cyclists, 6M, 25 y	LHTL	9.5 h/d, 23 consecutive d	3000 m FiO_2: 15.5%	LHTL: ↓ 3-O-methylfluorescein phosphatase (also depressed after exercise in C & LHTL). ⇆ Plasma K+ & [3H]ouabain binding. LHTL: ↓ $\dot{V}O_{2peak}$ but ⇆ work.
	As above, 7M, 25 y	C	Slept at home		
Aughey et al. (2006)	Cyclists & triathletes, 12M, 27 y	LHTL continuous	8 - 10 h/d, 20 consecutive d	2650 m FiO_2: 16.3%	LHTL continuous: ↓ 3-O-methylfluorescein phosphatase (after 5 d), below pre after 20 d.
	As above, 10M, 26 y	LHTL interspersed	8 - 10 h/d, 5 d LHTL, 2 d recovery for 26 d	2650 m FiO_2: 16.3%	LHTL interspersed: Initial ↓ reversed itself & ⇆ after 20 d. ⇆ plasma K+
	As above, 11M, 26 y	C	Slept in dormitories or own homes	Sea level	
Bonetti et al. (2006)	Sub-elite sprint kayak paddlers, 10M, 23 y	IHE	5 min hypoxia alternated with 5 min normoxia for 1 h, 5d/wk, 3wk	FiO_2: 12 – 10%	↑ Peak power, repeated sprint power, & Hb after IHE. ⇆ lactate, time trial, $\dot{V}O_{2peak}$ & exercise economy
	As above (crossover)	C	Placebo	Sea level	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Brugniaux et al. (2006b)	Elite middle distance runners, 5M, 24y	LHTL	14 h/d, 18 consecutive d	2500 - 3000 m FiO_2: 16.4%	LHTL: ↑$\dot{V}O_{2peak}$, maximal aerobic power, $\dot{V}O_2$ and ventilation at ventilatory threshold. ↓ HR_{submax} in 10 min run ↑ serum transferrin receptor ↑ Hb mass ⇆ red cell volume
	As above, 6M, 23 y	C	As above, normoxic placebo	Sea level	
Brugniaux et al. (2006a)	Cross country skiers, 3M3F, 23 y	LHTL	11 h/d, 18 d	2500 m for 6 d, 3000 m for 6 d, 3500 m for 6 d	⇆AMS, ↓SaO_2 in hypoxia to 90% at 3500 m. ↑ SaO_2 after acclimatization, (except at 3500). ⇆ leukocyte, or heart function.
	As above, 2M3F, 20 y	C	24 h/ d, 18 d	1200 m	
	Swimmers, 8M,1F, 20 y	LHTL	16 h/d, 13d	2500 m for 5 d, 3000 for 8 d	↑ HVR & smaller change in SpO_2) but ⇆ after 15 d.
	As above, 8M1F, 17 y	C	24 h/d, 13 d	1200 m	
	Runners, 6M, 25 y	LHTL	14 h/d, 18 d	2500 m for 6 d, 3000 m for 12 d	
	As above, 6M, 23 y	C	24 h/d, 18 d	1200 m	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Casas et al. (2000)	Elite climbers, 5M1F, 28 y	IHT*: 15 min / h at a HR of 120 - 130 beat·min-1 (based on 100W HR at sea level)	3 - 5 h/d, 17 d	4000 - 5500 m	↑ Packed cell volume, red blood cell & Hb concentration ↓ HR & Lactate vs exercise load curve shifted right (↑ aerobic endurance). ↑ Anaerobic ventilatory threshold, pulmonary ventilation, tidal volume, respiration frequency, $\dot{V}O_2$ CO_2 output & ventilatory equivalent to O_2 & Co_2 at ventilator threshold.
Clark et al. (2009)	Cyclists, 12 M, 22 y	LHTL	14 h /d, 3 wks	3000 m, FiO_2: 15.5%	↑ Hb mass (3 %), ⇆ $\dot{V}O_{2peak}$, 1% Hb ↑/week EPO peaked on day 2
Clark et al. (2004)	Triathletes and cyclists, 9 M, 27 y	LHTL continuous	9-10 h/3, 20 d	2650 m FiO_2: 16.3%	⇆ Lactate appearance in first 1 h exercise between groups. After 25 min at 85% $\dot{V}O_{2peak}$, lactate accumulation > 65%. $\dot{V}O_{2peak}$ (continuous less high). Lactate disappearance similar to lactate accumulation. ⇆ Buffering capacity in either.
	As above, 10M, 26 y	C	As above, normoxic placebo	Sea level	
	As above, 10M, 27 y	LHTL interspersed	9-10h /d, 5 consecutive d / wk, 4 wk	2650 m FiO_2: 16.3%	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Czuba et al. (2011)	Elite cyclists, 10 M, 22 y	IHT: 15 min: warm up / down. 30 - 40 min: 95% lactate threshold	60 - 70 min/d, 3 d/wk, 3 wk	2500 - 2600 m FiO_2: 15.2%	IHT: ↑ $\dot{V}O_{2peak}$, $\dot{V}O_2$ lactate threshold, work $rate_{max}$, work rate at lactate threshold & ↑ lactate concentration. Improved time trial. ⇆ Haematological variables.
	As above, 10 M, 24 y	C	As above	Sea level	
Dufour et al. (2006)	Highly trained distance runners, 9 M, 30 y	IHT: 1 X moderate, 1 X high intensity (with normal training) + 3 normoxic sessions	24 - 40 min/d, 2 d/wk, 6 wk	3000 m FiO_2: 14.5%	IHT: ↑ $\dot{V}O_{2peak}$ & time to exhaustion. ⇆ Haemodynamic. C: ⇆ any parameter
	As above, 9 M, 30 y	C	As above	Sea level	
Fu et al. (2007)	Collegiate Runners and Swimmers, 4M6F, 23 y	PHE*	3 h/d, 5 d/wk, 4 wk	4000 - 5000 m	⇆ HR, BP, cardiac output, stroke volume, total peripheral resistance, heart rate variability or cardiac vagal baroreflex action
	As above, 7M5F, 23 y	C	As above	Sea level	
Gore et al. (2006)	Swimmers and runners, 5M6F, 16 - 35 y	PHE*	3 h/d, 5 d/wk, 4 wk	4000 - 5000 m	↑ erythropoietin ⇆ Red cell volume, Hb, soluble transferrin receptor, or reticulocytes
	As above, 6M6F, 16 - 48 y	C	As above	Sea level	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Gore et al. (2001)	Triathletes, cross country skiers, road cyclists, 6M, 25 y	LHTL	9.5 h/d, 23 consecutive d	3000 m FiO_2: 15.5%	↑ muscle capacity ↓ $\dot{V}O_{2peak}$ (but work maintained) ↓ $\dot{V}O_{2submax}$ and ↑ efficiency
	As above, 7M, 25 y	C	Normal accommodation	Sea level	
Hamlin and Hellemans (2007)	Cyclists, runners, swimmers and kayakers, 5M7F, 32	IHE	5 min hypoxia: 5 min normoxia for 90 min, 5 d/wk, 3 wk	FiO_2: 10 - 12%	↓ 3km run time (-1.7% 2 d & 2.3% 17 d after IHE). ↑ reticulocytes (23.5% 2 d & 14.6 % 12 d later) in IHE vs C.
	As above, 8M2F, 32 y	C	Placebo	Sea level	
Hamlin et al. (2008)	Rugby Union players, 9MF, 22 y	IHE - tested at altitude	6 min IHE: 4 min normoxia for 1 h, 11 sessions in 15 d	3750 - 5000 m FiO_2: 10 - 13%	Improved HR_{submax} & Lactate speed ⇆ Other parameters. <u>In hypoxia:</u> ↓ Performance in all athletes ⇆ IHE effect.
	As above, 6MF, 26 y	C - tested at altitude	As above	Sea level	
	As above, 7MF, 21 y	C - Tested at SL	As above	Sea level	
Hamlin et al. (2010)	Cyclists (road, mountain bike, triathlete, and multisport athletes), 8M1F, 30 y	IHT: 60-70 HRR for 90 min followed by 2 Wingate tests (5 min recovery). Logged training	90 min / d, 10 consecutive d	SpO_2: 88 - 82%	<u>IHT</u>: ↑ Mean power ⇆ 30s peak power, 20km mean power, & 20 km oxygen cost. ↑ Lactate, RER & SPO_2 2 d post IHT.
	As above, 6M1F, 39 y	C	Placebo		

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Hendriksen and Meeuwsen (2003)	Triathlete, 12M, 30 y	IHT*: 105 min training on a cycle ergometer at 60 - 70% HRR	2 h/d, 10 consecutive d	2500 m	IHT: ↑ Maximal power output, anaerobic mean power, & anaerobic peak power& non-significant ↑ $\dot{V}O_{2peak}$.
	Crossover	C	Placebo	Sea level	C: ⇆ Maximal power output, $\dot{V}O_{2peak}$, anaerobic mean power, or peak power at sea level.
Hinckson and Hopkins (2005)	Runners and Triathletes, 11M, 31 y	LHTL	8 h/d, 7d/wk, 4 wk	2500 - 3000 m	↑ Running time, (1%, 1.4% & 1.9 % for 800 m, 1500 m & 3000 m), especially in athletes with I allele for angiotensin converting enzyme
	Crossover	C	no altitude tents	Sea level	⇆ Hb.
Hinckson et al. (2006)	Elite rowers, 2M5F, 23 y	IHE	6 min IHE: 4 min normoxia for 90 min, 7d/wk, 3 wk	SpO_2: 88 - 80%	IHE: ↑ 5000 m but ↓ mean power for 500 m time trial Major benefits unlikely
	Elite rowers, 1M4F, 20 y	C	Placebo	Normoxic placebo	
Hinckson et al. (2007)	Rugby players, 5M, 23 y	IHE	6 min IHE: 4 min normoxia for 1 h, 7d/wk, 2 wk	FiO_2: 10 - 15%	⇆ Maximum sprint time or speed. ↓ Peak power during scrum, offensive sprint & tackle
	Rugby players, 5M, 23 y	C	Placebo	Normoxic placebo	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Hinckson et al. (2005)	Competitive runners, 8M2F, 25 y	LHTL	10 h/d, 7 d/wk, 24 - 30 d	2500 - 3000 m	LHTL: ↑ Time to exhaustion (16 %). ⇆ Effect on genotype for angiotensin converting enzyme & change in Hb
	Competitive runners, 8M2F, 37	C	Normal accommodation	Sea level	
Julian et al. (2004)	Highly trained distance runners, 14M3F total, 25 y (groups not distinguished)	IHE	5 min hypoxia alternated with 5 min normoxia for 70 min, 5d/wk, 4wk	FiO_2: 10 - 12%	⇆ $\dot{V}O_{2peak}$, 3000 m time trial, EPO soluble transferrin receptors, or reticulocytes
	As above	C	Placebo	Normoxic placebo	
Katayama et al. (2003)	Endurance runners, 6M, 21 y	PHE*	90 min/d, 3d/wk, 3wk	4500 m	PHE: ↓ 3000 m running time. ↓$\dot{V}O_{2submax}$ ↑ Time to exhaustion in maximal test. Both: ⇆ cardiorespiratory parameters in exercise or resting haematological parameters ⇆ After 3-wk recovery.
	As above, 6M, 23 y	C	No treatment	Sea level	
Katayama et al. (2004)	Endurance runners, 8M, 22 y	PHE: 8	3 h/d, 7 d/wk, 2 wk	FiO_2: 12.3%	↓ $\dot{V}O_{2submax}$ & 3000 m time tended to improve ⇆ $\dot{V}O_{2peak}$ or resting haematological parameters.
	As above, 7M, 20 y	C: 7	No treatment	Sea level	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Katayama et al. (2005)	endurance runners, 7M, 20 y	PHE	3 h/d, 7 d/wk, 1 wk	FiO_2: 12.3%	⇆ Hypercapnic ventilatory slope after 7 d, ↑ after 14 d BOTH: ⇆ Hypercapnic ventilatory intercept & ↑ hypoxic ventilatory slope. ⇆ After 2 wk recovery
	As above, 7M, 22 y	C	No treatment	Sea level	
	As above, 8M, 22 y	PHE	3 h/d, 7 d/wk, 2 wk	FiO_2: 12.3%	
	As above, 8M, 20 y	C	No treatment	Sea level	
Katayama et al. (2007)	Endurance runners, 6M, 20y	PHE	1 h/d, 7 d/wk, 1 wk	2500 m FiO_2: 15.5%	⇆ Any parameter at FiO_2 15.5%. ↑ HVR at FiO_2 12.3% ⇆ HVR or SpO_2 during exercise.
	As above, 6M, 20 y	PHE	1 h/d, 7 d/wk, 1 wk	FiO_2: 12.3%	
	As above, 6M, 21 y	C	No treatment	Sea level	
Katayama et al. (2009)	Distance runners, 10M, 26 y	PHE	1 h/d, 7 d/wk, 1 wk	4300 m FiO_2: 12%	Both: ↑ HVR ⇆ Hypocapnic ventilatory response
	Crossover study	PHE	3 h/d, 7 d/wk, 1wk	4300 m FiO_2: 12%	
	Crossover study	C	No treatment	Sea level	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Kinsman et al. (2005)	endurance runners, 7M, 26 y	LHTL continuous	6 h/d, 7 d/wk, 15 d	2650 m	⇆ Between groups. Highly varied arousal response & respiratory disturbance (not significant).
	As above 7M, 26 y	LHTL interspersed	6 h/d, 5d/wk, 3 wk	2650 m	↓ SpO_2 during hypoxic sleep. ↑ Number of arousals at 1 & 2 wk into study, ↑ REM 1 wk into study.
Lecoultre et al. (2010)	Competitive cyclists, 7M, 29 y	IHT: 2 X HIIT + 15 min warm up/down; 1 X 100 min endurance	46 - 105 min/d, 3d/wk, 4 wk	3000 m	↑ Endurance performance but IHT ⇆ C ⇆ Lactate turnover C & IHT.
	As above 7M, 26 y	C	normoxic training	Sea level	↓ Glucose metabolism in IHT
Maciejczyk et al. (2012)	Elite Polish race walker, 1M, 30 y	C; LHTL; Real	LHTL: 8 h/d, 7 d/wk, 4 wk; Real 26 d continuous	LHTL: 2133 m, Real: 1800 m	<u>LHTL:</u> ↑ Haematological parameters & ↑ Ventilatory threshold ⇆$\dot{V}O_{2peak.}$ <u>Real altitude:</u> ↑$\dot{V}O_{2peak}$, ⇆ Ventilatory threshold ↓ Haematological measures.

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Manimmanakorn et al. (2012)	Well trained netball players, 10F, 20 y	Occlusion: As IHT	12 - 13 min/d, 5 d/wk, 5 wk	Kaatsu	↑ Maximal voluntary contraction, area under 20s force curve & number of 20% 1RM.
	As above, 10F, 20 y	IHT: 3 sets of knee extension & flexion to exhaustion (20% 1RM)	As above	SpO_2: 80%	↑ Cross sectional area in Kaatsu & IHT groups. ↑ Gains in Kaatsu vs IHT (except number of 20% 1RM).
	As above, 10F, 20 y	C: As IHT	As above	Normal	
Manimmanakorn et al. (2013)	Well trained netball players, 10F, 20 y	Occlusion	12 - 13 min/d, 5 d/wk, 5 wk	Kaatsu	↑ Electromyographic activity after Kaatsu vs to IHT and C
	As above, 10F, 20 y	IHT: 3 sets of knee extension & flexion to exhaustion (20% 1RM)	As above	SpO_2: 80%	
	As above, 10F, 20 y	C	As above	Normal	
Mounier et al. (2009)	Cyclists and triathletes, 10M, 24 y	IHT: 2 X interval, 3 X continuous: 1 h 60% $\dot{V}O_{2peak}$	26 - 60 min/d, 5d/wk, 3 wk	3000 m	↓ HIF-1a mRNA in skeletal muscle but ⇆ leukocytes
	As above 9M, 24 y	C	As above	Sea level	
Muza et al. (2009)	US Military personnel, 11M, 22 y	PHE + IHT; 2 h PHE followed by: 2 x 25 min cycling at 80% peak HR	2 h PHE & 1 h IHT, 7 d/wk, 1 wk	PHE: 4500 m IHT: 3000 m	<u>IHT+PHE</u> ⇆ all variables. <u>Real altitude</u>: improved time trial, ↓RPE
	As above, 10M, 21 y	Real	24 h/d, 6 d	2200 m	⇆HR & SaO2.

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Neya et al. (2007)	Collegiate middle and long distance runners, 10M, 21 y	LHTL	10 - 12 h/d, 7 d/wk, 4 wk	3000 m FiO_2: 14.5%	⇆ Total Hb, $\dot{V}O_{2peak}$, or time to exhaustion (any group) LHTL:↑ Running economy (5%)
	As above, 9M, 20 y	IHT: 30 min treadmill running for 12 of the 31 d intervention	30 min/d, 12 consecutive d	3000 m FiO_2: 14.5%	
	As above, 6M, 21 y	C	No treatment	Sea level	
Nordsborg et al. (2012)	Road cycling, triathlons, cycle cross, mountain biking, 15M1F, 39 y (groups not specified)	LHTL	16 h/d, 7 d/wk, 4 wk	3000 m	LHTL ⇆ C groups. ⇆ Mean power, arterial plasma pH, lactate, K+ & muscle buffer capacity, sarcolemmal H^+ transporters & NA^+K^+ pump subunits a1, a2 & b1 in submaximal or maximal exercise.
	As above	C	As above, normoxic placebo	Normoxic placebo	
Nummela and Rusko (2000)	400 m runners (Finnish national team), 6M2F, 22 y	LHTL	16.5 h/d, 10 consecutive d	2200 m FiO_2: 15.8%	LHTL: ↓ 400 m race time ↑ Speed at onset of blood lactate accumulation.
	As above, 10 M, 22 y	C	Sea level	Sea level	↑ Resting blood pH (6/ 8 LHTL participants)

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Pedlar et al. (2008)	Runners and Triathletes, 6M, 30 y	IHT: Treadmill running 75 min / d, below 2 mmol/L lactate	75 min / d, 8 consecutive d	2500 m FiO_2: 14.9%	<u>In hypoxia:</u> ↑ TT time <u>Both:</u> improved TT performance but not sig; better in IHT.
	As above, 6M, 29 y	C	Normoxic training	Normoxic placebo	↑ Individual variability
Ponsot et al. (2006)	Distance runners, 8M, 30 y	IHT: 2 x 12 min at beginning up to 2 x 20 min intervals at the second ventilatory threshold	24 - 40 min/d, 2 d/wk, 6 wk	3000 m FiO_2: 14.5%	<u>IHT</u>: ↑ Time to exhaustion, $\dot{V}O_2$ at the second ventilatory threshold & $\dot{V}O_{2peak}$; ⇆ muscle oxidative capacity
	As above, 7M, 31 y	C: as above, in normoxia	Normoxic training	Normoxic placebo	<u>IHT</u>: Mitochondria shifted to a more oxidative profile
Povea et al. (2005)	Swimmers and middle distance runners, 8M, 21 y	LHTL	11 - 16 h/d, 13 consecutive d	2500 - 3000 m FiO_2: 15.7-14.5%	↑ Ventilation & SNS in subsequent hypoxic exposures in the Hyp groups
	As above, 12M, 22 y	LLTL	No treatment	1200 m	
Pupiš and Čillík (2008)	Race walker, 1M, 28 y	IHE	6 min hypoxia alternated with 3 min normoxia for 90 min, 21 sessions over 14 d	FiO_2: 14 - 8% SpO_2: 90 - 70%	↓ 3km walk time after IHE ⇆ Haematological variables.
Ramos Campo et al. (2011)	Elite cyclists, 8 cyclists, 23 y	PHE	40 - 60 min/d, 4 d/wk, 8 wk	FiO_2: 14 - 11%; SpO_2: 90 - 85%	⇆ Haematological variables, no suggestion of erythropoiesis.
	As above, 8 cyclists, 27 y	C	Normoxia	Sea level	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Robach et al. (2006a)	Nordic skiers, 3M3F, 23 y	LHTL	11 h/d, 18 consecutive d	6 d 2500 m 6 d 3000 m 6 d 3500 m	↑ EPO & Soluble transferrin receptor response. ⇆ Reticulocytes or any haematological parameters.
	As above, 2M3F, 21 y	C	Normoxic placebo	1200 m	⇆ $\dot{V}O_{2peak}$, time to exhaustion. No advantage of altitude > 3500 m
Robach et al. (2006b)	Swimmers, 8M1F, 20 y	LHTL	16 h/d, 13 consecutive d	5 d 2500 m 8 d 3000 m	<u>LHTL:</u> ⇆Ret, sEPO, & Soluble transferring receptor; ↑ Red blood cells.
	As above, 8M1F, 17 y	C	No treatment	1200 m	↑ $\dot{V}O_{2peak}$ (not sig). ⇆ 2000 m time. <u>C</u>: ↓ Reticulocytes. ⇆ Performance/ haematological parameters after 2 wks.
Roberts et al. (2003)	Cyclists, 14M5F, 28 y groups not specified	LHTL: 5 d	8 - 10 h/d, 5 consecutive d	2650 m FiO_2: 16.3%	⇆ Groups so data pooled. ↑ Average maximal power output in 4 min, & maximum accumulated oxygen deficit (⇆ C). <u>Both:</u> ↑ $\dot{V}O_{2peak}$
		LHTL: 10 d	8 - 10 h/d, 10 consecutive d	2650 m FiO_2: 16.3%	
		LHTL: 15 d	8 - 10 h/d, 15 consecutive d	2650 m FiO_2: 16.3%	
		C	No treatment	Sea level	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Robertson et al. (2010a)	Elite swimmers, 11M7F, 21 y	Either 10 d of LHTL, or 5 d LHTL + 5 d LMTM for each 'block'. 4 blocks over 21 wks. Real: LMTM	9-10 h/d, 4 X 10 nights	2600 m	Large individual variation, Some physiological adaptation to altitude training but ↓transfer into improved competitive performance. Evidence of responders & non-responders.
Robertson et al. (2010b)	Middle distance and distance runners, 6M2F, 30 y	LHTL	14 h/d, 21 consecutive d; 5 wk wash out, repeated	3000 m	LHTL: ↑$\dot{V}O_{2peak}$ & Hb, ⇆ Time trial response. ⇆ Variability in the altitude blocks in time trial performance (LHTL ⇆C) LHTL: Reproducible ↑in $\dot{V}O_{2peak}$ (2.1%) & Hb (2.8%). ↑ LHTL > C after 1st block & ↑Hb > C in second block.
	As above, 5M3F, 30 y	C	Normoxia	Sea level	
Robertson et al. (2010c)	Middle distance runners, 6M2F	LHTL+H: Treadmill: 1 X moderate, 1 X long, 2X high intensity interval training.	LHTL: 14 h/d; 7 d/wk, 3 wk; IHT: 60 - 75 min/d; 4 d/wk, 3 wk	3000 m (living); 2200 m (IHT)	LHTL + IHT: ↑ $\dot{V}O_{2peak}$, Hb, & 3km time trial but ⇆ after 2 wks. IHT: Only ↑ $\dot{V}O_{2peak}$
	Middle distance runners, 7M2F	IHT: Treadmill: 1 moderate, 1 long, and 2 interval	IHT: 60 - 75 min/d; 4 d/wk, 3 wk	2200 m	
Rodríguez et al. (2000)	Well trained, 8M, 24 y	PHE*	90 min/d, 3d/wk, 3 wk	Started 4000 m, ↑ 500 m each day to 5500 m, 5500 m to end.	↑ All haematological indicators of red cells: packed cell volume, RBC count, reticulocytes, and Hb. ↑ SpO2 from 60 to 78 %

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Rodríguez et al. (2007)	Runners and Swimmers, 5M6F, 2 y	PHE*	3 h/d, 5 d/ wk, 4 wk	Started 4000 m, ↑ 500 m every second day to 5500 m, 5500 m to end	Both: ⇆ Time trial, $\dot{V}O_{2peak}$, ventilation, HR, ventilation, or $\dot{V}O_2$ at ventilatory threshold. ↑ Swimmers. $\dot{V}O_{2peak}$ & $\dot{V}O_2$ at ventilatory threshold
	As above, 6M6F, 23 y	C	As above, normoxic placebo	Normoxic placebo	
Rodriguez et al. (1999)	High altitude expedition members, 1M6F, 28 y	PHE*+IHT*; IHT*: 3 – 5 X 10 – 15 min light cycling (HR 120 - 130) + PHE for remainder	3 -5 h/d, 9 consecutive d	4000 m -5500 m	IHT+PHE ⇆ PHE ↑ Exercise time & maximal pulmonary ventilation during exercise$_{max}$, lactate velocity curved to the right (↑aerobic endurance), ↑ packed cell volume, RBC, Hb concentration & Reticulocytes
	As above, 8M2F, 28 y	PHE*	As above	4000 m -5500 m	
Roels et al. (2007a)	Cyclists and triathletes, 10M, 24 y	IHT: 3 X continuous: 60% $\dot{V}O_{2peak}$ for 1h. 2 X interval	56 - 90 min/d, 5 d/wk, 3 wk	3000 m	Both: ↑ Peak power output, ⇆$\dot{V}O_{2peak}$ In hypoxia: ↑ Peak power output after IHT. ↑ Average power output in the time trial in C
	As above, 8M, 24 y	C	Normoxic training	Sea level	
Roels et al. (2007b)	Cyclists and triathletes, 10M, 24 y	IHT: 3 X continuous: 60% $\dot{V}O_{2peak}$ for 1h. 2 X interval	56 - 90 min/d, 5 d/wk, 3 wk	3000 m	Both: ↑ Peak power output ⇆ $\dot{V}O_{2peak}$ IHT: changed mitochondrial substrate preference (↑ glutamate + malate & ↓ palmitate +malate during maximal ADP stimulated mitochondrial respiration
	As above, 9M, 24 y	C	Normoxic training	Sea level	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Roels et al. (2005)	Cyclists and triathletes, 11M, 22 y	IHT: 2 high intensity interval training sessions/wk	57 - 85 min/d, 2 d/wk, 7 wk	3000 m	Both: ↑ mean power output (not sig) ⇆ after 3 wks. IHT during warm up/ down + recovery: ↑ $\dot{V}O_{2peak}$. ⇆ Haematological variables
	Cyclists and triathletes, 11M, 23 y	IHT: (hypoxia during warm up and cool down but intervals in normoxia)	37 - 52 min/d, 2 d/wk, 7 wk	3000 m	
	Cyclists and triathletes, 11M, 33 y	C	Normoxic training	Sea level	
Saunders et al. (2004)	Elite distance runners, 10M, 25 y	LHTL	9 - 12 h/d, 5 d/wk, 4 w	2000 m -3100 m	LHTL: ↓ $\dot{V}O_{2submax}$, ventilation, respiratory exchange ratio, HR, Hb_{mass}, (not significant).
	As above, 10M, 24 y	LMTM	20 d real altitude	1570 m	
	As above, 13M, 25 y	LLTL	Sea level	Sea level	
Saunders et al. (2009)	Elite middle distance runners, 9 runners, 24 y	LHTL	9 h/d, 5 d/wk, 12 wk	2860 m	LHTL: ↓$\dot{V}O_{2submax}$, ↑Hb_{mass}, ↓HR_{submax} & trivial ↑ $\dot{V}O_{2peak}$
	As above, 9 runners, 27 y	C	Normal residence	Sea level	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Schmitt et al. (2006)	Cross country skiers, 3M3F, 23 y	LHTL	11 h/d, 18 consecutive d	6 d 2500 m 6 d 3000 m 6 d 3500 m	↑ $\dot{V}O_{2peak}$ LHTL & C groups ↑ Peak power LHTL > C. ⇆ $\dot{V}O_{2peak}$ after 15 d LHTL:
	As above, 2M3F, 21 y	C	Sea level	Sea level	↑ peak power output & power at respiratory compensation point
	Swimmers, 8M1F, 20 y	LHTL	16 h/d, 13 consecutive d	5 d 2500 m 8 d 3000 m	
	As above, 8M1F, 17 y	C	Sea level	Sea level	
	Runners, 5M, 24 y	LHTL	14 h/d, 18 consecutive d	6 d 2500 m 12 d 3000 m	
	As above, 6M, 23 y	C	Sea level	Sea level	
Siebenmann et al. (2012)	Road cycling, triathlon, cycle cross, mountain bike, 10 cyclists (15M1F, 29 y overall)	LHTL	16 h/d, 28 consecutive d	3000 m	⇆ $\dot{V}O_{2peak}$ (in normoxia & hypoxia). ⇆ Hb, mean power output & exercise economy in groups
	6 Cyclists	C	Sea level	Sea level	
Tadibi et al. (2007)	Endurance trained athletes 10M, 27 y	IHE	6 min hypoxia alternated with 4 min normoxia for 1 h, for 15 consecutive d	FiO_2: 10 - 11%	⇆ Peak power, mean power, $\dot{V}O_{2peak}$, lactate threshold, HR, ventilation, or submaximal variables.
	As above, 10M, 29 y	C	Normoxic placebo	Normoxic placebo	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Tiollier et al. (2005)	Cross country skiers, 3M3F, 23 y	LHTL	11 h/d, 18 consecutive d	6 d 2500 m 6 d 3000 m, 6 d 3500 m	↓ Secretory immunoglobin A (sIgA) significant in LHTL but not in C LHTL: ↑ Protein concentration negative correlation with sIgA at 3000 & 3500. ⇆ Cortisol
	As above, 2M3F, 21 y	C	Sea level	Sea level	
Townsend et al. (2002)	Cyclists and triathletes, 12M, 27 y	LHTL consecutive	8 - 10 h/d, 20 consecutive d	2650 m	↑ HVR LHTLcontinuous than C ↓$PETCO_2$ in LHTL groups
	Cyclists and triathletes, 10M, 27 y	LHTL interspersed	8 - 10 h/d, 5 d/wk, 4wk	2650 m	
	As above, 11M, 26 y	C	Sea level	Sea level	
Townsend et al. (2005)	Triathletes and cyclists, 11M, 26 y	LHTL continuous	8 - 10 h/d, 20 consecutive d		↑ Submaximal exercise ventilation in both LHTL groups, (not C). HVR correlated with exercise$_{submax}$ ventilation after LHTL
	Triathletes and cyclists, 9M, 26 y	LHTL interspersed	8 - 10 h/d, 5 d/wk, 4wk		
	As above, 11M, 26 y	C	Sea level		
Truijens et al. (2008)	Runners and Swimmers, 6M5F, 22 y	PHE*	3 h/d, 5 d/ wk, 4 wk	4000 m + ↑ 500 m every second day to 5500 m; 5500 m until end	⇆ Exercise economy, HR_{submax}, lactate, ventilation or velocity at VO_{2peak}
	As above, 6M6F, 23 y	C	As above	Normoxic placebo	

Authors	Participant description (cohort, MF, Age)	Group treatment	Frequency (sessions, per week, weeks)	Hypoxic severity	Overall outcome(s)
Truijens et al. (2003)	Swimmers, 3M5F, 29 y	IHT: 3 X High intensity training 3 X low / moderate normoxic training	3 h/d, 5 d/ wk, 4 wk	FiO_2: 15.3%	Both: ↑ 100 & 400 m freestyle; ↑ $\dot{V}O_{2peak}$
	As above, 3M5F, 29 y	C	normoxic training	Normoxic placebo	
Ventura et al. (2003)	Cyclists, 7 participants (11M1F, 25 y, groups not specified)	IHT: 30 min / session, Borg values > 15 & exercise HR recovery > 45 s	30 min/d, 3 d/wk 6 wk	3200 m	⇆ $\dot{V}O_{2peak}$, maximal power output (in hypoxia / normoxia) or hypoxic work capacity. In hypoxia: ↑ SpO_2 & blood lactate in IHT.
	As above, 5 participants	C	As above	560 m	In hypoxia both: ↓ Ferritin in (sig IHT); ↑ reticulocytes
Wood et al. (2006)	Hockey and Soccer players, 15M, 24 y	IHE	6 min hypoxia alternated with 4 min normoxia for 1 h, for 15 consecutive d	3600 – 6000 m	↑ $Speed_{max}$ & sprint speed (↑↑ last sprint), ↓ Lactate concentration, resting & exercising HR. ↑ Performance 9 d later.
	As above, 14M, 23 y	C	normoxic placebo	Sea level	

*: Hypobaric hypoxia; ↑ Increased; ↓ Decreased; ⇆ No change, or no difference; BP: Blood pressure; C: Control group; CO_2: Carbon dioxide; DBP: diastolic blood pressure; EPO: Erythropoeitin; F: Females; FiO_2: Fraction of inspired oxygen; Hb: Haemoglobin; HR: heart rate; HR_{submax}: heart rate measured during submaximal exercise; HRR: heart rate reserve; HDL: High density lipoprotein; HIIT: High intensity interval training; HVR: hypoxic ventilatory response; IHE: passive intermittent hypoxic exposure; IHT: intermittent hypoxic training (exercise in hypoxia); LDL: Low density lipoprotein; LHTL: Live high train low; LMTM: Live moderate train moderate; M: Males; O_2: Oxygen; PHE: Prolonged hypoxic exposure; RPE: Rating of perceived exertion; RBC: Red blood cells; Ret: Reticulocytes; RER: Respiratory exchange ratio; sEPO: Serum erythropoeitin SpO_2: peripheral oxygen saturation; SBP: Systolic blood pressure; SNS: Sympathetic nervous system; $\dot{V}O_2$: oxygen uptake; $\dot{V}O_{2peak}$: peak oxygen uptake; $\dot{V}O_{2submax}$: submaximal exercising oxygen uptake.

Chapter 3

Preface to Study 1: The effect of short term intermittent hypoxic exposure on heart rate variability in a sedentary population

The first study includes both a practical and academic purpose: from a practical point of view, the reasonably small study served to trial the recruitment protocols, participant management systems, testing and IHE and HRV protocols. The second purpose was to address some of the issues identified in the literature review. Specifically, regarding Research Gap I: The effect of IHE on cardiovascular fitness and the parasympathetic and sympathetic responses to simulated altitude; Research Gap II: A detailed assessment of the participant's subjective response to the IHE protocol; and by selecting sedentary, middle-aged participants, Research Gap III will also be addressed. The low cost, non-invasive and time efficient nature of heart rate variability (HRV) assessment made this measurement technique ideal for the purpose of the first study.

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Dr. Kathiravel and Dr. Elliott: Participant medical assessment and ongoing medical advisement. Input during design and feedback on draft documents.

Dr. Hellemans: Assistance with conceptual design, advisement on hypoxic protocol, ongoing medical advisement, feedback on draft documents.

Dr. Hamlin: Main supervisor to Catherine Lizamore, assistance and advisement in all phases of the study, primary feedback on draft documents.

3.1 Abstract

While the effects of instantaneous, single-bout exposure to hypoxia have been well researched, little is known about the heart rate variability (HRV) responses during or as an adaptation to repeated, short-term, high intensity intermittent hypoxic exposure (IHE) in a sedentary population. Resting HRV and exercise capacity was assessed in 16 participants (8 receiving IHE, (IHE) and 8 receiving placebo (C)) before and after a 4-week IHE intervention. Heart rate variability was also measured during an IHE session in the last week of the intervention. Post-intervention, the root mean squared successive difference (rMSSD) increased substantially in IHE (71.6 ± 52.5%, mean change ± 90% confidence limits) compared to C suggesting an increase in parasympathetic activity. However, aside from a likely decrease in submaximal exercise heart rate in the IHE group (-5.0 ± 6.4%) there was little evidence of improved exercise capacity. During the 4-week IHE measurement, there was a decline in parasympathetic activity during the hypoxic exposure (reduced R-R interval: –7.5 ± 3.2%; and rMSSD: -24.7 ± 17.3%), and reactivation during ambient breathing. It is possible that parasympathetic withdrawal and reactivation associated with the repeated hypoxic exposure and ambient air intervals was responsible for the increased rMSSD during rest. In summary, while 4 weeks of IHE is unlikely to improve maximal exercise capacity, it may be a useful means of increasing HRV in people unable to exercise.

Keywords: Autonomic nervous system, sedentary lifestyle, interval hypoxia, simulated altitude, physical fitness, health

3.2 Introduction

Heart rate variability (HRV) is the analysis of the variation in the beat-to-beat intervals in the heart rhythm, which reflects parasympathetic (vagal) and sympathetic actions on the sinus node (Stein and Kleiger, 1999). Typically, a lower resting HRV (or lower variation in RR intervals or cyclic oscillations in the beat-to-beat intervals) is associated with poorer health and cardiovascular disease (Dekker et al., 2000) while an increase in HRV is associated with improved health (Felber Dietrich et al., 2008, Rennie et al., 2003). Based on these associations, the assessment of HRV has provided a reasonably simple, non-invasive and cost effective means of assessing the sympathovagal balance in the autonomic control of the heart.

Heart rate variability is also responsive to the changes in the ANS associated with external stimuli such as real or simulated altitude (Saito et al., 2005). For example, an initially sharp increase in sympathetic activity is observed upon arrival at altitude (Guger et al., 2008, Vigo et al., 2010), which gradually declines through the acclimatization process (Jun et al., 2008, Sevre et al., 2001). The recovery of HRV to baseline levels upon return to normoxic ambient air depends on the length and severity of the initial exposure. That is, after 12 hours of continuous hypoxic exposure, a dampened effect on HRV is still evident an hour after returning to normoxic ambient air (Guger et al., 2008), but after a brief hypoxic exposure of 15 min, recovery is almost immediate (Roche et al., 2002).

While the acute effects of single or continuous hypoxic exposures have been well-documented, the HRV response during repeated bouts of intermittent hypoxic exposure (IHE), such as is used in simulated altitude procedures for athletes (Bonetti and Hopkins, 2009, Hamlin et al., 2009) and non-athletes (Burtsher et al., 2004, Haider et al., 2009) has received little attention. In addition, while IHE has been suggested as a safe means of improving exercise economy (Burtsher et al., 2004) and baroreflex sensitivity (Haider et al., 2009) in participants with cardiovascular disease, little is known about the training effect of IHE on HRV in a sedentary, middle-aged population. The aim of this research was to investigate the instantaneous and adaptive effects of 4 weeks of IHE on HRV in a sedentary, middle-aged population.

3.3 Methods

3.3.1 Participants and overview of the experimental procedure

Relatively inactive individuals who participated in less than 30 min of physical activity on most days of the week (Garber et al., 2011), who were generally healthy, aged between 45 – 60 years were eligible to participate. Exclusion criteria included: uncontrolled hypertension or cholesteraemia, any condition where S_pO_2 is already compromised, melanoma, pregnancy, or by their own or the screening physician's discretion.

Following a health assessment, all participants completed a baseline assessment during which HRV, BP and fitness were assessed. The 16 participants were then randomly selected into either a group receiving IHE treatments (IHE) (n=8; 5 females, 3 males; age: 56.5 ± 5.50 years; height: 171.0 ± 10.1 cm; weight: 82.3 ± 13.5 kg; body fat: 33.5 ± 4.5%; mean ± s) or a placebo (C) (n=8; 6 females, 2 males; age: 56.1 ± 5.1 years; height: 164.6 ± 8.4 cm; weight: 81.3 ± 27.2 kg; body fat: 35.2 ± 12.9%). During week 4 of the intervention, HRV was measured during the IHE and placebo treatments in order to measure the effects of hypoxia and the normoxic placebo against ambient air conditions. The week 4 measurements were not compared to baseline or post-intervention recordings as the recording durations and conditions were different. Following the conclusion of the 4-week intervention, a post-intervention assessment was conducted where the measurements taken at the baseline assessment were repeated. (See Figure 5 for a visual representation of the testing procedure). Participants remained blind to their groups throughout the study. All participants signed a written informed consent prior to participation. This study was approved by the local University Human Ethics Committee.

3.3.2 Baseline and post-intervention testing

Quantification of physical activity at baseline and post-intervention was completed using the International Physical Activity Questionnaire, long-format (IPAQ-LF), and a 4–day diary detailing nutritional intake, sleep, mood, medicine and physical activity. Participants were asked to replicate these on subsequent testing days. At baseline, participants were familiarised with the testing equipment and procedures, and anthropometric data were collected including body composition and weight (InBody230, Biospace Co. Ltd., Seoul, South Korea), height and age. An overview of the full experimental procedure is outlined in Figure 5.

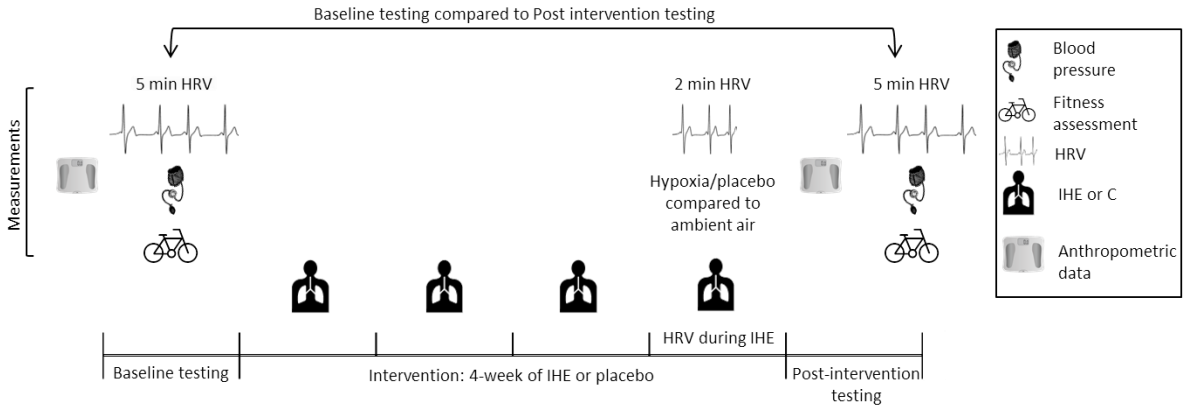

Figure 5: Experimental procedure including Baseline and Post-intervention, and the Week 4 HRV assessment

Heart rate variabilty.

Resting heart rate (HR), HRV and systolic blood pressure (SBP) were measured for 5 min following 5 min of supine rest in a quiet, semi-dark room. Heart rate variability was detected using a heart rate monitor (RS800CX, Polar Electro Oy, Kempele, Finland) and belt (Wearlink W.I.N.D, Polar Electro Oy, Kempele, Finland). The detection of RR intervals using similar Polar heart rate technology has been validated against 12-lead ECGs (Porto and Junqueira Jr, 2009). Following detection of the RR intervals, data were filtered and analysed using Polar software (Polar ProTrainer 5, Version 5.41.000) with the default settings of moderate filter power and minimum protection zone of 6 beat·min^{-1}. Data were then visually inspected and any remaining 'spikes' in the HR trace were removed and replaced with the next appropriate RR interval. Finally, some researchers have reported uncertainty regarding the ability of the Polar ProTrainer5 software's error correction function to detect non-sinus originating beats, which can result in inflated results (Wallén et al., 2012). Therefore, all data were screened for inflated values using the Median Absolute Deviation (Wilcox, 2010) method for outlier detection. Participants with greater or less than 2.5 x the population standard deviation as estimated by the median (Leys et al., 2013) were excluded from the dataset. The data were all log transformed prior to analysis to reduce skewness associated with HRV measurements and ensure a normal distribution for the statistical analyses.

The root mean square of successive differences in the RR interval (rMSSD) provides an indication of vagal stimulation from short term recordings, and is more resistant to breathing artefact than alternative analyses (Penttilä et al., 2001). Therefore, the rMSSD was used as the primary indicator of parasympathetic alteration. However, in order to present a more complete picture of any potential change in HRV, the standard deviation of normal to normal intervals (SDNN), as well as alterations in the high (HF, 0.15 – 0.4 Hz) and low frequency (LF, 0.04 – 0.15 Hz) bands, which were generated using autoregressive modelling in the respective bandwidths, are also reported.

The analysis of HRV can be unreliable during exercise due to non-neural influences associated with increased respiratory effort (Sandercock and Brodie, 2006), therefore no exercise-related HRV was analysed. Additionally, meaningfulness of the very low and ultra-low frequency in short term (5 min) HRV analysis is unclear (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996), and therefore these measurements have not been included.

Two participants in the IHE group were excluded from the resting HRV analyses due to equipment malfunction (n = 1), and an rMSSD outlier (n = 1).

Blood pressure.

Blood pressure was measured continuously throughout rest and exercise using plethysmography to detect brachial arterial pressure in the finger (MLE1054-V Finometer MIDI, Finapres Medical Systems, The Netherlands). Data were recorded using BeatScope Easy® software (Finapres Medical Systems, The Netherlands). The average of the last 5 min of the 10 min rest period was recorded as resting blood pressure.

Oxygen consumption during rest and exercise.

Respiration gases were collected through a facemask that allowed simultaneous respiration via the nose and mouth (Hans Rudolph, Kansas City, MO, USA), and measured using a breath-by-breath analysis system (MetaMax® 3B; Cortex Biophysik, Leipzig, Germany). Prior to each battery of tests, the gas analyser was calibrated against 15% oxygen and 5% carbon dioxide. Volume was calibrated daily (Hans Rudolph 5530 3 L syringe; Kansas City, MO, USA).

A modified submaximal Astrand protocol (Siconolfi et al., 1982) was used to measure submaximal oxygen uptake ($\dot{V}O_{2submax}$) and to predict $\dot{V}O_{2peak}$. Participants started pedalling at 50 rpm on a cycle ergometer starting at a workload of 25 W (Monark Ergomedic 818E, Sweden). Resistance increased by 25 W every 2 min until the participant reached 70% of their age predicted maximum heart rate ((220 – age)*0.7) (Siconolfi et al., 1982). Participants then continued to cycle at the same workload for an additional 2 min, and then until steady state (≤ 5 beat·min^{-1} between consecutive min) was achieved. See Figure 6 for a diagram of the exercise testing protocol.

Breath-by-breath data were exported to an Excel spreadsheet for visual inspection and any outlying data points were removed. The last 5 min of the 10 min resting interval, and the last 30 s of the 2 min 50 W exercise stage were selected, averaged and reported as oxygen consumption per min, per kg of body mass (ml·min^{-1}·kg^{-1}) for resting and $\dot{V}O_{2submax}$ measures respectively. Heart rate and RPE were measured at the end of each min during the 50W stage, and were used to indicate submaximal heart rate (HR_{submax}) and rating of exertion (RPE50w) respectively. The mean of the 70% age-predicted steady state HR recordings and the final workload were used to predict $\dot{V}O_{2peak}$ using the Astrand-Rhyming nomogram and age correction factor. Steady state heart rates of ≤ 112 beat·min^{-1} were excluded from the analysis as their predicted $\dot{V}O_{2peak}$ could not be accurately measured using the Astrand nomogram.

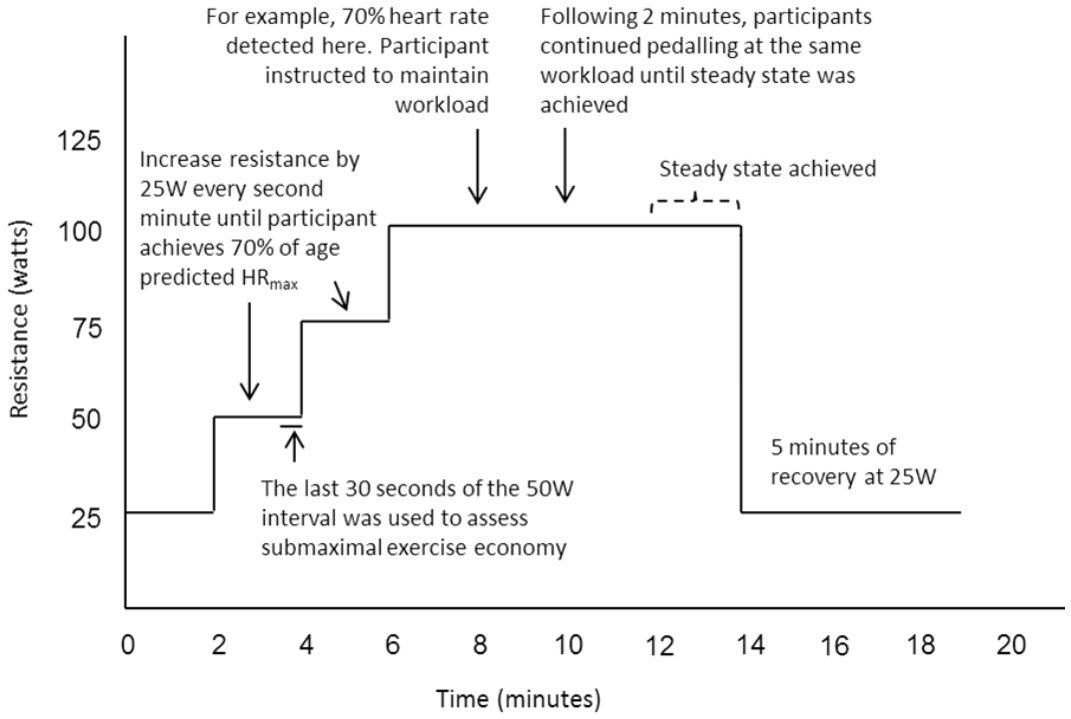

Figure 6: Exercise testing protocol

Intermittent hypoxic exposure.

During the 4-week intervention, participants received six 5-min intervals of hypoxic (or placebo) air delivered through a face mask (QuadraLite Facemask, Intersurgical, Intermed, Auckland, New Zealand) interspersed with six 5-min periods of breathing normoxic ambient air, 4 times per week during seated rest. Hypoxic air (Compressor Twin, GO2Altitude, Biomedtech, Victoria, Australia) was controlled such that: IHE: Week 1: SpO_2 = 95% (fraction of inspired oxygen (FiO2) ~ 0.18); Week 2: 90% (FiO_2 ~0.16); week 3: 85% (FiO_2 ~ 0.13); and Week 4: 80 - 85% (FiO_2 ~ 0.10 – 0.12); C: SpO_2 > 95% in Weeks 1 – 4. The slow progressive decline in SpO_2 in the test group was implemented to improve blinding of the participants in the test group to the hypoxic stimulus. SpO_2 was monitored using finger pulse oximetry (Nonin Pulse Oximeter, Plymouth, Minnesota). During Week 4, HRV and HR were recorded during each participant's intervention session to determine the parasympathetic and sympathetic interactions in response to a full IHE (or placebo) session. While 5 min recordings for the assessment of short-duration oscillations are recommended, we prioritised stationarity of the HR recordings and therefore elected to use the last 2 min of each 5 min hypoxic and normoxic interval where the participant was most likely to have stabilised to the new condition. The average of the 6 hypoxic (or normoxic) intervals and the average of the 6 ambient air intervals were then compared.

Throughout the intervention period participants kept a diary to record any intervention-related comments. Recurring comments between participants were identified and the total number of

referrals to the 'captured' comment was counted, as was the greatest contribution made by one participant. This was done to identify where repetitive comments by one participant could have skewed the data.

3.3.3 Statistical analyses

Statistical analysis was performed using a baseline – post parallel groups spreadsheet designed to compute the magnitude of the smallest worthwhile change between groups using a 90% confidence interval (Hopkins, 2006). The smallest worthwhile change was determined using Cohen's value of 0.2 (i.e. the difference between means is at least greater than the between-participant standard deviation at baseline, divided by 0.2) to indicate that the true effect is at least small. The confidence interval indicates the range of uncertainty of the true value. For a mechanistic inference (in the case of IPAQ-LF, HRV and HR) if the confidence interval overlaps both substantially increased and substantially decreased values, the effect is 'unclear'. In all other cases, the outcome is 'clear' and statement based on the percent chance of the outcomes is given (increased/trivial/decreased). Probabilistic inferences for the qualitative outcome were assigned using the following criteria: <0.5% = most unlikely; 0.5 – 5% = very unlikely; 5 – 25% = Unlikely; 25 – 75% = possibly; 75 – 95% = likely, or 95 – 99.5% = very likely, >99.5% = most likely (Hopkins et al., 2009).

For variables directly affecting health or performance (exercise economy, SBP and predicted $\dot{V}O_{2peak}$), a clinical analysis was performed. In this analysis, if the potential for benefit was acceptable but the risk of harm was too high, the effect is 'unclear'. An odds ratio of benefit: harm was accepted if the ratio was > 66%. In the case of a clear outcome, a statement of the likelihood that the changes were beneficial/ trivial/ harmful was made. In this way, the risk of making a Type 1 clinical error (using a harmful effect) is prioritised over a Type 2 error (not using a beneficial effect).

Data were log transformed prior to analysis to reduce non-uniformity of error. Baseline values were used as a covariate in all analyses to avoid the effect of regression to the mean, and bias associated with uneven baseline averages (Hopkins, 2006). Unless otherwise specified, all results are expressed as mean change (%) ± 90% confidence interval. The raw baseline and post-intervention values, as well as the qualitative outcome and the percent chances that the change has substantially increased/ is trivial/ has substantially decreased (in the case of the mechanistic analyses) or is beneficial/ trivial/ harmful (in the case of clinical analyses) are reported in Table 7.

3.4 Results

Raw data are presented in Table 7.

3.4.1 Differences between baseline and post-intervention measurements

Resting HR in the IHE group decreased compared to the C group, (-10.4 ± 8.7%, mean change ± 90% confidence interval) and the RR interval increased (7.8 ± 8.1%) when Post-intervention data were compared to baseline values. (However, when looking at the raw data, there was only a 1 beat.min^{-1} decrease in resting HR in the IHE group, compared to a 2 beat.min^{-1} increase in the C group. Therefore, the statistically worthwhile effect in this measure may have limited practical value.) Additionally, markers of parasympathetic contribution to HRV increased substantially in the IHE group compared to the C group (rMSSD: 71.6 ± 52.5% (See Figure 7); and HF: 242.1 ± 147.2%) following the 4 weeks of IHE treatment. There were no clear changes in SDNN (11.8 ± 41.9%), LF (-7.1 ± 127.4%), SBP (-3.4 ± 8.4%) or resting oxygen consumption (-0.7 ± 16.9%) between the groups following the 4-week intervention.

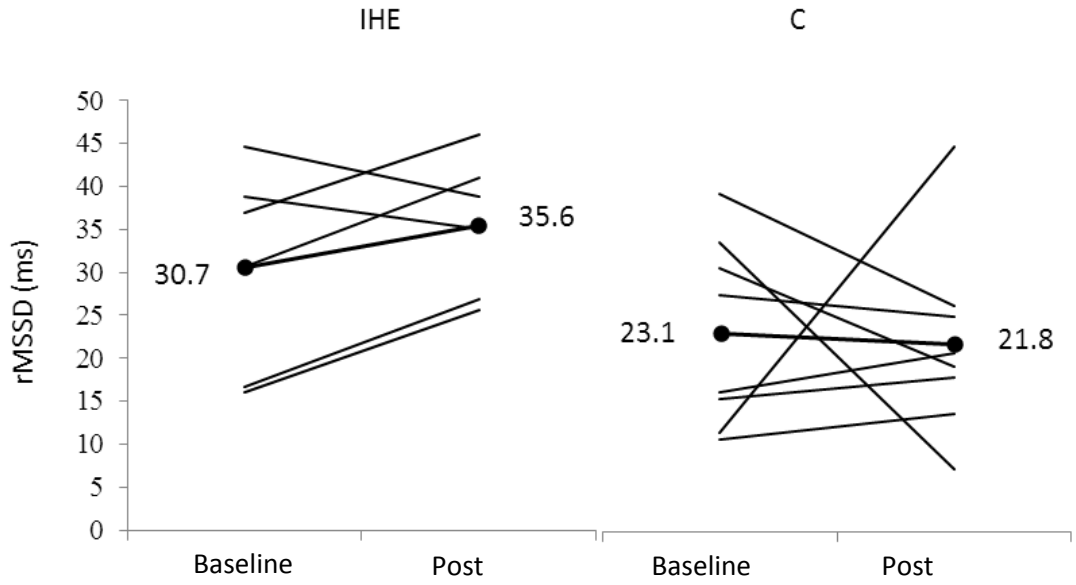

Figure 7: Root mean square of successive differences (rMSSD) at baseline and post-intervention
Thin lines indicate individual responses; thicker lines indicate the group average (which is also indicated numerically on either end of the mean line). IHE: group receiving hypoxic air during intermittent hypoxic treatments; C: Control Group receiving normoxic (placebo) air during intermittent hypoxic treatment; rMSSD: root mean square successive difference.

One participant in the IHE group was excluded from all exercising oxygen consumption measurements due to missing post-intervention data. All participants completed the 50 W submaximal stage. There were no clear changes in $\dot{V}O_{2submax}$ (-0.1 ± 13.9%) or RPE_{50W} (-5.0 ± 13.0%) between groups at a 50 W workload. However, the IHE group demonstrated a likely decrease in HR_{submax} (-5.0 ± 6.4%) compared to the C group. Two participants in each group were excluded from the peak oxygen uptake ($\dot{V}O_{2peak}$) predictions as their steady state heart rates were less than 112

beats·min^{-1}. Changes in estimated $\dot{V}O_{2peak}$ between groups as a result of IHE training were trivial (0.8 ± 7.0%).

3.4.2 Week 4 assessment of 2 min HRV during IHE or placebo treatments.

During the hypoxic exposure there was a decrease in rMSSD (30.0 ± 2.6 ms to 25.4 ± 1.8 ms) and the RR-interval (976 ± 20 ms to 883 ± 16 ms), and an increase in HR (62.4 ± 1.5 beat·min^{-1} to 68.2 ± 1.3 beat·min^{-1}) compared to ambient air. The C group remained relatively stable when alternating between placebo and ambient air (rMSSD: 18.2 ± 1.5 ms to 20.3 ± 2.1 ms; RR-interval: 970 ± 16 ms to 950 ± 35 ms; HR: 62.4 ± 1.2 beat·min^{-1} to 63.6 ± 2.6 beat·min^{-1}). When the hypoxic interval was compared to the placebo, there was a very likely decrease in rMSSD (-24.7 ± 17.3%, 1/1/98, mean change (%) ± 90% confidence interval, % chances of increase/trivial/decrease) and RR-interval: -7.5 ± 3.2%, 0/0/100) and a most likely increase in HR (8.8 ± 3.4%, 100/0/0), see Figure 8.

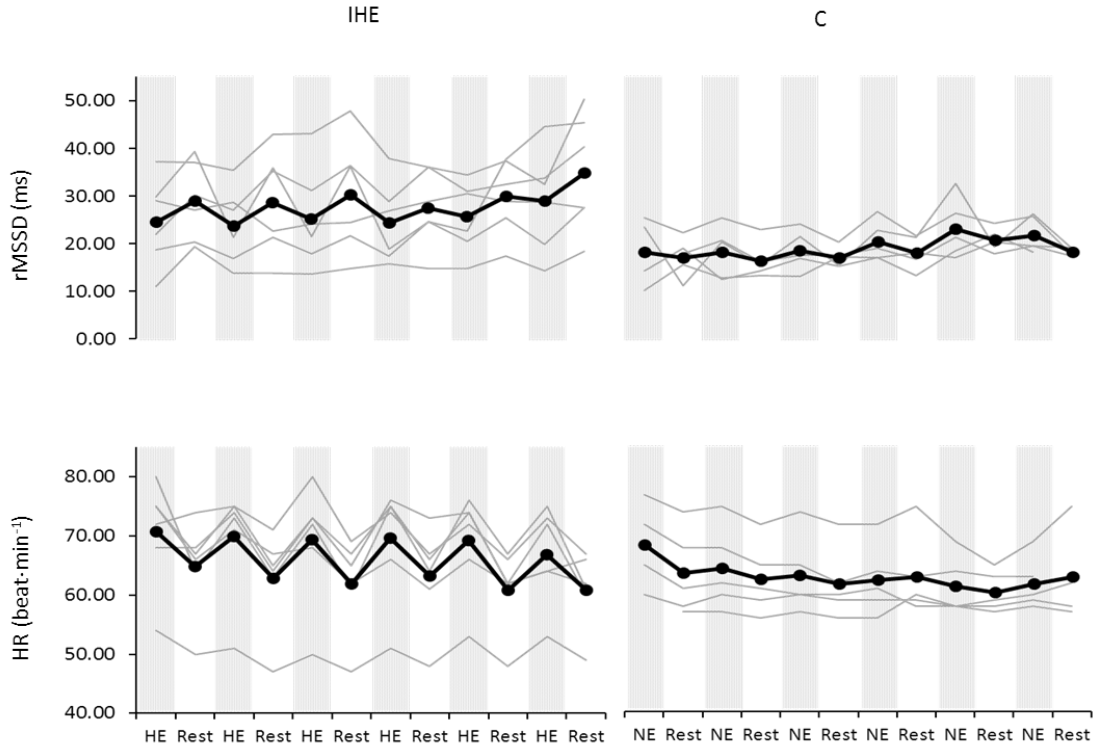

Figure 8: Heart rate and rMSSD responses during a typical Week 4 IHE session.
IHE: group receiving hypoxic air during intermittent hypoxic treatments; C: Group receiving normoxic (placebo) air during intermittent hypoxic treatment. rMSSD: Root mean square successive difference; HR: Heart rate; HE: 5-min hypoxic exposure interval; Rest: 5-min ambient air interval; NE: 5-min normoxic (placebo) interval. Shaded bars indicate the hypoxic or placebo intervals. Thin grey lines indicate individual participant results, whereas the thicker black lines indicate the group mean.

Table 7: Raw Baseline and post-intervention resting and exercising data, including mechanistic and clinical outcomes

	IHE baseline	IHE post	C baseline	C post	Qualitative outcome
Rest					
Mechanistic variables					
HR (beat·min^{-1})	59.2 ± 7.4	57.8 ± 6.2	66.7 ± 9.8	67.4 ± 8.2	Likely decreased (1/6/94)
RR-interval (ms)	1029 ± 135	1049 ± 118	955 ± 164	921 ± 116	Likely increased (83/16/2)
rMSSD (ms)	30.7 ± 11.9	35.6 ± 8.1	23.1 ± 10.9	21.8 ± 11.0	Very likely increased (95/3/1)
SDNN (ms)	39.2 ± 11.0	45.0 ± 13.5	30.0 ± 11.0	35.1 ± 15.9	Unclear (56/27/17)
HF (ms^2)	325.2 ± 263.5	467.6 ± 242.7	234.1 ± 150.7	235.6 ± 255.4	Very likely increased (97/2/1)
LF (ms^2)	251.3 ± 87.8	380.7 ± 167.8	355.1 ± 261.2	492.1 ± 491.7	Unclear (25/39/36)
Clinical variables					
SBP (mmHg)	120.7 ± 19.5	122.6 ± 21.0	109.4 ± 12.7	117.0 ± 11.8	Unclear (9/37/54)
$\dot{V}O_{2rest}$ (ml·min^{-1}·kg^{-1})	3.1 ± 1.1	3.3 ± 0.5	2.9 ± 0.7	3.4 ± 0.7	Unclear (24/46/30)
Fat mass (kg)	27.5 ± 5.3	28.7 ± 7	31.1 ± 21.2	31.4 ± 21.0	Very likely trivial (1/99/0)
Muscle mass (kg)	30.7 ± 6.6	30.2 ± 5.9	28.1 ± 5.3	28.1 ± 28.1	Very likely trivial (1/95/3)
Exercise					
$\dot{V}O_{2submax}$ (ml·min^{-1}·kg^{-1})	10.0 ± 1.8	11.0 ± 1.7	11.3 ± 1.8	12.1 ± 2.2	Unclear (31/38/32)
HR_{submax}	90.3 ± 8.7	86.3 ± 7.9	90.8 ± 14.0	91.0 ± 12.7	Likely decreased (2/23/75)
RPE_{50W}	10.5 ± 1.3	10.0 ± 1.3	10.0 ± 1.9	10.3 ± 1.6	Unclear (12/29/60)
Predicted $\dot{V}O_{2peak}$ (ml·min^{-1}·kg^{-1})	23.6 ± 2.5	23.5 ± 2.6	25.0 ± 10.0	24.9 ± 10.8	Likely trivial (9/86/5)

Data are mean ± SD, outcomes are expressed as: % chance of increase/trivial/decrease for mechanistic variables, and % chances of beneficial/trivial/harmful in clinical variables. IHE: Intermittent hypoxic exposure group; C: control group; baseline: recording prior to intervention; Post; 1 – 3 days post-intervention; HR: Heart rate; RR- interval: Average interval between R to R peaks in the HR trace; rMSSD: Root mean square successive difference; SDNN: Standard deviation of normal to normal R-peak intervals; HF: high frequency; LF: low frequency; SBP: Resting Systolic blood pressure; $\dot{V}O_{2rest}$: Oxygen uptake during rest; $\dot{V}O_{2submax}$: oxygen uptake during the last 30 s of the 50W interval; HR_{submax}: average heart rate taken at the end of each min during the 50W exercise interval; RPE_{50W}: average rating of perceived exertion taken that the end of the 50W exercise interval; predicted $\dot{V}O_{2peak}$: peak oxygen uptake predicted using Astrand nomogram

The most frequently reported comments relating to the IHE sessions were “fine” and “tired”. More participants in Hyp than C reported breathing discomfort (usually regarding an absence of “enough air”) or headaches (Figure 9).

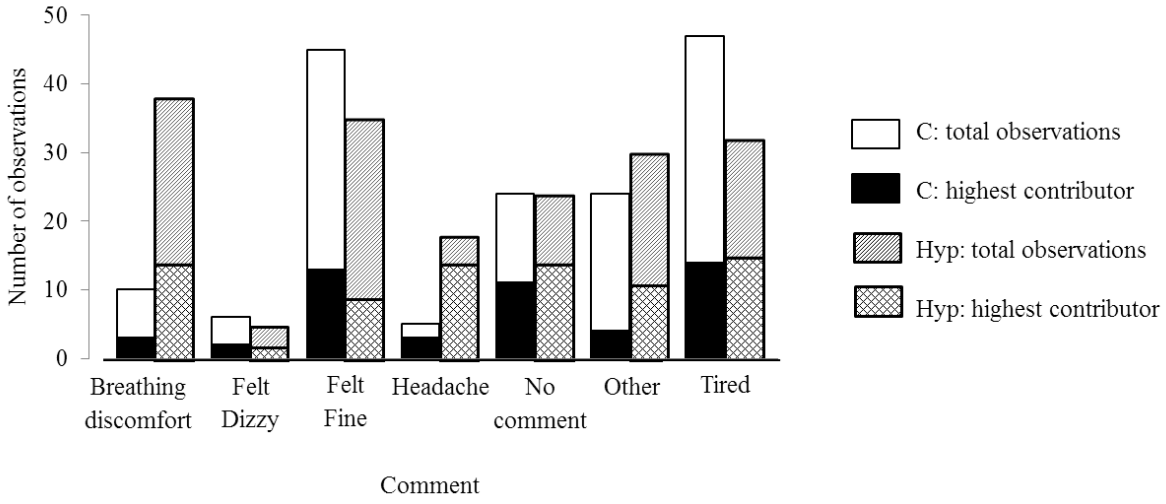

Figure 9: Frequencies of recurring comments from participants regarding the hypoxic/normoxic exposure recorded in notebooks over the course of the 4-week intervention period.
Black and white bars on the left represent proportions from the control group, while lined and crossed bars on the right represent proportions from the hypoxic group. As these bars indicate the total number of times the comment was made over the 4-week intervention, irrespective of whether it was from 1 person repeatedly, or many people once, the value of the highest contribution by one person has been indicated (black and crossed sections respectively). C: control group receiving normoxic (placebo) air during intermittent hypoxic treatments. Hyp: group receiving hypoxic air during intermittent hypoxic exposure

There were no clear changes in physical activity (total METS) between groups over the course of the study. Food and activity diaries indicated that participants had not deviated substantially in their day-to-day nutrition, medication or physical activity habits.

3.5 Discussion

The results of this study indicate that although the typical response to breathing hypoxic air is associated with a decrease in HRV, the subsequent adaptation following 4 weeks of IHE is associated with increased HRV. As lower HRV is associated with impaired health (Stein and Kleiger, 1999), the results of this study would indicate an improvement in parasympathetic activity during rest and possibly improved health in the IHE group. While some studies have invesigated the immediate response of hypoxic intervals on HRV in a young healthy population (Wadhwa et al., 2008), to our knowedge, this is the first study to look at both the instantaneous and adaptive response of the ANS to repeated hypoxic exposure intervals in a sedentary, middle-aged population.

The overall increase in resting HRV following 4 weeks of IHE (post-intervention compared to the baseline measurement) is possibly the result of an adaptive response to the sympathetic and parasympathetic alterations observed during the Week 4 hypoxic intervals. That is, during the hypoxic intervals there was a decrease in rMSSD and an increase in HR suggesting parasypathetic withdrawal and an increase in the proportion of sympathetic activity. This was followed by an increase in rMSSD and a decrease in HR with return to ambient air, suggesting sympathetic withdrawal and the return of parasympathetic activity. While a substantial vagal withdrawal during hypoxic exposure has been noted in several studies (Guger et al., 2008, Kanai et al., 2001, Koelwyn et al., 2013, Saito et al., 2005), there is only one other study (to our knowledge) that has investigated the actions of repeated hypoxic intervals on HRV. Wadhwa et al. (2008) monitored the interaction between hypoxic and normoxic interventions between eight 4-min hypoxic intervals separated by a 5-min normoxic recovery in young males and females (24 years). As in our study, Wadhwa et al. (2008) reported a decrease in parasympathetic activity during hypoxia. However, Wadhwa and colleagues also observed a progressively greater decline in parasympathetic contribution with sucessive hypoxic intervals. We did not detect a similar progressive decline with sucessive hypoxic exposures, but the inter-individual variation in the IHE group in our study was high, and therefore we may have missed finer HRV patterns. Alternatively, fitter individuals have reported greater sensitivity to hypoxia (Woorons et al., 2007), which may infer that although participants in our study and in Wadwha et al. (2008) received a similar hypoxic dosage, the younger cohort may have reacted more severely to the hypoxic dosage. Finally, aside from a brief familiarization the day before the data collection, participants in Wadhwa and colleagues's study were not experienced with the hypoxic intervention. Therefore, it is possible that the 3 weeks of hypoxic treatments prior to the Week 4 measurement could have inferred a level of resistance to the hypoxic stimulus. Indeed, in a similar study in healthy army recruits 2 weeks of IHE resulted in reduced vagal withdrawal during the hypoxic exposure suggesting that repeated, short term hypoxic exposure can develop tolerance to future hypoxic episodes (Bernardi et al., 2001).

Balykin et al. (2004) also reported a reduction in the strain index of autonomic regulation (a reduction in markers of sympathetic activity and an increased parasympathetic activity) in young adult males following 10 IHE sessions, but no comments regarding possible mechanisms for this adaptation were discussed. In contrast, Bernardi and colleagues' (2001) study showed no overall changes in resting HRV between baseline and post-intervention. Unfortunately Balykin et al. (2004) did not specify whether the 10 sessions were on consecutive days, or whether they were spread over 1 month as some of the other groups in their study were. If the 10 IHE sessions were indeed spread over 1 month, it is possible that the absence in a change in HRV at post-intervention in Bernardi et al.'s (2001) study could be due to the comparatively shorter short 2-week intervention.

There were no worthwhile changes in SDNN or LF in the post-intervention compared to the baseline in the IHE compared to the C group. As the LF reflects both sympathetic and parasympathetic activity (and is therefore difficult to interpret), and the SDNN is an assessment of all long- and short-term HRV components (which may be limited by the 5 min recording), conclusions drawn from these variables are limited.

While we did not detect any improvement in estimated $\dot{V}O_{2peak}$ or $\dot{V}O_{2submax}$, we did notice lower HR during submaximal (50 W) exercise. This finding is supported by Burtscher et al. (2009) who, in addition to improved $HR_{submax,}$ also reported significantly lower SBP, HR, and RPE in the group receiving IHE following his 3-week intervention. The greater improvement in $\dot{V}O_{2submax}$ in Burtscher et al.'s (2009) participants compared to our participants may be attributed to the lower baseline health of the individuals in their study. Similarly to Burtscher et al. (2009), despite the improvement in HR_{submax}, we did not detect any change in $\dot{V}O_{2submax}$ between the groups. These findings suggest that IHE seems to improve submaximal HR but has an unclear effect on oxygen efficiency. The decreased HR_{submax} may be associated with the increased parasympathetic activity at rest. Alternatively, haemodynamic adaptation to the hypoxic exposure may account for the reduction in HR_{submax}. For example, Burtscher et al. (2009) detected a significant increase in total haemoglobin (Hb) mass in his participants following 5 IHE sessions per week for 3 weeks. It is possible that an increase in Hb mass in our participants would allow sustained oxygen delivery at a reduced HR_{submax}. However, it would be expected that with improved oxygen-carrying capacity, improvement in both $\dot{V}O_{2submax}$ and $\dot{V}O_{2peak}$ would result (Calbet et al., 2006). Therefore, in our participants, it is possible that the reduced HR_{submax} with no apparent change in $\dot{V}O_{2submax}$ or $\dot{V}O_{2peak}$ is as a result of greater vagal preservation during light or moderate exercise.

There was wide variation in the subjective reponse of the participants to the IHE treatment. For example, while most of the participants tolerated the hypoxic intervals well (see "fine" or "no comment" in Figure 9), a number of the Hyp group detailed mild side effects during the hypoxic interval (such as having an urge to breath faster or more deeply, see "breathing" in Figure 9). One participant noted headaches, chest pain and blurry vision during the hypoxic exposure interval (note the much higher "highest contributor" portion in the "headache" and "other" bars in Figure 9). On the advice of the physician, the participant in question stopped the hypoxic interval at the onset of any of these symptoms and only continued with the IHE session following full recovery, and if so desired by the participant. On all occasions, the symptoms abated following a short return to ambient air and the participant was happy to continue the session. The session was continued at a previously well-tolerated F_iO_2.

The absense in any meaningful changes in body composition supports the absence of a change in body composition noted by Balykin et al. (2004). It is likely that the frequency and the duration of the hypoxic stimulus would need to be greater than the mild dose administered to participants in this study before any changes in body composition were to be observed.

3.5.1 Limitations

The measurement of rMSSD reflects vagal modulation, which is largely related to the respiratory sinus arrhythmia and, therefore, can be influenced by breathing frequency (Hirsch and Bishop, 1981). Given that there can be substantial individual variation in the hypoxic ventilatory response (Hirshman et al., 1975), the change in breathing frequency with hypoxic exposure may be responsible for the decrease in rMSSD. However, while Penttilä et al. (2001) did note a decrease in rMSSD with increased breathing frequency, the change in rMSSD was not statistically significant compared to paced breathing conditions. Therefore the decrease in rMSSD during hypoxic exposure in our study is likely to predominantly reflect vagal withdrawal, rather than change in respiration.

There was reasonably high intra-individual variation in the HRV measurements, particularly in the control group, which suggest that some individuals decreased their HRV following the intervention period. The higher level of intra-individual variation in the control group suggests that a high level of day-to-day variation in this measurement and that cautiousness would be appropriate when drawing meaning from the present findings.

The small sample sizes (Hyp: n = 8, and C: n = 8), and the necessity to exclude some of the data due to erronous or incomplete datasets increase the risk of Type 1 errors, and reduce the extent to which these results can be applied to other populations. Therefore, caution should be used when interpreting these results until the findings can be repeated in a larger population.

Participants were encouraged to be as inclusive as possible when using their comment books, but this notetaking was not monitored. Consequentially, some participants may have recorded data unrelated to the hypoxic exposure, while others neglected to complete the comment books altogether. A focus group debriefing session or individual open-ended interview would provide more structured feedback.

3.6 Conclusion

Four IHE sessions per week for 4 weeks had a positive response on the parasympathetic contribution to HRV in sedentary, middle-aged adults, and lowered their submaximal exercising HR. However, the IHE intervention did not demonstrate any beneficial effect on aerobic efficiency or capacity as there were no substantial changes in $\dot{V}O_{2submax}$ or estimated $\dot{V}O_{2peak}$ in the Hyp group. If these findings are confirmed in a larger cohort, IHE may prove to be a useful technique for improving HRV in people who are unable to exercise, such as those confined to bed rest, but 4 IHE sessions per week are unlikely to improve oxygen uptake characteristics in a sedentary middle-aged population. Our study demonstrated a high level of variation in the participant's physiological and subjective response to the hypoxic exposure session which may suggest that some participants may have adapted better to hypoxic exposure than others.

Chapter 4

Preface to Study 2: The minimum dosage of intermittent hypoxic exposure per week for health improvement in sedentary, middle-aged individuals.

The results from the previous study indicated a beneficial effect of IHE on HRV. However, changes in predicted $\dot{V}O_{2peak}$ and rating of perceived exertion were unclear. There are two reasons for the absence in any change in cardiovascular fitness (aside from lower submaximal exercising heart rate). The first reason could be associated with the choice of measurement techniques. That is, the estimated $\dot{V}O_{2peak}$ may not have been sensitive enough to detect subtle improvements in cardiovascular function. In addition, there are also inherent inaccuracies with estimation of $\dot{V}O_{2peak}$ compared to maximal testing. The following studies have, accordingly, used a maximal (contraindication-free) fitness assessment in the assessment of cardiovascular fitness. Furthermore, a more sensitive cardiovascular risk factor (highly sensitive C-reactive protein) has been included. The second reason could lie with the treatment. In the 4-week intervention, only the last 2 weeks were designed to yield an oxygen saturation below 90% which may have been insufficient time for meaningful HIF-1α related adaptations to have taken place. The following study has been designed as a 5-week intervention to allow further time for adaptation. Finally, this study seeks, primarily, to address Research Gap IV: the effect of different frequencies of IHE per week on cardiovascular risk factors. And, as a sedentary middle-aged population were recruited for this study, Research Gap III is also touched on.

Authorship:

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Dr. Kathiravel and Dr. Elliott: Participant medical assessment and ongoing medical advisement. Input during design and feedback on draft documents.

Dr. Hellemans: Assistance with conceptual design, advisement on hypoxic protocol, ongoing medical advisement, feedback on draft documents.

Dr. Hamlin: Main supervisor to Catherine Lizamore, assistance and advisement in all phases of the study, primary feedback on draft documents.

4.1 Abstract

Objectives: Determine the effect of different frequencies of IHE per week on novel (HRV and hs-CRP) and traditional (fitness and systolic blood pressure) cardiovascular risk factors in a sedentary population. **Method:** Twenty nine untrained participants (12 male; aged: 55.9 ± 5.0; BMI: 28.0 ± 4.9, mean ± SD) were randomly allocated to groups receiving intermittent hypoxic exposure (IHE) treatments 2-3 times/week (IHE3); IHE treatments 5 times/week (IHE5) or a control group receiving normoxic placebo 2-3 times/week (C). Prior to the fitness assessments, highly sensitive C-Reactive Protein (hs-CRP) and Haemoglobin concentration (Hb) were collected. During baseline and post-intervention assessments, participants rested for 16 minutes in a quiet, semi-dark room. Between minutes 5 – 10 heart rate variability (HRV) was recorded under spontaneous breathing conditions. The participants then breathed in time to a metronome set to 12 breathes per minute for the last 6 minutes while HRV was recorded under controlled respiration. The average of 2 systolic blood pressure (SBP) measurements (immediately following spontaneous and controlled breathing periods respectively) were recorded as resting SBP. Following the resting measurements, participants completed a modified Astrand protocol on a stationary bicycle. Participants pedalled at a set cadence of 50rpm, while resistance increased every 2 minutes. At 40 – 50% heart rate reserve, participants maintained the workload for 6 minutes while submaximal oxygen uptake ($\dot{V}O_{2submax}$) was measured. The protocol was then resumed and resistance increased every 2 minutes until a pre-defined cut off criteria was reached. The time between the steady state exercise and the maximal exertion ($time_{ex}$), the maximum resistance achieved for a minimum of 30 seconds (res_{max}), and maximal oxygen uptake ($\dot{V}O_{2peak}$) were measured. During 5 weeks of treatment, IHE participants breathed hypoxic air through a nose and mouth mask connected to hypoxicators to slowly reduce peripheral blood oxygen saturation (S_pO_2) from > 95 % in week 1 to 80 % in weeks 4 – 5. The control participants breathed normoxic placebo air through identical nose and mouth masks also connected to the hypoxicators, but such that week 1 - 5: S_pO_2 > 95 %. All sessions included alternating intervals of 5 minutes hypoxia /placebo with 5 minutes of ambient air intervals for 1 hour while seated at rest. Magnitude based inferences were used to analyse the data for practical significance, using the mean change ± 90 % confidence interval. **Results:** Following 5 weeks of IHE, participants in the IHE5 group demonstrated improved rMSSD under both spontaneous and controlled breathing conditions (36.7 $\pm$ 42.7% and 47.3 $\pm$ 41.6% respectively, mean change $\pm$ 90% confidence interval) but the RR-interval was only likely increased under controlled breathing (6.2 $\pm$ 6.9%). The IHE3 group demonstrated no clear changes in HRV parameters under spontaneous breathing, but both RR-interval (4.6 $\pm$ 5.7%) and rMSSD (30.3 $\pm$ 39.2%) were increased when breathing was controlled at 12 breaths per minute. Regarding hs-CRP, there were no clear change IHE3 (7.6 $\pm$ 58.6%) following the intervention.

However, the IHE5 group demonstrated a possibly trivial increase in the inflammation marker (12.7 ± 48.7%).

The participants in the IHE5 group improved their $time_{ex}$ (34.5 ± 36.8%) and their res_{max} (14.4 ± 14.9%) compared to the control group, and despite an increase in Hb (2.7 ± 2.8%) there were only trivial changes in $\dot{V}O_{2submax}$ (0.0 ± 8.0%) and no clear changes in $\dot{V}O_{2peak}$ in this group. Conversely, the IHE3 group demonstrated no clear change in $time_{ex}$ or res_{ex} but did demonstrate a likely increase in both $\dot{V}O_{2submax}$ (5.5 ± 6.8%) and $\dot{V}O_{2peak}$ (12.6 ± 9.3%), despite no clear change in Hb.

Both IHE3 and IHE5 demonstrated possibly decreased SBP (IHE3: -5.4 ± 8.1 %; IHE5: -4.0 ± 7.0 %) at rest compared to the control group, however the clinical outcome was unclear for both groups.

Conclusions: Attending 5 IHE sessions per week for 5 weeks resulted in greater benefit in novel and traditional risk factors (primarily regarding improved exercise tolerance and HRV). However, participants with already high hs-CRP should be restricted to 2 – 3 IHE sessions per week until more research is conducted concerning the effects of IHE on inflammation. Interestingly, 2 – 3 IHE sessions per week for 5 weeks were also associated with an improvement in $\dot{V}O_{2peak}$, and possibly HRV. IHE may be a useful in preparing inactive participants for exercise, and may reduce some cardiovascular disease risk factors.

Keywords: heart rate variability; intermittent hypoxic exposure; sedentary; heart disease risk factors

4.2 Introduction

Cardiovascular disease has long been a major cause of death and disability worldwide (Kannel et al., 1976, Perk et al., 2012). Given the severity of this disease, a number of atherogenic (such as cholesteraemia, hypertension, glucose intolerance) and lifestyle (physical inactivity, poor diet or cigarette smoking) risk factors for heart disease have been identified (Conen et al., 2009, Chobanian et al., 2003, Myers et al., 2004, Kannel et al., 1976) in the hopes of interrupting the progression of cardiovascular disease (Dzau et al., 2006). More recently, additional risk factors such as reduced heart rate variability (HRV) and elevated highly sensitive C-Reactive protein (hs-CRP) have been highlighted as independent predictors of heart disease and mortality (Rifai and Ridker, 2001, Ridker, 2001, Dekker et al., 2000, Tsuji et al., 1996). An increase in the basal level of hs-CRP, a sensitive marker of inflammation, can indicate the presence of atherosclerosis which is an inflammatory process which results in the formation of atherosclerotic plaques in the arterial wall (Ross, 1999). Therefore, an increase in hs-CRP can indicate reduced arterial health and a higher risk of an atherosclerotic event.

Heart rate variability (HRV) measures the variance in the beat-to-beat intervals of the heart rhythm which provides insight into the sympathovagal balance of the autonomic nervous system (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). An increase in sympathetic activity as indicated by lower HRV is generally associated with poorer health outcomes, while higher HRV is associated with improved autonomic function (either through increased parasympathetic contribution or reduced sympathetic innervation) and improved health (Felber Dietrich et al., 2008).

Evidence suggests that physical activity plays an important role in maintaining lower basal hs-CRP (Lavoie et al., 2010) and higher HRV (Rennie et al., 2003). However, exercise tolerance is often reduced in those at risk for, or with, cardiovascular disease. This can limit the effectiveness of exercise-based interventions. In an attempt to improve exercise tolerance, several researchers have investigated the use of simulated altitude techniques. It is hypothesised that the improved oxygen delivery and stress tolerance inferred by adaptation to the hypoxic stimulus will result in improved exercise tolerance in a population with compromised health (Burtscher et al., 2009, Burtsher et al., 2004, Meerson, 1993). For example, passive intermittent hypoxic exposure (IHE; Haider et al., 2009, Saeed et al., 2012), and hypoxic exposure during exercise, also known as intermittent hypoxic training (IHT; Bailey et al., 2000), have both been used to improve the cardiovascular health of both asymptomatic individuals (Bailey et al., 2000) and patients with cardiovascular disease (Haider et al., 2009, Saeed et al., 2012). Indeed, the results of a recent review of the literature suggest that IHE and IHT interventions demonstrate greater improvements in exercise tolerance in participants with

compromised health, such as those with COPD, compared with healthy participants (Burtscher et al., 2010).

Despite the increasing use of IHE and IHT in both asymptomatic and higher risk participants, little is known about the minimum required frequency of intermittent hypoxic exposure per week for health benefit, and, to date, there has been no guidance on the minimum appropriate length or intensity of each IHE session. For example, while the intervention durations for Saeed et al. (2012) and Haider et al.'s (2009) participants were a similar length (approximately 3 weeks), and both adopted the IHE approach, the severity of the hypoxic exposure intervals were quite different. That is, Saeed et al.'s (2012) participants underwent 10 X 3 – 4 hour continuous hypoxic exposure sessions at a simulated height of 2700 m (S_pO_2 average at Week 4 = 91.1 %), while Haider et al.'s (2009) participants were exposed to 15 X 18 – 40 minute IHE sessions (SpO2 = 80% at Week 4). To date, no research has been conducted investigating the minimum number of IHE sessions per week for improvement in cardiovascular risk factors. Therefore, the aim of this study was to determine the effects of different frequencies of IHE per week (either 2-3 times per week, or 5 times per week) on traditional (physical fitness and systolic blood pressure) and novel (HRV and hs-CRP) risk factors for cardiovascular disease in a sedentary population.

4.3 Methods

4.3.1 Participants.

Forty-nine volunteers from the local communities surrounding the host university responded to the recruitment material. After learning more about the study, 19 decided not to participate, and 1 male participant in the C group withdrew their participation from the study in the third week of the intervention due to the time commitment. Only the data for the 29 participants who completed the study have been reported (see Table 8 for baseline characteristics). All participants were medically assessed prior to enrolment in the study. Participants were sedentary (did not undertake any regular, or planned physical activity) and were free of any medication or illness that could be compromised by the intervention. Prescription to any medication was sufficiently stable such that the dosage was unlikely to change over the course of the intervention. Exclusion criteria included the presence of any melanoma, unstable medical condition, or if the participant was advised against participation by their medical practitioner for any other reason. All participants provided written informed consent. This study was approved by the local University Human Ethics Committee.

4.3.2 Experimental design.

This was a randomized placebo controlled single-blind study. (While participants were aware of how many times per week they were attending sessions, they were unaware of whether or not they were

receiving hypoxic or placebo treatments). All individuals expressing interest in the study were medically assessed prior to inclusion. If the individual was suitable, and had no contra-indications to participation, they proceeded with the baseline blood sample. Participants then underwent a familiarisation session, followed one week later by a maximal baseline assessment. Following the fitness assessment, the participant was then randomly assigned into either a group receiving 5 IHE sessions per week (IHE5, n = 10); 2 - 3 IHE sessions per week (IHE3, n = 10), or 2 - 3 normoxic placebo sessions per week (C, n = 9). The total hypoxic dosage per session was 30 min of hypoxic at a targeted SpO_2 of 80%.

Table 8: Physical characteristics of groups

	C (n=9)	IHE3 (n=10)	IHE5 (n=10)
Age (yr)	54.8 ± 5.4	57.0 ± 5.9	55.7 ± 5.0
Males/females	3M/6F	4M/6F	5M/5F
Body mass (kg)	79.5 ± 11.5	81.6 ± 20.0	76.2 ± 13.7
Height (cm)	168.7 ± 3.5	171.4 ± 10.2	163.7 ± 6.8
BMI ($kg.m^{-2}$)	27.9 ± 4.1	27.6 ± 5.2	28.5 ± 5.6
$\dot{V}O_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	26.8 ± 3.3	23.7 ± 6.0	27.2 ± 7.5
SBP (mmHg)	107.5 ± 17.9	118.8 ± 17.7	111.7 ± 22.8
On permanent medication [†]	6 (66.7%)	7 (70%)	5 (50%)

Values are mean ± SD, except for permanent medication: n (% of group).
[†]Excluding supplements and occasional medication (e.g. for migraines).

All participants completed 5 weeks of the intervention treatment. The post-intervention venous blood sample was collected within 7 days of the final IHE/placebo session, and was taken at any time before or at least 1 day after the post – intervention fitness assessment (this was to ensure an adequate time for re-hydration prior to the blood sample). All measurements were taken at the same time of day ± 2 hours as the baseline assessment to reduce biological variation (Huikuri et al., 1994). Prior to all blood samples and fitness assessments, participants were asked to record their physical activity and dietary intake for the day before and the day of the measurement, and were asked to match their routines and caloric intake prior to each assessment as closely as possible. Participants were also advised to avoid any strenuous exercise, caffeine or alcohol for at least 12 hours prior to their assessments. See Figure 10 for a diagram of the order of testing.

4.3.3 Food and activity monitoring

During the baseline and post-intervention testing weeks, the participants were given a pedometer to wear, and were instructed to log their steps over 3 typical days. The average of the 3 day recordings was used as the baseline and post-intervention record of habitual daily activity. Similarly, participants were asked to record all food and medicine ingested, and to describe any physical activity for 2 days prior to measurement sessions.

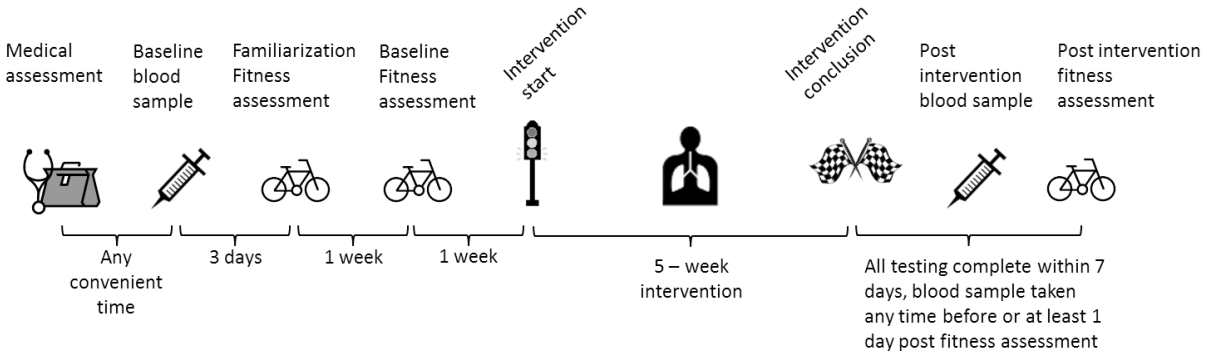

Figure 10: Study timeframe
The familiarisation, baseline and post-intervention fitness assessments included resting anthropometric measurements, resting heart rate, heart rate variability and blood pressure. This was followed by a maximal fitness assessment conducted on a cycle ergometer

4.3.4 Venous blood samples.

All blood samples were taken in a rested state and in a seated position. Samples were drawn from the antecubital vein at a local medical facility by a trained phlebotomist. Venous blood samples were then analysed at an accredited laboratory (Canterbury Health Laboratories, Christchurch). High sensitivity C-reactive protein (hs-CRP) was measured using CardioPhase® hs-CRP (Siemens Healthcare Diagnostics, Marburg, Germany). As hs-CRP is sensitive to inflammation or infection (Chambers et al., 1991), participants were also asked to postpone the blood sample collection if they were feeling unwell or had sustained any injuries within 7 days (Pearson et al., 2003). All hs-CRP measurements >10 gm/L were excluded, as were results consistent with acute inflammation or infection based on the medical doctor's opinion. Each participant's hs-CRP results were classified into risk-stratified quintiles defined by Ridker (2001), (1: 0.1 – 07 gm/L low; 2: 0.7 - 1.1 gm/L mild; 3: 1.2 - 1.9 gm/L moderate; 4: 2 - 3.8 gm/L high; 5: 3.8 – 15 gm/L highest) and then averaged into a group mean to reduce the effects of normal within subject variability and to provide greater risk stratification (Ridker, 2001).

Haemoglobin was also drawn to help explain any exercise-related improvements (or lack thereof) following the intervention. Haemoglobin was estimated using the SLS-Hb method (Sulfolyer reagent system, Sysmex XE-2100, Kobe, Japan). Two participants in C were excluded from the Hb analysis (blood sample taken > 7days after last IHE session (n=1); and no post-intervention measurement (n = 1)) and 1 participant in IHE3 opted out of the blood sample collection.

No participants had hs-CRP > 10 mg/L. However, 3 participants in C, 1 participant in IHE3 and 2 participants in IHE5 were removed from the dataset for reasons unrelated to the study. Reasons included: dentistry or migraines earlier in the week, blood samples taken > 7 days following final IHE session, or missing post intervention samples.

4.3.5 Resting anthropometric and cardiovascular data.

Following explanation of the testing procedure and familiarisation with the equipment, height (Mechanical Stadiometer, Surgical & Medical Products, Mentone, Australia) and body composition (InBody230, Biospace Co. Ltd., Seoul, South Korea) were measured. Resting heart rate and heart rate variability were measured continuously over a 10 minute supine resting period in a semi-dark room (Polar RS800CX and Polar WearLink® Transmitter, Kempele, Finland). Immediately following this 10-minute resting interval, blood pressure (described below) and instantaneous heart rate (HR) were recorded. Participants remained supine and were instructed to breathe in time to a metronome at 12 breaths.min^{-1} for an additional 6 minutes while HRV and HR rate were measured. (Refer to "4.3.7 Heart rate variability analysis" for more detail regarding breathing frequency). No attempt was made to control tidal volume, participants were simply asked to breathe as normally as possible. Systolic blood pressure was measured again at the end of the controlled breathing interval. Throughout the 16-minute resting period, breath by breath analysis of respiration rate and gaseous exchange was monitored using a calibrated metabolic cart (MetaMax®3B, Cortex Biophysik, Leipzig, Germany). The controlled breathing period was later reviewed, and any participant who had a breathing frequency of > 12 ± 0.5 breaths.min^{-1}, or a SD of > 1.0 over the selected 5 minute interval was excluded from the HRV analysis. Based on these criteria, 1 participant in C and 1 participant in IHE3 were excluded from the controlled breathing dataset due to non-conformity with the controlled breathing requirements. An additional 2 participants in IHE3 and 1 participant in IHE5 were excluded from all HRV analyses due to unclear recordings. The last 5 minutes of the 10 minute spontaneous breathing interval and the last 5 minutes of the 6-minute controlled breathing interval were selected for HRV analysis.

4.3.6 Blood pressure.

Resting and exercising systolic blood pressure was measured using a sphygmomanometer coupled with a pressure transducer that converts the applied pressure to an electronic signal (MLT1100/D, ADInstruments, Dunedin, New Zealand). A piezoelectric element was used to detect finger pulse (MLT1010/D Pulse Transducer, ADInstruments, Dunedin, New Zealand). The finger pulse and sphygmomanometer readings were recorded via a data acquisition unit (ML856 PowerLab 26T, Dunedin, New Zealand) and displayed concurrently on graphical recordings (LabChart®, ADInstruments, Dunedin, New Zealand). The blood pressure cuff was manually calibrated before each session. The cuff was inflated until the finger pulse was no longer detected by the Pulse Transducer, and thereafter an additional 20 mmHg was applied. Pressure was slowly released from the cuff, and the SBP was read off the sphygmomanometer graph at the point where the finger pulse returned. This technique was used to avoid bias associated with manual blood pressure recordings, particularly in single-blind studies, and to improve accuracy. To further improve the accuracy of the baseline and post-intervention blood pressure recordings the average of 2 blood pressure readings,

separated by 5 minutes, were taken during the rest interval. The first was taken immediately following the spontaneous breathing interval, and the second was taken immediately following the controlled breathing interval. Controlled breathing has no statistically relevant effect on SBP (Pinna et al., 2006). The average of these values was used as the baseline and post-intervention resting blood pressure.

4.3.7 Heart rate variability analysis.

Heart rate variability was measured during the supine rest period, under spontaneous and controlled-breathing conditions. A breathing frequency of 12 breaths per minute (or 0.2 Hz) was selected for the controlled breathing as this is comfortably within the range of the HF band (0.15 – 0.4 Hz), but may prevent the mild hyperventilation observed by Pinna et al. (2006) when respiration was paced at 15 breaths / minute (or 0.25 Hz). Along with the average time between the R to R peaks (RR interval), the root mean square of successive differences in the R to R peaks (rMSSD) was used as the primary measurement of HRV as it is highly correlated to the high frequency measurement in the spectral domain (Tsuji et al., 1996, Sinnreich et al., 1998) but is more resistant to the effect of alterations in respiration on HRV (Penttilä et al., 2001). However, for greater comparability with other studies, the standard deviation of normal to normal R peak intervals (SDNN) and the high frequency (0.15 – 0.4 Hz) and low frequency (0.04 – 0.15 Hz) in the spectral domain are also reported. The rMSSD and HF are used to estimate the short term components, or parasympathetic modulation of the HRV analysis, while the SDNN represents overall HRV. The interpretation of the LF measurement is more difficult as it is thought to reflect both sympathetic and parasympathetic interactions (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996).

Heart rate variability was detected using a transmitter belt (Wearlink W.I.N.D, Polar Electro Oy, Kempele, Finland) and recorded on a Polar heart rate watch (RS800CX, Polar Electro Oy, Kempele, Finland) set to capture RR interval data within an accuracy of 1 ms. A contact electrode gel (Sigma Crème®, Clectrode cream, Parker Laboratories Inc. Fairfield, New Jersey, USA) was used to optimise the contact between the electrodes of the transmitter belt and the skin. Heart rate data were saved on the watch until it could be uploaded (external IrDA USB 2.0) to the Polar ProTrainer5™ software (Version 5.40.172) onto a laptop. HR data were automatically filtered for ectopic beats using the "error correction" function in the Polar ProTrainer5™ software. Filters were set to the default 'Moderate' filter power and Minimum Protection Zone of 6 beat·min^{-1}. Following the filtering process, any remaining 'spikes' in the data were manually removed prior to analysis. Spontaneous HR data were selected from the last 5 minutes of the 10 minute spontaneous breathing rest period. Data

for the controlled breathing interval were selected using the 5-minute portion of the 6 minute controlled breathing period, starting 30 seconds into the paced breathing.

To avoid including erroneous data in the final dataset, participants with particularly high HRV recordings (which can occur when non-sinus originating beats go undetected by the error correction software (Wallén et al., 2012)) were excluded from the analysis. The median absolute deviation (MAD) method was used to detect outliers (Leys et al., 2013) from the combined groups. This method is far less sensitive to the effect of outliers than protocols relying on the mean $\pm$ SD (Wilcox, 2010). A moderately conservative approach was used (Leys et al., 2013) and all data 2.5 X the SD as estimated from the median were considered outliers and were removed from the dataset. See Table 9 for the number of participant data excluded at each stage of the data filtering process.

Table 9: HRV data filtering process

	Total data collected		
	IHE3: n = 10	IHE5: n = 10	C: n = 9
			
	Excluded due to: **Equipment / measurement error**		
	IHE3: n = 10	IHE5: n = 9	C: n = 8
			
	Excluded due to: **Outlying data (2.5*MAD)**		
Data for Spontaneous breathing dataset:	IHE3: n = 8	IHE5: n = 8	C: n = 8
			
	Excluded due to**: Non-compliance with controlled breathing**		
Data for Controlled breathing dataset:	IHE3: n = 7	IHE5: n = 8	C: n = 7

4.3.8 Fitness assessments.

A modified Astrand protocol performed on a manually calibrated cycle ergometer (Monark Ergomedic 818E, Sweden) was used to estimate oxygen consumption and fitness level during moderate and high intensity exercise during familiarization, baseline and post-intervention assessments. Cadence was displayed between the handle bars of the cycle ergometer and participants were instructed to maintain a cadence of 50 rpm throughout the assessment. Participants initially pedalled at a resistance of 25 W. After 2 minutes, blood pressure and instantaneous heart rate were recorded while participants continued to pedal. Every second minute, resistance was increased by 25W until a target heart rate of 40 – 50% heart rate reserve (HRR), or a rating of perceived exertion (RPE) of 13-14 on the 20-point Borg RPE Scale was achieved. Participants continued for 5 minutes at the targeted 'moderate' intensity workload while heart rate and oxygen consumption were recorded. Following this, the workload increased by 25 W every second minute until one of the following cut off criteria were achieved: HR within 10 beats of age predicted heart rate maximum; RPE of ≥ 19; participant's volitional exhaustion; or an inability to maintain the required cadence (Siconolfi et al., 1982). The test was abandoned if any of the following occurred: SBP ≥ 210 mmHg; cramp; chest pain; dizziness or light headedness; signs of poor perfusion; or any other pain. A recovery interval of 5 minutes pedalling at a workload of 25 W and 50 rpm followed. See Figure 11 for a description of the baseline and post-intervention testing protocols.

Three participants were restricted to submaximal fitness assessments due to high blood pressure (n = 2) and a back injury (n = 1). Submaximal data were reported as an average of all data recorded during the 5 minute moderate intensity interval. A change in submaximal fitness was assessed using the change in submaximal exercise heart rate (HR_{submax}), systolic blood pressure (SBP_{submax}), and oxygen uptake ($\dot{V}O_{2submax}$) in IHE3 and IHE5 compared to C.

Maximal fitness capacity was determined using maximum oxygen consumption ($\dot{V}O_{2peak}$) measured over the last 30 seconds of maximal exertion, the time from the end of the moderate intensity steady state period to maximal exertion ($Time_{ex}$), and the resistance of the last complete minute (Res_{max}). Refer to Figure 11 for a diagrammatic representation of the fitness assessment.

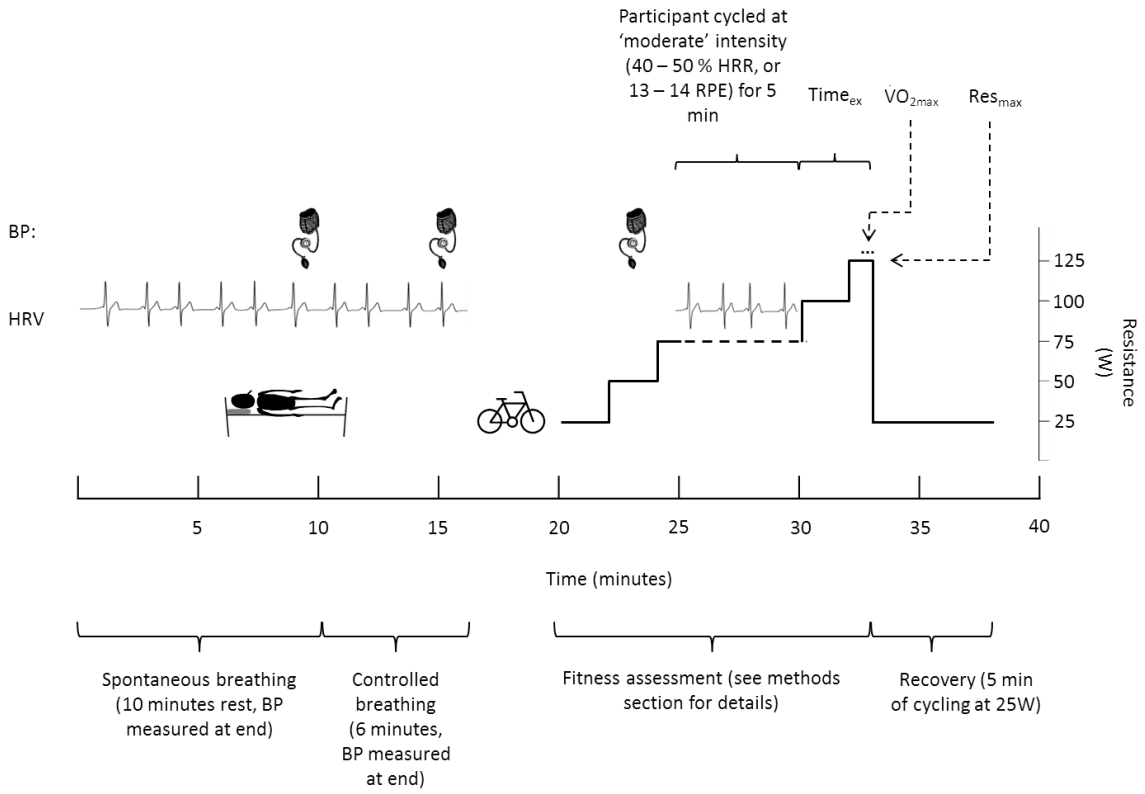

Figure 11: Protocol for baseline and post-intervention fitness assessment

4.3.9 5 week intermittent hypoxic exposure treatment.

Participants received 5 minute intervals of either normoxic placebo air (C) or hypoxic air (IHE3 and IHE5) delivered through their own hand held face mask (QuadraLite Facemask, Intersurgical, Intermed, Auckland, New Zealand) fitted with an antibacterial filter (HMEF Clear-therm, Intersurgical, Intermed, Auckland, New Zealand) which was connected to a Hypoxicator System (GO2Altitude, Biomedtech, Victoria, Australia). The 5-minute normoxic/hypoxic intervals were separated by 5-minute ambient air intervals, repeated for 1 hour (total of 30 minutes of hypoxic/placebo exposure per session). Heart rate and peripheral oxygen saturation (SpO_2) were monitored continuously throughout the IHE session using fingertip pulse oximeters (Nonin Pulse Oximeter, Plymouth, Minnesota) built into the hypoxicators. Participants in the IHE3 and IHE5 groups received a fraction of inspired oxygen (FiO_2) to yield weekly SpO_2 values as follows: Week 1: 95%, Week 2: 90%, Week 3: 85%, and weeks 4 and 5: 80%. The C participants received a normoxic FiO_2 such that during all weeks $SpO_2 > 95\%$. Participants were blinded to their SpO_2 and HR data during the breathing sessions.

4.3.10 Statistical analyses.

Any changes in pre- and post-intervention measurements in the IHE3 and IHE5 groups were compared to changes in C. Magnitude based inferences were used to assess the change in pre- and post-intervention variables. A spreadsheet (Hopkins, 2007) which analyses the magnitude of the

change in means against a smallest worthwhile change was used to provide a quantitative and qualitative assessment of the changes in the test groups (IHE3 and IHE5) against the control. Cohen's smallest worthwhile change of 0.20 standardised units, that is, the inter-individual standard deviation (SD) between all participants at baseline divided by 0.2 (Cohen, 1988), was applied to test whether the true changes in variables were at least small. To reduce the effects of non-uniformity of error, and non-normal distributions (which are particularly prevalent in HRV analyses), the natural logarithm of the dependent variables was used in the statistical analysis (Hopkins et al., 2009).

The practical significance of the results were reported using mechanistic and clinical inferences. The mechanistic inference relates to where the likely range of the true value lies relative to the smallest worthwhile change (Batterham and Hopkins, 2006). The likely range of the true value is expressed using the percentage change (from the natural logarithm) ± 90 % confidence interval, and the percent chances for substantially increased / trivial / substantially decreased outcomes (Hopkins, 2007). Where the confidence interval spans all three possibilities (increased, trivial and decreased), the result was deemed unclear. In all other cases, a mechanistically clear outcome is achieved (i.e. the confidence interval spans either 1, or a maximum of 2 possibilities, i.e. beneficial/trivial, or harmful/trivial) and a qualitative assessment of the magnitude of the change can be given. To determine the qualitative outcome the following scale was used: < 0.5 % = most unlikely; 0.5 – 5 % = very unlikely; 5 – 25 % = unlikely; 25 – 75 % = possibly; 75 – 95 % = likely, or 95 – 99.5 % = very likely, > 99.5 % = most likely).

In cases where the variable has a direct effect on health or performance a clinical analysis was performed (these included SBP, hs-CRP, exercise efficiency and maximal oxygen uptake in the present study). In clinical analysis, the risk of harm is also included in the analysis, and results are only considered acceptable for use when the potential for benefit is substantially greater than the risk of harm. In this way avoiding harm is prioritised over neglecting to use a beneficial effect (Hopkins et al., 2009, Hopkins, 2007). For this study the benefit: harm ratio was considered acceptable if it was greater than 66 %.

In all cases the baseline measurement was included as a covariate to reduce the impact of regression to the mean, and neutralise uneven starting values between groups (Hopkins, 2006). Results are presented as the % change in the mean from the natural log $\pm$ 90% confidence interval; % chance that the change substantially increased/trivial/decreased; followed by the qualitative outcome.

4.4 Results

All raw baseline and post-intervention testing data are presented in Table 23 in Appendix C.

4.4.1 Resting heart rate variability

The HRV results are presented in Table 10. Refer to Table 9 for the filtering process and final number of participant data included for each group.

Table 10: Baseline and post-intervention resting HR and HRV results

	Mean change (%) and 90% confidence interval	Chances of increase/ trivial /decrease	Qualitative outcome
Spontaneous breathing			
RR interval (ms)			
IHE3 – Control	-1.6 ± 7.5	19 / 35 / 46	Unclear
IHE5 – Control	2.1 ± 8.1	50 / 33 / 18	Unclear
rMSSD (ms)			
IHE3 – Control	13.5 ± 49.8	52 / 32 / 16	Unclear
IHE5 – Control	36.7 ± 42.7	86 / 11 / 3	Likely increased
SDNN (ms)			
IHE3 – Control	-1.9 ± 36.9	32 / 29 / 39	Unclear
IHE5 – Control	5.3 ± 41.1	49 / 21 / 29	Unclear
HF (ms^2)			
IHE3 – Control	58.2 ± 123.9	70 / 22 / 8	Unclear
IHE5 – Control	101.5 ± 95.0	91 / 7 / 2	Likely increased
LF (ms^2)			
IHE3 – Control	74.0 ± 76.2	89 / 8 / 3	Likely increased
IHE5 – Control	55.0 ± 67.9	86 / 10 / 4	Likely increased
Controlled breathing			
RR interval (ms)			
IHE3 – Control	4.6 ± 5.7	77 / 20 / 3	Likely increased
IHE5 – Control	6.2 ± 6.9	84 / 13 / 3	Likely increased
rMSSD (ms)			
IHE3 – Control	30.3 ± 39.2	81 / 15 / 4	Likely increased
IHE5 – Control	47.3 ± 41.6	93 / 5 / 2	Likely increased
SDNN (ms)			
IHE3 – Control	15.3 ± 23.2	73 / 22 / 5	Possibly increased
IHE5 – Control	22.2 ± 26.7	86 / 10 / 4	Likely increased
HF (ms^2)			
IHE3 – Control	46.4 ± 96.8	67 / 26 / 7	Unclear
IHE5 – Control	62.3 ± 104.3	78 / 15 / 7	Unclear
LF (ms^2)			
IHE3 – Control	222.5 ± 228.2	93 / 2 / 4	Likely increased
IHE5 – Control	39.3 ± 633.7	58 / 11 / 31	Unclear

Spontaneous breathing: Participants were instructed to breathe as normally as possible for 10 minutes, the last 5 minutes of this 10 minute interval were used to assess heart rate variability measures; Controlled breathing: participants breathed in time to a metronome which controlled respiration at 12 breaths per minute for 6 minutes. Heart rate variability was measured for 5 minutes starting 30 seconds after the start of the metronome. RR Interval: time interval between R – R peaks of the QRS complex; IHE3 and IHE5: Groups receiving intermittent hypoxic exposure treatments 2 – 3 or 5 times per week respectively; rMSSD: root mean square successive difference; SDNN: standard deviation of normal to normal beats; HF: high frequency; LF: low frequency

4.4.2 Fitness

Submaximal and maximal results are presented in Table 11.

Table 11: Differences in submaximal and maximal exercise as a result of the 5 week intervention

	Mean change (%) and 90% confidence interval	Chances of increase/ trivial /decrease	Qualitative outcome
Submaximal exercise			
$\dot{V}O_{2submax}$ *(ml·min^{-1}·kg^{-1})*			
IHE3 – Control	5.5 ± 6.8	68 / 29 / 2	Clinically possibly harmful
IHE5 – Control	0.0 ± 8.0	17 / 65 / 18	Clinically possibly trivial
SBP (mmHg)			
IHE3 – Control	3.6 ± 6.1	53 / 44 / 3	Clinically possibly harmful
IHE5 – Control	- 1.7 ± 6.2	8 / 61 / 31	Unclear
HR_{submax} *(*beat·min^{-1}*)*			
IHE3 – Control	- 0.9 ± 5.8	26 / 28 / 46	Unclear
IHE5 – Control	- 0.3 ± 5.5	30 / 32 / 38	Unclear
Maximal exercise			
$\dot{V}O_{2peak}$ *(ml·min^{-1}·kg^{-1})*			
IHE3 – Control	12.6 ± 9.3	92 / 7 / 0	Clinically likely beneficial
IHE5 – Control	0.1 ± 11.0	20 / 58 / 20	Clinically possibly trivial
Res_{max} *(watts)*			
IHE3 – Control	4.4 ± 16.1	52 / 30 / 18	Unclear
IHE5 – Control	14.4 ± 14.9	88 / 9 / 3	Likely increased
$Time_{ex}$ *(sec)*			
IHE3 – Control	16.6 ± 48.1	63 / 21 / 16	Unclear
IHE5 – Control	34.5 ± 36.8	91 / 5 / 4	Likely increased

Submaximal exercise: Participants were instructed to maintain a 'moderate' workload for 5 minutes while submaximal variables were recorded; $\dot{V}O_{2submax}$: average oxygen uptake over the 5 minutes of moderate exercise; SBP_{submax}: average of all systolic blood pressure recordings taken during the 5 minute moderate workload interval; submaximal oxygen uptake; HR_{submax}: average heart rate during the 5 minute moderate workload interval; Maximal exercise: workload increased every 2 minutes until any of the pre-defined criteria for maximal exertion were achieved; $\dot{V}O_{2peak}$: maximal oxygen uptake during last 30 seconds of peak exercise; Res_{max}: Maximum resistance tolerated for at least 30 seconds; $Time_{ex}$: Time from the end of the 5 minute moderate workload to maximal exertion; IHE3: Group receiving intermittent hypoxic exposure treatments 2 - 3 times per week; IHE5: Group receiving intermittent hypoxic exposure 5 times per week; rMSSD: root mean square successive difference.

4.4.3 Blood samples:

hs-CRP.

There were no clear changes in hs-CRP between IHE3 and C (7.6 ± 58.6 %; 24/65/11; unclear). The IHE5 group demonstrated possibly trivial clinical changes when compared to C (12.7 ± 48.7 %; 23/72/5; possibly trivial). When the participants' hs-CRP results were divided into the quintiles defined by Ridker (2001), there were no differences between the groups.

Hb.

There were no clear changes in Hb in the IHE3 group compared to the C group (0.8 ± 3.3 %; 33/58/10, unclear). However, the IHE5 group demonstrated a possible increase in Hb compared to the C group (2.7 ± 2.8 %; 68/32/1; possibly increased).

4.4.4 Resting blood pressure

Both the IHE3 group and the IHE5 groups demonstrated a clinically unclear change in blood pressure from baseline to post-intervention measurements (IHE3 vs C: -5.4 ± 8.1 %; 4/27/69; unclear; IHE5 vs C: -4.0 ± 7.0 %; 3/44/53; unclear).

4.4.5 Muscle mass and fat mass

There were no meaningful changes between groups regarding fat mass or muscle mass as a result of the intervention. (Fat mass: IHE3 vs C: -3.1 ± 3.1 %; 0/100/0; most likely trivial; IHE5 vs C: 4.3 ± 6.0 %; 13/87/0; likely trivial. Similarly for muscle mass: IHE3 vs C: -0.2 ± 1.7 %; 0/100/0; most likely trivial; IHE5 vs C: -1.5 ± 2.8 %; 0/88/12; likely trivial).

4.4.6 Pedometer and food diaries

There were no clear differences between groups on steps taken before and after the intervention in any group.

Food and activity diaries were inspected and none of the participants varied substantially in their daily routines pre- and post- intervention.

4.5 Discussion

The purpose of this study was to determine the effect of different frequencies of IHE on traditional and novel cardiovascular risk factors in a sedentary, but otherwise health population. The key finding of the study is that while there were a greater number of beneficial adaptations associated with the IHE5 group (greater increases in the parasympathetic components of HRV, improved time to exhaustion in the maximal fitness assessment, increased maximum workload, and improved Hb)

there was also evidence of improved HRV and fitness measurements in the IHE3 group (improved HRV under controlled conditions, and increased $\dot{V}O_{2peak}$). To our knowledge, this is the first time that the effect of the number of IHE sessions per week has been tested on selected cardiovascular risk factors in a sedentary population.

4.5.1 Novel risk factors

HRV

As rMSSD and the RR interval were the primary measures of HRV in this study, the results of this study suggest a more prominent HRV response to IHE in the group receiving more frequent hypoxic stimulus. Indeed, the results of this study support the findings of an earlier study run by our research lab which demonstrated an increase in rMSSD following a 4-week intervention of 4 IHE sessions / week (see Study 1, Chapter 3). In this earlier study, the improvement in HRV at rest was attributed to an adaptive response to the repeated parasympathetic withdrawal and reactivation during hypoxic exposure. The results of the present study develop this finding further in that it appears that a minimum number of hypoxic exposure sessions per week are necessary before increases in HRV become clear. That is, there were small increases in rMSSD in IHE3 (compared to the control group, and only under controlled breathing conditions), and a clear increase in rMSSD in IHE5 under both spontaneous and controlled breathing conditions. Taken with the findings from the 4-week 2010 study, our results suggest that a minimum of 4 – 5 IHE sessions per week for at least 4 weeks are required before an increase in resting parasympathetic activity is detected in a sedentary, middle-aged population.

The rMSSD in our participants was just within the normal range outlined by Nunan et al. (2010), which likely reflects the healthy but sedentary nature of the middle-aged participants in this study, compared to the 'healthy adults' in their meta-analysis. However, the controlled breathing interval in our study did not produce increased HRV values as reported in the aforementioned article (See raw values presented in Table 23 in Appendix C). Rather, our outcomes are similar to those of Pinna et al. (2006) who noted very little difference in markers of parasympathetic and sympathetic activity between spontaneous or controlled breathing frequencies in their middle-aged participants. There are some notable differences between the study by Pinna et al. (2006) and our study. The first difference is that we controlled breathing at a frequency at 0.2 Hz to reduce any hyperventilation, compared to Pinna et al. (2006) who used 0.25 Hz which is more comfortably within the high frequency bandwidth of 0.15 – 0.4 Hz (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). Secondly, the time domain (rMSSD) was the primary indicator of parasympathetic activity in the present study, rather than the spectral domain (high and low frequency). The rMSSD reported in the present study supports the findings of

Pinna et al. (2006) in that the controlled breathing did not appear to have a substantial effect on rMSSD. However, the HF reported in the present study was substantially higher than Pinna et al.'s (2006) study, and did appear to increase with paced breathing. For example, group means of 278, 238, and 234 Hz under spontaneous breathing conditions, and 282, 313, and 391 Hz (C, IHE3, IHE5 respectively) under controlled conditions were reported at the baseline recordings of the present study. Pinna et al. (2006) reported group means of 90 Hz (range: 46 – 145 Hz) under spontaneous and 70 Hz (35 – 170 Hz) under controlled conditions. Using data gleaned from the practice and baseline assessments of the present study, we found HF and LF measurements to be the least reliable measures of HRV in this cohort (see draft paper in Appendix B), and therefore would urge caution in drawing conclusions from the spectral components of HRV reported in this study.

The high variation in the HF and LF reported in the present study is not unique. In the current literature, there is a wide range of reported HRV recordings, particularly regarding spectral analyses. For example, in Nunan et al. (2010) systematic review to determine normative values for HRV measures, high frequency reports ranged from 98 Hz (Notarius et al., 1999) to 1246 Hz (Sandercock et al., 2004) under spontaneous breathing, and from 82 Hz (Piccirillo et al., 2004) to 1506 Hz (Sandercock et al., 2004) under controlled breathing, refer to Supplementary table in Nunan et al. (2010). This high variation in reported HRV may depend on the equipment used to capture the RR intervals, the orientation of the participant during the measurement, the length of the HRV recordings, the population under investigation, and the level of the participant's familiarity with the equipment, researchers and procedures (and therefore anxiety). Therefore, in assessing the differences between paced and spontaneous breathing, it is important that the measurements being compared are from the same participants, measured under the same conditions and ideally, by the same researchers. Unfortunately there were only two such studies that measured both paced and spontaneous breathing in the review by Nunan et al. (2010). Of these two studies, one reported an increase in HF and rMSSD values with paced breathing (Sandercock et al., 2004), while the other reported little difference between the paced and spontaneous breathing (Sinnreich et al., 1998). The systematic review for normal values of HRV by Nunan et al. (2010) is an important and long overdue 'puzzle piece' in the understanding of HRV, and indeed is useful in detecting where one's participants fit in the greater scheme of the reported research. However, the methodological causes of variation discussed above, and the restricted number of large population studies meeting the requirements for inclusion in the review may limit the more specific generalisations that can be drawn from the findings.

Hs-CRP

While the hs-CRP in the IHE3 group remained relatively consistent throughout the trial, the hs-CRP demonstrated a small increase in the IHE5 group. While this increase was trivial, and not sufficient to increase the IHE5 group from the 4th into the 5th quintile of 'highest relative risk' for future cardiovascular disease as defined by Ridker (2001), it is important to address the possible causes of this increase. As mentioned earlier, highly sensitive C-reactive protein is a sensitive marker of inflammation that has become useful in the detection and monitoring of the early atherosclerotic stages of cardiovascular disease (Koening et al., 1999). However, an increase in C-reactive protein (CRP) has also been associated with hypoxic exposure, both in disease, such as in patients with Obstructive Sleep Apnoea (Yokoe et al., 2003), and environmentally such as during sojourns to high altitude (Hartmann et al., 2000). The inflammation response to the hypoxic environment is delayed, with very little increase in CRP in the first (Pavlicek et al., 2000) or second (Hartmann et al., 2000) days at high altitude, but is substantially increased on days 3 and day 4 (Hartmann et al., 2000). The participants in the IHE5 group were exposed to daily doses of hypoxia to a target SpO_2 of 80%, which is approximately equivalent to 4,500 m (Hartmann et al., 2000). While these intervals were brief (total of 30 minutes of hypoxic exposure), the additive effect of the daily exposure may have slowly increased systemic inflammation. While Hartmann et al. (2000) did not present any data regarding the recovery time of the elevated hs-CRP to normal resting values following the return to sea level, when the patients with obstructive sleep apnoea syndrome were given continuous positive airway pressure devices to eliminate the hypoxic episodes during sleep, the C-reactive protein was decreased substantially (Yokoe et al., 2003). Therefore, it is unlikely that the marginally elevated hs-CRP will persist for any extended period of time following cessation of the hypoxic training sessions. However, as C-reactive protein may propagate cardiovascular disease rather than just indicate its presence (Li and Fang, 2004) caution should be used when recommending IHE to participants who might already have elevated levels of hs-CRP.

4.5.2 Traditional risk factors

Exercise tolerance

As the submaximal workload was controlled using HR, the uniformity of the submaximal exercise heart rate between baseline and post-intervention measurements in all three groups indicates good consistency in the exercise test between baseline and post-intervention trials. The within group differences between baseline and post-intervention oxygen efficiency, as assessed by $\dot{V}O_{2submax}$, suggested that the IHE3 remained largely unchanged following the 5-week intervention, and the IHE5 group improved efficiency (see Table 23 in Appendix C). As the decrease in $\dot{V}O_{2submax}$ in IHE5 (-1.2 $ml \cdot min^{-1} \cdot kg^{-1}$) was similar to the change in the control group (-0.8 $ml \cdot min^{-1} \cdot kg^{-1}$), it is unlikely that the improved oxygen efficiency could be attributable to the intermittent hypoxic exposure. Despite

including a familiarization session, the improvements in the Control group could be attributed to improved familiarity with the researchers and exercise testing protocols. Thus, neither group (IHE3 nor IHE5) demonstrated any advantage attributable to the IHE treatment over the control group during submaximal exercise.

During the maximal assessment, the results were somewhat conflicting. The IHE3 group likely improved $\dot{V}O_{2peak}$ but did not demonstrate any other improvement to support an improvement in exercise tolerance (such as improved $time_{ex}$, or res_{max}). Conversely, the IHE5 group did demonstrate increased $time_{ex}$ and res_{max} but did not demonstrate any improvement in $\dot{V}O_{2peak}$, despite an increase in Hb. The findings from the IHE5 group are similar to those of Burtscher et al. (2009) who reported improved exercise time, time to anaerobic threshold, and increased total haemoglobin mass, but no clear improvement in $\dot{V}O_{2peak}$ in his population of patients at risk for coronary pulmonary obstructive disease. Burtscher et al. (2009) suggests, that the exercise time reflects greater sensitivity to training effects than $\dot{V}O_{2peak.}$ So, while participants in the IHE5 group did not improve exercise tolerance to the point of improving $\dot{V}O_{2peak}$, exercise capacity was improved sufficiently to alter more sensitive markers of exercise tolerance. Contrary to the findings of other researchers (Calbet et al., 2006), the 1.75% increase (within group) in Hb in the IHE5 group following the 5 weeks of IHE treatment was not sufficient to alter the $\dot{V}O_{2peak}$ of our participants in a meaningful way. Similarly, Clark et al. (2009) reported no change in $\dot{V}O_{2peak}$ in their cohort of well-trained male cyclists following an increase of 3.3% $Hb_{mass,}$ and only a trivial correlation (r = 0.09) between the change in Hb_{mass} and $\dot{V}O_{2peak}$. Our research supports the idea of a minimum dose required in a hypoxic environment for an increase in haemoglobin (the Control and IHE3 groups exhibited no change, but there was a clear, possible increase in Hb_{mass} in IHE5). However, it does not support an automatic improvement in $\dot{V}O_{2peak}$ with an increase in Hb_{mass}. The question arises of whether in addition to recommendations for a minimum time spent in hypoxic exposure for an increase in Hb_{mass} (Rusko et al., 2004), there is also a minimum worthwhile increase in Hb_{mass} required before a practical increase in $\dot{V}O_{2peak}$ is achieved.

Alternatively, the increased workload and higher time to exhaustion in the IHE5 group may indicate a greater anaerobic tolerance. Findings such as these have also been observed by other researchers (Hamlin et al., 2010) who posit that mechanisms such a shift to glycolytic energy pathways (Hamlin et al., 2010), and improved skeletal muscle buffer capacity (Mizuno et al., 1990) are responsible for the improvements in anaerobic performance.

The improvement of $\dot{V}O_{2peak}$ in the IHE3 group without any other supporting evidence of improved exercise tolerance is confusing. It is possible that 'upstream changes' in response to hypoxic exposure (Garvican et al., 2011) may be responsible for the increase in $\dot{V}O_{2peak}$ in the IHE3 group in the current study. That is, while 2-3 IHE sessions per week are insufficient to simulate an increase in

Hb, they may be sufficient to stimulate other, non-haematologically based adaptations, such as the adaptations associated with an up regulation of HIF-1α and an adaptation of the hypoxic sensing system (Vogt et al., 2001, Hoppeler et al., 2003). However, if this were the case, it is unlikely that these adaptations would occur in the group receiving 2 – 3 IHE sessions per week, and not in the group receiving 5 IHE sessions per week.

Blood pressure

Resting systolic blood pressure decreased in both the IHE3 (9.6 mmHg) and IHE5 (5.2 mmHg) groups compared to negligible change in the control group (0.2 mmHg). However, when the baseline was added as a covariate the changes between groups were unclear. The improved HRV as indicated by increased parasympathetic activity (rMSSD) in IHE3 (small) and IHE5 (moderate – large) following the 5 weeks of IHE may be linked to the improved resting SBP in the IHE3 and IHE5 groups. However, as the SBP at baseline in the IHE3 and IHE5 groups was substantially higher than the C group, it is possible that the improvements reflect a regression to the mean, as opposed to a true change (Hopkins, 2006). Therefore, the 'unclear' outcome is appropriate in this regard, and more information is needed before a confident statement can be made regarding the effect of IHE on SBP.

Muscle mass and fat mass

There were no meaningful differences between groups in either the fat mass or muscle mass measurements following the intervention when compared to the control group. The absence in any notable changes between groups pre- and post-intervention supports both the study by Balykin et al. (2004) and our earlier study (see Chapter 3) and suggests that an IHE protocol in this format, without an additional exercise component, is insufficient to induce any beneficial alteration in muscle mass or fat mass.

4.5.3 Dosage

As with real altitude exposure, the frequency, and length of the IHE intervention are important for altitude related adaptations. However, in both studies run by our research group, the IHE sessions were fixed at 5-minute hypoxic exposure intervals separated by 5 minutes of ambient air for one hour and the intervention period ran for a period of 4 - 5 weeks. As different intervention lengths or hypoxic exposure intervals were not assessed, the minimum hypoxic exposure or intervention length is still unknown.

Our research protocol was similar to that of Tadibi et al. (2007) but we used a lower frequency of hypoxic exposure intervals per week (3 and 5 in our study, compared to 7 in Tadibi et al.'s (2007) protocol), but longer intervention length (5 weeks in our study compared to 2 weeks in Tadibi et al.'s (2007) protocol). This suggests that the length of the intervention may be as important as the

frequency or intensity of IHE. For example, in Beidleman et al.'s (2009) study, 1 week of severe hypoxic exposure and hypoxic training did not yield any meaningful change in the hypoxic group when compared to the placebo in submaximal or maximal exercise parameters (Beidleman et al., 2009). While this is by no means a full review of the literature, and there are some exceptions (Beidleman et al., 2008), it is likely that an intervention of greater than 3 weeks in duration will be beneficial for improvement associated with IHE (Haider et al., 2009, Saeed et al., 2012).

The results from this study and our earlier 2010 study suggest that a minimum IHE frequency of 4 - 5 times per week are required before clear physiological changes take place. However, as little as 2 – 3 sessions per week may result in small increases in parasympathetic activity at rest, but these responses are likely to be of a smaller magnitude than more frequent exposures per week.

4.5.4 Limitations

The nature of this study lends itself to Type 1 errors, or declaring that an effect is worthwhile, when it is in reality a product of chance. Both the small group sizes and the large number of variables are important factors when considering the chances of a false positive (Hopkins, 2000a). This increase in the risk of Type 1 errors may account for the unexpected improvements in the $\dot{V}O_{2peak}$ data in the IHE3 group. No attempt was made to correct for these potential errors as all measurements were pre-planned and have been openly reported.

This study was a single blind, randomised control trial. Strictly speaking however, there was no control for the group coming 5 times per week, as the control participants in this study all came 3 times per week. Following the recruitment period, and after all recruitment strategies had been exhausted, there were not enough participants to divide into 4 groups, as the group sizes would have been too small to make meaningful inferences (Pyne and Hopkins (2012) suggest a minimum of 10 individuals per group). Thus, when deciding between whether to assign a control group to the IHE3 or IHE5 group, the researchers felt the best participant retention would be in the IHE3 group.

Iron is an important part of haemoglobin production, and to ensure anaemia does not interfere with aerobic adaptation some researchers recommend the inclusion of iron supplementation during altitude training (Brownlie et al., 2002, Maughan, 1999). Unfortunately, due to budgetary constraints we were unable to provide iron supplementation to the participants in this study. However, none of the participants presented with low Hb_{mass} at baseline, and as none of them were participating in strenuous exercise at the time, it is unlikely that the participants would have been iron deficient.

Some of the studies used in the introduction and discussion are specific to well-trained individuals, and/or are focussed on real rather than simulated altitude. These techniques and populations may well respond differently to the sedentary cohort in this study. However, the inclusion of these studies

in the discussion and introduction of this chapter help to understand the mechanisms behind some of the adaptations reported in the current investigation.

In any exercise assessment, motivation will play a large role in the extent to which a participant is willing to endure the exercise load. Furthermore, ratings such as the Borg Rating of Perceived Exertion scale are subjective and require the participant to assess themselves. To reduce the effects of variations in extrinsic motivation, the researcher leading the fitness assessment paid attention to maintain a similar level of verbal encouragement between all participants at all measurement sessions. However, some variations in the level of encouragement supplied the motivation of the participant on the day and the participant's subjective rating of their exertion could have confounded the results of the fitness assessment.

4.6 Conclusion

Intermittent hypoxic exposure appears to have a beneficial effect on both novel and traditional cardiovascular risk factors in a sedentary population. Greater improvements in HRV and exercise tolerance were observed in the IHE5 group (improved $time_{ex}$, res_{max}, Hb and HRV) than the IHE3 group; however the IHE3 group did demonstrate improvement in $\dot{V}O_{2peak}$ and a small increase in HRV (only when compared to the control and under controlled conditions only). While within group change suggested a decrease in SBP with IHE, there were no clear changes in SBP compared to C. Caution is recommended when advising participants with high levels of hs-CRP to engage in more than 3 IHE sessions per week. Intermittent hypoxic exposure sessions at least 4 – 5 times per week are recommended.

Chapter 5

Preface to Study 3: Does IHE enhance the cardioprotective effect of exercise in a sedentary population?

The previous two studies have demonstrated improvements in both novel and traditional cardiovascular risk factors with IHE. While Study 2 demonstrated greatest improvement in cardiovascular risk factors with 5 IHE sessions per week, there was some health benefit in the group attending 2 – 3 IHE sessions per week. Therefore, the aim of the following study was to determine whether 2 – 3 IHE sessions per week in addition to 3 exercise training sessions per week would result in greater improvement in cardiovascular risk profile than exercise only. The time frame of the exercise intervention was extended to 10 weeks as this is the time frame used in the government funded "Green Prescription" programme. The advantage of aligning the time frame of the current research programme to the Green Prescription time frame is that it opens the door to future comparisons between 'real world' interventions. Additional risk factors, such as arterial stiffness, total cholesterol and high density lipoprotein were selected for greater risk assessment profiling. Additionally, the findings in Study 2 indicated a possibly trivial increase in hs-CRP following 5 IHE sessions per week. Typically, an increase in basal hs-CRP indicates systemic inflammation which is usually associated with atherosclerosis. By including an assessment of arterial stiffness, we can better understand the effects of IHE on immediate and longer-term vascular health. Finally, as walking / jogging formed the majority of the aerobic component in the exercise training, a treadmill fitness assessment, rather than the bicycle assessment was used to improve the testing protocol with the functional adaptation to the exercise intervention.

This study has been written in research paper format but it has not been submitted to a journal. It is likely that this research will be divided into 2 research papers (one focussing on arterial stiffness and the other focussing on the cardiovascular risk profile). However, in the interests of avoiding unnecessary repetition (particularly in the methods section), all information has been included into one paper.

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Dr. Kathiravel and Dr. Elliott: Participant medical assessment and ongoing medical advice.

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5.1 Abstract

Exercise is one of the best means of reducing cardiovascular disease risk in a sedentary population. As simulated (or real) altitude training is thought to improve exercise capacity in an athletic cohort, several health-focussed researchers have investigated whether simulated altitude would improve the effectiveness of a traditional exercise programme in reducing cardiovascular risk factors. However, most research has focussed on individual risk factors, rather than overall cardiovascular risk profile. In addition to this, research has largely focussed on exercise training in hypoxia, rather than training in normoxia and receiving an additional passive hypoxic stimulus. **Objectives:** Determine the effects of exercise supplemented with passive intermittent hypoxic exposure (IHE) on individual risk factors, and overall cardiovascular disease risk profile compared to exercise alone. **Methods:** All participants were medically screened prior to inclusion in the study. Following acceptance, 34 participants were divided into either a group receiving exercise and IHE (IHE3 + Ex, n = 16; 6 males, 10 females; Age: 56.7 $\pm$ 6.4 years; weight: 78.6 $\pm$ 12.4 kg; height: 168.0 $\pm$ 8.8 cm) or exercise alone (Ex, n = 18, 5 males, 13 females; Age: 56.4 $\pm$ 6.5 years; weight: 81.2 $\pm$ 15.9; height: 167.3 $\pm$ 8.42). Both groups received exercise training 3 d/ week for 10 weeks. Training was for an hour and included, a 10 min light aerobic warm up, 20 min aerobic (walking/ jogging outdoors), 15 min strength (including all major leg, core and arm muscle groups) and a 15 min warm down comprising light aerobic cool down and stretching. All participants trained with heart rate monitors and aimed for a an intensity of 70 – 75% of their heart rate maximum in Week 1 and 75 – 80% heart rate maximum for weeks 2 – 10. In addition to the exercise training, the IHE3 + Ex group also received IHE sessions for 2 – 3 d/ week, which involved breathing 5 min hypoxic air alternated with 5 min ambient air for 1 h while seated comfortably. The fraction of inspired oxygen (FiO_2) was controlled by the researcher to yield an SpO_2 of 90%, 85%, and 80% in weeks 1, 2 and 3 – 10 respectively. Testing took place before (Baseline), after (Post) and at 4 and 8 weeks following the intervention. Each testing period included: a blood sample (high density lipoprotein, HDL, and total cholesterol, TC). Blood samples were not collected at the 4-week follow up.; An arterial stiffness assessment (pulse wave velocity, PWV, and pulse wave analysis, PWA using the augmentation index, AIx); and a fitness assessment (including resting heart rate variability (HRV) and systolic blood pressure (SBP) and a maximal treadmill test to exhaustion). Heart rate variability was measured using the average R peak to R peak (RR) interval and the root mean square of the successive difference (rMSSD) between beats. Overall 5-year risk of cardiovascular disease was assessed using the New Zealand Guidelines Chart which combines SBP, age, sex, TC:HDL ratio, and diabetic status to assess an overall risk. Additionally, 10-year cardiovascular risk was assessed using the Framingham risk stratification process. **Results:** In all analyses, the mean change from the baseline measurement between IHE3 + Ex and Ex was assessed. Therefore, a negative result represents a decrease in the IHE3 + Ex compared the Ex group in the

change score from baseline. Following 10 weeks of IHE, TC decreased in both groups, but was higher in the IHE3 + Ex group than the Ex (Post: 4.4 ± 6.5%, possibly harmful; and 8-wk: 6.5 ± 7.2% likely harmful, mean change ± 90% confidence interval, qualitative outcome). The higher TC in IHE3 + Ex was due to a likely beneficial increase in HDL in the IHE3 + Ex group compared to Ex at Post and 8-wk (Post: 8.0 ± 8.0%; 8-wk: 10.0 ± 8.5%). Accordingly, the ratio between TC and HDL was likely beneficial at Post (-77.2 ± 50.5%) but unclear at 8-wk (-24.7 ± 52.4%). Systolic blood pressure decreased more so in the IHE3 + Ex group immediately following the intervention (Post: -3.4 ± 3.4%, possibly beneficial), was possibly trivial at 4-wk (0.5 ± 3.5%), and possibly beneficial at 8-wk (-3.5 ± 3.7%). Overall cardiovascular risk using the New Zealand Guidelines Chart for risk assessment demonstrated subtle improvements between risk categories in both groups. There were no clear changes in PWV at any time point (Post: -0.3 ± 11.2%; 4-wk: -4.2 ± 11.5%; 8-wk: -8.1 ± 9.3%), however PWA demonstrated an unclear decrease in AIx at Post (-6.1 ± 18.4%), and a likely and very likely harmful increase at 4-wk and 8-wk respectively (4-wk: 11.8 ± 18.4%; 8-wk: 24.8 ± 19.7%) compared to Ex. Both groups showed increased RR interval and rMSSD as a result of the 10-week intervention, but the changes between groups were most likely trivial at all test periods in the RR interval (Post: 3.1 ± 5.1%; 4-wk: 0.0 ± 5.3%; 8-wk: 3.4 ± 5.4%) and unclear in rMSSD (Post: 18.0 ± 24.0%; 4-wk: 14.0 ± 25.2%; 8-wk: 13.0 ± 25.6%). Changes in cardiovascular fitness showed different, but beneficial responses in both groups. While the differences between groups in $\dot{V}O_{2peak}$ remained unclear at post (3.1 ± 7.7%) there was a likely beneficial response in the IHE +Ex group at 4 and 8-wk follow up (4-wk: 9.4 ± 8.0%, 8-wk: 7.9 ± 8.3) suggesting improved aerobic capacity. Time to exhaustion (measured as the time taken from the beginning of Stage 1 to voluntary exhaustion) was similar in both groups (Post: 3.8 ± 6.3%; 4-wk: -1.3 ± 6.5%; 8-wk: 5.0 ± 6.8%). **Conclusion:** Exercise with IHE resulted in greater reduction in individual risk factors (improved SBP, $\dot{V}O_{2peak}$, HDL, and a tendency towards increased HRV) but showed no substantial benefit in 5- or 10 year risk of cardiovascular disease. Changes in cardiovascular fitness were more difficult to interpret. There was an unclear decrease in PWV in the IHE3 + Ex group, but the AIx measurements demonstrated an unclear decrease at Post, but then a likely and very likely increase at the 4- and 8-wk assessments respectively. The increase in AIx could be accounted for by an increase in the pulse pressure with little change in the augmentation pressure, or from a down-regulation of the endothelial nitric oxide synthase following cessation of the hypoxic exposures (resulting in increased vascular tone and more intense reflection waves). More research is needed regarding the long-term effects of IHE on vascular health before IHE can be considered a safe intervention. Whether a less severe dose of hypoxia would generate the cardio-protective adaptations without the apparent increase in arterial stiffness should be explored in either a healthy, younger cohort or in animal models.

Keywords: heart rate variability; intermittent hypoxic exposure; sedentary; heart disease risk factors

5.2 Introduction

It has been well established that exercise is a highly effective means of reducing cardiovascular disease and its risk factors (Garber et al., 2011). In an attempt to enhance the health benefits of exercise, some researchers have investigated the use of either exercise supplemented with passive simulated altitude exposure (Balykin et al., 2004) or exercise in a hypoxic environment (Friedmann et al., 2003, Geiser et al., 2001). The motivation behind the inclusion of a hypoxic stimulus to the exercise training is related to the adaptations associated with the actions of the alpha subunit of the transcription factor, Hypoxic Inducible Factor – 1 (HIF-1α). In hypoxia, HIF-1α acts to stimulate genes responsible for adaptations such as angiogenesis and erythropoiesis (Semenza, 2009), adaptations which are thought to have a beneficial effect on exercise, resulting in greater improvements in exercise capacity than when training in normoxia.

Indeed, previous research conducted in a sedentary middle-aged population by our research group has demonstrated improved HRV, time to exhaustion in a maximal exercise test on a cycle ergometer test, and increased haemoglobin following 5 weeks of passive simulated altitude exposure. These outcomes support the cross-adaptive effects of hypoxia (without concurrent exercise training) to exercise capacity. However, research regarding exercise training in hypoxia in a sedentary population has returned mixed findings. For example, some studies have reported improved muscle and mitochondrial volume and greater capillary length density (Geiser et al., 2001), improved vascular health (Nishiwaki et al., 2011), and greater $\dot{V}O_{2peak}$ (Katayama et al., 1998). Other studies have reported either no worthwhile effect of training in hypoxia compared to normoxic training on strength (Friedmann et al., 2003) or mitochondrial function (Pesta et al., 2011). Some research has even reported a detrimental effect on erythrocytes structure and stress tolerance immediately following an exercise intervention in hypoxia (Mao et al., 2011). Others have found that although the hypoxic stimulus resulted in adjustment at the molecular level, this did not transfer into a functional improvement in exercise capacity more so than that of exercise in normoxia (Vogt et al., 2001).

To our knowledge, there has only been one study which has compared the effect of passive hypoxic exposure as a supplement to exercise training in a sedentary population. Balykin et al. (2004) examined the effects of an intermittent hypoxic exposure (IHE) technique to simulate altitude in a population of overweight, sedentary males. In this protocol, the participants were seated and passively inhaled 5 min of hypoxic air alternated with 5 min of normoxic air, for 1 h for a total of 10 IHE sessions. The groups receiving IHE in conjunction with 30 min of aerobic exercise on a cycle ergometer at a resistance of 100 W (either immediately following IHE or on alternative days) demonstrated increased parasympathetic and decreased sympathetic activity in the autonomic control of the heart and greater improvements aerobic capacity compared to either exercise training

or IHE on its own. Given the promising results from this study, further research using IHE as a supplement to exercise should be investigated in a sedentary population who may already exhibit autonomic dysfunction and poor cardiovascular fitness.

There are several limitations in the literature investigating the potential health benefits of IHE. For example, most studies, including Balykin's (2004) study, have examined the effects of IHE on specific risk factors. While this is useful in that it allows the researcher to pinpoint specific responses as a result of adaptation to the hypoxic stimulus, cardiovascular disease is the result of combination of several risk factors, rather than just one (Dahlöf, 2010). Therefore, when assessing the effects of IHE and exercise on cardiovascular health it is useful to examine the overall cardiovascular profile of the participant in addition to individual risk factors. Additionally, most research on altitude training has been conducted in healthy, fit individuals (for example, the participants in the Balykin et al. (2004) study were healthy men between the ages of 18 – 20 years). As sedentary, middle-aged participants have a higher cardiovascular risk than a healthy young cohort, and therefore have more to benefit from such a treatment than a healthy young cohort, more research regarding the effects of IHE on health in an older, inactive population is warranted. Finally, the long-term effects of IHE interventions are seldom measured, and therefore it is unknown how long any potential adaptation associated with hypoxic exposure will last.

In response to these limitations in the literature, the purpose of this investigation is to determine the effect of adding IHE to a standard exercise programme on cardiovascular health in sedentary, middle-aged participants. This will be assessed using both individual risk factors for heart disease such as arterial health (pulse wave analysis, PWA and pulse wave velocity, PWV), heart rate variability (HRV), cardiovascular fitness (time to exhaustion and peak oxygen uptake, $\dot{V}O_{2peak}$), and an overall 5 – year, and 10 -year risk of cardiovascular disease as estimated using the New Zealand Guidelines Group (2009) year risk assessment chart, and Framingham 10 year risk assessment (D'Agostino et al., 2008). See Figure 31 in Appendix E for a copy of the New Zealand Guideline's 5 – year cardiovascular risk assessment chart.

5.3 Methods

5.3.1 Participants.

Men and women who were doing less than NZ physical activity guidelines of 30 minutes of moderate physical activity on most (<5) days of the week (Ministry of Health, 2008), and who were between the ages of 45 – 70 were recruited from the local community. All interested candidates were required to undergo a medical screening prior to inclusion. Participants were excluded if they had an uncontrolled medical condition (uncontrolled hypertension, hypercholesterolemia etc.) were

smokers, had any melanoma, cardiovascular disease or were recommended against participation by the assessing medical practitioner or their general practitioner. Participants on medication were included provided the condition was stable and the medication dose was unlikely to change over the course of the trial.

As outlined in Figure 12, two participants in Ex (n=18) and three participants in IHE3 + Ex (n=16) withdrew their participation in the first week of the training programme, and have been excluded from the group descriptions and analyses. Participant descriptions can be found in Table 12. All participants were informed of the procedures and risks and provided written informed consent. Human Ethics approval was granted by the Lincoln University Human Ethics Committee.

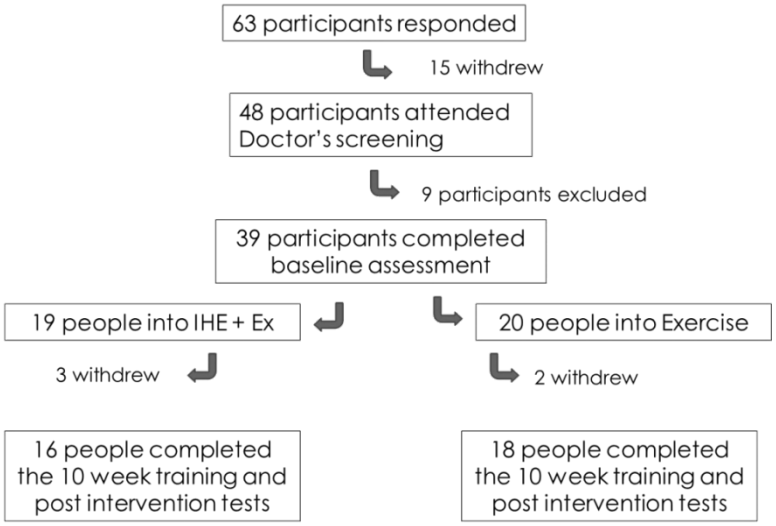


Figure 12: Participant recruitment process

5.3.2 Experimental design.

Following inclusion into the study, participants underwent a baseline testing period followed by a 10-week intervention period. Testing periods then took place within 3 days of the conclusion of the 10-week interval (Post) and 4- and 8-weeks (4-wk and 8-wk respectively) following the conclusion of the intervention.

Each testing period required the participants to attend 3 separate appointments. The first assessment was to collect a blood sample, from which total cholesterol (TC) and high density lipoprotein (HDL) were measured (but not at 4-wk follow up). The second assessment was an arterial health assessment and included PWV and PWA analyses. The last assessment was a fitness assessment which included anthropometric, HRV, and exercise ($\dot{V}O_{2peak}$ and time to exhaustion) measurements. Arterial stiffness assessments were completed within 1 weekend, and all fitness assessments were completed within the adjacent following week (participants attended sessions at the same time of day for each returning assessment), see Figure 13. Following baseline assessments,

participants were matched for age and sex and were then allocated into 1 of 2 groups. (The allocation of participants to groups was random except for 3 participants who specifically requested the exercise only group due to the time commitment associated with the exercise and simulated altitude group). One group received 3 exercise sessions per week (Ex) while the second group received 3 IHE sessions per week in addition to 2-3 of sessions (IHE3 + Ex) per week. Participants in the IHE3 + Ex and Ex groups all trained together. After the 10-week intervention, all the baseline assessments were repeated immediately and at 4 and 8-wk follow up assessments. See Figure 13 for the research timeframe.

Table 12: Anthropometric data of participants

	IHE3 + Ex (n=16)		**Ex (n=18)**	
	Female (n=10)	**Male (n=6)**	**Female (n=13)**	**Male (n=5)**
Age (years)	56.9 ± 5.8	56.3 ± 7.9	55.4 ± 6.2	59.0 ± 7.3
Weight (kg)	74.0 ± 12.5	86.1 ±8.5	79.2 ± 17.7	86.5 ± 8.8
Height (cm)	163.6 ± 6.3	175.3 ± 7.5	164.6 ± 8.1	174.2 ± 4.7
Body fat (%)	37.5 ± 7.2	23.6 ± 1.2	39.5 ± 5.8	26.4 ± 4.2
Resting heart rate (beats·min^{-1})	65.4 ± 10.0	63.8 ± 8.9	62.0 ± 10.3	58.8 ± 14.4
SBP (mmHg)	124 ± 11.0	126.9 ± 14.1	125.7 ± 17.6	119.1 ± 29.7
DBP (mmHg)	71.1 ± 7.6	73.6 ± 3.7	72.3 ± 8.0	69.0 ± 16.5
TC	5.3 ± 1.1	5.7 ± 0.5	5.9 ± 0.5	5.9 ± 1.1
TC:HDL	3.8 ± 1.3	5.1 ± 1.1	4.3 ± 0.7	5.4 ± 2.1
$\dot{V}O_{2peak}$ (mL·min^{-1}·kg^{-1})	26.8 ± 6.0	34.8 ± 5.0	25.0 ± 3.9	25.4 ± 7.7

IHE3 + Ex: Group receiving intermittent hypoxic exposure in addition to exercise during the intervention period. Ex: Group receiving exercise only during the intervention period. SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TC: Total cholesterol; HDL: High density lipoprotein; TC: HDL: Total cholesterol to high density lipoprotein ratio; $\dot{V}O_{2peak}$: peak oxygen uptake during exercise to exhaustion.

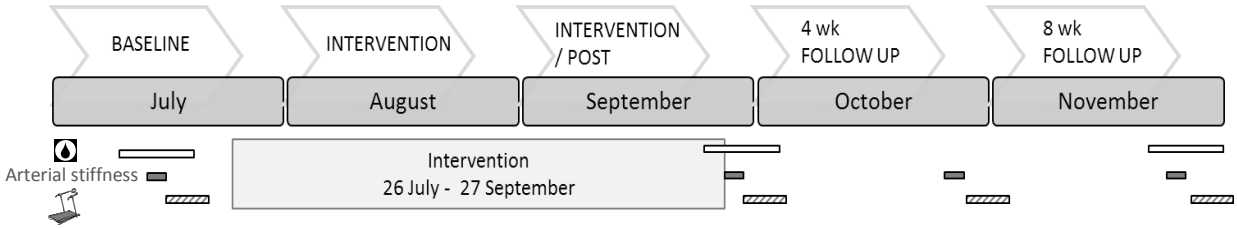


Figure 13: IHE3 + Ex versus Ex only research timeframe
The bars indicate where various measurements took place, and the interval for measurement. Open bars indicate the period for blood collection, solid bars indicate where arterial stiffness measurements were taken (over 1 weekend), and striped bars outline where fitness assessments were measured (over 1 week, immediately following the arterial stiffness assessment.

5.3.3 Food and activity monitoring

Participants were asked to record all food and medicine ingested, and to describe any physical activity for 2 days prior to measurement sessions.

5.3.4 Assessment 1: Venous blood samples.

All blood samples were taken following an overnight fast. Participants reported to a participating local medical facility, were seated and had their blood drawn from the antecubital vein by a trained phlebotomist. Venous blood samples were then analysed at an accredited laboratory (Canterbury Health Laboratories, Christchurch). High density lipoprotein (HDL) and total cholesterol (TC) were analysed using an enzymatic essay performed using the Abbott c8000 analyser (Abbott Laboratories. Abbott Park, Illinois, U.S.A) with Abbott reagents.

5.3.5 Assessment 2: Arterial stiffness

There are several different ways of monitoring or assessing arterial health. Two of the most convenient, non-invasive, and well-validated methods are the assessment of pulse wave velocity and pulse wave analysis.

Pulse wave analysis

Pulse wave analysis (PWA) provides a systemic appraisal of vascular health, including the ventricular-vascular interaction and an assessment of endothelial function (Stoner et al., 2012). The augmentation index (AIx) is a PWA assessment which indicates arterial stiffness based on the augmentation of the pressure wave generated during systole (Stoner et al., 2012), and is also an independent risk factor for coronary artery disease (Weber et al., 2004). Following ejection of the ventricular load, the forward moving pressure wave (incident wave) travels through the low-resistance aorta and arteries towards the periphery. When the incident wave reaches the highly

resistant arterioles, a reflection wave is generated that travels back up along the arteries and aorta towards the heart (Vlachopoulos and O'Rourke, 2000). A fast travelling pulse wave (associated with stiffer arteries) will arrive back at the heart during systole, which in turn will have two important effects on the vascular-ventricular interaction. First, by interacting with the incident wave earlier on during systole where the pressure is higher, an even higher second systolic peak is created. And second, by arriving back at the heart during systole (rather than diastole) both ventricular ejection and coronary blood flow are inhibited (Vlachopoulos and O'Rourke, 2000). The amount of pressure added to the incident wave by the reflected wave is known as the augmentation pressure (derived using maximum systolic peak of the pulse wave minus the pressure at the inflection point where the forward and reflected waves interact). The augmentation index is the augmentation pressure expressed as a percentage of the pulse pressure (Stoner et al., 2012), see Figure 14.

Pulse wave analysis was assessed using a SphygmoCor device (SphygmoCor, AtCor Medical, Sydney, Australia) and integrated software (SCOR Px 7.1, AtCor Medical, Sydney, Australia) which automatically interprets and displays the AIx. Measurements were taken on the radial artery, with the arm fully supported and following a minimum of 20 min undisturbed rest in the supine position (Stoner et al., 2013). To reduce the impact of heart rate on the AIx (Wilkinson et al., 2000), all data were normalised to a heart rate of 75 beats per minute prior to analysis. A minimum of two recordings were taken. If AIx differed by 4% between the two recordings a third recording was taken, and the average of the closest two measurements were used. The quality of the recordings was assessed using the default setting specifications (average pulse height: 80 units, pulse height variation: 5 %, Diastolic variation: 5 %, and quality index: >80 %) and recordings were only accepted if they fell within these limits.

Pulse wave velocity

The carotid – femoral pulse wave velocity is considered the gold standard in the measurement of arterial stiffness (Laurent et al., 2007), as it reflects the velocity of the pulse wave down the aorta (regional aortic pressure). The velocity of the pulse wave is determined by the stiffness of the aorta, whereby the stiffer the aorta, the faster the pulse wave. However detecting a clear and reliable femoral pressure wave using this technique can be difficult in participants with metabolic syndrome or obesity (Laurent et al., 2007). Additionally, as the distance between measurement sites is needed to assess the blood distance travelled, individuals with abdominal obesity (particularly men) or large breast size may introduce additional error into these measurements (Laurent et al., 2007). As the participants in our study were likely to have one or more of these limitations, PWV was assessed between the carotid and radial arteries.

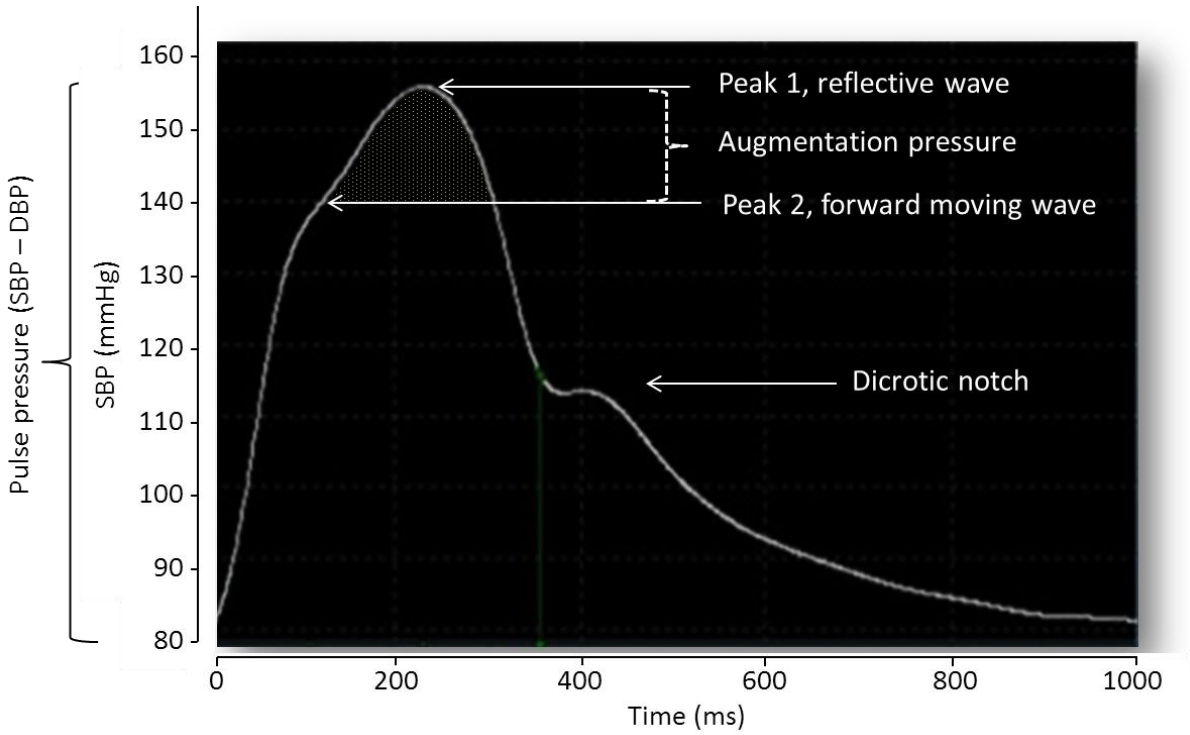


Figure 14: Pulse wave analysis assessed using the augmentation index at the radial artery
The above waveform was captured using radial tonometry. The speed at which the pressure wave travels through the arterial system determines where the forward moving wave (Peak 2) interacts with the reflected wave (Peak 1). In the above example, taken from a sedentary middle-aged participant, the reflected pressure wave has interacted with the forward moving wave near the systolic peak which has resulted in a reasonably high augmentation of the pressure wave. The augmentation index is the ratio between the augmentation pressure and the pulse pressure (%). For example, in the wave form above, the augmentation index is: (156-140)/(156-80) *100 = 21.1%

Following palpation to detect the region of strongest pulse, pressure waves at the carotid and radial sites were recorded sequentially using a high-fidelity tonometer. The 'foot', or the steep rise of the pressure wave at the end of diastole, of each pressure pulse was used as the locus for the pulse to pulse measurement. The R wave of a simultaneously recorded ECG (using a lead II configuration on the participant's wrist and ankle) provided a timing reference. At least 2 ½ screens of a clear ECG and tonometry recording was required for each site. The mean time of the distance between the R wave of the ECG and the foot of the carotid pulse minus the time between the R wave of the ECG and the foot of the radial pulse was used to determine transit time. The distance covered by the pressure wave was estimated by using a tape measure to measure the distance between the measurement site at the radial artery and the suprasternal notch, and the supersternal notch and the measurement site at the carotid artery (to the nearest mm). Pulse wave velocity is equal to the distance covered over the mean difference in time (Laurent et al., 2007, AtCor Medical, 2008). Diastolic blood pressure/Mean arterial pressure was used to correct the data.

5.3.6 Assessment 3: Anthropometric, SBP, HRV and Cardiovascular fitness

Athropometric data

Upon entering the exercise lab, participants were assessed for height (Mechanical Stadiometer, Surgical & Medical Products, Mentone, Australia) and body composition (InBody230, Biospace Co. Ltd., Seoul, South Korea), including total body fat percentage and lean muscle mass.

Blood pressure.

Resting blood pressure was assessed during the supine rest period of the HRV analysis. To ensure an accurate average resting blood pressure recording was captured, the average of two blood pressure (Omron, HEM-907XL, Matsuzaka City, Japan) measurements, one min apart were taken following 10 min, and 16 min of supine rest respectively. The reported systolic blood pressure was the average of the 10 and 16 min recordings.

Heart rate variability analysis.

Heart rate variability is the assessment of the beat – to – beat variation between the R – R intervals (RR interval) of a heart rate (HR) recording, and reduced HRV has been correlated with impaired health or risk of future cardiovascular disease (Tsuji et al., 1996). An analysis of this beat-to-beat variation reflects the influence of the parasympathetic and sympathetic branches of the autonomic control of the heart rhythm. A lower variation (or lower HRV) reflects more sympathetic activity, and higher variation (or higher HRV) reflects greater parasympathetic activity, or sympathetic withdrawal (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). Details regarding the measurement and assessment of HRV have been reported in detail in the previous chapter, and therefore will not be repeated here.

Briefly, alterations in the RR intervals were detected using a Polar transmitter belt (Wearlink W.I.N.D, Polar Electro Oy, Kempele, Finland) applied with contact electrode gel (Signa Crème®, Electrode cream, Parker Laboratories Inc. Fairfield, New Jersey, USA) to improve skin contact, and relayed and stored on a HR watch (RS800CX, Polar Electro Oy, Kempele, Finland).

The participant rested in a supine position for 10 min while they breathed freely, followed by 6 min of paced breathing (12 breaths.min^{-1} or 0.2 Hz). As the heart rate recording was started as the participant began the rest period, minutes 5 to 10 of the recorded trace were used as the spontaneous breathing HRV data. The 5 min portion 30 s following the start of the controlled breathing period was selected for analysis. Selected heart rate data were filtered for ectopic beats ('moderate filter power', minimum protection zone of 6 beat·min^{-1} and 'remove spikes' selected) and analysed using Polar ProTrainer software (Version 5.40.172). Parasympathetic activity was measured using the root mean square of successive differences (rMSSD) and the RR interval (Task Force of The

European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996) under spontaneous and controlled breathing. Unfortunately an insufficient number of participants (10 in IHE3 + Ex, but only 6 in Ex) were able to breathe in time to the metronome satisfactorily (breathing frequency of 12 $\pm$ 1; and a standard deviation for the breathing frequency of < 1.5) on more than 2 occasions and therefore the paced results were not analysed. The standard deviation of all normal-to-normal beats (SDNN, R-peaks can be referred to normal, or N peaks following editing procedures to denote the differences between filtered and unfiltered data). Low frequency (LF) and high frequency data were also reported to enable greater comparability with other studies. However as we have found these measurements to be less reliable in the population under investigation, these are not used as primary indicators of HRV alteration in the pre- and post-intervention discussion.

The median absolute deviation (MAD) method was used to detect outliers (Leys et al., 2013) from the combined groups in order to eliminate inflated HRV results that may be related to recording error (Wallén et al., 2012). The MAD method for outlier detection is more resistant to the effects of outliers than protocols relying on the mean $\pm$ SD (Wilcox, 2010). All data 2.5 X the SD as estimated from the median were considered outliers and were removed from the dataset (Leys et al., 2013).

Cardiovascular fitness.

Maximal oxygen uptake assessments were conducted using an incremental treadmill test to exhaustion. Two additional stages were added to the Standard Bruce Protocol to cater for the older, more sedentary population in this study. The protocol for this Modified Bruce Protocol (MBP) is outlined in Table 13.

Table 13: Modified Bruce Protocol

Stage	**Time (min)**	**Elevation (degrees)**	**Speed (km/h)**
1	1 - 3	0	2.7
2	4 – 6	2.8	2.7
3	7 – 9	5.7	2.7
4	10 – 12	6.8	4
5	13 – 15	7.9	5.5
6	16 – 18	9.1	6.8
7	19 – 21	10.2	8

Every three minutes the workload was increased until the participants reached voluntary exhaustion. The third stage of the Modified Bruce Protocol corresponds to the first stage of the standard Bruce Protocol, thereafter the protocols are identical.

Maximal oxygen consumption was assessed using a metabolic cart (MetaMax® 3B; Cortex Biophysik, Leipzig, Germany) which was calibrated daily for volume (Hans Rudolph 5530 3 L syringe; Kansas City, MO, USA), and between each testing period for gas composition (15% O_2 and 5% CO_2). The oxygen sensor was calibrated against ambient air prior to each participant's testing session. Participants were fitted with leak proof face masks (Hans Rudolph, Kansas City, MO, USA) with a small dead space (approximately 70 mL) that allowed breathing through the nose or mouth. Expired breath was directed to the volume and gas analysers which were housed in plastic fitting attached to the face mask. The same Polar HR monitor and belt used in the HRV assessment were used to record maximum HR during the fitness assessment. The participant was supervised continuously throughout the MBP and the test was stopped immediately if any contra-indications to exercise, as outlined in the American College of Sports Medicine guidelines for exercise testing and prescription, became evident (Thompson et al., 2010). Maximal exertion was achieved when the participant satisfied 2 or more of the following criteria: HR > 90% of age-predicted HR max (220 – age); respiratory exchange ratio of >1.15; a rating of perceived exertion of 19 or 20 on the Borg Scale, or the participant wished to stop due to exhaustion. A 5 min warm down at Stage 1 intensity followed. Participants were supervised until the researcher was satisfied that they had recovered sufficiently.

All breath – to – breath data were collected and exported to an excel spreadsheet for analysis. The last 30 s prior to maximal exhaustion were used to determine $\dot{V}O_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$). Time to exhaustion was measured as the taken from the start of Stage 1 to voluntary exhaustion (min).

Determination of cardiovascular risk

Cardiovascular risk was assessed using 2 different risk profiling assessments. The first was assessed using the New Zealand Guidelines Group 5- year risk stratification. In this analysis systolic blood pressure, TC, HDL, age, diabetes status and sex were used to determine each participant's overall 5 – year cardiovascular risk (See Appendix E). Following the risk assessment, participants were divided into 4 different categories:

- >2.5 % chance in the next 5 years
- 2.5 – 5 % chance in the next 5 years
- 5 – 10 % chance in the next 5 years
- 10 – 15 % chance in the next 5 years

The second analysis used the Framingham overall cardiovascular risk assessment to determine 10 year risk. In this assessment, each risk factor is awarded either positive (for adverse health measurements such as high blood pressure) or negative points for beneficial health indicators (such as low blood pressure). The overall points accumulated are then associated with a percentage risk for general cardiovascular disease in the next 10 years (D'Agostino et al., 2008). Risk factors included in

the Framingham Risk Assessment include age, sex, TC, HDL, SBP (and whether this is treated or not) diabetes status and smoking status.

5.3.7 10 week intervention.

All participants attended 3 exercise sessions per week. Exercise sessions included a mix of aerobic and strength training and typically included a 10 minute warm-up (65 – 70 % of HR maximum as assessed during the $\dot{V}O_{2peak}$ assessment), followed by another 15 - 20 minutes of walking or jogging at 70 – 80% of maximum HR in Week 1, and thereafter at 75 – 80% of maximum HR. Participants then completed 20 minutes of strength training which targeted major muscle groups and included free weights (bicep and tricep curls, anterior and lateral raises) resistance bands (as an alternative to free weights), lunges, calf raises, sit-ups, back extension exercises; lateral, front and back straight leg raises, and stepping up and down on a step. Where participants had physiological restrictions to prescribed exercises, alternative exercises were provided. The exercise sessions were concluded with 10 minutes of light aerobic activity and stretching. All exercise sessions were supervised and the participants were encouraged to communicate with the lead researcher regarding any injury, or discomfort experienced in any of the prescribed training exercises or intensities.

In addition to the above training programme, half of the participants completed 2 – 3 simulated altitude training sessions per week using the IHE protocol of 5 min hypoxia alternated with 5 min normoxia for 1 h. Hypoxic air was delivered through a hand-held face mask (QuadraLite Facemask, Intersurgical, Intermed, Auckland, New Zealand) fitted with an antibacterial filter (HMEF Clear-therm, Intersurgical, Intermed, Auckland, New Zealand). Each participant had their own face masks and antibacterial filter, and all facemasks and filters were discarded at the end of the intervention period. Hypoxic air (Era®-II Hypoxic Air Generator, GO2Altitude, Biomedtech, Victoria, Australia) was delivered to a main control computer which allowed the researcher to control the fraction of inspired oxygen (FiO_2). Hypoxic dose was monitored using SpO_2 measured at the finger with a pulse oximeter (Nonin Pulse Oximeter, Plymouth, Minnesota). The FiO_2 was progressively lowered to yield the following SpO_2 targets: Week 1: 90%, Week 2: 85%, and Week 3 - 10: 80%.

5.3.8 Statistical analyses.

A mixed modelling procedure (Proc Mixed) was used to analyse the repeated measures (Statistical Analysis System, v. 9.3, SAS Institute, Cary, NC). To reduce the effects of non-uniformity of error, the natural logarithm of each dependent variable was used in the data analysis (Hopkins et al., 2009). Fixed effects were the time points (Baseline, Post, 4-wk, 8-wk) and the group (IHE3 + Ex and Ex) and their interaction. Dependent variables included, SBP, $\dot{V}O_{2peak}$, Time to exhaustion, PWV, PWA, HDL, TC, and HDL:TC ratio and Framingham Risk Assessment Score. Random-effects parameters, or

variance components, were also included in the model as covariance components, and were estimated using the restricted maximum likelihood (REML) method. A mixed (including both fixed and variance components) linear model was then fitted to the data which allowed greater statistical inference that is resistant to unbalanced data (SAS Institute Inc., 2008).

Effect estimates and p-values were then converted to magnitude-based inferential statements using a spreadsheet (Hopkins, 2007). Changes from baseline to Post, 4-wk and 8-wk in the IHE3 + Ex group were compared to changes in Ex only using the smallest worthwhile change identified by Cohen (1988), that is, 0.2 multiplied by the between subject SD at baseline.

Clinical assessments were performed in parameters that have a direct effect on health or performance, which included all variables except HRV in this study. As the outcome of the clinical parameter will have a direct effect on health, the risk of harm was considered after the likely range of the true value (90% confidence interval) had been estimated. Results were only considered beneficial when the potential for benefit was substantially greater than the risk of harm. In this way avoiding the risk of harm, rather than neglecting to use a beneficial effect was prioritised (Hopkins et al., 2009, Hopkins, 2007). For this study a benefit: harm ratio of > 66% was considered acceptable. Where the clinical effect was unclear, but there was a clear mechanistic outcome, the mechanistic outcome was reported in brackets.

Heart rate variability was included as a mechanistic variable as changes in this measurement can relate to stimuli unrelated to risk stratification. Unlike the clinical assessments, the mechanistic inference simply relates to where the likely range of the true value lies relative to the smallest worthwhile change (Batterham and Hopkins, 2006), and does not consider the potential for harm.

No statistical analyses were performed on the ordinal categorical data retrieved from the 2 groups across the 3 time points from the New Zealand Guidelines risk assessment chart. Instead the outcomes of these analyses have been presented in pie charts to clearly represent changes between risk categories at each assessment.

For both the clinical and mechanistic assessments, the likely range of the true value is expressed using the percentage change (from the natural logarithm) ± 90 % confidence interval, and the percent chances for substantially increased / trivial / decreased outcomes (Hopkins, 2007). Where the confidence interval spanned all three possibilities (increased, trivial and decreased), the result was deemed unclear. In all other cases (the confidence interval spans either 1, or a maximum of 2 possibilities, i.e. increased/trivial, or decreased/trivial), a clear outcome was reported and a qualitative assessment of the magnitude of the change was given. To determine the qualitative outcome the following scale was used: < 0.5% = most unlikely; 0.5 – 5% = very unlikely; 5 – 25% =

unlikely; 25 – 75% = possibly; 75 – 95% = likely, or 95 – 99.5% = very likely, > 99.5% = most likely). For clinical analyses, the descriptors “Beneficial”, “trivial”, “harmful” were used, and for mechanistic analyses, the descriptors, “Increased”, “trivial” or “decreased” were used. Results are presented as: mean % change (from the natural log) ± 90% confidence interval; % chance that the change substantially increased/trivial/decreased; followed by the qualitative outcome. See Table 14 for an overview of participants excluded from analyses and the motivations for exclusion.

5.4 Results

Food and activity diaries were examined following the 10-week intervention, and participants did not deviate substantially from their habitual food intake and physical activity. Raw data (mean ± SD) are presented in Table 24 in Appendix D. Results for anthropometric data (body fat % and muscle mass) are presented in Table 12. Independent risk factors (PWV, PWA, and cardiovascular fitness, blood lipids, SBP and overall cardiovascular risk) have been presented in Table 15. HRV changes have been reported in Table 16, while changes in fat and muscle mass have been reported in Table 17. Changes in cardiovascular risk category are presented in Figure 15.

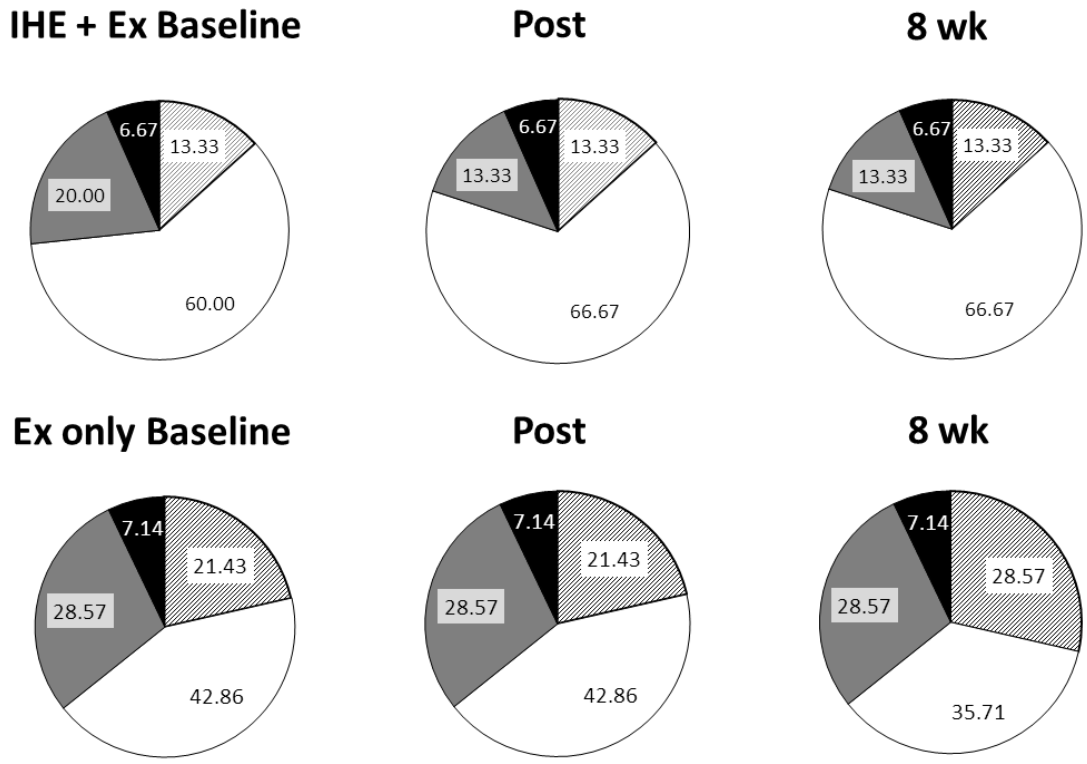


Figure 15: 5-year cardiovascular risk assessed using the New Zealand Guidelines chart
Pie graphs representing the percentage of participants in pre-defined risk categories as defined by the New Zealand Guidelines Group. Striped portions indicate <2.5% risk; White portions indicate between 2.5 – 5% risk of cardiovascular disease in the next 5 years; Grey portions indicate 5 – 10% risk; and Black portions indicate 10 – 15% risk of cardiovascular disease in the next 10 years.

Table 14: Number of participants excluded from respective analyses

	IHE3 + Ex (n)	Ex (n)	Reason for exclusion
Anthropometric data			
Body fat (%)	0	0	
Muscle mass (kg)	0	0	
Arterial Health			
Pulse wave velocity (ms)	6	3	Unavailability at the time of recording:
Pulse wave analysis (AIx)	5	3	PWV & PWA: IHE3 + Ex: n = 4; Ex: n = 1 PWV: > 2 Inconclusive results: IHE3 + Ex: n = 2; Ex: n = 4 PWA > 2 Inconclusive results: IHE3 + Ex: n = 1; Ex: n = 5
Heart rate variability			
RR interval (ms)	1	4	RR- Interval & rMSSD: Unclear recording: IHE3 + Ex: n = 1; Ex: n = 4.
rMSSD (ms)			
Cardiovascular fitness			
$\dot{V}O_{2peak}$ *(ml·min^{-1}·kg^{-1}) &* *Time to exhaustion (min)*	1	3	Test terminated due to hypertension: IHE3 + Ex: n = 1; Ex: n = 2
Blood lipids			
TC, HDL & TC:HDL	1	3	Elected not to have blood samples drawn: IHE3 + Ex: n = 1; Ex: n = 3. An additional 2 (forgot/ not available) in Ex and 5(2 excluded due to lipid lowering drugs between measurements, 3 forgot/not available) in IHE3 + Ex were unable to submit blood samples at the 8-wk follow up.
Systolic blood pressure			
SBP	1	1	On hypertension medication but BP fluctuated substantially between time points: IHE3 + Ex: n = 1; Ex: n = 1
Cardiovascular risk			
NZGG & Framingham assessments	1	4	Missing either SBP and / or blood sample

All data are reported as a mean change from baseline in IHE3 + Ex relative to the Ex group (i.e. IHE3 + Ex – Ex at each time point). IHE3 + Ex: Group receiving intermittent hypoxic exposure and exercise during the intervention period. Ex: Group receiving exercise only during the intervention period. PWA: pulse wave analysis; PWV: Pulse wave velocity; RR interval: average distance between R – R peaks; rMSSD: root mean square successive difference; $\dot{V}O_{2peak}$: peak oxygen uptake during exercise to exhaustion; TC: Total cholesterol; HDL: High density lipoprotein; TC: HDL: Total cholesterol to high density lipoprotein ratio; SBP: Systolic blood pressure; NZGG assessment: 5 – year risk assessment according to the New Zealand Guidelines Group.

Table 15: Baseline, Post, 4-wk and 8-wk analyses for independent risk factors and overall cardiovascular risk

	Mean change (%) ± 90% confidence interval	Chances of increase/ trivial /decrease	Qualitative outcome
Arterial Health			
Pulse wave velocity (ms)			
Post – baseline	-0.25 ± 11.2	47 / 3 / 50	Unclear
4-wk – baseline	-4.2 ± 11.5	26 / 2 / 72	Unclear
8-wk – baseline	-8.1 ± 12.1	13 / 1 / 86	Unclear
Pulse wave analysis (AIx)			
Post – baseline	-6.1 ± 18.4	24 / 11 / 65	Unclear
4-wk – baseline	11.8 ± 18.4	82 / 7 / 11	Likely harmful
8-wk – baseline	24.8 ± 19.7	97 / 1 / 2	Very likely harmful
Cardiovascular fitness			
$\dot{V}O_{2peak}$ *(ml·min^{-1}·kg^{-1})*			
Post – baseline	3.1 ± 7.7	65 / 17 / 18	Unclear
4-wk – baseline	9.4 ± 8.0	95 / 3 / 2	Very likely beneficial
8-wk – baseline	7.9 ± 8.3	90 / 6 / 4	Likely beneficial
Time to exhaustion (min)			
Post – baseline	3.8 ± 6.3	81 / 5 / 13	Unclear
4-wk – baseline	-1.3 ± 6.5	33 / 8 / 59	Unclear
8-wk – baseline	5.0 ± 6.8	87 / 4 / 9	Unclear
Blood lipids			
Total cholesterol			
Post – baseline	4.4 ± 6.5	86 / 2 / 12	Likely harmful
8-wk – baseline	6.5 ± 7.2	93 / 1 / 6	Likely harmful
High density lipoprotein			
Post – baseline	8.0 ± 8.0	95 / 0 / 5	Likely beneficial
8-wk – baseline	10.0 ± 8.5	97 / 0 / 3	Very likely beneficial
Total cholesterol: High density lipoprotein ratio			
Post – baseline	-77.2 ± 50.5	1 / 0 / 99	Very likely beneficial
8-wk – baseline	-24.7 ± 52.4	21 / 1 / 78	Unclear
Systolic blood pressure			
SBP (mmHg)			
Post – baseline	-3.4 ± 3.4	0 / 43 / 57	Possibly beneficial
4-wk – baseline	0.5 ± 3.5	12 / 83 / 5	Possibly trivial
8-wk – baseline	-3.5 ± 3.7	0 / 42 / 58	Possibly beneficial
Cardiovascular risk			
Framingham assessment			
Post – baseline	-3.1 ± 15.1	30 / 13 / 57	Unclear
8-wk – baseline	6.6 ± 16.9	69 / 10 / 21	Possibly harmful

All data are reported as a mean change from baseline in the group receiving exercise and intermittent hypoxic exposure relative to the group receiving exercise only. Post, 4-wk and 8-wk: measurements taken immediately, 4-wk and 8-wk following the intervention compared to baseline assessments; AIx: Augmentation index; $\dot{V}O_{2peak}$: peak oxygen uptake during maximal exercise; SBP: Systolic blood pressure.

Table 16: Changes in HRV between baseline and post - intervention measurements

	Mean change (%) ± 90% confidence interval	Chances of increase/ trivial /decrease	Qualitative outcome
Heart rate variability			
RR interval (ms)			
Post – baseline	3.1 ± 5.1	0 / 100 / 0	M: Most likely trivial
4-wk – baseline	0.03 ± 5.3	0 / 100 / 0	M: Most likely trivial
8-wk – baseline	3.4 ± 5.4	0 / 100 / 0	M: Most likely trivial
rMSSD (ms)			
Post – baseline	18.0 ± 24.0	87 / 5 / 8	M: Unclear
4-wk – baseline	14.0 ± 25.2	78 / 7 / 15	M: Unclear
8-wk – baseline	13.0 ± 25.6	76 / 7 / 17	M: Unclear
SDNN (ms)			
Post – baseline	1.7 ± 23.1	48 / 12 / 39	M: Unclear
4-wk – baseline	-9.9 ± 23.9	20 / 10 / 70	M: Unclear
8-wk – baseline	0.9 ± 24.9	46 / 12 / 42	M: Unclear
HF (ms^2)			
Post – baseline	52.7 ± 50.0	69 / 31 / 0	M: Possibly increased
4-wk – baseline	31.1 ± 52.9	41 / 57 / 2	M: Possibly trivial
8-wk – baseline	31.7 ± 53.1	42 / 56 / 2	M: Possibly trivial
LF (ms^2)			
Post – baseline	-11.0 ± 51.5	4 / 81 / 15	M: Likely trivial
4-wk – baseline	27.9 ± 54.2	31 / 67 / 2	M: Possibly trivial
8-wk – baseline	7.4 ± 54.9	14 / 80 / 6	M: Unclear
LF:HF			
Post – baseline	-86.4 ± 56.2	0 / 1 / 99	M: Very likely decreased
4-wk – baseline	-15.0 ± 58.3	23 / 22 / 55	M: Unclear
8-wk – baseline	1.3 ± 59.6	40 / 23 / 37	M: Unclear

Data are mean change from baseline in IHE3 + Ex relative to the Ex group (i.e. IHE3 + Ex – Ex at each time point; Post, 4-wk, 8-wk: Measurements taken immediately after, 4- and 8-wk following the intervention; M: mechanistic inference; RR interval: average distance between R – R peaks; rMSSD: root mean square successive difference; SDNN: Standard deviation of normal to normal peaks; HF: high frequency; LF: low frequency; LF:HF: low frequency: high frequency ratio (LF/HF*100)

Table 17: Changes in body fat % and muscle mass between baseline and Post, 4-wk and 8-wk

	Mean change (%) ± 90% confidence interval	Chances of increase/ trivial /decrease	Qualitative outcome
Muscle mass (kg)			
Post – baseline	1.4 ± 1.5	56 / 43 / 0	Possibly beneficial
4-wk – baseline	1.2 ± 1.5	46 / 53 / 0	Possibly beneficial
8-wk – baseline	0.6 ± 1.5	23 / 75 / 2	Unclear
Fat mass (kg)			
Post – baseline	-0.7 ± 3.9	12 / 59 / 29	Unclear
4-wk – baseline	2.3 ± 3.9	56 / 21 / 3	Possible harmful
8-wk – baseline	4.1 ± 4.0	81 / 18 / 1	Likely harmful

All data are reported as a mean change from baseline in IHE3 + Ex relative to the Ex group (i.e. IHE3 + Ex – Ex at each time point; Measurements taken immediately after, 4- and 8-wk following the intervention.

5.5 Discussion

The purpose of this study was to assess whether a small number of IHE sessions per week (2 – 3) over 10-week period would supplement an exercise training programme and yield greater reduction in independent cardiovascular risk factors and overall risk of cardiovascular disease than exercise alone. Contrary to our expectations, while there was a tendency for an increase in rMSSD in the IHE3 + Ex group compared to the Ex only group, there was no clear 'additive' effect of IHE on HRV. The key findings from this study are the greater improvements in HDL, SBP and $\dot{V}O_{2peak}$ following IHE3 + Ex when compared to exercise only. However, while the arterial stiffness as assessed using the augmentation index appeared to decrease slightly compared to Ex immediately post-intervention, there was a likely and very likely increase at the 4-wk and 8-wk follow up. While this could have been due to a decrease in the pulse pressure (attributed to the decrease in SBP in IHE3 + Ex) relative to the augmentation pressure, more research is needed on the long-term effects of IHE on vascular health before hypoxic-based intervention can be described as 'safe'.

5.5.1 Cardiovascular risk parameters and overall cardiovascular risk profile

Changes in the overall cardiovascular risk profiles were subtle. The proportion of participants in different risk categories (presented in pie graphs in Figure 15) suggest that both the Ex and the IHE3 + Ex groups demonstrate greater effectiveness in the 'middle' risk categories. That is, in the IHE3 + Ex group, there was a small change between the 2.5 – 5% category (increased) and the 5 – 10% category (decreased) at Post which persisted to the 8-wk assessment but there were no changes in portion size in either the 10 – 15% or the >2.5% categories following the 10-week intervention. Additionally, there were no changes in the Ex group between Post and Baseline, but there was an increase in the <2.5% risk portion, and a decrease in the 2.5 – 5% portion. Based on these observations, it appears that the 10-week intervention had a negligible effect on the most (<2.5% risk) and least (10-15% risk) healthy participants, but a possibly beneficial effect on those between the 2.5 and 10% risk categories. It is likely that below 2.5% risk, there is little more that can be done to improve overall risk (due to non-modifiable risk factors such as age and sex), and at higher risk, perhaps a more aggressive intervention is required before health benefit is observed. However, the sample size for each group were relatively small (IHE3 + Ex: n=15; and Ex: n = 14) and so each one person represents approximately 7%. Therefore caution should be used when drawing meaning from the pie charts. These results should be repeated in a larger cohort before conclusive statements can be made.

The absence of more substantial changes in the overall cardiovascular risk assessments is likely due to the magnitude of the changes needed before a change in risk category is observed (see Figure 31 in Appendix E). For example, each SBP increment in the New Zealand Guidelines chart spans 20 mmHg, and HDL:TC changes in 1 mmol/L increments. Indeed as the risk categories can include

several increments of HDL:TC and SBP, the 5-year risk assessment chart is unlikely to have been sensitive enough to detect smaller changes in overall cardiovascular risk as a result of a relatively short intervention.

Changes in 10-year risk of cardiovascular disease as determined using the Framingham study indicated no clear benefit of one intervention over the other at Post-intervention, but there was a possibly harmful effect of using IHE3 + Ex in the 8-wk follow up. On closer inspection of the data, this increased risk score in the IHE3 + Ex group was primarily due to the increase in total cholesterol. As discussed below, the increase in total cholesterol was due to the increase in high density lipoprotein in the IHE3 + Ex group compared to Ex, and therefore, the relevance of the "possible harmful" outcome is limited.

Systolic blood pressure

The greater decrease in SBP at Post in the IHE3 + EX group compared to Ex supports earlier research by our lab where we have reported a tendency for a decrease in SBP following a 5-week IHE intervention (without additional exercise training). The decrease in SBP in our study may be related to several of the other findings in this study, particularly the tendencies for decreased PWV and increased rMSSD.

The close relationship between SBP and PWV has long been investigated (Gribbin et al., 1976), with conventional wisdom suggesting that increased SBP is the cause and the correlated increase in PWV is simply the reflection of the arterial dysfunction associated with the sustained strain on the arterial system (Franklin, 2005). As highlighted by Franklin (2005), the longitudinal study by Dernellis and Panaretou (2005), and the more recent study by Najjar et al. (2008) have challenged this assumption by indicating that the increase in PWV may precede the increase in SBP. The predictive ability of increased PWV in normotensive individuals suggests that PWV is the risk factor, rather than the risk marker. Therefore, the unclear decrease in PWV in the IHE3 + Ex group in the current study may have contributed to the decrease in SBP in the same group. Alternatively, both the lowered SBP and PWV may be related to the tendency towards an increase in the parasympathetic component (rMSSD), or sympathetic withdrawal in the autonomic nervous system (Franklin, 2009, Palatini and Julius, 2004). That is, as both SBP (Guyenet, 2006) and arterial compliance (Grassi et al., 1995, Boutouyrie et al., 1994) are subject to autonomic control. The contribution of sympathetic activity on both the SBP and the PWV was recently demonstrated in patients with resistant hypertension who underwent renal sympathetic denervation (Brandt et al., 2012). Following the renal denervation, patients demonstrated substantial reductions in both blood pressure and arterial stiffness. Therefore, the tendency towards improved sympathovagal balance (unclear increase in rMSSD) in the IHE3 + Ex may also have attributed to the lower SBP in the IHE3 + Ex group. While the improvements in rMSSD and

PWV were unclear, the combined interaction of these variables likely contributed to the clear improvement in SBP in the IHE3 + Ex group compared to Ex.

Total cholesterol and high density lipoprotein

Tin'kov and Aksenov (2002) posit that the modification of blood lipid profile by IHE is attributable to greater oxidation in the monooxygenase pathway in cholesterol biosynthesis. To support this claim, these authors report an increase in the activity of 7-α-hydroxylase, an important initiating and rate limiting enzyme in the catabolism of cholesterol into bile salts (Russell, 2009). However, no data other than changes in HDL, TC, low and very low density lipoprotein and triglycerides were reported. Contrastingly, Johnson (2008) outlines a possible inhibiting action of hypoxia on cholesterol 7-α-hydroxylase in patients with obstructive sleep apnoea. Johnson (2008) suggests that the shift to glycolytic metabolism in response to the repeated hypoxic intervals associated with obstructive sleep apnoea promotes an increase in nicotinamide adenine dinucleotide + hydrogen (NADH) which has an inhibitory effect on Sirtuin. In turn, the inhibitory action of Sirtuin decreases active proliferator-activated receptor-ɣ co-activator-1 α which is needed for co-activation of cholesterol 7-α-hydroxylase. In this view, IHE would result in a decreased expression of cholesterol 7-α-hydroxylase and inhibition of the conversion of cholesterol into bile salts. However, there are several physiological responses to obstructive sleep apnoea that appear reversed following shorter, controlled periods of IHE, such the increase in SBP associated with obstructive sleep apnoea (Møller et al., 2003), but not with IHE (Serebrovskaya et al., 2008). The differences in responses are usually thought to be due to an adaptive response to the short term stress (in the case of IHE), rather than maladaptation to long-term distress (in obstructive sleep apnoea). Therefore, it is unclear whether the pathway described by Johnson (2008) would have a negative effect on cholesterol metabolism following IHE, or a short term inhibition of cholestrol catabolism following IHE, followed by a compensatary 'catch up' during recovery.

At first glance, the increase in the HDL in the IHE3 + Ex (0.1 mmol/L increase at Post which was sustained at 8-wk follow up, compared to no change in Ex) appears to support the findings of Tin'kov and Aksenov (2002) who reported an increase of 0.08 mmol/L following 22 days of 3 h hypoxic exposure intervals, with a further 0.05 mmol/L increase at the 3 month follow up. However, upon closer examination of the TC:HDL results in the present study, a different conclusion can be reached for our participants. That is the overall decrease in TC in Ex was substantially higher than IHE3 + Ex (0.3 mmol/L immediately following the intervention in the Ex group of the present study, and a further decrease of 0.1 mmol/L at the 8-wk follow up, compared to no change in IHE3 + Ex at Post and a decrease of 0.1 mmol/L at 8-wk follow up). Therefore, it appears that the likely proportion of non-HDL cholesterol was lower in the Ex group compared to the IHE3 + Ex group following the intervention, and more supportive of the inhibitory mechanism proposed by Johnson (2008). As

Tin'kov and Aksenov (2002) did not use a control group, it is possible that the decrease in TC and increase in HDL that these researchers observed was due to normal fluctuations in blood lipid profile associated with a change in season (Ockene et al., 2004) rather than the influence of hypoxia. However, in the current controlled study, participants in the IHE3 + Ex group demonstrated a likely beneficial increase in HDL at the Post-intervention assessment, compared to no change in the Ex group. As these participants were traversing the seasons simultaneously, it appears that IHE may indeed facilitate an increase in HDL. It is likely that this increase in HDL was also responsible for the 'possibly harmful' response in TC, and the very likely beneficial response in the TC:HDL ratio.

It is important to note that there were a number of participants in this study who were unable to complete the 8-wk follow up (5 in the IHE3 + Ex group and 2 in the Ex group). This was either due to missing or erroneous data, see Table 14.

While a modelling-based statistical analysis was utilised to reduce the impact of missing values, there is no doubt that the integrity of a full dataset is lacking. Therefore the results of the 8-wk follow up may not reflect the true change between groups. In this regard, the results of this study do not help to clarify whether there is a beneficial long-term effect of IHE on blood lipid profile as proposed by Tin'kov and Aksenov (2002), or a negative response as proposed by Johnson (2008).

5.5.2 Arterial stiffness

While pulse wave velocity and pulse wave analysis (specifically, the augmentation index), are both measures of arterial stiffness, they cannot be used interchangeably. Pulse wave velocity is a measure of the speed of the pressure wave along the artery (measured as the distance (mm) divided by the transit time (s)). The PWV reflects the stiffness of the segment of the artery that has been measured (so, as the brachial artery was assessed in the current study, we can only make inferences regarding the arterial stiffness of the upper body), and is inversely related to the arterial distensibility (Van Bortel et al., 2002). An increase in the arterial stiffness is usually caused by an increase in collagen, a decrease in elastin and endothelial dysfunction which can occur with increases in inflammation or arterial pressure (Zieman et al., 2005, Wang et al., 2008). On the other hand, the augmentation index is a composite measurement (derived using the ratio of the pulse pressure and the augmentation pressure) that reflects systemic arterial stiffness. Therefore, the augmentation index is influenced by both the central arteries which influence the speed of the pulse wave (i.e. the PWV), and factors influencing the peripheral conduit arteries (which then alter the reflected wave) such as peripheral vascular tone (Kelly et al., 2001).

Several researchers have reported an improvement in arterial stiffness (using PWV) following exercise in a hypoxic environment compared to normoxic exercise (Nishiwaki et al., 2011, Shi et al.,

2013) which suggests that a hypoxic stimulus enhances the effect of exercise alone on arterial health. In the presence of hypoxia, HIF-1α triggers an increased expression of endothelial NO synthase which, via several steps, initiates a vasodilatory response (Beall et al., 2012). The exogenous application of glyceryl trinitrate (which is metabolised to NO in the vascular wall) has also been found to decrease PWV (Wilkinson et al., 2002b). Conversely, inhibition of NO synthase (via the vasoconstrictor L-N^G-monomethyl arginine) has resulted in increased PWV and AIx (Wilkinson et al., 2002a). As endothelial function (and thus endothelium- derived NO control) is strongly correlated to arterial stiffness (McEniery et al., 2006), it is possible that NO plays an important role in arterial stiffness. Therefore, the intermittent up-regulation of NO during the hypoxic exposure is likely to have had a beneficial effect on arterial compliance. Indeed, the vasodilatory effects of NO during hypoxic intervals may also account for the improvement in flow mediated dilation observed in the group training in hypobaric hypoxia compared to normobaric normoxia reported by Nishiwaki et al. (2011).

Pulse wave velocity in the current study

Pulse wave velocity decreased in both groups following the 10- week intervention. While the IHE3 + Ex group demonstrated a seemingly lower PWV at the 4-wk and 8-wk follow up testing period, there were no clear differences between IHE3 + Ex and Ex. This is contrary to other researchers who have reported a decrease in PWV in post-menopausal women (Nishiwaki et al., 2011) and in healthy men (Shi et al., 2013). The differences in the outcomes between our study and that of Nishiwaki et al. (2011) and Shi et al. (2013) could be due to either differences in the measurement sites or the hypoxic dosage.

Regarding the PWV measurements, both Nishiwaki and colleagues and Shi et al. (2013) used brachial–ankle PWV to assess arterial stiffness, compared to the carotid – radial PWV in our study. As the carotid-radial PWV reflects the arterial compliance of the upper body (specifically the brachial artery) and that the majority of the training completed by the participants in this study involved the lower limbs (walking and running in addition to leg strength, compared to arm strength exercise only), a clearer decrease in PWV may have been observed using a brachial – ankle PWV assessment.

Alternatively, the intensity of the hypoxic stimulus may be responsible for the different PWV outcomes between the current study and those by Nishiwaki and colleagues, and Shi et al. (2013). In the current study supplementary passive IHE was included in addition to a traditional normoxic exercise programme while the studies by Shi and colleagues and Nishiwaki and colleagues used exercise in a hypoxic environment. As exercising in hypoxia would provide a higher stress stimulus, the passive exposure to normobaric hypoxia for 1-h may have been insufficient to induce clear changes to endothelial function, or collagen and elastin regulation. Indeed, the differences and

similarities between these 3 studies meant that we can tease out some of the effects of the intervention length, hypoxic dosage and participants recruited on the relative effect of the simulated altitude on the PWV outcome. For example, the current study was a 10-week intervention with a 1-h passive hypoxic exposure which yielded an unclear PWV outcome in sedentary middle-aged participants. On the other hand, the participants in the study by Shi and colleagues used a 4-week intervention period in healthy young men, and while the exercise prescription was similar to the current study, it was completed in hypoxia followed by an additional 30 min of passive hypoxic exposure. The participants in the study by Nishiwaki et al. (2011) had arguably the highest dose of hypoxia and was run over a similar period of time to the current study (10-weeks in the current study, and 8-weeks in the study by Nishiwaki and colleagues) and with similar participants to the current study. In the intervention by Nishiwaki and colleagues, a combination of exercise in hypobaric hypoxia with an additional 90 min of passive hypobaric hypoxic exposure for an 8-week intervention period was used. Taken together, these differences suggest that the hypoxic dosage, rather than the intervention length (longer than 4 wk) has a greater influence on the PWV.

Augmentation index in the current study

Immediately following the 10-wk intervention there was a qualitatively unclear decrease in the augmentation index in the IHE3 + Ex group compared to the Ex. It is possible that an increase in vascular endothelial growth factor in response to HIF-1α activation resulted in angiogenesis and therefore increased absorption of the pressure waves, and an associated reduction in AIx. However, Geiser et al. (2001) only reported an increase in capillary length density (length of capillary in 1 mm^3) following high intensity exercise in hypoxia, and not in moderate-intensity exercise (let alone passive hypoxic exposure). Therefore, it is unlikely, that angiogenesis would be responsible for the reduction in the post-intervention AIx, and the actions of NO (as discussed above) provide a more likely explanation for any alterations in vascular compliance.

However, in the 4- and 8-wk follow up assessments, the augmentation index was reversed (i.e. increased) in the IHE3 + Ex group compared to Ex suggesting a possible mal-adaption to the hypoxic intervention. These results are intriguing as they appear to conflict with the other measured responses to IHE (such as the decrease in SBP and unclear improvements in rMSSD and PWV in the IHE3 + Ex compared to Ex).

It is possible that the increase in augmentation index could be due to a stiffening of the smaller arterioles resulting in a larger intensity of the reflected wave (Kelly et al., 2001). Speculatively, an up-regulation of the endothelial NO synthase pathways during the intervention may have resulted in down-regulation of endothelial NO synthase activity in the post-intervention phase, and subsequently increased arterial vasoconstriction. Kelly et al. (2001) suggest that as the central

arteries are predominantly elastic, acute increases in vascular tone may not be readily detected with PWV, but would increase the intensity of the reflected wave and in so doing, increase AIx. As such, while the immediate effects of IHE on arterial stiffness are promising, future research should examine the longevity of changes associated with arterial health (particularly regarding the AIx) that are associated with IHE or hypoxic exercise. In addition PWV measurements should be tailored to the primary segment involved in the intervention-based training (i.e. carotid-radial in upper-body interventions, and carotid-ankle for lower-body intervention, or carotid-femoral for risk stratification) to get a better indication of overall changes in vascular health.

5.5.3 Heart rate variability

Earlier research by our lab detected improvements in HRV following a 4- and 5-week IHE intervention. In these studies we suggested that the increase in parasympathetic activity demonstrated by the participants receiving IHE treatments was due to improved stress tolerance conferred via the repeated parasympathetic withdrawal and reactivation between hypoxic intervals. Therefore, we expected that the addition of IHE to an exercise programme would have an additive effect on the post- intervention HRV compared to the group receiving exercise only. However, while rMSSD did appear to increase in the IHE3 + Ex group more so than Ex immediately post- intervention, this was effect was unclear.

The absence of any clear advantage of IHE +Ex compared to Ex on HRV may be related to the small dosage of hypoxia. Indeed, in our earlier 5-week study, the improvement in HRV in the group receiving IHE 2 – 3 times per week was only evident under controlled breathing conditions. As exercise has also been known to increase the RR interval and rMSSD (Jurca et al., 2004, Sandercock et al., 2005a), the exercise stimulus may have out-weighed any clear additional benefit of IHE to the parasympathetic activity of the nervous system.

It is also important to consider the wide variability associated with HRV, particularly in individuals with compromised health (Sandercock et al., 2005b). To address this variability, recent research has demonstrated far clearer and more reliable results when the HRV is measured over the course of a week and reported as a weekly average, rather than using a single measurement (Le Meur et al., 2013, Plews et al., 2013). Therefore, future research regarding the use of IHE in a sedentary population should consider using the average of a week's worth of HRV data, rather than a single measurement, in order to elucidate any beneficial (or harmful) effects of IHE on HRV.

5.5.4 Cardiovascular fitness

The IHE3 + Ex group showed a small increase in $\dot{V}O_{2peak}$ at each of the time points (except at the 8-wk follow up where $\dot{V}O_{2peak}$ returned to baseline). On the other hand, the Ex group remained unchanged

between Baseline and Post, increased at 4-wk follow up but then decreased to below the baseline value at the 8-wk follow up. We had anticipated a larger increase in the $\dot{V}O_{2peak}$ in both groups as a result of the combined action of the intervention (Murias et al., 2010) and the seasonal change from winter to spring (Ingemann-Hansen and HalkjÆr-Kristensen, 1982). Additionally, the decrease to below baseline in the 8-wk assessment was also surprising and is difficult to account for as any alterations in environmental or testing conditions would have also affected the IHE3 + Ex group. One would anticipate a decrease in $\dot{V}O_{2peak}$ following a time interval of more than 2 weeks of inactivity (Neufer, 1989) but this detraining effect is typically halted at approximately baseline values. One possible explanation for the substantially lower $\dot{V}O_{2peak}$ in the Ex group could be attributed to participant motivation (or lack thereof). For example, Andreacci et al. (2002) demonstrated an increase in $\dot{V}O_{2peak}$ of approximately 5 $ml \cdot kg^{-1} \cdot min^{-1}$ when participants were given frequent verbal encouragement compared to when they received no or infrequent encouragement. Despite an effort to maintain frequent and similar encouragement for all participants during all maximal exercise tests in the current study, if the participants in the Ex group were intrinsically less motivated at the final follow up assessment, this could explain the lower $\dot{V}O_{2peak}$. The counter-argument to this explanation is that there was not an associated decrease in the time taken to complete the test, which means that motivation as an explanation for the decrease in $\dot{V}O_{2peak}$ in the Ex group is unsatisfactory.

Nevertheless, the difference between the groups, while small, indicates an additive effect of the IHE on peak oxygen uptake compared to exercise alone. The improvement in $\dot{V}O_{2peak}$ in the IHE3 + Ex group could be attributed to haematological adaptation, such as an increase in haemoglobin, acting to increase the oxygen-carrying capacity of the blood, as has been reported following other IHE-based interventions (Burtsher et al., 2004) and in our earlier study (following 5 IHE sessions per week for 5 weeks, see Chapter 4, Study 2). While there were no clear increases in haemoglobin in the participants attending 2-3 IHE sessions per week in our earlier study, the inclusion of exercise into the current study may have produced sufficient oxidative stress to induce erythropoiesis in the IHE3 + Ex group. Despite the statistically beneficial outcome of the IHE3 + Ex on $\dot{V}O_{2peak}$, there were no clear changes between groups in the time taken to complete the exercise tests. The absence of a clear change in time to complete the test questions the practical benefit of the relative $\dot{V}O_{2peak}$ increase in the IHE3 + Ex group.

5.5.5 Fat mass and muscle mass

When one is exposed to hypoxia, oxygen sensors such as HIF-1α act to reduce the dependency of the body on oxygen-dependent systems such as oxidative phosphorylation. This requires a metabolic shift to anaerobic glycolytic pathways that do not require the break-down of fats for energy provision. In addition, the active cellular switch to glycolysis avoids the accumulation of excessive levels of reactive oxygen species that occur under oxidative stress (Semenza, 2009). Additionally, exposure to hypoxia up-regulates the leptin gene which is associated with appetite suppression (Ambrosini et al., 2002). The alteration in metabolism and the propensity for reduced caloric intake has prompted several researchers to assess the effects of exercise training in hypoxia on body composition (Haufe et al., 2008, Netzer et al., 2008, Wiesner et al., 2010). One of these studies, like the current study, assessed the effect of passive IHE in conjunction with exercise (Balykin et al., 2004). All of the above-mentioned studies have reported greater improvements in body fat mass reduction following exercise in hypoxia compared to normoxic exercise.

While both groups decreased body fat mass over the course of the 10-week intervention, and continued to decrease until the 4-wk follow up, the declines in body fat were substantially greater in the Ex group than the IHE3 + Ex group. Additionally, the IHE3 + Ex group had returned to baseline levels at the 8-wk follow up whereas the Ex group continued to decrease. Therefore, unlike (Balykin et al., 2004) the participants in the IHE3 + Ex group did not demonstrate greater body composition improvement with hypoxia and exercise compared to exercise alone. This difference in result could be attributed to the differences in population groups. Balykin's participants were all young (19 y) overweight males, whereas the current participants were older (56 y) and heterogeneous (being overweight was not required for inclusion, nor were overweight or obese excluded based on their body composition). It is possible that individuals with more fat mass (and therefore greater scope for improvement in body composition) or who are younger (potentially greater sensitivity to hypoxia) may demonstrate greater responsiveness to hypoxic interventions.

Unlike the changes in fat mass, the IHE3 + Ex groups demonstrated likely beneficial improvements in muscle mass. The findings from this study support previous findings from our research group who demonstrated an increase in muscle cross-sectional area following 5 weeks of either blood occlusion or inspired hypoxia during strength training in female netball players (Manimmanakorn et al., 2013). These increases in CSA were attributed to an earlier switch in the recruitment pattern from the lower-powered aerobically fuelled Type I fibres to the greater force-generating, anaerobic Type II muscle fibres with the hypoxic stimuli during exercise (Manimmanakorn et al., 2013). As the participants in the current study received a passive dose of hypoxia, it is unlikely that the increase in muscle mass in our participants was due to this reason. Instead, perhaps, the shift to glycolytic

metabolism, and associated increases in lactate production during the IHE sessions facilitated accelerated improvements buffering capacity allowing for greater exertion during the strength training portion of the exercise intervention.

One limitation of the body composition analysis in the current study was the use of bioelectrical impedance to estimate muscle and fat mass, rather than a more reliable measurement such as hydrostatic weighing. As the changes between the groups were reasonably small, caution should be applied when interpreting the results.

5.5.6 Limitations

Given the timeframe of the study, there was a considerable change in the ambient conditions from the mid-winter baseline temperatures to the summer 4- and 8- week follow up temperatures. It is possible that this seasonal variation may have had an impact on some of the results. However, as this was a controlled trial, we would expect any variation in seasons to affect both groups equally.

The participants in the study were all sedentary and middle aged when they were recruited into the study. However, despite being sedentary, the participants were in good health. It is possible that a 'ceiling effect' of sorts limited the effectiveness of the prescribed intervention and that a greater change in cardiovascular risk factors would have been evident in a population with a greater severity of cardiovascular risk.

In any maximal tests, particularly in fitness assessments, motivation to continue will pay a substantial role in the drive to continue exercising to exhaustion and attaining a true $\dot{V}O_{2peak}$. Additionally, the length of a maximal fitness assessment should be <10 min (Astorino et al., 2004, Yoon et al., 2007). However, given the wide range of fitness abilities, and the fixed protocol of the MBP, some participants took considerably longer than this, and therefore the $\dot{V}O_{2peak}$ results in the current study may be under-estimated for some participants. While this makes it difficult to compare the absolute $\dot{V}O_{2peak}$ with other studies, the results should still demonstrate changes in fitness in the individual as the protocol was kept consistent between time points.

5.6 Conclusion

The inclusion of IHE into a traditional exercise programme resulted in slightly bigger improvements in SBP, $\dot{V}O_{2peak}$ and HDL compared to exercise alone. While there was some indication of increased resting parasympathetic activity (assessed using rMSSD) in the IHE3 + Ex group compared to the Ex group, none of these changes were clear. Additionally, there were no clear differences between the groups in the time taken to complete the maximal exercise test. Importantly, while the immediate arterial response to IHE3 + Ex seems to improve augmentation index, the longer term effects of IHE3 + Ex on arterial stiffness demonstrated an opposite, increase in augmentation index. It is possible that this increased augmentation index was simply the result of a reduction in the pulse pressure without an associated decrease in the augmentation pressure. Still, further research should test the long-term effects of hypoxic exposure on vascular health before IHE can be considered a safe intervention in a sedentary population. Finally, the inclusion of IHE into a traditional exercise programme may benefit some independent cardiovascular risk parameters, but the protocol we have used is unlikely to improve the overall cardiovascular risk profile or exercise capacity more so than exercise alone in a our sedentary middle-aged participants. Furthermore, when the additional time and travel costs of the participant are considered, the practical application of IHE as an effective supplement to exercise must be questioned.

Chapter 6

Final discussion and Conclusion

Regular exercise plays a vital role in both maintaining and improving health. Active individuals demonstrate lower risk of coronary heart disease (Tanasescu et al., 2002), reduced blood pressure (Whelton et al., 2002), improved glycemic control in diabetics (Thomas et al., 2006), and lower levels of depressions and other metal illness (Stathopoulou et al., 2006). Indeed, the adage attributed to the late Robert Butler, former Director of the National Institute on Aging, that "if medicine was a pill, it would be the single most widely prescribed and beneficial medicine in the nation" elequently encapsulates the medical importance of regular physical activity. However, for those for whom exercise is not a possibility, or for those who can only exercise in limited doses –such as those with arthritis, injuries or illness, an alternative intervention is lacking. The overarching thesis statement was to determine the effects of intermittent hypoxic exposure on cardiovascular risk factors in a sedentary population. To address this, the following aims were defined:

1) Identify gaps in the literature regarding the use of simulated altitude for health improvement. (Addressed in Chapter 2)

2) Informed by findings in the literature review, determine the effects of simulated altitude on selected cardiovascular disease risk factors in a sedentary population.
(Addressed in Chapters 3 and 4)

3) Informed by findings in the literature review, determine whether simulated altitude is effective as an accompaniment to exercise on selected cardiovascular disease risk factors in a sedentary population
(Addressed in Chapter 5)

Following an extensive literature review, the following gaps in the literature regarding the use of simulated altitude in a sedentary population were identified:

I. Further investigation to support or challenge the reports of improved sympathovagal balance in the autonomic nervous system (primarily through increased vagal activity), vascular health, and blood lipid profile associated with simulated altitude. Additionally, a full cardiovascular risk profile, rather than individual risk factors should be used to detect improvement in cardiovascular health.

II. Few studies report the participant's subjective response to the simulated altitude treatments.

III. Despite promising reports of health improvement, only a small portion of simulated altitude research is focussed in a clinical or sedentary population.

IV. No research has assessed the effects of different frequencies of simulated altitude sessions per week on health (assessed via modulation of cardiovascular risk factors).

V. More research should be conducted on the effects of passive simulated altitude in conjunction with exercise (versus exercise alone or exercise in hypoxia) in a sedentary, middle-aged group.

Informed by these gaps in the literature, three studies took place to address the 2nd and 3rd aims, that is, the effects of simulated altitude without, and with exercise on cardiovascular risk factors in a sedentary population.

6.1 What are the effects of simulated altitude (without the use of exercise) on selected risk factors in a sedentary population?

The first and second studies (in chapters 2 and 3 respectively) sought to contribute to this question. In doing so, research gaps II, III and IV were addressed, as was the effect of simulated altitude on the sympathetic and parasympathetic contribution to autonomic cardiac variation as indicated in research gap I. The novel findings in this portion of the thesis research relate to the improvements in HRV. While several studies have investigated the instantaneous response of HRV to hypoxic exposure, this is the first research that has demonstrated an improvement in the parasympathetic contribution to HRV following 4 or 5 weeks of IHE in a sedentary, middle-aged population. Additionally, this is the first time the effects of different weekly frequencies of IHE were measured on independent cardiovascular disease risk factors.

Based on the findings of studies 1 and 2, IHE has potential to increase resting HRV, primarily through an increase in the indicator for parasympathetic activity, rMSSD. While 2 – 3 IHE sessions per week yielded a small increase in rMSSD, these changes were only detectable under paced breathing. The impact of IHE on rMSSD was most clearly observed following 4 or 5 IHE sessions per week (in both controlled and spontaneous breathing conditions).

There were no substantial improvements following IHE on submaximal exercise efficiency. While 4 IHE sessions per week seemed to improve submaximal exercise tolerance as indicated by a lower heart rate at the same exercise intensity at post-intervention compared to baseline (Study 1, Chapter 2), this was not evident following either 2-3 or 5 IHE sessions per week (Study 2, Chapter 3). There

were some differences between these studies that compromise the ability to directly compare changes in submaximal exercise between groups. For example, the submaximal exercise intensity in Study 2 was controlled by heart rate (therefore it was relative to the individual) whereas in Study 1, an absolute submaximal intensity of 50 W was used. This HR-defined submaximal workload remained consistent between baseline and post-intervention submaximal exercise assessments and was set at 40 - 50% heart rate reserve. It is possible that 40 – 50% heart rate reserve would have been too high to detect changes in submaximal efficiency, compared to the lighter 50 W workload. Given the absence in any worthwhile changes in $\dot{V}O_{2submax}$ following any frequency of IHE per week (2 – 5), it is unlikely that IHE on its own had a beneficial influence on moderate, submaximal exercise efficiency in the participants in the current study.

The effects of IHE on maximal exercise capacity are more difficult to interpret. While 2 – 3 IHE sessions per week (Study 2, Chapter 3, possibly Study 3, Chapter 4) increased $\dot{V}O_{2peak}$, there were no changes in $\dot{V}O_{2peak}$ following 4 (estimated $\dot{V}O_{2peak}$, Study 1) or 5 (measured $\dot{V}O_{2peak}$, Study 2) IHE sessions per week. While no improvements in $\dot{V}O_{2peak}$ were observed following 5 IHE sessions per week, participants receiving this dosage took longer to complete the exercise test, and tolerated a higher maximum workload, suggesting improved tolerance to maximal exertion. This improvement in high intensity exercise tolerance is possibly due to improvements in buffering capacity (Gore et al., 2007) or a shift to glycolytic energy pathways (Semenza, 2001).

Changes in SBP were unclear in Study 1, but did demonstrate a tendency to decrease with IHE in Study 2. Furthermore, when IHE was included in an exercise programme, there was a possibly beneficial effect on SBP compared to exercise alone. Therefore the effect of IHE on SBP warrants further attention in a larger cohort, receiving a dosage of 3 - 5 IHE sessions per week for a minimum of 4 weeks. (Participants in the IHE group in Study 1 effectively received IHE for 3 weeks, as the first week was a very mild dose of hypoxia and primarily used to familiarize participants and facilitate blinding).

Overall, the participants tolerated the hypoxic exposures well, with only one participant demonstrating moderate side effects across all 3 studies.

6.2 Does IHE enhance the cardio-protective effects of a traditional exercise programme compared to exercise alone?

The final study (Study 3 in Chapter 4) addressed the effectiveness of IHE as an accompiament to exercise for the purpose of reducing cardiovascualr risk factors. In doing so, Research Gap V was prioritised. Additionally, the participants recruited into each of the studies were all sedentary and middle-aged and therefore contributed to Research Gap III. Finally, the effects of IHE and exercise

were measured against changes in arterial stiffness, blood lipids (limited to high density lipoprotein and total cholesterol) and overall cardiovascualr risk profile, thus informing Research Gap I.

Overall, neither the exercise only nor the exercise with IHE groups had a substantial effect on overall cardiovascular risk (as assessed using the New Zealand Guidelines 5 – year cardiovascular risk assessment chart, and the 10 – year Framingham assessment) immediately following the 10-week intervention. While there was a possibly harmful outcome at the 8-wk follow up in the IHE3 + Ex group compared to Ex, this was caused by an increase in total cholesterol (which was inflated due to an increase in high density lipoprotein). Additionally, the absence of a full dataset at the 8-wk follow up owing to missing blood sample data (either not collected or excluded due medication that would have altered the lipid profile between the post and 8-wk time points), further compromised the accuracy of the 8-wk follow up overall cardiovascular risk profile.

Regarding the independent risk factors, the group receiving IHE in addition to exercise demonstrated increased high density lipoprotein and maintained $\dot{V}O_{2peak}$ (compared to a decrease in Ex only) at all post-intervention measurements. Additionally, there was a tendency for lower pulse wave velocity (particularly at 4- and 8-wk post) and increased HRV (increased rMSSD, at all post-intervention time points) in the exercise and IHE group compared to exercise only, however none of these changes were clear. If these findings become clearer in a larger study, these adaptations may suggest superior caridioprotective adapation with IHE and exercise compared to exercise only in a sedentary population.

However, while the indicator for systemic arterial stiffness, the augmentation index, appeared to decrease immediately post-intervention, there was a likely and very likely harmful outcome in the 4- and 8-wk follow ups. Because the augmentation index is a ratio between the augmetation pressure and the pulse pressure (SBP – diastolic blood pressure), the increase in the AIx could have been caused by a decrease in the pulse pressure with a smaller decrease, no change, or increase in the augmentation pressure. It is also possible that the increase in AIx is a side effect of the repeated up-regulation of the endotheial NO systhase activity during the hypoxic intervals resulting in a down-regulation following the hypoxic exposure. The down-regulation of the endothelial NO synthase activity would result in increased vascular tone and a greater reflected waves. Therefore, until further research can disqualify any long-term vascular maladaptation to IHE, this protocol should not be recommended for beneficial health application in a sedentary population.

6.3 Possible side effects

The participants receiving IHE 5 times per week in Study 2 demonstrated a likely trivial increase in hs-CRP. While this increase was not sufficient to change the risk category of the participants, it is worth

investigating further. Additionally, when IHE was included in an exercise programme, the participants in the IHE3 + Ex group initially demonstrated an unclear decrease in the augmentation index suggesting an improvement in arterial compliance. However at the follow up assessments, the augmentation index had increased substantially at 4 and 8-wk respectively. Whether the slightly elevated hs-CRP noted in Study 2 was related to the delayed increase in arterial stiffness in noted in the IHE3 + Ex group in Study 3 is uncertain.

Before IHE can be freely recommended as a useful tool for improving cardiovascular health, a greater understanding of the long-term effects of IHE on arterial health and systemic inflammation is required. Future research should probe further into this potential side-effect in a healthy young cohort, or possibly in animal models, and avoid using sedentary, middle-aged participants to prevent any possible further declines in vascular health that participants in this population are likely to have. The findings of potentially increased systemic inflammation and a delayed increase in arterial stiffness noted in Studies 2 and 3 may challenge the 'safe' description of IHE as a potentially therapeutic intervention.

6.4 Proposed mechanisms behind adaptations observed with IHE in Studies 1, 2 and 3.

Many of the adaptations observed in the 3 studies completed can be related back to adaptations associated with HIF-1α and peripheral chemoreceptor activity (particularly the carotid bodies). Figure 16 attempts to resolve the research outcomes from the current studies with the adaptations known to occur with the HIF-1 α and peripheral chemoreceptor activation in hypoxia. As no direct measures of HIF-1 α activity, such as vascular endothelial growth factor, erythropoietin or carbonic anhydrase concentrations were taken, these are all hypothesised mechanisms for the observed changes that have been based on research by others. There are some questions that arise, and seeming conflicts between this proposed model and some of the adaptations.

Figure 16 demonstrates the stimulation of genes associated with up-regulating erythropoiesis and carbonic anhydrase, as well as stimulating alterations in vascular tone and an active cellular shift to glucose metabolism (Semenza, 2001). From these, improvements in in oxygen carrying capacity; carbon dioxide, bicarbonate ions and hydrogen transport to the lungs (Geers and Gros, 2000); vascular compliance (Wilkinson et al., 2002a, Wilkinson et al., 2002b) is produced. One important consequence of the shift to glycolysis is the increase in nicotinamide adenine dinucleotide + Hydrogen which (following several steps) inhibits 7-α-hydroxylase, a rate limiting enzyme in the conversion of cholesterol to bile salts (Johnson, 2008) which cause an increase in plasma cholesterol.

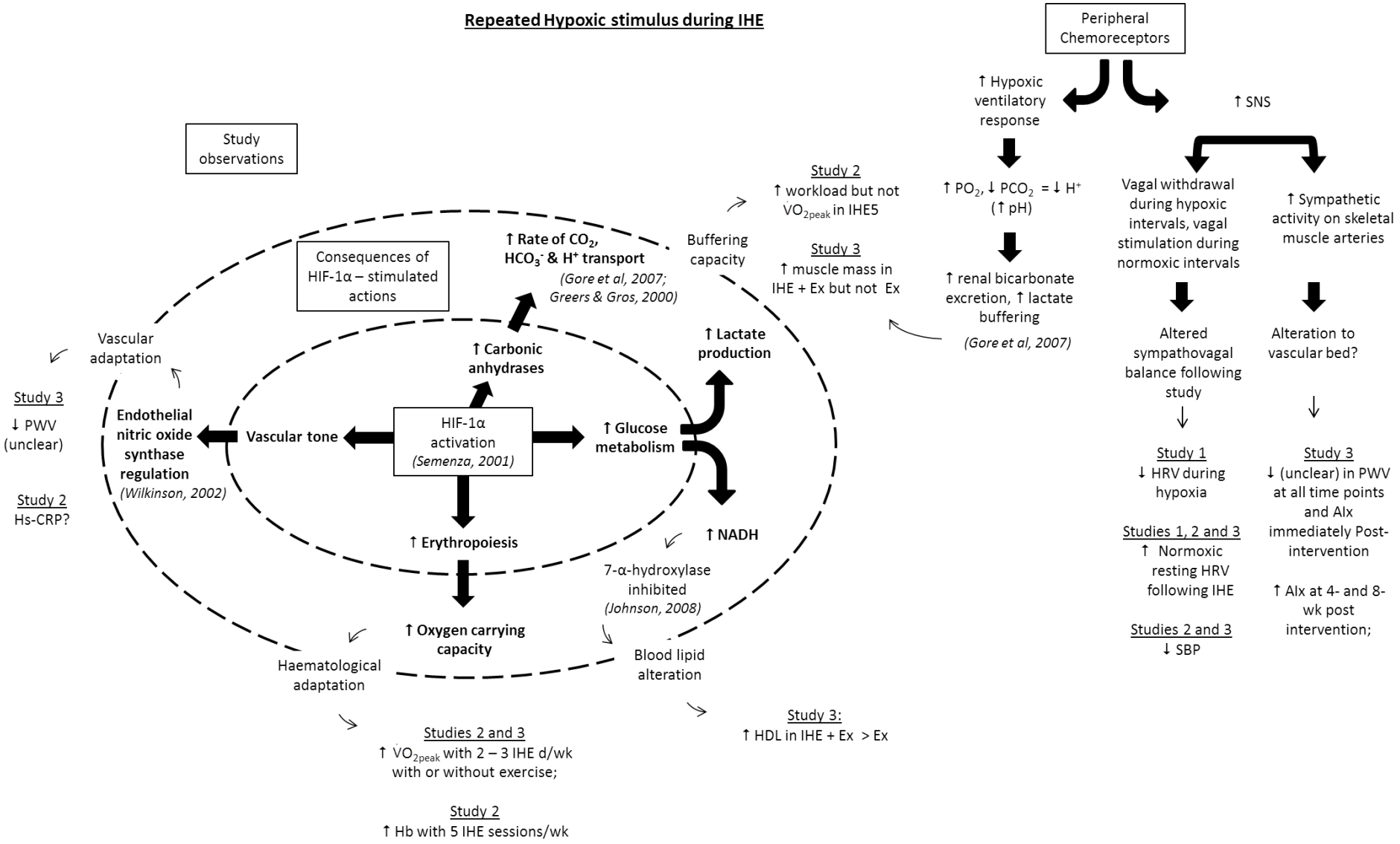


Figure 16: Integration of adaptations associated with IHE interventions to the actions associated with HIF-1 α and peripheral chemoreceptor activation

Upon cessation of the hypoxic stimulus and reversion to oxidative phosphorylation the relatively higher cholesterol concentration in the blood may then trigger an up-regulation of 7-α-hydroxylase resulting in an overall decrease in cholesterol. The point of interest, however, is that the IHE3 + Ex group in Study 3 demonstrated an increase in high density lipoprotein while total cholesterol remained stable over the course of the study (and subsequently decreased at the 8-wk follow up). In comparison, the Ex group decreased total cholesterol throughout the study and at the 8-wk follow up, but had no change in the high density lipoprotein. It seems, then that the effect of the IHE treatments may have been more related to the increase in high density lipoprotein, than the decrease in non-high density lipoprotein.

There are a few 'puzzle pieces' that don't seem to fit in the diagram outlined in Figure 16. For example, while an increase in $\dot{V}O_{2peak}$ was observed in both studies using IHE 2 – 3 times per week (the IHE3 group in Study 2 and the IHE3 + Ex group in Study 3), there was no increase in estimated or measured $\dot{V}O_{2peak}$ following 4 or 5 IHE sessions per week (the Hyp group in Study 1 and IHT5 group in Study 2 respectively). Additionally, it is possible that the short term influence of the increased endothelium nitric oxide activity (the unclear decrease in pulse wave velocity, and augmentation index) may have long-term mal-adaptations such as an increase in augmentation index and hs-CRP.

The effect on an increase in sympathetic nervous activity associated with hypoxia on vascular health is complicated. For example, despite an increase in the sympathetic innervation to skeletal muscle arteries (which would typically be associated with an increase in vasoconstriction), there is a vasodilatory response in the skeletal muscle arteries (Hudson et al., 2011). This may suggest that other, local factors (possibly due to metabolic alterations) may play a larger role on vascular augmentation than the increase in sympathetic innervation.

6.5 Practical lessons

Part of the aims outlined in the first study in Figure 1: Decision making process for research progression" in Chapter 1 was to assess the best ways of running the studies, specifically regarding participant recruitment, management and testing scheduling. The following practical and invaluable learnings through the research have been gathered through the research process.

When it comes to recruitment, any form of personal communication was more effective than notices. For example, most of the participants were successfully recruited following information stalls at Farmers Markets (most effective), or presentations at community groups such as Rotary Clubs. Information drops in mail boxes, posters on community notice boards or small notices in the newspaper were not as effective.

Regarding participant management, it was difficult to find the balance between giving the participants enough information so that they were fully informed, but not too much so that they 'switched off' and did not read the information packs. Having a clear and concise summary at the beginning of the information pack was helpful. Reminding participants of their medical and testing appointments the day before the appointment, including any preparatory detail (such as avoiding caffeine and strenuous exercise etc.) improved the flow of the testing procedure.

Having a full run through and familiarization of the baseline testing procedures made a substantial difference to the quality of the baseline data. For example, in the HRV analyses in Study 2, there were considerably fewer outliers detected in the baseline testing session compared to the familiarization. Unfortunately, due to logistical and time restrictions, a full familiarization was not conducted in Studies 1 and 3, and participants were familiarized with the testing procedures for approximately 30 min prior to baseline collection. It is unclear what effect this would have had on the baseline values in these studies.

6.6 Practical considerations regarding the use of IHE as a means of reducing cardiovascular risk factors

The findings of this thesis demonstrate several cardio-protective responses to an IHE intervention. However, it is important to recognise the considerable time and travel commitments that are also required and therefore one should consider the time cost to health benefit trade-off when recommending the use of an IHE treatment. It is likely that a similar or improved cardiovascular risk response can be achieved by a moderate exercise intervention. In this regard, IHE may not be appropriate for individuals who are able to exercise for the government-recommended time of 30 minutes on most days of the week, even if the exercise dose is mild. The use of IHE as a tool for cardiovascular risk reduction may be best suited for those who are unable to exercise sufficiently or at all.

6.7 Future research

The most pressing area for investigation is to investigate the long-term effects of IHE on vascular health. In particular, future research should focus on markers of inflammation, such as hs-CRP or measures of arterial compliance such as the augmentation index which may indicate mal-adaptation to the repeated hypoxic intervals. As the group receiving 2 -3 IHE sessions per week did not demonstrate any change in hs-CRP following the 5-week intervention, mediating the potentially harmful effects of IHE may have to do with the dosage. Therefore, research investigating different intensities of hypoxia (for example, reducing SpO_2 to 90 - 85% instead of 80%), or using a lower

frequency of IHE sessions per week may avoid the observed increases in hs-CRP and delayed increase in arterial stiffness.

Should a safe mode of IHE be established, additional research, in a larger cohort should examine the effects of simulated altitude (4 – 5 times per week, for 4 weeks) on changes in systolic blood pressure. Finally, other methods of simulated altitude, such as the live high, train low protocol using altitude tents, or IHT should be investigated for their influence on reducing cardiovascular risk factors in a sedentary population.

6.8 Conclusion: The effect of IHE on cardiovascular risk factors in a sedentary population

The use of IHE either alone, or accompanying exercise may prove to be a useful intervention for improving HRV, high intensity exercise tolerance, increasing high density lipoprotein and possibly reducing systolic blood pressure. Additionally, the findings of this research suggest an IHE intervention 4 or 5 times per week, for 4 weeks (at the target SpO_2) is the dosage most likely to result in health improvement. However, potential side effects, such as increased hs-CRP and the long-term effect on arterial stiffness following IHE need to be investigated further before IHE can be described as 'safe'. Given the additional intervention hours required by the participants in the IHE3 + Ex group, the time commitment required for IHE may be better spent engaging in physical activity more regularly in a healthy population. However, should the concerns regarding the long-term effects of IHE on vascular health and systemic inflammation be unfounded, IHE may be a useful intervention for individuals for whom additional exercise is not an option. Target populations could include those in surgical or injury rehabilitation programmes, or even those new to exercise training.

Appendix A

A beginner's guide to heart rate variability

Heart rate variability (HRV) is a widely used tool in the assessment of the autonomic control of the heart. However, there is little practical guidance regarding the measurement of HRV. The aim of this guide is to try and fill this gap by providing some practical steps and considerations in the measurement of HRV using a Polar heart rate monitor and software. While there are a number of ways to measure HRV, this guide will focus on short term (5 minutes), time and spectral domain analyses measured using a polar heart rate monitor (HRM), measured in a laboratory setting. The authors welcome any further contribution by other researchers, particularly those with experience in longer term (24 hours) or alternative methods of HRV (e.g. geometric, fractal, course graining spectral analyses etc.).

A.1 What, exactly, is heart rate variability (HRV)?

Heart rate variability is how much the interval between each of your heart beats vary from one to another (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). While heart rate measures an average of the number of times your heart beats in one minute, HRV lets us know how uniform the time interval between each of the beats was. The more uniform the time intervals, the lower the heart rate variability. For example, in the graph below (from a 3-lead ECG, recorded using Beatscope software), notice that none of the intervals between the heart beats are exactly the same. This is because your body is always responding to both internal and external stimuli by up-regulating (sympathetic stimulation increase and/or parasympathetic withdrawal) or down-regulating (increased parasympathetic stimulation via the vagal nerve and/or sympathetic withdrawal) the autonomic nervous system to maintain homeostasis (Porges, 1992).

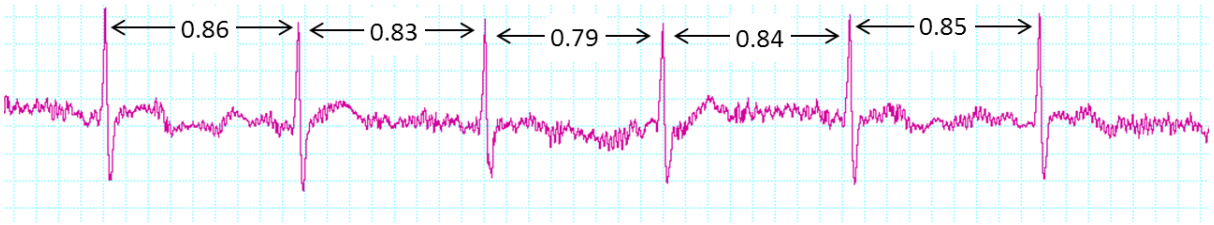
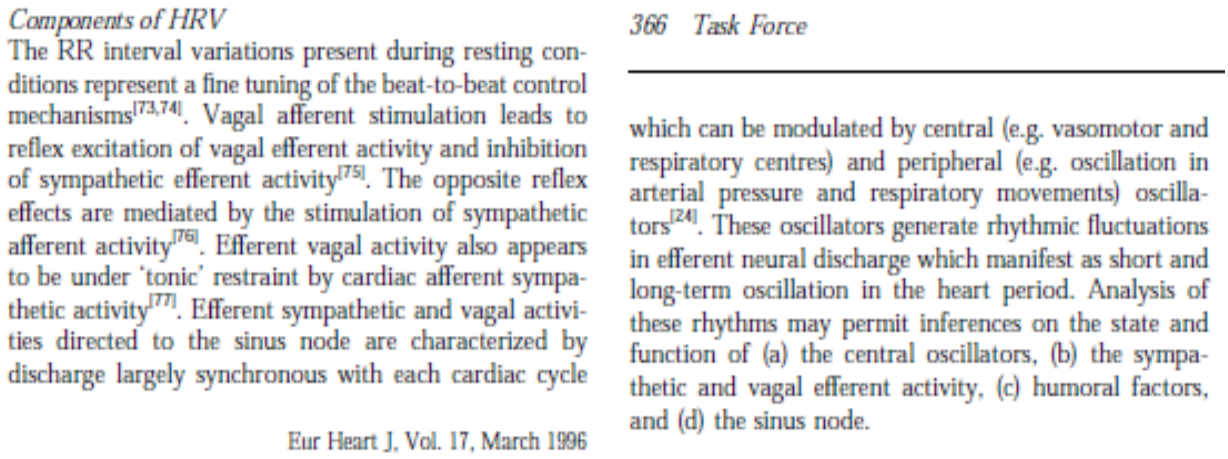


Figure 17: Fluctuations in heart beat intervals

The fluctuations between up- and down-regulation occur at different frequencies. For example, the fastest fluctuation would be related to your respiratory sinus arrhythmia – when your heart beat speeds up as you breathe in, and then slows down as you breathe out. The purpose of the

respiratory sinus arrhythmia is to increase the amount of blood sent to your lungs while there is fresh oxygen available (while inhaling), and reduces the amount of blood sent to your lungs while there is little fresh oxygen available (while exhaling) (Yasuma and Jun-ichiro, 2004). The extent to which your heart rate speeds up and slows down during your respiratory sinus arrhythmia is controlled by your parasympathetic nervous system via the vagus nerve (Porges, 1992), and as it fluctuates with every breath, it occurs at a high frequency (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996).

In 1996 a taskforce (The European Society of Cardiology and the North American Society of Pacing and Electrophysiology) was established to describe the various HRV techniques, define the correct nomenclature, and to standardise the best practice for the measurement of HRV. The Taskforce guidelines (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996) explain the interaction of the ANS and HRV very well (See guidelines p. 366)



Components of HRV

The RR interval variations present during resting conditions represent a fine tuning of the beat-to-beat control mechanisms[73,74]. Vagal afferent stimulation leads to reflex excitation of vagal efferent activity and inhibition of sympathetic efferent activity[75]. The opposite reflex effects are mediated by the stimulation of sympathetic afferent activity[76]. Efferent vagal activity also appears to be under 'tonic' restraint by cardiac afferent sympathetic activity[77]. Efferent sympathetic and vagal activities directed to the sinus node are characterized by discharge largely synchronous with each cardiac cycle

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which can be modulated by central (e.g. vasomotor and respiratory centres) and peripheral (e.g. oscillation in arterial pressure and respiratory movements) oscillators[24]. These oscillators generate rhythmic fluctuations in efferent neural discharge which manifest as short and long-term oscillation in the heart period. Analysis of these rhythms may permit inferences on the state and function of (a) the central oscillators, (b) the sympathetic and vagal efferent activity, (c) humoral factors, and (d) the sinus node.

Figure 18: Excerpt from the Taskforce Guidelines (1996) explaining the interaction between sympathetic and vagal activity

A.2 What the abbreviations mean

As mentioned above, the focus of this guide is on short term time and spectral domain heart rate variability measured using Polar heart rate monitors. Therefore, only the time and frequency domain parameters are reported in Table 18.

Pinna et al. (2007) have compiled an excellent review on the reliability, repeatability and estimated samples sizes needed to detect changes in these variables.

Table 18: The abbreviations, full titles and meanings of some of the time and spectral domain HRV analyses

Time domain	Units	Unabbreviated	What it measures
RR interval	ms.	The average time interval between R-peaks.	Much like heart rate, it gives an overall indication of the SNS/PNS balance. Just as a lower heart rate indicates higher PNS, higher RR interval indicates higher PNS. Always report this in your studies.
SDNN	ms	Standard Deviation of Normal – Normal intervals. (intervals between normal QRS complexes)	Because overall variance = overall power, SDNN measures overall cyclic power of the variability. The length of the SDNN recording greatly affects the SDNN value, so ensure the recording length is consistent (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996)
rMSSD	ms	Root mean square successive difference	Parasympathetic component (largely related to the respiratory sinus arrhythmia). rMSSD reflects cardiac vagal activity.
PNN50	%	The proportion of the number of pairs of adjacent NN intervals that are greater than 50 ms in the total number of NN intervals recorded.	Parasympathetic component
Frequency domain	Units	Unabbreviated	What it measures
TP	ms^2	Total power	Overall variance of the 5 minute segment.
HF	ms^2	High frequency (0.15 – 0.4 Hz)	Parasympathetic component (via respiratory sinus arrhythmia)
LF	ms^2	Low frequency (0.04 – 0.15 Hz)	Sympathetic and parasympathetic (some respiratory sinus arrhythmia, also blood pressure related changes)
VLF	ms^2	Very low frequency (0.003 – 0.04 Hz)	Unsure – do not use this in short term recordings.
HF or LF	n.u.	High frequency and low frequency expressed in normalised units	Measuring HF and LF in normalised units (nu) allows the researcher to see the proportion of each out of 100%. (When expressed in nu, the values added together will equal 100%). This allows the researcher to see whether the SNS or PNS was more dominant. (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996)
LF/HF		Low frequency to high frequency ratio	Dominance of SNS

A.3 Using the right tool for the job

Each of the different HRV measurements is used to draw different conclusions. Therefore, it is important to decide which is the most useful 'tool' or measurement before you start to ensure you are able to answer the research question effectively. By selecting specific variables you avoid conflicting results and avoid using in-appropriate measurements for your study.

Spectral methods have been recommended for short term recordings (Sandercock et al., 2005b, Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). However, some researchers suggest that spectral analyses can be less reliable than time domain analyses (Al Haddad et al., 2011), while others report that no one measure is more reliable than the others (Sandercock et al., 2005b). The rMSSD appears to be the most reliable time domain measure that reflects vagal activity and, importantly, does not appear to be influenced by breathing patterns (Penttilä et al., 2001).

The HF and LF measurements can give a better insight into the interplay between the sympathetic and parasympathetic systems. The HF component is a good reflection of the parasympathetic nervous system and represents short term fluctuations on the sinus node. Because the LF component is a mix of both parasympathetic and sympathetic nervous systems interpreting the cause of the change in LF is somewhat more challenging and the LF can be difficult to interpret.

A.4 How does the polar HRM compare to an ECG

The Polar HRMs have been validated against 2 lead (Gamelin et al., 2006), 12 lead ECG (Porto and Junqueira Jr, 2009) (amongst others) and have been accepted as a good, non-invasive means of detecting HRV. Still, ECGs remain the gold standard and should be used where possible.

A.5 How to set up the equipment

Electrode Gel on the HRM belt

To improve the contact of the HRM belt to the participant's skin, it is recommended that you use some electrode cream under the belt of the HRM strap.

Figure 19: Apply some electrode gel to the electrodes of the HRM belt to improve contact with the skin

Recording frequency on the watch

Ensure that the Polar HRM watch is set to record the RR Intervals (check box is ticked, see image below). The RR Interval function measures the variation in heart beats to within 1 ms (Polar Electro Oy, 2008). If you find that the memory is filling up too fast, you can set the recording rate to 5 seconds instead of 1 second. Just make sure you keep it consistent between participants.

Figure 20: Ensure that the HRM watch is set to record the RR interval. Use the top right button (A) to scroll through options and the bottom red button (B) to select an option. Use the bottom left button to return to the previous menu (C)

A.6 How to take the measurement

Supine, Sitting, Standing or during exercise

This will depend entirely on what you're trying to measure. For example, if you wanted to measure whether an intervention altered the normal resting state of the participant, you would position your participant such that the SNS stimulation is reduced, and the parasympathetic component is enhanced (a supine position in a quiet, 'safe' space). While supine rest appears to be the most commonly used posture for the collection of HRV data, some researchers suggest adopting a seated posture. The Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology (1996) guidelines do not specify a posture, only that it is consistent.

As the parasympathetic contribution is measured primarily using the respiratory sinus arrhythmia, the participant should not talk during the measurement as this alters breathing frequency and

therefore the respiratory sinus arrhythmia. The participants should be encouraged to breathe as normally as possible.

Measuring HRV during exercise is very difficult for a couple of reasons.

a) For an accurate measurement of HRV, the recording needs to be taken during a physiological stable state (unless you're measuring HRV during the Valsalva manoeuvre or head tilt and so on – I have not used these methods, so cannot comment here). Achieving a steady state during exercise over a period of 5 minutes can be difficult, particularly in a sedentary, middle-aged population. The HR stationarity can also become compromised in protocols that require a consistent workload (as is required in most ramp protocols). Completing such measures on athletes is probably easier but its best to try things out in a pilot study first.

b) There are a lot of non-neural stimuli that affect HRV during exercise, so the accuracy of the HRV measurement using time and spectral domain analyses is compromised (coarse graining spectral analyses and non-linear methods may be more appropriate) (Sandercock and Brodie, 2006).

c) Short-term HRV recordings should be 5-minutes long, as recommended by the guidelines (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996), so that the results of your study can be compared with other studies. The length of the HR recording influences the HRV value, so if the recording length is shorter or longer than this, it cannot be compared to other research or even different recording lengths between participants in the same study. (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996)). Recordings shorter than 5 minutes are also considerably less reliable than the 5-minute recommendation (Schroeder et al., 2004).

A.7 Using Paced / Controlled breathing or spontaneous breathing?

Controlled breathing can improve the clarity and reliability of the heart rate recording (Pinna et al., 2007); however, spontaneous breathing can also produce reliable data, particularly if you're using the rMSSD (Penttilä et al., 2001). The participants should rest for 5 minutes prior to the 5 minutes HRV recording to ensure the participants are relaxed.

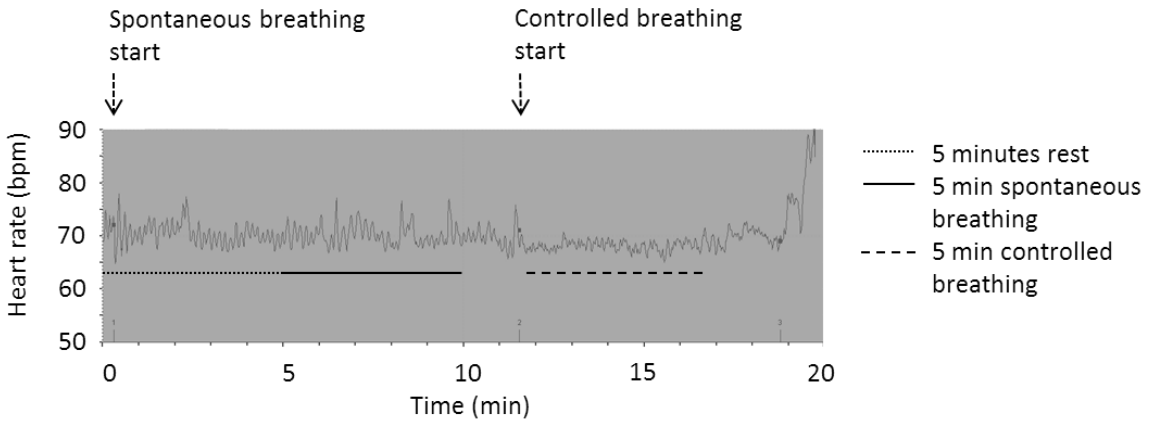

Figure 21: Spontaneous and controlled breathing on HR

Measuring controlled HRV can present a few practical issues for the researcher to consider:

a) People have a wide range of natural breathing frequencies, so they may find the 'set' breathing frequency uncomfortable. The participants can also hyperventilate if they breathe too deeply or too fast, so it's important to encourage the participants to breathe as normally as possible.

b) Even if you set the metronome to a frequency similar to the participant's natural breathing rate, it is often still uncomfortable for the participant to breathe at a controlled pace for an extended period of time.

c) It is difficult to monitor adherence to the controlled breathing protocol.

d) After already lying supine for 5 - 10 minutes, the continuous metronome can put the participants to sleep. As soon as the participants fall asleep, their breathing rate changes, and their HRV is also altered between sleep and wake states (Shinar et al., 2006).

The use of a metronome in conjunction with a visual cue of sorts may reduce the tendency for the participant to fall asleep. A seated position might keep the participants awake better while only introducing a small increase in the sympathetic component (Chan et al., 2007).

A.8 Rest time, lighting and noise.

Related to section 3.2 above, a 5 minute rest period can provide the participant with some time to 'unwind' from the stress of getting to the lab (possibly rushing to do so), getting set up with possibly intimidating equipment, and being in an unfamiliar place. However, a rest period of longer than 5 minutes may increase the likelihood of the participant falling asleep. Practically, while many researchers recommend the use of a quiet, semi-dark room, a room that is too quiet, dark and comfortable readily induces sleep, and therefore keeping the lights on and not removing all white

noise may be beneficial. Sudden or sharp noises will increase the participant's heart rate suddenly and should be avoided.

A.9 How to analyse the data using Polar Protrainer5

Which sample to select?

The 5 minutes of rest (to reduce the impact of any initial white coat syndrome or elevated HR due to unfamiliar equipment or rushing to the exercise laboratory) immediately followed by the 5 minutes of recording means that the participant will be resting for a period of 10 minutes. If you have started your HRM as the participant starts their rest period, you should select the last 5 minutes of the 10 minute rest period for your HRV analysis as this is the most reliable way of collecting data. Before actually testing this (unpublished preliminary research), we assumed that selecting the 'most consistent' or 'tidiest' 5 minutes of data in the 10 minute period would be best. However, by doing this, you are selecting unusually 'high/low/unvaried' data which actually reduces the repeatability of the measurement.

A.10 How to select your sample

After the HR data are uploaded into Polar Protrainer5™ it will need to be filtered for ectopic beats.

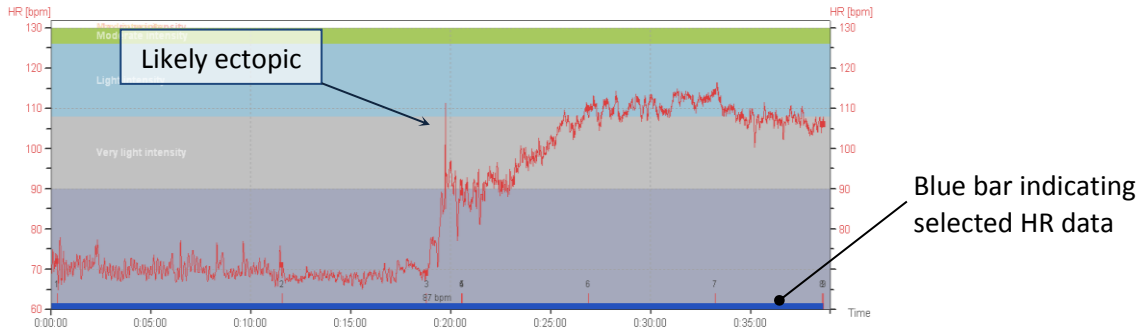

Figure 22: Selecting the data

After filtering the data, you will need to select the portion that you wish to analyse. To do both of these, you will need to select the relevant portions of data. By right clicking on the y-axis, you can choose to display the RR interval instead of HR. This gives you an inverted HR graph. This is entirely personal preference and will not affect the outcome of the data.

The blue bar at the bottom of the trace indicates which data has been is selected. To remove the selection, right click under the graph. To add a selection, left click and drag over the interval you wish to analyse. The HRV analyses will only work when there is only one piece of data selected. If there are no values when you display the HRV data (see section A.12 below) it might be because you have accidently selected more than one interval for analysis.

A.11 Filtering your data

To filter the data, select the full segment (the full segment should be selected when you open the file for the first time). Right click anywhere on the graph and select “error correction”.

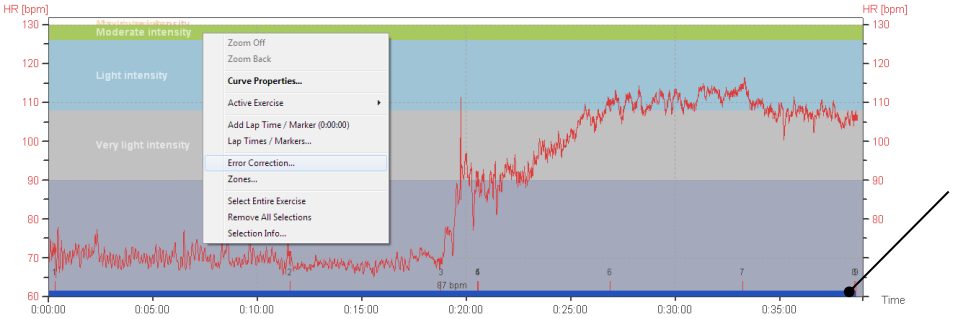

Figure 23: Choose the "error correction" function

Use the default settings of “moderate filter power”; “minimum protection zone” of 6 beat·min^{-1}; and make sure that the scissors tick box has been selected. Click preview to see the changes, and then click OK.

Figure 24: Filter the data
The purple line is the filtered trace. Notice that the tall peak as the participant started exercising (in the above figure) will be removed following filtering.

A.12 Measuring HRV

After the data have been filtered, remove the blue selection bar under the graph by right clicking under the graph. Select the portion of data you wish to analyse by left clicking and dragging underneath the X-axis of the graph over the required portion. If you are using spontaneous breathing, this portion should be the 5 minutes following your 5 minute rest interval. If you are using controlled breathing, use the 5 minute interval shortly after the start of the metronome (or whatever you are using to control breathing frequency).

For example, in the graph used in Figure 22 - Figure 25 the participant rested for 5 minutes, HRV under spontaneous breathing conditions was then recorded for 5 minutes, followed by controlled

breathing for 5 minutes. The participant then started the exercise portion of the fitness assessment. The start of each new period was separated using the 'lap' function on the heart rate watch. When the function is pressed, it creates a small vertical line and number on the X-axis when after the HR has been downloaded. For example, to analyse the controlled breathing portion, clear the selection by right clicking under the graph, and select the data shortly after the '2' marker by dragging the curser over the following 5 minutes.

Once you have selected the portion for analysis, right click anywhere on the graph and select "Selection Info…" This will bring up a table of the HRV results which can be copied and pasted into an Excel Spreadsheet.

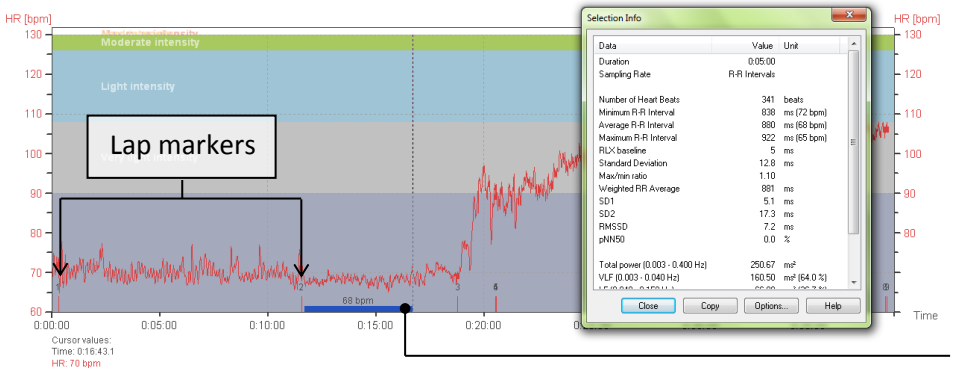

Figure 25: Perform HRV analysis using the "Selection Info..." option

A.13 How to improve the accuracy of the data.

Our research group has found rMSSD to be the most reliable measurement (unpublished preliminary data) in a sedentary, middle-aged population. The HF spectral recording is also reasonably reliable, but the typical error associated with this reading is large, so you need to interpret your data carefully (Al Haddad et al., 2011). If your data are within the typical error of measurement, consider plotting the change scores from Time 1 and Time 2 to see if there's a general trend. Ensure that you mention the high typical error in your discussion.

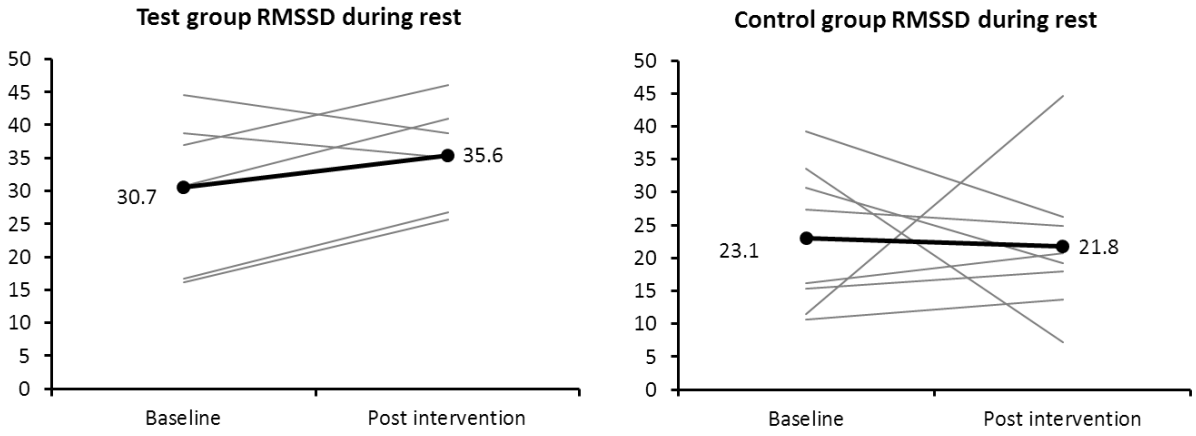

Figure 26: The use of change scores to illustrate the change in HRV

The data in the above graphs are an excerpt from a study conducted in November 2009 by our lab. The pre/post-intervention changes in rMSSD indicate a more uniform result in the test group compared to the control group.

A.14 Dealing with outliers

Unfortunately, the Polar ProTrainer5 may not pick up non-sinus originating beats (which would be excluded in an ECG) which can result in inflated HRV values (Wallén et al., 2012). The use of outlier detecting methods can be useful in deciding whether an inflated HRV result is likely to be 'normal'. Importantly, the outlier detection strategy should be resistant to the effects of outliers. For example while including all data that are 2 standard deviations (SD) from the mean is a common form of outlier detection (Leys et al., 2013), it is flawed in that the technique itself is susceptible to the effect of the outlying data. The use of the median absolute deviation (MAD) or Interquartile range methods to detect and remove outliers are two robust techniques that are resistant to the effects of outlying data (Wilcox, 2010) . Unfortunately there has not yet been a study that directly assesses whether outlier removal improves the reliability of the results. However, it is not unusual to receive some particularly high HRV recordings (see Figure 27 below). Some reasons why the high value could be false include: the belt losing contact with the skin; mis-interpreting components of the QRS complex (Gamelin et al., 2006); or incorrect automatic filtering for ectopic or non-sinus originating beats (Wallén et al., 2012). Regardless of whether the outlier removal improves reliability, it is likely to improve the integrity of the data.

How you detected the outliers, the number of outliers detected and removed, and possibly what the outliers were should be noted in your publication.

For more information on outlier detection and removal, read: Wilcox, R. R. 2010. The normal curve and outlier detection. In *Fundamentals of modern statistical methods: Substantially improving power and accuracy*. New York: Springer.

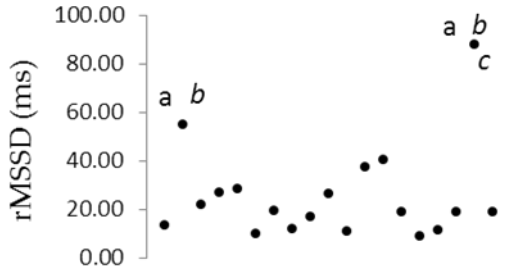

Figure 27: Outliers detected by visual inspection (a), Median Absolute Deviation (b), and 2X SD (c) methods of outlier detection (interquartile range not shown)

While one of the outlying data points were selected by the 2 X SD method, the MAD method was slightly more sensitive and picked up a data point at 60 ms (a, b), where most of the data for this population were below 40 ms. However the data are analysed, it is essential that an objective and consistent approach is used in the data filtering process.

A.15 When to use the natural logarithm

Some authors recommend always using the log transformed data as the nature of HRV measurements, particularly the rMSSD, is usually skewed (Plews et al., 2012).

If you are doing a repeatability study, and your data are heteroscedastic or follows a non-normal distribution, you'll need to log transform the data before you analyse it.

A.16 What is the advantage of Kubios?

Kubios is free software that can assist with the assessment of HRV. Kubios, unlike the Polar software can also produce Poincare plots (rather than just the SD1 and SD2 values returned by the PolarProTrainer5 software). The Kubios software can be download from: http://kubios.uef.fi/. A registration with the developers of the software is required. Following registration, an activation link for the download will be emailed. The registration field requires a valid email address and it appears after selection of the appropriate software version (Linux or Windows) of Kubios has been made.

There is very little difference in the HRV reports between the two software programmes when identical strips of heart rate data (recorded from a Polar device) are analysed Kubios and ProTrainer. Kubios may be more relevant for other heart rate monitors that are not compatible with PolarProTrainer5, or ECG recordings that have been converted into a text file.

A.17 Practice measurements

Full 'practice' measurements can minimise the number of outliers and improve the quality of HR recordings. The improvement in the quality of the data could be due to:

a) The participants being more relaxed and more comfortable with the researcher, the lab, and the equipment.

b) The researcher will be more relaxed, have a better understanding of how the timing between participants will work, what each of the participants requires, and (importantly) how to explain the session to the participants.

A.18 Using the average of several recordings

Plews et al (2013) recommends using the average of HRV measurements taken every day for one week, rather than just one measurement on one day (Plews et al., 2013, Plews et al., 2012). While this is a useful means of improving the sensitivity of the data, it might not be practical for larger studies. Schroeder et al (2004) noticed that taking multiple 1-minute recordings during one rest period vastly improves reliability (Schroeder et al., 2004). More research to assess whether taking the average of 2 or 3 5-minute recordings improved reliability, or whether HRV recordings several times per week (rather than every day) improve reliability.

A.19 Are there normal values?

Nunan et al (2010) have recently published some normative values of HRV (Nunan et al., 2010). However, one must be careful when trying to decide whether your population demonstrate 'normal' data as reliability and HRV values vary considerably across different populations (Sandercock et al., 2005b). For example, we have consistently found that our middle-aged, sedentary participants who are usually on some form of stable medication, have lower HRV values (and reliability) than the averages reported by Nunan et al (2010).

Appendix B

Repeatability and outlier detection of HRV measurements

The purpose of this paper was to assess the variability of HRV measurements. However, there were several important drawbacks with this research that prevented us using the assessed variations in the current research. The main drawbacks of the current research include are the small sample size (n = 19), and the variation and length long between measurements (1 – 2 weeks).

B.1 Abstract:

Background: While recommendations exist regarding the appropriate usage, filtering and analysis of heart rate variability (HRV) data, there are no recommendations regarding the selection of appropriate data segments or outlier detection. **Aim**: Determine whether subjectively selected ('most stable'), predefined ('last 5 minutes'), or 'controlled breathing' 5-minute data segments produce the highest HRV reliability in a sedentary population. **Method**: Eight males (age: 56.3 $\pm$ 4.6; BMI: 26.4 $\pm$ 4; on medication: n=6) and 11 females (age: 55.5 $\pm$ 6.7; BMI: 26.8 $\pm$ 5.6; on medication: n=6; mean $\pm$ standard deviation) attended 2 measurements 1 - 2 weeks apart. Supine resting HRV (Polar RS800CX) was assessed during spontaneous breathing (10 minutes) and controlled breathing (6 minutes). Visual inspection, the Median Absolute Deviation, and 2X Standard Deviation were considered for outlier detection. **Results**: The 'last 5 minutes' demonstrated the best relative reliability in heart rate (HR: 0.82 (0.58 – 0.92 intraclass correlation coefficient and 95% confidence limits)), root mean square successive difference (rMSSD: 0.82 (0.56 – 0.93)), high frequency (HF: 0.76 (0.43 – 0.91)) and low frequency (LF: 0.42 (-0.08 – 0.75). The standard deviation of NN intervals (SDNN: 0.57 (0.13 – 0.82)) was most relatively reliable in the 'most stable' segment. The 'controlled breathing' and 'most stable' data segments produced best absolute reliability for HR (5.0 (3.8 – 7.5) coefficient of variation (95% Confidence Limits)) and LF (56.4 (37.8 – 109.3)); and SDNN (28.4 (20.4 – 46.3)) and HF (61 (41.7 – 111.9)) respectively. The 'last 5 minutes' produced the best absolute reliability for rMSSD (24.4 (17.5 – 40.2)). **Discussion**: High frequency and rMSSD were sufficiently reliable in this population. However, the typical error is still large, and results, (especially SDNN, LF and HF) should be interpreted with caution. **Conclusion**: The rMSSD from the last five minutes of a 10 minute resting period during spontaneous breathing is recommended for this population.

Key words: Polar heart rate monitor, median absolute deviation, rMSSD, inactive

B.2 Introduction:

Heart rate variability (HRV) has been used in both health and sports science contexts as it is a simple means of estimating the contributions of the parasympathetic and sympathetic contributions to the autonomic control of the heart period (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). While the variation in the RR peaks has traditionally been detected using ECGs, the use of commercial devices such as the Polar heart rate monitors and automatic error correction functions provides a far more convenient means of measuring HRV. The use of these devices to detect RR intervals has been validated against 2 (Gamelin et al., 2006) and 12 lead ECGs (Nunan et al., 2008).

Although heart rate monitors have been validated against traditional ECGs, some researchers have debated their accuracy. Gamelin et al. (2006) identified the two highest causes of error in the heart rate monitor when compared to ECG recordings include missing beats (resulting in much longer RR intervals), or detecting too many beats (resulting in smaller RR intervals). The authors suggest that these errors could be caused by the heart rate monitor belt either lifting away from the skin, resulting in a missed beat, or the heart rate monitor belt misinterpreting a T-wave or P-wave for an R-wave and effectively doubling a beat (Gamelin et al., 2006). In this regard, it is essential that the raw HR data are corrected prior to HRV analysis. However, the automatic error correction with the Polar ProTrainer5^{TM} is not always sufficient in detecting these errors, or other non-sinus originating beats (Wallén et al., 2012). These errors result in higher HRV measurements (Wallén et al., 2012) and HRV parameters that differ from those derived from traditional ECG recordings (Nunan et al., 2008). This emphasises the responsibility of the researcher to carefully examine the data post-filtering for instances where data may be erroneous. It is thus important that a robust and objective outlier detection method is employed to identify any potential outlying data points.

Since the HRV Guidelines were published (1996), several authors have made recommendations to improve the repeatability and reliability of the HR data. For example, Penttilä et al., (2001) recommends using the root mean square of successive difference (rMSSD) rather than spectral analyses. Others recommend that if one is unable to use the recommended 5 minute recordings (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996), the average of multiple 10 second or 2 minute recordings rather than single ultra-short recordings substantially improves reliability (Schroeder et al., 2004). Plews et al (2013) has recently advocated the use of the average of HRV parameters recorded daily over approximately one week to improve the reliability. Finally, Nunan et al (2008) recommends that, where possible, ECGs should be used instead of Polar devices.

Breathing frequency also has a substantial effect on the spectral measures of HRV. For example, a particularly low breathing frequency (less than 6 breaths/min) will shift the 'respiration' peak, or HF band, into the LF range (0.04 – 0.15 Hz). Conversely, a particularly high breathing frequency (above 24 breaths per minute) may shift the HF peak outside of the typical HF range (0.15 – 0.4 Hz) (Penttilä et al., 2001). In order, to minimise the influence of different breathing patterns, the use of controlled respiration, typically between 12 – 15 breaths per minute, has become commonplace.

While several reliability studies have emerged in recent years, most of these have been conducted in reasonably young, healthy, and physically active populations (Gamelin et al., 2006, Guijt et al., 2007, Pinna et al., 2007). For example, the average age in Pinna et al.'s (2007) study was 38 years (ranging from 26 – 56 years) and all the participants were free from medication and chronic disease (Pinna et al., 2007). However, as there is a high prevalence of sedentary behaviour and medication use in the general population (Bertoldi et al., 2006), the findings from this study may not be representative of a typical middle-aged, sedentary population. In addition, Pinna et al (2007) used custom-designed software as opposed to readily available commercial equipment to detect and analyse the RR intervals. Consequently, the findings of their study may not translate well for commercially available equipment. Therefore, the aim of this research is to determine the reliability of various short term HRV measurements in a slightly older, sedentary population. To make the results as reflective of the population as possible, we minimised exclusion based on medication or medical conditions. We also used readily available equipment (Polar RS800CX) and analysis software (Polar Protrainer5^{TM}) to improve the relevance of the results of the study to other researchers. In addition to this, we aimed to determine whether there are alternative means of increasing the reliability of the HRV recording. For example, if HRV is recorded over a 10 minute resting period, would it be better to select an objectively pre-defined 5 minute interval (for example, the last 5 minutes of the 10 minute HR recording), or use researcher discretion to select the clearest, 'most stable' 5 minute portion in the 10 minute HR recording?

B.3 Method

Following a medical examination, 19 participants from the local community (8 males, 11 females) were recruited into the study. Participants were aged between 45 – 70 years, and sedentary (did not meet the guidelines of 30 minutes of planned physical activity on most days of the week (Garber et al., 2011)). Exclusion criteria included any current cardiovascular disease or a history of cardiac events, and any uncontrolled medical condition such as hypertension, or cholesteraemia. Most (n=12) of the participants were on medications however these medications were stable and were unlikely to change over the course of the study thereby reducing the influence of a change in

medication or dosage on HRV. All participants signed a written informed consent prior to participation and this study was approved by the Lincoln University Human Ethics Committee.

Table 19: Anthropometric data for HRV reliability study

	Male (n=8)	Female (n = 11)
Age (y)	56.3 ± 4.6	55.5 ± 6.7
Weight (kg)	76.9 ± 11.5	73.2 ± 14.7
Height (cm)	170.8 ± 6.8	165.4 ± 6.6
BMI	26.4 ± 4	26.8 ± 5.6
Body fat (%)	24.5 ± 6.6	35.3 ± 10.7
SBP (mmHg)	114.1 ± 15.5	109.9 ± 16.5
DBP (mmHg)	77.1 ± 12.9	69.1 ± 69
Medications n(%)	6 (75)	6 (54.5)

Medications were for asthma (Ventolin (1M), Flixotide (2F) and Serevent (1F)); Cholesterol (Atorvastatin (1M, 1F) and Simvastatin (1M)). Other medications included Esterin cream (1F), Thyroxine (1F), Asprin (1M), Betaloc (1M), Cilazapril (1M), CPAP (1M), Ropinirole (restless legs, 1M), Radiaodine (1F), Prednisone (1M), Panadeine (1M), Codeine (1F), Paracetamol (1F) and Citalopram (1F). Medications exclude supplements (2 females, 1 male) and occasional medication (such as for migraines, 1 female). (M: male; F: female). Results are mean ± standard deviation, unless otherwise specified.

Participants were advised to avoid caffeine, alcohol or strenuous exercise for 24 hours, and any large meals 2 hours prior to the assessments. Participants were allowed to book their appointments at any time convenient to themselves, provided the follow-up appointment was at the same time of day, 1 – 2 weeks following the first assessment. Upon arrival at the testing laboratory, the protocol was explained and the participants were familiarised with the equipment and the metronome-controlled breathing protocol. Height (Mechanical Stadiometer, Surgical & Medical Products, Mentone, Australia), weight and body composition (InBody230, Biospace Co. Ltd., Seoul, South Korea) were then measured.

Participants were fitted with a Polar transmitter belt (Wearlink W.I.N.D, Polar Electro Oy, Kempele, Finland) applied with contact electrode gel (Signa Crème®, Electrode cream, Parker Laboratories Inc. Fairfield, New Jersey, USA) to optimise the contact between the electrodes of the transmitter belt and the skin. Heart rate data were recorded and stored on a Polar heart rate watch (RS800CX, Polar Electro Oy, Kempele, Finland) set to capture RR interval data with an accuracy of 1ms. A facemask (Hans Rudolph, Kansas City, MO, USA) was placed over the participant's nose and mouth to monitor adherence to the controlled breathing interval. Respiration data were analysed using a breath-by-breath gas analysis system (MetaMax® 3B; Cortex Biophysik, Leipzig, Germany). All equipment was calibrated as per manufacturer instruction.

Participants rested in a supine position in a semi-dark room while resting heart rate and heart rate variability were recorded continuously for 16 minutes. For the first 10 minutes, participants breathed spontaneously. Participants were then instructed to breathe in time to a metronome set at 12

breaths per minute (inhale and exhale for 4 seconds each) for an additional 6 minutes. No attempt was made to control tidal volume, participants were simply asked to breathe as normally as possible.

HR data were uploaded to the Polar ProTrainer5^{TM} (Version 5.40.172) interface. All data were automatically filtered for ectopic beats using the "error correction" function in the Polar ProTrainer5^{TM} software (using default settings: moderate filter power, minimum protection zone of 6 beat·min^{-1}, and 'cut' peaks in the data). Three different 5-minute segments of the HR trace were selected for HRV analysis (Figure 28**Error! Reference source not found.**). The 3 segments included: the most 'stable' segment (determined visually by the researcher), the last 5 minutes of the 10 minute spontaneous breathing resting period, and 5 minutes of the controlled breathing period, starting 30 seconds after the start of the metronome.

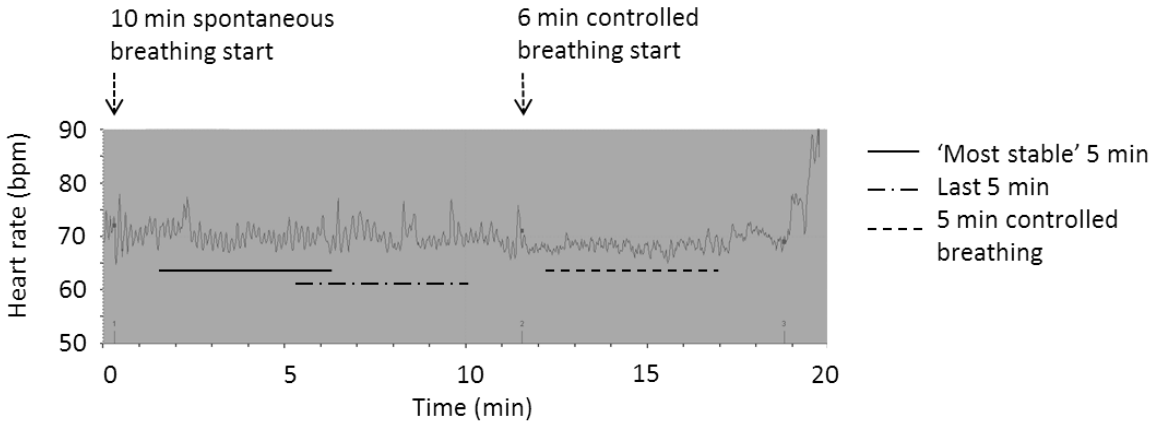

Figure 28: 5 minute selections for HRV analysis
Three 5-minute heart rate segments were extracted from the 16 minute heart rate recording. During the first 10 minutes participants breathed spontaneously, followed by 6 minutes of breathing in time to a metronome at 12 breaths per minute. The Three 5 minute segments included the 'most stable' 5 minutes during the 10 minute spontaneous interval, the 'last 5 minutes' of the 10 minute spontaneous breathing interval and 5 minutes starting 30 seconds in to the controlled breathing period.

Time domain analysis included the root mean square of successive differences (rMSSD), and the natural logarithm of this measure (Ln rMSSD), as well as the standard deviation of the NN interval (SDNN). Spectral analyses included both high (HF, 0.15 – 0.4 Hz) and low frequency (LF, 0.04 – 0.15 Hz).

Statistical analyses

To determine the most appropriate means of detecting outliers, the initial data from Time 1 of the 'most stable' dataset was scrutinised for outliers using three different methods. The first was a visual inspection of the data. In this method, the researcher plotted the data in a scatter graph, and selected data that appeared outside the normal range or grouping of the other observations (i.e. data that appeared to be outliers). The advantage of this method is that is a simple and convenient

method of identifying and removing erroneous data. However, the technique is highly subjective (and thus may vary from researcher to researcher), and it may be difficult to make a decision regarding some borderline data points.

The second method used to inspect the data for outliers is based on the Standard deviation (SD) as estimated from the median value, namely the Median Absolute Deviation (MAD) (Wilcox, 2010). In this method the raw data are ranked, and the median is determined. Each observation is then subtracted from the median value. The absolute values of the differences between the observation and the median are then ranked again, and the median of the absolute differences is the MAD. The MAD divided by the constant 0.6745 is used to estimate the population standard deviation (SD) (Wilcox, 2010). (This constant is sometimes written as MAD X 1.4826 (Leys et al., 2013) which is simply $\frac{1}{0.6745}$)). Based on the work of Miller (1991), Leys et al (2013) recommends using an outlier threshold of 2.5 X estimated population SD. This creates a moderate level of conservativeness which reduces the chances of excluding a true value (which is more likely when multiplying by 2), while reducing the risk of including erroneous data (which is more likely when multiplying by 3) (Leys et al., 2013). Thus in our sample, an outlier was identified if:

$$|X - M| > 2.5 \frac{MAD}{0.6745}$$

Where X is the individual observation, M is the population's median value, MAD is the absolute difference between the observation and the median, 0.6745 is the constant applied to estimate the sample mean, and where data falling within 2.5 times the estimated sample mean are accepted. (Variations using 2 and 3 X estimated population median SD is also presented in Figure 29. Because this method is centred on the median rather than the mean, it is highly tolerant of outliers, and thus has a very high level of resistance before the method breaks down. That is, more than 50% of the data would have to be spurious before the median is affected. The limitation of the method is that using the median to estimate the population mean is not as accurate as the true mean and standard deviations of a normally distributed population (Wilcox, 2010).

The third method is based on the standard deviations from the mean, and is one of the most frequently used means of reporting outliers (Leys et al., 2013). In this method, data that are outside a pre-defined number of standard deviations (usually 2 - 5) is excluded. As 2 times the SD appears to be the minimum number of standard deviations used to detect outliers, this is what we have used in our analysis. While this method is quick and easy to apply, the biggest limitation is that the method itself is subject to the influence of the outlying data.

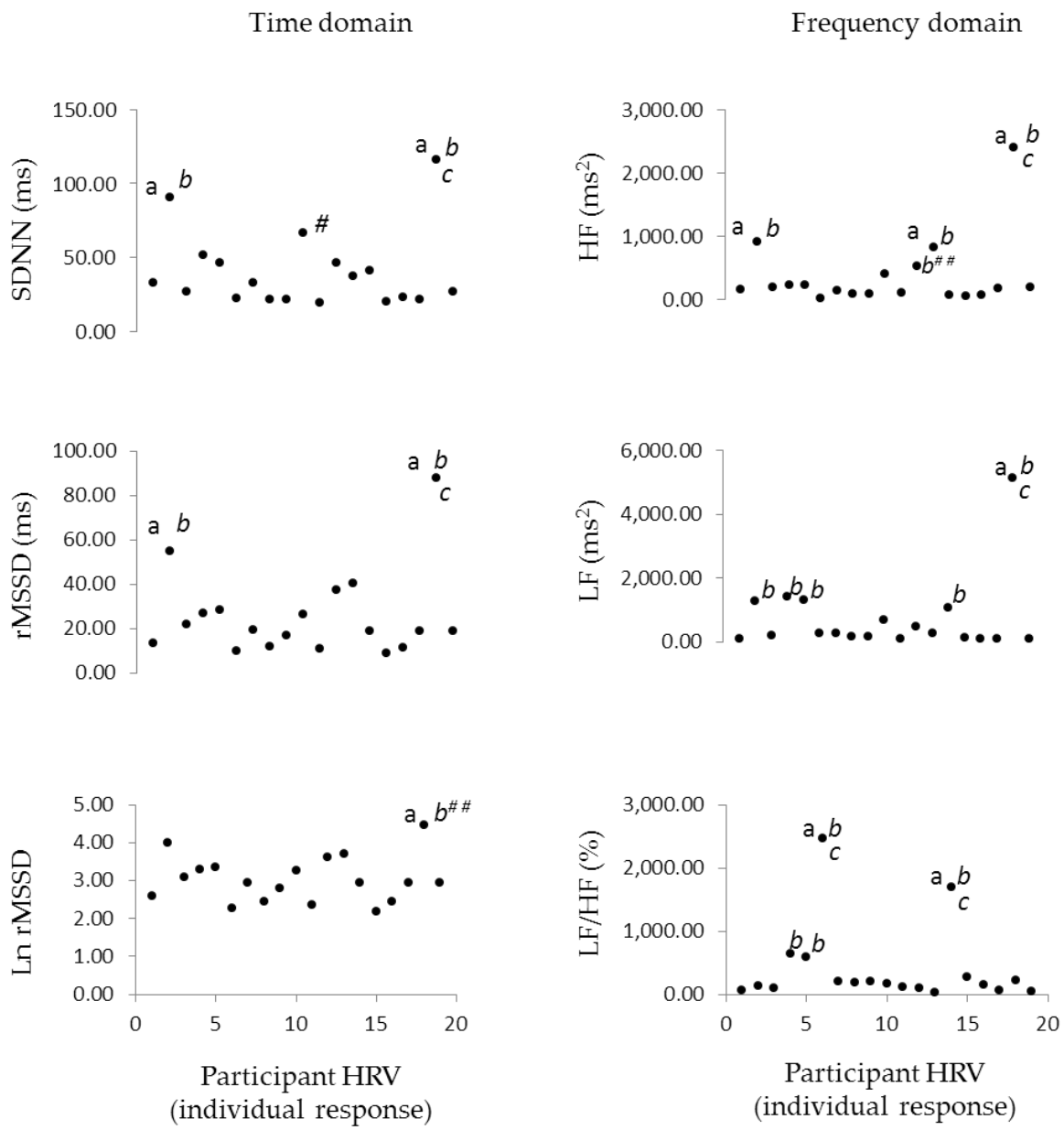

Figure 29: Outliers detected by visual analysis, MAD and SD methods (using the most consistent 5 min dataset)

To determine the closest, objective outlier detection, the HRV data were initially screened by 3 different methods. **A)** Visual inspection of the data, outliers detected using this method are indicated by the symbol "a". **B)** Median Absolute Deviation (MAD) which detects outliers based on their proximity to the median value (2, 2.5 or 3 times the standard deviation (SD) estimated by the median) "b" denotes outliers detected at all three strengths, "*b*$^{\#\#\prime}$" denotes observations detected as an outlier only at the 2.5 and 2 X SD range, and $^{\#}$ denotes observations that were only detected at the 2 X SD range). **C)** Outliers detected if they were more than 2 SD from the sample mean. Outliers detected by this method are denoted as "*c*". SDNN: Standard deviation of NN intervals; (Ln) rMSSD: (natural logarithm of the) Root mean square successive difference; HF: High frequency; LF: Low frequency; LF/HF: low frequency to high frequency ratio; HRV: heart rate variability

Results were filtered for outliers using the MAD method (which was the most sensitive to outlying data), and erroneous data were removed prior to further analysis. Each dataset, i.e. 'most stable', 'last 5 min' and 'controlled breathing' were analysed individually and data excluded from one data set were not necessarily excluded from the other. If an rMSSD observation was highlighted as an outlier, the corresponding Ln rMSSD measurement was also removed. As the absolute HF and LF measurements were measured, as opposed to the normalised units, the exclusion of one did not exclude the other.

Bland Altman plots were then constructed where by the difference between the Time 1 and Time 2 measurements were plotted against the mean of the 2 measurements (Figure 30). This is a useful method for qualitatively displaying the agreement between the two time measurements, and to determine whether there is any relationship between mean measurement and the differences between the two time measurements (Bland and Altman, 2010).

Reliability can be assessed in both a relative and an absolute manner. For example, relative reliability is used to assess how much a participant varies in their measurement relative to the rest of the participants and is usually measured using an intraclass correlation coefficient, (ICC) (Hopkins, 2000b). On the other hand, absolute reliability informs us how much that individual's measurement fluctuates in repeated measurements under consistent conditions. Absolute reliability is usually given as the standard error of measurement (SEM) or typical error (Hopkins, 2000b), or coefficient of variation (CV) (Atkinson and Nevill, 1998, Hopkins, 2000b)).

However, because the typical error and ICC calculations and are based on the assumption that the difference in time 1 and time 2 recordings is random (homoscedastic) (Hopkins, 2000b, Atkinson and Nevill, 1998), and as the 95% limits are based on the assumption of normality (Bland and Altman, 1999), the data needed to be tested for heteroscedasity and non-normality. The differences between the two measurements were assessed for normality using a spreadsheet designed by Guth (2006) to display a normal quantile plot. Normality was rejected if the correlation coefficient (r) between the Z score and the observation was less than the critical value of $\alpha = 0.05$.

To assess for heteroscedasity, the absolute difference in the measurements was plotted against their mean, and the correlation coefficient was calculated. If the heteroscedasticity correlation is close to zero, we can assume the data are homoscedastic (or normally distributed). However, even if a weak relationship exists, it is beneficial to log transform the data prior to analysis (Atkinson and Nevill, 1998).

Spreadsheets designed by (Hopkins, 2000b) were used to calculate the ICC (as a measure of relative reliability) and the typical error (as a measure of absolute reliability and given as a coefficient of

variation). As there are several ways of determining the ICC, each of which giving slightly different answers (Atkinson and Nevill, 1998), it is valuable to state how data were analysed. In this study, ICC was determined as follows:

$$ICC = \frac{SD^2 - sd^2}{SD^2}$$

Where SD^2 = between-subject standard deviation, and the sd^2 is the within-subject standard deviation (Hopkins, 2000b). We have added slightly to categories defined by Pinna et al. (2007) used to interpret the strength of the ICC. Based on the recommendations of several authors, Pinna et al (2007) identified ICC > 0.8 as good - excellent, and ICC > 0.6 as substantial. Based on these categories, the following interpretation of the ICC will be used: ICC > 0.9: Excellent; ICC > 0.8: Good; ICC > 0.7 substantial; ICC > 0.6: Reasonable; ICC <0.6: Poor

Typical error was calculated as follows:

$$\frac{time\ 1 - time\ 2}{\sqrt{2}}$$

All data were log transformed, so the coefficient of variance (CV) was derived from the typical error and was expressed as a percent. The 95% upper and lower confidence limits were also determined. The confidence limits represent the likely range of the systemic change (Hopkins, 2000b). To assess the magnitude, or appropriateness of the typical error, the normal fluctuations of the HRV parameters were determined in a subset of 10, randomly selected participants. In these participants, the 2 successive 5-minute intervals from the 10 minute spontaneous breathing period were selected and analysed using the Polar ProTrainer5™ software. The typical error of the log transformed data were determined. This gave the expected percentage fluctuation of the assumedly 'steady state' recording. If the typical error for the Time 1 and Time 2 recordings fell within the expected fluctuation of the first 5 minute and second 5 minute measurement, the typical error was considered 'ideal', if it fell within 2X the expected fluctuation, it was considered 'good', and if it fell within 3X the expected fluctuation it was considered 'acceptable'.

The RR interval and heart rate are highly correlated, thus, heart rate was reported instead of the RR interval to increase the relevance of our findings to studies not using HRV parameters. While the RR data will not be presented explicitly in this paper, it can be extrapolated by using the relationship between frequency and period ($T = f^{-1}$) where T is period and f is frequency. (Period is used to solve for the RR interval).

As HR is measured in beat·min^{-1}, the frequency is thus $\frac{HR}{60}$, and since the RR interval (in ms) = T (in seconds) *1000, this equation resolves to:

$$RR\ interval = \left(\frac{60}{HR}\right) * 1000$$

As HR is rounded off to a whole number, results using this formula for HR < 100 beat·min^{-1} will be accurate up until the last digit (when the RR interval is expressed in ms).

B.4 Results

Outlier detection

A comparison of the outcomes for the different methods of detection is displayed in Figure 29 and the outlier values are given in Table 20. The MAD method was the most sensitive, followed by visual inspection, and the 2XSD method. The MAD method was selected as the method for outlier detection for the remainder of the conditions. There were approximately twice as many outliers detected from the Time 1 dataset as there were from the Time 2 dataset. A considerable number of HF and LF data points were highlighted as outliers which left an insufficient number of high and low frequency pairs to compare the reliability of the LF: HF ratio.

Normality and homoscedasity

While most of the differences in Time 1 and Time 2 measurements followed normal distributions (HR, rMSSD, Ln rMSSD, HF in all conditions, and LF in controlled breathing only), most of the data were heteroscedastic (see Table 21), and required log transformation prior to analysis. Since it would be redundant to report the log transformed data twice, only the SDNN and rMSSD data are reported (omitting the Ln rMSSD). Heteroscedasity was also evident in the Bland Altman plots (Figure 30) where most of the HRV measures exhibit an increasing difference with an increasing mean measurement (see especially the spectral analyses).

Table 20: Outlier values

	Most stable spontaneous	Last 5 minutes spontaneous	Controlled breathing
	SDNN (ms)		
Time 1 (n; outlier value(s))	2(90.3[1]; 116.4[2])	2 (138.6[2]; 174.1[3])	0
Time 2 (n; outlier value(s))	0	0	0
	rMSSD (ms)		
Time 1 (n; outlier value(s))	2 (54.7[1]; 88[2])	3 (55.7[1]; 68.0[3]; 92.5[2])	1 (65.7[1])
Time 2 (n; outlier value(s))	1 (52.00[4])	0	0
	Ln rMSSD (ln ms)		
Time 1 (n; outlier value(s))	1 (4.48[2])	1 (4.53[2])	0
Time 2 (n; outlier value(s))	0	0	0
	HF (Hz)		
Time 1 (n; outlier value(s))	4 (524.59[5]; 823.39[4]; 906.93[1]; 2406.86[2])	4 (857.69[4]; 989.74[1]; 1258.29[3]; 2229.29[2])	3 (568.44[4]; 656.46[5]; 1765.47[1])
Time 2 (n; outlier value(s))	1 (1083.31[4])	1 (864.23[4])	1 (1057.56[4])
	LF (Hz)		
Time 1 (n; outlier value(s))	5 (1072.84[6]; 1278.56[1]; 1319.9[7]; 1398.96[8]; 5157.83[2])	2 (5335.08[3]; 7143.11[2])	4 (783.18[5]; 877.28[7]; 1286.98; 1691.25[1])
Time 2 (n; outlier value(s))	3 (572.4[7], 574.37[6], 739.95[5], 1669.79[8])	1 (1669.79[8])	2 (553.68; 578.13[7])

Superscript numbers $^{(1-8)}$ indicate data from one participant. In the 'most stable' dataset, it is possible that the 'most stable' data were indeed the last five minutes, which is why, for example, there are identical LF outliers in both the 'last 5 min' and 'most stable' data sets. For the data analysis, if data were considered an outlier both the participant's Time 1 and Time 2 observations were removed. This table ignores this rule and examined Time 1 and Time 2 datasets independently. There were no outliers detected for heart rate. Time 1: measurement taken during the first assessment; Time 2: measurement taken during the second measurement at the same time of day but 1 - 2 weeks following the first measurement; SDNN: Standard deviation of NN interval; (Ln) rMSSD: (natural logarithm) root mean square successive difference; HF: high frequency; LF: low frequency

Table 21: Heteroscedasity correlation coefficient

	HR	SDNN	rMSSD	Ln rMSSD	LF	HF
Most stable	-0.13	0.40	0.39	0.12	0.81	0.84
Last 5 min	-0.25	0.50	0.24	-0.13	0.53	0.56
Controlled breathing	0.43	0.44	0.30	-0.36	0.60	0.49

Heteroscedasity was assessed by plotting the (Time 1 – Time 2) difference against the mean of the two measurements and then determining the correlation coefficient of the relationship between the two. The closer the correlation is to zero, the more homoscedastic (random) the change in the variables is. As you can see from the above table, all of the HRV measurements has at least a weak (in the case of Ln rMSSD and HR) and in some cases a strong (HF in the most stable condition) correlation, which indicates heteroscedasity, or some relationship between the magnitude of the difference between the Time 1 and Time 2 measurement and the mean. HR: heart rate; SDNN: Standard deviation of NN interval; (Ln) rMSSD: (natural logarithm) root mean square successive difference; HF: high frequency; LF: low frequency

Relative and absolute reliability

The results from the ICC and the typical error (expressed in percent as CV) are presented in Table 22. The typical error in HRV parameters from the two successive 5-minute analyses are as follows: HR: 1.4%; SDNN 20.8%; rMSSD 10.9%; LF: 33.6%; HF: 27.2%. Thus, if the typical error was less than or equal to the above mentioned typical value for the respective measurement, the reliability was considered 'ideal'. The typical error was considered 'reasonable' if it was between "ideal" and 2 X this value, and "acceptable" if the typical error was within 2 – 3 X this value. Typical errors greater than 3X the expected fluctuation during one measurement were considered "poor".

All participants breathed in time with the metronome during the controlled breathing segment. That is, participants maintained an average breathing rate of 12 $\pm$ 0.5 breaths per minute, with an individual standard deviation of less than 1 breath per minute). Breathing frequency data are as follows: Time 1 (12.11 $\pm$ 0.12, mean $\pm$ SD), Time 2 (12.14 $\pm$ 0.19).

Table 22: A table comparing the relative and absolute reliability of the various HRV parameters

	Most stable spontaneous	Last 5 minutes spontaneous	Controlled breathing
HR (beat·min^{-1})			
Time 1 (mean ± SD)	64.3 ± 8.6	64.9 ± 8.3	65.2 ± 7.2
Time 2 (mean ± SD)	65.6 ± 7.7	65.1 ± 7.1	65.3 ± 7.0
n	19	19	19
ICC (95% CL)	0.76 (0.48 – 0.9) #	0.82 (0.58 – 0.92)*	0.81 (0.58 – 0.92)*
Typical error as CV (95% CL)	6.7 (5.0 – 10.1)	5.6 (4.2 – 8.3)	5.0 (3.8 – 7.5)
SDNN (ms)			
Time 1 (mean ± SD)	32.8 ± 13.6	41.0 ± 18.6	35.0 ± 16.5
Time 2 (mean ± SD)	34.0 ± 12.1	37.3 ± 11	31.8 ± 8.8
n	17	17	19
ICC (95% CL)	0.57 (0.13 – 0.82)	0.48 (0.01 – 0.77)	0.49 (0.06 – 0.77)
Typical error as CV (95% CL)	28.4 (20.4 – 46.3) $	32.3 (23.2 – 53.2) $	33 (24.0 – 52.4) $
rMSSD (ms)			
Time 1 (mean ± SD)	18.7 ± 8.1	21.3 ± 9.8	20.2 ± 9.1
Time 2 (mean ± SD)	20.5 ± 9.6	23.7 ± 11.6	22.4 ± 8.8
n	16	16	18
ICC (95% CL)	0.78 (0.48 – 0.92) #	0.82 (0.56 – 0.93)*	0.73 (0.41 – 0.89) #
Typical error as CV (95% CL)	24.8 (17.8 – 40.8) ^	24.4 (17.5 – 40.2) ^	26.5 (19.3 – 42.3) ^
HF (Hz)			
Time 1 (mean ± SD)	140.2 ± 98.4	181.5 ± 133	192.5 ± 147.0
Time 2 (mean ± SD)	190.7 ± 167.4	245.3 ± 202.6	272.7 ± 196.9
n	15	15	16
ICC (95% confidence limit)	0.74 (0.39 - 0.91) #	0.76 (0.43 – 0.91) #	0.64 (0.23 – 0.86) ‡
Typical error as CV (95% CL)	61 (41.7 – 111.9) ^	63.5 (43.3 – 117.1)	65.0 (44.7 – 117.0)
LF (Hz)			
Time 1 (mean ± SD)	199.3 ± 159.4	434 ± 358.4	175.8 ± 148.0
Time 2 (mean ± SD)	217.3 ± 125.1	349.2 ± 222.8	165.8 ± 106.8
n	13	16	14
ICC (95% confidence limit)	0.21 (-0.36 – 0.67)	0.42 (-0.08 – 0.75)	0.64 (0.16 – 0.87) ‡
Typical error as CV (95% CL)	68.1 (45.1 – 135.8) ^	84.5 (57.2 – 158) ^	56.4 (37.8 – 109.3)

Relative absolute reliability was categorised as follows: *Good relative reliability (ICC >0.8); #Substantial relative reliability (ICC > 0.7); ‡ reasonable relative reliability (ICC > 0.6). Absolute reliability was categorised relative to the naturally occurring typical error as measured from consecutive 5 minute HR recordings. Absolute reliability is categorised as follows: $good absolute reliability (within 2 X naturally occurring typical error); and ^acceptable absolute reliability (within 3 X naturally occurring typical error). HR: heart rate measured in beats per minute; Time 1: measurement taken during the first assessment; Time 2: measurement taken during the second measurement at the same time of day but 1 - 2 weeks following the first measurement; SD: standard deviation; ICC: intraclass correlation coefficient; CV: coefficient of variation; CL: confidence limit; SDNN: Standard deviation of NN interval; (Ln) rMSSD: (natural logarithm) root mean square successive difference; HF: high frequency; LF: low frequency

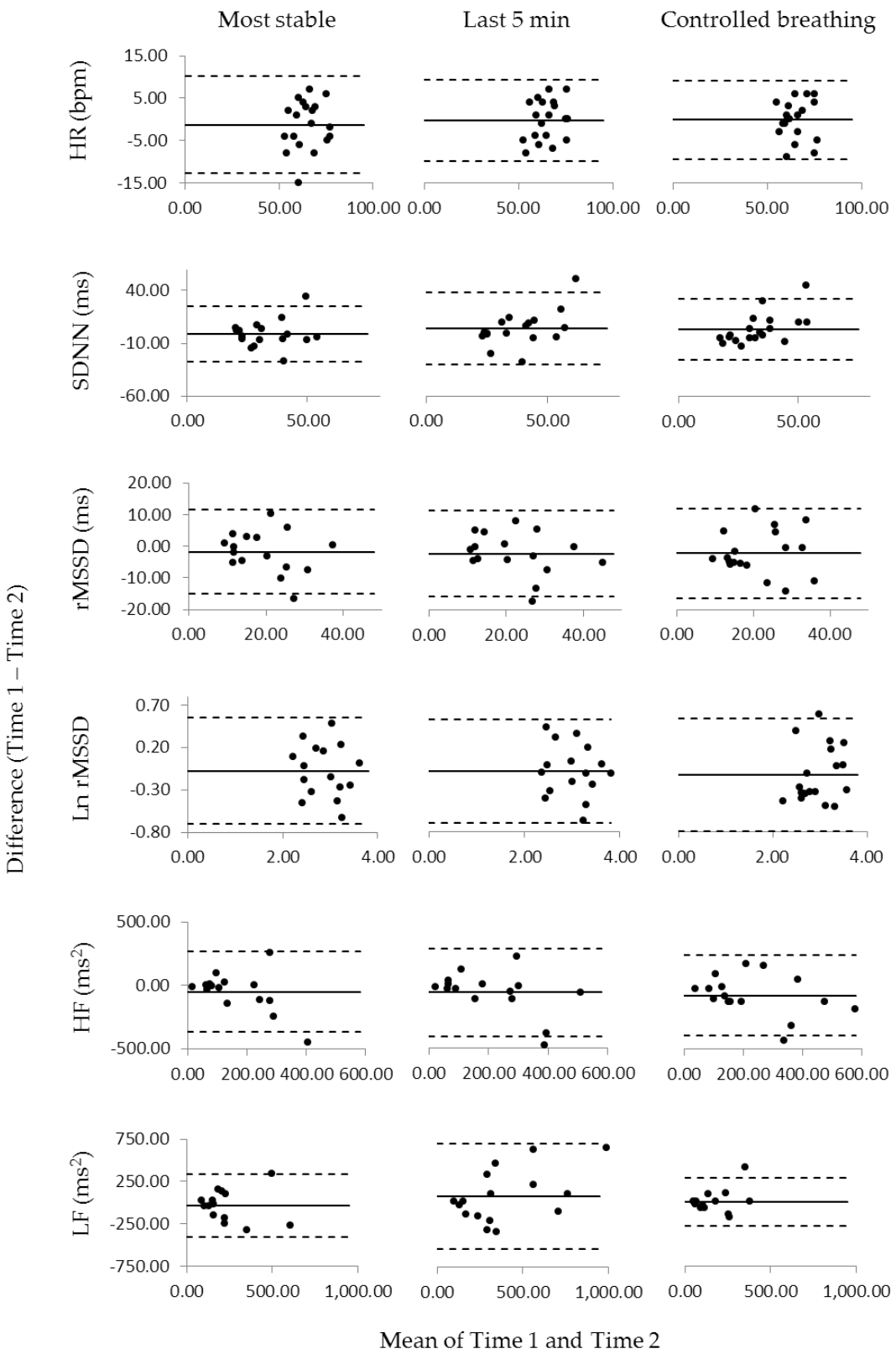

Figure 30: Bland Altman plots of the HRV measurements across the different conditions
The Bland Altman plot is used to detect any relationship between the difference in the 2 time measurements and their mean. The HR and Ln rMSSD are homoscedastic, while SDNN, LF and HF (particularly under the 'most stable' condition are heteroscedastic). Time 1: measurement taken during the first assessment; Time 2: measurement taken during the second measurement at the same time of day but 1 - 2 weeks following the first measurement; SD: standard deviation; SDNN: Standard deviation of NN interval; (Ln) rMSSD: (natural logarithm) root mean square successive difference; HF: high frequency; LF: low frequency.

B.5 Discussion

We expected that selecting the 'most stable' 5 minute interval of spontaneous breathing HR would improve the relative reliability when compared to the fixed 'last 5 minutes', and display results similar to those of the controlled breathing period. Therefore, we found it surprising that the 'most stable' 5 minute segment was very similar, but slightly less relatively reliable than the 'last 5 minutes' in HR, rMSSD, HF and LF, with the 'most stable' 5 minutes only more reliable in SDNN (however, LF and SDNN both had poor relative reliability). Interestingly, the last 5 minutes was also more relatively reliable than the controlled breathing period in the HR, rMSSD, and HF measurements. Controlled breathing improved LF (reasonable relative reliability) and SDNN (poor relative reliability). Both the LF and SDNN are markers that are sensitive to both sympathetic and parasympathetic contributions. In contrast, HF and rMSSD specifically reflect parasympathetic innervation (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). As the vagal response is far quicker than the sympathetic response, the poor reliability in SDNN and LF may be a function of the relatively short time interval.

To assess the absolute reliability, we compared the typical error to the naturally occurring typical error associated with the measurement, rather than using pre-defined arbitrary criteria. For example, if the measure under investigation is a highly dynamic measurement, such as the LF and HF measures, it would be reasonable to expect a wide 'normal' range of resting measures, and a higher typical error. On the other hand, if a measurement is typically a very stable measurement, such as HR or the RR interval, even a small deviation from the normal range could be considered a large or meaningful change. To this end, the typical error was assessed for two consecutive 5 minute measurements during one uninterrupted 10 minute resting period. By basing the acceptability of the typical error from Time 1 to Time 2 on the 'normal steady state fluctuations' of the consecutive 5 minute intervals, we were able to get relative indices of acceptability for the absolute reliability of the HRV parameters. While this improves our ability to gauge the repeatability of the measurements, the researcher must be aware that changes derived from inherently dynamic variables in the assessment of intervention-based change must be interpreted with caution.

By using the above described absolute reliability assessment criteria, HR was not considered absolutely reliable under any of the conditions even though it had the lowest typical error. The SDNN component was the most absolutely reliable time domain component, which was surprising when considering its low relative reliability. This dichotomy is probably due to the high naturally occurring typical error associated with the SDNN, which likely improved the repeatability description but also resulted in reduced relative reliability.

Controlled breathing improved the typical error of the LF to within 2X the steady state typical error which indicates good reliability. All of the other spectral domain measures exhibited an acceptable (3X the steady state) typical error. However, as the typical errors for the spectral domain analyses were large, even though the result is reliable, the researcher should interpret changes in this value with caution. Unlike the relative reliability, selecting the 'most stable' 5 minutes and using controlled breathing improved the absolute reliability. For example, the typical error was lowest for HR and LF under controlled breathing, and the typical error for SDNN and HF were lowest when the 'most stable' data were selected.

While controlled breathing improved reliability (both absolute and relative) in the low frequency measurements, it did not exhibit a substantially different response in other measurements. These findings are similar to those of Penttilä et al (2001) who demonstrated no change in rMSSD with controlled breathing but did report alterations in the spectral domain (although these researchers used a breathing frequency of 15 breaths per minute, rather than the 12 breaths per minute in our study). However, when compared to the improved relative reliability under the 'last 5 minutes', these differences in absolute reliability, do not seem big enough to warrant the use of either controlled breathing, or selecting the most stable 5 minute period instead of using the pre-defined last 5 minutes. Thus, regarding the segments of data to be analysed from the overall HR trace, our data support the use of the last 5 minutes of a 10 minute resting period for the analysis of HRV, particularly for HR, (Ln) rMSSD, and HF. The LF data were most reliable under controlled breathing, and SDNN was most reliable when the most stable 5 minute segment was selected, however in both these measurements the reliability was poor.

While using the average rMSSD taken over one week as suggested by Plews et al. (2013) may considerably improve reliability, a lack of time and resources may not make this possible in large studies. However, it would be worth investigating whether the average of 2 or 3 consecutive 5 minute HRV analyses extracted from one 15 minute HR recording would improve relative and absolute reliability in test – retest protocols. For example, Schroeder et al (2004) demonstrated substantially improved reliability in ultra-short HR recordings when the average of several ultra-short HRV recordings were used, when compared to poor reliability when just 1 measurement was taken.

Our data confirms the findings of Pinna et al. (2007) who reported large typical error measurements, particularly in the spectral domain. However, our study reports a much lower repeatability than Pinna et al (2007) which is likely due to both the population sampled (younger, medication-free individuals compared to sedentary, unhealthy older adults), and measurement technique (ECG and manual editing versus polar heart rate monitor and automated error correction).

The MAD method of outlier detection appeared to be the most sensitive, and 2 SD of the mean did not identify clearly outlying data. While some data points rejected as outliers by MAD were not identified as outliers by visual inspection, as soon as the outlying data were removed and the axes readjusted, the initially accepted points were quite clearly different from the rest of the data. This was particularly evident in the spectral analyses. For example, in the LF analysis (using the 'most stable 5 min' dataset, Figure 29) one outlying data point extended the axes to up to 6000 ms^2 while most of the data were less than 200 ms^2. Thus when the extreme observation was removed, the initially accepted data points at 1000 ms^2 were considered likely erroneous. We recommend, however, that when data are excluded, a list of the identified outliers is made available.

Limitations

The test re-test protocol did not include a full protocol before the two measurements were taken, and so a substantial proportion of the variability in the 2 measurements could be due to familiarization and systematic bias associated with mental stress (Bernardi et al., 2000) rather than by random error association with true equipment and biological variation (Atkinson and Nevill, 1998, Hopkins, 2000b).

B.6 Recommendations:

The number of outliers detected was approximately halved in the Time 2 dataset. This is possibly due to the participant feeling more comfortable with the equipment and procedures and was more relaxed during the measurement. This suggests that having a full practice assessment prior to the baseline measurements substantially improves the quality of recording in this population, and should be included in any test-retest protocol.

Regarding outlier detection, the Median Absolute Deviation (MAD) may provide a reasonable and objective method of identifying outliers. However, we would recommend using this method in association with visual inspection to determine whether to use 2, 2.5 or 3 X $\frac{MAD}{0.6745}$. For example, where a relatively small change is expected (such as in changes in resting values), a coefficient of 2 might improve reliability, however, when larger changes are possible, a more conservative threshold for exclusion might be necessary and a coefficient of 3 might be more appropriate.

The results of this study recommend the use of rMSSD (and/or the natural logarithm of the rMSSD signal) as the most reliable measure of HRV in short term recordings in a sedentary, middle-aged population. High Frequency (absolute units) in the spectral domain can be also be used, but the researchers should be aware that although the relative reliability is reasonable (0.76 (0.43 – 0.91)), the absolute reliability is unclear (65% (43.3 – 117.1), Typical error as CV, 95% confidence interval).

Measurements sensitive to both parasympathetic and sympathetic innervation such as SDNN and LF should be avoided in this population in short term recordings.

The findings of this study also recommend the use of a fixed, rather than selected 5 minute period for the analysis of HRV.

B.7 Conclusion

While the HRV indices in our sedentary, older population with various medical complaints was somewhat less reliable than the reliability reported in younger, healthy, active cohorts, the absolute and relative reliability for rMSSD, Ln rMSSD and HF was still acceptable. We recommend the use of rMSSD or Ln rMSSD in the assessment of HRV in this population. Researchers should be aware that the typical error associated with all HRV measurements is reasonably high, particularly regarding spectral measures. As such, interpretation of intervention-related changes in HRV using HF and LF measurements should be assessed with caution. The median absolute deviation (MAD) method of outlier detection is a robust, sensitive and objective means of outlier identification.

Appendix C

Raw data for Study 2: The minimum dosage of intermittent hypoxic exposure per week for health improvement in sedentary, middle-aged individuals.

Table 23: Raw baseline and post-intervention data for Study 2

	C		IHE3		IHE5	
	Baseline	**Post**	**Baseline**	**Post**	**Baseline**	**Post**
Anthropometric data						
Age (y)	54.8 ± 5.4		57.0 ± 5.9		55.7 ± 5.0	
M/F	3M6F		4M6F		5M5F	
Height (cm)	168.7 ± 3.5		171.4 ± 10.2		163.7 ± 6.8	
Weight (kg)	79.5 ± 11.5	79.2 ± 11.4	81.6 ± 20.2	80.9 ± 19.9	76.2 ± 13.7	76.4 ± 13.8
BMI	27.9 ± 4.1	27.8 ± 4.0	27.6 ± 5.2	27.4 ± 5.2	28.5 ± 5.6	28.7 ± 5.7
Body fat (%)	34.3 ± 7.0	34.2 ± 7.1	31.8 ± 11.4	30.4 ± 12.4	32.9 ± 10.4	32.5 ± 11.3
Muscle mass (kg)	28.8 ± 3.9	28.9 ± 4.2	30.7 ± 8.5	29.7 ± 8.7	28.6 ± 5.6	28.2 ± 4.7
HRV: Spontaneous breathing						
RR interval (ms)	928 ± 114	927 ± 123	966 ± 61	942 ± 74	986 ± 67	984 ± 76
rMSSD (ms)	23.3 ± 10.7	20.9 ± 11.6	26.2 ± 14.8	23.9 ± 10.9	26.4 ± 7.3	29.6 ± 10.0
SDNN (ms)	40.4 ± 11.2	34.8 ± 13.0	32.7 ± 9.8	30.6 ± 9.1	37.2 ± 9.5	35.2 ± 13.1
HF (ms^2)	277.5 ± 247.0	155.0 ± 194.0	237.5 ± 220.6	228.3 ± 177.9	233.8 ± 107.7	296.5 ± 135.8
LF (ms^2)	258.3 ± 105.7	143.1 ± 107.2	278.2 ± 257.7	228.1 ± 166.6	297.7 ± 209.4	181.3 ± 68.3

	C		IHE3		IHE5	
	Baseline	**Post**	**Baseline**	**Post**	**Baseline**	**Post**
HRV: Controlled breathing						
RR interval (ms)	938 ± 123	913 ± 125	970 ± 43	967 ± 36	970 ± 63	992 ± 75
rMSSD (ms)	21.9 ± 9.9	18.1 ± 10.2	23.2 ± 11.1	23.6 ± 8.1	26.4 ± 5.1	31.3 ± 10.1
SDNN (ms)	32.2 ± 7.3	30.1 ± 6.2	27.5 ± 9.8	31.4 ± 8.5	35.8 ± 8.9	39.0 ± 12.4
HF (ms^2)	281.9 ± 250.3	223.5 ± 238.3	313.1 ± 329.6	299.0 ± 260.1	390.8 ± 154.4	443.9 ± 235.6
LF (ms^2)	144.9 ± 24.1	147.1 ± 174.1	119.2 ± 89.17	226.7 ± 145.8	212.4 ± 147.8	246.2 ± 126.6
Resting BP						
SBP (mmHg)	110.4 ± 17.2	110.6 ± 15.8	122.7 ± 18.4	113.11 ± 15.5	114.2 ± 23.1	109.00 ± 23.9
Blood Samples						
Hb (g/L)	139.3 ± 14.9	138.4 ± 13.8	137.6 ± 8.3	138.1 ± 10.3	142.2 ± 13.2	144.7 ± 11.4
Hs-CRP (mg/L)	1.6 ± 2.6	1.6 ± 2.1	1.5 ± 1.5	1.6 ± 1.2	2.1 ± 2.3	2.6 ± 3.0
Hs-CRP (quintiles)	4	4	4	4	4	4
Submaximal exercise						
HR_{submax} ($beat \cdot min^{-1}$)	105.5 ± 5.6	106.9 ± 7.7	108.1 ± 7.3	108.1 ± 10.0	106.8 ± 7.5	107.5 ± 9.3
$\dot{V}O_{2submax}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	15.5 ± 2.3	14.7 ± 14.7	15.6 ± 3.0	15.5 ± 3.1	17.1 ± 3.9	15.9 ± 3.2
SBP_{submax} (mmHg)	148.8 ± 23.2	141.6 ± 14.0	157.4 ± 24.1	151.1 ± 15.7	140.6 ± 23.7	134.9 ± 26.5
Maximal exercise						
$\dot{V}O_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	26.8 ± 3.3	24.8 ± 3.9	23.7 ± 6.0	24.5 ± 5.7	27.2 ±7.5	25.3 ± 7.5
$Time_{ex}$ (min)	5.4 ± 1.2	5.0 ± 2.3	5.0 ± 1.8	5.2 ± 2.3†	5.1 ± 1.4	5.8 ± 1.7
Res_{max} (watts)	146.4 ± 26.7	142.9 ± 40.1	142.5 ± 29.0	142.5 ± 33.4	136.1 ± 28.3	147.22 ± 29.2

Values are mean ± SD; C: Control group; IHE3: Group attending 2 – 3 IHE sessions / week; IHE5: Group attending 5 IHE sessions/week; Baseline: measurement taken at baseline; Post: measurement taken within 1 week of the last IHE session; M: male; F: female; BMI: Body mass index; RR interval: average time interval between R to R peaks; SDNN: standard deviation of normal to normal heart beat intervals; HF: high frequency; LF: low frequency; SBP: Systolic blood pressure; Hb: haemoglobin; Hs-CRP: highly sensitive C-Reactive protein; HR_{submax}: Average heart rate taken over the last 5 minute of the steady state exercise period; $\dot{V}O_{2submax}$: oxygen uptake during submaximal exercise; SBP_{submax} systolic blood over the steady state submaximal exercise period; $\dot{V}O_{2peak}$: oxygen uptake over the last 30 seconds of peak physical exertion; $Time_{ex}$: Time from the end of submaximal steady state exercise to exhaustion; Res_{max}: maximum sustained (for at least 30 sec) resistance.

Appendix D

Raw data for Study 3: Does IHE enhance the cardioprotective effect of exercise in a sedentary population?

Table 24: Raw baseline, post, 4-wk and 8-wk data for Study 3

	IHE + Ex				Ex			
	Baseline	**Post**	**4-wk**	**8-wk**	**Baseline**	**Post**	**4-wk**	**8-wk**
Anthropometric data								
Age (y)	56.7 ± 6.4				56.4 ± 6.5			
M/F	6M10F				5M13F			
Height (cm)	168.0 ± 8.8				167.3 ± 8.4			
Weight (kg)	78.6 ± 12.4	78.5 ± 12.1	79.5 ± 11.8	79.7 ± 11.4	81.2 ± 15.9	81.0 ± 16.1	79.3 ± 15.5	80.4 ± 15.4
Body fat (%)	32.3 ± 8.9	31.3 ± 8.7	31.2 ± 8.3	31.9 ± 8.8	35.9 ± 8.0	35.2 ± 8.4	34.7 ± 8.6	34.2 ± 9.4
Body fat (kg)	25.3 ± 8.5	24.5 ± 8.3	24.7 ± 25.3	25.3 ± 8.1	29.5 ± 5	29.0 ± 11.6	27.8 ± 10.9	28.0 ± 11.7
Muscle mass (kg)	29.8 ± 7.1	30.3 ± 6.9	30.7 ± 6.8	30.5 ± 7.0	28.8 ± 6.0	28.9 ± 6.0	28.6 ± 6.3	29.2 ± 6.0
Arterial health								
PWV (ms)	7.91 ± 1.0	7.5 ± 1.4	7.3 ± 0.8	7.2 ± 1.3	7.8 ± 1.3	7.3 ± 1.3	7.4 ± 1.1	7.8 ± 7.7
PWA	20.7 ± 8.7	17.2 ± 6.6	21.3 ± 7.5	23.2 ± 8.0	27.7 ± 7.1	26.0 ± 5.3	27.3 ± 5.9	26.5 ± 6.7
Heart rate variability								
RR interval (ms)	903.6 ± 106.1	993.2 ± 148.3	943.5 ± 128.9	1019.2 ± 137.5	981.1 ± 109.3	1032.9 ± 159.1	1033.4 ± 129.6	1034.0 ± 157.0
rMSSD (ms)	21.7 ± 9.4	32.2 ± 13.7	30.6 ± 15.7	31.0 ± 16.3	28.9 ± 9.8	33.4 ± 13.3	32.5 ± 16.9	33.6 ± 15.3
SDNN (ms)	37.3 ± 10.9	43.7 ± 14.2	40.3 ± 14.1	44.0 ± 11.5	37.3 ± 11.8	43.2 ± 14.5	45.1 ± 17.0	45.4 ± 16.5
HF (ms^2)	240.7 ± 208.2	462.4 ± 342.6	311.0 ± 248.8	430.8 ± 369.7	269.3 ± 173.3	392.4 ± 213.9	258.0 ± 141.1	374.0 ± 241.1
LF (ms^2)	342.7 ± 229.6	493.2 ± 316.6	531.8 ± 294.0	418.2 ± 218.8	311.1 ± 213.8	490.7 ± 273.8	407.0 ± 273.5	454.1 ± 265.6
LF:HF ratio	103.0 ± 50.5	79.4 ± 39.2	156.6 ± 108.8	130.8 ± 74.1	105.9 ± 58.9	151.2 ± 69.7	158.4 ± 72.4	130.1 ± 80.7

	IHE + Ex				Ex			
	Baseline	**Post**	**4-wk**	**8-wk**	**Baseline**	**Post**	**4-wk**	**8-wk**
Cardiovascular fitness								
$\dot{V}O_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	29.5 ± 6.8	29.8 ± 7.8	30.1 ± 5.7	29.3 ± 8.0	26.9 ± 5.7	26.9 ± 6.4	27.4 ± 6.4	25.5 ± 6.8
Time to exhaustion (min)	14.0 ± 1.9	16.0 ± 1.9	15.7 ± 1.4	16.7 ± 2.3	14.0 ± 2.3	15.5 ± 2.5	16.5 ± 1.8	16.1 ± 2.2
Systolic blood pressure								
SBP (mmHg)	125.3 ± 11.9	121.9 ± 13.4	120.8 ± 13.9	122.7 ± 12.0	124.7 ± 17.6	125.4 ± 18.1	121.0 ± 15.8	125.0 ± 15.5
Blood Samples								
Total cholesterol	5.8 ± 1.2	5.8 ± 1.3		5.5 ± 1.0	5.9 ± 0.7	5.6 ± 0.9		5.5 ± 1.1
HDL	1.3 ± 0.3	1.4 ± 0.3		1.5 ± 0.4	1.3 ± 0.2	1.3 ± 0.2		1.3 ± 0.3
TC: HDL ratio	4.6 ± 1.4	4.5 ± 1.5		3.9 ± 1.2	4.7 ± 1.4	4.6 ± 1.1		4.4 ± 1.2
Cardiovascular risk								
Framingham risk	9.6 ± 7.2	9.6 ± 7.5		9.9 ± 7.9	10.6 ± 7.9	10.0 ± 7.1		10.0 ± 6.9

Values are mean ± SD; IHE + Ex: Group receiving intermittent hypoxic exposure treatments in addition to 3 exercise sessions per week; Ex: Group receiving 3 exercise sessions per week. Baseline, Post, 4-wk and 8-wk: Measurements taken immediately before, immediately after and then 4 and 8 weeks following the 10-week intervention respectively; M/F: Male and female participants included in the study; PWV: Pulse wave velocity; PWA: Pulse wave analysis; RR interval: Average time interval between R peaks; rMSSD: Root mean square of successive differences in the heart's beat-to-beat intervals; SDNN: Standard deviation of all normal to normal R intervals; HF: High frequency; LF: Low frequency; $\dot{V}O_{2peak}$:oxygen uptake during the last 30 seconds of maximal exertion; SBP: Systolic blood pressure; HDL: High density lipoprotein; TC:HDL ratio: The ratio between total cholesterol to high density lipoprotein; Framingham risk assessment protocol estimating an individual's 10 year risk of cardiovascular disease.

Appendix E

New Zealand Guidelines Group: 5-year risk assessment chart

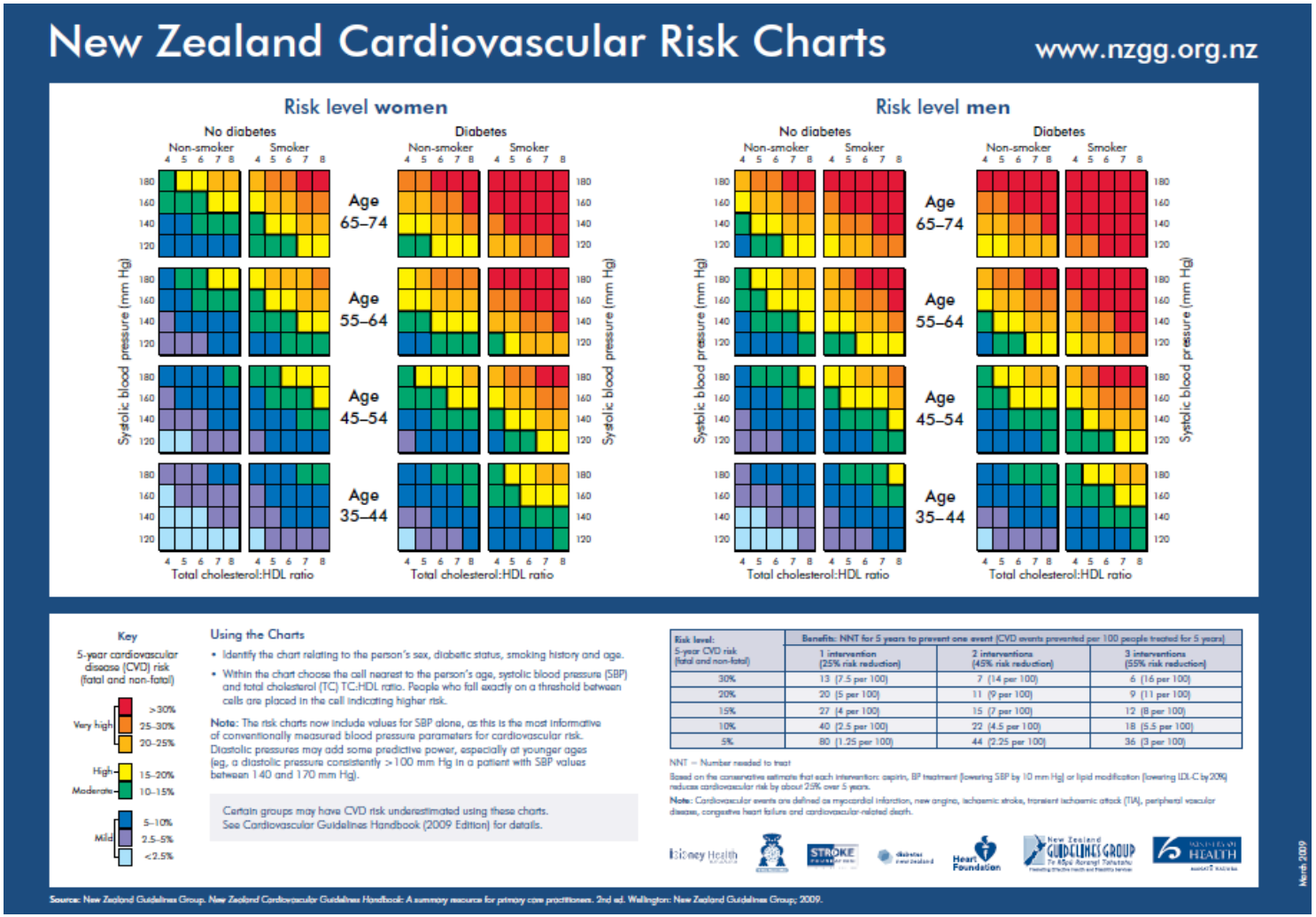

Figure 31: New Zealand 5 Year Risk Assessment Chart

◀ **Source**: New Zealand Guidelines Group: New Zealand Cardiovascular Guidelines Handbook: A summary resource for primary care practitioners. 2nd ed. Wellington: New Zealand Guidelines Group; 2009.

Appendix F

Explaining the code used to assess qualitative outcomes in clinical analyses using spreadsheets designed by Will Hopkins

IF(benefit to harm ratio>66,*value if true:* IF(smallest important harmful change > 0, *value if true:* use the qualitative outcome from the substantially negative block, *value if false:* use the qualitative outcome from the substantially positive block) and add the descriptor" beneficial", *value if false:* IF(smallest important harmful change>0, *value if true:* **IF(**percent chance for a substantially negative outcome >minimum chance of benefit (25%) , *value if true:* **IF(**percent chance for a substantively positive outcome <Maximum chance of harm (50%), *value if true:* insert the qualitative outcome for the substantially negative (beneficial) response and add the descriptor" beneficial", *value if false:* insert "unclear; get more data"**)**, *value if false:* **IF(**percent chance for substantially positive outcome>25, *value if true:* insert the qualitative outcome from the substantially positive block and add the descriptor " harmful", *value if false:* use the qualitative outcome from the trivial block and add the descriptor " trivial"**))**, *value if false:* **IF(**the percentage for a substantially positive outcome>minimum chance of benefit (25%), *value if true:* IF(the percent chance for a substantially negative outcome <maximum risk of harm (50%), *value if true:* use the qualitative outcome from the substantially positive block and add the descriptor " beneficial", *value if false:* "unclear; get more data"), *value if false:* **IF(**the percent chance for a substantially negative outcome >25, *value if true:* insert the qualitative outcome from the substantially negative block and include the descriptor " harmful", *value if false:* use the qualitative outcome from the trivial block and add the descriptor " trivial"**)))**

Basically, what this means is:

IF the odds ratio is greater than 66:

Use the substantially beneficial qualitative outcome and add the word “beneficial”

IF the odds ratio is less than 66:

And the chances of benefit are greater than 25%:

AND the percent for a substantially harmful outcome is LESS than the maximum risk of harm of 50%, then use the qualitative outcome from the substantially beneficial block and add the word “**beneficial**"

BUT the percent for substantially harmful outcome is MORE than the maximum risk of harm of 50% display the words "**unclear; get more data**"

And if the chances of benefit are less than 25%:

AND the value for a substantially harmful outcome is greater than 25, use the qualitative outcome from the substantially harmful block and add the word **"harmful"**.

BUT the value for a substantially harmful outcome is less than 25, use the trivial block's qualitative outcome and add the word **"trivial"**

(The rest of the formula is repeated for a smallest important harmful change of <0)

For example, after an intervention, the differences in total cholesterol were 86 / 2 / 12 for percent chances of increase / trivial/ decrease at Post – intervention, and then 93 / 1 / 6 at the 8-wk follow up.

Because of the wide overlap in beneficial and harmful outcomes, a mechanistic outcome would return a qualitative outcome of "unclear". On the other hand, the clinical assessment considers the extent of benefit (it must be at least 25% beneficial) and harm (the maximum risk of harm should be less than 50%) and therefore a clear statement can be made. In both these situations, the potential for benefit is less than 25% and the risk of harm is greater than 50% and therefore the qualitative outcome is "likely harmful" for both time points.

The qualitative outcome is based on the following scale:

< 0.5% = most unlikely

0.5 – 5% = very unlikely

5 – 25% = unlikely

25 – 75% = possibly

75 – 95% = likely

95 – 99.5% = very likely

> 99.5% = most likely

Appendix G

Further publications arising from this thesis

This paper has been submitted to the Medicine and Science in Sports and Exercise Journal and is currently under review.

Title: Does arterial health affect $\dot{V}O_{2peak}$ and muscle oxygenation in a sedentary cohort?

Authors: Lizamore, Catherine Anne[1], Stoner, Lee[2], Lucas, Samuel John Edwin. [3, 4], Lucero, Adam[2], Hamlin, Michael John[1]

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Corresponding Author: Dr. Mike Hamlin

Running title: Relationship between AIx, $\dot{V}O_{2peak}$ and TOI

Disclosure of funding: The lead researcher receives a Doctoral Scholarship from the National Heart Foundation of New Zealand

Conflicts of interest: None. Results of this study do not constitute endorsement by ACSM.

G.1 ABSTRACT

Purpose: An association between peak oxygen consumption ($\dot{V}O_{2peak}$) and arterial health has been demonstrated, however little is known about how arterial health influences muscular oxygenation during exercise. The purpose of this study therefore, was to gain insight into the relationships between arterial health, $\dot{V}O_{2peak}$ and muscle oxygenation in a sedentary population. **Methods**: Radial augmentation index (AIx; via Pulse Wave Analysis) of 21 sedentary, middle-aged participants (15 females, 6 males; age: 54.7 $\pm$ 5.4 years; BMI: 29.0 $\pm$ 4.7, mean $\pm$ SD) was assessed, and on another day (<7 days) participants completed a Modified Bruce Protocol (MBP). Using near infra-red spectroscopy, total oxygenation index (TOI) of the left flexor carpi ulnaris and the left vastus lateralis was monitored throughout the MBP. Individual and average (arm + leg) percentage decrease in TOI between stage 1 of the MBP and at maximal exertion (TOI_{diff}) was calculated. Changes between dependent variables were correlated using Pearson product-moment correlations; interpreted as: *r*>0.5: strong, 0.5>*r*>0.3: Moderate; *r*<0.3: weak. **Results**: We observed a moderate negative correlation between AIx and $\dot{V}O_{2peak}$ (*r* = -0.34, -0.63 to -0.03; Pearson correlation, 90% Confidence Limits), and a strong negative correlation between AIx and average TOI_{diff} (*r* = -0.58, -0.78 to - 0.27). The $\dot{V}O_{2peak}$ and average TOI_{diff} were strongly correlated (*r*= 0.55, 0.23 to 0.77). Individual arm and leg correlations were similar to the combined average, but slightly smaller. **Conclusion**: Arterial health appears to be an important determinant of muscle oxygenation during exercise. In turn, the muscle oxygenation during exercise is strongly related to the $\dot{V}O_{2peak}$. Developing training modalities to prioritise arterial health outcomes may be a useful way of improving $\dot{V}O_{2peak}$ in this population.

Keywords

Near infrared spectroscopy, total oxygenation index, augmentation index, exercise capacity

G.2 Introduction

The process of aging is usually associated with various decrements in cardiovascular health (Lakatta, 2002) and physical performance (Rogers et al., 1990). For example, aging is associated with increased arterial and ventricular stiffening (Redfield et al., 2005) and a steady decline in peak oxygen uptake ($\dot{V}O_{2peak}$) (Astrand et al., 1973). However, the deleterious effects of aging on $\dot{V}O_{2peak}$ and arterial stiffness are reduced with healthy lifestyle habits such as regular physical activity (Rogers et al., 1990, Tanaka et al., 1998, Lee et al., 1999). This associated decline in $\dot{V}O_{2peak}$ and increased arterial stiffness with age and concurrent improvement with healthy lifestyle habits have led to several investigations aimed at determining whether arterial stiffness and $\dot{V}O_{2peak}$ are related. For example, it is possible that the decline in arterial health is linked to the decline in $\dot{V}O_{2peak}$ as an impaired vascular system would limit the capacity to deliver oxygenated blood to the working muscle. To assess the arterial stiffness, researchers have used the augmentation index (AIx), which provides a systemic assessment of arterial health based on the augmentation of the pressure wave generated during systole (Stoner et al., 2012). Indeed, researchers have noted an inverse relationship between $\dot{V}O_{2peak}$ and arterial stiffness (Vaitkevicius et al., 1993, Binder et al., 2006). For example, Binder et al. (2006) noted a decrease of 1 mL/min/kg for every 10% increase in arterial stiffness (2006).

However, in addition to an efficient oxygen delivery system, enhanced $\dot{V}O_{2peak}$ also requires adequate pulmonary, circulatory, tissue, and mitochondrial diffusion of oxygen (2003). While the previously reported inverse relationship between arterial stiffness and $\dot{V}O_{2peak}$ provides some insight into the relative importance of the oxygen delivery network to overall $\dot{V}O_{2peak}$, the relative importance of oxygen diffusion to $\dot{V}O_{2peak}$ is unknown in this sedentary, middle-aged population. Furthermore, it is unknown whether the health of the blood vessels is related to oxygen uptake in muscle tissue.

Therefore, the purpose of this study was to determine the relationship between muscular oxygen uptake, arterial stiffness and $\dot{V}O_{2peak}$. We hypothesise that an increase in oxygen uptake from the capillaries to the muscle will be strongly associated with an increase in $\dot{V}O_{2peak}$. In addition, we hypothesise that improved arterial health will be associated with greater oxygen uptake at the muscle. The findings of this study will provide insight into currently unexplored downstream effects of changes in arterial health associated with a sedentary lifestyle.

G.3 METHODS

Participants.

Twenty-one middle-aged participants who were not actively engaged in planned physical activity were recruited from the local community (see Table 25 for anthropometric data). Exclusion criteria included the presence of any overt or uncontrolled cardiovascular disease, smoking or uncontrolled

medical condition such as undiagnosed hypertension. All participants were screened by a medical practitioner prior to the study, and all participants provided written informed consent. This study was approved by the Lincoln University Human Ethics Committee.

Table 25: Anthropometric data for participants

	Participant data
Total n (M/F)	21 (6 M / 15 F)
Age (years)	54.7 ± 5.4
Height (cm)	167.5 ± 8.4
Weight (kg)	80.9 ± 11.9
BMI (average)	29.0 ± 4.7
Body fat (%)	34.9 ± 8.9
SBP (mm Hg)	121.1 ± 11.9
DBP (mm Hg)	71.8 ± 7.0
Resting HR (beats·min^{-1})	64.4 ± 9.2
HR_{max} (beats·min^{-1})	166.0 ± 12.7
$\dot{V}O_{2peak}$ (ml·min^{-1}·kg^{-1})	28.1 ± 6.0
AIx (%)	24.2 ± 8.9

Values are mean ± SD. Total n: final number of males and females included in the study, BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; Resting HR: heart rate taken during 16 min of rest; HRmax: Average heart rate over last 30 seconds of maximal exertion; $\dot{V}O_{2peak}$: peak oxygen consumption during maximal exertion; AIx: augmentation index normalised to a heart rate of 75 beats·min^{-1}

Protocol.

Participants attended two sessions. The first session included the collection of anthropometric and arterial stiffness data and was conducted in a quiet, temperature controlled room (18 - 25°C). Anthropometric data included body composition, height, total mass, body fat percentage (InBody230, Biospace Co. Ltd., Seoul, South Korea) and body mass index (BMI). Blood pressure was recorded using an automated machine (Omron, HEM-907XL, Matsuzaka City, Japan) following 20 minutes of supine rest. The average of two recordings was used, with a third measure taken if readings differed by >5 mmHg. In this case the average of the closest two readings was used.

Session two was completed within one week of session one, and included assessments of peak oxygen uptake (using a metabolic cart) and skeletal muscle oxygen uptake (using near infrared spectroscopy) during a maximal treadmill protocol. Participants were asked to refrain from caffeine or alcohol for 12 hours, and eating for 4 hours prior to the assessment.

Augmentation Index.

Pulse wave analysis (SphygmoCor, AtCor Medical, Sydney, Australia) was used to measure the augmentation index (AIx) as an assessment of systemic arterial stiffness (Oliver and Webb, 2003, Mackenzie et al., 2002). All measurements were taken on the radial artery with a tonometer following 20 minutes of undisturbed supine rest (Stoner et al., 2013). The AIx is the augmentation pressure (maximum systolic peak of the pulse wave minus the pressure at the inflection point) expressed as a percentage of the central pulse pressure (Laurent et al., 2007) and was derived using the integrated software (SCOR Px 7.1, AtCor Medical, Sydney, Australia). As AIx is influenced by heart rate (Wilkinson et al., 2000) all AIx data were normalised to a heart rate of 75 beats per minute (AIx@75) prior to analysis. Two recordings were taken; if AIx differed by 4% between the two recordings a third recording was taken, and the average of the closest two measurements was used. Recordings were assessed for quality and were only considered acceptable if they were within the default setting specifications (average pulse height: 80 units, Pulse height variation: 5 %, Diastolic variation: 5 %, and quality index: >80 %).

Peak Oxygen Uptake.

After being fitted with the required equipment, participants rested for 16 minutes while baseline oxygen uptake and muscle oxygenation were recorded. Following the rest period, participants transferred to the treadmill (Rodby™, RL 1600E, Enhorna, Sweden) for the fitness assessment.

Maximal oxygen uptake assessments were conducted using a Modified Bruce Protocol (MBP). Two stages were added to the beginning of the standard Bruce protocol to cater for older or less fit individuals (the third stage corresponded to the first stage of the standard Bruce Protocol). The workload was increased every three minutes either by increasing the gradient and/or the speed of the treadmill until maximal exertion.

Breath by breath analysis was used to determine maximal oxygen consumption (MetaMax® 3B; Cortex Biophysik, Leipzig, Germany); and was calibrated for volume and gas composition (15% O_2 and 5% CO_2) prior to all testing sessions. Expired breath was directed to the volume and gas analyses through a leak-free face mask (Hans Rudolph, Kansas City, MO, USA) with small dead space (approximately 70 mL) that minimised restriction to breath via the nose or mouth. A heart rate (HR) monitor (RS800CX, Polar Electro Oy, Finland) and belt (Wearlink W.I.N.D, Polar Electro Oy, Finland) were used to record HR and determine maximal HR (HR_{max}). The test was stopped if there were any exercise-related contra-indications as outlined by the American College of Sport Medicine guidelines (Thompson et al., 2010). Maximal exertion was achieved when the participant met one of the following criteria: HR >90% of age-predicted HR_{max} (220 – age); respiratory exchange ratio of > 1.15; or a rating of perceived exertion of 19 or 20 on the Borg Scale (Borg, 1982). Participants then walked

at a low intensity for 5 minutes to warm down. All data were collected and exported to an excel spreadsheet for analysis. The average of the last 30 seconds of the respective datasets associated with the maximal assessment was used to determine $\dot{V}O_{2peak}$ and $HR_{max.}$

Total Oxygenation Index.

To measure skeletal muscle oxygen uptake, we monitored the change in the total oxygenation index (TOI) in the flexor carpi ulnaris of the forearm and in the vastus lateralis of the thigh. The participant's skin was prepared for the NIRS assessment by removing any excess hair, exfoliating and sterilizing the probe sites. A set of NIRS probes consisting of a light emitting laser diode, and a light detecting photodiode were then placed over the belly of the participant's flexor carpi ulnaris (5 cm distal from the lateral epicondyle), and vastus lateralis (6 cm proximal of the midway point between the lateral condyle and the lateral border of the patella), following the line of the muscle fibres. All NIRS measurements were conducted using a commercially available NIRS system (NIRO-200; Hamamatsu Photonics KK; Hamamatsu, Japan). The emitting laser diode and the light detecting photodiode were 5 cm apart to ensure adequate penetration in to skeletal muscle (Yoxall and Weindling, 1997) and were housed in a black casing and covered with tape to minimize extraneous light (Lucas et al., 2012). The NIRO-200 uses a modified Beer-Lambert method and spatially resolved spectroscopy to calculate changes in oxy- and deoxygenated haemoglobin (oxy-Hb and Hb respectively), and total haemoglobin from the light attenuation at three different wavelengths (Hamamatsu Photonics K.K., 2005, Boushel et al., 2001, Lucas et al., 2012) in real time (see Figure 32). The TOI is determined as follows: TOI % = oxy-Hb/t-Hb x 100 (Lucas et al., 2012).

To assess the TOI during rest and exercise, either 30-second or 5-second bins were used (refer to Figure 32). The average of two 30-second intervals at rest in a supine position was taken (after 10 minutes and 16 minutes supine rest, respectively) to assess inactive, resting tissue TOI. During exercise, 30-second intervals were used during steady state conditions (the first 3 stages of the Modified Bruce Protocol, and 2 and 5 minutes into the recovery following maximal exertion), or where there was a large amount of noise in the signal (during the 4 consecutive 30-second recordings in the 2 minutes immediately before maximal exertion). Five-second intervals were used to elicit the TOI at peak exercise, and then the last 5 seconds every 10 seconds for the first minute of recovery.

Near infrared spectroscopy is better suited to monitoring a change rather than the absolute value of the chromophores (Yoxall and Weindling, 1997). In addition to this, because irrelevant, but unavoidable physiological differences, such as the thickness of the adipose tissue (McCully and Hamaoka, 2000), have a profound effect on the NIRS measurement, data were normalised to a change score (TOI_{diff}) from a baseline value rather than using an absolute TOI value. The participant's

TOI during the last 30 seconds of the first stage of the Modified Bruce protocol was used as the baseline exercising reference as the signal position of the NIRS probe can shift and therefore alter the measurement as participants change position (McCully and Hamaoka, 2000) such as from supine rest to treadmill walking. To determine TOI_{diff}, the percentage drop in the TOI between the last 30 seconds of the first stage of the Modified Bruce Protocol and the 5-second maximal exertion recording between the forearm and thigh was determined for each participant. The arm TOI_{diff} and the leg TOI_{diff} were independently correlated with AIx and $\dot{V}O_{2peak}$. In addition to this, as both the $\dot{V}O_{2peak}$ and AIx are 'whole body' measures, the average of the forearm and thigh TOI_{diff} were used as an estimate of the systemic change in TOI.

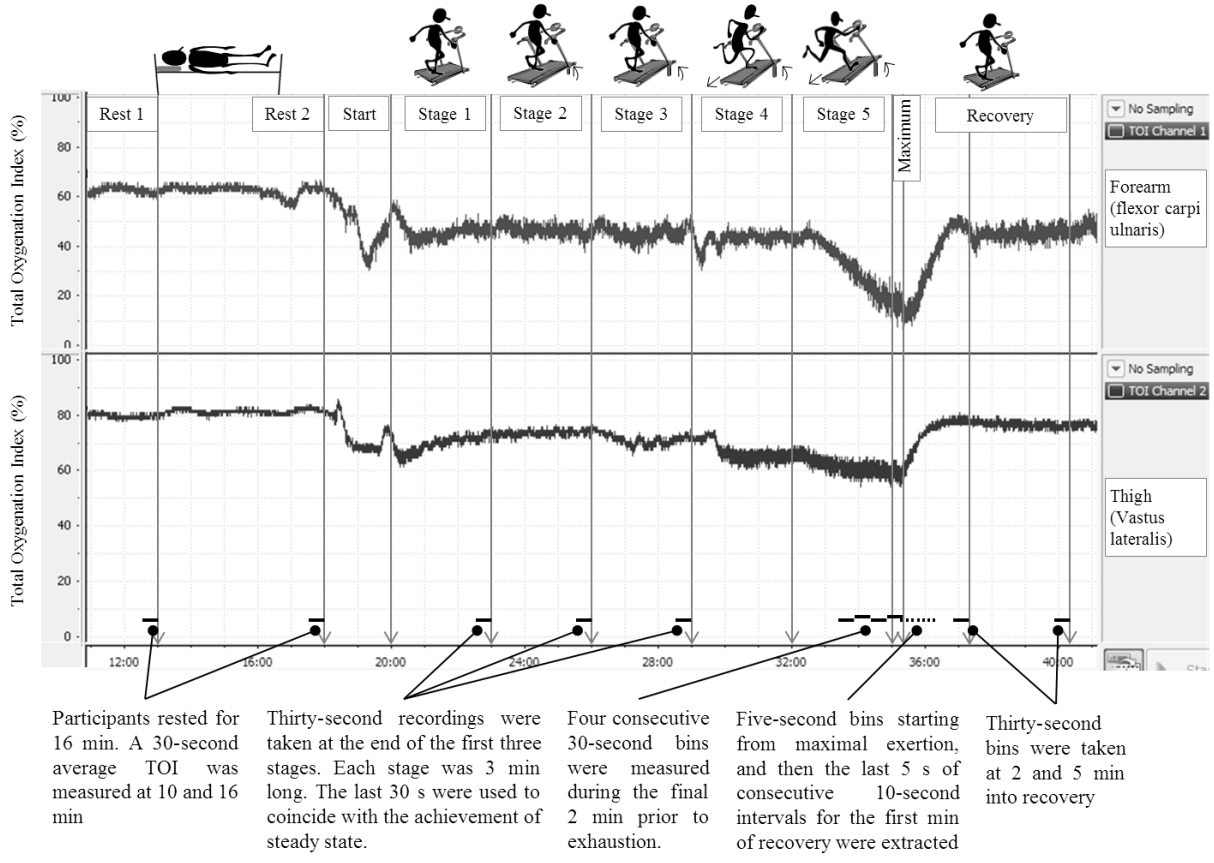

Figure 32: Continual measurement of total oxygenation index in the forearm and thigh of a participant

Statistical analyses.

Pearson product-moment correlation coefficients were used to assess the relationship between TOI_{diff}, $\dot{V}O_{2peak}$ and arterial stiffness (AIx), and AIx and TOI_{diff}. As BMI, age, heart rate and height have demonstrated strong relationships with AIx (Binder et al., 2006), an additional partial correlation controlling for these variables was also conducted. Results are recorded as the *r* score ± 90% confidence interval. A correlation of $r > 0.50$ was considered strong; the correlation was considered

'moderate' if 0.5 > *r* > 0.3, and weak if *r* < 0.3 (Hopkins, 2000a). Correlations were analyses using SAS 9.3 software (SAS Institute, Cary, North Carolina, USA).

The change in the TOI in the arm and the leg during Stage 1 (S1: last 30 seconds) and at maximal exertion (M: 5 seconds at maximal exertion) of the Modified Bruce Protocol, and at minutes 2 and 5 of the recovery period (R2 and R5, 30 seconds respectively) was also measured (see Figure 33). The differences in these time points were converted to their natural logarithms to avoid any effects of non-uniformity of error, and were then assessed using magnitude based inferences (Hopkins, 2006, Hopkins, 2007, Batterham and Hopkins, 2006). The likely range of the change in the true value (expressed as the 90% confidence interval) between time points (S1 – M; S1 – R2; S1 – R5) were measured against the smallest meaningful change using Cohen's standardised units (change in mean divided by 0.2 of the between subject SD at baseline) (Cohen, 1988). The descriptors: increased, trivial or decreased were used to describe the direction of the change. Where the confidence interval spanned all three possibilities (increased, trivial and decreased), the result was deemed unclear. In all other cases, such as no overlap, or an overlap between 2 possibilities (trivial and increased, or trivial and decreased) a clear result was achieved. Finally, the magnitude or probability of the change was assessed using a qualitative scale defined as: < 0.5 %: almost certainly not; < 5 %: very unlikely; < 25 %: unlikely/probably not; 25 – 75 %: possibly, possibly not; > 75 %: likely, probably; > 95 %: very likely; and > 99.5 %: almost certainly.

Results are expressed using the percentage change (from the natural logarithm) ± 90 % confidence limit, the % chance the variable has increased / is trivial / has decreased followed by the qualitative outcome.

G.4 RESULTS

Participant characteristics are summarised in Table 25.

Arm and leg oxygenation during exercise.

The TOI decreased substantially between stage 1 (S1) and maximal exertion of the MBP in both the arm (-22.8%; CL: -30.5 to -14.2%, 0 / 0 / 100, most likely decreased) and the leg (-14.0%; CL: -22.1 to -5.2%, 0 / 4 / 96, very likely decreased, Figure 33). In the arm, there was a slight increase in the TOI at 2 min (R_2) and 5 min (R_5) into the recovery compared to S1 (arm R_2: 4.5%, CL: 1.2 to 7.9%, 26 / 74 / 0, possibly increased; R_5: 6.4%, CL: 3.0 to 10.0%, 64 / 36 / 0, possibly increased). However, there was little difference in the TOI in the leg at S1 compared to R_2 (leg: 3.7%, CL: 1.0 to 6.4%, 20 / 80 / 0, likely trivial) and R_5 (leg: 3.0%, CL: -0.2 to 6.2%, 14 / 86 / 0, likely trivial, see Figure 33).

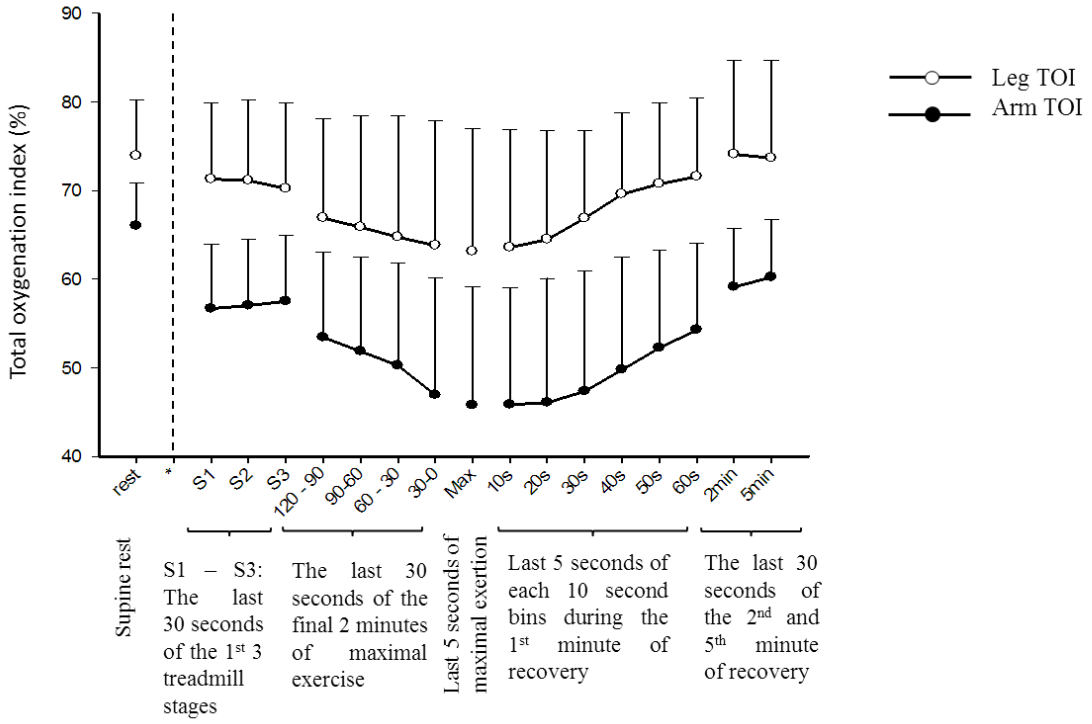

Figure 33: Total oxygenation index of the arm and the leg during rest, exercise and recovery

Correlations between AIx, $\dot{V}O_2$peak, and TOIdiff.

The arm and leg TOI_{diff} were both independently correlated to AIx and $\dot{V}O_{2peak}$ (See Table 26). However, average (arm + leg) TOI_{diff} demonstrated an even stronger correlation with AIx and $\dot{V}O_{2peak}$ (See Table 26, Figure 34A and Figure 35). The AIx was moderately correlated to $\dot{V}O_{2peak}$ *(r* = -0.34; CL: -0.63 to 0.03; see Figure 34B).

Table 26: Independent arm and leg correlations with VO2peak and AIx

Correlation between	*r*	90% Confidence Limits	Qualitative Outcome
$\dot{V}O_{2peak}$ and			
Arm TOI_{diff}	0.41	0.05 – 0.68	Moderate
Leg TOI_{diff}	0.41	0.05 – 0.68	Moderate
Average (Arm + Leg) TOI_{diff}	0.55	0.23 – 0.77	Strong
AIx and			
Arm TOI_{diff}	-0.38	-0.66 – -0.02	Weak/moderate
Leg TOI_{diff}	-0.49	-0.73 – -0.15	Moderate/strong
Average (Arm + Leg) TOI_{diff}	-0.58	-0.78 – -0.27	Strong

Qualitative outcome was assessed as: *r*>0.5: strong, 0.5>*r*>0.3: Moderate; *r*<0.3: weak; $\dot{V}O_{2peak}$ was measured over the last 30 seconds of maximal exertion (using a Modified Bruce Protocol), TOI_{diff}: change in total oxygenation index between stage 1 of the Modified Bruce Protocol and maximal exertion, measured in the flexor Carpi ulnaris (Arm TOI_{diff}) and vastus lateratis (Leg TOI_{diff}) independently and averaged over both sites (Average (Arm + Leg) TOI_{diff}); AIx: Augmentation index normalised to a heart rate of 75 beats·min^{-1}

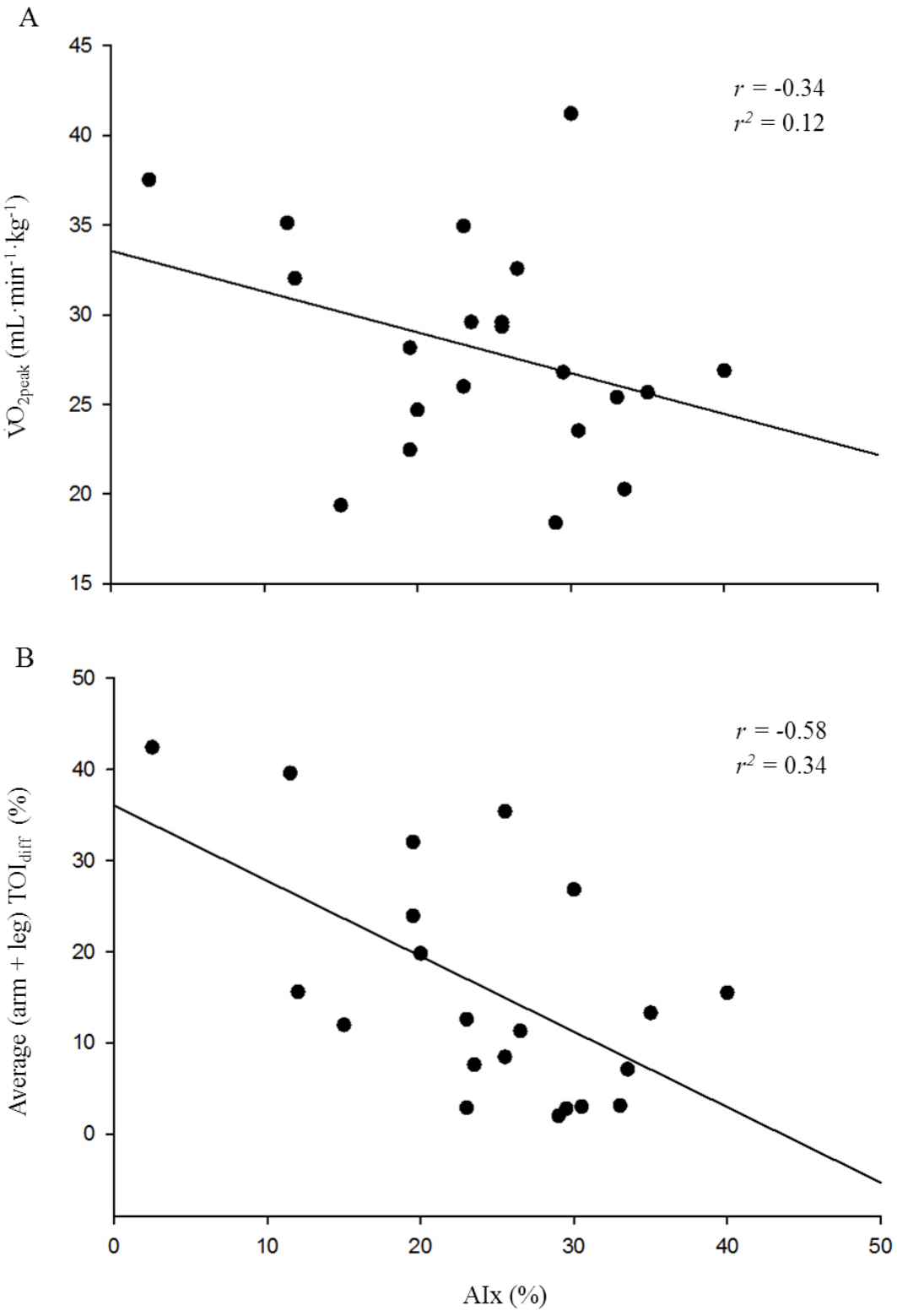

Figure 34: Correlations between (A) AIx and $\dot{V}O_{2peak}$ and (B) AIx and average (arm + leg) TOIdiff

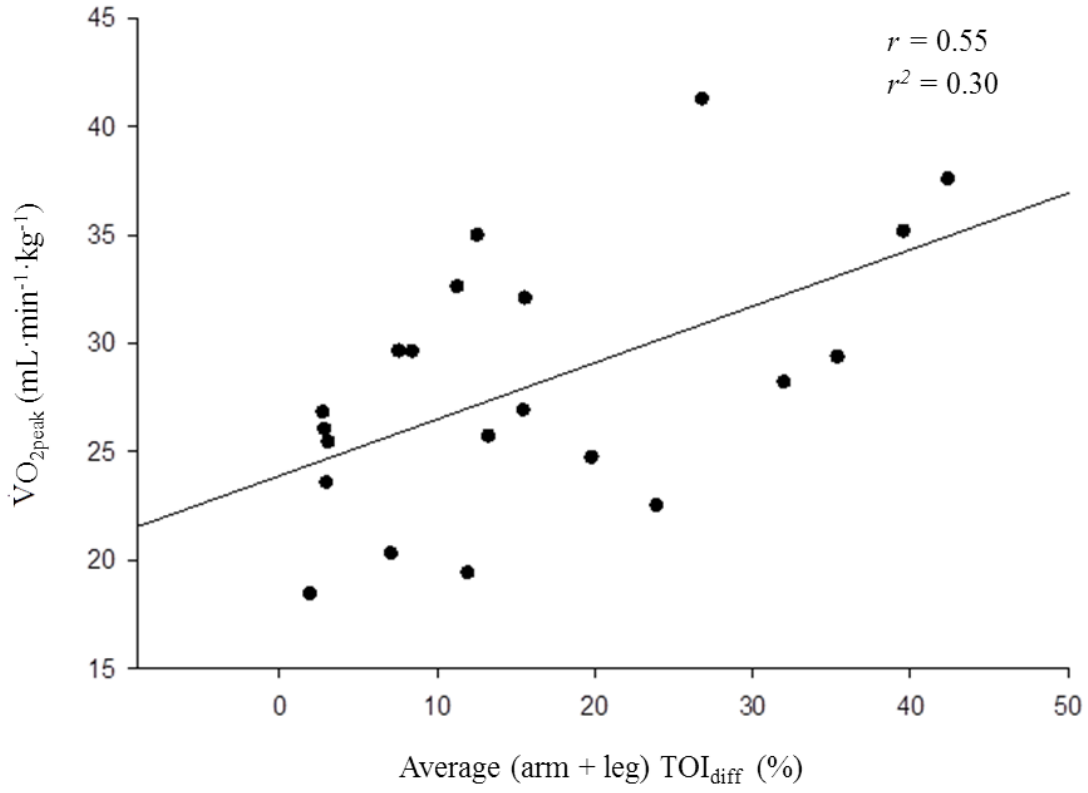

Figure 35: Correlation between the average (arm and leg) decrease in total oxygenation index (TOIdiff) and peak oxygen consumption ($\dot{V}O_{2peak}$)

Accounting for BMI, age, resting HR and height in the analysis slightly weakened the correlation between average (arm + leg) TOI_{diff} and $\dot{V}O_{2peak}$ from a strong to a moderate relationship (r = 0.40, CL: 0.04 to 0.67), while the correlation between average (arm + leg) TOI_{diff} and AIx became stronger (r = -0.63, CL: -0.81 to -0.34) after controlling for these variables. The correlation between AIx and $\dot{V}O_{2peak}$ remained similar to the unadjusted correlation (r = -0.31, CL: -0.61 to 0.07).

G.5 DISCUSSION

While several authors have examined the relationship between arterial stiffness and peak oxygen consumption, to our knowledge, this is the first study that has investigated the relationship between arterial stiffness, muscle oxygen uptake, and peak oxygen consumption ($\dot{V}O_{2peak}$). Our results support those of previous studies which have found a negative correlation between arterial health and $\dot{V}O_{2peak}$ (Binder et al., 2006, Tanaka et al., 1998, Vaitkevicius et al., 1993). The novel finding in this study was that arterial health also appears to play an important role in the extent to which oxygen dissociates from haemoglobin, and thereby may be an important determinant in the uptake of oxygen by the skeletal muscle in a sedentary, middle-aged, but otherwise healthy population.

$\dot{V}O_{2peak}$ and AIx.

The results of our study support the findings of Vaitkevicius et al. (1993), Tanaka et al. (1998) and Binder et al. (2006), who all found an inverse relationship between the AIx and $\dot{V}O_{2peak}$ in sedentary, middle-aged individuals (Binder et al., 2006, Tanaka et al., 1998, Vaitkevicius et al., 1993). The moderate correlation between AIx and $\dot{V}O_{2peak}$ in our study (r = -0.34) was somewhat lower than the strong correlation reported by Vaitkevicius et al. (1993) who reported r = -0.54 for men and r = - 0.74 for women. The participants in the Vaitkevicius et al. (1993) study, although sedentary and of a similar age to the participants in this study, were screened for any forms of cardiovascular disease or severe hypertension (blood pressure >160/95), and none of the participants were on any cardiovascular medication. Likewise, Tanaka et al. (1998) demonstrated a strong correlation between carotid AIx and $\dot{V}O_{2peak}$ of r = -0.53 in their cohort of (combined) active and sedentary middle-aged women. Similar to Vaitkevicius and colleagues' study, the participants in the Tanaka et al. (1998) study were free of any overt cardiovascular disease, and aside from hormone replacement, did not take any regular medications. We suspect that the lower correlation in our study could be due to a possibly more heterogeneous cohort. By increasing participant diversity, factors in addition to vascular function may be responsible for limiting the physiological capacity to deliver, take up and use oxygen. In this regard, our population was more like the sample of Binder et al.(2006), which consisted of sedentary middle-aged men and included participants who smoked, were hypertensive and who were on medications such as statins, insulin and oral hypoglycaemic agents (2006). While none of our participants were smokers or had uncontrolled medical conditions, we did not exclude participants who were on hypertension or cardiovascular-related medication. Following adjustment for age, heart rate, height and body mass index, Binder et al (2006) reported a correlation of r = -0.22 between AIx and $\dot{V}O_{2peak}$. The correlation in our study and the health profiles of our participants fit between the healthier groups tested by Vaikevicius et al. (1993) and Tanaka et al (1998) and the more diseased participants tested by Binder et al. (2006).

It is possible that the site of the AIx measurement could have played a role in the sensitivity of the relationship between AIx and $\dot{V}O_{2peak}$. That is, both Vaitkevicius et al (1993) and Tanaka et al. (1998) used the carotid artery as the measurement site for AIx, while the present study and that of Binder et al. (2006) measured AIx at the radial artery. The radial site requires use of generalized transfer function to estimate the pressure waveform at the level of the aorta, while the carotid artery is closer to the aorta and does not require a transfer function (Laurent et al., 2006). However, the technical differences between the sites are unlikely to explain the lower correlation in our study as the AIx at each of these sites has been previously reported to infer similar information (Sugawara et al., 2007, Sugawara et al., 2008).

While the nature of a correlation study does not lend itself to causality, the ability of the arterial system to dilate and facilitate blood flow to skeletal muscle is likely to be related to oxygen uptake (Vaitkevicius et al., 1993). Accordingly, as the arteries stiffen the ability of the blood vessels to deliver oxygen to working skeletal muscle becomes impaired, which would contribute to the $\dot{V}O_{2peak}$ decline

TOI and AIx.

In the current study, TOI_{diff} was used to indicate the oxygen taken up by the musculature (change in TOI between light and maximal exercise). A strong negative relationship was found between AIx and TOI_{diff}. Additionally, both the arm and the leg independently demonstrated negative correlations between AIx and TOI_{diff}. The strength of the correlation between AIx and TOI_{diff} became progressively stronger as the included musculature increased. That is, the correlation between the AIx and arm TOI_{diff} was weaker than the correlation between AIx and leg $TOI_{diff,}$ both of which were weaker than the correlation between the AIx and the average (arm + leg) TOI_{diff}.

In older adults, there is progressive stiffening of the central arteries, while the peripheral arteries remain relatively stable (Mitchell et al., 2004, Safar et al., 2003). The increased central stiffness as compared to the peripheral vasculature results in later reflection sites, and more of the pulsatile energy of the forward wave being transferred to the microcirculatory vessels, potentially damaging these structures (Mitchell et al., 2004, Mitchell, 2008). This disruption to the microvascular structure and/or function appears to occur independently of traditional cardiovascular risk factors (Mitchell et al., 2005) and therefore may have already been present in our sedentary but otherwise healthy population. While skeletal muscle tissue may be less sensitive to the pulsatile energy increases (Mitchell, 2008), the minor microcirculatory damage may be sufficient enough to reduce oxygen uptake. That is, the conduit arteries may be able to deliver oxygenated blood to the active skeletal muscle, but damage to the microvasculature may impede the process of diffusion from the capillary to the site of the skeletal muscle cells, thereby limiting oxygen diffusion and aerobic metabolism.

Alternatively, the correlation between AIx and TOI_{diff} could be a spurious relationship. That is, both the decline in the magnitude of the TOI_{diff}, and apparently associated increased AIx could be caused by a third variable such as physical (in)activity. For example, the absence of regular physical activity in our participants could have resulted in a decline in the oxidative properties of the mitochondria (2001, Layec et al., 2012), which would result in a reduced capacity to utilize oxygen. Therefore, while increased arterial stiffness and decreased mitochondrial function are both manifestations of a sedentary lifestyle, it may be that mitochondrial function, not arterial stiffness is explaining impaired $\dot{V}O_{2peak}$.

TOI and $\dot{V}O_{2peak}$.

There was a strong positive correlation between the average (arm + leg) TOI_{diff} and $\dot{V}O_{2peak,}$ (this correlation was 'moderate' and identical in the arm and leg independently) which indicates that the capacity of the muscle to take up and use oxygen (a-vO_2diff) is an important determinant of $\dot{V}O_{2peak}$. Interestingly, the contribution of the average TOI_{diff} to the $\dot{V}O_{2peak}$ in our study (r^2 = 0.30) is similar to the estimation by di Pampero (2003) that 25 – 30 % of the $\dot{V}O_{2peak}$ is attributable to the muscle and mitochondrial 'resistors' to oxygen diffusion (di Prampero, 2003).

Most of the oxygen desaturation (TOI_{diff}) is due to the decrease in capillary and venous oxyhaemoglobin (Belardinelli et al., 1995). With continuous exercise above the lactic acid threshold, increases in H^+ (increased PCO_2 from aerobic metabolism and the bicarbonate ion buffering of lactic acid) and consumption of bicarbonate ions (increased lactic acid) lowers the pH and shifts the oxyhaemoglobin dissociation curve to the right (Stringer et al., 1994). In addition, an increase in muscle temperature during heavy exercise also acts to promote oxygen dissociation from haemoglobin (Severinghaus, 1966), however the effect of temperature on the oxyhaemoglobin dissociation curve is comparatively small compared to the decrease in pH (Stringer et al., 1994). As NIRS measures the change in absorption by chromophores rather than the oxygen molecules per se (Yoxall and Weindling, 1997), this results in a lower TOI. Therefore, it would be expected that participants with a greater lactate tolerance (and therefore lower pH at maximal exertion) would also demonstrate lower $TOI_{diff.}$

TOI during rest and exercise.

There were considerable differences between the TOI measured in the forearm and in the leg (see Figure 33) whereby the arm demonstrated lower TOI throughout the measurement period when compared to the leg. During whole body exercise, particularly where the legs provide the primary musculature activity, sympathetically-mediated vasoconstrictor signals act to oppose the metabolic vasodilatory stimuli in the arms and prioritise blood flow to the legs (Calbet et al., 2007). This enhanced vasoconstrictor response results in reduced vascular conductance in the arms and forces maximal oxygen uptake in the available blood (Calbet et al., 2007). As the TOI reflects the proportion of oxyhaemoglobin in the blood, rather than the absolute volume of oxygen taken up by the musculature, this vascular restriction may account for the considerably lower TOI in the arms compared to the legs during exercise. In addition, reduced vascular conductance as reported in other studies (Calbet et al., 2007, Volianitis and Secher, 2002) likely impeded lactate clearance in the arms, further reducing local pH and facilitation of oxygen dissociation from haemoglobin (Volianitis and Secher, 2002).

During recovery the TOI in the arms increased to beyond the recorded levels during light exercise, while the legs returned to a slightly elevated (but only trivially) TOI. The reversal of the sympathetically mediated vasoconstriction in the arms at the end of maximal exercise, and return of the metabolically driven local vasodilation may account for this enhanced recovery in the arms compared to the legs.

Limitations.

The small sample size of the current population (n = 21) increases the risk of Type 1errors whereby a trivial or meaningless relationship is assessed as significant. To this end, further research is needed to validate and further explore the relationships between arterial health, oxygen uptake and $\dot{V}O_{2peak}$ in a sedentary population. As our sample was inclusive of a wide age range of heterogeneous participants (i.e. we did not exclude based on ongoing medication) we also increase the chance of any change, or correlation being attributed to a spurious variable. However, as our findings are consistent with those of others (Binder et al., 2006, Vlachopoulos and O'Rourke, 2000) these results are likely to be reliable.

In this study, we used the TOI to estimate oxygen uptake and utilisation. There are several limitations with the conclusions we can draw from this assumption, for example, as the TOI represents a ratio between oxyhaemoglobin and total haemoglobin, we cannot make any inferences regarding the volume of blood flow of the volume of oxygen dissociated from haemoglobin. By using the average of arm and leg TOI we likely reduced the impact of volume discrepancies in the arm and leg, however more research specifically examining the relationship between arterial health and blood flow, blood volume and changes in oxy haemoglobin volumes is needed to explain our preliminary findings.

Finally, this is a cross-sectional study which does not lend itself to causal conclusions and caution must be used when interpreting the data, as such, further research is needed to establish these causations via longitudinal and/or intervention-based studies.

G.6 CONCLUSIONS

The ability to monitor oxygen saturation continuously during exercise offers a unique opportunity to observe the oxidative demand of skeletal muscle tissue. We observed a strong relationship between the augmentation index and the average percentage decrease in total oxygenation index, indicating that arterial health may have an important role in the uptake of oxygen by working skeletal muscle. Arterial health was also negatively related to the $\dot{V}O_{2peak}$. However, this relationship was not as strong. Oxygen uptake at the muscle appears to be an important determinant for $\dot{V}O_{2peak}$, the contribution of which is similar to di Pampero's estimation of 30 % (di Prampero, 2003). Further

research should determine the relative contributions of global arterial health, microvascular supply and mitochondrial capacity to $\dot{V}O_{2peak}$ in this cohort.

Acknowledgements

We gratefully acknowledge the support from the New Zealand National Heart Foundation who pays a stipend to the lead researcher of this study. We would also like to express our thankful appreciation to the participants who willingly and cheerfully volunteered their time to this study. No other funding, grants, or financial support was used for this study.

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