Aspects of respiratory physiology and autonomic function after extreme respiratory stress and in brainstem disease

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PUBLICATIONS

Peer reviewed publications from this Thesis (reproduced in Appendix C)

- Seccombe LM, Rogers PG, Jenkins CR, Peters MJ. Maintenance of vital capacity during repetitive breath-hold in a spearfishing competition. Respirology. 2012;17(2):350–3.
- Seccombe LM, Jenkins CR, Rogers PG, Pearson MA, Peters MJ. Evidence of respiratory system remodelling in a competitive freediver. Eur Respir J. 2013 Mar;41(3):760-2.
- Seccombe LM, Giddings, HL, Rogers PG, Corbett AJ, Hayes MW, Peters MJ, Veitch EM. Abnormal ventilatory control in Parkinson's disease – further evidence for non-motor dysfunction. Respir Physiol Neurobiol. 2011 Dec 15; 179(2-3):300-4.
- Seccombe LM, Rogers PG, Hayes MW, Farah CS, Veitch EM, Peters MJ. Reduced hypoxic sympathetic response in mild Parkinson's disease: further evidence of early autonomic dysfunction. Parkinsonism Relat Disord. 2013 Jul;19:1066-8.

Abstracts from this Thesis (reproduced in Appendix D)

- Seccombe LM, Rogers PG, Puthucheary Z, Wong C, Jenkins CR. Repetitive breath-hold diving during a spearfishing competition does not affect vital capacity. Respirology. 2011;16(Suppl. 1):4.
- Seccombe LM, Jenkins CR, Rogers PG, Pearson MA, Peters MJ. Evidence of respiratory system remodelling in a competitive freediver. Respirology. 2013;18(Suppl. 2):3.
- Seccombe L, Rogers P, Hayes M, Farah C, Veitch E, Peters M. Patients with mild Parkinson's disease have reduced sympathetic response to hypoxia. Respirology. 2013;18(Suppl. 2):6. Awarded the Excellence in Respiratory Measurement Prize
- Seccombe LM, Ellyett KM, Rogers PG, Farah C, Veitch E, Peters M. Detrended fluctuation analysis of heart rate demonstrates autonomic dysfunction during progressive hypoxia in patients with Parkinson's disease. Am J Respir Crit Care Med. 2014;(189):A5058.

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LIST OF ABBREVIATIONS

ABG	Radial arterial blood gas
AHVR	Acute hypoxic ventilatory response
AST	Altitude simulation test
atm	Atmosphere
BGB	Blood gas barrier
BP	Blood pressure
BTPS	Body temperature pressure saturated
CO_2	Carbon dioxide
COMT	Catechol-O-methyl transferase
CT	Computed tomography
DAT	Dopamine transporter
DFA	Detrended fluctuation analysis
DMV:	Dorsal motor nucleus of the vagus
ECG	Electrocardiography
$FECO_2$	Fraction of expired carbon dioxide
$FEF_{25-75\%}$	Mid expiratory flow
$F_{ET}CO_2$	Fraction of end-tidal carbon dioxide
FEV_1	Forced expiratory volume in one second
$FICO_2$	Fraction of inspired carbon dioxide
FIO_2	Fraction of inspired oxygen
FRC	Functional residual capacity
FVC	Forced vital capacity
GI	Glossopharyngeal insufflation
H&Y	Hoehn and Yahr staging scale
HCVR	Hypercapnic ventilatory response
HR	Heart rate
HRV	Heart rate variability
HVD	Hypoxic ventilatory decline
HVR	Hypoxic ventilatory response
iHCVR	Isoxic hypercapnic ventilatory response
K_{CO}	Transfer factor for carbon monoxide corrected for alveolar volume

LLN	Lower limit of normal
MAO-B	Monoamine-oxidase-B
MEP	Maximal expiratory pressure
MIGB	Metaiodobenzylguanidine
MIP	Maximal inspiratory pressure
MSNA	Muscle sympathetic nerve activity
NN	Normal-to-normal
O_2	Oxygen
OSA	Obstructive sleep apnoea
P_{100}	Occlusion pressure in the first 100ms of an inspiratory effort against an occluded airway
P_ACO_2	Pressure of alveolar carbon dioxide
P_AO_2	Pressure of alveolar oxygen
$PaCO_2$	Pressure of arterial carbon dioxide
PaO_2	Pressure of arterial oxygen
PB	Personal best
PD	Parkinson's disease
PE	Maximal expiratory pressures
PEF	Peak expiratory flow
$P_{ET}CO_2$	Pressure of end-tidal carbon dioxide
$P_{ET}O_2$	Pressure of end-tidal oxygen
pHVR	poikilocapnic hypoxic ventilatory response
PI	Maximal inspiratory pressures
PM	Pneumomediastinum
PTT	Pulse transit time
R	R wave of electrocardiogram
RER	Respiratory exchange ratio
RMS	Root mean square
RR	Respiratory rate
RSA	Respiratory sinus arrhythmia
RV	Residual volume
SBP	Systolic blood pressure
S_CO_2	Calculated oxygen saturation

SCUBA	Self-contained underwater breathing apparatus
SD	Standard deviation
SPECT	Single photon emission computed tomography
SpO_2	Arterial pulse oximetry
TLC	Total lung capacity
TL_{CO}	Transfer factor for carbon monoxide
UPDRS	Unified Parkinson's disease rating scale
V_A	Alveolar volume
VC	Vital capacity
VCO_2	Carbon dioxide uptake
$\dot{V}_E$	Minute ventilation
$\dot{V}O_2$	Oxygen uptake
V_T	Tidal volume
V_{TG}	Thoracic gas volume

ABSTRACT

Competitive freedivers repeatedly tolerate physiological extremes of intra-thoracic pressure, lung volume and arterial blood gas tensions - both with alveolar hyperbaria during glossopharyngeal insufflation (GI) at sea level and with extrinsic hyperbaria as they dive. Lung size changes acutely with these pressures and repetitive exposure to this over time may alter lung function and structure. The associated tolerance of severe hypoxia and hypercapnia may affect ventilatory control and cardiac autonomic function. Similarly, abnormal ventilatory and autonomic function may also be a feature of early Parkinson's disease (PD). A recent hypothesis suggests that PD originates in the medulla rather than the midbrain, which may result in central ventilatory effects and disorders of autonomic parameters prior to the classical motor manifestations.

The aim of this Thesis was to describe ventilatory function and control in elite freedivers that voluntarily endure the limits of hypoxia and in PD subjects that may have early central ventilatory dysfunction. Importantly, a method was derived to concomitantly measure ventilatory and autonomic responses to differentiate voluntary override of the ventilatory response from intrinsic deficits.

Lung volume measurements were collected acutely following repetitive bouts of hyperbaric compression and longitudinally following many years of freediving participation. The respiratory function of patients with moderate and mild PD was measured including respiratory muscle strength. Ventilatory and autonomic responses to progressive hypoxia and hypercapnia were assessed in all study subjects and compared to healthy, non-diving controls.

Lung volumes were not affected by repeated intra-day exposure to hyperbaria, however freedivers who perform GI have increased total lung capacity without evidence of reduced lung compliance. This suggests that the increased lung volume is related to reduced chest wall recoil. Freedivers displayed voluntary ventilatory override during the ventilatory control studies, with a normal autonomic response as reflected in pulse transit time and heart rate variability. Subjects with PD had normal lung volumes, with a reduced ventilatory drive in moderate, but not mild disease. However in mild PD, a

reduced autonomic response to hypoxia was identified that was not affected by PD dopaminergic therapy.

The derived method gave important insight both into subjects that were at the limit of human endurance and in patients with brainstem disease. The concomitant analysis of ventilatory and autonomic function differentiated voluntary override in the freedivers, from subtle intrinsic deficits in patients with mild PD. This analysis has potential for wide application across disease states that are affected by primary or ancillary autonomic dysfunction.

CHAPTER 1

INTRODUCTION

Competitive freedivers repeatedly tolerate the physiological limits of intra-thoracic pressure, lung volume and arterial blood gas tensions - both with alveolar hyperbaria during glossopharyngeal insufflation (GI) at sea level and with extrinsic hyperbaric compression as they breath-hold dive. A background series of previously conducted studies described the acute physiological effects of GI; a manoeuvre that uses the glossopharyngeal structures to increase entrained gas above usual total lung capacity (TLC). Immediately following GI, the divers experience elevated lung volumes and alveolar pressures, sufficient to alter thoracic structure and pulmonary perfusion (1-3). Freedivers stay submersed for extended periods on a single breath and are unique in their ability to volitionally withstand significant hypoxia and hypercapnia.

Repetitive exposure to elevated intrathoracic pressure and ventilatory suppression has the potential to acutely and/or longitudinally alter resting lung function, structure, ventilatory control and closely related cardiac autonomic function.

Altered ventilatory and autonomic function may be an early feature of Parkinson's disease (PD). Braak's hypothesis suggests that PD originates in the brainstem, which may involve the respiratory control centres that lie within the medulla and pons prior to the classical motor manifestations (4). The study of ventilatory control and autonomic function in patients with PD can be valuable in clarifying the findings in freedivers but also separately of intrinsic interest.

The aim of this Thesis was to describe ventilatory function and control in elite freedivers that voluntarily endure the limits of intrathoracic pressure and hypoxia, and in PD subjects that may have early central ventilatory dysfunction. Ventilatory parameters can be significantly affected by subjective influences; therefore measures of autonomic function were included to elucidate voluntary override from intrinsic deficits.

The acute effect of repetitive bouts of hyperbaria on lung function is addressed in **Chapter 2.** Previous case reports suggested that breath-hold dives to varying depths possibly result in acute lung barotrauma due to thoracic compression (5). Competitive spearfishers dive to modest depths repetitively over many hours. Pulmonary function, medical symptomatology and diving statistics were collected onsite at a five-hour spearfishing competition. The hyperbaric exposure typical for such an event, and

consequent signs and/or symptoms of adverse events in this general community of breath-hold divers are described (6).

The longitudinal effect of freediving and GI participation on resting lung function and structure is addressed in **Chapter 3**. The analysis was of complex respiratory function collected on an elite freediver over an eight-year period. This included associated thoracic imaging (7).

Chapter 4 describes the method developed to concomitantly measure ventilatory and autonomic function during progressive poikilocapnic hypoxia and isoxic hypercapnia inclusive of data for responses in healthy normal subjects. Pulse transit time (PTT) and heart rate (HR) were included to measure cardiovascular autonomic function. The analysis of these methods performed on elite freedivers is presented in **Chapter 5.**

A description of respiratory function and ventilatory control in patients with mild-moderate PD is presented in **Chapter 6** (8). **Chapter 7** describes the responses in subjects with exclusively mild PD, to investigate whether ventilatory and/or autonomic impairment were identifiable in the early stages of disease. This chapter also addresses the potential effect of standard clinical dopaminergic therapy, to elucidate whether the responses were affected by PD treatment that had been previously shown to suppress ventilation (9).

As an additional assessment of autonomic function, **Chapter 8** describes the comprehensively analysis of the electrocardiograph (ECG) data collected during hypoxia in all subject groups. Heart rate variability (HRV) was measured via detrended fluctuation analysis (DFA).

The resulting publication citations from this Thesis are listed in the preliminary pages (v) with full manuscripts and abstracts in **Appendix C** and **D** respectively. Relevant background material is included in the complete publications list in **Appendix E**. In writing this Thesis, some of the data was re-analysed and therefore some results may slightly differ to the published data.

CHAPTER 2

THE EFFECT OF REPETITIVE BOUTS OF HYPERBARIA (SPEARFISHING) ON RESPIRATORY FUNCTION

2.1 Background

There is a paucity of data pertaining to the acute and longitudinal effects of freediving and GI participation on respiratory function and structure. It was recently postulated that repeated and/or extreme exposure to hyperbaric compression might cause acute pulmonary barotrauma in breath-hold divers (5, 10-12). Adverse events in individual cases have been reported after hyperbaric exposure from 15 m depth following "underwater fishing" and spearfishing (11, 12). Competitive spearfishing competitions are regular occurrences during the Australian summer. Such events allow the opportunity to elucidate the frequency and severity of potential lung injury with repetitive breath-hold diving in the general community. This Chapter describes a study site visit to a five-hour spearfishing competition.

2.1.1 Professional and recreational breath-hold diving

Historically, breath-hold diving has been used in the pursuit of seafood and pearls. Accounts of Japanese and Korean "Ama" seafood and pearl divers date back 2000 years and they still continue today. Korean female divers (cachido) and Japanese male divers (funado) have similar diving regimens. Their diving sessions can last from 2-5 h a day, possibly completing more than 100 dives in that time. Each submersion usually lasts less than one min, averaging 4-10 m depth (13).

85 % of Australians live within 50 km of the coastline (14) and spearfishing (otherwise known as underwater fishing or skindiving) is a common and traditional recreational activity. There is a regulatory body for the sport of spearfishing, established in 1948, which developed standards promoting both environmental and safety criteria. Its "sporting spirit" bans the use of self-contained breathing apparatus (SCUBA), with all fish taken to be only used for one's immediate needs (Figure 2.1). Organised competitions are required to run from 4-7 h where the aim is to spear different varieties of accepted (non protected) species (15). Spearfishing competition diving statistics, such as number of dives and average diving depths, have not been previously described.

Figure 2.1. A spearfishing subject. *Competitive spearfishing is single-catch, breath-hold diving only. Image provided by the subject, W.Judge.*

2.1.2 The physiological effects of hyperbaria

Participants are enticed by the many challenges associated with spearfishing; including fluctuations in weather, water temperature, water currents, sun exposure and marine hazards (such as sharks, stingrays, jellyfish and blue bottles). Another associated risk with breath-hold diving relates to hyperbaric compression.

Pressure from the surrounding water increases linearly with increasing depth; equivalent to one atmosphere (atm) of pressure every 10 m; creating a compressive effect as expressed by Boyles Law (16). Sea level is standard at 1 atm (760 mmHg); thus 10 m depth below sea level exerts 2 atm of pressure (1520 mmHg) and so on.

During descent, several mechanisms may cause a relative increase in pulmonary capillary and pulmonary artery pressure with increasing atmospheric pressure:

i. Increase in intrathoracic blood content

With submersion, the dive reflex and cold exposure causes a redistribution of blood flow from the periphery to the thorax, which increases intrathoracic blood content. This increase can be as much as 1.5 L and elevates pulmonary artery pressure (17-19).

Historically it was thought that the limit to human diving depth was the point where lung volume was compressed to the size of residual volume (RV) (20, 21), or the ratio of non-compressible to compressible air containing spaces (21). Any further descent to

where lung volume is less than the non-compressible space should result in "thoracic squeeze" and pulmonary barotrauma. Typically a total lung capacity (TLC)/RV ratio is 5:1, therefore by Boyles Law, the diving limit would be 40 m, or 5 atm. Upon recognition of the achievement of Bob Croft, who completed a dive to 65 m in 1967, Albert Craig claimed that *"The physiologist finds himself in the embarrassing position of being proven wrong by the sports divers"* and responded with a theory of increased thoracic blood flow to allow lung volume to be compressed below RV (16). He estimated that to achieve 65 m, there needed to be a 570 mL blood shift from the peripheral to central circulation. This theory was confirmed nearly ten years later, with a maximum 1.5 L increase in intrathoracic blood content measured during hyperbaria (17, 19). The current "no limits" world record of 214 m can be therefore be rationalised. Tragically however, the holder of this record recently attempted 240 m and experienced catastrophic brain injury from cerebral gas embolism (22). While the exact limit to diving depth has not been defined, thoracic compression and pulmonary capillary stress failure is an important limitation.

ii. Increased circulatory demand from physical activity

As the divers fish, there is strong potential for intense exercise with increased oxygen demand from the exercising muscles. As they breath-hold, alveolar oxygen is depleted and this alveolar hypoxia, via the compensatory mechanism of hypoxic pulmonary vasoconstriction elevates pulmonary vascular resistance. Both effects increase pulmonary artery pressure (23). Bakovic and colleagues demonstrated elevated pulmonary vascular resistance following repeated face immersion breath-holds in both experienced freedivers and healthy controls (24). Genetically determined "patchy" pulmonary capillary vasoconstriction following altitude-related hypoxaemia is associated with high altitude pulmonary oedema (25), which occurs within minutes of exposure to hypobaric hypoxia (26). It is unknown whether this also relates to susceptibility of pulmonary oedema with breath-hold diving.

iii. Pulmonary hyperinflation from prior glossopharyngeal insufflation (GI)

While glossopharyngeal insufflation (GI) is more commonly used in competitive freediving (as described in Chapter 3.1.2), some spearfishers may also use this manoeuvre prior to submersion to extend breath-hold time. GI may further increase the

permeability of the pulmonary capillaries if significant pulmonary hyperinflation occurs (27).

Any combination of these effects may place stress on the blood gas barrier (BGB). An increase in transmural pressure of at least 24 mmHg (33 cmH_2O) can lead to stress failure (23) (Figure 2.2), leading to a fluid shift into the alveolar spaces resulting in pulmonary oedema or haemorrhage. Signs and symptoms of this include a restrictive pattern in pulmonary function, a reduction in oxygen saturation, chest pain, dyspnoea, cough and haemoptysis.

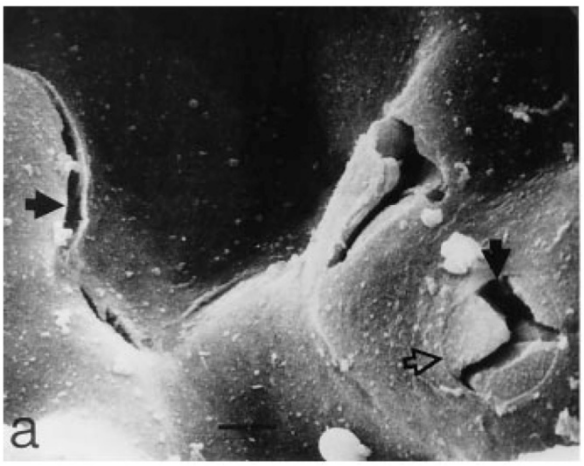


Figure 2.2. Scanning electron micrograph of a capillary at a transmural pressure of 52.5 cmH_2O. Arrows show stress failure of the pulmonary capillaries. *Image from West 1992* (28).

2.1.3 Incidence of pulmonary barotrauma in breath-hold divers

Signs and symptoms of pulmonary barotrauma have been recently reported in more than 60 % of competitors at a freediving competition (5) where, unlike spearfishing, the aim is to achieve the greatest depth in various disciplines. Following single deep dives ranging from 25-75 m, 19 competitors experienced a mean 8 % fall in forced vital capacity (FVC), 4 % fall in pulse oximetry (SpO_2) and three competitors experienced haemoptysis. The extent of hyperbaria experienced that day related to the fall in measured FVC and worsening symptoms of cough and chest pain. Four of 57 (7 %) competitors at another freediving competition were reported to have experienced

haemoptysis however this did not relate to depth achieved that day (maximum 70 m depth) (10).

Freedivers generally achieve far greater depths than pearl divers and spearfishers however there have been six case reports of haemoptysis following repetitive dives to relatively shallower depths ranging from 15-35 m. Five of these cases were spearfishing just prior to the haemoptysis and also experienced cough and dyspnoea (11, 12).

A statement made by Liner and colleagues in the Journal of Applied Physiology was a prime motivation for this study. They observed pulmonary restriction (as measured by a fall in FVC) following freediving and concluded that elite freedivers were at significant risk of pulmonary oedema. Within the text of their discussion, they stated: *".. it remains to be investigated whether repeated diving to shallower depths, e.g., spear fishing, can provoke similar changes.."* (5).

2.1.4 Aim

The aim of this study was to investigate whether repetitive breath-hold dives to moderate depths over an extended period would elicit changes in respiratory function or cause respiratory symptoms.

2.2 Methods

2.2.1 Subjects

Participants of the "Sydney Cup" spearfishing competition were approached via an on-line forum and on-site on the day of competition. The event was held in a salt-water estuary (Gunnamatta Bay) south of the metropolis of Sydney (Figure 2.3) between the hours of 9 am and 2 pm. A data collection station was set up adjacent to the boat ramp where competitors started and finished the competition, with assistance provided by Dr Christopher Wong (Medical student) and Dr Zudin Puthucheary (Respiratory Registrar). Participants dived from powered boats that visited reefs and sand bars within the estuary and in the Pacific Ocean. It was summer when seawater temperatures average 21-25 °C.

Figure 2.3. The salt-water estuary of Gunnamatta Bay. *The circle depicts the location of the boat ramp where measurements were taken.*

Healthy normal (non diving) subjects were recruited from staff at Concord Hospital for the assessment of device and subject variability over the competition time period. Testing took place on a typical working day where the subject did not participate in any physical activity (including diving).

Subjects for the "healthy normal (non diving)" group were excluded if they reported any previous or current respiratory, cardiac or neurological disease or were current smokers.

2.2.2 Questionnaire

Prior to competition

Immediately prior to the event, a stadiometer and weight scale was used to record the subject's demographic details (Dr Christopher Wong). An attending Physician (Dr Zudin Puthucheary) documented any previous relevant medical and diving history (Appendix A i).

Immediately following competition

Immediately after visiting with the competition judges, subjects were asked to provide details of the day's diving statistics (Appendix A i). This information was sourced from

the subjects' own pressure-recording dive watches (e.g. in Figure 2.4a). An attending Physician (Dr Zudin Puthucheary) documented any relevant medical symptoms that were directly due to the day's activities and recorded details of any enhancing techniques used.

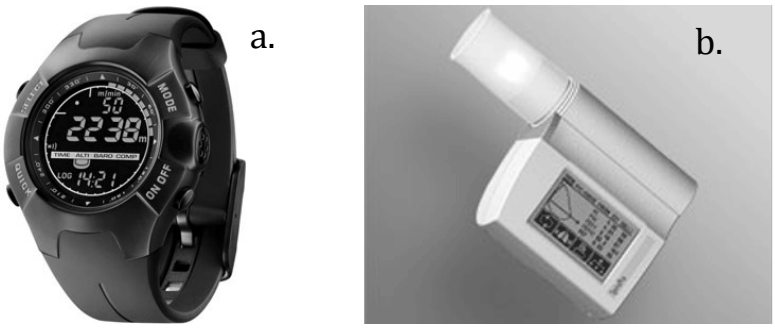


Figure 2.4. An example of a pressure-recording waterproof watch (a) and a portable spirometer to measure respiratory function (b).

2.2.3 Respiratory function

Spirometry was measured (SpiroPro, Sensormedics, Yorba Linda, CA [Figure 2.4b]) according to American Thoracic Society/European Respiratory Society criteria (29) prior to and immediately following the competition. The same operator performed all tests (myself, L.S.). Periodically, a 1 L syringe was used to verify volume measurement stability. If verification failed (±3 %), the internal pneumotachometer was replaced. Predicted values were derived from the recommendations of Hankinson and colleagues [NHANES III] (30).

Healthy normal (non-diving) subjects were studied using the same device and by the same operator (myself, L.S.). Spirometry was performed approximately five h apart (within the hospital grounds), a time period similar to the spearfishing competition.

The study was reviewed and approved by the Human Ethics Review Board of the Sydney South West Area Health Service (New South Wales, Australia). Each subject gave written informed consent.

Statistical Analysis

Results are expressed as mean (SD) unless otherwise stated. A paired t-test was used to

assess the spearfishers' and healthy normal (non diving) group's spirometry before and after the five hour time period. An unpaired t-test was used to compare the demographic data of the healthy normal (non diving) group with that of the spearfishers. A linear correlation investigated any associations between spearfishing history, diving statistics on the day, baseline spirometry and spirometry changes recorded during the competition. A P value <0.05 was considered significant.

2.3 Results

2.3.1 Subjects

29 (2 female) spearfishing competitors agreed to participate in the study. This was a large (>50 %) portion of the total competitors on the day. One subject did not return for post diving spirometry because of other commitments. Three subjects' spirometry did not meet the required criteria due to lack of within-test repeatability. One subject did not have details of the number of dives completed during the competition and one did not have details of the average duration of dives.

25 (2 female) spearfishing subjects were therefore included in the analysis. 10 (3 female) healthy normal subjects were studied.

2.3.2 Questionnaire

Subject demographics are summarised in Table 2.1. There was no difference in age, height or weight between the two groups (P>0.9). Spearfisher diving statistics for the competition are presented in Table 2.2. In five hours, the divers completed 76 (33) dives to 10 (3) m [max depth 17 (4) m] that lasted 0.9 (0.3) min [54 (18) s].

Table 2.1. Subject demographics for 25 spearfishers and 10 healthy normals.

	Spearfishers	Healthy normal	P value
Age *years*	33 (11)	32 (7)	0.78
Gender *male:female*	23:2	7:3	-
Height *cm*	176 (8)	173 (12)	0.28
Weight *kg*	80 (16)	72 (12)	0.10
BMI	25.9 (3.9)	23.9 (3.3)	0.17

Data are mean (SD). BMI; body mass index.

Table 2.2. Competition diving statistics for 24 spearfishers.

	Spearfishers
Breath-hold dives	76 (33)
Average depth *m*	10 (3)
Max depth *m*	17 (4)
Average duration *min*	0.9 (0.3)

Data are mean (SD).

The spearfishers had been participating in the sport for 16 (10) years. Five spearfishers reported a previous history of asthma. There were no reports of previous or current cardiac disease or previous pneumothorax. Four had a past history of smoking and none were current smokers.

13/25 (52 %) of competitors used either hyperventilation or deep breathing prior to diving. Two competitors used GI.

Three subjects reported clinical adverse symptoms following the competition that all related to sinus/middle ear discomfort. One reported sinusitis, one experienced earache and the third reported an inability to equalise his left ear.

2.3.3 Respiratory function

The spearfishers' spirometry values before and after the competition are presented in Table 2.3, Figure 2.5. There was no change in any spirometric parameter following five h of spearfishing except for mid expiratory flow ($FEF_{25-75\%}$) which increased by 0.16 (0.34) L ($P<0.04$). The pneumotachometer was replaced twice due to excessive moisture on the mesh.

Table 2.3. Respiratory function in 25 spearfishers before and after a five-hour competition.

	Before Competition	After competition	Change	P value
FEV_1 *L*	4.83 (0.82)	4.89 (0.87)	0.06 (0.20)	0.15
FVC *L*	5.96 (1.22)	5.97 (1.24)	0.02 (0.20)	0.67
FEV_1/FVC *%*	82 (6)	83 (7)	1 (3)	0.19
PEF *L/s*	11.5 (1.7)	11.4 (1.7)	-0.1 (0.9)	0.54
$FEF_{25-75\%}$ *L/s*	4.68 (1.01)	4.85 (1.16)	0.16 (0.34)	0.03

Data are mean (SD). FEV_1; forced expiratory volume in 1 s, FVC; forced vital capacity, PEF; peak expiratory flow, $FEF_{25-75\%}$; mid expiratory flow.

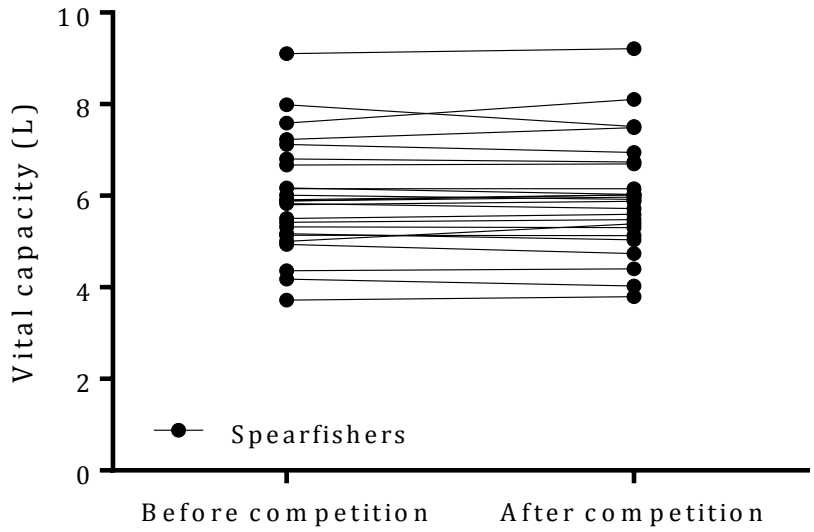


Figure 2.5. Vital capacity before and after a five-hour competition in 25 spearfishers.

The healthy normal (non diving) group's spirometry at baseline was within normal limits (Table 2.4). There was no change in any spirometric parameter following five h except for $FEF_{25-75\%}$ that increased by 0.31 (0.28) L ($P<0.02$) and FEV_1/FVC % that increased by 1 (1) % ($P<0.02$).

Table 2.4. Respiratory function at baseline and after five hours in 10 healthy normals.

	Baseline	After 5 hours	Change after 5 hours	P value
FEV_1 *L*	4.16 (0.48)	4.12 (0.46)	-0.04 (0.14)	0.41
FVC *L*	4.87 (0.66)	4.69 (0.53)	-0.02 (0.13)	0.64
FEV_1/FVC *%*	86 (4)	88 (3)	1 (1)	0.01
PEF *L/s*	10.4 (2.0)	10.5 (2.2)	0.1 (0.5)	0.71
$FEF_{25-75\%}$ *L/s*	4.54 (0.76)	4.90 (0.82)	0.31 (0.28)	0.01

Data are mean (SD). FEV_1; forced expiratory volume in 1 s, FVC; forced vital capacity, PEF; peak expiratory flow, $FEF_{25-75\%}$; mid expiratory flow.

The spearfishers had larger expired volume than the healthy normal (non diving) group in terms of measured FVC (P<0.02) (Table 2.5).

Table 2.5. Respiratory function in 25 spearfishers and 10 healthy normals.

	Spearfishers	Healthy normal	P value
FEV_1 *% predicted*	115 (10)	106 (14)	0.06
FVC *% predicted*	115 (14)	101 (12)	0.01
PEF *% predicted*	118 (13)	114 (16)	0.68
$FEF_{25-75\%}$ *% predicted*	115 (25)	115 (24)	0.71

Data are mean (SD). FEV_1; forced expiratory volume in 1 s, FVC; forced vital capacity, PEF; peak expiratory flow, $FEF_{25-75\%}$; mid expiratory flow.

There were no associations between spearfishing history, diving statistics that day, baseline respiratory function and change in spirometry. Figure 2.6 demonstrates no correlation between the change in FVC following the competition and depth achieved on the day (R^2=0.03).

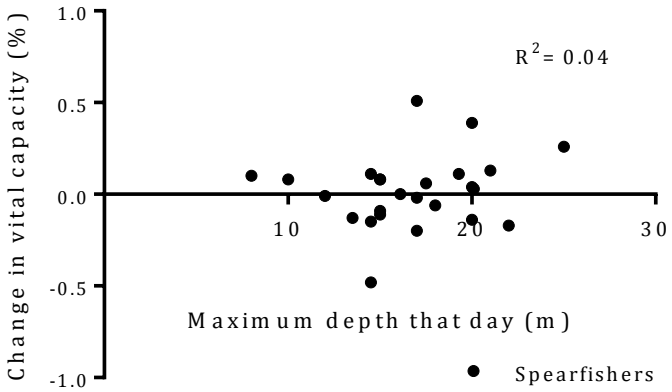


Figure 2.6. Correlation between change in vital capacity before and after a five-hour spearfishing competition and depth achieved on the day in 25 spearfishers.

2.4 Discussion

An assessment of respiratory function and a medical survey taken prior to, and immediately following, a spearfishing competition, failed to elucidate any adverse respiratory signs and/or symptoms. The group had large lungs as compared to predicted values and underwent significant physical activity during the five h competition.

2.4.1 Previous observations of barotrauma with breath-hold diving

Signs and symptoms of pulmonary barotrauma have been previously reported following breath-hold dives ranging from 15-75 m depth, or 2-9 atm (5, 10-12). These observations were predominantly case reports and there is currently a paucity of epidemiological data regarding the incidence of adverse events among the general community of freedivers and spearfishers.

On-site competition surveys can be challenging. The locations of these events are often remote from medical facilities and equipment. Therefore physiological measurement devices need to be portable, possibly battery charged and mostly waterproof. When adverse medical signs are present, clinical follow-up can be difficult as the competitors are not always local residents and typically commence their return travel immediately.

Current studies have mostly assumed BGB stress failure from secondary markers such

as pulmonary restriction, desaturation and haemoptysis. Among these studies cited above, only Boussuges and colleagues confirmed alveolar bleeding in their case reports via bronchoalveolar lavage (12).

Recent discussion of the "signs and symptoms" of barotrauma in the literature suggests that lung injury or pulmonary oedema is a frequent and concerning diving risk. Without a physiological and epidemiological basis, this concern may be misleading.

2.4.2 Study findings

In this spearfishing population, no adverse respiratory signs or symptoms were observed following many dives to depths between 10-25 m, or 2-4 atm. Variation in respiratory function over the five-hour competition was similar to the healthy normal (non diving) group; with very little change in any parameter. An increase in $FEF_{25-75\%}$ both in the spearfishers and in the healthy normal group was identified. The significance of this is unclear, but is likely to be due to diurnal variation with little functional importance. Previous population studies have identified diurnal variation, with an increase in measured lung volume later in the day both in normals and in those with respiratory symptoms (31), however no change was seen in FVC. International guidelines minimise the importance of $FEF_{25-75\%}$ in regards to clinical interpretative strategies in comparison to other parameters because of its known variability (32). In this study, the variability does not appear effort dependent as peak expiratory flow (PEF) and FVC did not change.

In this study, spirometry was completed in all 25 competitors within (approximately) 20 min following the end of their event. Pulmonary oedema following intense ocean swims results in pulmonary restriction for several days (33), thus it is unlikely that any effect was "missed" in this time period.

There were only three adverse symptoms reported following the competition. All were related to discomfort in the middle ear and sinuses. As discussed in Chapter 3.1.2, the inability to equalise the sinuses is a significant limitation to diving depth.

GI prior to diving and the presence of involuntary diaphragmatic contractions following hypoxaemia while immersed increase the prevalence of haemoptysis (11, 34). Only two of the competitors in this study reported using GI and the average diving time

experienced by the competitors suggest that they would not experience the level of hypoxaemia associated with involuntary diaphragm contractions. GI is not popular with spearfishers presumably for its buoyancy effect at these relatively shallow depths. Therefore, this study is not informative on risks of submersion and hyperbaria in divers that employ GI at moderate depths to stay submersed for extended periods.

Interestingly, eight (32 %) of the subjects reported hyperventilating prior to diving, despite their regulatory body strongly discouraging this technique (15). Reducing the pressure of arterial carbon dioxide ($PaCO_2$) with hyperventilation delays the hypercapnic ventilatory drive and therefore potentially increases breath-hold time. However, as the judgement of when to surface is now impaired, there is an increased risk of shallow water blackout (immersed syncope). The consequences of this may be serious and fatalities do occur (35).

2.4.3 Comparison to other breath-hold diving populations

The diving statistics of a typical Australian spearfishing competition had not been previously reported. The diving patterns of participants in this competition were similar to previously described patterns of the "Ama" (13). In five hours, approximately 80 dives were completed, averaging 10 m depth and were less than one min in duration. The maximum depth achieved was 25 m, approximately 10m deeper than the average "Ama" dive. The competition does appear to pose a significant physical challenge with several potential sources for elevating both pulmonary artery and pulmonary capillary pressure however there was no evidence in this study to suggest BGB stress failure.

Also similar to the "Ama", the spearfishers in this study have large lungs as suggested by predictive values with mean spirometry parameters in the upper portion of the normal distribution curve (36). This has been previously observed in competitive freedivers (1, 37) and also in professional SCUBA instructors (38). Whether this is directly due to their diving experience or because of a pre-selecting aptitude to the activity cannot be ascertained. The absence of an association between FVC as a per cent of predicted and years of spearfishing in this study may favour aptitude over an effect of diving but this is uncertain. Skogstad and colleagues demonstrated an increase in baseline VC following three years of professional SCUBA diving, but these divers also initially presented with large lungs (38). The following Chapter 3 is a longitudinal study of respiratory function

in an elite, competitive freediver that has participated in the sport for more than 10 years. This subject is also a spearfisher and a competitor (and a test subject) in this study. While it is logical that those with an aptitude for breath-holding would be more likely to continue participation, longitudinal respiratory function monitoring may elucidate if long-term participation has had any direct impact on pulmonary function and structure.

A recent study of 11 male competitive “fish-catchers” during a competition in the much colder waters of the Mediterranean (12 °C), also reported a mean near 80 dives lasting 51 s duration (39). The average maximal depth was a little deeper at 20 m, which likely reflects the ecology of reefs/sandbars in that area. An hour after the competition, the authors performed echocardiographs (requiring travel to a laboratory). The main findings were a fall in left ventricular preload due to significant dehydration, with no discussion of barotrauma.

Generally the subjects in this study presented as very healthy. They had negligible or no respiratory or cardiac history, were non-smokers with favourable demographics (normal body mass index) covering a wide age range. When considering the risk factors for BGB stress failure as described by West (23), the potential for pulmonary oedema is present even at these moderate depths. The dive reflex causes an increase in intrathoracic blood content and peripheral vascular constriction. The divers are exercising intensely as they swim and fish. Hypoxic vasoconstriction and increased pulmonary vascular resistance occur with extended submersion. If GI is employed, the permeability of the pulmonary capillaries can be altered by pulmonary hyperinflation. This study however was unable to detect any evidence of BGB failure that was large enough to be measured by spirometric parameters or relevant clinical symptoms. It remains possible that the combination of these effects may have caused BGB stress failure in these subjects detectable only when using more sensitive analysis, such as with blood gas analysis and imaging.

2.5 Conclusion

In summary, in keeping with previous studies of swimmers and divers, competitive spearfishers have large lungs compared to predicted values. There was no significant difference in FVC before and after a five-hour competition. There was however an

unexplained increase in $FEF_{25-75\%}$ which was also observed in the healthy normal (non diving) group. There was no association between the extent of hyperbaria experienced on the day and change in spirometry.

No adverse acute respiratory signs or symptoms were observed following multiple dives between 10-25 m, or 2-4 atm. Pulmonary oedema or other lung injury associated with repetitive breath-hold diving is either infrequent or so minor as to not cause respiratory symptoms or change in pulmonary function.

2.6 Resulting publication (Appendix C)

Seccombe LM, Rogers PG, Jenkins CR, Peters MJ. Maintenance of vital capacity during repetitive breath-hold in a spearfishing competition. Respirology. 2012;17(2):350–3.

CHAPTER 3

A LONGITUDINAL STUDY OF AN ELITE COMPETITIVE FREEDIVER

– RESPIRATORY FUNCTION AND STRUCTURE

3.1 Background

The previous Chapter 2 studied the acute effects of repetitive bouts of hyperbaric compression, experienced while breath-hold diving during a spearfishing competition. This Chapter monitors longitudinal respiratory function in an elite competitive breath-hold diver (freediver) who has consistently employed the technique of glossopharyngeal insufflation (GI). The significant increase in lung volume and intrathoracic pressure associated with a GI manoeuvre, when used repeatedly over time, may affect resting pulmonary function and structure.

3.1.1. The sport of freediving

Freediving is a highly competitive, increasing popular, extreme sport. Participants continually push their physiological limits in relation to diving depth and duration of submersion on a single breath. There are several freediving categories and disciplines and with the recent increase in popularity, world records for each have improved dramatically over the last few decades (40). These categories include: "constant weight", where the diver achieves the greatest ocean depth possible without using any assistance except for fins (Figure 3.1a); "no limits", where the diver can achieve ocean depth by any means, such as with motorised sleds; "dynamic apnoea", performed in a 50 m pool, the diver achieves the greatest horizontal distance with the assistance of fins; "static apnoea", is submersed breath-hold time with the diver passive in a pool (Figure 3.1b).

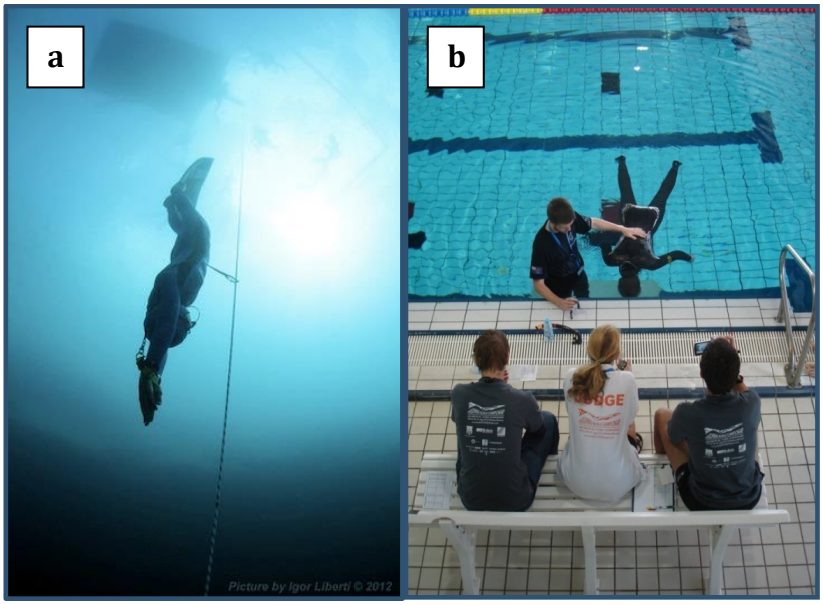


Figure 3.1. An example of the freediving category "constant weight" (a) and "static apnoea" (b). *Picture by Igor Liberti 2012.*

There is an international controlling body, AIDA International, which recognises official times and distance records only when they are conducted in accordance to their strict guidelines and criteria. There needs to be at least two AIDA accredited judges present at each record attempt and they will "pass" an attempt only if the diver completes a defined surface protocol; showing no adverse neurological signs while completing basic motor tasks, within 15 s of surfacing. Their achievements are impressive. The "no-limits" world record is currently 214 m depth, where the diver experiences 22 atm. "Dynamic" and "static apnoea" world records currently stand at 273 m and 11:35 min respectively (41).

In Australia, most competitive freedivers have had previous experience with recreational spearfishing (personal communication, LS), a sport described in Chapter 2.1.1. After an aptitude for breath holding is recognised, these individuals are encouraged to join local freediving clubs. In Sydney, on the East Coast of New South Wales, the "Sydney Freedivers" (est. 2007) have 50 current members and train up to five times weekly across several locations in the greater metropolitan area. The "145 Club" was developed to distinguish members that are at an elite level. To qualify, competency across several freediving disciplines is necessary. Divers must be able to swim at least 100 m "dynamic apnoea", achieve 40 m "constant weight" ocean depth and breath-hold for 5 min "static apnoea" (42) . Highly ranked club members regularly represent the club at national and international AIDA competitions.

Similar to any elite or extreme sportsperson, freedivers regularly train and develop strategies to improve their performance. They improve their diving efficiency via altering (streamlining) their technique, so that less oxygen consumption and carbon dioxide production are required to produce the same work. They develop a greater volitional tolerance to hypoxaemia and hypercapnia and are able to withstand the involuntary diaphragmatic contractions that occur in an attempt to force the diver to breathe during severe hypoxaemia (43). Tolerance to the discomfort of hyperbaric compression is improved with sinus equalisation techniques (44) and increasing intrathoracic gas content prior to diving (1), which will be discussed in the next section (3.1.2). With experience and training, the inherent autonomic diving reflex is augmented; with bradycardia, redistribution of blood flow to the vital organs and

splenic contractions to increase circulating red blood cells (45, 46). Further discussion of the effects of hyperbaric compression is within Chapter 2.1.2.

3.1.2. Glossopharyngeal insufflation (GI)

The technique GI is commonly used by freedivers to augment performance. Some of the immediate physiological effects of this were first described in this population in 2005-6 (1, 37). GI improves performance via several mechanisms; it increases available alveolar oxygen content, it assists in protecting the thorax against the compressive effects of external hyperbaria and is commonly used as an effective method of sinus equalisation at depth.

GI is a process that employs the glossopharyngeal structures to force air into the lungs above usual TLC. The manoeuvre has been described as a pump-like action of the mouth, tongue, pharynx, soft palate and larynx to force boluses of air into the lungs, with the vocal cords functioning as a valve (47) (Figure 3.2). It was first described to assist patients with diaphragm weakness to enhance tidal breathing and improve cough effectiveness in the era when polio was common (48). To avoid daytime mechanical ventilation, those with respiratory muscle weakness perform GI at functional residual capacity (FRC). Freedivers have modified the technique and perform GI at TLC. This is to increase lung gas prior to apnoea (1, 47), and is effective in improving duration and distance achieved while submersed independent of other training effects (49).

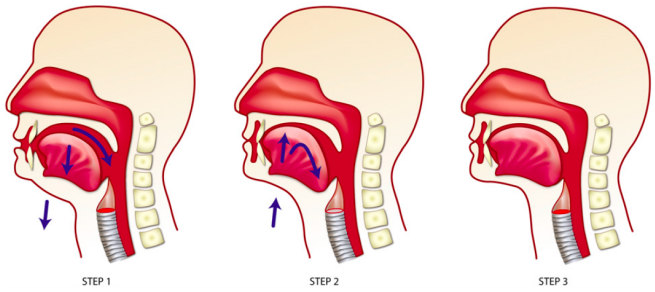


Figure 3.2. Schematic representation of glossopharyngeal insufflation. The mouth is open; air enters the oral and pharyngeal cavities (Step 1). The mouth is sealed by the lips or the tongue; muscles of the tongue and pharynx are used to propel small volumes of air into the lower airways through the open glottis, which is raised during the manoeuvre (Step 2). Closure of the glottis ensures that the air is trapped into the lungs while preparing for the next gulp of air (Step 3). *Reproduced from Maltais, 2011 (50).*

GI can also be used in "reverse" as a method of equalising the sinuses at great depth. During descent, a greater than ≈150mmHg pressure differential across the tympanic membrane causes inward bulging, where air must be moved into the sinuses to equalise. A failure to equalise the sinuses results in significant pain, oedema, haemorrhage or even eardrum rupture. The tensile strength of the tympanic membrane (directly measured post-mortem) has a mean rupture pressure of ≈1.2 kp/cm^2 (51). Sinus pain is commonly reported as a primary limitation to diving depth (personal communication, LS) and was the only symptom reported by the competitive spearfishers in Chapter 2.3.2. Interestingly, in other diving mammals (cetaceans), the sinus cavities are lined with venous plexuses that become engorged with blood as they descend to prevent barotrauma (52). As the freediver descends to a point where the thorax is compressed to volumes below RV, the respiratory muscles are no longer effective in compressing the thorax further. Therefore, to fill the cheeks with air to supply the sinuses for equalisation divers commonly use "reverse" GI to "suck" a bolus of air from the lungs to use for equalising (53).

3.1.3. The effect of GI on the respiratory system

Lung volumes

It has been repeatedly shown that GI significantly increases exhaled lung volumes in experienced freedivers by 21-36 % above baseline (1, 37, 53, 54). With measurements of pulmonary volume, alveolar pressure, pulmonary perfusion and computed tomography (CT) during GI, it has been calculated that that the increase in exhaled air is partially a result of gas compression (approximately 30 %) but is predominately via geometric (Euclidian) lung expansion (1, 2). From CT images, the greatest Euclidian changes were consistently seen at the level of the diaphragm, or inferior lung regions (2).

Alveolar and transpulmonary pressure

Both alveolar and transpulmonary pressure are significantly elevated during GI, as high as 80 cmH_2O (1, 53). These pressures significantly impact the surrounding anatomical structures. A study using transthoracic echocardiography during GI manoeuvres demonstrated significant reductions in ejection fraction and cardiac output, resulting in significant tachycardia and hypotension, (54). The authors concluded that the observed left ventricular systolic dysfunction was from a combination of impaired diastolic filling

and decreased contractility. This is consistent the observation of mediastinum compression following GI measured by CT (2).

Pulmonary perfusion

The concomitant analysis of pulmonary perfusion during GI using single photon emission CT (SPECT) identified a significant alteration in pulmonary perfusion. There was a marked reduction in perfusion intensity in the anterior and inferior lung regions (2). It was postulated that during GI, alveolar pressure exceeds pulmonary artery pressure creating lung zones that were not perfused, or Zone 1 lung units as described by West (55). This had not been demonstrated previously in otherwise healthy subjects.

Lung compliance

An increase in measured VC within one min of a maximal GI manoeuvre as compared to baseline (1) reflects a possible transient reduction in lung elasticity. Similarly, Loring and colleagues reported reduced transpulmonary pressures at TLC lung volumes following a GI manoeuvre (53). Tetzlaff and colleagues reported a transient reduction in static compliance in five freedivers following a maximal GI manoeuvre (56) however the same group where unable to repeat these observations in a follow-up study of four of the five original subjects three years later (57). The acute reduction in compliance, transpulmonary pressure and increase in lung volume immediately following GI may be explained by a transient reduction in pulmonary blood volume, as reflected in the SPECT CT imaging (2). In a study of healthy normal subjects that experienced positive airway pressure assistance and negative pressure assistance at the chest wall (reflecting lung hyperinflation) an acute reduction in lung distensibility was attributable to reduced pulmonary blood content (58). Maximal transpulmonary pressure in this study however was 10 cmH_2O, much lower than the pressure exerted by GI.

3.1.4. Long-term effects of GI

The significant acute physiological effects of GI on both the lung and the cardiovascular system is now well described; however there has been limited investigation into the long-term effects of freediving and GI on respiratory function and structure. In Tetzlaff and colleagues three year follow-up study of five freedivers, static compliance values were within normal limits after six years of freediving participation (57). There have

been two studies assessing lung volume changes following use of GI after six weeks and three years respectively. Nygren-Bonnier and colleagues reported a small increase in VC of 100-200 mL in normal subjects after six weeks of GI training (59). However, Walterspacher and colleagues found no change in the lung function of four freedivers after three years follow-up (57).

Evidence of alveolar barotrauma has been recently associated with GI (3, 60). Of the six freediving subjects that underwent CT imaging following a maximal GI manoeuvre (2), five had evidence of pneumomediastinum (PM) as identified by a radiologist (3). Baseline scans were performed at least three days later, where all PM had either resolved or were significantly smaller. The single subject without a PM was later shown not to be proficient at GI. All subjects were asymptomatic.

A case report by Jacobson described a similar event (60), where a PM was evident in a freediver who had just previously performed a maximal GI manoeuvre with elevated recorded alveolar pressures. It was postulated that the elevated transpulmonary pressures created by GI was directly responsible for the resulting barotrauma.

Generally, the long-term effects of GI on respiratory function and structure are unknown. The previous literature as described above suggests that GI causes a subtle increase in lung volumes with reduced lung compliance. This observation however was not consistent and follow-up studies spanned a maximal period of three years.

Any extent of cumulative alveolar injury from episodes of barotrauma and/or subtle general or regional increases in lung compliance (reduced lung elasticity), would be detrimental at the physiological extremes in the competitive use of GI. After use of GI, there were marked disturbances of ventilation/perfusion and this could be further compromised by lung injury reducing extraction of alveolar oxygen during apnoea. For athletes contemplating GI as a strategy to increase TLC for putative benefit, the net effect could be a negative if a reduction in lung elasticity elevates operational lung volumes and increased work of breathing, as seen in patients with emphysema (61).

3.1.5. Aim

The aim of this chapter was to characterise the nature of any changes in the respiratory system that may be associated with GI. The longitudinal physiological data from a

healthy competitive freediver who practised regular GI training, that was demonstrated to be effective in achieving acute increase in lung volumes, were analysed.

3.2 Methods

3.2.1. Subject

The subject first presented to the Respiratory Laboratory, Concord Hospital, in 2004. He was then 25 years old, 186cm height, 90kg weight, and very healthy with no known respiratory or cardiac disease. He was not taking any medications and was a non-smoker.

He had been a recreational spearfisher since 1999 and had taken up competitive freediving and regular GI training in 2002. He was strongly involved in the "Sydney Freedivers". He was a "145 Club" member (described in 2.1.1.), participated in regular training sessions and represented the club at national and international meets. Training sessions were typically three times weekly from 2009 that which was mostly maintained through to 2012, and he consistently competed at a highly ranked national and international level.

Personal best freediving statistics were documented at every visit (Appendix A ii). There had been improvement in his freediving performance over the eight-year period. The subject's "static apnoea" performance (submersed breath-hold time) had increased from 7 min in 2004 to 8 min in 2012 and there had been a 37 m increase in "constant weight" (ocean depth achieved unassisted with fins) to 88 m (Table 3.1). He had been in good health in this period without any symptoms or evidence of barotrauma.

Table 3.1: Competitive statistics in a healthy freediver.

Date *(DD/MM/YYYY)*	06/09/2004	17/01/2009	20/10/2009	07/05/2012
Subject age *years*	25	30	31	33
Years freediving	2	6	7	10
Static apnoea *PB*	7:00 min	-	7:15 min	8:00 min
Dynamic apnoea *PB*	-	-	194 m	196 m
Constant weight *PB*	51 m	-	57 m	88 m

PB; personal best at time of visit, Static apnoea; submersed breath-hold, Dynamic apnoea; distance while submersed with fins, Constant weight; ocean depth while submersed unassisted with fins.

3.2.2. Respiratory function

Complex respiratory function tests prior to, and immediately following a maximal GI manoeuvre were collected over a period of eight years. This data was sourced and reviewed. Measurements were taken on four occasions (from age 25 to 33 years). All tests were performed according to American Thoracic Society / European Respiratory Society criteria (29, 62, 63) and were conducted by the same scientific officer (myself, L.S.). There was one equipment upgrade (Vmax Encore, Sensormedics, Yorba Linda, Ca, USA) in that time that demonstrated no changes in measurement using biological controls as per laboratory accreditation procedures.

Sitting body plethysmography was recorded prior to and immediately following maximal GI (TLC_{GI}) using a technique developed previously (1). Once enclosed in the body plethysmograph (Figure 3.3a), with tracing initiated by tidal volume (V_T), the subject was instructed to come off the mouthpiece to perform the typical manoeuvre that allowed him to achieve what he believed to be his maximal lung volume with GI, nose clip in situ. Then, without air leak and with a closed glottis, he returned to the mouthpiece, then once sealed opened the glottis to perform a slow expiratory VC manoeuvre to RV (VC_{GI}). Panting manoeuvres against a closed shutter were then performed for thoracic gas volume (V_{TG}); the measurement was performed after a small inhalation (ERV_I), immediately after the VC manoeuvre.

TLC_{GI} was calculated by: TLC_{GI} (BTPS) = V_{TG} - ERV_I + VC_{GI}.

As measurements were made using a plethysmograph, exhaled gas volumes were recorded rather than Euclidian (geometric) volumes, and thus were free of a gas compression effect.

Reference values were derived from European Community for Coal and Steel (64).

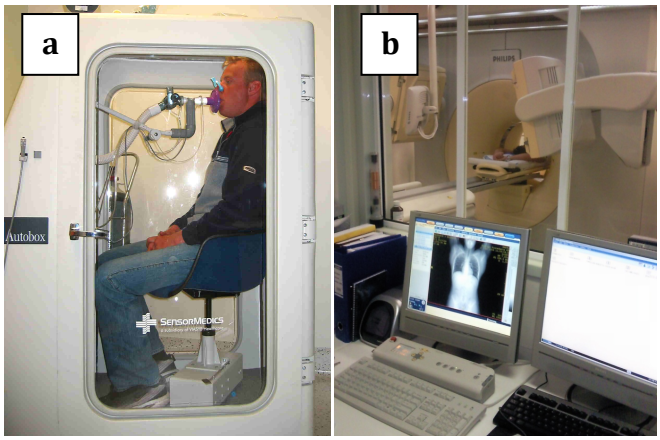


Figure 3.3 Lung volume measurements using plethysmography (a.) and computed tomography (b.) in a healthy freediver.

3.2.3. Computed tomography (CT)

Non-contrast CT images of the thorax were previously acquired in 2009. This subject was originally to be included in the previously published pulmonary perfusion study (2). A protocol variation however omitted this data from that study and hence was not included in any previous publication.

A hybrid dual-headed camera (Precedence; Philips Medical Systems, Milpitas, Ca, USA), acquired a six slice CT of the thorax (15 s at 32 steps per head over an interval of 360 °) with the subject in the supine position with arms extended behind the head (Figure 3.3b). Images were captured on two occasions, firstly during breath-hold (≈80 s) following a maximal GI manoeuvre ("TLC_{GI}") and secondly during baseline tidal breathing ("Baseline") seven weeks later for comparison. For "TLC_{GI}" the volume, slice width, interval, pitch and field were 50 mA (90 kV), 5 mm, 5 mm, 1 and 35 cm ~1.1 mSv. These measurements were the same for "Baseline" with the exception of field which was 25 cm ~0.8 mSv. Images were analysed independently (Astonish; Philips Medical System).

The CT images were then segmented for 3-dimensional analysis of lung tissue. This analysis was done with the assistance of Dr Clayton Frater and Mr Mark Pearson (Nuclear Medicine Scientists), Nuclear Medicine, Concord Hospital.

Lung tissue was segmented from the CT images, with volumes calculated (ITK-SNAP, University of Pennsylvania, PA.) and then rendered as a 3-dimensional image (Blender 2.63a, Holland, The Netherlands).

The study was reviewed and approved by the Human Ethics Review Board of Sydney South West Area Health Service (NSW, Australia) with written informed consent obtained from the subject.

3.3. Results

3.3.1. Respiratory function

There was no change in forced expiratory volume in one s (FEV_1) over the eight year period, however FVC increased significantly by over 900 mL, resulting in a reduced FEV_1/FVC ratio (Table 3.2).

Table 3.2: Spirometry in an elite freediver over eight years

Date of test	06/09/2004	17/01/2009	20/10/2009	07/05/2012	Increase from 2004 (%)
FEV_1 *L*	6.20	5.87	6.18	6.28	8 mL (1 %)
FVC *L*	8.11	8.62	9.09	9.02	910 mL (11 %)
FEV_1/FVC *%*	76	68	68	70	-6

FEV_1; forced expiratory volume in 1 s, FVC; forced vital capacity.

Transfer factor for carbon monoxide (TL_{CO}) is a measure of the surface area of the lung that is available for gas exchange (a "window" on the pulmonary microcirculation). This had remained stable and was within normal limits. However K_{CO} (TL_{CO} corrected for alveolar volume [V_A]) was abnormal and fell during the period of observation (Table 3.3).

Table 3.3. Diffusing capacity in an elite freediver over eight years.

Date of test	06/09/2004	17/01/2009	20/10/2009	07/05/2012
TL_{CO} adj	40.4	-	35.1	37.8
mL.mmHg.min	(104 % pred)			(103 % pred)
K_{CO} adj	4.24	-	3.65	3.88
mL.mmHg.min	(66 % pred)			(63 % pred)

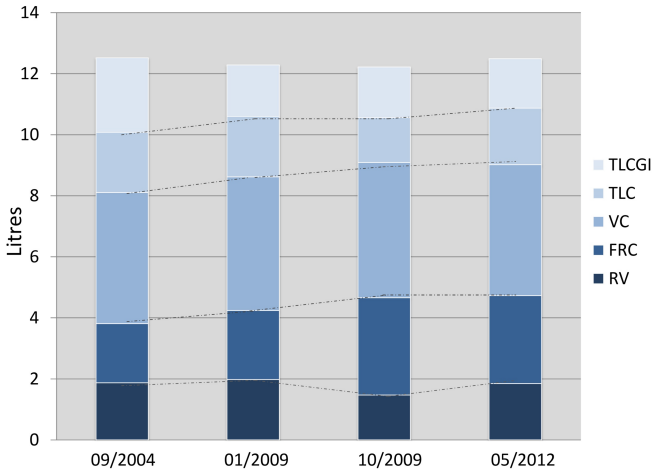
TL_{CO} adj; transfer factor for carbon monoxide adjusted for haemoglobin, K_{CO}; TL_{CO} adj corrected for alveolar volume.

TLC and FRC had significantly increased by 790 mL and 920 mL respectively, however with no change in RV (Table 3.4, Figure 3.4).

Table 3.4. Plethysmography in an elite freediver over eight years.

Date of test	06/09/2004	17/01/2009	20/10/2009	07/05/2012	Increase from 2004 (%)
TLC *L*	10.08	10.60	10.56	10.87	790 mL (8 %)
FRC *L*	3.81	4.24	4.66	4.73	920 mL (24 %)
RV *L*	1.87	1.98	1.47	1.85	-20 mL (-1 %)

TLC; total lung capacity, FRC; functional residual volume, RV; residual volume



GI; glossopharyngeal insufflation, TLC; total lung capacity, VC; vital capacity, FRC; functional residual capacity, RV; residual volume

Figure 3.4. Lung volumes in a competitive freediver over eight years.

When considering the effect of GI, he was adept at the manoeuvre, and in 2004 he was able to entrain an additional 2.4 L of air, increasing his measured FVC by 30 %. By 2012 the additional lung gas volume from GI had reduced to 1.62 L, which was in proportion to the increase in FVC. Thus TLC with GI was stable, despite ongoing, regular GI training (Table 3.5).

Table 3.5. The effect of glossopharyngeal insufflation on lung volumes in an elite freediver.

Date of test	06/09/2004	17/01/2009	20/10/2009	07/05/2012
FVC *L* (predicted)	8.11 (142 %)	8.62 (158 %)	9.09 (166 %)	9.02 (167 %)
Increase with GI	2.44L (30 %)	1.69L (20 %)	1.66L (18 %)	1.62L (18 %)
TLC_{GI} *L*	12.42	12.29	12.22	12.49

FVC; forced vital capacity, GI; glossopharyngeal insufflation, TLC; total lung capacity.

The subject initially presented with significantly large lungs when compared to predicted values. His initial FVC was 142 % of predicted in 2004 and 167 % of predicted in 2012 (Table 3.5).

3.3.2. Computed tomography (CT)

The CT images were initially volume scaled for direct comparison. A comparison of CT images during tidal breathing and during breath-hold at TLC_{GI} is presented in Figure 3.5.

The TLC_{GI} CT images demonstrate significant hyperinflation with compression of the mediastinum and a flattening of the central part of the diaphragm (and accentuated costo-phrenic angle) as compared to baseline (Figure 3.5, 3.6).

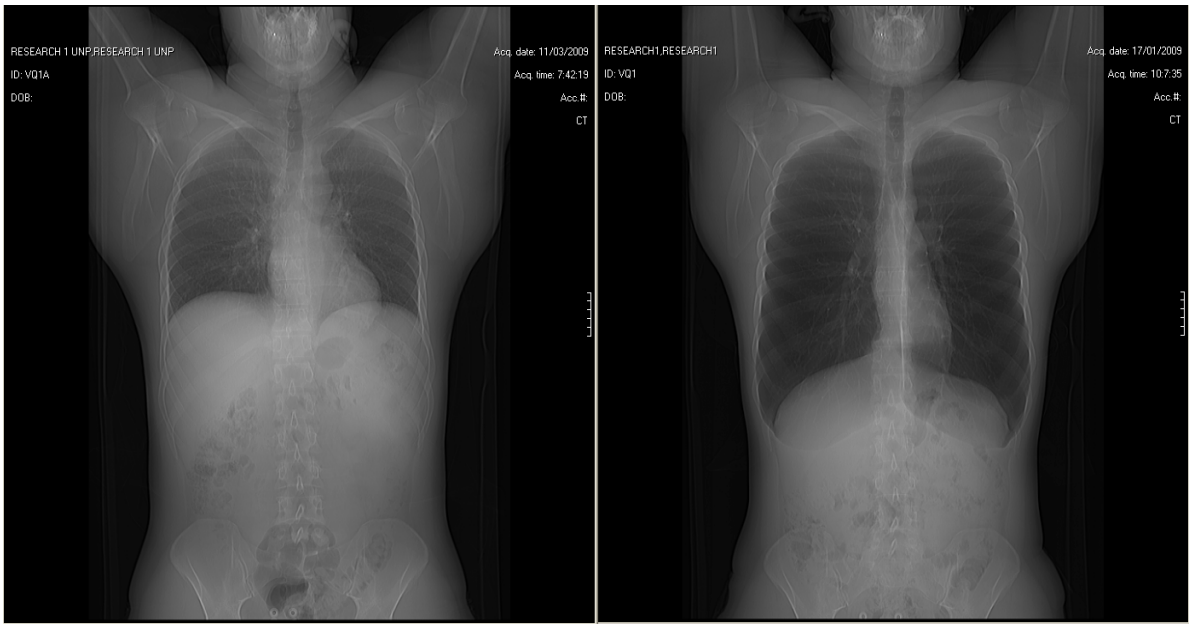


Figure 3.5. Computed tomography during tidal breathing (left) and following a maximal GI manoeuvre (right) in a competitive freediver. *Significant lung hyperinflation with compression of the mediastinum is evident.*

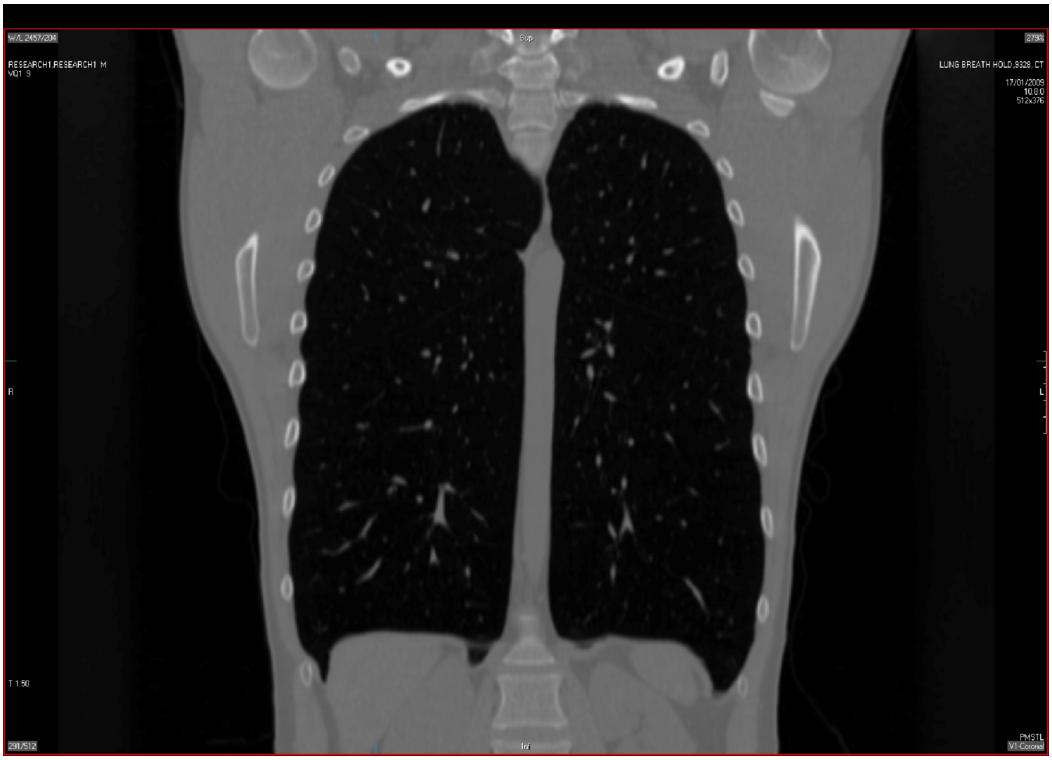


Figure 3.6. Computed tomography following a maximal glossopharyngeal insufflation manoeuvre. *A flattening of the central part of the diaphragm with accentuated costo-phrenic angle is evident.*

Snapshots of the 3-dimensional images are presented in Figure 3.7. A video capturing all views is presented in the supplementary material (Video 1). The 3-dimensional images demonstrate the significantly increased Euclidian lung volume following GI, predominately inferior and posterior.

Video 1. Video of rendered 3-dimensional images of lung tissue from CT scans taken during tidal breathing (on right) and following maximal glossopharyngeal insufflation (on left) from the same competitive freediver. Images are scaled for direct volume

Other noteworthy features evident in the TLC_{GI} 3-dimensional images include intercostal bulging of lung tissue, mediastinal distortion, a flattening of the central part of the diaphragm and an accentuated costo-phrenic angle as compared to the baseline image (volume scaled for direct comparison). A crowding of the thoracic inlet during TLC_{GI} can be seen in the inferior and anterior views.

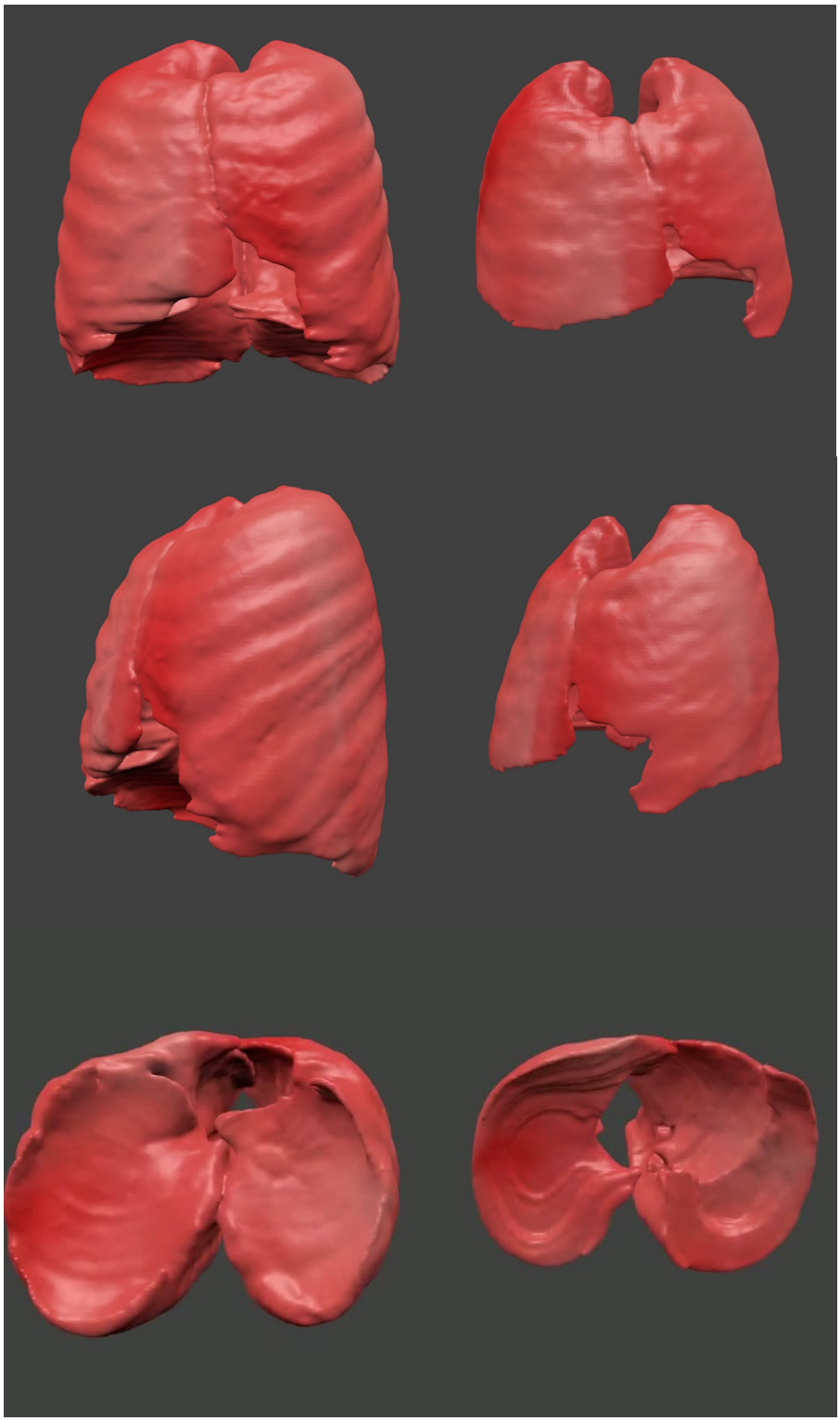


Figure 3.7. Snapshot of rendered 3-dimensional images of lung tissue from CT scans taken during tidal breathing (on right) and following maximal glossopharyngeal insufflation (on left) from the same competitive freediver [coronal, sagittal and inferior views]. *Images are scaled for direct volume comparison.*

A PM was evident in the "TLC_{GI}" scan, which was not present in the "Baseline" scan (Figure 3.8).

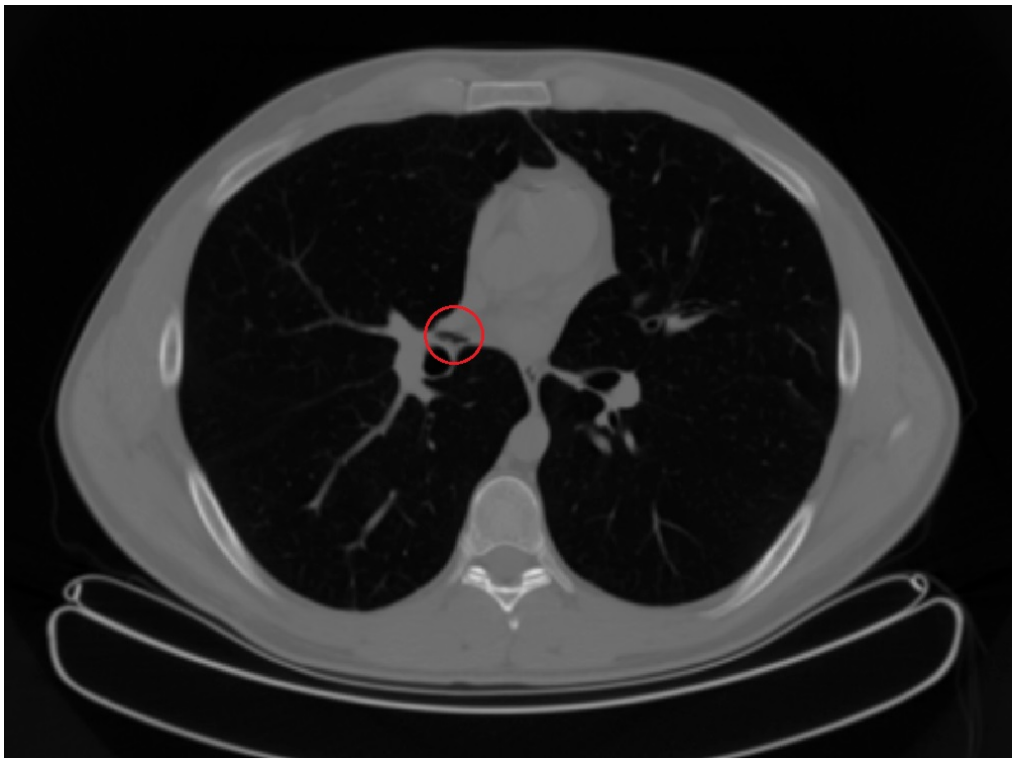


Figure 3.8. Computed tomography following a maximal glossopharyngeal insufflation manoeuvre. *A pneumomediastinum is circled in red.*

3.4. Discussion

In an elite freediver, over a period of eight years, an increase in measured lung volumes was evident with a greater than 800 mL increase in FVC, FRC and TLC. There was no evidence of gas trapping, as RV remained unchanged. These changes represent an increment over any pulmonary effect of GI performed before the initial test in 2004. Importantly, TL_{CO} was stable, suggesting a preservation of the total volume of the pulmonary-capillary bed and no development of high ventilation/perfusion lung units. The subject was adept at GI however there was an observed limit to TLC_{GI} despite ongoing GI training. CT rendered images demonstrate the significant distortion that was experienced by the lung and surrounding structures during GI.

Previous studies of freedivers, spearfishers and SCUBA divers have noted overall large lung volumes when compared to normal (1, 6, 38, 53). It is generally unknown as to

whether this represents a preselection for the sport or if it is directly due to altered physiology as a result of their participation. This subject had very large lungs, with an initial FVC of 142 % of predicted in 2004, which increased further to 167 % of predicted in 2012. A study of 65 SCUBA divers that initially presented with large lungs at an introductory diving course, demonstrated no change in FVC after 3 years of follow-up (38). This suggests that while initial large lungs were a factor in this subject's success and continuing interest in the sport of freediving, GI had contributed to further increased lung volume over time.

This pattern of change – increase in FVC, FRC and TLC over time with low K_{CO} and high V_A resulting in normal and stable TL_{CO} – is most consistent with a reduction in chest wall recoil at higher lung volumes as a consequence of repeated GI. In the absence of more complex transpulmonary pressure measurements, this does not discount a possible smaller contribution of changes in the lung itself.

A reduction in lung elasticity was not evident. Reduced lung elastic recoil is often accompanied by an elevated RV, which remained stable in this case. This is in keeping with the observations of Walterspacher and colleagues (57) who found no change in dynamic or static compliance in four freedivers after three years of GI participation. Nygren-Bonnier and colleagues demonstrated small increases in VC in healthy normals following six weeks of GI training, also with no change in RV (59).

Interestingly TLC_{GI} remained stable despite ongoing GI training. Whether this was due to the inability of the lung to expand further irrespective of the behaviour of the chest wall, or to an intrinsic limit imposed by the chest wall is not known. The stable TL_{CO} is consistent with no effect on the pulmonary capillary blood volume and suggests a similar distribution of ventilation.

Despite a limit to TLC_{GI}, performance had improved suggesting the importance of additional training attributes such as diving efficiency, subjective CO_2 tolerance and possible augmentations of the dive reflex. This is addressed further in Chapter 5. Whether such alteration in lung mechanics from GI on athletic performance would be advantageous in any other sport is a matter for speculation.

The 3-dimensional rendered CT scans were extremely helpful in quantifying the mechanical stresses placed on the lung during GI. The significantly increased Euclidian

lung volumes seen on the TLC_{GI} CT image reflects the great mechanical force on the respiratory system with GI. There was intercostal bulging of lung tissue, mediastinal distortion, flattening of the central part of the diaphragm with an accentuated cost-phrenic angle (Figure 3.5, 3.6, 3.7 and Video 1). This pattern of lung hyperinflation is different to that seen in emphysematous patients. There was also an observed crowding of the thoracic inlet. This may contribute to the significant reduction in cardiac out and frequent syncopal episodes that have been associated with GI (2, 54, 65).

A PM was evident on this subject's CT scan immediately following GI that was not evident during tidal breathing seven weeks later. The significance of this is unclear. Similar observations have been reported immediately following GI (3, 60), suggesting that the elevated alveolar pressures are responsible for the acute lung injury. This subject was asymptomatic, with no signs of acute or previous lung injury seen on the baseline, follow-up scan.

3.5. Conclusion

Longitudinal monitoring of a competitive freediver spanning this time period had not been previously presented. As demonstrated in this case, repeated exertion of the great force that GI imposes on the respiratory system can alter its mechanics. The increase in lung volumes appears to be associated with use of a technique specifically used to enhance athletic performance. This had been achieved without developing functionally important macroscopic lung damage, at least as evidenced by the CT scans, and measures of gas exchange.

3.6 Resulting publication (Appendix C)

Seccombe LM, Jenkins CR, Rogers PG, Pearson MA, Peters MJ. Evidence of respiratory system remodelling in a competitive freediver. Eur Respir J. 2013 Mar;41(3):760-2.

CHAPTER 4

METHOD FOR DETERMINING CHEMOSENSITIVITY TO HYPERCAPNIA AND HYPOXIA

4.1 Background

The following Chapters address the measurement of ventilatory control in elite freedivers that voluntarily endure the limits of intrathoracic pressure and hypoxia, and in PD subjects that may have early central ventilatory dysfunction. As ventilatory parameters can be significantly affected by subjective influences, measures of autonomic function were necessary to elucidate voluntary override from intrinsic deficits.

Careful dissertation on the pro's and con's of ventilatory response protocols were critical when deciding which method to use and which parameters to monitor. For the goals of this study there was a strong emphasis of minimising the influences of subjective factors. Also, the method had to be achievable for those with possible neurological impairment. This Chapter addresses the history and variants in ventilatory control protocols. The chosen method, with the healthy normal response is presented.

4.2 Ventilatory control in normal respiration

Despite widely varying demands for O_2 uptake and CO_2 output during daily life, the arterial pressure of O_2 (PaO_2) and CO_2 ($PaCO_2$) are tightly regulated and are very stable in health. This is achieved via the control of ventilation where the central and peripheral chemoreceptors feed information to the respiratory centres located in the pons and medulla within the brainstem. Here the information is coordinated and commands are sent to the respiratory muscles that mechanically cause ventilation.

Chemoreceptors

The central and peripheral chemoreceptors respond to a change in the chemical composition of the blood or the fluid around it. The central chemoreceptors, near the ventral surface of the medulla, respond primarily to changes in $PaCO_2$ that alters the pH of the cerebrospinal fluid (66-68). When blood $PaCO_2$ rises, CO_2 diffuses into the cerebrospinal fluid from the cerebral blood vessels, liberating H^+ ions (Figure 4.1) (69). H^+ or dissolved CO_2 changes ventilation within seconds. The central chemoreceptors also regulate cerebral blood flow, with cerebrovascular reactivity and ventilatory response being tightly linked. Hypercapnia causes cerebral vasodilation to attenuate the rise in central PCO_2, and similarly hypocapnia causes cerebral vasoconstriction (70).

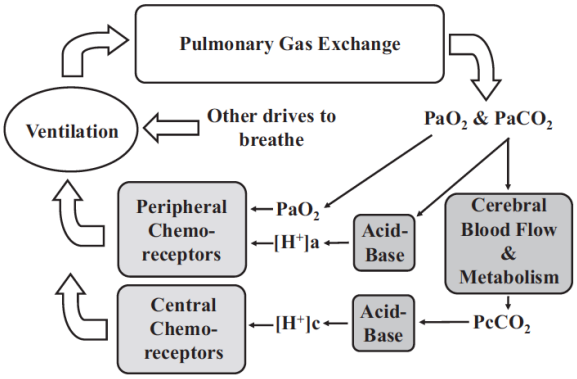


Figure 4.1. A simplified block diagram illustrating the respiratory chemoreflex control of ventilation. *Reproduced from Duffin* (69).

Peripheral chemoreceptors are located in the carotid bodies at the bifurcation of the common carotid arteries, and in the aortic bodies above and below the aortic arch (71). The carotid bodies are the primary receptors and contain type I and II glomus cells. Type I contain large aliquots of dopamine and are in close apposition to endings of the afferent carotid sinus nerve. The precise mechanism is unclear however it is believed that the glomus cells are the sites of chemoreception and that modulation of neurotransmitter release from the glomus cells by physiological and chemical stimuli affects the discharge rate of the carotid body afferent fibres. The carotid bodies send their afferent input to the brain stem via the central nervous system (glossopharyngeal nerve).

The peripheral chemoreceptors respond to decreases in PaO_2 and pH, and increases in $PaCO_2$ (Figure 4.1). They are primarily responsible for the increase in minute ventilation ($\dot{V}_E$) in response to arterial hypoxaemia. This is the dominant means for mammals to compensate for an acute decrease in O_2 availability (72). In parallel, peripheral chemoreceptors also provoke an increase in sympathetic nervous system activity (73, 74). Tissues with morphology similar to carotid and aortic bodies are also in the thorax and the abdomen, which may serve as ancillary chemoreceptors (75, 76).

In health, $PaCO_2$ is the primary factor that controls ventilation under normal conditions and the response is highly sensitive. The response to hypoxaemia becomes more important in certain environmental and physiological circumstances, such as travel to

high altitude where $PaCO_2$ is significantly reduced, certain lung diseases that result in chronic hypercapnia, and in response to certain pharmacological agents.

4.3 The variants of chemosensitivity protocols

4.3.1 Hypercapnic ventilatory response (HCVR)

Typically past a threshold (≈45mmHg), $\dot{V}_E$ increases linearly with increasing pressure of end-tidal CO_2 ($P_{ET}CO_2$). Therefore an individual's hypercapnic ventilatory response (HCVR) can be measured by rebreathing from a reservoir such that the fraction of inspired CO_2 ($FICO_2$) increases gradually.

Originally, steady-state methods were used in the assessment of hypercapnic sensitivity. They required 10-20 min of $PaCO_2$ equilibrium between arterial blood and chemoreceptor tissues and it became clear that this was unsuitable for clinical testing. It was extremely uncomfortable and impractical for patients with pulmonary, cardiac and metabolic disorders. This led to the development of a progressive hypercapnia challenge by Read in 1967 (77), a variant of a technique that was initially described by Haldane and Smith in 1892 (78), as below;

"During rebreathing, expired CO_2 is constantly returned to the lungs; as metabolism continues, and CO_2 accumulates, the PCO_2 of all body fluids rises progressively. As the PCO_2 in the region of the chemoreceptors rises, a progressive CO_2 stimulus to ventilation develops. A high initial concentration of O_2 in the rebreathing bag provides O_2 for metabolism and prevents the development of any hypoxic stimulus to ventilation" (78).

Read's adaptations were to initiate the rebreathing at a $PaCO_2$ near that of the brain tissue and mixed venous blood (≈7 %), and to use a small rebreathing bag (6 L). This was intended to create a theoretical linear relationship between ventilation and $PaCO_2$, and to provide rapid equilibrium between venous blood, arterial blood and gas both within the lung and in the rebreathing bag. This protocol also initiates rebreathing with an elevated O_2 concentration (hyperoxia; FIO_2 0.50), allowing PaO_2 to remain at ≈200 mmHg after 4 min.

This method remains the most commonly used for the assessment of the HCVR. Duffin recently included a measure of the initial breakpoint in the $\dot{V}_E$ response, interpreted as the threshold for either the peripheral chemoreflex or the central chemoreflex

ventilatory response (69). This requires 5 min of prior voluntary hyperventilation to assess the response in hypocapnic range. Prior hyperventilation however may influence the subsequent ventilatory response via reduced cerebral blood flow, respiratory fatigue, changes in psychological state and cardiac output (79) and may be uncomfortable for those with already reduced respiratory reserve.

Pressure of end-tidal oxygen during progressive hypercapnia

A lowered $P_{ET}O_2$ and subsequent hypoxia during a HCVR increases the sensitivity of the $\dot{V}_E$ response (80). Therefore the slope of the linear response to progressive hypercapnia becomes steeper, with considerable variation between subjects (77, 81). The method of Read avoids hypoxaemia but PIO_2 and PaO_2 are not stable. An alternative challenge is isoxic hypercapnia during which the fraction of inspired (FIO_2) is stable throughout the challenge by the addition of small aliquots of O_2 continually introduced to the rebreathing circuit to maintain a constant FIO_2 of 0.21.

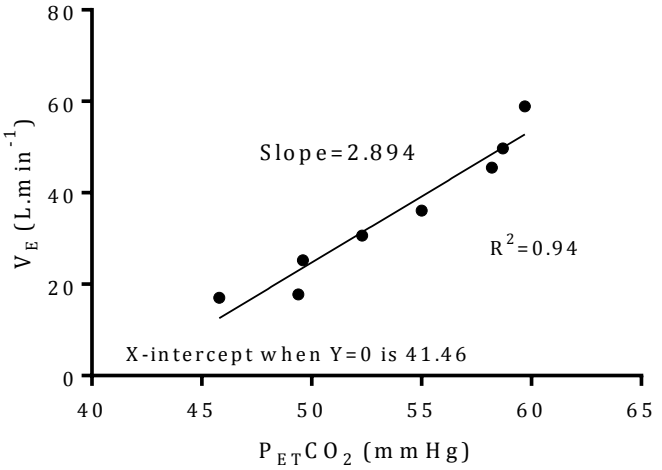
Duffin advocates rebreathing at two isoxic levels, $P_{ET}O_2$ 50 and 150 mmHg, to estimate the contribution of the peripheral chemoreceptors (69). He states that initiating rebreathing with hyperoxia ($P_{ET}O_2$ 150-250 mmHg) will "silence the peripheral chemoreflex response to CO_2" (82). This method has been criticised as the fast peripheral component of the ventilatory response has still been identified in humans at a $P_{ET}O_2$ >200 mmHg (83) also hyperoxia can affect central drive via the Haldane effect, reducing brain blood flow (84).

Analysis and expression of the HCVR

Fast, accurate flow and gas analysers allow for breath-by-breath analysis of end-tidal gases that can be plotted directly against $\dot{V}_E$. $P_{ET}O_2$ and $P_{ET}CO_2$ are essentially the pressure of alveolar gases (P_ACO_2 and P_AO_2 respectively), that strongly reflect $PaCO_2$ and PaO_2, even in the presence of respiratory disease (85).

As the HCVR is not typically initiated until $PaCO_2$ exceeds 45 mmHg, data points are plotted for $\dot{V}_E$ and $P_{ET}CO_2$ in the ranges of 45-70 mmHg $P_{ET}CO_2$. In health, it has been consistently shown that the response is near linear. Therefore each subject's response is defined by calculating the equation of the line of least squares regression: $m\chi+b$. The slope "m" ($L.min^{-1}.mmHgP_{ET}CO_2^{-1}$) reveals information about the hypercapnic

"sensitivity" of the respiratory centre (77) and is the primary outcome measure. An additional measure commonly analysed is the point where the regression line crosses the x-axis; i.e. $P_{ET}CO_2$ at $\dot{V}_E = 0$ (Figure 4.2).



$\dot{V}_E$; minute ventilation, $P_{ET}CO_2$; pressure of end tidal carbon dioxide.

Figure 4.2. An example of the expression of the hypercapnic ventilatory response in a healthy subject. *Slope is the linear regression of data points.*

The optimal combination of tidal volume (V_T) and respiratory rate (RR) typically follows the law of minimal work output under the conditions of rest and exercise as described by Otis, Fenn and Rahn (86). The increase in $\dot{V}_E$ until an $FICO_2$ 0.05-0.07 is achieved primarily through V_T. With further hypercapnia, a greater contribution is seen from an elevation in RR. Individuals with larger lung volumes tend to have larger V_T and have a less sensitive HCVR (87). The analysis of ventilatory components may be helpful in identifying specific responses to hypercapnia and how these responses may differ between individuals.

4.3.2. Hypercapnic occlusion pressure response (P_{100})

Occlusion pressure (P_{100} or $P_{0.1}$) is the pressure generated at the mouth during the first 100 ms of an inspiratory effort against an occluded airway. While less commonly studied, it can assist in distinguishing between central and peripheral respiratory impairment.

P_{100} is seen to be a measurement of ventilatory drive independent of the mechanical properties of the lung. As no airflow occurs during the occlusion, the interference from mechanical abnormalities, such as increased resistance or decreased compliance, is omitted. Therefore the pressure generated reflects the neural output of the medullary centres that drive the rate and depth of breathing (88). The rate of the rise of inspiratory muscle activity at the beginning of inspiration is independent of the level of vagal activity (89).

West describes those with central nervous system or neuromuscular inadequacy as those that "won't breathe" (central impairment) and those with mechanical abnormalities (peripheral impairment) as those that "can't breathe" (90). The P_{100} can assist in distinguishing between these two processes. For example, a subject that has central impairment will experience both a reduced $\dot{V}_E$ and P_{100} response to hypercapnia, however a subject with peripheral impairment will experience a reduced $\dot{V}_E$ response, but with a normal P_{100} response.

P_{100} is most reliable when performed near functional residual capacity (FRC) and when the subject is unaware when the occlusion will occur (91). Therefore the airway is typically occluded randomly, with a pressure-time line recorded (Figure 4.3).

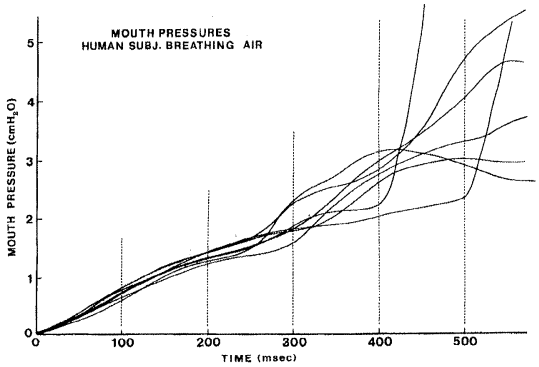
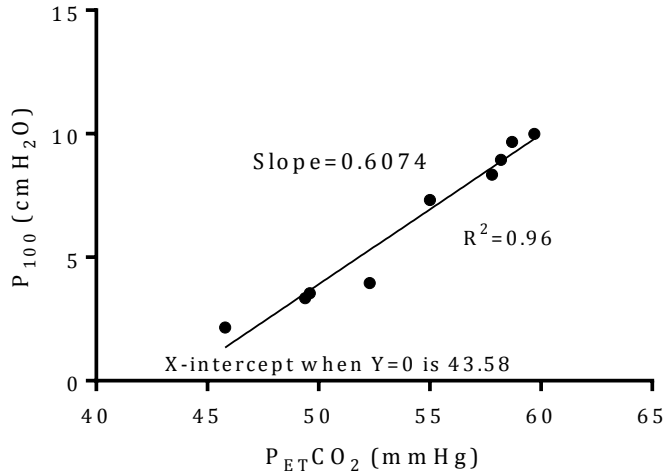


Figure 4.3. Repeatability of occlusion pressure waves up to 250 ms in the same subject. *Reproduced from Whitelaw* (89).

Analysis and expression of P_{100}

The expression of the response to changing $P_{ET}CO_2$ is similar to $\dot{V}_E$ as it typically elicits a linear response above 45 mmHg$P_{ET}CO_2$ (Figure 4.4).



$P_{ET}CO_2$; pressure of end tidal carbon dioxide.

Figure 4.4. An example of the expression of the occlusion pressure (P_{100}) response to progressive hypercapnia in a healthy subject. *Slope is the linear regression of data points.*

4.3.3 Acute hypoxic ventilatory response (AHVR)

There is much variation in the literature regarding the appropriate method for determining the acute hypoxic ventilatory response (AHVR). The protocol variants encompass

i. step, single steady-state or progressive protocols
ii. the duration of the hypoxic exposure
iii. the levels of interacting $FICO_2$.

These factors are discussed briefly below.

i. Step, single steady-state or progressive

There are typically three specific experimental AHVR procedures; 1. progressive 2. incremental step or sawtooth 3. single immediate steps. The most popular are the progressive and step procedures as they quantify the ventilation/oxygen response. Steady-state type procedures are typically longer in duration and therefore can be contaminated by hypoxic ventilatory decline (HVD) that will result in a central depressive effect. Step tests are technically difficult, as they generally require either

complex servo-type mechanisms that control FIO_2 and $FICO_2$ to within 0.5 % or expensive pre-mixed gases. For progressive tests, continuous, fast monitoring of flow and gas concentrations is required as the typical exponential $\dot{V}_E$ response can be sudden.

ii. Test duration

The timing of the rebreathing protocol is important, primarily to describe the response that is not affected by HVD. With tests exceeding five min, the potential for systematic errors due to HVD contamination increase, that is thought to represent peripheral chemoreceptor desensitisation (92) and/or other centrally mediated mechanisms, including an initial stage of acclimatisation (93). The recommendation is to complete testing within 5-8 min (94, 95) and HVD is less pronounced with poikilocapnic hypoxia (95).

iii. Pressure of end-tidal carbon dioxide during progressive hypoxia

It is clear that any increase in $P_{ET}CO_2$ augments the AHVR. $\dot{V}_E/P_{ET}O_2$ slopes are higher in hypercapnia and isocapnia than poikilocapnia (96). A modulating effect of CO_2 on the response could originate from three sources: the carotid bodies, a central-peripheral interaction, and/or central O_2-CO_2 interaction but the precise mechanisms are unclear.

Failing to control CO_2 (poikilocapnia - from the Greek “poikilos”, meaning varied) typically causes a further, concomitant fall in P_aCO_2 due to the resulting increase in $\dot{V}_E$ from the progressive hypoxia (97). This will counter the hypoxic drive to breathe, therefore poikilocapnia typically induces a reduced AHVR. While these protocols produce more complicated results due to the interactive effects of hypoxia and concomitant hypocapnia, they are more representative of certain environmental conditions, such as high altitude and pharmacological effects. Therefore, although the determination of the mechanisms contributing to the responses becomes more difficult to interpret, the responses themselves are more readily assessed for the applied significance (98).

Investigators that raise $FICO_2$ for hypercapnic hypoxia protocols (e.g. $PaCO_2$ +5 mmHg from baseline) presumably do so to prevent a possible confounding influence of central-peripheral interaction (95). This forms the basis of initiating hypoxic rebreathing

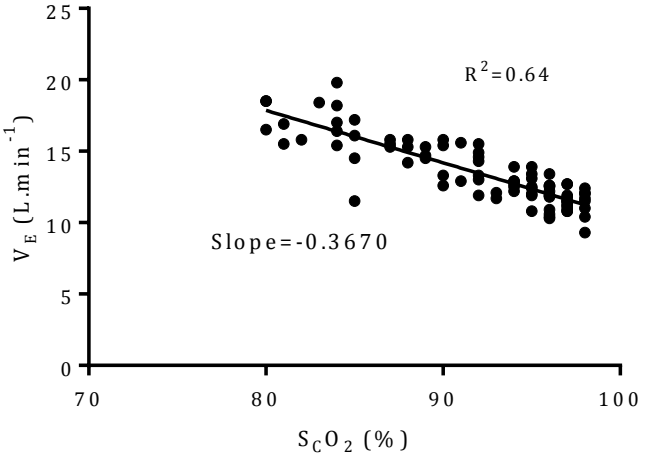
challenges with hyperoxia ($P_{ET}O_2$ 150-250 mmHg) to "silence the peripheral chemoreflex response to CO_2" as previously discussed (82).

Currently there is no consensus on defining the test end-tidal or arterial $PaCO_2$ during a hypoxic challenge. If there is a gold standard, it is to measure the AHVR at varying $PaCO_2$ levels that requires multiple tests. It is generally accepted however that the AHVR should commence at a normoxic baseline level (95).

Expression of the response

It is challenging to describe progressive hypoxic sensitivity as the $\dot{V}_E$ response to $P_{ET}O_2$ is typically hyperbolic rather than linear. However, when the response is expressed as a saturation (rather than $P_{ET}O_2$), it can be considered a linear function (99). It is important to calculate saturation from $P_{ET}O_2$ (S_CO_2), as Steinback and Poulin demonstrated that erroneous calculations of the AHVR were made when using pulse oximetry (SpO_2). There is a significant discrepancy between ScO_2 and SpO_2 at the time of peak V_E. Contributory factors includes the temporal delay to the periphery, and confounding factors of the oxygen dissociation curve (e.g. pH and temperature as with the Bohr effect) where oximetry is tested on a digit (100). S_CO_2 represents the true chemoreceptor response and is less affected by other physiological artefacts.

In healthy normal subjects, Godfrey and colleagues found the correlation coefficient of the reciprocal regression lines for $\dot{V}_E$ and S_CO_2 in progressive hypoxia to be significant, using least squares method for linear regression (94). Therefore, similarly to the HCVR, a linear response curve is plotted from $\dot{V}_E$ and S_CO_2, where the negative slope "m" is representative of the response (Figure 4.5).



$\dot{V}_E$; minute ventilation, S_CO_2; calculated oxygen saturation

Figure 4.5. An example of the expression of the hypoxic ventilatory response in a healthy subject. *Slope is the linear regression of data points.*

As per the HCVR, the assessment of ventilatory components (V_T and RR) may reveal specific adaptations to hypoxia and how these adaptations may differ between individuals. Ventilation increases both to significant increases in V_T and RR during isocapnic hypoxia however during poikilocapnic hypoxia the increase is primarily due to an increase in V_T (98), in keeping with an attenuated $\dot{V}_E$ response.

4.3.4. Sympathetic/cardiovascular response to progressive hypoxia

The neuronal control of ventilation and HR are closely linked, both anatomically and functionally. This is known as cardiorespiratory coupling. Autonomic cardiovascular control also resides within the brainstem and it has been proposed that respiratory sinus arrhythmia (RSA) is caused by the same neural processes that control breathing (discussed further in Chapter 8.1.1) (101). Certain disease states can disrupt this coupling, resulting in dysautonomia. Proposed mechanisms range from developmental immaturity of cardiorespiratory physiologies, to brainstem abnormalities (possibly Parkinson's disease) to sympatho-vagal imbalance (101).

Much work has been done on the observation of an increase in central nervous system gain during chronic and intermittent hypoxia; a phenomenon known as "sympathetic activation". As one example, chronic intermittent hypoxia, such as experienced with

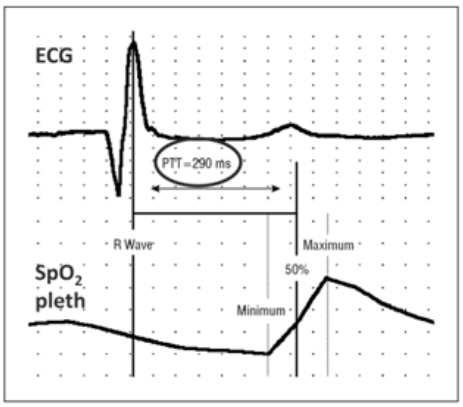
obstructive sleep apnoea (OSA), leads to an increased hypoxic ventilatory sensitivity, an increase in sympathetic activity and increased blood pressure (BP) (73). With acute exposure to high altitude, healthy travellers experience sympathetic excitation, with an increase in cardiac output primarily via HR (102). A study of HR variability following ascent to 4300 m, demonstrated a dominance of sympathetic activity and concurrent decrease in parasympathetic activity in healthy subjects (103).

During acute, progressive hypoxia, Ainslie and colleges presented a strong association between ventilation, cerebral blood flow and BP, irrespective of $FICO_2$ (96). They suggest this interaction accounts for the inter-subject variability in ventilation during progressive hypoxia.

4.3.5. Pulse transit time

Pulse transit time (PTT) is a measure of vascular tone and allows beat-by-beat, non-invasive estimation of systolic BP (SBP). It has been used primarily in Sleep Medicine as a cardiovascular marker in association with arousals and respiratory disturbances (104) but PTT may also be a useful, objective measure of autonomic function during progressive hypoxia.

PTT refers to the time taken for a systolic pulse wave to travel between two arterial sites. The speed at which the arterial pressure wave propagates has been said to be directly proportional to SBP changes (105-107). As an acute rise in SBP causes vascular tone to increase, the arterial wall stiffens, the pressure wave magnitude increases, and PTT decreases. The reverse is seen with a reduction in SBP. PTT is calculated as the time from the R wave in an electrocardiograph (ECG) (central) to a peripheral measure of oximetric photoplethysmography (the arrival of the pulse wave at the periphery). This measurement is typically taken as the point on the pulse waveform that is 50 % of the height of the maximum value (Figure 4.6) (108).



ECG; electrocardiography, PTT; pulse transit time, S_pO_2 pleth; pulse oximetry

Figure 4.6. Calculation of pulse transit time. *Modified from Bradley* (108).

As PTT is a derived measure, it is more useful/appropriate as an assessment of the changes relative to baseline, rather than a surrogate directly for SBP in a clinical setting (107). It reflects spontaneous fluctuations in SBP attributable to autonomic nervous system influences; i.e. PTT decreases with predominate sympathetic activity, and increases with predominate parasympathetic activity. As SBP is dependent on both vascular function and ventricular contraction, PTT is a composite measure of both vascular and cardiac activity. PTT is measured beat-to-beat, is non-invasive with subjects unaware of measurement, unlike sphygmomanometery or by Finapres.

Measuring BP and HR during progressive hypoxia can give insight into vasomotor activity and importantly, are associated with clinical outcomes. As hypoxia typically excites the ANS – these markers can be considered reflective of SNS activity.

Limitations of PTT

PTT measured with a single peripheral site does not correct for the pre-ejection period. This reflects the time taken between depolarisation of the heart and opening of the aortic valve (106, 107), or the onset of electrical cardiac activity and the start of mechanical ventricular ejection. This period is comprised both of the electromechanical delay and the period of isovolumetric contraction, and is not constant. It has been suggested that the pre-ejection period can account for approximately 12 % of PTT in humans. However Ochiai and colleagues showed that both PTT corrected and not corrected for the pre-ejection period, correlated linearly with changes in BP (106).

There have been age related observations of increased SBP due to decreased proximal aortic compliance. Generally, large conduit arteries (proximal elastic, less muscular) lose their compliance with age more so than muscular peripheral arteries. In keeping with this, Cameron and colleagues found no association in pulse wave velocity (aorta to the finger) and age in healthy normals (109) suggesting that PTT is unlikely to be affected by age.

4.3.6. Previous normative data and individual variability

Previous normative data demonstrates a large individual variation in both the HCVR and AHVR. In HCVR studies, the $\dot{V}_E$ "m" predominantly lies in the range of 2-5 $L.min^{-1}.mmHgP_{ET}CO_2^{-1}$. P_{100} normative data during the HCVR had been less studied, however "m" lies in the range 0.2-0.6 $cm.H_20.mmHgP_{ET}CO_2^{-1}$ (Table 4.1).

Table 4.1. Previously reported hypercapnic ventilatory and occlusion pressure responses in healthy normal subjects.

Author, year (reference #)	N = (male:female)	$\dot{V}_E$ $L.min^{-1}.mmHgP_{ET}CO_2^{-1}$	$P_{ET}CO_2$ at $\dot{V}_E = 0$	P_{100} $cmH_2O.mmHgP_{ET}CO_2^{-1}$
Read, 1967 (77)	21 (16:5)	2.65 (1.16-6.18)	41.7 (34.6-48.6)	-
Whitelaw, 1975 (89)	10 (10:0)	3.36 (2.0-4.7)	43.4 (37.8-50.1)	0.37 (0.12-0.64)
Hirshman, 1975 (110)	44 (40:4)	2.69 (1.00-5.95)	33.1 (27.9-45.0)	-
Sahn, 1977 (81)	8 (6:2)	4.74 (3.5-5.54)	-	-
Manning, 1992 (111)	8 (8:0)	2.95 (2.48-3.68)	-	0.37 (0.14-0.67)
Lin, 1998 (112)	7 (7:0)	1.93	-	0.51

Data are mean (range) unless otherwise stated, $\dot{V}_E$; minute ventilation, P_{100}; occlusion pressure, $P_{ET}CO_2$; pressure of end tidal carbon dioxide, N; subject number included in study.

It is difficult to generalise the "typical" normal AHVR response because of the variation in methods of assessment as described. Intra-subject assessment and group responses to an intervention require precise protocol replication.

The HCVR and AHVR can be affected by sleep, circadian rhythm, age, genetic, personality and environmental factors, and work of breathing. Inter-subject variation is significant and is driven by a range of these factors, but intra-subject variability is relatively low when exposed to standardised subject environmental and physiological conditions (87, 95, 113).

Intra-individual variation in AHVR and HCVR is further reduced when repeat testing is performed on the same day as compared to different days (81). This is related to daily and diurnal physiological changes such as metabolic rate, circadian rhythm and progesterone levels in females. No significant systematic change in mean HCVR or AHVR occurs over a 2 h period (81), with a large effect of circadian rhythm on the HVCR (114), thus individual variation can be minimised by testing on the same time of day and particularly by performing sequential measurements within 30min -2 h (81). An important benefit of performing the test within a short time frame on the same day is that other subjective factors that may affect ventilatory response are also minimised. These include technical, emotional and environmental variation that can significantly alter both inter- and intra-subject variability (81, 95, 115). This suggests a lack of a training effect or an interaction between sequential measurements of ventilatory response.

It would be feasible to expect that age may affect the ventilatory response to hypoxia and hypercapnia as age results in a decline in function at many levels of the neuromechanical link between chemosensors, brain stem and ventilatory pump. Generally across many studies however, the ventilatory response is marginally affected at old age, which suggests that the chemoreceptor function remains unaltered (116, 117).

4.3.7 Specific methodological considerations for the current study

The method needed to be robust, to be easily replicated, and also able to be performed by subjects that have some physiological limitation (as with disease of the brain stem – Parkinson's disease) and also subjects that have potentially high subjective influences (i.e. freedivers). The decision on the chosen method was made in consultation with Peter Rogers, Senior Scientist, Concord Hospital.

Following the review of the literature as above, it was decided to minimise subject discomfort and subjective influences by omitting prior hyperventilation and initiating both rebreathing tests with medical air. Therefore the initial breakpoint in the $\dot{V}_E$ response was not calculated. The initial volume of the rebreathing bag was adjusted based on each subject's vital capacity so that the test would be completed within 10 min

(estimated) to avoid the contribution of HVD in the AHVR and to elicit a linear response in the HCVR.

To allow assessment of the effect of pharmacological intervention, it was decided not to control the $FICO_2$ during AHVR; allowing $P_{ET}CO_2$ to vary naturally (poikilocapnia). It has been demonstrated that poikilocapnic hypoxia elicits similar (relative) associations between ventilation and sympathetic markers as hyper- and isocapnic hypoxia (96). FIO_2 was to be kept constant during the HCVR rather than initiating the breathing with hyperoxia and allowing FIO_2 to vary.

The measurement of P_{100} during HCVR was included to assess both peripheral and central involvement. HR and single-site PTT were measured during the AHVR to determine the utility of these markers as a measure of autonomic function. This was also in consideration of determining the response in subjects that may be significantly influenced by subjective factors (i.e. freedivers with familiarity and control of sensations of hypercapnia and hypoxia). These markers are monitored continuously, are non-invasive and are collected without subject awareness.

The finalised methodological procedures are comprehensively described below.

4.4 Study protocol procedure

4.4.1. Equipment and maintenance

Testing was conducted within the Exercise Laboratory, Department of Thoracic Medicine, Concord Hospital (50 m elevation above mean sea level [7th floor]). Ventilation was continuously sampled breath-by-breath for gas concentration and flow using a cardiopulmonary exercise system including a mass flow sensor. P_{100} was measured using a pressure transducer while the airways were occluded (VMax Encore, Sensormedics, Yorba Linda, CA) (Figure 4.7a). Finger probe SpO_2 (Nonin, Plymouth, MN) and 5-lead ECG (Alice PDx, Philips Respironics, Murrysville, PA) were measured continuously for the determination of HR and PTT (Figure 4.7b).

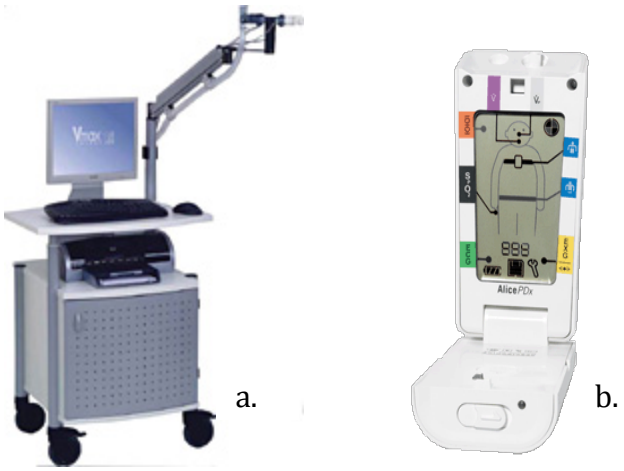


Figure 4.7. Cardiopulmonary exercise system for continuous gas analysis (a) and device for continuous collection of electrocardiography and pulse oximetry for the determination of pulse transit time (b).

The cardiopulmonary exercise system was calibrated immediately prior to each test, with current ambient temperature, barometric pressure and humidity entered into the calibration programme. Calibration included variable flow rates (3 L [±0.4 %] syringe, Hans-Rudolph, Kansas City, MO) and a 2-point gas calibration (16 % O_2, 4% CO_2 and 26 % O_2, 0 % CO_2, BOC gases, Australia). A biological control (myself, LS) was tested on testing days for respiratory flow and volume, and monthly for steady state oxygen uptake ($\dot{V}O_2$), carbon dioxide uptake ($\dot{V}CO_2$) and $\dot{V}_E$ at 50, 75 and 100 watts. A variation of < 9 % in $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ was considered acceptable. Following the rebreathing tests, a verification of the current gas analyser calibration was conducted, which was acceptable for all subjects included in the analysis. All tests and subsequent analysis was conducted by myself (L.S.), data collection was performed with the assistance of Peter Rogers, Respiratory Scientist, Concord Hospital.

4.4.2. Subject preparation

Subjects were asked to refrain from caffeine and heavy exercise prior to testing. Upon arrival, the procedures were explained to the subject with written consent obtained. The subject was set-up with a 5-lead ECG (Figure 4.8) and pulse oximeter probe placed on the index finger. After verification of signals, the leads were tidily arranged - attached to a neck strap so that the subject was comfortable.

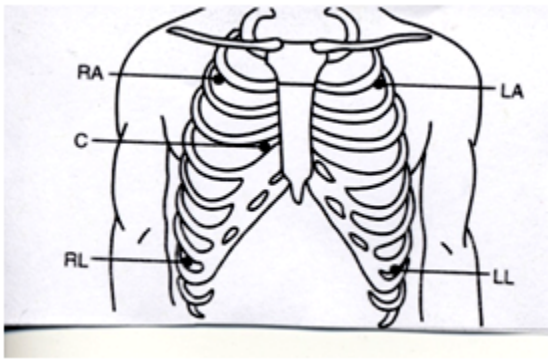


Figure 4.8. Placements for 5-lead electrocardiography.

The subject instruction was consistent. Firstly, the subject was familiarised with the methods and the breathing circuit. The circuit was situated so that they could look out of a large bright window with a pleasant view of the surrounding suburbs. The subject was taught the "thumbs up" (yes), "thumbs down" (no) method to communicate. While at a comfortable seat height the subject was then instructed to relax, look at the view and "breathe naturally" on the mouthpiece. The room was kept quiet and the instruction was somewhat "vague" to minimise subjective stimuli. Without being too descriptive, the subject was told that they might feel "air hunger" or feelings or claustrophobia.

Prior to all tests, the subject was allowed to rest, while seated breathing room air for at least 20 min. The subject initially entered the circuit on the mouthpiece and breathed room air (with nose clip in situ) until respiratory exchange ratio (RER) was below 0.9 and $\dot{V}O_2$ was below 4 $mL.min^{-1}.kg^{-1}$; reflecting resting basal metabolism (4-8 min) (118). Resting parameters were considered as the average of the last 2 mins breath-by-breath and beat-to-beat data while breathing room air prior to the initiation of the rebreathing protocol. The resistance of both circuits as described below were measured at less than 2 cmH_2O using a manometer at normal tidal flows.

4.4.3. Progressive isoxic hypercapnia (iHCVR)

As described by Ruppel (88), the subject re-breathed on a closed circuit consisting of a 20 L gas bag (Erich Jaeger GmbH, Höchberg, Germany) connected via a large bore two-way tap* (Collins, Braintree, MA) until the fraction of end tidal CO_2 exceeded 8 % or until volitional fatigue. *The tap enables the subtle switch from breathing room air to the rebreathing circuit. The rebreathing bag was filled with medical air with consideration

of the subject's vital capacity so that time of test would not exceed 10 min to allow a linear increase in $FICO_2$.

During rebreathing, small aliquots of 100 % O_2 were added to the circuit to maintain a FIO_2 near 0.21. This was monitored with an online, continuous display. P_{100} was collected, following at least three tidal breaths at a stable FRC. P_{100} was recorded randomly (from breath 1 to 6 following) via an occlusion valve (Figure 4.9).

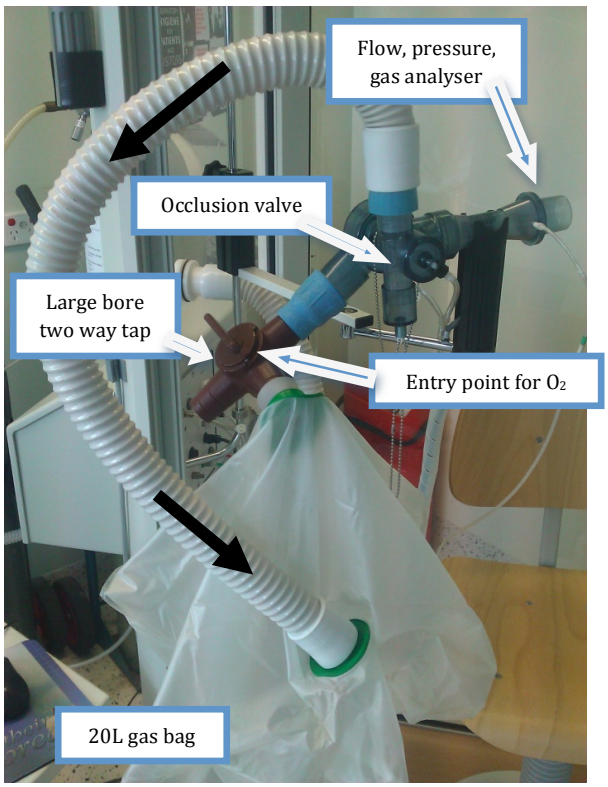


Figure 4.9. Rebreathing circuit for the hypercapnic ventilatory response. *Arrows indicate direction of gas flow.*

Data analysis

For each subject $\dot{V}_E$ and P_{100} were plotted in the range above 45 mmHg $P_{ET}CO_2$. Individual responses of $\dot{V}_E$ and P_{100} to $P_{ET}CO_2$ were assessed using Pearson's correlation. If a significant correlation was evident, the responses were described using linear regression. When parameters did not correlate with $P_{ET}CO_2$ ($P>0.05$), the response was not included in the analysis. The slope "m" of the linear regression was recorded for each response. Any artefact or significant outliers were omitted (Figure 4.2, 4.4).

4.4.4. Progressive poikilocapnic hypoxia (pHVR)

As described by Fan and colleagues (99), subjects rebreathed on a closed circuit that consisted of a non-rebreathing valve (T-shape 2600, Hans Rudolph Inc, Kansas City, MO) connected to a 20 L gas bag (Erich Jaeger GmbH, Höchberg, Germany) via large bore tubing. CO_2 and moisture were scavenged using soda lime and silica gel respectively (Collins, Braintree, MA) (Figure 4.10).

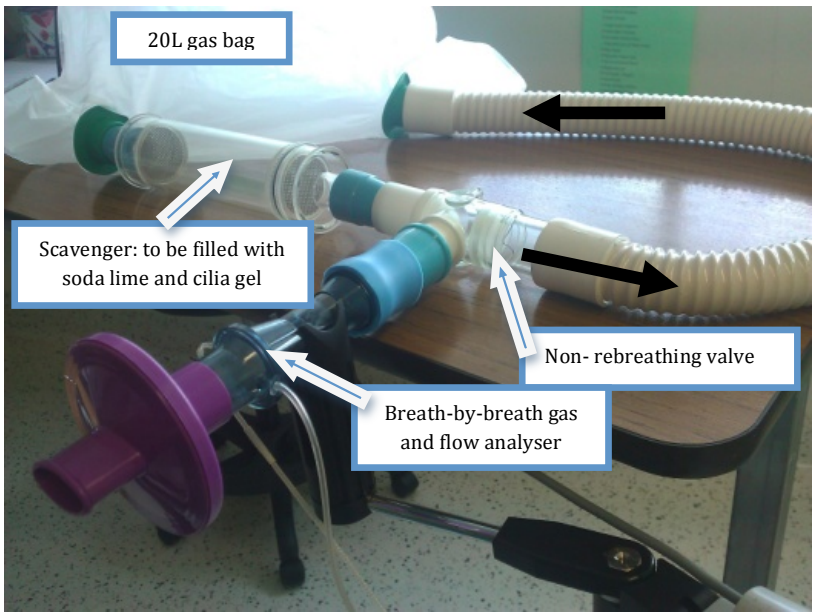


Figure 4.10. Rebreathing circuit for the hypoxic ventilatory response. *Arrows indicate direction of gas flow.*

Testing was terminated when $P_{ET}O_2$ fell below 43 mmHg. $P_{ET}CO_2$ was not manipulated and allowed to vary naturally (poikilocapnia). The rebreathing bag was filled with medical air with consideration of the subject's VC to allow a linear reduction in $P_{ET}O_2$, so that time of test would not exceed 10 min (Figure 4.11).

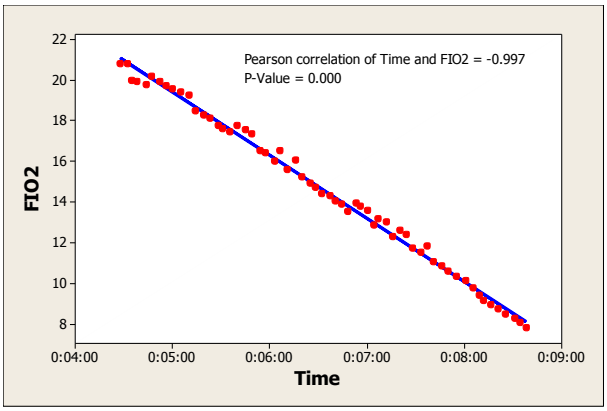


Figure 4.11. The typical linear fall in FIO_2 using the described rebreathing circuit method.

Five-lead electrocardiography and finger probe SpO_2 were concurrently measured for the determination of PTT and HR. The time-of-day on the PTT device was synchronised with the cardiopulmonary exercise system prior to data collection.

Data analysis

Beat-to-beat PTT was derived (post-processing) from ECG and SpO_2 plethysmograph pulse wave (Figure 4.12) as a continuous digital trace that was uploaded to a statistics programme (excel). PTT and HR data points were time matched with gas analysis data and plotted against ScO_2 (calculation described below). Significant outlier values and artefact were manually eliminated (PTT < 200ms, PTT> 400ms) (119).

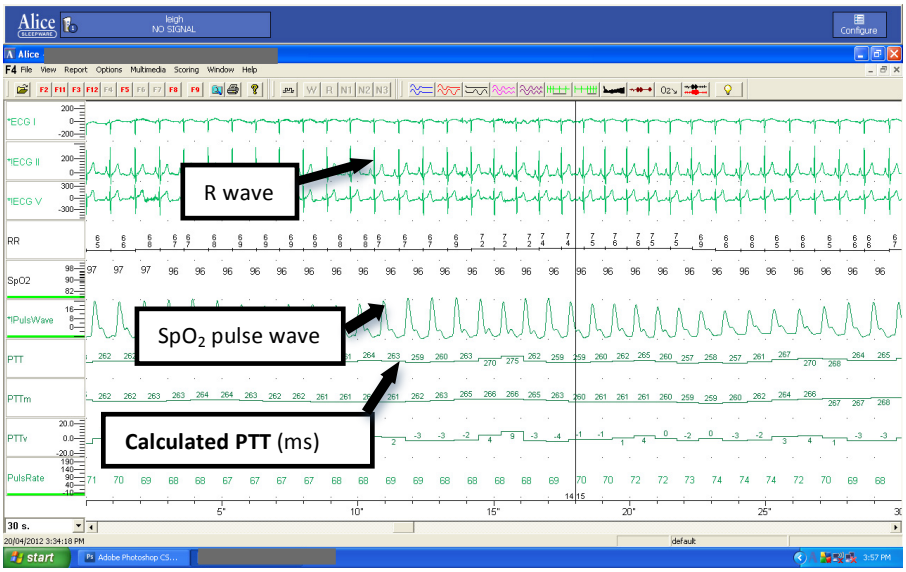


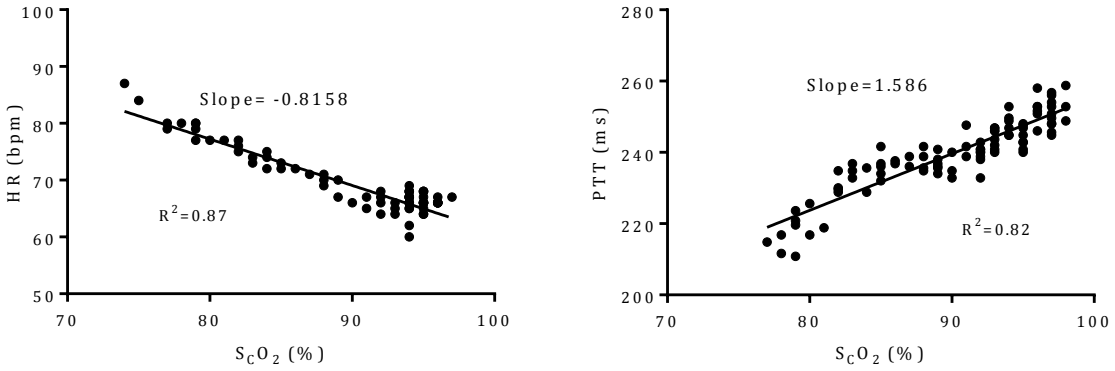
Figure 4.12. An example of calculated pulse transit time (PTT) from the R-wave of the electrocardiograph to the corresponding pulse wave from finger pulse oximetry (SpO_2) in a healthy subject.

To express $\dot{V}_E$ and PTT changes as a linear function of the hypoxic stimulus, oxygen saturation was calculated from breath-by-breath $P_{ET}O_2$ (S_CO_2) using the calculation from Severinghaus (120) in a statistics programme:

$$S_CO_2 = \left[(P_{ET}O_2^3 + 150P_{ET}O_2)^{-1} x\ 23{,}400 + 1\right]^{-1} \text{ x } 100$$

$P_{ET}O_2$ was substituted for PO_2 from Severinghaus's original equation. $P_{ET}O_2$ is essentially P_AO_2, strongly reflective of PaO_2 (85). This method is currently used in the literature for its ability to plot breath-by-breath oxygen saturation non-invasively against ventilation (96, 99).

Individual responses of $\dot{V}_E$, HR and PTT to changing S_CO_2 were assessed using Pearson's correlation. If a significant correlation was evident, the responses were described using linear regression. When parameters did not correlate with S_CO_2 (P>0.05), the response was not included in the group analysis. $\dot{V}_E$, PTT and HR were plotted against S_CO_2 and linear response curves were generated for each response (Figure 4.5, 4.13) and the slope "m" recorded.



S_CO_2; calculated oxygen saturation

Figure 4.13. An example of the expression of the heart rate (HR) and pulse transit time (PTT) response to progressive hypoxia in a healthy subject. *Slope is the linear regression of data points.*

4.5 Protocol validation in healthy normals

4.5.1. Aim

The aim was to determine the normal response of the chosen methods for iHCVR and pHVR. A broad range of subjects were sourced to identify any age effect. The utility of PTT and HR as a measure of sympathetic/cardiovascular response and their connection to ventilation was investigated.

4.5.2. Methods

Subjects

Healthy normal subjects were recruited from staff and associates from Concord Hospital and the Woolcock Research Institute. Subjects were excluded if they had any clinically significant respiratory or cardiovascular disease, neurological disease, a significant smoking history (>10 pack years), structural abnormalities of the chest wall, recent upper respiratory tract infections or claustrophobia.

Respiratory function

Spirometry was measured according to American Thoracic Society/ European Respiratory Society criteria (29). Predicted values were derived from the recommendations of the European Community for Coal and Steel (64).

Subjects attended the laboratory on a single visit. Following spirometry, the two rebreathing tests were performed at least one hour apart. The order of tests was not controlled.

The study was reviewed and approved by the Human Ethics Review Board of the Sydney Local Health District (New South Wales, Australia). Each subject gave written informed consent.

4.5.3. Results

24 (eight female) healthy normal subjects were recruited. Subject demographics are presented in Table 4.2.

Table 4.2. Subject demographics for 24 healthy normals.

	Mean (SD)	Range
Male:female	17:7	
Age *years*	42 (15)	24-71
Height *cm*	173 (10)	160-192
Weight *kg*	72 (13)	52-105
SBP *mmHg*	125 (6)	115-130
DBP *mmHg*	75 (9)	60-85

SBP; systolic blood pressure, DBP; diastolic blood pressure

Spirometry parameters are presented in Table 4.3. All values were above the lower limit of normal.

Table 4.3. Spirometry in 24 healthy normals.

	Healthy normal	Range
Male:female	17:7	
FEV_1 *L*	3.68 (0.89)	2.36-5.37
FEV_1 *% predicted*	105 (13)	83-135
FVC *L*	4.62 (1.20)	2.98-7.47
FVC *% predicted*	109 (14)	89-141
Ratio *%*	81 (8)	64-99

Data are mean (SD). FEV_1; forced expiratory volume in one s, FVC; forced vital capacity.

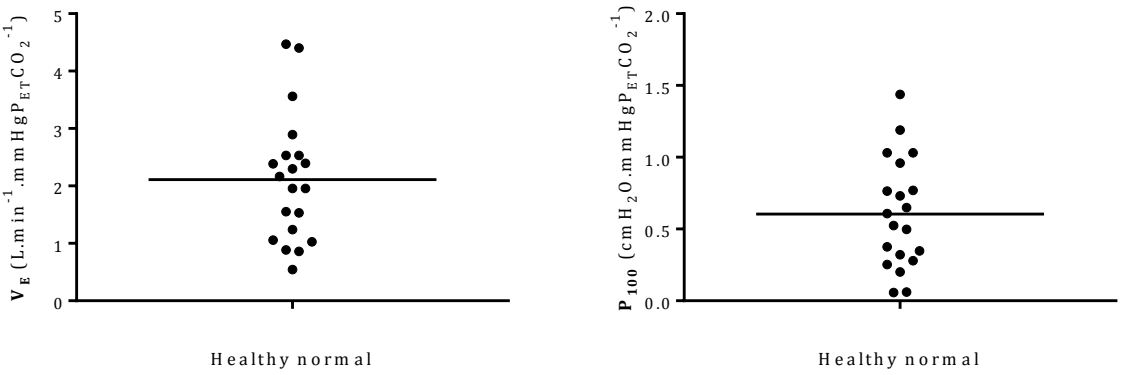
Progressive isoxic hypercapnia (iHCVR)

Three subjects did not perform the hypercapnic challenge due to other commitments, and one subject's ventilatory response was non-interpretable (non-significant Pearson's correlation) that appeared to be due to subjective influences. Therefore, 20 (7 female) subjects were included in the analysis.

The iHCVR in the healthy normal subjects was 2.11 (1.11) $L.min^{-1}.mmHgP_{ET}CO_2^{-1}$ [range 0.5-4.5], with a P_{100} response of 0.6 (0.4) $cmH_2O.mmHgP_{ET}CO_2^{-1}$ [range 0.1-1.4]. The x-intercept ($P_{ET}CO_2$ when V_E=0) was 38.0 (4.9) mmHg [range 22.5-43.2]. Individual data

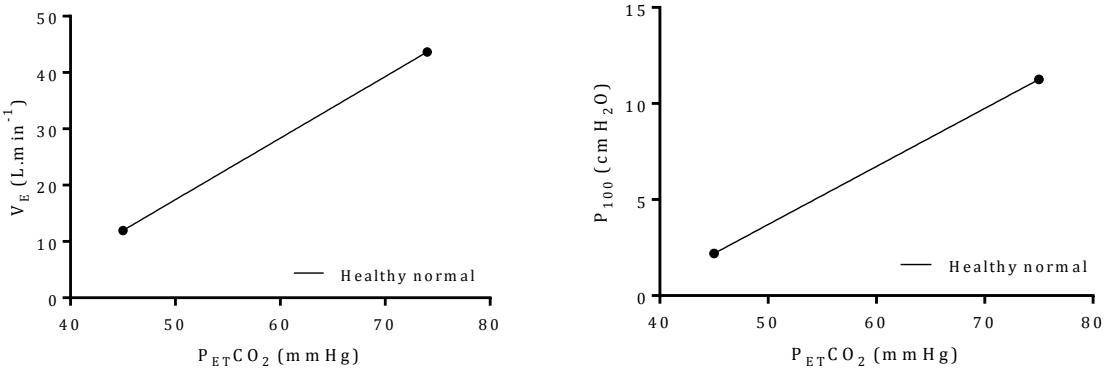
points are presented in Figure 4.14. The group mean of the individual linear regression at 45 and 70 mmHg$P_{ET}CO_2$ for $\dot{V}_E$ and P_{100} are presented in Figure 4.15.

There were no associations between $\dot{V}_E$ and P_{100} response and age (P= 0.9 and 0.9 respectively).



$P_{ET}CO_2$; pressure of end-tidal CO_2

Figure 4.14. Mean (bar) and individual regression slopes for minute ventilation ($\dot{V}_E$) and occlusion pressure (P_{100}) during progressive hypercapnia in 20 healthy normals.



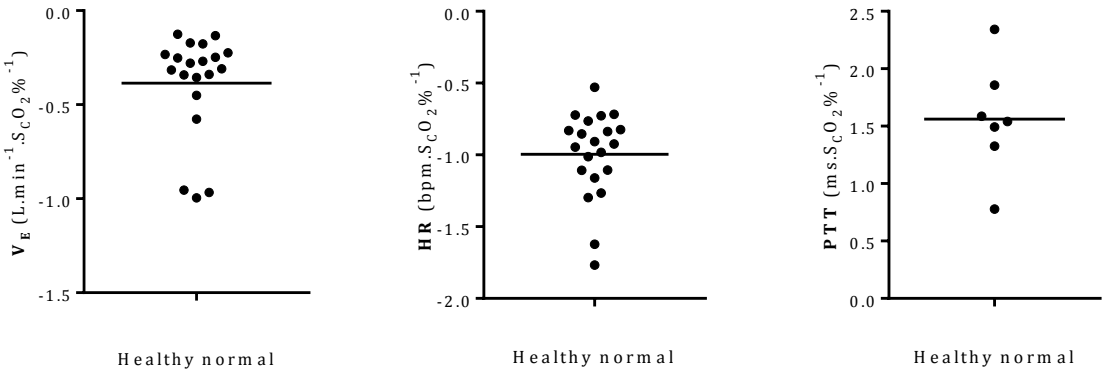
$P_{ET}CO_2$; pressure of end-tidal CO_2

Figure 4.15. The group mean of the individual linear regression at 45 and 70 mmHg$P_{ET}CO_2$ for minute ventilation ($\dot{V}_E$) and occlusion pressure (P_{100}) during progressive hypercapnia in 20 healthy normals.

Progressive poikilocapnic hypoxia (pHVR)

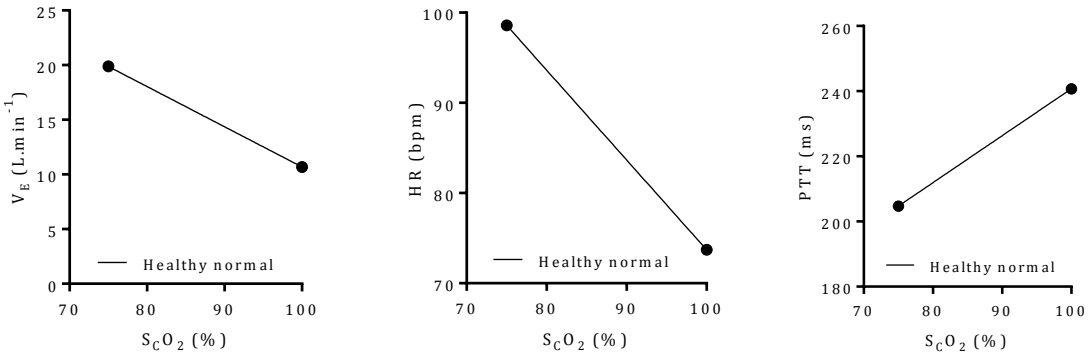
Two subjects did not perform the hypoxic challenge due to other commitments, therefore the responses of 22 (6 female) healthy normal subjects were analysed. Two subjects ventilatory data was non-interpretable (non-significant Pearson's correlation), that appeared to be due to subjective influences and was thus excluded from the $\dot{V}_E$ analysis [20 (6 female) subjects]. Due to a change in protocol, the implementation of PTT measurement was introduced after 10 subjects had already been studied; therefore this analysis included 12 (2 female) subjects.

The pHVR in the normal group was -0.39 (0.27) $L.min^{-1}.\%S_CO_2^{-1}$. HR and PTT response was -1.00 (0.30) $bpm.\%S_CO_2^{-1}$ and 1.56 (0.48) $ms.\%S_CO_2^{-1}$ respectively. Individual data points are presented in Figure 4.16. The group mean of the individual linear regression at 75 and 100 S_CO_2% for $\dot{V}_E$, HR and PTT are presented in Figure 4.17.



S_CO_2; calculated oxygen saturation. Horizontal bars indicate mean values. *N=20 $\dot{V}_E$, 12 PTT*

Figure 4.16. Mean (bar) and individual regression slopes for minute ventilation ($\dot{V}_E$), heart rate (HR) and pulse transit time (PTT) during progressive hypoxia in 22 healthy normal subjects.



S_CO_2 calculated oxygen saturation. *N=20 $\dot{V}_E$, 12 PTT*

Figure 4.17. The group mean of the individual linear regression at 75 and 100 S_CO_2% for minute ventilation ($\dot{V}_E$), heart rate (HR) and pulse transit time (PTT) during progressive hypoxia in 22 healthy normals.

There were no significant associations between age and $\dot{V}_E$, HR and PTT response (P=0.77, 0.36, 0.89 respectively). A strong correlation between the $\dot{V}_E$ and HR response to S_CO_2% was seen (R^2= 0.64, P<0.0001) (Figure 4.18). There was no association between PTT and $\dot{V}_E$ (P=0.92) or HR (P=0.53) response.

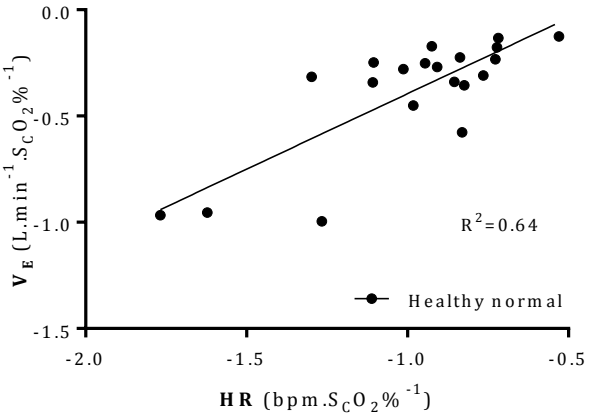


Figure 4.18. The relationship between ventilation ($\dot{V}_E$) and heart rate (HR) response to calculated oxygen saturation (S_CO_2) in 20 healthy normal subjects.

4.6. Conclusion

The protocols presented in this Chapter were generally well tolerated. There was one non-interpretable $\dot{V}_E$ response during hypercapnia (5 %) and two non-interpretable $\dot{V}_E$

responses during hypoxia (9 %) that appeared to be due to subjective influences. There were no significant symptoms reported during or following any tests. No tests were terminated before pre-determined physiological criteria were met.

The normative data for the iHCVR protocol was similar to previous HCVR studies (Table 4.4).

Table 4.4. Previously reported hypercapnic ventilatory and occlusion pressure responses in healthy normal subjects, including the current data.

Author, year (reference #)	N = (male:female)	$\dot{V}_E$ $L.min^{-1}.mmHgP_{ET}CO_2^{-1}$	$P_{ET}CO_2$ at $\dot{V}_E = 0$	P_{100} $cmH_2O.mmHgP_{ET}CO_2^{-1}$
Read, 1967 (77)	21 (16:5)	2.65 (1.16-6.18)	41.7 (34.6-48.6)	-
Whitelaw, 1975 (89)	10 (10:0)	3.36 (2.0-4.7)	43.4 (37.8-50.1)	0.37 (0.12-0.64)
Hirshman, 1975 (110)	44 (40:4)	2.69 (1.00-5.95)	33.1 (27.9-45.0)	-
Sahn, 1977 (81)	8 (6:2)	4.74 (3.5-5.54)	-	-
Manning, 1992 (111)	8 (8:0)	2.95 (2.48-3.68)	-	0.37 (0.14-0.67)
Lin, 1998 (112)	7 (7:0)	1.93	-	0.51
***Seccombe, 2013** (9)*	***20 (13:7)***	***2.11 (0.55-4.47)***	***38.0 (22.5-43.2)***	***0.6 (0.1-1.4)***

Data are mean (range) unless otherwise stated, $\dot{V}_E$; minute ventilation, P_{100}; occlusion pressure, $P_{ET}CO_2$; pressure of end tidal carbon dioxide, N; subject number included in study.

The progressive, linear reduction in S_CO_2 elicited a similarly linear reduction in PTT and increase in HR. This suggests a positive utility of PTT and HR as a measure of autonomic function during hypoxia. While two subjects $\dot{V}_E$ response during pHVR were non-interpretable, the PTT and HR response were linear and within 1SD of the group response. This suggests that PTT and HR were less influenced by subjective influences than $\dot{V}_E$. In keeping with previous observations, no age effect was seen in $\dot{V}_E$, HR or PTT response in the pHVR and $\dot{V}_E$ and P_{100} in the iHCVR (109, 116, 117).

CHAPTER 5

VENTILATORY CONTROL IN ELITE FREEDIVERS

5.1 Background

Chapters 2 and 3 investigated the acute and long-term effects of participation in freediving on resting respiratory function and structure. The analysis related to the effect of extreme intrathoracic pressure experienced both at the surface with glossopharyngeal insufflation (GI), and during descent with hyperbaric compression. There was no evidence of any adverse peripheral physiological consequences.

Competitive, elite freedivers are unique in their ability to repeatedly endure significant hypoxia and hypercapnia. The repeated voluntary suppression of ventilation has the potential to affect ventilatory control and cardiac autonomic function. This Chapter seeks to determine whether freediving participation and its associated training have any effect on central ventilatory and autonomic responses.

5.1.1. The effect of freediving on arterial blood gases

The current world record for "static apnoea" (immersed breath-hold time) is 11:30 min and assisted ocean depth ("no limits") is 214 m, a dive that takes 4-5 min to complete (41). These extended apnoea's, with or without physical exertion, have significant consequences on arterial blood gas tensions.

There have been several studies that have collected either end-tidal or arterial blood gases' at the completion of freediving efforts. Following immersed apnoea's averaging 5:00 min, end-tidal gas analyses have demonstrated an average P_AO_2 of 27 mmHg (n=9) (121) and 25 mmHg (n=7) (49). P_ACO_2 was 38 and 56 mmHg respectively. Muth and colleagues collected arterial samples from two divers that were submersed within a pressurised water tank simulating 3 atm, or 20 m depth. After being submersed for 4:30 min, they reported a similar level of hypoxaemia with a mean PaO_2 of 30 mmHg and $PaCO_2$ of 43 mmHg (122). Andersson and colleagues also took arterial samples following apnoea of up to 6:00 min in nine freedivers. PaO_2 was significantly reduced to 28 mmHg, with a $PaCO_2$ of 45 mmHg (123).

Interestingly, these levels of arterial oxygen pressure were similar to those that have been collected near the summit of Mt Everest, and are considered to be at the limit of human tolerance. Arterial samples from five acclimatised climbers at 8400 m demonstrated a mean PaO_2 of 25 mmHg however with significantly lower $PaCO_2$ of

13 mmHg (124). This is attributed to the significant hyperventilation from the hypoxic ventilatory response (HVR) and this difference in $PaCO_2$ highlights the extent of voluntary suppression of ventilation that the divers achieve. Arterial oxygen saturation (SaO_2) at these significantly reduced arterial pressures of oxygen was calculated at ≈50 %.

5.1.2. Adverse events in freediving related to hypoxaemia

Adverse hypoxaemic events are common, both in training and in freediving competition. Freedivers often continue to breath-hold despite experiencing involuntary diaphragmatic contractions (43) and significant discomfort from rising $PaCO_2$. Therefore typical "warning" signs that trigger inhalation are ignored. Neurological events, colloquially known as "sambas", are commonplace. These events have not been described pathophysiologically. They appear to be pre-syncopal episodes with involuntary muscle contractions a feature (personal observations, LS) but whether they are primarily related to circulation, diffuse cerebral hypoxia or were epileptiform is unknown. The divers call them "sambas" as a reference to a macabre Latin American dance. A medical survey conducted at a world championship competition in 2004 reported 25 such "sambas" from 57 divers (10).

Syncopal events while the diver is still immersed are clearly serious and are associated with fatalities. These are known as "shallow water blackout" (35) because they generally occur within a few metres from the surface. During hyperbaric compression, PaO_2 is elevated, as high as 220 mmHg at 3 atm (122). Even as exercise in apnoea continues, this can preserve a gradient from circulation to tissue markedly depleting arterial oxygen content. As the diver ascends, moving to a more hypobaric environment, the combined effects of this depletion of oxygen stores and the loss of the compressive enhancement of tissue oxygenation gradient results in a dramatic, and exponential, reduction in PaO_2 and peripheral oxygen uptake. Therefore the greatest risk of syncope is just prior to reaching the surface. During the same survey as mentioned above, there were three reported episodes of immersed syncope (10). Recently, one of the research subjects in this research program experienced an immersed syncopal episode lasting more than 40 s during a record-breaking attempt (personal communication, LS). These individuals were otherwise healthy and symptoms generally subside quickly. Due to the common

occurrences of these events, freediving policy is to never dive alone and to be proficient at resuscitation and rescue techniques.

Those that travel to high altitude experience persistent cognitive impairment for many months due to cerebral hypoxaemia (125). There has been limited investigation into the long-term cognitive consequences of freediving. Andersson and colleagues reported elevated serum levels of the brain damage marker S100B in nine freedivers after simulating a "typical" training session (123). This included breath-holds of up to 6 min resulting in a mean PaO_2 of 28 mmHg. The authors suggest that these levels of hypoxaemia may possibly result in negative long-term cerebral effects. Two case reports similarly report a transient neurological disorder following freediving (126, 127).

5.1.3. Ventilatory control and sympathetic activation in patients with chronic intermittent hypoxia

Patients with untreated obstructive sleep apnoea (OSA) experience chronic intermittent hypoxaemia. It is well established that repeated alterations in blood gas tensions results in heightened sympathetic activity, leading to further physiological impairment. This includes endothelial dysfunction and arterial hypertension that is associated with an increase in cardiovascular morbidity and mortality (128, 129). Sympathetic activation in patients with OSA persists during the day (awake hours), despite arterial saturation returning to stable, normal levels. This is explained by an underlying alteration of the autonomic nervous system. An augmented chemo-reflex and attenuated baro-reflex have been shown to be the major reflex alteration for evoking persistent sympathetic excitation by intermittent hypoxia (73). In addition, there is an increase in the synthesis and release of various vasoactive hormones leading to vasoconstriction (73). The augmented arterio-chemoreceptor reflex and hyperactive carotid body function have a significant effect on ventilatory control. Typically, the response to hypoxia and hypercapnia in those that experience chronic intermittent hypoxia is augmented (130).

The HVR is the primary physiological compensation to a reduced pressure of inspired oxygen when travelling to high altitude. Andean Indians and Sherpas born and living at high altitude generally have blunted ventilatory responses to hypoxia (131, 132). While not a definitive risk factor, a reduced AHVR is associated with a higher incidence of high altitude pulmonary oedema (133).

5.1.4. Ventilatory control in breath-holding populations

The question is posed as to whether repeated bouts of voluntary apnoea have similar physiological consequences as with pathological or other environmental intermittent hypoxia.

The ventilatory responses to hypoxia and hypercapnia have been investigated previously in several breath-hold diving populations. These include competitive freedivers, underwater hockey players, synchronised swimmers, spearfishers and the "ama" pearl divers.

A study of 20 active "ama", who have similar diving patterns to spearfishers (Chapter 2), were studied by Song and colleagues who demonstrated a blunted response to varying CO_2, but no change in hypoxic response when compared to "normal housewives" (36). The "Funado" (assisted breath-hold divers of Japan) however have an attenuated response to both hypoxia and hypercapnia (134).

A reduced response to CO_2 has been identified in 34 underwater hockey players as compared to dry-land athletes (135). 10 synchronised swimmers demonstrated a blunted ventilatory response to hypoxia but with a normal hypercapnic response (136).

Delapille and colleagues found a reduced chemosensitivity to progressive hyperoxic hypercapnia in experienced "breath-hold divers" as compared to healthy non-divers but there was no change in occlusion pressure response (137), which typically represents central respiratory drive (discussed further in Chapter 4.2.2). This suggests a similar central ventilatory response in divers and non-divers. Thus a subjective influence (suppression) of ventilation by the trained breath-hold subjects cannot be discounted. With a normal occlusion pressure response, the reduced ventilatory response to hypercapnia could otherwise only be explained by peripheral, mechanical respiratory impairment. This is unlikely in healthy breath-hold divers. Grassi and colleagues have also reported a blunted response to hypercapnia, however the three freediving subjects studied were from the same family (138).

Walterspacher and colleagues found a reduced response to elevated steady-state CO_2 in freedivers as compared to previously studied healthy control subjects (57). They suggested that this reflected a trainable adaptation to hypercapnia. Similarly Ivancev

and colleagues reported a blunted ventilatory response to hypercapnia, however cerebrovascular circulation was preserved. The authors described this as being a protective mechanism against chronic intermittent hypoxia (139). Again, this also may reflect a possible subjective influence on minute ventilation due to their familiarly with the sensations of hypercapnia.

Dujic and colleagues included measures of sympathetic response in their studies of ventilatory control in spearfishers. They found no evidence of sympathetic activation as measured via muscle sympathetic nerve activity (MSNA) or altered response to progressive hypercapnia in 11 experienced spearfishers ("fish catchers") (140). This suggests that the repeated episodes of hypoxia experienced in competitive spearfishing were not sufficient to cause sympathetic activation in the absence of additional co-morbidities. They reported maximal apnoea duration of 4 min and diving depth of 32 m. This was more profound than our previously observed average apnoea duration of 1 min and depth of 20 m in 24 spearfishing subjects over a five-hour competition (Chapter 2) (6).

The same group also tested isocapnic hypoxia in what appeared to be the same spearfishing subjects (141). Hypoxia did not elicit any difference in ventilation or MSNA in this group, with normal baseline blood pressure and MSNA values.

While there was some inconsistency, the majority of previous literature suggests that breath-hold diving groups demonstrate blunted ventilatory response to progressive and steady-state hypercapnia. However, the limited evidence in relation to concurrent assessments of cardiovascular and sympathetic activity suggests that these responses were preserved. A plausible explanation for this is volitional control or override of the ventilatory response. Analysis of markers that were less affected by subjective factors during hypoxia and hypercapnia would elucidate as to whether impairment was due to volitional override or intrinsic deficits.

In health, the neuronal control of breathing and HR are closely linked, functionally as well as anatomically (101). This is vital for homeostatic regulation of blood gases and regulation of the central nervous system. Alterations in this function, or dysautonomia, can negatively affect cardiac health and/or respiratory control. This has been demonstrated during progressive hypoxia, with a strong association between

ventilatory response and cerebrovascular blood flow and blood pressure (96). It appears that concomitant analysis of these processes is required for comprehensive description of any possible adaptive alterations in ventilatory control.

Freedivers typically experience greater levels of hypercapnia and hypoxaemia than other breath-hold diving groups. Often, freedivers are originally spearfishers, and with an observed aptitude to the sport (and apnoea) continue on to competitive freediving, as discussed in Chapter 3.1.1. Their "aptitude" is most likely multi-factorial, one aspect of which may be a greater tolerance of hypercapnia. In the 1950's the sensitivity to CO_2 was used as a physiological selection test. It was observed that the "efficient" underwater swimmers and divers belonged in a group of slow, deep breathers with a high tolerance to increased CO_2 and lowered O_2 (87). This raises the question as to whether success in freediving is related to increased tolerance to hypercapnia and hypoxia, or whether the athletes' chemosensitivity is constitutively different from healthy normal subjects.

5.1.5 Aim

The aim of this study was to assess the ventilatory responses to progressive hypoxia and hypercapnia in elite, competitive freedivers. Cardiovascular parameters were concurrently measured as markers of the autonomic response. The responses were compared to healthy normal, non-diving subjects.

5.2. Methods

5.2.1 Subjects

Elite freediving subjects were recruited from the "Sydney Freedivers". The divers were in an advanced training state, in preparation for a national and international competition within months of testing. Personal best freediving statistics were documented (Appendix A ii). All subjects were members of the "145 Club" which are considered to be at a high level across several freediving disciplines (Chapter 3.1.1).

The subjects attended the Respiratory Function Laboratory, Concord Hospital, on a single occasion. The rebreathing tests were performed at least one h apart to allow basal metabolism to return to normal values. Subjects were asked to abstain from caffeine and had not participated in any freediving training practises prior to attending the

laboratory on the day of testing. Testing and analysis was performed by the same scientific officer (myself, L.S.).

For each freediving subject, two healthy normal subjects, matched for age (+/- 1 year), height and sex were selected from the 24 subjects that describe the healthy normal response presented in Chapter 4.4.

Subjects were excluded if they had any clinically significant respiratory or cardiovascular disease, a significant smoking history (>10 pack years), structural abnormalities of the chest wall, recent upper respiratory tract infections or claustrophobia.

5.2.2 Respiratory function

Spirometry and lung volumes measured by plethysmography were performed according to American Thoracic Society / European Respiratory Society guidelines (29, 63) (Vmax Encore, Sensormedics, Yorba Linda, CA). Predicted equations were derived from the recommendations of the European Community for Coal and Steel (64). The healthy normal subjects performed spirometry only.

5.2.3 Progressive isoxic hypercapnia (iHCVR)

A full description of method development, analysis and expression of data for the iHCVR is provided in Chapter 4.3.3.

5.2.4 Progressive poikilocapnic hypoxia (pHVR)

A full description of method development, analysis and expression of data for the pHVR is provided in Chapter 4.3.4.

Statistical analysis

Results are expressed as mean (SD) unless otherwise stated. Individual responses of $\dot{V}_E$, HR and PTT to changing S_CO_2 and individual responses of $\dot{V}_E$ and P_{100} to $P_{ET}CO_2$ were assessed using Pearson's correlation. If a significant correlation was evident, the responses were described using linear regression. When parameters did not correlate with S_CO_2 or $P_{ET}CO_2$ ($P>0.05$), the response was not included in the group analysis. Freediver responses were compared to the healthy normals using unpaired, two-tailed,

t-tests. A P value <0.05 was considered significant. $\dot{V}_E$ response was plotted against HR response in each group with linear response curves generated.

The study was reviewed and approved by the Human Ethics Review Board of the Sydney South West Area Health (New South Wales, Australia). Each subject gave written informed consent.

5.3 Results

5.3.1 Subjects

Five (1 female) elite freedivers and 12 (2 female) healthy normal subjects were studied. Demographics and personal best freediving statistics are presented in Table 5.1. There was no difference in age, height and weight between the groups (P>0.34).

Table 5.1. Subject demographics and competition statistics for 5 elite freedivers and 12 matched healthy normals.

	Freedivers	Matched normals	P value
Male:female	4:1	10:2	
Age *years*	32 (11)	33 (6)	0.82
Height *cm*	182 (10)	177 (10)	0.35
Weight *kg*	76 (11)	75 (15)	0.87
Static apnoea PB *min:s*	6:58 (0:38)	-	
Dynamic apnoea PB *m*	193 (26)	-	

Data are mean (SD). Static apnoea; immersed breath-hold, Dynamic apnoea; immersed distance with fins, PB; personal best.

Two freediving subjects did not employ the technique of GI. Two subjects were the current male and female Australian Champion, able to breath-hold for 8 and 6 min respectively. Three were able to swim more than 200 m on a single breath in the "dynamic apnoea" discipline.

5.3.2 Respiratory function

Spirometry and lung volumes are presented in Table 5.2. FEV_1 and PEF were not different between groups (P>0.08). FVC was significantly larger in the freediving

subjects compared to the healthy normals by 1.72 L (P<0.04). TLC was elevated at 124 (25) % predicted in the freediving group. RV was normal at 86 (18) % predicted.

Table 5.2. Respiratory function in 5 elite freedivers and 12 matched healthy normals.

	Freedivers	Matched normals	P value
male:female	4:1	10:2	
FEV_1 *L*	4.95 (1.12)	4.04 (0.87)	0.10
FEV_1 *% predicted*	116 (22)	101 (11)	0.09
FVC *L*	6.78 (1.48)	5.06 (1.21)	0.03
FVC *% predicted*	133 (27)	106 (11)	0.02
PEF *L/s*	9.53 (3.03)	11.50 (2.47)	0.20
TLC *L*	8.97 (1.28)	-	
TLC *% predicted*	124 (25)	-	
RV *L*	1.62 (0.29)	-	
RV *% predicted*	86 (18)	-	

Data are mean (SD). FEV_1; forced expiratory volume in one s, FVC; forced vital capacity, PEF; peak expiratory flow, TLC; total lung capacity, RV; residual volume.

5.3.3 Progressive isoxic hypercapnia (iHCVR)

A $\dot{V}_E/P_{ET}CO_2$ correlation was not seen in 3 of the 5 freediving subjects (P>0.05). In these subjects a reduced/variable respiratory rate also precluded a P_{100} measurement in the time allotted for data collection (requires at least three stable tidal breaths and then recorded randomly from breath 1 to 6 following). An example is provided in Figure 5.1, a screenshot of data collection in freediving subject #5. There is a significantly elevated $P_{ET}CO_2$ of 70 mmHg, with hypoventilation at ≈13 $L.min^{-1}$ (predicted calculated at ≈50 $L.min^{-1}$).

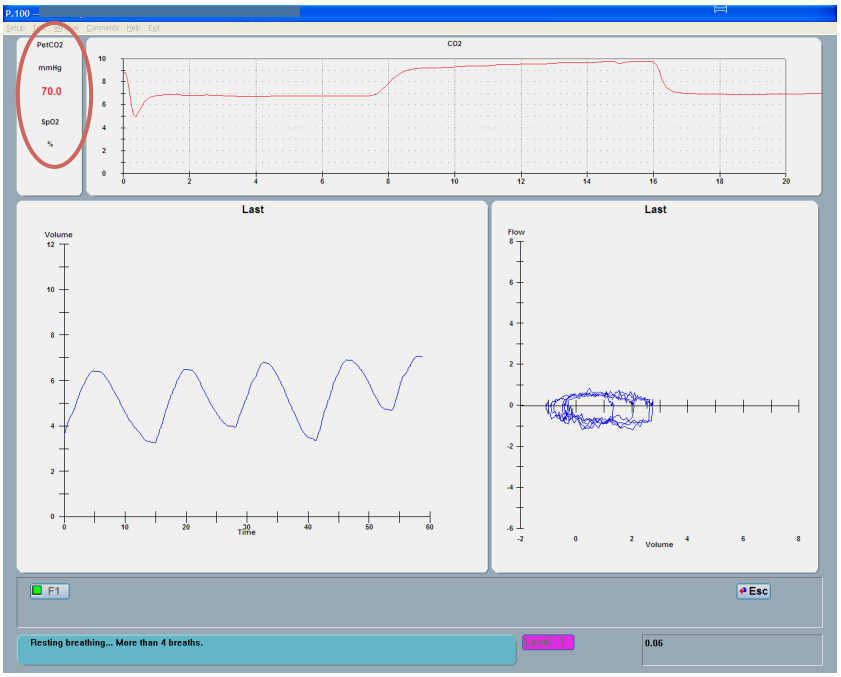


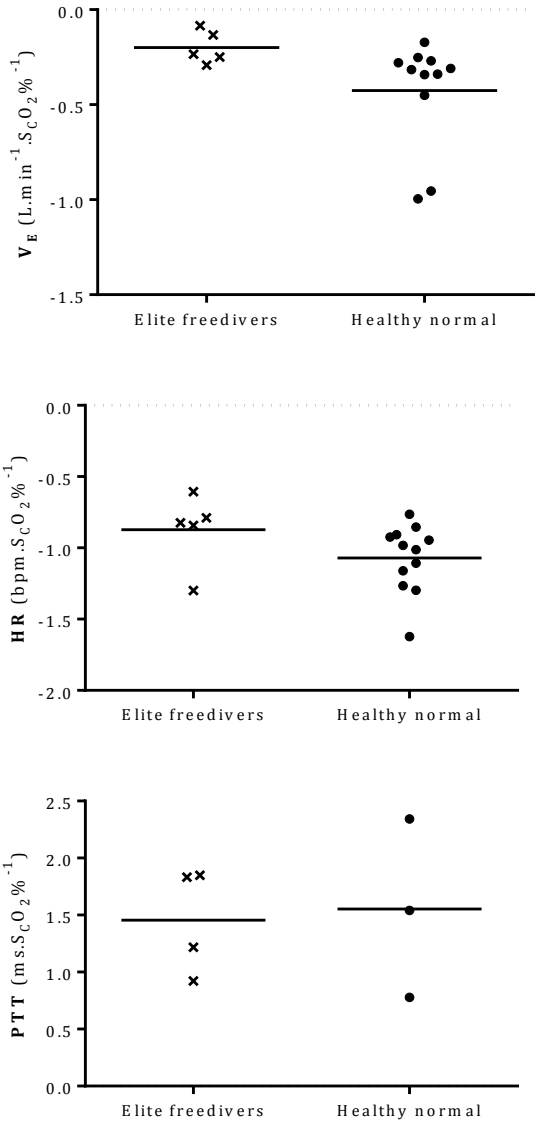
Figure 5.1. Screen shot of ventilation (volume) at $P_{ET}CO_2$ of 70 mmHg (circled in red) in freediving subject #5. *There is significant hypoventilation.*

The iHCVR in the two subjects with acceptable responses was 1.36 and 1.13 $L.min^{-1}.mmHgP_{ET}CO_2^{-1}$, with a P_{100} response of 0.44 and 0.31 $cmH_2O.mmHgP_{ET}CO_2^{-1}$ respectively. These values were lower than the mean of the age and height matched normals at 2.36 (1.02) $L.min^{-1}.mmHgP_{ET}CO_2^{-1}$ and 0.67 (0.42) $cmH_2O.mmHgP_{ET}CO_2^{-1}$, however formal comparative statistical analysis was not possible due to the necessary exclusion of the three remaining subjects with ventilatory responses that did not correlate with $P_{ET}CO_2$.

5.3.4 Progressive poikilocapnic hypoxia (pHVR)

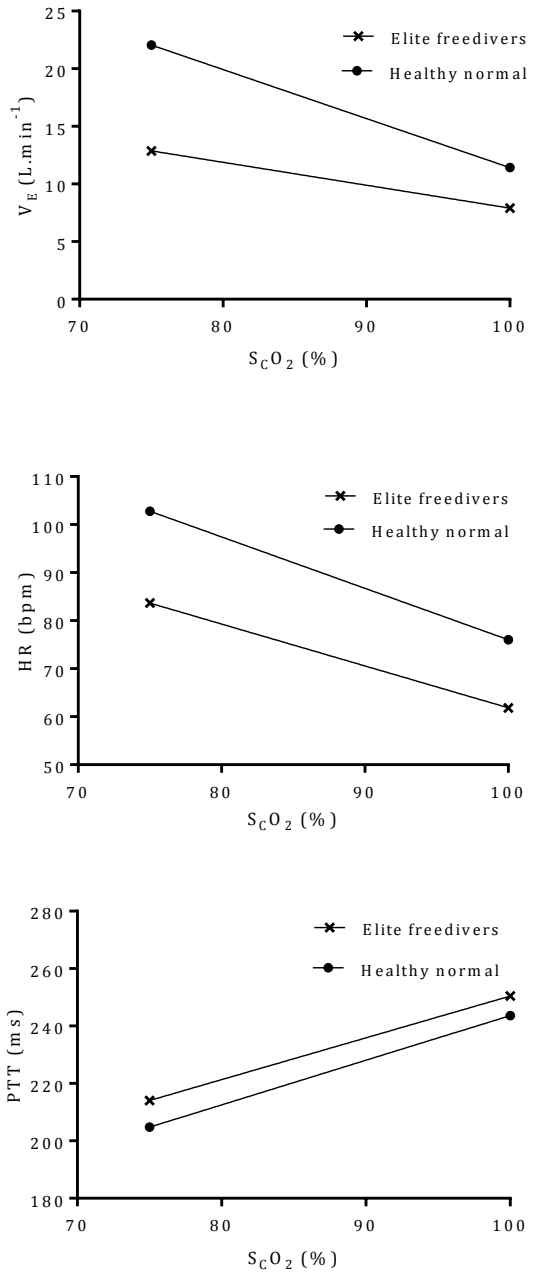
One subject's ventilatory response was excluded, as it did not correlate with S_CO_2 ($P>0.05$). This subject's HR and PTT response did however correlate with S_CO_2 and was included in those analyses. Mean and individual regression slopes for $\dot{V}_E$, HR and PTT during progressive hypoxia were -0.20 (0.09), -0.87 (0.26) and 1.46 (0.46) respectively. Height and age matched healthy normals were -0.43 (0.28), -1.07 (0.24) and 1.55 (0.78) respectively and are presented in Figure 5.2. Graphical representations of the mean group responses are presented in Figure 5.3. Ventilation was trending lower in the freedivers as compared to the normal group, however this was not significant ($P=0.10$).

HR and PTT response in the freedivers was not different to the healthy normals (P=0.14, 0.84). HR was lower in the freediving subjects at 100 and 75 % S_CO_2 (P<0.04), but change in HR during progressive hypoxia was not different from the healthy normals (P=0.14).



S_CO_2, calculated oxygen saturation. Horizontal bars indicate mean values. N=4 for $\dot{V}_E$ (freedivers), PTT (healthy normal).

Figure 5.2. Mean and individual regression slopes for minute ventilation ($\dot{V}_E$), heart rate (HR) and pulse transit time (PTT) during progressive hypoxia in five freedivers and 12 healthy normals.



S_CO_2, calculated oxygen saturation. N=4 for VE (freedivers), PTT (healthy normal).

Figure 5.3. Mean group response for minute ventilation ($\dot{V}_E$), heart rate (HR) and pulse transit time (PTT) during progressive hypoxia in five freedivers and 12 healthy normals.

Figures 5.4 and 5.5 provide individual subject examples of $\dot{V}_E$ and PTT response to S_CO_2 in a healthy subject (Figure 5.4) and in a freediver (Figure 5.5).

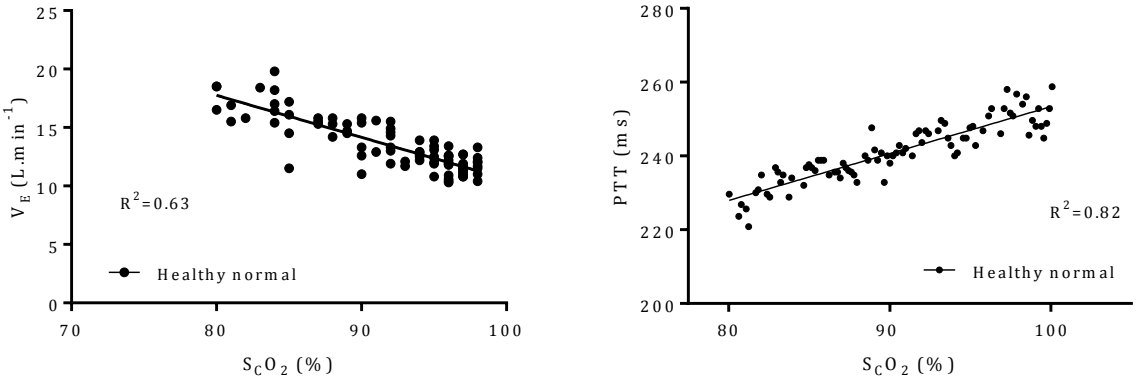


Figure 5.4. An example of normal ventilatory ($\dot{V}_E$) and pulse transit time (PTT) response to calculated oxygen saturation (S_CO_2) (Normal subject #11).

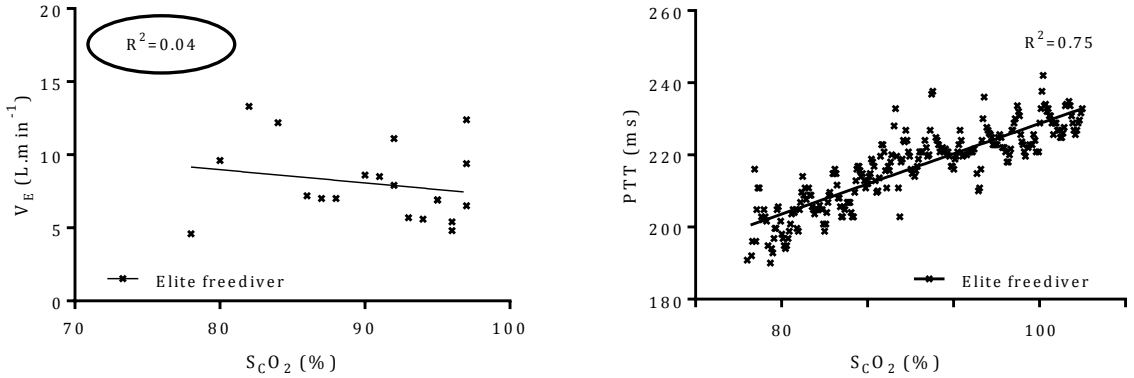


Figure 5.5. An example of poor correlation with ventilation ($\dot{V}_E$) and strong correlation with pulse transit time (PTT) to calculated oxygen saturation (ScO_2) (Freediving subject # 4).

In both subjects there was a significant and strong correlation between PTT and S_CO_2, however $\dot{V}_E$ was only significantly correlated with S_CO_2 in the healthy normal subject, with a reduced (and variable) $\dot{V}_E$ response in the freediver.

The associations of HR and $\dot{V}_E$ response as an indication of cardiorespiratory coupling in the healthy normal group and the freediving group are presented in Figure 5.6. A strong correlation was seen in the healthy normal subjects (R^2=0.6) that was not evident in the freedivers (R^2=0.2).

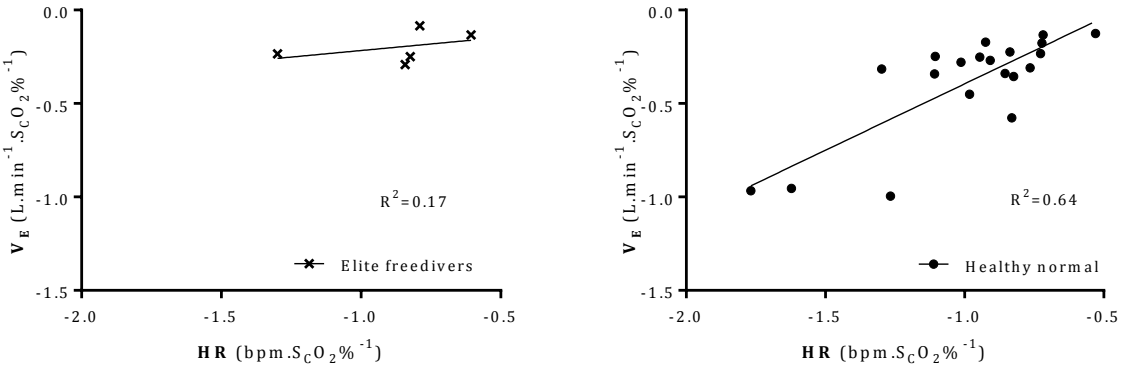


Figure 5.6. The relationship between ventilation ($\dot{V}_E$) and heart rate (HR) response to hypoxia in elite freedivers and healthy normal subjects.

5.4 Discussion

The measurement of ventilatory control was challenging in this population of elite freedivers. They were familiar with the sensations of hypercapnia and hypoxia. A common training practise is a "CO_2 table" exercise, where the athlete withstands long breath-holds with progressively shorter recovery times (without allowing CO_2 to return to baseline), which becomes increasingly uncomfortable. Notwithstanding prior instructions, their trained responses to tolerate these sensations, led to what appeared to be a voluntary override (suppression) of ventilation.

In contrast, markers of cardiovascular and sympathetic activity suggest that these autonomic responses were normal. Ainslie and colleagues have reported a strong link between ventilatory and cardiovascular responses during progressive hypoxia (96). While this was evident in the healthy subjects, there was no association in the freediving subjects. It may be expected that if there were a central "adaptation" or alteration in chemosensitivity, both processes would be similarly affected. It was likely that this was representative of voluntary hypoventilation rather than dysautonomia.

The elite freediving subjects had large lungs when compared to predicted values. FVC was 133 (27) % predicted in comparison to the healthy (non diving) normals of 106 (11) %. This was in keeping with previous observations of large lungs in those that freedive (1, 2) and spearfish (6). The longitudinal study of a freediver with progressively increasing lung volumes (Chapter 3), suggests that freediving participation and its

related training practises may be partially responsible for this observation (7). This small group were at an elite level. Interestingly the current Australian male and female champions had the “largest” lungs at 167 % and 157 % predicted.

A healthy normal group was selected that were similar in age, height and weight. This was due to the suggestion the body surface area (138) has an effect on ventilatory response as measured by minute ventilation. However others have found no effect of age on carotid sensitivity (116, 117) and there was no age effect seen across the complete group of healthy normals, discussed further in Chapter 4. Despite no difference in age, height and weight between the freedivers and healthy normals, measured lung volumes were significantly larger in the freedivers. This may have had the potential to attenuate the absolute ventilatory response to both hypoxia and hypercapnia in the freedivers, however both were not different as compared to the healthy normals.

There was a single female freediving subject in this elite group. Freediving and spearfishing is predominantly a male oriented sport and there are few female divers at the elite level. The majority of previous studies do not identify a difference in the ventilatory response to hypoxia between males and females (142-144).

As highlighted in the results, in three freediving subjects there was a clear voluntary override of the hypercapnic response. Ventilation was ≈80 % below predicted at a $P_{ET}CO_2$ of 70 mmHg. The extent of hypoventilation relative to that expected was not as great during progressive hypoxia. As mentioned in the results, freedivers are very familiar with the signs and symptoms of hypercapnia. They are less familiar with isocapnic hypoxaemia. The signs and symptoms of hypoxia are subtle. The main descriptor is of a “white out”, as it negatively affects peripheral vision, that has been described by jet pilots (145). A similar situation of isocapnic hypoxia in freediving is when the divers hyperventilate prior to breath-hold, which is discussed in Chapter 2.4.2.

In this study, ventilatory responses that did not correlate with changing end-tidal gases were excluded from analysis. Inclusion of these response curves would have resulted in significantly reduced ventilatory responses in this group and therefore would have supported other studies that have contributed to beliefs that responses were constitutively abnormal (57, 135, 137-139). Exclusion of the data, based on defined criteria, was supported by the concurrently measured, normal autonomic responses.

The two previous studies that included non-subjective markers of MSNA and cerebral blood flow during hypercapnia, reported a normal response of autonomic function during hypercapnia (139, 140). This strongly suggests that the study of ventilatory control must include measurements that are less affected by subjective influences, particularly in this unique population of breath-hold divers. The assessment of ventilatory parameters alone may underestimate the actions of central and peripheral chemoreceptors. Assessment of these particular measures had not been combined previously during progressive hypoxia in elite freediving subjects.

5.5 Conclusion

In freedivers, despite repeated experience of extremes of hypercapnia and hypoxia, autonomic responses to both clearly remain normal. Including all data, attenuated responses to hypercapnia and hypoxia were certainly seen but the data suggest that these were so because of voluntary override. Linked analysis of ventilatory and autonomic responses was essential to study this group and the previous data suggesting blunted responses may be in error.

CHAPTER 6

HYPERCAPNIC VENTILATORY RESPONSE AND RESPIRATORY FUNCTION IN MILD-MODERATE PARKINSON'S DISEASE

6.1 Background

The previous Chapters measured respiratory function, ventilatory and autonomic responses in elite freedivers, otherwise healthy subjects that repeatedly endure significantly elevated intrathoracic pressure, hypoxia and hypercapnia. The inference drawn, that previous beliefs may be incorrect because of the failure to detect abnormal responses in both ventilatory and autonomic responses, must be made with some caution unless it can be shown in another relevant group of subjects that this methodology was sensitive enough to detect abnormality when present.

The next two chapters (Chapter 6 and 7) describe the measurement of these responses in a subject group that may experience early pathological impairment of these processes.

Recent theories suggest early, medullary involvement in PD may impair ventilatory and autonomic control prior to the classical motor signs and symptoms (4). This Chapter measures respiratory function, the iHCVR and the response to steady-state hypoxia, in subjects with mild-moderate PD. It was postulated that the current method would identify any early impairment, and differentiate early central involvement from peripheral (mechanical) factors

6.1.1 Pathophysiology of Parkinson's disease

PD is a progressive degenerative illness affecting the human central, peripheral, and enteric nervous systems. Neurons within the substantia nigra become impaired which leads to a lack of dopamine production within the midbrain. This occurs, especially in the basal ganglia, which are responsible for organising movement commands. Symptoms typically appear when approximately 70 % of the dopamine-producing cells are damaged (4, 146). Although classified as a movement disorder due to the typical features of bradykinesia, tremor and rigidity, PD also exhibits non-motor features.

The pathology of PD typically consists of degeneration of the neuronal cytoskeleton and the accumulation of proteinaceous inclusions called Lewy bodies. Although previously thought to occur principally in dopaminergic neurons in the mid-brain (sustantia nigra), the Braak staging hypothesis based on pathology studies (146) suggests that the earliest evidence of disease is in the medulla, enteric nervous system and olfactory bulb. It is

proposed that the disease then slowly spreads trans-neuronally to the mid-brain, causing the classical motor features, and finally to the cortex with progressive cognitive and limbic symptoms. The hypothesis implies that there may be pre-motor, autonomic and olfactory dysfunction and there is now accumulating evidence supporting this, particularly relating to postural hypotension, cardiac denervation, bowel motility and hyposmia (147, 148). The anatomy of the brainstem is presented in Figure 6.1.

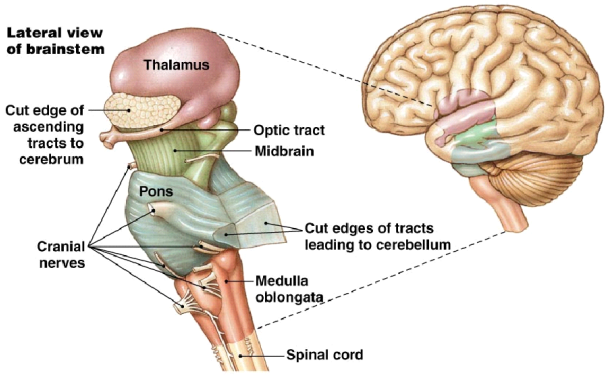


Figure 6.1. The anatomy of the brainstem.

6.1.2 Respiratory involvement in Parkinson's disease

PD has always been thought to involve a disturbance of respiration. James Parkinson observed in 1817 that his patients *"fetched their breath rather hard"* in his monograph, "An essay on the shaking palsy" (149). Despite this early recognition of involvement of the respiratory system, the exact abnormalities and their causation remain unclear.

Respiratory complications, primarily aspiration pneumonia, are the most common cause of mortality in PD (150, 151). It was suggested that the lack of airway protection from sensory deficits or diminished cough reflex contributes greatly to this (152).

Respiratory involvement in PD can also occur through peripheral mechanisms. Motor manifestations such as rigidity, tremor and weakness can affect the upper airway and the respiratory muscles. Literature since the 1950's describing respiratory function in PD has yielded disparate findings. Peripheral involvement studies have demonstrated normal lung volumes (153, 154), restrictive flow volume characteristics (155-157), upper airway dysfunction (154, 157, 158) and reduced peak expiratory flow (155, 157,

159). Maximal inspiratory and expiratory pressures have been shown to be reduced (154, 158) that related to both disease severity and upper airway dysfunction (160).

Upper airway dysfunction was determined in these studies from flow volume loops; with abnormal forced expiratory/inspiratory ratios and/or appearance of "sawtoothing" (Figure 6.2). The above studies included subjects with severe disease (154, 160) or from a broad selection of disease severity (158, 159).

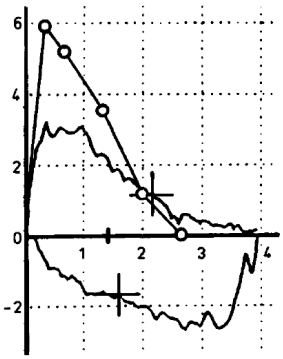


Figure 6.2. An example of "sawtoothing" of the respiratory flow volume curve. *From Hovestadt* (154).

6.1.3 The effect of Parkinson's disease on ventilatory control

Early non-motor brain stem involvement may have an effect at the level of respiratory control, which resides mainly in the medulla. The evolution of the PD pathology identifies Stages 1 and 2 ("mild" disease) with initial legions within the medulla and pontine tegmentum (4). Investigations into brainstem mediated function in PD have been limited. This is presumably because respiratory symptoms are not prominent in mild PD. The effects of cardiac denervation may also have minimal symptomatic impact but metaiodobenzylguanidine (MIBG) scanning, which reflects the degree of post-ganglionic cardiac sympathetic denervation, is one of the most useful diagnostic tests in early PD, particularly in countries where dopamine transporter (DAT) scanning is not available (161). The dorsal motor nucleus of the vagus (DMV) in the medulla is one of the earliest regions to accumulate alphasynuclein pathology (4). The dorsal medullary respiratory centre including the nucleus of the tractus solitarius (which lies immediately lateral to the DMV) receives afferent input from carotid and aortic chemoreceptors, which are pivotal in ventilatory control as discussed in Chapter 4.1. Vagal efferent axons

from pre-ganglionic parasympathetic DMV terminate on post-ganglionic neurons associated with respiratory (as well as cardiac and GI) function.

The limited evidence on respiratory control in PD includes subjects with moderate disease. A reduced sensitivity to progressive hypoxia was demonstrated (153, 162) however with no effect on progressive hypercapnia in a single investigation (153). An additional measurement of central respiratory drive (occlusion pressure) would elucidate as to whether any observed impairment was due to mechanical (reduced respiratory muscle weakness) or central factors.

Abnormality of responses to hypoxia may have broader significance in patients with mild PD if they travel to altitude or, more commonly, by commercial aircraft. At aircraft cruise altitudes of up to 13000m (≈43000ft), intolerable hypoxaemia would be experienced were the cabin not pressurised. Regulations require cabin pressure to be no less than barometric pressure equivalent to an altitude of 2438 m above sea level (8000 ft, P_B 574 mmHg) (163). Short-haul flights commonly have mean cabin pressures close to this minimum (164) whereas pressures are commonly higher in long-haul wide-body aircraft. A mean cabin pressure altitude of 1600 m (5250 ft) has been reported in Boeing 747 flights averaging 10 hours in duration (165). A reduced sensitivity to the reduced pressure of inspired oxygen experienced in-flight has the potential to result in significant hypoxaemia.

6.1.4 Aim

The aim of this study was assess the ventilatory and occlusion pressure response to progressive isoxic hypercapnia and steady-state hypoxia in subjects with mild-moderate PD. Complex respiratory function measurement was included to comprehensively describe any possible concomitant peripheral respiratory involvement.

6.2 Methods

6.2.1. Subjects

Patients with a diagnosis of idiopathic PD were recruited from the Concord Hospital PD Outpatient Clinic with assistance from the patient's treating Neurologist (Dr Michael Hayes and Dr Alastair Corbett) and medical student (Dr Hugh Giddings). Exclusion criteria included any clinically significant respiratory or cardiovascular disease,

neurological disease other than PD, a significant smoking history (>10 pack years), structural abnormalities of the chest wall or recent upper respiratory tract infection.

In this arm, all testing was performed with the patient taking their usual medications; the timing of medication use in relation to the tests was not controlled. All testing and analyses were conducted by the same Scientific Officer (myself, L.S.). Data collection was made with the assistance of Peter Rogers, Respiratory Scientist, Concord Hospital. Methodological procedures were performed during a single subject visit.

A PD history, including current medications, was documented (Appendix A iii). An assessment of disease severity was sourced from the subject's treating neurologist that included the motor examination section of the unified PD rating scale (UPDRS) in the "on-state" (166) and the Hoehn and Yahr scale (H&Y) (150). The assessments were completed within six months of recruitment to this study.

Healthy normal subjects were recruited as presented in Chapter 4.4.

6.2.2 Respiratory flow, volume and muscle function

Spirometry, diffusing capacity and lung volumes (determined by plethysmography) were performed according to American Thoracic Society / European Respiratory Society criteria (Vmax Encore, Sensormedics, Yorba Linda, CA) (29, 62, 63). Predicted values were derived from the recommendations of the European Community for Coal and Steel (64). Respiratory muscle strength was determined using methods and predictive reference equations described by Black and Hyatt (167).

6.2.3 Progressive isoxic hypercapnia (iHCVR)

After resting, while seated breathing room air for at least 20 min, subjects performed the iHCVR as described in Chapter 4.3.3.

6.2.4 Mild steady-state hypoxia

Subjects rested breathing room air while seated for at least 20 min prior to commencing the steady-state hypoxic challenge. The response to mild hypoxia was assessed using an altitude simulation test (AST), that replicates the aircraft cabin environment, the using the technique described by Gong and colleagues (168).

A radial arterial blood gas (ABG) sample was taken at rest ("FIO_2 0.21") and following 20 min breathing a hypoxic gas mix containing 15.1 % (+/- 0.2 %) O_2, balance nitrogen ("FIO_2 0.15") (BOC Gases, Australia). Subjects breathed through a two way, non-rebreathing valve (T-shape 1410, Hans Rudolph Inc, Kansas City, MO) from a reservoir (30 L non-diffusing gas bag; Hans Rudolph Inc, Kansas City, MO). HR and SpO_2 were continually monitored. Testing was terminated if SpO_2 fell below 80 % or due to volitional fatigue.

Statistical analysis

Results are expressed as mean (SD) unless otherwise stated. Individual responses were assessed using linear regression. Responses were compared to healthy normal subjects as described in Chapter 4, using unpaired two-tailed, t-tests. Outlier fell outside two SD from the mean. The response was also presented as compared to previously published normative data by Hirshman and colleagues for ventilatory response (110) and Lopata and colleagues for occlusion pressure (169).

The group response for the steady-state hypoxic challenge was compared to previously published normative data (170). Group responses were assessed using unpaired, two-tailed, t-tests.

A linear correlation was used to determine associations between PD severity, respiratory function and ventilatory response. A P value <0.05 was considered significant.

The study was reviewed and approved by the Human Ethics Review Board of the Sydney South West Area Health (New South Wales, Australia). Each subject gave written informed consent.

6.3 Results

6.3.1 Subjects

19 subjects (2 female) with a diagnosis of idiopathic PD were recruited. Respiratory function and iHCVR data were compared to 20 (7 female) healthy normal subjects as described in Chapter 4. Demographics and disease history and severity are presented in Table 6.1.

Table 6.1. Subject demographics, disease history and disease severity in 19 subjects with Parkinson's disease and 20 healthy normals.

	Parkinson's disease	Normal	P value
Male:Female	17:2	13:7	
Age *years*	66 (8)	39 (12)	0.01
Height *cm*	174 (7)	174 (10)	0.96
Weight *kg*	80 (11)	73 (14)	0.09
Duration of symptoms *years*	8 (5)	-	-
Time since diagnosis *years*	7 (5)	-	-
UPDRS *(motor examination)[0-52]**	19 (8)	-	-
H&Y score *[0-5]***	2.5 (0.5)	-	-

Data are mean (SD), BMI; body mass index, UPDRS; unified Parkinson's disease rating scale (motor section), H&Y; Hoehn and Yahr. *N=15 **N=16

Disease severity

By UPDRS motor examination, average scores reflect mild-moderate impairment in the PD subjects (166). H&Y staging (150) classified subjects as having unilateral disease (Stage 1: n=2), unilateral disease plus axial involvement (Stage 1.5: n=1), bilateral disease, without impairment of balance (Stage 2: n=4), mild bilateral disease (Stage 2.5: n=4) or mild-moderate bilateral disease (Stage 3: n=5); 3 were unclassified.

Medication use

All 19 subjects reported taking levodopa or carbidopa and 11 subjects were taking a form of dopamine agonist (9 on pramipexole, 2 on cabergolide, 1 on an apomorphine infusion overnight). 10 subjects were taking entacapone (catechol-O-methyle transferase inhibitor). Two subjects were taking amantadine. One subject had undergone deep brain stimulation.

6.3.2 Respiratory flow, volume and muscle function

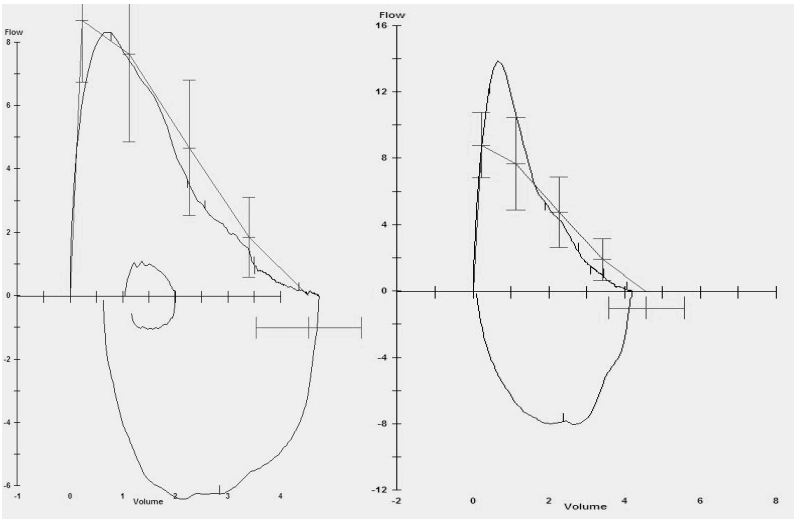
Spirometry, diffusing capacity and lung volume parameters are shown in Table 6.2.

Table 6.2. Respiratory function in 19 subjects with Parkinson's disease and 20 health normals.

	Parkinson's disease	Healthy normal	P value
FEV_1 *L*	3.28 (0.71)	3.73 (0.90)	0.09
FEV_1 % Predicted	107 (15)	102 (11)	0.31
FVC *L*	4.06 (0.89)	4.65 (1.22)	0.10
FVC % Predicted	104 (14)	109 (14)	0.54
FEV_1/FVC *%*	81 (6)	81 (9)	0.96
PEF *L/s*	9.01 (2.00)	10.36 (2.74)	0.09
PEF % Predicted	115 (23)	-	-
TLC *L*	6.07 (1.01)	-	-
TLC % Predicted	90 (12)	-	-
FRC *L*	3.38 (0.71)	-	-
FRC % Predicted	96 (18)	-	-
RV *L*	1.97 (0.36)	-	-
RV % Predicted	81 (16)	-	-
RV/TLC *%*	33 (6)	-	-
TL_{CO} Adj *mL/mmHg/min*	25.0 (5.1)	-	-
TL_{CO} % Predicted	93 (14)	-	-

Data are mean (SD), FEV_1; forced expiratory volume in 1 s, FVC; forced vital capacity, PEF; peak expiratory flow, TLC; total lung capacity, FRC; functional residual volume, TL_{CO} Adj; single breath lung carbon monoxide transfer factor corrected for haemoglobin.

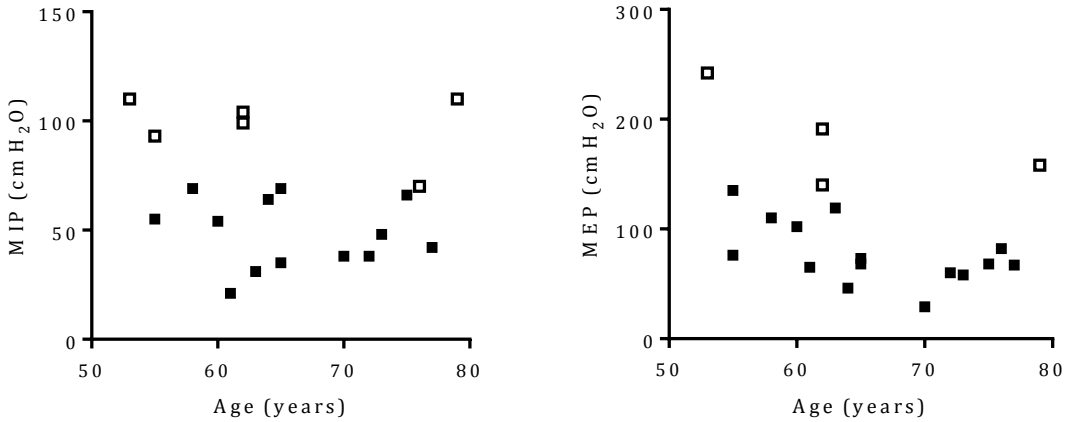
16/19 (84 %) had respiratory function within normal limits. One subject had mild airflow limitation as determined by GOLD criteria (171) and two were classified as restricted by European Respiratory Society guidelines (32) with total lung capacity 65 % and 78 % of predicted. No upper airway abnormalities, such as oscillation or "sawtoothing" were observed on the flow-volume curves (e.g. Figure 6.3).



Curve with error bars is predicted.

Figure 6.3. Examples of normal flow volume curves in subject #2 and #3. *There is no evidence of "sawtoothing".*

The results for maximal inspiratory (MIP) and expiratory (MEP) pressures are shown in Figures 6.4. There was a wide range of values when considered as a % of predicted (Figure 6.5). 13/19 (68 %) fell below the lower limit of normal (LLN) for MIP and 15/19 (79 %) for MEP (167).



Filled data points fall below the lower limit of normal.

Figure 6.4. Maximum inspiratory (MIP) and expiratory (MEP) pressures in 19 subjects with Parkinson's disease.

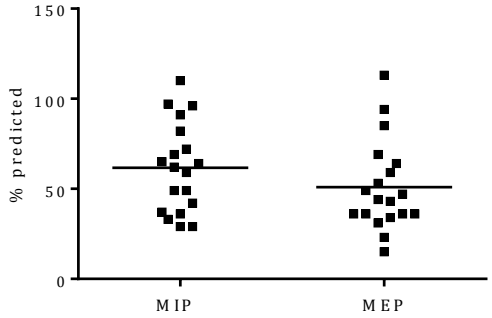
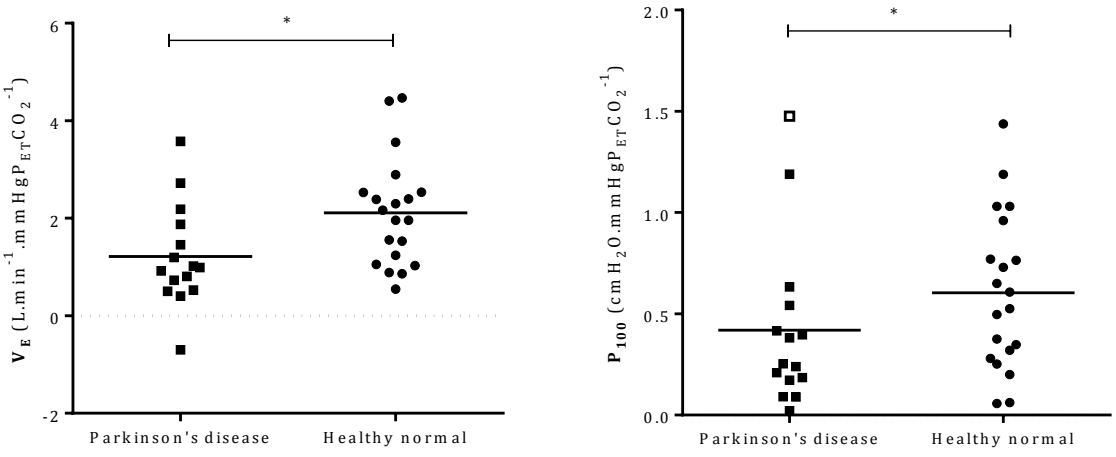


Figure 6.5. Maximum inspiratory (MIP) and expiratory (MEP) pressures in 19 subjects with Parkinson's disease as % predicted.

6.3.3 Progressive isoxic hypercapnia (iHCVR)

Four subjects were unable to maintain an adequate mouth seal due to muscle weakness and were excluded from analysis. $\dot{V}_E$ and P_{100} at baseline was 13.4 (2.9) L.min^{-1} and 2.5 (0.5) cm.H_2O respectively. The slope of the ventilatory response ($\dot{V}_E$ L.min^{-1}.mmHg $P_{ET}CO_2$ mmHg^{-1}) was 1.04 (0.84), R^2 = 0.90 (0.10). Occlusion pressure response (P_{100} cmH_2O.mmHg $P_{ET}CO_2^{-1}$) was 0.34 (0.30), R^2 = 0.85 (0.21). Compared to healthy normals, there was a significantly lower $\dot{V}_E$ (normals; 2.11 [1.11], P<0.005) and P_{100} (normals; 0.6 [0.4], P<0.05) in the mild-moderate PD group. Individual regression slopes for $\dot{V}_E$ and P_{100} are presented in Figure 6.6.

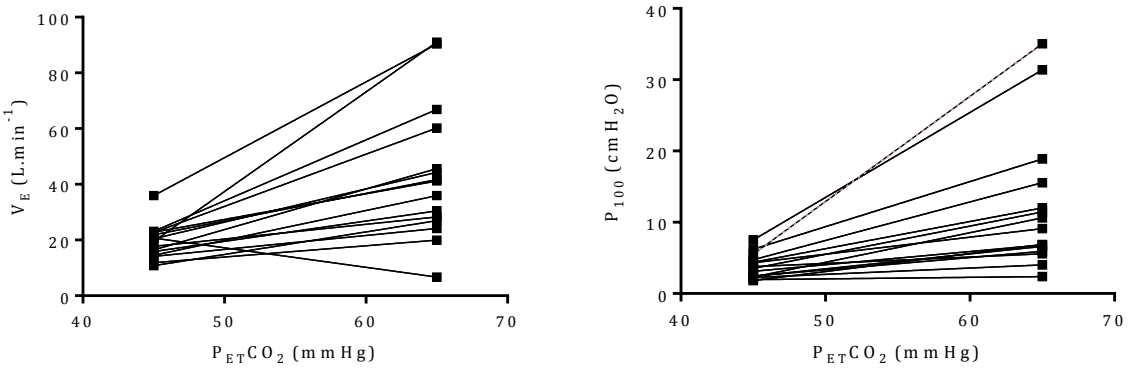


$P_{ET}CO_2$; pressure of end-tidal CO_2. * P<0.05. Open data point is outlier.

Figure 6.6. Mean (bar) and individual regression slopes for minute ventilation ($\dot{V}_E$) and occlusion pressure (P_{100}) during progressive hypercapnia in 15 subjects with Parkinson's disease and 20 healthy normals.

When compared to predicted values, 7/15 (47 %) subjects had an abnormal ventilatory response (<1 L.min^{-1}.mmHg $P_{ET}CO_2^{-1}$) (110) and 11/15 (73 %) patients had an abnormal occlusion pressure response (<0.5cmH_2O.mmHg $P_{ET}CO_2^{-1}$) (172).

$\dot{V}_E$ and P_{100} at 45 and 65 mmHg $P_{ET}CO_2$ (derived from the linear response curve) for each subject are presented in Figure 6.7.



Broken line is outlier.

Figure 6.7. Ventilation ($\dot{V}_E$) and occlusion pressure (P_{100}) at 45 and 65 mmHg pressure of end tidal carbon dioxide ($P_{ET}CO_2$) in 15 subjects with Parkinson's disease.

6.3.4 Mild steady-state hypoxia

The majority of subjects had ABG's within normal limits when breathing room air, at rest. "FIO_2 0.21" PaO_2 was 94.9 (8.7) mmHg and $PaCO_2$ 40.5 (4.3) mmHg. One subject with a mildly reduced PaO_2 (77 mmHg) and two subjects had a mildly elevated $PaCO_2$ (47 and 50 mmHg). One subject declined ABG sampling.

Following the AST, ABG's were able to be collected on 16 subjects. Individual values for PaO_2 at "FIO_2 0.21" and "FIO_2 0.15" respectively are presented in Figure 6.8. PaO_2 at "FIO_2 0.15" was 60 (4) mmHg. This was more favourable than previous normative values of 58 (4) mmHg (170). No subject's PaO_2 fell below what was considered to be acceptable for safe commercial air travel by medical guidelines (50 mmHg) (173).

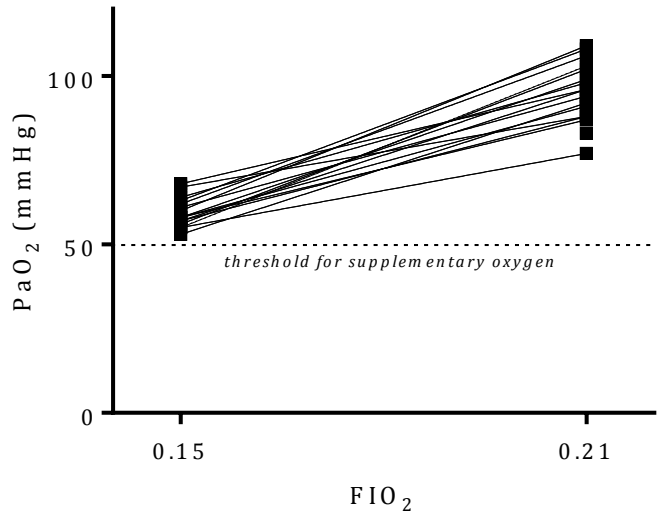


Figure 6.8. Individual values for pressure of arterial oxygen (PaO_2) at sea level, room air (FIO_2 0.21) and while breathing a low oxygen gas mix (FIO_2 0.15) in 16 subjects with Parkinson's disease.

Correlations

There were no associations between PD severity, respiratory function or iHCVR. There was no association between MIP max % predicted and P_{100} response to hypercapnia (R^2= 0.08) or P_{100} at 65 mmHg $P_{ET}CO_2$ (R^2=0.08).

6.4 Discussion

6.4.1 Main findings

This patient group with mild-moderate PD, on optimal treatment, had normal respiratory function as measured by respiratory flow and volumes. Respiratory muscle weakness was common, but insufficient as to cause pulmonary restriction. Ventilatory response to hypercapnia however was abnormal. There was a marked impairment of the ventilatory and occlusion pressure response to progressive hypercapnia but not to mild steady-state hypoxia. The hypercapnic protocol identified an abnormality of central respiratory drive that was consistent with the Braak hypothesis, suggesting early brainstem involvement in PD (146).

6.4.2 Subject selection as compared to previous studies

The finding of normal respiratory flow and volume suggest that the subjects in this study had not progressed to the point where pulmonary restrictive abnormalities were evident as per previous observations. Direct comparison of the relative severity of PD in these patients with those previously studied however was difficult. The PD assessment tools (150, 166) employed in this study were not always used or available previously. In contrast to some previous studies, it was ensured that only patients who had idiopathic PD were included and that subjects with other respiratory disease or significant past smoking were excluded. Importantly, if restrictive lung volume changes are a marker of disease progression as suggested (159), these data suggest that abnormality of ventilatory control may be a relatively early event in the clinical course of PD.

The healthy normal group was younger than the PD group. Correlation analysis did not identify an age effect within the normal group (Chapter 4), which was consistent with previous evidence that hypoxic ventilatory drive and carotid body function is unaltered with age (116, 117). Importantly, there was no difference in measured lung volumes between groups.

6.4.3 Impaired respiratory drive to hypercapnia

The results demonstrate an impaired respiratory drive in response to hypercapnia. The abnormal occlusion pressure response indicates central drive impairment independent of mechanical factors (such as muscle weakness). However Whitelaw and Derenne (91) have since acknowledged that respiratory muscle myopathy can also have an inhibitory effect on this “central” measure.

It is relevant to consider whether the measures of ventilatory response to hypercapnia in this study were abnormal only because of reduced muscle strength.

Firstly, although the subjects in this study did have respiratory muscle pressure below the lower limit of normal, it was not to the level where extra-pulmonary restriction was seen. Secondly, there was no relationship between maximal respiratory muscle strength and the linear occlusion pressure response or occlusion pressure at 65 $P_{ET}CO_2$ mmHg. Finally, across the group, the average value for P_{100} at 65 $P_{ET}CO_2$ mmHg was 27 % of PI max, suggesting that there was considerable capacity for incremental inspiratory muscle

pressure generation if brain stem drive was higher.

There are limited published data in relation to the HCVR in PD. In contrast to this study, Onodera and colleagues (153) found a normal hypercapnic response in PD patients while taking dopaminergic medication. However, a reduction in chemosensitivity to progressive hypoxia has been demonstrated previously in PD (153, 162). The mild hypoxic challenge used in this study did not elicit an abnormal response. The need for further study monitoring central and/or sympathetic drive with ventilation during progressive hypoxia was apparent and is addressed in the following Chapter 7.

6.4.4 Early brainstem involvement in PD

It has recently been recognised that some of the earliest pathological changes of PD are seen in the brainstem, rather than the extra-pyramidal grey matter nuclei of the substantia nigra and pars compacta. Braak and Braak (146) describe six pathological stages, with the typical motor manifestations of PD only starting to be seen in stages 3-4. Loss of smell and constipation are seen early, often pre-dating the typical motor findings, corresponding with early involvement of the olfactory bulb and DMV nerve. Along with these phenomena, abnormalities of respiratory control may also be amongst the earliest changes in PD. Mild disease is regarded as Stages 1 and 2; classified with initial lesions within the medulla and pontine tegmentum (4). The study of those with exclusively mild disease has not been reported; also addressed in the following Chapter 7.

6.4.5 Effect of therapeutic regimes

All subjects in this study were on dopamine receptor activators during assessment. The effect of dopamine on ventilatory control on those with or without PD is varied. Hsiao and colleagues (174) showed that ventilatory control to both hypoxia and hypercapnia was elevated in cats following domperidone, while Olson and colleagues (175) demonstrated that healthy subject's ventilatory response to hypercapnia decreased following dopamine infusions, that was reversed with the dopamine-receptor blocking agent prochlorperazine (176). Importantly, Blain and colleagues (177) recent work on dogs identified a strong connection between the central and peripheral chemoreceptor's, suggesting there is not a clear distinction between the two processes as previously thought. Which of these observations may be important in interpreting

this data is not clear. Whilst dopamine D1 receptor agonists augment respiratory drive (178), the dopamine agonists used for PD treatment are active at D2 receptors. Nonetheless, an effect of treatment cannot be excluded.

The possibility of re-assessing these subjects after medication withdrawal or in the "off" state for those who experience on-off phenomenon was explored. There were practical difficulties with mouthpiece seal and retention. Therefore accurate, reliable data could not be generated. In addition, the "off" state is discomforting for subjects and their carers, particularly in a public setting, raising both ethical issues and practical difficulty with travel to visits. During these initial trials, the subjects were also unable to communicate effectively (with hand signals or otherwise) while "off", which also raised safety concerns during rebreathing.

To explore the question of an effect of PD treatment further, it was concluded that studying a group with more mild PD severity would be necessary, both before and after their dopaminergic therapy and this is presented in Chapter 7.

6.5 Conclusion

In summary, the isoxic hypercapnic protocol identified an abnormal ventilatory response to CO_2 in subjects with mild-moderate PD independent of peripheral respiratory function. Idiopathic PD and other Parkinsonian syndromes are associated with excess morbidity and mortality from respiratory causes, with pneumonia continuing to be the leading cause of death in end-stage PD (150, 151). Impaired respiratory drive in acute illness may contribute to adverse outcomes independent of the contribution of respiratory muscle weakness and any structural lung disease. The ventilatory response seen at moderate altitude was preserved and commercial aircraft flight was therefore safe but questions remain about the normality of responses at altitudes significantly above 2500 m.

6.6 Resulting publication (Appendix C)

Seccombe LM, Giddings, HL, Rogers PG, Corbett AJ, Hayes MW, Peters MJ, Veitch EM. Abnormal ventilatory control in Parkinson's disease – further evidence for non-motor dysfunction. Respir Physiol Neurobiol. 2011 Dec 15;179(2-3):300-4.

CHAPTER 7

CONCURRENT ASSESSMENT OF VENTILATORY AND AUTONOMIC RESPONSES TO HYPOXIA AND HYPERCAPNIA IN MILD PARKINSON'S DISEASE

7.1 Background

Respiratory function measurements and the isoxic hypercapnic protocol in the previous Chapter 6 identified an abnormal hypercapnic response in subjects with mild-moderate PD due to central, rather than peripheral, impairment. The response to mild steady-state hypoxia, as measured by arterial oxygenation, however was normal.

Three main questions arose from the study;

i. Is ventilatory control impairment identifiable in subjects with exclusively mild PD?

ii. Are measures of sympathetic and cardiovascular responses during progressive, rather than steady-state, hypoxia sensitive measures of early autonomic dysfunction?

iii. Does standard, clinical dopaminergic therapy affect ventilatory and sympathetic control?

This Chapter directly addresses these questions with the same methods used to distinguish voluntary override from intrinsic deficits in the elite freedivers.

7.1.1 PD disease severity and autonomic dysfunction

PD is a degenerative illness affecting the human central, peripheral, and enteric nervous systems as discussed in Chapter 6.1.1. Neurons within the substantia nigra become impaired which leads to a lack of dopamine production within the midbrain. This occurs especially in the basal ganglia that are responsible for organising movement commands. Symptoms typically appear when approximately 70 % of the dopamine-producing cells are damaged (4, 146).

The Braak staging hypothesis in PD suggests that the earliest evidence of disease is within the medulla (146). This has been recently supported with neuroimaging. White and grey matter volume reduction in the brain stem, between the pons and the medulla, has been identified in mild PD patients with an H&Y stage of 1-2 (Figure 7.1) (179). It suggests that brain stem damage may be the first identifiable stage of PD neuropathology.

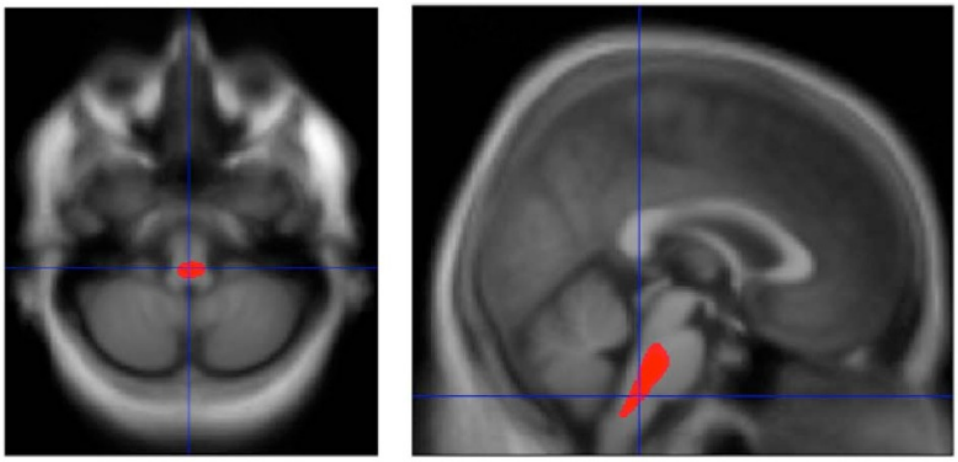


Figure 7.1. Localisation of atrophy in mild Parkinson's disease. *Red mark is cluster of significant volume reduction in the brain stem as compared with healthy controls. Image from Jubault, 2009* (179).

Abnormalities in central respiratory control may be identifiable in mild PD due to this early pathological involvement of the pons and the medulla, where the respiratory centres are situated. At this stage of the disease, the peripheral motor manifestations of PD (including rigidity, tremor, postural abnormalities and muscle weakness), which can later impair ventilatory-muscle function and upper airway integrity (180), are not evident.

Previous studies that have reported an abnormal chemosensitivity to hypoxia and hypercapnia in PD included patients with predominately moderate disease severity. Serebrovskaya and colleagues studied 7 subjects with H&Y between 2-2.5 (162). Onodera and colleagues studied 25 subjects with H&Y between 2-3 (153). Similarly in the previous Chapter, subjects with a mean H&Y of 2.5 were recruited (8). A group with exclusively mild disease has not been studied. This would identify any ventilatory control impairment from autonomic dysfunction in the very early or mild stage of the disease as per Braak's hypothesis. "Mild" PD is considered with an H&Y scale between stages 0-2. This includes those with *"no signs of disease"*, *"unilateral disease"* and *"bilateral disease without impairment of balance"* (150).

A subject group with exclusively mild PD was sought to determine whether any impairment in ventilatory and autonomic control are identifiable at this stage of disease

severity. This would indicate autonomic dysfunction from brain stem involvement before development of clinically evident motor manifestations.

7.1.2 Sympathetic and cardiovascular responses during progressive hypoxia as a measure of autonomic function

In the previous Chapter 6, PD subjects with mild-moderate disease severity had a normal arterial oxygen response to mild steady-state hypoxia (FIO_2 0.15). However previous studies of subjects with similar, or more severe PD severity, have demonstrated an abnormal response to both progressive (153) and steady-state (FIO_2 0.11) (162) hypoxia. Sympathetic/cardiovascular measures during a progressive hypoxia challenge may identify subtle autonomic changes that may not be evident with ABG's or ventilation, particularly in those with mild PD severity.

Sympathetic/cardiovascular markers during hypoxia have not been previous reported in PD subjects. As discussed in Chapter 4.1, respiration is largely regulated by chemoreflex mechanisms. Peripheral chemoreceptors located in the carotid bodies respond primarily to hypoxia. The response to hypoxia includes an increase in ventilation and augmentation of sympathetic nerve activity (72-74). In health, the cardiovascular and ventilatory responses during progressive hypoxia are strongly linked. Ainslie and colleagues have demonstrated a strong correlation between ventilation, peak cerebral blood flow velocity and mean arterial blood pressure during progressive hypoxia (96).

A well-established response to acute exposure to hypoxia, such as with ascent to altitude, is an increase in ventilation (the HVR). Concomitantly there is an increase in sympathoadrenal activity. Cardiac output is increased, via both increased HR and increased myocardial contractility (102). In health, the sympathetic nervous system is dominant to the parasympathetic nervous system during hypoxia (103).

Afferents from both respiratory and cardiovascular systems share common neuronal pathways that interact in the brainstem (181). In mild PD, autonomic dysfunction includes post-ganglionic sympathetic cardiac denervation. This forms the rationale for MIBG scintigraphy for the diagnosis of Lewy body-related disorders (161, 182). It is postulated that any impairment in chemosenstivity to hypoxia in mild PD should also affect the cardiovascular /sympathetic response.

7.1.3 The effect of dopamine on ventilatory control

The previous studies by other investigators that identified abnormal ventilatory control in PD included subjects that were taking their usual medications and the timing of their medication regime was not controlled.

PD is characterised by a lack of dopamine production within the midbrain and clinical dopaminergic therapy is standard in PD. The effect of therapeutic dopaminergic agents on dopamine receptors within the carotid body is not totally understood but based on pharmacology, it could affect both ventilatory and sympathetic control. Dopaminergic therapy may also modulate the sensitivity of the peripheral arterial chemoreceptors (183).

Human and animal type I cells (glomus cells) of the carotid bodies contain abundant quantities of dopamine. Dopamine intervenes in the regulation of respiration at the level of the carotid bodies, and also in the neurons of the nucleus tractus solitarius, where afferents from the carotid bodies end. Hypoxia increases carotid body tyrosine hydroxylase activity, resulting in increased dopamine synthesis and release (183). This suggests that dopamine can play a role in the regulation of ventilation.

There is strong evidence from animal and human studies that low-dose dopamine infusion decreases the normal ventilatory response to hypoxia (92, 175, 184-189) by inhibiting the chemosensory discharge rate. Similarly, the peripheral dopamine D2 receptor antagonist domperidone improves ventilatory sensitivity (189, 190). Jansen and colleagues assessed exercise ventilatory response following lose-dose dopamine infusion and observed a reduced minute ventilation response to end-tidal CO_2 (191).

There has been less investigation into the effect of dopaminergic therapy in those with disease. Van de Borne and colleagues demonstrated ventilatory inhibition after low-dose dopamine infusion in hypoxic cardiac patients (192). Cardiac patients are often prescribed dopaminergic therapy for its vasopressor effect. This has important clinical implications as they are weaned off supplemental oxygen, as a reduced hypoxic drive may lead to respiratory failure.

Dopaminergic therapy may also have an effect on respiratory function and structure. Herer and colleagues assessed pulmonary function in 22 subjects with PD before and

after a 12 hour withdrawal of dopaminergic therapy (H&Y Stage 2-4). In those subjects that observed upper airway dysfunction ("saw toothing") in the "off" state, dopaminergic therapy improved forced expiratory flow measures (193).

In addition to potentially affecting ventilatory function and control, there is some evidence that dopaminergic therapy effects pulmonary circulation. Huckauf and colleagues demonstrated a significant reduction in PaO_2 following dopamine in patients with left heart failure. They attributed this to a vasodilatation of the pulmonary capillaries, leading to perfusion of poorly ventilated lung units, therefore increasing ventilation-perfusion mismatch (194). They did not report any ventilatory changes in these subjects following dopamine. This is supported by Polak and colleagues who observed a reduced pulmonary vascular resistance, and therefore reduced pulmonary artery pressure, in mice following fenoldopam, a dopamine receptor agonist (195).

Clinical use of dopamine

Dopaminergic therapy is primarily used in clinical medicine for its vasopressor and inotropic actions in the management of critically ill patients with circulatory failure (183, 196). Dopaminergic therapy is also an early, standard therapy in patients with PD due to the intrinsic pathophysiology of the disease as previously discussed. Common dopaminergic medications used in PD and their actions are listed in Table 7.1.

Table 7.1. Common dopaminergic medications and their actions.

Levodopa	Is converted to dopamine by the action of the enzyme dopa-decarboxylase
Dopamine receptor agonists	Stimulate the dopamine receptors
MAO-B inhibitor	Prolongs the action of dopamine by blocking its metabolic pathway
COMT inhibitor	Prolongs the action of dopamine by blocking its metabolic pathway

MAO-B; Monoamine-oxidase-B, COMT; Catechol-O-methyl transferase

These previous studies mentioned above predominantly assessed the effects of *infused* dopamine. It is difficult to compare this with standard, regular oral dopaminergic therapy. However, the evidence raises valid questions as to the contribution of PD medication on ventilatory and sympathetic control. In response to Onodera and

colleagues report of a reduced AHVR in subjects with moderate PD (153), Roggia posed this question to the editor of The Lancet:

> *"All patients were taking their regular dopaminergic medications…. we cannot be sure whether the disease itself or the treatment is causal for these findings."* (197)

This important question is addressed in this Chapter, at least for those with mild PD, by assessing ventilatory and sympathetic/cardiovascular responses before and after the subject's usual dopaminergic therapy.

Considerations of dopaminergic therapy withdrawal

As mentioned in Chapter 6.4.5, testing subjects with moderate PD severity using rebreathing methods was challenging while in the "off" state. Subjects with mild PD were generally still able to maintain an adequate mouth seal and communicate effectively when "off" medication. However, there were still ethical considerations in withholding levodopa medication in these subjects for extended periods. The timing of testing and medication withdrawal was designed to be the least disruptive to the patient's usual regimen.

The subjects were asked to arrive at the laboratory very early in the morning prior to their first morning levodopa dose (around 7-8 am). The last evening dose was typically around 7 pm. Therefore subjects were initially tested following a minimum 12 h dopaminergic therapy washout. Levodopa has a short plasma half-live of approximately 50 min, and when combined with carbidopa, is extended to approximately 90 min. To monitor the effect of medication, tests were repeated at least one h, and no longer than 120 min, after directly observed therapy.

The responses, in the presence and absence of usual dopaminergic therapy, were studied in each subject on the one day. Within-day individual variability is significantly less than between-day variability (81). In addition, Spengler found a strong circadian effect on the HCVR (113), with Sahn and colleagues finding no significant systematic change in the hypoxic or hypercapnic response over a two h period (81). This suggests the lack of a training effect or an interaction between measurements, and when tests are performed on the same day within a short time period, environmental, psychological and metabolic variability are kept to a minimum.

7.1.4 Aim

The aim of this study was to investigate whether abnormalities in central respiratory drive are identifiable in subjects with exclusively mild PD by standard criteria. The ventilatory and cardiovascular/sympathetic response to hypoxia and ventilatory response to hypercapnia were compared to healthy normal subjects. In the PD patients, the effect of dopaminergic medication on these responses was assessed.

7.2 Methods

7.2.1 Subjects

Patients with a diagnosis of mild idiopathic PD were recruited from Concord Hospital PD Outpatient Clinic, following direct identification by their treating Neurologist (Dr Michael Hayes). Patients were excluded if they had any clinically significant respiratory or cardiovascular disease, neurological disease other than PD, a significant smoking history (>10 pack years), structural abnormalities of the chest wall, recent upper respiratory tract infections or claustrophobia.

All testing and subsequent data analysis was conducted by the same Scientific Officer (myself, L.S.). Data collection was made with the assistance of Peter Rogers, Respiratory Scientist, Concord Hospital.

Healthy normal subjects were recruited with exclusion criteria as per PD subjects as described in Chapter 4.4.

7.2.2 Disease severity assessment scales

A PD history was documented by a Physician (Dr Claude Farah) (Appendix A iii). This included details of their current medications, time of last medication dose, time since onset of PD symptoms and time since PD diagnosis. The same Physician performed a PD disease severity assessment using the motor examination section of the unified PD rating scale (UPDRS) (166), the (modified stage 0-5) H&Y scale (150) and the modified Schwab and England Activities of Daily Living Scale (198). These were performed on the day of testing immediately following medications.

7.2.3 Respiratory flow, volumes and muscle function

Spirometry and lung volumes (determined via plethysmography) were measured according to American Thoracic Society/ European Respiratory Society criteria (Vmax Encore, Sensormedics, Yorba Linda, CA) (29, 63). Predicted values were derived from the recommendations of the European Community for Coal and Steel (64).

Respiratory muscle strength was determined using methods and predictive reference equations as described by Black and Hyatt (167).

7.2.4 Progressive poikilocapnic hypoxia (pHVR)

After resting, while seated breathing room air for at least 20 min, subjects performed the pHVR protocol as described in Chapter 4.3.4.

7.2.5 Progressive isoxic hypercapnia (iHCVR)

After resting, while seated breathing room air for at least 20 min, subjects performed the iHCVR protocol as described in Chapter 4.3.3.

7.2.6 Protocol design

Patients attended the laboratory on a single visit. They were asked to arrive early in the morning, prior to their first standard dopaminergic therapy of the day. Patients were also asked to abstain from caffeine that morning.

After respiratory function measurements and rebreathing tests as described above (at least one h apart), the patient took their usual morning medications (directly observed). The two rebreathing tests were then repeated more than one h later and at least one h apart.

Healthy normal subjects performed spirometry and the two rebreathing tests at least one h apart.

Statistical analysis

Results are expressed as mean (SD) unless otherwise stated. Individual responses were assessed using Pearson's correlation and linear regression as per Chapter 4. Responses in the PD subjects before and after dopaminergic therapy were assessed using paired,

two-tailed, t-tests. PD responses were compared to the healthy normals using unpaired, two-tailed, t-tests. The association between $\dot{V}_E$ and HR response to progressive hypoxia was determined using a correlation analysis. An association between age and PTT, HR, and $\dot{V}_E$ response was determined using correlation analysis. A P value <0.05 was considered significant.

The study was reviewed and approved by the Human Ethics Review Board of the Sydney South West Area Health District (New South Wales, Australia). Each subject gave written informed consent.

7.3 Results

7.3.1 Subjects

Twelve (two female) subjects with PD and 24 (seven female) healthy normal subjects as described in Chapter 4 were studied. All subjects' demographics and baseline parameters are presented in Table 7.2. There was no difference in height, weight or resting blood pressure between either group (P>0.05). The PD group was older than the normal group (P<0.02).

Table 7.2. Subject demographics and resting parameters in 12 subjects with mild Parkinson's disease and 24 healthy normals.

	Mild Parkinson's	Healthy normal	P value
Male:female	10:2	17:7	
Age *years*	63 (5)	42 (15)	0.01
Height *cm*	175 (7)	173 (10)	0.70
Weight *kg*	80 (14)	72 (13)	0.08
SBP *mmHg*	126 (12)	125 (6)	0.78
DBP *mmHg*	84 (7)	75 (9)	0.05

Data are mean (SD). SBP; systolic blood pressure, DBP; diastolic blood pressure

7.3.2 Disease severity assessment scales

Disease severity classifications averaged the group as very mild, in the early stage of disease. Subjects had been first diagnosed with PD 5.7 (3.0) years prior.

H&Y scales averaged 1.5 (0.7), with a range of 0-2.5 [scale range Stage 0 – Stage 5]. A H&Y of 1.5 relates to a stage of *"unilateral disease including axial involvement, prior to impairment in balance"*.

UPDRS motor section averaged 9.3 (4.6), with a range of 4-18 [scale range 0 -56]. Schwab and England score was an average 90 (10) % with a range of 70-100 % [scale range 0 – 100 %]. A Schwab and England score of 90 % assesses the patient as *"completely independent, able to do chores with some degree of slowness, difficulty and impairment - beginning to be aware of difficulty"*.

All subjects were currently taking levodopa. Supplementary PD medications included dopamine agonists (n=7), anti-cholinergics (n=1) and MAO-B inhibitors (n=2).

7.3.3 Respiratory flow, volumes and muscle function

Spirometry data are presented in Table 7.3. Spirometry values both absolute and as a per cent predicted for the PD subjects are were not different to the normal group (P>0.24).

Table 7.3. Spirometry in 12 subjects with mild Parkinson's disease and 24 healthy normals.

	Mild Parkinson's	Healthy normal	P value
Male:female	10:2	17:7	
FEV_1 *L*	3.29 (0.62)	3.68 (0.89)	0.10
FEV_1 *% predicted*	106 (15)	105 (13)	0.83
FVC *L*	4.27 (0.82)	4.62 (1.20)	0.23
FVC *% predicted*	109 (15)	109 (14)	0.98
Ratio *%*	78 (7)	81 (8)	0.31

Data are mean (SD). FEV_1; forced expiratory volume in one s, FVC; forced vital capacity.

All PD plethysmography parameters are presented in Table 7.4. All values were within normal limits, except for two subjects with a residual volume below the lower limit of normal. Maximal respiratory pressures were below the lower limit of normal in nine PD subjects (75 %).

Table 7.4. Respiratory function in 12 patients with mild Parkinson's disease.

	Absolute	**% predicted**
TLC *L*	6.47 (1.49)	98 (12)
RV *L*	2.13 (0.87)	89 (29)
MIP cmH_2O	64 (22)	65 (20)
MEP cmH_2O	97 (50)	57 (25)

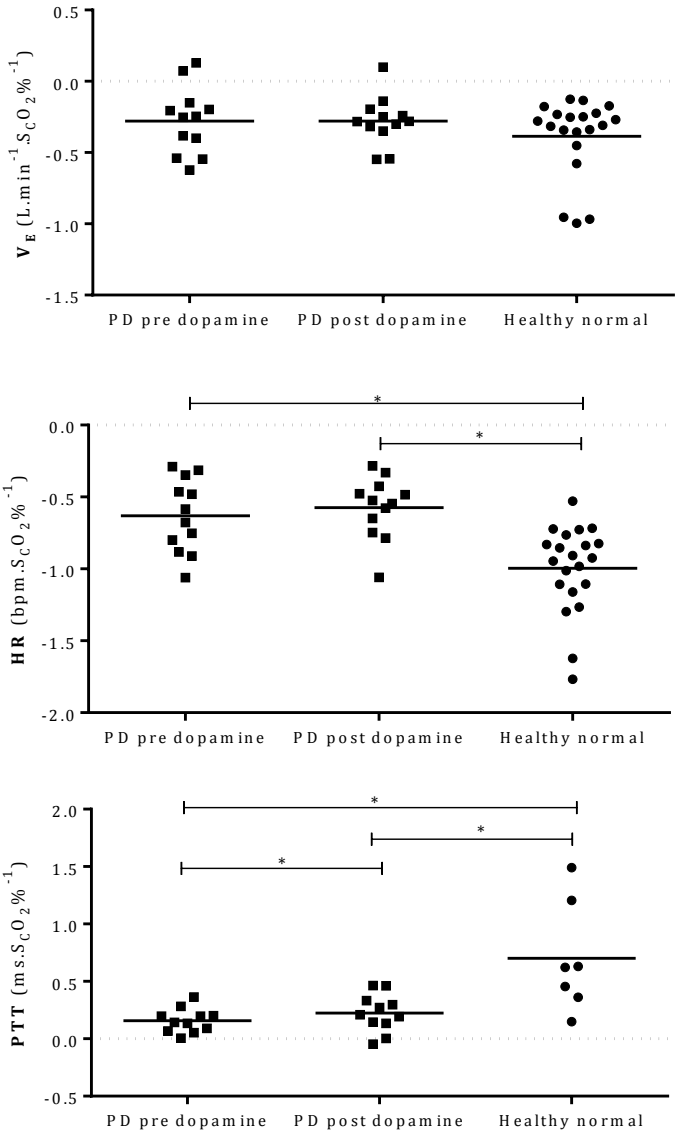
Data are mean (SD). TLC; Total lung capacity, RV; residual volume, MIP; maximal inspiratory pressure, MEP; maximal expiratory pressure

7.3.4 Progressive poikilocapnic hypoxia (pHVR)

The pHVR response was determined in 12 (2 female) mild PD (before and after dopamine) and 22 (6 female) normal subjects, with ventilatory response interpretable in all PD subjects and 20 (6 female) subjects. Resting ventilatory parameters were not different between or within groups prior to the rebreathing tests (P>0.15). These parameters included $\dot{V}_E$, $\dot{V}O_2$, $P_{ET}CO_2$, $FECO_2$ and RER. In the mild PD group the response to S_CO_2 before ("pre") and after ("post") dopaminergic therapy for $\dot{V}_E$ was -0.28 (0.23) Vs -0.28 (0.17) $L.min^{-1}.S_CO_2\%^{-1}$ [P=0.98], for HR was -0.63 (0.26) Vs -0.57 (0.21) $bpm.S_CO_2\%^{-1}$ [P=0.47] and PTT was 0.57 (0.40) Vs 0.96 (0.51) $ms.S_CO_2\%^{-1}$ [P<0.03].

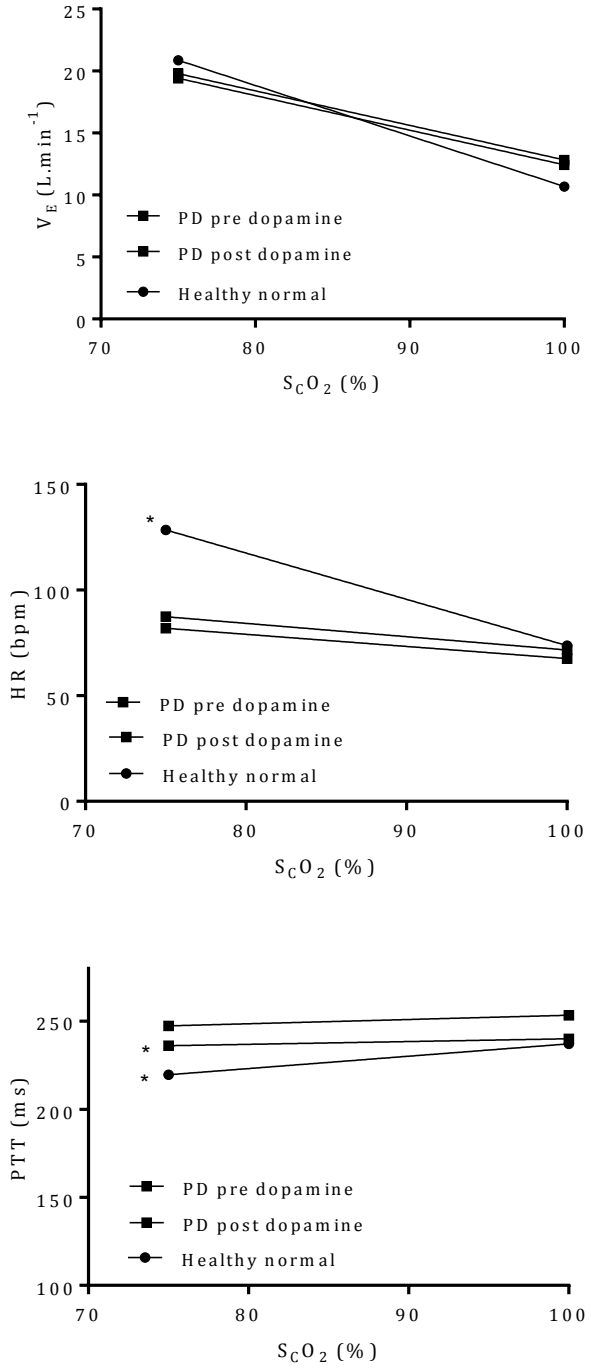
Compared to normal subjects, the ventilatory responses in mild PD "pre" were not different (-0.28 Vs -0.39 $L.min^{-1}.S_CO_2\%^{-1}$, P=0.27), but there were a significantly reduced HR (-0.63 Vs -1.0 $bpm.S_CO_2\%^{-1}$, P<0.01) and PTT (0.57 Vs 1.56 $ms.S_CO_2\%^{-1}$, P<0.001) response in the PD group as compared to the normals.

The mean and individual regression slopes for $\dot{V}_E$, HR and PTT during progressive hypoxia are presented in Figure 7.2. The group mean of the individual linear regression at 75 and 100 S_CO_2% for $\dot{V}_E$, HR and PTT are presented in Figure 7.3.



S_CO_2; calculated oxygen saturation, PD; Parkinson's disease. Horizontal bars indicate mean values. *P<0.03, *N=12 PTT, 20 $\dot{V}_E$ healthy normal.*

Figure 7.2. Mean (bar) and individual regression slopes for minute ventilation ($\dot{V}_E$), heart rate (HR) and pulse transit time (PTT) during progressive hypoxia in 12 subjects with mild Parkinson's disease before (pre) and after (post) dopaminergic therapy and 22 healthy normals.



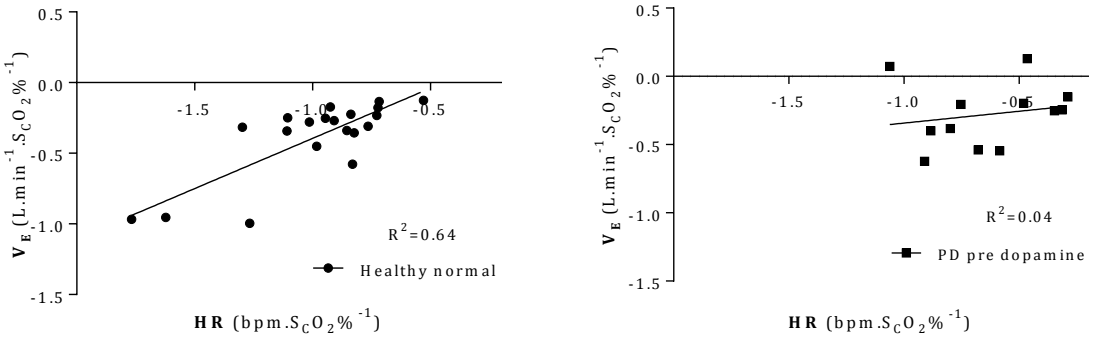
S_CO_2 calculated oxygen saturation, PD; Parkinson's disease. *Vs PD "pre" (P<0.03). *N=12 PTT, 20 $\dot{V}_E$ healthy normal.*

Figure 7.3. Mean group response for minute ventilation ($\dot{V}_E$), heart rate (HR) and pulse transit time (PTT) during progressive hypoxia in 12 subjects with mild Parkinson's disease before (pre) and after (post) dopaminergic therapy and 22 healthy normals.

Compared to normal subjects, the ventilatory responses in mild PD were not different, but there were a significantly reduced HR (P<0.01) and PTT (P<0.001) response in the PD group as compared to the normals. There was no change in $\dot{V}_E$ or HR response before or after dopaminergic therapy in the PD group (P>0.5). PTT response increased following dopaminergic therapy (P<0.03), however "post" PTT response was still significantly reduced when compared healthy normals (P<0.03).

Correlation analysis between age and $\dot{V}_E$, HR and PTT response amongst the normal group were non significant (P=0.77, 0.36, 0.89 respectively).

A strong correlation between the $\dot{V}_E$ and HR response to S_CO_2 % was seen in the normal group (R^2= 0.64, P<0.0001), but not the PD group, either before (R^2= 0.04, P=0.55) or after dopaminergic therapy (R^2= 0.07, P=0.4) (Figure 7.4).



S_CO_2 calculated oxygen saturation, PD; Parkinson's disease.

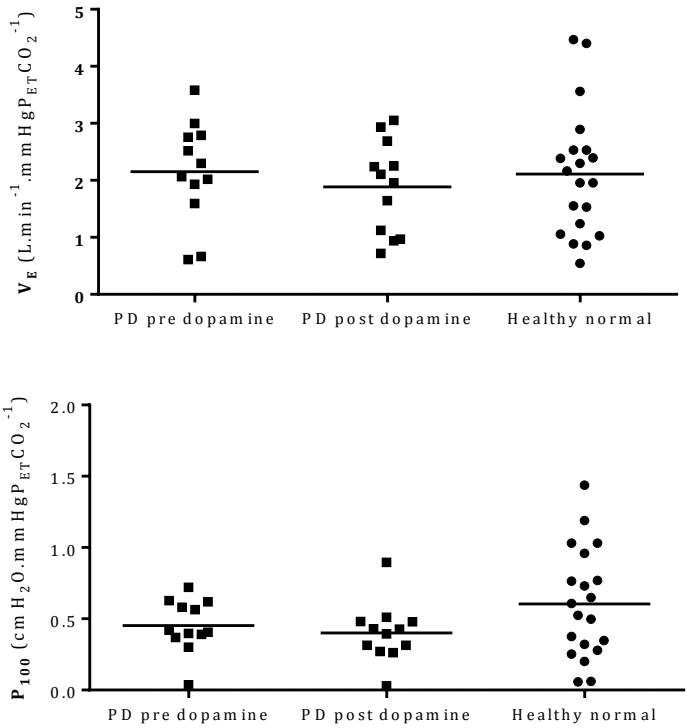
Figure 7.4. The relationship between ventilation ($\dot{V}_E$) and heart rate (HR) response to calculated oxygen saturation (S_CO_2) in 20 healthy normal subjects and 12 patients with mild Parkinson's disease before (pre) dopaminergic therapy.

7.3.5 Progressive isoxic hypercapnia (iHCVR)

iHCVR was determined in 12 (2 female) mild PD subjects ("pre" and "post" dopaminergic therapy) and 20 (7 female) healthy normal subjects.

$\dot{V}_E$ response in the mild PD group was not different "pre" or "post" dopaminergic therapy (2.15 [0.98] Vs 1.89 [0.81] L.min^{-1}.mmHg$P_{ET}CO_2^{-1}$, P=0.19). P_{100} was also not different "pre" or "post" dopaminergic therapy (0.45 [0.18] Vs 0.40 [0.20] cmH_2O.mmHg$P_{ET}CO_2^{-1}$, P=0.45). Compared to the normal group, $\dot{V}_E$ (normal 2.11 [1.11]

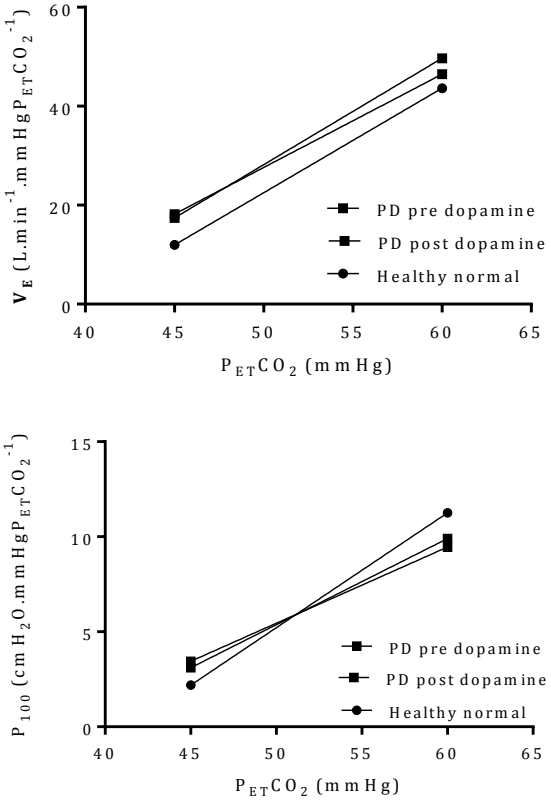
L.min^{-1}.mmHg$P_{ET}CO_2^{-1}$, P=0.92, 0.54) and P_{100} (normal 0.60 [0.38] cmH_2O.mmHg$P_{ET}CO_2^{-1}$, P=0.21, 0.10) were not different to mild PD subjects either "pre" or "post" dopaminergic therapy. Individual data points are presented in Figure 7.5.



$P_{ET}CO_2$; pressure of end-tidal CO_2, PD; Parkinson's disease.

Figure 7.5. Mean (bar) and individual regression slopes for minute ventilation ($\dot{V}_E$) and occlusion pressure (P_{100}) during progressive hypercapnia in 12 subjects with mild Parkinson's disease before (pre) and after (post) dopaminergic therapy and 20 healthy normals.

The group mean of the individual linear regression at 45 and 70 mmHg$P_{ET}CO_2$ for $\dot{V}_E$ and P_{100} are presented in Figure 7.6.



$P_{ET}CO_2$; pressure of end-tidal CO_2, PD; Parkinson's disease.

Figure 7.6. Mean group response for minute ventilation ($\dot{V}_E$) and occlusion pressure (P_{100}) during progressive hypercapnia in 12 subjects with mild Parkinson's disease before (pre) and after (post) dopaminergic therapy and 20 healthy normals.

7.4 Discussion

The sympathetic/cardiovascular response to progressive hypoxia was reduced in those with mild PD as compared to healthy normals. The ventilatory and cardiovascular response to hypoxia and the ventilatory response to hypercapnia in subjects with mild PD were not affected by their usual clinical dopaminergic therapy.

7.4.1. Ventilatory limitation in mild Parkinson's disease

Spirometry and lung volumes in the PD subjects were all within normal limits and were not different to the healthy normal group. Respiratory muscle strength was below the lower limit of normal, but not to the extent where pulmonary restriction was seen. In other clinical settings, this degree of muscle weakness would not be expected to cause

ventilatory impairment. Therefore this group with mild PD did not present with any peripheral ventilatory limitation.

The mild PD group in this study had similar ventilatory response to both hypoxia and hypercapnia to healthy normal subjects. The previous observations of reduced ventilatory chemosensitivity to hypoxia and hypercapnia have been demonstrated in more severe PD (8, 153, 162).

7.4.2. Sympathetic/cardiovascular responses during progressive hypoxia

In contrast to the normal ventilatory responses to hypoxia and hypercapnia, there was a reduced sympathetic/cardiovascular response to progressive hypoxia in mild PD. This had not been previously reported but is consistent with Braak's hypothesis that autonomic dysfunction can be an early feature of PD. This is likely to be a consequence of the post-ganglionic sympathetic cardiac denervation that had been previously observed in mild PD (182). In health the cardiovascular and ventilatory responses during progressive hypoxia are strongly linked (96). The discordance between HR and $\dot{V}_E$ responses in mild PD in this study is consistent with early autonomic cardiac changes.

A fall in PTT and an increase in HR were reliable markers of increased sympathetic and cardiovascular activity. A decrease in PTT correlates well with increasing systolic blood pressure, is representative of both vascular and cardiac activity and can be considered a measure of sudden transient hemodynamic changes (105-107) (as discussed in Chapter 4.2.5). The strong linear response observed between PTT and S_CO_2 in both the normal and PD subjects (mean R^2=0.6) indicate the utility of PTT as an effective, non-invasive, beat-to-beat measure of vascular response that had not been previously monitored during rebreathing protocols.

7.4.3. The effect of dopaminergic therapy

Low dose *infused* dopamine can alter the regulation of ventilation and sympathetic nerve activity at the level of the carotid bodies (183) and inhibits the hypoxic chemosensory discharge rate in healthy normal subjects (92, 175, 184, 185). Olson and colleagues reversed this depressant effect with the dopamine-receptor blocking agent prochlorperazine mesylate (176). Previous reports of reduced chemosensitivity in PD did not discount an effect from the subjects' clinical dopaminergic therapy. In the

previous Chapter 6, patients were tested “on” medication due to ethical and technical reservations about testing those with moderate PD in the “off” state (8).

All mild PD subjects in this study had their medication, which included levodopa, withheld for a minimum of 12 h. Ventilatory parameters before and after dopaminergic therapy, at rest, during hypoxia and hypercapnia, were unchanged. HR response to hypoxia was also unchanged before and after dopaminergic therapy however there was an increase in PTT response. The significance of this is unclear however both responses were significantly reduced when compared to healthy normals. It can be concluded that at the doses used in mild PD, dopaminergic therapy does not have an effect on ventilatory control.

7.4.4. Protocol considerations

The sympathetic/cardiovascular measures during progressive hypoxia in this study appeared to be more sensitive in detecting early autonomic dysfunction than minute ventilation. This may be partially explained by the decision not to control carbon dioxide during the progressive hypoxic protocol. During poikilocapnic hypoxia, the ventilatory response was attenuated in comparison to iso- or hypercapnic hypoxia (98, 153). This was due to the concomitant effects of hypocapnia from hypoxic-induced hyperventilation. However, poikilocapnic (i.e. uncontrolled inspired carbon dioxide) hypoxia has greater applicability to a clinical physiological and pharmacological intervention. Poikilocapnic hypoxia elicits similar (relative to ventilation) changes in HR and mean arterial blood pressure as isocapnic hypoxia (199).

The healthy normal group was younger than the PD group. Correlation analysis did not identify an age effect within the normal group, which is consistent with previous evidence that hypoxic ventilatory drive, carotid body function and pulse wave velocity is unaltered with age (95, 109, 116, 117).

All tests of each PD subject were performed on the same day within a 2-3 h period to minimise environmental, physiological, technical and emotion variation (81). The ventilatory and HR responses to progressive hypoxia and hypercapnia in the PD subjects before and after therapy were not different. This reflects low intra-subject variability.

7.5 Conclusion

In conclusion, sympathetic/cardiovascular responses were reduced in subjects with mild PD and these markers appear more sensitive than ventilatory responses in detecting the effects of non-motor brainstem involvement in mild PD. Dopaminergic therapy used n mild PD does not affect ventilatory or HR response to hypoxia or ventilatory response to hypercapnia. These novel observations from the chosen methodology provide further evidence that autonomic dysfunction is an early feature of PD and that the abnormalities seen in mild PD were an effect of PD and not its treatment.

7.6 Resulting publication (Appendix C)

Seccombe LM, Rogers PG, Hayes MW, Farah CS, Veitch EM, Peters MJ. Reduced hypoxic sympathetic response in mild Parkinson's disease: further evidence of early autonomic dysfunction. Parkinsonism Relat Disord. 2013 Jul;19:1066-8.

CHAPTER 8

DETRENDED FLUCTUATION ANALYSIS OF HEART RATE DURING HYPOXIA

– A FURTHER MEASURE OF AUTONOMIC FUNCTION

8.1 Background

Sympathetic/cardiovascular responses measured during progressive hypoxia were less affected by volitional override in elite freedivers (Chapter 5) and were more sensitive in detecting early autonomic dysfunction in mild PD (Chapter 7) than ventilatory parameters.

The study of heart rate variability (HRV) provides insight into cardiac autonomic regulation, both in health and in disease. This Chapter provides a comprehensive analysis of the electrocardiograph (ECG) data that was collected on the study subjects as an additional measure of autonomic function. It is postulated that the analysis of HRV at rest and during hypoxia induced sympathetic vasomotor activity will support the previous observation of cardiac autonomic dysfunction in subjects with mild PD (that is not affected by dopaminergic therapy) and normal autonomic function in elite freedivers. The utility of the non-linear method for the analysis of HRV, detrended fluctuation analysis (DFA), as a simple, short-duration and non-invasive measure of autonomic function is considered.

8.1.1 Clinical utility of heart rate variability analysis

In the past three decades, HRV has emerged as a useful marker to quantify autonomic activity, as a description of vagal function. HR and rhythm are largely under the control of the autonomic nervous system. The parasympathetic influence of HR is mediated via release of acetylcholine by the vagus nerve. The sympathetic influence is mediated by the release of epinephrine and norepinephrine (200). Under resting conditions variations in HR are largely dependent on vagal modulation and the vagal and sympathetic activities constantly interact. Modulation occurs by central (e.g. vasomotor and respiratory centres) and peripheral (e.g. oscillation in arterial pressure and respiration) oscillators (201).

A reduction of HRV has been observed in several pathological conditions. It has been primarily used in patients with cardiac disorders, where a strong association between reduced HRV and increased cardiac mortality has been recognised (200). HRV is a strong and independent predictor of mortality following an acute myocardial infarction (202-204). In cardiac disorders, it is thought that the depressed HRV reflects a decrease

in vagal activity that leads to a predominance of sympathetic activity. Similarly, patients with cardiac failure have reduced HRV, with sympathetic activation, elevated HR's and high levels of circulating catecholamines (205). Therapeutical interventions to augment HRV are to improve cardiac electrical stability.

A reduction in HRV precedes the clinical expression of autonomic neuropathy in patients with diabetic neuropathy, and therefore is seen as an early marker of disease (206). Patients with both neuropathy and tetraplegia that have reduced HRV have a minimal low frequency component of HR using spectral analysis which suggests reduced sympathetic activity (200).

For the analysis presented in this Chapter, it was postulated that early cardiac denervation in patients with PD may display reduced HRV due to autonomic dysfunction that is not affected by dopaminergic therapy. It is also postulated that elite freedivers will have a robust HRV, reflective of good health.

8.1.2 History of the analysis of heart rate variability

The invention of the "Physician's Pulse Watch" in 1707 (a watch with a second hand that could be stopped) allowed the accurate assessment of cardiac pulse rate changes. In 1847, Carl Ludwig first described respiratory sinus arrhythmia (RSA). He observed the temporal fluctuation in HR that exhibit a marked synchrony with respiration; increasing during inspiration and decreasing during expiration. The advent of digital signal processing techniques in the 1960's allowed the complex investigation of HRV and its relationship to health and disease (207).

Hon and Lee described the clinical relevance of HRV in 1965. They noted that foetal distress was preceded by alterations in inter-beat intervals prior to any appreciable changes in HR (208). By the 1980's it was confirmed that HRV was a strong and independent predictor of mortality following an acute myocardial infarction (202-204).

Due to the precision required, HRV is measured from the R wave of the QRS complex (rather than from a pulse wave), representing ventricular depolarisation, as recorded by ECG (Figure 8.1). HRV refers to the beat-to-beat variation in the R-R interval over time (Figure 8.2). A robust periodic change in the R-R interval is a hallmark of health (200).

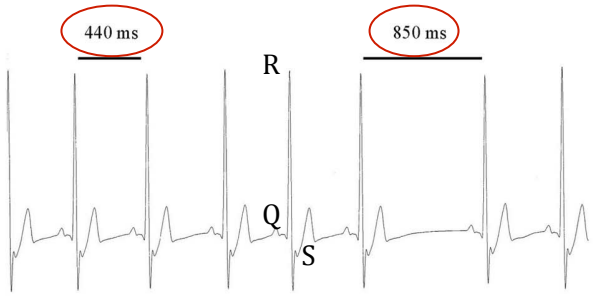


Figure 8.1. An example of varying R-R intervals recorded from a QRS complex using electrocardiography.

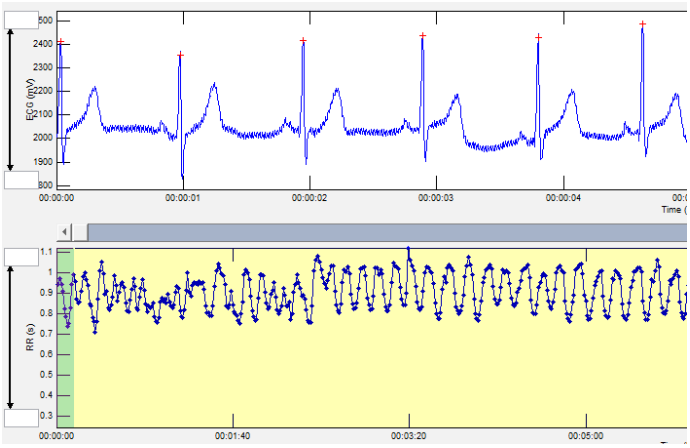


Figure 8.2. An example of R-R interval variability over a 6 min period in a healthy subject. Digital processing identifies the R wave (+) and plots R-R interval with time. *Respiratory sinus arrhythmia is evident.*

Both HRV and RSA are thought to reflect changes in cardiac autonomic regulation. The mechanism underlying the fluctuation and the exact contributions of the parasympathetic and sympathetic divisions of the autonomic nervous system to HRV are not totally understood, but appear to be related primarily to countervailing neuroautonomic inputs. The non-linear interaction (competition) between the sympathetic and parasympathetic branches of the autonomic nervous system is the postulated mechanism for the type of erratic HRV recorded in healthy subjects (207). Similarly, autonomic failure is suggested with a reduced HRV as discussed in 8.1.1.

8.1.3 Quantification of heart rate variability

The application of HRV for quantifying autonomic functions has gained recent popularity, particularly because of its non-invasiveness and ease of measurement. Techniques have been developed to provide insight into these processes and their contribution to cardiac autonomic regulation. The primary linear methods of HRV quantification are time and frequency domain analysis. Recently, non-linear methods have been introduced that do not require a stationary signal.

i. Time domain analysis

"Simple" time domain variables are calculated from the normal-to-normal (NN) intervals (QRS complexes). These include the mean NN interval, mean HR, the difference between the longest and the shortest NN interval, the difference between states (e.g. night and day). Other measures include variations in instantaneous HR secondary to autonomic stimulation; such as valsalva manoeuvres, head-tilt and certain medications (200).

Statistical methods of time domain analysis are more complex and are calculated from longer time periods, traditionally over 24 hours. The most common variable is the standard deviation of the NN interval (SDNN), i.e. the square root of the variance. As the total variance of HRV increases with the length of the analysed recording, comparisons can only be made from similar recording durations (207).

Geometric methods involve converting the series of the NN intervals into a geometric pattern. This includes the sample density distribution of NN durations and sample density distribution of the differences between adjacent NN intervals. Geometric methods require long recording durations, preferably 24 h (200).

ii. Frequency domain analysis

Linear HRV in frequency domain employs mathematical algorithms for frequency assignment. Power spectral density analysis is a commonly used frequency domain analysis that provides the basic information of how power (variance) distributes as a function of frequency. Total or absolute power (ms^2) is decomposed into high (RSA-typically parasympathetic), low and very low frequency bands (207, 209).

iii. Non-linear methods

In the 1980's, evidence indicated that HR was not the product of a regular periodic oscillator (sine wave) but displayed complex non-linear dynamic behaviour. Analytical approaches based on Chaos Theory and fractal maths were employed to evaluate HRV. Chaos Theory describes an underlying order in a seemingly random varying sequence of events. There is seemingly aperiodic behaviour, however with a subtle but regular pattern (210). Importantly, these techniques do not measure HRV magnitude, but provide an estimate of its complexity (207). The analysis of nonparametric, nonlinear, short-time series includes DFA.

8.1.4 Detrended fluctuation analysis (DFA)

DFA has proven to be an effective research tool across many disciplines, uncovering long-range power–law correlations in bioinformatics, economics, meteorology, material science and ethology. Peng and colleagues described DFA as a method for quantifying long-range correlation behaviour properties in beat-to-beat fluctuation in HR in a non-stationary physiological time series (211).

DFA is a modified root mean square analysis of a "random walk" (a path that consists of a succession of random steps), aimed to differentiate between sources of HR heterogeneity; i.e. whether HR fluctuation arise from a complex nonlinear dynamical system (intrinsic) or from environmental stimuli. It was considered that only the fluctuation arising from the dynamics of the complex, multi-component system should show long-range correlations. Other responses, or "noise", although physiologically important, can be treated as "trend" – giving rise to a short-scale fluctuation. The method proposed by Peng and colleagues was validated on control time series and initially applied to detect long-range correlations in highly heterogeneous DNA sequences (211).

Calculation

Briefly, DFA is a scaling analysis method to represent the correlation properties of a signal. The calculated time series is separated into equal boxes of length "n", and in each box, the local trend is calculated and is subtracted from the time series. The calculated root-mean-square (RMS) of this integrated detrended series is called "F(n)". Scaling or

fractal correlation is present if the data are linear on a graph of log F(n) versus log(n) (211). The slope of the graph has been termed the scaling exponent. Usually, more than one scaling exponent can be derived from a graph (Figure 8.3).

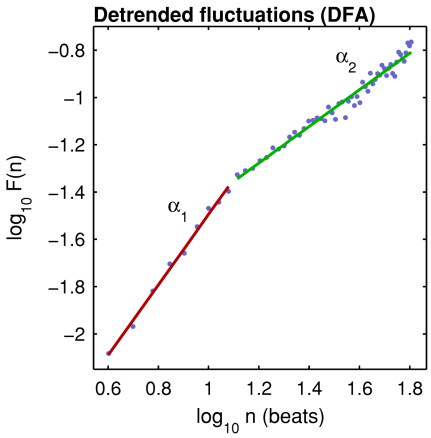


Figure 8.3. An example of scaling components or fractal correlations on a graph of log F(n) versus log(n) in a healthy subject. *Two scaling exponents, α1 and α2 are evident.*

The advantage of DFA over other measures of HRV (including spectral analysis) is that it detects long-range correlations embedded in a (seemingly) non-stationary time series. It also avoids the spurious detection of apparent long-range correlations that are an artefact of the non-stationary (207).

Crossover phenomenon

When using substantially shorter recording time periods in their initial applications, Peng and colleagues observed a crossover phenomenon associated with a change in short and long scaling exponents (Figure 8.3, 8.4) (211).

Alpha 1 (α1) is the exponent estimated from short-scale fluctuation (α1; 2≤n≤16 beats), and alpha 2 (α2) is estimated from long-range correlations (α2; 16≤n≤64 beats). It is postulated that in health, over short-range scales, the physiologic inter-beat interval fluctuation is dominated by the relatively smooth RSA, thus giving a high α1 (<1). For longer scales, the inter-beat fluctuation reflects the intrinsic dynamics of a complex system (Figure 8.3). A value close to 1 indicates the existence of fractal like correlations. The loss of fractal-like HR dynamics may indicate a divergence from normal HR behaviour (101).

In pathology however, the crossover pattern is different. In the short-range scales, the fluctuation is quite random and uncorrelated (close to white noise: α1 ≈ 0.5). In long-range scales, the fluctuation becomes smoother (approaching Brownian noise α2 ≈1.5) (Figure 8.4). Although the mechanisms are unclear, the differences in these scaling properties are suggested to be of use in distinguishing healthy from pathological data sets. Certain disease states may be accompanied by alterations in this scale-invariant (fractal) correlation property. Marked changes in short-range HR dynamics were observed by Peng and colleagues in heart failure patients as compared to normal (Figure 8.4) (211).

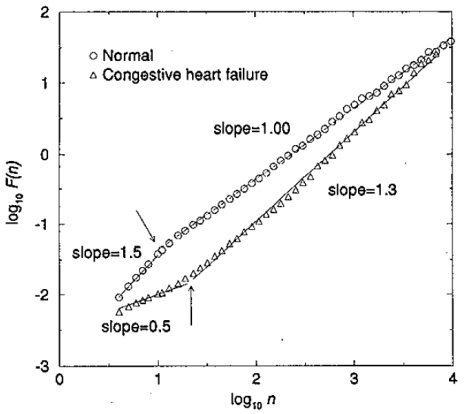


Figure 8.4. Plot of log F(n) Vs log (n). *Arrows indicate "crossover" point in scaling, reflecting a change in short and long scaling exponents. Image from Peng* (211)

When assessing the scaling components relationship (α1/α2), there was a good separation between the healthy and the heart disease subjects (Figure 8.5). A "reverse" crossover of these scaling components was termed when the α1/α2 ratio was <1. This occurred in > 70 % of the subjects with heart disease (211).

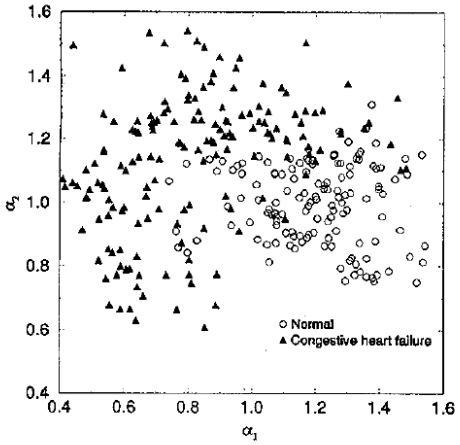


Figure 8.5. Scatterplot of scaling exponents α1 and α2. *Note clustering of data points separating the healthy (α1/α2 >1) and heart failure (α1/α2<1) subjects. Image from Peng and colleagues* (211).

α1 has been identified as an independent predictor of mortality in patients with heart failure (212) and of non-fatal acute cardiac events following myocardial infarction (213). In these studies, an α1 of <0.9 and ≤1.025 respectively, related to an elevated risk ratio. Echeverria and colleagues found repeatability of α1 measurements collected ≈ 60 days apart in children with congenital hypothyroidism (214).

Short- versus long-term recordings

The analysis of HRV using linear methods typically requires 24 h ECG recordings. For DFA, group mean α1calculated from short segments (<300 beats, 5-10 min) have good agreement with long-term recordings (24 h) (215). Due to increased statistical variation with short segments, they should not be used for individual risk stratification, however for group comparisons, segments of < 300 beats were suitable (216). Scaling exponents from short segments were repeatable if performed at a similar time of day due to an identified diurnal variation (217).

The effect of autonomic stimulation.

Vagal tone influences α1. There is an association between accentuated sympathovagal interaction and reduced short-term fractal organisation of HR in health. There is evidence that α1 decreases with vagal activation; during cold face immersion (218) and

noradrenalin infusion (219). Similarly, in health α1 is consistently elevated with reduced vagal and enhanced sympathetic activation; during cold hand immersion (218), passive head-up tilt, exercise (220) and vagal blockade by atropine (221). During coactivation of sympathetic and vagal outflow, HR behaviour is more towards random dynamics in healthy subjects (218).

DFA has been tested during varying physiological conditions using lumped parameter models (222). Consistent with human studies as described above, α1 increased with sympathetic activation or vagal deactivation, however there was no change in α2.

Limitations of DFA

Respiratory parameters can profoundly alter R-R interval variability independent of changes in cardiac autonomic regulation. It has been well established that increases in respiratory frequency reduce the amplitude of HRV. Increases in V_T or static lung volume provoke an increase in the R-R interval variability (207).

Ectopic beats, arrhythmic events, missing data, and noise effects affect HRV. Wherever possible, recordings that are free of ectopic beats, missing data, and noise should be used. The acceptance of only ectopic-free, short-term recordings however may introduce significant selection bias so it is important that recording areas be defined in a standardised fashion before analysis.

An age-related loss of fractal organisation in heart beat dynamics has been recognised. In a study of 10 young (21-34 years) and 10 older subjects (68-81 years), α1 was reduced in the older subjects (0.90 Vs 1.12) (223). The authors suggested that an age-related loss of fractal organisation in heartbeat dynamics may reflect the degradation of integrated physiological regulatory systems. Recently, a high α1 was demonstrated in older subjects that maintain good aerobic capacity. In subjects >65 years, those that regularly undertook aerobic exercise had a higher α1 than sedentary, with mean values similar to that seen in young subjects (> 1.1) (224).

While a seemingly simple tool, with many modern devices able to produce automated measurements of HRV, the significance and meaning is complex, and there is potential for incorrect conclusions.

8.1.5. Heart rate variability in Parkinson's disease

Autonomic nervous system dysfunction is a hallmark of PD as discussed in Chapter's 6 and 7. The accumulation of Lewy bodies is associated with neuronal loss to the sympathetic system structures of the central nervous system, the postganglionic sympathetic system and heart intrinsic neurons and to the parasympathetic system structures of the DMV. The study of HRV in those with PD is predominantly consistent with previous observations of autonomic dysfunction. 24 hour spectral and time domain analysis identified a decrease in HRV that progressed with PD severity (225-227). Kallio and colleagues however reported no difference between PD subjects of mild-moderate severity (H&Y 1-3) and healthy normals using DFA of 10 min resting HR samples (228). As previously mentioned α1 is influenced by vagal tone; increasing with reduced vagal and enhanced sympathetic activation. This was previously induced by cold water hand immersion and head-up tilt manoeuvres. It is postulated that hypoxia induced sympathetic vasomotor activity (229) may identify subtle differences in autonomic function in mild PD.

8.1.6. Heart rate variability in freedivers

There have been several studies investigating HRV in freedivers. It was postulated that the repeated activation of the autonomic nervous system through the dive response, might alter cardiac autonomic function. 24 h time and frequency domain analysis demonstrated higher HRV in elite freedivers as compared to controls. Both sympathetic and parasympathetic tone was higher than controls with a significantly lower mean HR over the 24 h period (230).

HRV as measured by time domain analysis and DFA were measured during static and dynamic apnoea in nine freedivers. While apnoea resulted in some blunting of cardiac vagal activity, not surprisingly, dynamic apnoea presented greater sympathetic activity than static apnoea (231). Time domain analysis during a single apnoea in five freedivers elicited a "biphasic" HR response. The authors explained this as baroreflex stimulation in freedivers as compared to controls (232) however these findings are difficult to interpret.

In healthy subjects who experience hypoxia, as with ascent to altitude, there is increased sympathetic activity as commonly identified with an elevated HR and cardiac output

(102). HRV analysis of three subjects following ascent to 4300 m (Pike's Peak, Colorado) identified both an increase in sympathetic activity and a concomitant decrease in parasympathetic activity (103). During shorter exposures (10 min) to normobaric hypoxia (FIO_2 of 0.15), spectral analysis of the R-R interval identified an increase in sympathetic vasomotor activity in 18 healthy males, however with no change detected in parasympathetic activity (229). Cardiac sympathetic dominance was seen to be responsible for the elevated HR during hypoxia.

There is a paucity of data assessing autonomic function using DFA of HR during sympathetic vasomotor activity comparing healthy subjects with those that may display dysautonomia. DFA is advantageous over other HRV analysis, as it requires only short time series that are not necessarily stationary, therefore do not require steady-state.

8.1.7 Aim

The aim of this study was to determine the utility of DFA in assessing autonomic function in elite freedivers and subjects with mild PD before and after dopaminergic therapy. HR fluctuation at rest and during increasing sympathetic vasomotor activity with progressive hypoxia was compared to healthy, non-diving subjects.

8.2 Methods

8.2.1 Subjects

The data collected on all research subjects that underwent a progressive hypoxic challenge was reviewed and analysed by myself, L.S. in consultation with Dr Kevin Ellyett, Respiratory Physiologist, Auckland District Health Board, New Zealand. Those subjects that had continuous, artefact and arrhythmia free ECG data were included in the analysis. This included healthy normal subjects, elite freedivers, and subjects with mild PD with inclusion/exclusion criteria as described in Chapters 4.4.2, 5.2.1 and 7.2.1 respectively. Exclusion criteria included the use of any medication that may affect the interpretation of HR (e.g. beta-blockers).

8.2.2 Detrended fluctuation analysis (DFA)

Five-lead ECG was collected at rest, room air and continuously during progressive poikilocapnic hypoxia (Alice PDX, Respironics, Murrysville, PA) as previously described

(Chapter 4.3.4). In mild PD subjects, testing was performed prior to, and repeated after (>1 h, <2 h) their usual morning medications (directly observed) as described in Chapter 7.2.6. ECG was stored as a continuous digital trace (sampling rate 200Hz) and was uploaded through a compiler (MATLAB, Version 7.17) for analysis of the R-R interval.

DFA of the R-R intervals, including short- ($\alpha1$; $2 \leq n \leq 16$ beats) and long-range ($\alpha2$; $16 \leq n \leq 64$ beats) scale exponents were calculated using a HRV programme (Kubios HRV V2.1, Finland). Artefact free periods of at least 64 beats were collected while at rest breathing room air ("room air") and during the progressive hypoxia challenge ("hypoxia"). A log F(n) versus log(n) graph was generated for each subject (Figure 8.6).

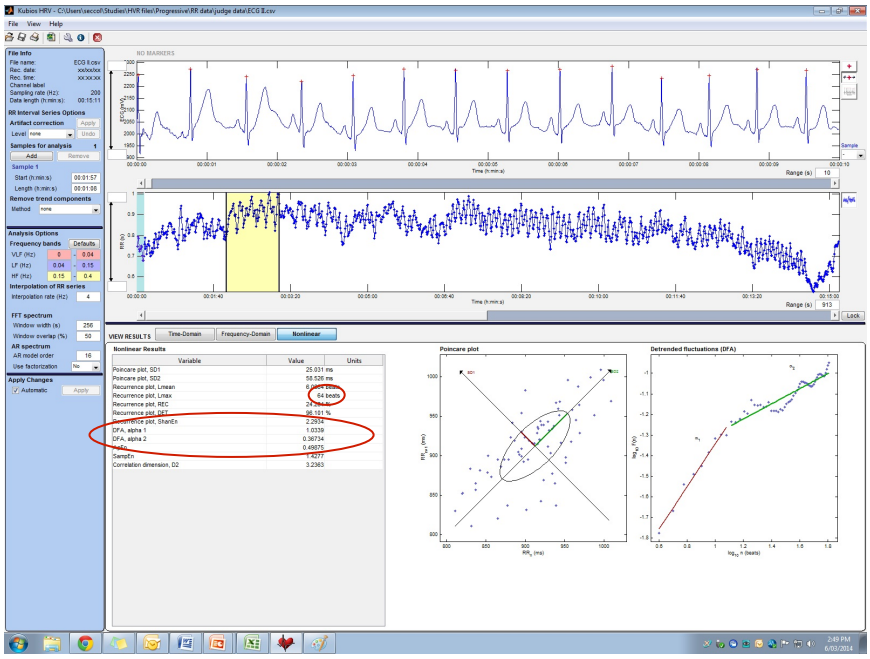


Figure 8.6. An example of a subject's continuous ECG trace uploaded and analysed by the HRV programme. *Yellow shaded area denotes the minimum analysed section (64 beats).*

Statistical analysis

$\alpha1$, $\alpha2$ and mean HR during "room air" and "hypoxia" in the elite freedivers and mild PD subjects were compared to healthy normal subjects using an unpaired, two-tailed, t-test. Within group "room air" and "hypoxia" and dopaminergic therapy comparison was made using a paired, two-tailed, t-test. A correlation analysis determined associations

between α1, α2 and age. A P value<0.05 was considered significant.

8.3 Results

8.3.1 Subjects

The ECG data of 9 healthy normal, 5 elite freedivers and 10 mild PD subjects were included in the analysis. Two PD and three healthy normal subjects were excluded due to excessive ECG artefact. Subject demographics are presented in Table 8.1.

Table 8.1. Subject demographics for 9 healthy normals, 5 elite freedivers and 10 subjects with mild Parkinson's disease.

	Healthy normal	Elite freedivers	Mild Parkinson's disease
Male:female	7:2	4:1	8:2
Age, years	48 [16]	32 [11]*	63 [5]*
Height, cm	176 [9]	182 [10]	175 [8]
Weight, kg	79 [14]	76 [11]	80 [14]

Data are mean [SD]. *Vs Normal (P<0.02)

Subject demographics were not different between groups except for age, which was higher in the subjects with mild PD (P<0.01) and lower in the elite freedivers (P<0.02) when compared to the healthy normal subjects.

8.3.2 Detrended fluctuation analysis (DFA)

R-R interval analysis blocks for "room air" and "hypoxia" were maximised to reduce statistical error, while remaining free of artefact and arrhythmia. Blocks were a mean 276 beats in duration.

HR increased linearly with falling calculated oxygen saturation (S_CO_2) in all groups during the pHVR (mean R^2 = 0.8). However, the HR response was reduced in the PD subjects "pre" dopamine as compared to healthy normals (-0.64 bpm.$S_CO2\%^{-1}$, -0.97 bpm.$S_CO2\%^{-1}$ respectively, P<0.03). HR response was not different "pre" as compared to "post" dopamine (P=0.54). HR response was not different in the elite freedivers (-0.87 bpm.$S_CO2\%^{-1}$) as compared to healthy normal subjects (P=0.41).

Short- and long-range scaling exponents (α1, α2) as calculated from DFA at rest, room air and during hypoxia for the mild PD subjects "pre" and "post" dopaminergic therapy

compared to healthy normal subjects are presented in Table 8.2. Table 8.3 presents the results for the elite freedivers as compared to healthy normal subjects.

Table 8.2. Scaling exponents (α1 and α2) from detrended fluctuation analysis of heart rate at rest, room air and during hypoxia in 10 subjects with mild Parkinson's disease before (pre) and after (post) dopaminergic therapy and 9 healthy normals.

	Mild PD "pre"			Mild PD "post"			Healthy normal		
	α1	α2	α1/α2	α1	α2	α1/α2	α1	α2	α1/α2
Room air	0.83* [0.26]	1.02 [0.16]	0.84* [0.36]	1.05*† [0.25]	1.28*† [0.17]	0.85* [0.24]	1.36 [0.22]	0.89 [0.19]	1.59 [0.46]
Hypoxia	0.86* [0.28]	1.06* [0.18]	0.83* [0.35]	0.98 [0.27]	1.21* [0.21]	0.83* [0.27]	1.23 [0.35]	0.85 [0.21]	1.54 [0.68]

Data are mean [SD], *Vs Normal (P<0.04), †Vs PD "pre" (P<0.02)

Table 8.3. Scaling exponents from detrended fluctuation analysis of heart rate at rest, room air and during hypoxia in 5 elite freedivers and 9 healthy normals.

	Healthy normal			Elite freedivers		
	α1	α2	α1/α2	α1	α2	α1/α2
Room air	1.36 [0.22]	0.89 [0.19]	1.59 [0.46]	1.30 [0.26]	0.53* [0.26]	3.42 [2.90]
Hypoxia	1.23 [0.35]	0.85 [0.21]	1.54 [0.68]	1.36 [0.27]	0.61 [0.25]	3.16 [2.96]

Data are mean [SD], *Vs Normal (P<0.02)

Patients with mild PD (both "pre" and "post" dopamine) had altered scaling exponents as compared to healthy normal subjects (Table 8.2). There was no appreciable difference between "room air" and "hypoxia" in all groups (P>0.7). All parameters were not different in PD subjects "pre" dopaminergic therapy as compared to "post" except for room air α1 and α2 which were elevated (P<0.02).

α1/α2 was consistently reduced in the mild PD subjects as compared to healthy normals (P<0.01). All healthy subjects and elite freediving subjects α1>α2, however there was "reverse crossover" (α1<α2) in 8/10 PD subjects at rest, room air and 7/10 PD subjects during hypoxia before dopaminergic therapy (Figure 8.7). Freedivers were not different

to the healthy normal subjects except for α2 during hypoxia, which was reduced (P<0.02).

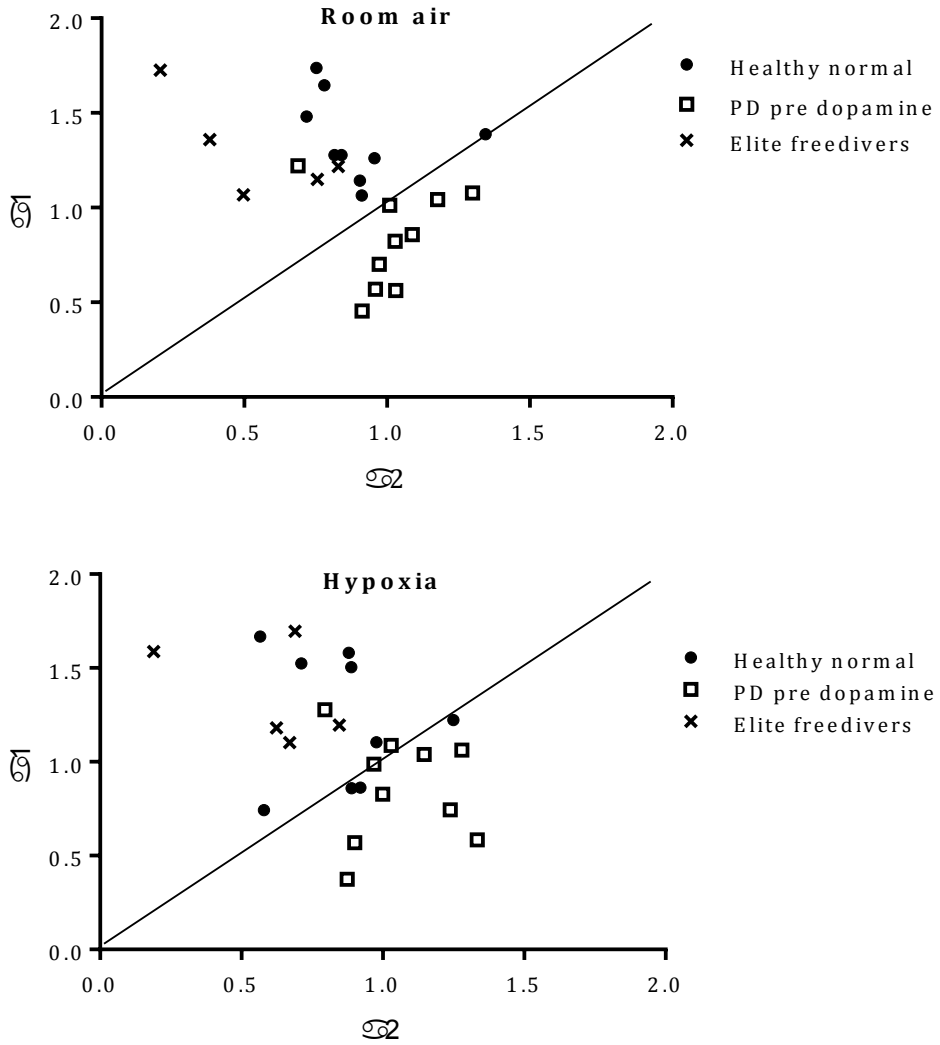


Figure 8.7. Relationship between scaling exponents α1 and α2 while at rest, room air (Room air) and during hypoxia (Hypoxia). *Line is equivalence.*

An example of the calculated log F(n) versus log(n) graph for a normal subject (#5), an elite freediver (#2) and a mild PD subject (#8) during hypoxia is presented in Figure 8.8. The mild PD subject displays a "reverse crossover".

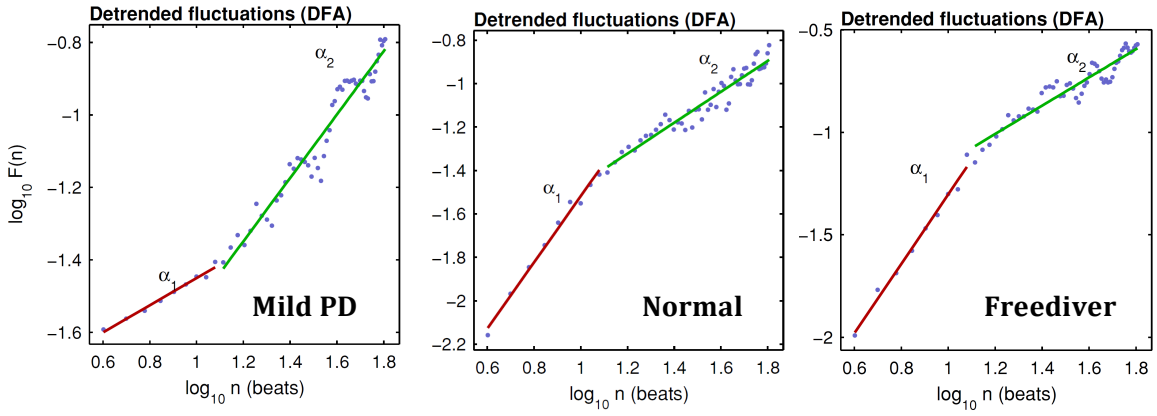


Figure 8.8. Calculated log F(n) versus log(n) graph for a normal, elite freediver and mild Parkinson's disease (PD) subject at rest, room air. *The mild PD subject has a "reverse crossover", where α1<α2.*

There was no correlation between α1, α2 and age at room air in the healthy normal group (P>0.5), however there was a weak correlation between α1 and age following hypoxia ($p<0.05$, $R^2=0.48$).

8.4 Discussion

8.4.1. DFA of heart rate in subjects with mild Parkinson's disease

The results of this analysis show that subjects with mild PD have altered fractal correlation properties of the R-R interval when compared to healthy normal subjects. DFA of HR identified a reduced α1 at rest breathing room air and during hypoxia in subjects with mild PD. The "reverse crossover" pattern (α1<α2) observed in the majority of the mild PD subjects was similar to that observed in patients with dysautonomia (211). DFA of HR was a simple, non-invasive measure that identified a clear distinction between the mild PD and the normal subjects in this study. There was a separation of data points between groups (Figure 8.7) that was not affected by dopaminergic therapy.

A reduced α1 as seen in the mild PD subjects in this study, represents random and uncorrelated fluctuation (211) and was consistent with observations of reduced HRV in PD of varying disease severity using spectral analysis over a 24 h period (225-227). The mechanism by which α1 and/or HRV was reduced and predictive of pathological risk is

not yet defined, but it is likely to involve derangements in neural activity of cardiac origin. One hypothesis suggests that the changes in the geometry of a beating heart due to diseased and noncontracting segments may abnormally increase the firing of sympathetic afferent fibres by mechanical distortion of the sensory endings (233). This sympathetic excitation attenuates the activity of vagal fibres directed to the sinus node. Another explanation is the reduced responsiveness of sinus nodal cells to neural modulations (234).

The observation of abnormal fractal properties of HR in this study of patients with exclusively mild PD suggests DFA as a possible co-marker of early disease and/or disease progression. Haensch and colleagues found no association with HRV and myocardial adrenergic function as measured by MIBG scanning in patients with more severe disease (235). Further investigation of the associations between early myocardial denervation, medullar dysfunction and alterations in HRV is required.

It was postulated that the scaling exponents might be influenced by hypoxia induced sympathetic vasomotor activity. In this study the scaling exponents were not different during hypoxia as compared to at rest breathing room air. Previous studies have shown an increase in α1 following other autonomic stressors such as head-up tilt, cold hand immersion and vagal blockade using atropine (218, 220, 221). While acute hypoxia increases sympathoadrenal response, it did not affect α1 or α2 in this study.

Kallio and colleagues found no difference in α1 in PD subjects of varying disease severity and healthy normals at rest (228). Their normal group, with a broad age range (36-85 years), presented with a lower α1 (mean 0.86) than in this study and as expected with smooth RSA as described by others (>1.0) (211, 215). A reduced α1 of a mean 0.9 has been demonstrated in much older healthy subjects of ages 68-81 (223).

Effects of dopaminergic therapy

All parameters were predominately unchanged in the presence and absence of dopaminergic therapy. There was an observed increase in α1 and α2 while breathing room air “post” dopaminergic therapy that was still different to the healthy normal subjects. The subject’s responses were performed on the same day within a short time period, to minimise environmental, psychological, diurnal and metabolic variability (81, 217). “Pre” dopaminergic therapy was considered after a minimum 12 h washout.

Levodopa has a short plasma half-life of approximately 50 min, and when combined with carbidopa, is extended to approximately 90 min. This was consistent with the previous observation that ventilatory response to progressive hypoxia was not affected by dopaminergic therapy in these subjects (Chapter 7). Therefore, these results suggest that any observed impairment was attributable to PD and not therapeutic regimes at this disease severity.

8.4.2. DFA of heart rate in elite freedivers

The freedivers were not different when compared to healthy normal subjects except for α2 while breathing room air. The significance of this is unclear but may relate to their younger age and aerobic training status. A previous finding of high HRV in freedivers using frequency domain analysis (230) was thought to have reflected elevated aerobic exercise capacity. Regular exercise training is thought to modify the autonomic balance. There is an enhanced cardiac vagal activity with suppressed sympathetic tone at rest and during submaximal exercise. Studies on competitive cyclists using frequency domain analysis have demonstrated enhanced HRV as compared to controls (236, 237), and thus cardiac autonomic modulation. The results of this assessment were in keeping with the previous observation of a cardiovascular response during progressive hypoxia that was not different to healthy normal (non-diving) subjects (Chapter 5) and therefore not reflective of any autonomic impairment/adaptation.

8.4.3. Possible limitations

R-R interval variability can be altered by large changes in minute ventilation and HR independent of cardiac autonomic regulation (207, 222). Both parameters increased during hypoxia compared to room air. The HR response was lower in the mild PD subjects as compared to the normal and freediving subjects following exposure to hypoxia. There has also been a recognised age-related loss of fractal organisation in heart beat dynamics (223) that may be avoided with the maintenance of good cardiovascular aerobic capacity (224). The scaling exponents did not correlate with age except for a weak association with α1 during hypoxia. Whether the differences in HR and age were enough to produce interference with the calculation of α1 is uncertain but unlikely.

HRV is also affected by ECG artefact, ectopic beats and arrhythmic events. Subjects with large blocks of artefact and/or arrhythmias were omitted from the analysis. Blocks were selected that were free of small episodes of artefact, ectopic beats and arrhythmia which may introduce selection bias. The analysed segments were ≈280 beats. There is good agreement between short (<300 beats) and long (24 h) recordings in group α1 responses despite the increased statistical variation (216).

Castiglioni and colleagues have recently challenged the use of multiple scaling exponents. They support the single DFA exponent α(n) or α(t) as a more appropriate description of the whole spectrum (238, 239). They suggest that defining multiple exponents with short time series may oversimplify the complex continuous spectrum. Due to the clear distinction of α1 and α2 in the current data, α(n) was not calculated, but may provide further insight in longer time series and requires further study.

8.5 Conclusion

DFA of HR in mild PD subjects displayed altered correlation properties of the R-R interval that were consistent with autonomic dysfunction. Elite freedivers were predominately not different from healthy normal subjects. Unlike other analyses of HRV, DFA was robust over short, non-stationary periods and may provide further insight into multi-system control mechanisms that regulate cardiac dynamics during hypoxia.

CHAPTER 9

SUMMARY

9.1. Introduction

The commencement of this research program, culminating in the completion of this Thesis, was triggered by important research questions arising from the investigator's background research conducted on competitive freedivers. The initial aim was to investigate whether participation in freediving and its related training practises may acutely and/or chronically alter respiratory function, structure and ventilatory control. During the course of the candidature, in response to the results, the research plan evolved to focus on the study of autonomic function during hypoxia. This was relevant in the study of these unique subjects that volitionally suppress ventilation, but also in patients with brainstem pathology.

The following sections summarise the key and novel findings with suggested future directions that arise from each set of observations.

9.2. Short and long-term respiratory consequences of participation in freediving

Previous and largely anecdotal reports of acute barotrauma following breath-hold dives to varying depths had suggested a high incidence of acute lung injury. These had not been studied with precision and, in particular, the dive statistics that would provide an indication of the extent and duration of hyperbaric and hypoxic exposure were not provided.

This was addressed by studying competitors in a five-hour long spearfishing competition. Competitors endured significant physical activity with an average of 80 dives to ≈ 10 m depth, each requiring ≈ 1 min breath-holds. In this general spearfishing community, repetitive breath-hold dives to moderate depths did not elicit any signs or symptoms of acute pulmonary barotrauma and there was otherwise no observed change in respiratory function.

These results do not support previous statements suggesting lung injury is common amongst recreational breath-hold divers when diving to relatively shallow depths. An effect at depths greater than those experienced by the subjects during this competition (≈ 25m, <4 atm) cannot be excluded, nor an alteration in respiratory function that is not detectable by a combination of spirometry and clinical examination. If this effect exists, it must be of relatively small magnitude.

Evaluating a more subtle, acute effect would require on-site study of complex respiratory function with a significant number of competitors exposed to greater atmospheric pressure. In the meantime, some reassurance is provided by the observations made here that there is no evidence of macroscopic lung damage in the novel longitudinal study of a single elite competitive freediver. However the repeated use of GI did alter respiratory system mechanics, with an observed increase in lung volumes over an eight-year period.

9.3. Ventilatory and autonomic control in freedivers - *the need for a new method of analysis*

Previous authors had reported a reduced ventilatory response to hypoxia and hypercapnia in breath-hold divers including underwater hockey players and pearl divers. They postulated that repetitive tolerance of significant hypoxia and hypercapnia during training and while diving alters central ventilatory response. When studying ventilatory responses to hypoxia and hypercapnia in elite competitive freedivers, it was immediately evident that the results were influenced by voluntary override. Ventilation was suppressed to the point where the results were non-interpretable.

Therefore, a new method was required to confirm the presence or absence of brainstem adaptation. Non-invasive, continuous measures of PTT and HRV were employed to assess autonomic function, which had not been previously monitored during progressive hypoxia. This new method was first validated in a group of healthy normal subjects across a broad age range. A strong intra-subject association between ventilatory and autonomic responses was identified.

In the elite freedivers, despite there being a non-interpretable ventilatory response to progressive hypoxia, the cardiovascular/autonomic response was normal. These athletes have the skill, aptitude and determination to inhibit ventilation in response to hypoxia/hypercapnia. It was clear that the ventilatory responses were strongly affected by voluntary ventilatory suppression. This calls into question previous reports of altered ventilatory control in this population in studies performed without the concomitant analysis of autonomic function. To alternatively quantify the extent of subjective influences, a further study of ventilatory response during sleep in these subjects. A normal response would support this hypothesis.

9.4 Ventilatory and autonomic control in Parkinson's disease

With the freedivers demonstrating a normal autonomic response to progressive hypoxia, it was important to test the method on a subject group that may have central ventilatory and autonomic dysfunction due to brainstem pathology. Had this not been done, the proposition that the method was insensitive to brainstem pathology would have been plausible. While autonomic dysfunction is a known feature of advanced PD, a recent hypothesis suggests that there is early involvement of the medulla, prior to the midbrain. Previous studies of ventilatory control in PD did not include patients with mild disease, did not include autonomic measures and the timing of the subjects medications was not controlled. PD patients seemed a reasonable group to examine in order to detect autonomic impairment.

When developing the new method to measure ventilatory and autonomic control, clinical laboratory practicalities were an important consideration. Previously described complex methods, principally performed on healthy normal subjects, would be challenging for those with pathological limitations. The new method was tolerated well and elicited an interpretable response in 87% of Parkinson's disease subjects who were graded as mild to moderate severity.

Ventilatory and occlusion pressure response to progressive hypercapnia were reduced in moderate, but not mild PD. However, the subjects with mild PD had a reduced autonomic response during progressive hypoxia. These novel findings support the theory that PD presents with early, pre-motor autonomic dysfunction. The autonomic measures were sensitive in detecting early pathological changes in those with mild disease, where the ventilatory parameters were not different to the healthy normal subjects.

Separately, it had been suggested that standard clinical PD dopaminergic therapy might attenuate measures of ventilatory control due to a vasopressor effect however this had not been directly studied. In this Thesis, patients with mild PD were studied before and after dopaminergic therapy with strong intra-subject repeatability. This suggests that any observed impairment is attributable to PD and not therapeutic regimes at this disease severity.

The developed method has potential for wide application across disease states that have autonomic dysfunction as a primary or secondary feature. The method is easily replicated and there is high repeatability even in patients with pathological limitations. Alterations in responses may assist with confirmation of a suspected diagnosis in the early stages of disease, longitudinal monitoring of disease progression and screening of those with susceptibility, such as family members.

9.5 A measure of HRV to assess autonomic function

DFA of the R-R interval had been proposed as a non-invasive, relatively simple clinical measure of dysautonomia, first identified in patients with heart failure. The comprehensive analysis of all subject group's ECGs at rest and during hypoxia provided further support to the observed reduced autonomic function in mild PD and normal autonomic function in elite freedivers. Subjects with mild PD had altered fractal correlation properties of the R-R interval when compared to healthy normal subjects; both at rest and following hypoxia.

While the exact mechanisms of these observations are unknown, DFA is a simple and non-invasive investigation that identified clear differences between mild PD and healthy normal subjects. This method may have important future clinical application in cardiac risk prediction and early disease detection across disease states that are affected by cardiac denervation.

Further studies are required to compare this method with other clinical markers of autonomic function that are presently used to quantify disease severity and monitor disease progression. Although the mathematical concepts are complex, the implementation is quite simple and it may have wide application as a non-invasive, low cost and radiation-free alternative to current testing methods.

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APPENDICES

A. SUBJECT QUESTIONNAIRES

i. Spearfisher

Date

NAME: HEIGHT:

DOB: WEIGHT:

SEX:

Medical history

Do you currently, or have you suffered from:

Asthma?

Heart disease ?

Previous pneumothorax?

Any other respiratory disease?

Do or have your smoked? If so how many/day?

Diving history

How long have you been freediving for?

How often do you freedive?

Any diving related incidents?

Today's competition statistics (diving watch? y/n)

How many dives did you complete today?

What was your average depth today?

What was your maximum depth today?

What was your average dive duration today?

Did you use any enhancing techniques to increase your breath hold time today?

ii. Elite freediver

Date:

Name: Age: Sex:

DOB: Height: Weight:

Smoking history:

☐ Never ☐ Past ☐ Current

Age you started/stopped smoking:

Average number of cigarettes per day:

Have you ever been diagnosed with:

- Chronic respiratory disease:
- Heart conditions (Congestive heart failure) :
- Other neurological diseases:
- Chest wall or thoracic cage deformity:
- Claustrophobia:
- Another other medical conditions? Please specify:

Diving history

How long have you been freediving for?

How often do you freedive?

Any diving related incidents?

Personal best competition statistics

Static apnoea

Dynamic apnoea (with and without fins)

Constant weight (with and without fins)

Other categories

Did you use any enhancing techniques or dive today?

iii. Parkinson's disease

Date:

Name: Age: Sex:

DOB: Height: Weight:

Smoking history:

☐ Never ☐ Past ☐ Current

Age you started smoking:

Age you stopped smoking:

Average number of cigarettes per day:

Have you ever been diagnosed with:

- Chronic respiratory disease:
- Heart conditions (Congestive heart failure) :
- Other neurological diseases:
- Chest wall or thoracic cage deformity:
- Claustrophobia:
- Another other medical conditions? Please specify:

Duration of Parkinson's disease (PD) symptoms:

Time since diagnosis of PD:

Duration of drug treatment for PD:

Past and current PD medications:

Time of last dose:

Any upper respiratory tract infections within the last month:

B. ETHICS APPROVAL NOTED BY MACQUARIE UNIVERSITY

i. Ethics approval for the study of ventilatory control- **#5201100840 D**

From: Ethics Secretariat
Sent: Friday, 21 October 2011 14:39
To: Prof Matthew Peters
Cc: Ms Leigh Seccombe
Subject: External Approval Noted- Peters (5201100840 D) Dear Prof Peters

Re: "The Sydney Local Health District Human Research Ethics Committee"

The above application was considered by the Executive of the Human Research Ethics Committee. In accordance with section 5.5 of the National Statement on Ethical Conduct in Human Research (2007) the Executive noted the final approval from the Sydney Local Health District and your right to proceed under their authority.

Please do not hesitate to contact the Ethics Secretariat if you have any questions or concerns.

Please do not hesitate to contact the Ethics Secretariat at the address below, if you require a hard copy letter of the above notification.

Please retain a copy of this email as this is your official notification of external approval being noted.

Yours sincerely

Dr Karolyn White
Director of Research Ethics
Chair, Human Research Ethics Committee

ii. Ethics approval for the site study of spearfishers - **#5201100243**

---------- Forwarded message ----------
From: **Ethics Secretariat** <ethics.secretariat@mq.edu.au>
Date: 11 March 2011 09:44
Subject: External Approval Noted- Peters (5201100243)
To: Prof Matthew Peters <matthew.peters@mq.edu.au>
Cc: Ms Leigh Seccombe <Leigh.seccombe@students.mq.edu.au>

Dear Prof Peters

Re: "Competition spearfishing divers can transiently expand vital capacity"

The above application was considered by the Executive of the Human Research Ethics Committee. In accordance with section 5.5 of the National Statement on Ethical Conduct in Human Research (2007) the Executive noted the final approval from the Concord Repatriation General Hospital and your right to proceed under their authority.

Please do not hesitate to contact the Ethics Secretariat if you have any questions or concerns.

Please do not hesitate to contact the Ethics Secretariat at the address below, if you require a hard copy letter of the above notification.

Please retain a copy of this email as this is your official notification of external approval being noted.

Yours sincerely

Dr Karolyn White
Director of Research Ethics
Chair, Human Research Ethics Committee

iii. Ethics approval for the longitudinal study of lung function and thoracic imaging in a freediver- **#5201100247**

---------- Forwarded message ----------
From: **Ethics Secretariat** <ethics.secretariat@mq.edu.au>
Date: 11 March 2011 09:55
Subject: External Approval Noted- Peters (5201100247)
To: Prof Matthew Peters <matthew.peters@mq.edu.au>
Cc: Ms Leigh Seccombe <Leigh.seccombe@students.mq.edu.au>

Dear Prof Peters

Re: "Training exercises in competition freedivers can transiently expand VC (CH62/6/2004-028)"

The above application was considered by the Executive of the Human Research Ethics Committee. In accordance with section 5.5 of the National Statement on Ethical Conduct in Human Research (2007) the Executive noted the final approval from the Concord Repatriation General Hospital and your right to proceed under their authority.

Please do not hesitate to contact the Ethics Secretariat if you have any questions or concerns.

Please do not hesitate to contact the Ethics Secretariat at the address below, if you require a hard copy letter of the above notification.

Please retain a copy of this email as this is your official notification of external approval being noted.

Yours sincerely

Dr Karolyn White
Director of Research Ethics
Chair, Human Research Ethics Committee

C. PEER-REVIEWED PUBLICATIONS FROM THIS THESIS

Pages 173-187 of this thesis have been removed as they contain published material. Please refer to the following citation for details of the articles contained in these pages.

Seccombe, L. M., Rogers, P. G., Jenkins, C. R., & Peters, M. J. (2012). Maintenance of vital capacity during repetitive breath-hold in a spearfishing competition. *Respirology*, 17, 350-353.
doi: 10.1111/j.1440-1843.2011.02090.x

Seccombe, L. M., Jenkins, C. R., Rogers, P. G., Pearson, M. A., & Peters, M. J. (2013). Evidence of respiratory system remodelling in a competitive freediver. *European Respiratory Journal,* 41, 760-762.
doi: 10.1183/09031936.00176412

Seccombe, L. M., Giddings, H. L., Rogers, P. G., Corbett, A. J., Hayes, M. W., Peters, M. J., & Veitch, E. M. (2011) Abnormal ventilatory control in Parkinson's disease – further evidence for non-motor dysfunction. *Respiratory Physiology and Neurobiology,* 179, 300-304.
doi:10.1016/j.resp.2011.09.012

Seccombe, L. M., Rogers, P. G., Hayes, M. W., Farah, C. S., Veitch, E. M. & Peters, M. J. (2013) Reduced hypoxic sympathetic response in mild Parkinson's disease: further evidence of early autonomic dysfunction. *Parkinsonism and Related Disorders,* 19, 1066-1068.
doi: 10.1016/j.parkreldis.2013.07.006

D. ABSTRACTS FROM THIS THESIS

Respirology (2011) **16** (Suppl. 1), 1–4

AO 13

REPETITIVE BREATH-HOLD DIVING DURING A SPEARFISHING COMPETITION DOES NOT AFFECT VITAL CAPACITY

L SECCOMBE, P ROGERS, Z PUTHUCHEARY, C WONG, C JENKINS
Thoracic Medicine, Concord General Hospital, NSW, Australia

Cough, desaturation and a reduction in vital capacity have recently been reported following single breath-hold dives to depths of 25–75 m (4–8 atm). We sought to investigate whether repetitive dives to more moderate depth of less than 20 m (3 atm) would elicit the same effects.

Methods Interested participants in the "Sydney Cup" spearfishing competition were recruited. Subjects performed spirometry before and after the 5-hour event on-site. Subject demographics were collected including a medical and diving history and details of dives undertaken that day. Following the competition subjects were asked to report any respiratory symptoms. Spirometry parameters immediately before ("Pre") and immediately after ("Post") competition were compared using a paired *t*-test.

Results 29 subjects were studied. 24 subjects (2 female) met ATS criteria (mean (SD) age 33 (11) years). During the competition, subjects dived 79 (32) times, to an average depth of 11 (3) m, lasting 1.0 (0.4) minute. Maximum depth was 17 (4) m. 2/29 subjects experienced sinusitis or ear ache, with no other symptoms reported.

N = 24	**Pre**	**Pre** (% predicted)	**Post**	**Change** (Pre-Post)
FEV_1, L	4.75 (0.73)	114 (9)	4.81 (0.78)	–0.06 (0.21)
FVC, L	5.82 (1.05)	113 (12)	5.80 (1.08)	0.03 (0.28)
Ratio, %	82 (6)	–	84 (7)	–
PEF (L/sec)	11.4 (1.6)	118 (13)	11.3 (1.8)	0.0 (0.8)
$FEF_{25–75}$ (L/sec)	4.66 (1.02)	–	4.82 (1.18)*	–0.16 (0.35)

All results expressed as mean (SD). *Vs Pre; $p < 0.05$.

Conclusion Repetitive breath-hold diving to average depths equivalent to 2 atm did not affect spirometry apart from $FEF_{25–75}$. In this group of subjects there were no measurable respiratory symptoms following a 5-hour spearfishing competition.

Key Words lung function, breath-hold diving, spirometry.

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AO09

EVIDENCE OF RESPIRATORY SYSTEM REMODELING IN A COMPETITIVE FREEDIVER: A COMPETITIVE ADVANTAGE?

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Introduction: Glossopharyngeal insufflation (GI) is commonly employed by freedivers to increase oxygen stores for depth and duration events. Lung barotrauma has been associated with GI, which raises the possibility that use of this technique results in lung damage and physiological impairment. Paradoxically, these alterations in the respiratory system may be performance enhancing. We sought to characterize the nature of any changes in the respiratory system that may be associated with GI, using physiological measurement and imaging.
Methods: The research data from a healthy competitive freediver who practised regular GI training was reviewed. Lung function was sequentially measured, including volumes achieved with a maximal GI manoeuvre. Non-contrast computed tomography (CT) of the thorax taken at baseline and following GI on a single occasion were segmented for 3D analysis of lung tissue.
Results: Lung function was measured over a period of eight years (from age 25 to 33 years) on four occasions. There was progressive baseline lung hyperinflation with a total >800 mL increase in baseline VC, FRC and TLC. There was no evidence of gas trapping as RV remained unchanged, and TL_{CO} was preserved. The subject was adept at GI; however, there was an observed limit to maximal absolute volume achieved above traditional TLC despite ongoing GI training. Rendered 3D images of lung tissue demonstrated significant lung hyperinflation following GI, with intercostal bulging of lung tissue, mediastinal distortion and flattening of the diaphragm. A pneumomediastinum was evident on the GI scan.
Conclusion: We present a healthy freediving subject with progressively increasing baseline lung volumes associated with repeated performance of an intervention used to enhance athletic performance. However, the upper limit of TLC with GI was stable. The repeated use of GI over time in this case appears to have altered respiratory system mechanics without any functionally important macroscopic lung damage, at least as evidenced by CT scans and measures of gas exchange.
Key words: hyperinflation, breath-hold diving, lung function.
COI: None

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AO17

PATIENTS WITH MILD PARKINSON'S DISEASE HAVE INTRINSIC IMPAIRMENT IN SYMPATHETIC RESPONSE TO HYPOXIA

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Braak's hypothesis emphasizes early brainstem, non-motor involvement in Parkinson's disease (PD). Patients with moderately severe PD have impaired ventilatory responses to hypercapnia. Dopamine is a standard therapy in PD which may have an inhibitory effect on ventilation via the activation of specific receptors within the carotid body. We sought to study the hypoxic and hypercapnic ventilatory and sympathetic responses in patients with mild PD, before and after their standard dopamine therapy and compared to healthy normals.
Methods: Patients with mild PD with no known respiratory disease were recruited. Ventilatory response to progressive poikilocapnic hypoxia and isoxic hypercapnia were assessed via closed circuit rebreathing methods. Ventilatory parameters were collected using breath-by-breath gas analysis. Pulse transit time (PTT) and heart rate (HR) were measured as markers of sympathetic response. Tests were performed before and after dopamine therapy (Rx) on the same day. V'E, PTT and HR response to changing pressure of end-tidal gases (P_{ET}) and calculated oxygen saturation were compared using a Pearson's correlation and linear regression. Healthy normal subjects were recruited for comparison.
Results: Eight (2 female) mild PD [mean (SD) age 64 (5) yrs] and 24 (8 female) normal subjects [age 42 (15)] were studied. Baseline ventilation and cardiovascular parameters were similar prior to all rebreathing tests. PTT and HR response to progressive hypoxia was reduced in mild PD compared to normal subjects ($p < 0.02$). In the PD subjects, all hypoxic and hypercapnic rebreathing ventilatory and sympathetic parameters were not different before and after Rx.
Conclusion: The sympathetic response to progressive hypoxia was reduced in mild PD despite a normal ventilatory response. Impairment of VR in more severe PD is unlikely to be related to standard dopamine therapy.
Key words: ventilatory response, hypoxia, dopamine, hypercapnia.
Conflict of Interest: None.

[Poster Board # E94] Detrended Fluctuation Analysis Of Heart Rate Demonstrates Autonomic Dysfunction During Progressive Hypoxia In Patients With Parkinson's Disease, [Publication Number: A5058]

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Rationale: Detrended fluctuation analysis (DFA) can detect scaling alterations in pathological states during non-stationary, short time measures. This includes the assessment of the fractal characteristics of heart rate (HR). HR fluctuations relate to countervailing neuroautonomic inputs. Patients with Parkinson's disease (PD) have early non-motor, medulla involvement that may impair autonomic HR control. The aim of this study was to assess the utility of DFA in identifying autonomic dysfunction in mild PD. HR fluctuations at rest and during increasing sympathetic activation with progressive hypoxia were compared to healthy normals.

Methods: Electrocardiography was measured at rest, room air and during progressive poikilocapnic hypoxia in subjects with mild PD and healthy controls. Using DFA of the R-R intervals, short-term (α1) and long-term (α2) scale exponents were calculated (Kubios HVR V2.1, Finland). Groups were compared using an unpaired t-test.

Results: 10 (2 female) mild PD (mean [SD] age 63 [5] years) and 8 (2 female) healthy subjects (52 [16] years) were studied. 8 PD subjects (80%) had a α1/α2 ratio <1 ("reverse crossover") at rest, room air. All healthy subjects had a α1/α2 ratio>1.

	Normal			Mild Parkinson's disease		
	α1	α2	HR	α1	α2	HR
Room air - 64 beats	1.19 [0.24]	0.60 [0.19]	71 [12]	0.83* [0.35]	1.12* [0.33]	72 [11]
Hypoxia - last 64 beats	1.35 [0.29]	0.81 [0.34]	84 [14]	1.02* [0.25]	1.29* [0.24]	80 [13]

*Vs Normal (P<0.03)

Conclusions: Subjects with mild PD have altered correlation properties of the R-R interval when compared to normal. There was an observed low heart rate variability at rest in PD that was unaffected by hypoxia, which is consistent with autonomic dysfunction. Unlike other analysis of heart rate variability, DFA is robust over short periods (64 beats) and may provide further insight into multi-system control mechanisms that regulate cardiac dynamics during hypoxia.

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