HYPOXIA AND AUTONOMIC CONTROL

by

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ABSTRACT

Humans have a remarkable ability to cope with and survive exposure to hypoxia. Some have suggested a benefit in certain physiological systems in response to such exposure. However, the physiological response to hypoxia is multifaceted and includes an orchestrated response from many autonomic mechanisms. Thus, the purpose of this thesis was to more fully understand the human autonomic response to hypoxia as an integrated unit. Furthermore, pathological models of hypoxia provide evidence that suggests hypoxia can result in an autonomic response that outlasts the hypoxic stimulus. However, the persistent effect of hypoxia is only evident in certain reflexes, although comorbidities that accompany a pathological model complicate interpretation. Therefore, employing a healthy human model with continued measurement of physiological measures in the post-hypoxia period provides a more complete understanding of the integrated human physiological response to hypoxia. This Doctoral thesis is comprised of four separate investigations, each focusing on autonomic control both during and following an acute hypoxic exposure. In the first study (Chapter 2), the microneurography technique was used to demonstrate that the chemoreflex plays an important role in persistent sympathoexcitation following acute isocapnic hypoxia. With the use of the spontaneous baroreflex analysis technique, the follow-up study (Chapter 3) implicated a resetting of the arterial baroreflex that works to permit the persistent sympathoexcitation. The focus of the third study (Chapter 4) was on cerebrovascular control during fluctuations in blood pressure via bolus injections of vasoactive drugs. There was an improvement in cerebral autoregulation to increases in blood pressure following acute isocapnic hypoxia. The final study (Chapter 5) considered the role of carbon dioxide on hypoxic cerebral autoregulation, and found an impairment in isocapnic hypoxia but no effect in poikilocapnic hypoxia. The findings from this series of studies demonstrate the acute and persistent effects of short-term hypoxia, and the integrated nature in which autonomic mechanisms orchestrate the human physiological response to hypoxia.

PREFACE

The project presented in Chapter 2 received ethical approval from the UBC Clinical Research Ethics Board (Certificate #H06-70060). A version of Chapter 2 has been previously published as: Querido JS, Kennedy PM, Sheel AW (2010) Hyperoxia attenuates muscle sympathetic nerve activity following isocapnic hypoxia in humans. J Appl Physiol 108(4): 906-912. I identified the research question, designed the study, analyzed the data, and wrote the manuscript for publication. Data collection was shared between Kenned PM and myself. Sheel AW provided intellectual input during formulation of the research question, and also provided editorial feedback on the manuscript.

The project presented in Chapter 3 received ethical approval from the UBC Clinical Research Ethics Board (Certificate #H10-00225). A version of Chapter 3 has been previously published as: Querido JS, Wehrwein EA, Hart EC, Charkoudian N, Henderson WR, Sheel AW (2011) Baroreflex control of muscle sympathetic nerve activity as a mechanism for persistent sympathoexcitation following acute hypoxia in humans. Am J Physiol Regul Integr Comp Physiol 301(6): R1779-R1785. I identified the research question, designed the study, analyzed the data, and wrote the manuscript for publication. Data collection was shared between Henderson WR and myself. Wehrwein EA, Hart EC, and Charkoudian N assisted in data analysis. All coauthors provided editorial feedback on the manuscript.

The project in Chapter 4 received ethical approval from the UBC Clinical Research Ethics Board (Certificate #H10-00225).

The project presented in Chapter 5 received ethical approval from the UBC Clinical Research Ethics Board (Certificate #H11-01-593).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CA	Cerebral autoregulation
CBF	Cerebral blood flow
CC	Carotid chemoreceptors
cm	Centimetre(s)
CO_2	Carbon dioxide
CVR	Cerebrovascular resistance
DBP	Diastolic blood pressure
ET-1	Endothelin-1
F_b	Breathing frequency
h	Hour(s)
HR	Heart rate
kg	Kilogram(s)
MAP	Mean arterial blood pressure
MCAV	Middle cerebral arterial blood velocity
min	Minute(s)
mmHg	Millimetres of mercury
MSNA	Muscle sympathetic nerve activity
N_2	Nitrogen
NTS	Nucleus tractus solitarius
O_2	Oxygen
OSA	Obstructive sleep apnoea
$PaCO_2$	Arterial partial pressure of carbon dioxide
PaO_2	Arterial partial pressure of oxygen
PE	Phenylephrine
$P_{ET}CO_2$	End-tidal partial pressure of carbon dioxide
$P_{ET}O_2$	End-tidal partial pressure of oxygen
$\dot{Q}$	Cardiac output
s	Second(s)
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of the mean
SNP	Sodium nitroprusside
SpO_2	Oxyhaemoglobin saturation with pulse oximetry
SV	Stroke volume
T50	Diastolic blood pressure with a 50% likelihood of a burst occurring
$\dot{V}_I$	Minute ventilation
VLM	Ventrolateral medulla
V_T	Tidal volume
yr	Year(s)

CHAPTER 1: INTRODUCTION

Hypoxia poses a significant physiological stress causing homeostatic imbalance, which initiates compensatory mechanisms to ensure survival. The autoregulatory mechanisms are numerous and there is considerable complexity in the physiological response. In brief, an autonomic mechanism is a reactive response that works to mitigate a stimulus. Acute exposure to hypoxia (i.e. a stimulus) leads to a whole-body response which includes modifications in respiratory and cardiovascular control; ultimately working to compensate for the reduction in arterial oxygen pressure (PaO_2). Environmental hypoxia at high-altitude lasting from days to lifelong exposure demonstrates the ability to survive a chronic reduction in PaO_2. Previous studies have demonstrated physiological adjustments in long-term residents of high-altitude that help cope with the hypoxia (e.g. haematopoiesis). However, hypoxia is not always constant, but rather intermittent. For example, repeated sojourn to altitude for occupational purposes is common among South American and Asian countries. At sea-level, individuals who suffer from obstructive sleep apnoea (OSA) are exposed to apnoea-induced hypoxia during sleep for ~20 s which can happen upwards of 60 times an hour. Interestingly, the autonomic reflexes in this pathological model of hypoxia differ from the typical acute or high-altitude hypoxia models, which is likely due to the differences in the hypoxic paradigm (i.e. sustained vs. intermittent) (Peng and Prabhakar 2004). However, although humans have the ability to manage and survive hypoxia, the physiological consequences of such exposure are still not fully defined. Furthermore, the vast majority of research on the human autonomic response to acute hypoxia has focused on physiological measures during the hypoxia exposure, but autonomic function following the hypoxic stimulus is still uncertain and has not been considered in detail.

There is evidence to suggest that hypoxia exposure is physiologically beneficial. Certainly, many elite athletes use various ‘live high, train low’ hypoxia paradigms with the intention of improving exercise performance (Stray-Gundersen et al. 2001). Furthermore, various lines of evidence suggest a cardioprotective effect of repeated hypoxic exposure (via ischaemia reperfusion models) (Serebrovskaya 2002; Anderson and Honigman 2011). Improvement in functional measures in patients with incomplete spinal injury has also been reported, as

demonstrated by an increase in ankle plantar flexion torque following a single session of repeated hypoxia exposures (Trumbower et al. 2012). Lastly, personal hypoxia machines ('*hypoxicators*') are marketed to the health-conscious person with promises of improved health and fitness.

Despite the above, the human physiological response to hypoxia is complex and involves reflexes from many autonomic mechanisms. Thus, it is important to consider the possibility for an adjustment in one autonomic control system to subsequently alter control in a related autonomic system. For instance, the ventilatory chemoreflex is responsible for increasing minute ventilation ($\dot{V}_I$) in hypoxia; however, the hypoxia-induced hyperventilation leads to a reduction in arterial pressure of CO_2 ($PaCO_2$) which reduces cerebral blood flow. Studies performed in sea-level residents at high-altitude have demonstrated an impairment in cerebrovascular control (i.e. a greater influence of peripheral pressure on cerebral blood flow) (Ainslie et al. 2008), which is also evident in natives of the Nepalese Himalayas (Jansen et al. 2000), suggesting that there is no improvement with chronic hypoxia exposure. Furthermore, could alterations in autonomic function outlast the hypoxic stimulus? High-altitude exposure in sea-level residents induces sympathoexcitation which persists for days following descent to sea-level (Hansen and Sander 2003). The physiological consequences of hypoxia are not only demonstrated at high-altitude. For instance, hypertension, impaired cerebral autoregulation, and increased vasomotor outflow have all been documented in patients with OSA (Carlson et al. 1993; Hla et al. 1994; Urbano et al. 2008); and hypoxia has been implicated as the major causative factor (Lavie 2003; Lavie 2005). It is important to note that these pathological outcomes are present in patients with OSA during the daytime, while in normoxia. Therefore, the autonomic response to hypoxia is not limited to the exposure alone; rather, hypoxic exposure appears to result in adjustments in autonomic function that persist in normoxia.

Given that the physiological consequences of hypoxia are variable, ranging from health to disease, a more comprehensive understanding of the underlying mechanisms that are stimulated in hypoxia is warranted. This introduction will provide a brief overview of the different

autonomic responses to hypoxia, followed by the persistent effect of hypoxia on autonomic reflexes, and finally the objectives of the thesis.

1.1 Effect of hypoxia on cardiorespiratory measures

The cardiorespiratory response to an acute hypoxic exposure is multifaceted, and consists of complex interplay between several distinct autoregulatory systems. Much information on the mechanisms of the autoregulatory response to hypoxia has been obtained from studies that have used isolated preparations. For instance, investigations using isolated carotid bodies of the rat have demonstrated the important role of the carotid bodies in the hypoxic chemoreflex, the importance of the hypoxic paradigm in inducing alterations in the sensitivity of the carotid bodies, and the biochemical mechanisms responsible for these changes (Prabhakar 2001; Peng and Prabhakar 2004). However, the carotid bodies do not operate in isolation; rather, they are modulated by other autonomic mechanisms such as lung stretch receptors (Heistad et al. 1975). Therefore, by incorporating the findings from these studies using isolated preparations into an integrated approach using non-anaesthetised animals or humans, we are able to more fully understand and appreciate the interrelation of the autonomic systems in evoking a 'whole-body' response.

One of the most well-known and observable autonomic mechanisms stimulated in hypoxia is the ventilatory chemoreflex which serves as the first line of defence to hypoxia to meet the oxygen requirements of cells. Acute exposure to hypoxia leads to a dose-response increase in $\dot{V}_I$, mainly owing to an increase in tidal volume (V_T) (Powell et al. 1998). However, the effect of the chemoreflex on ventilation can be cooperatively mediated by many other systems (e.g. baroreflex, lung stretch receptors) (Heistad et al. 1975). Peripheral chemoreceptors located in the bifurcation of the carotid arteries and aortic arch are the major sensors of arterial oxygen pressure (PaO_2) in the blood; whereas central oxygen sensors appear to be less sensitive to hypoxia (Guyenet 2000). A decrease in PaO_2 stimulates an immediate increase in ventilation, which is mediated by the peripheral chemoreceptors (Dejours 1962). Afferent signals are integrated in the respiratory centres of the brainstem, and lead to increased efferent outflow via

the phrenic nerve to the respiratory muscles (Guyenet 2000). In contrast, hypocapnia, a result of hypoxia-induced hyperventilation, inhibits ventilation due to the subsequent alkalosis (Smith et al. 1995). It is important to note that the peripheral and central chemoreceptors do not work in isolation of one another, but rather there is cross-talk between the two chemosensitive areas (Blain et al. 2010). The ventilatory response to hypoxia is also variable within a subject (Sahn et al. 1977), and can be augmented with previous hypoxia exposure. Progressive augmentation, increased ventilatory magnitude with successive hypoxia exposures, is observed in disease models of hypoxia (Narkiewicz et al. 1999), and demonstrates the plasticity of ventilatory control. Thus, although hyperventilation in hypoxia is a consistent finding in the literature, the degree of hyperventilation is variable.

The peripheral chemoreceptors also play a role in vascular tone. Upon exposure to hypoxia, there is a concurrent increase in vasomotor constrictor outflow with ventilation, which is mediated by the peripheral chemoreceptors (Guyenet 2000). However, the sympathetic profile is also subject to respiratory modulation. In humans, stimulation of lung stretch receptors, a consequence of the increase in V_T in hypoxia, inhibit vasomotor outflow during inspiration (Seals et al. 1993; St Croix et al. 1999). Furthermore, the hypoxia-induced sympathoexcitation observed in freely-breathing humans during hypoxia is reduced compared to apnoeic hypoxia (Somers et al. 1989). The respiratory-neural synchronization provides another example of the orchestrated response of autonomic reflexes in producing a whole-body effect. Similar to the progressive augmentation of ventilation, the magnitude of sympathoexcitation in hypoxia is increased following acute hypoxia of a duration as brief as 20 min (Morgan et al. 1995). It has been suggested that the potentiation of sympathetic outflow originates from a hypoxia-induced sensitization of the peripheral chemoreceptors, and is the cause for the elevated levels of vasomotor outflow in patients with OSA during night-time apnoeic (hypoxic) episodes (Narkiewicz et al. 1999; Prabhakar and Kumar 2010).

Acute hypoxia provides a direct vasodilating effect on cerebral, coronary, and peripheral vascular beds (Heistad and Abboud 1980); however, sympathoexcitation to many vascular beds opposes the dilation. Thus, the net effect of hypoxia on mean arterial blood pressure (MAP) is a

function of the direct vasodilating effect of hypoxia, and the degree of sympathetic outflow (Heistad and Abboud 1980; Rowell and Blackmon 1987). The effect of acute hypoxia on MAP is inconsistent, with previous studies demonstrating an increase (Lusina et al. 2006), decrease (Rowell and Blackmon 1986), or no effect (Van Mil et al. 2002). Part of the inconsistency may be the result of using different degrees of hypoxia, species differences, and the vascular bed investigated to represent MAP (Rowell and Blackmon 1987). Regardless of whether MAP changes in hypoxia, the mechanisms that control MAP are altered in hypoxia. In particular, the increases in sympathetic outflow have already been discussed. Also, although hypoxia causes bradycardia in isolated heart preparations or in apnoeic animals, the bradycardia is overcome by vagally-mediated pulmonary stretch receptors (Deburghdaly and Scott 1964; James and Deburghdaly 1969). Consequently, the arterial baroreflex is thought to be reset in acute hypoxia to elevated levels of sympathetic outflow and increased heart rate (HR) (Halliwill and Minson 2002; Halliwill et al. 2003).

The cerebral vasculature is also sensitive to hypoxia. Although not fully understood, reductions in PaO_2 cause vessel dilation and increased cerebral blood flow (CBF), whereas hyperoxia can result in a reduction in CBF (Lennox and Gibbs 1932; Shapiro et al. 1970). An important mediator of the CBF response to hypoxia is the background level of $PaCO_2$ (Betz 1972; Ainslie and Poulin 2004). If $PaCO_2$ levels fall in hypoxia due to hyperventilation, the typical increase of CBF in hypoxia can be blunted or completely abolished (Ainslie and Poulin 2004). Neural influence on cerebrovascular tone is controversial (Strandgaard and Sigurdsson 2008; van Lieshout and Secher 2008), but recent findings suggest that they do play a role (Zhang et al. 2002). Cerebral vessel vasodilation in isocapnic hypoxia helps preserve adequate perfusion and oxygen delivery to cerebral tissue, but the significance of CBF changes in hypoxia is also unclear. In particular, clinical measures such as acute mountain sickness is not related to CBF or its regulation in hypoxia (Subudhi et al. 2010).

Given this brief review, it is clear that exposure to hypoxia causes a complex physiological response. Although traditionally thought of as distinct and unrelated systems, the respiratory and

cardiovascular systems reciprocally mediate the autonomic response to hypoxia and are inextricably linked.

1.2 Persistent effect of acute hypoxia exposure

Although the cardiorespiratory response to acute hypoxia has been well-documented, the potential persistent effect of this chemical stress is often overlooked. Hypoxia exposure can elicit persistent changes in autoregulatory mechanisms under different conditions. For instance, patients with OSA demonstrate increased levels of vasomotor outflow and are at an increased risk of complications such as hypertension, endothelial dysfunction, and stroke (Hla et al. 1994; Lavie 2003; Arzt et al. 2005). It is important to note that these clinical outcomes are evident throughout the day, while the patient is freely breathing and normoxic. OSA is often associated with other comorbidities (e.g. obesity), so healthy human models of hypoxia are useful in isolating the physiological response to hypoxia. Acute exposure to hypoxia in healthy humans can lead to autonomic adjustments that are similar to that observed in the pathology of OSA. For example, an acute exposure to hypoxia (20 min) in healthy humans results in sensitization of the carotid chemoreceptors to subsequent hypoxia, and sympathoexcitation that outlasts the hypoxic stimulus (Morgan et al. 1995; Xie et al. 2001; Lusina et al. 2006). However, the continued sympathoexcitation post-hypoxia is not necessarily accompanied with a change in peripheral vascular resistance or MAP; although interpretation is difficult due to differences in the length and severity of the hypoxia exposure, and whether isocapnia was maintained (Morgan et al. 1995; Xie et al. 2001; Tamisier et al. 2004; Tamisier et al. 2005; Gilmartin et al. 2006; Lusina et al. 2006; Tamisier et al. 2007). In contrast, the large increases in $\dot{V}_I$ and heart rate (HR) that occur in hypoxia are quickly normalized upon termination of the hypoxic stimulus in humans (Xie et al. 2001; Lusina et al. 2006).

The majority of the studies investigating the effects of hypoxia have neglected the post-hypoxia period, or used experimental approaches that confound the results (e.g. high-altitude hypocapnia, hypercapnia and obesity of OSA). Therefore, the overarching objective of my Doctoral thesis was to use a healthy human model to more fully understand the coordinated function of

autonomic reflexes in acute hypoxia, with a particular focus on the post-hypoxia period. This objective was accomplished by concentrating on the following autonomic systems in, and following acute hypoxia:

- The sympathetic arm of the chemoreflex (Chapter 2)
- The baroreflex (Chapter 3)
- Cerebral autoregulation (Chapter 4)
- Cerebral autoregulation – chemoreflex interactions (Chapter 5)

Each research chapter begins with a focused introduction and accompanying hypotheses. The introductions are followed by detailed explanation of the experimental approaches, experimental data, and discussion sections that interpret the findings. The final chapter of this document (Chapter 6) integrates the findings with the literature by providing overall conclusions and perspectives.

CHAPTER 2: HYPEROXIA ATTENUATES MUSCLE SYMPATHETIC NERVE ACTIVITY FOLLOWING ACUTE ISOCAPNIC HYPOXIA

2.1 Introduction

Acute exposure to hypoxia elicits abrupt increases in respiratory and circulatory measures. In particular, large increases in muscle sympathetic nerve activity (MSNA) are observed in hypoxia, and are dose-dependent (Morgan et al. 1995). The proposed mechanism is a hypoxia-mediated increase in afferent signals from the oxygen-sensitive carotid chemoreceptors (CC), which in turn increase ventilatory drive and vasomotor outflow (Guyenet 2000). Consequently, the CC play an obligatory role in mediating the cardiorespiratory and sympathetic responses to acute hypoxia (Lugliani et al. 1973; Fletcher et al. 1992; Timmers et al. 2003).

Although many investigations have characterized the sympathoexcitation that accompanies acute hypoxia (Saito et al. 1988; Somers et al. 1989; Somers et al. 1989; Xie et al. 2001; Tamisier et al. 2004), relatively little is known about the response after the hypoxia exposure has ended. However, it appears that once the acute exposure to hypoxia is terminated there is an uncoupling between hypoxia and sympathetic outflow, in that MSNA remains elevated for at least 20 min despite a rapid return of other cardiorespiratory measures to resting values (Xie et al. 2001; Cutler et al. 2004; Lusina et al. 2006). Three lines of evidence suggest that the persistent sympathoexcitation is due to hypoxia-induced modulation of CC activity. First, continued sympathoexcitation following chemostimulation is inherent only to hypoxia; chemical stimulation of the CC with hypercapnia does not result in long-lasting sympathoexcitation (Xie et al. 2001). Second, healthy human and pathological models of intermittent hypoxia modulate CC activity which results in heightened MSNA in normoxia and hypoxia (Lusina et al. 2006; Imadojemu et al. 2007). Third, CC sensitivity may be enhanced following hypoxic stimulation, as demonstrated by a trend for an increase in the magnitude of the ventilatory and sympathetic outflow responses to acute isocapnic hypoxia (Morgan et al. 1995). Taken together, these findings suggest that sustained sympathoexcitation following hypoxia is dependent on elevated CC activity; however, no study has systematically investigated this possibility. Therefore, the

purpose of the present investigation was to determine the contribution of the chemoreflex on persistent sympathoexcitation following isocapnic hypoxia. Accordingly, MSNA was recorded in healthy humans during brief bouts of hyperoxia before and after 20 min of sustained isocapnic hypoxia. It was hypothesized that transient CC inhibition with hyperoxia would lower sympathetic activity following acute isocapnic hypoxia.

2.2 Materials and methods

Subject characteristics. Seven healthy subjects (30 ± 3 yrs; 2 female) of normal height (176 ± 3 cm) and weight (73 ± 5 kg) who were free from cardiovascular, pulmonary, and neurological diseases participated in the study. A history of smoking, hypertension, or sleep apnoea, altitude exposure within 6 months prior to testing, or participation in breath-hold activities excluded participation. On an initial visit, written informed consent was obtained and subjects underwent a familiarization session where all experimental procedures, excluding microneurography, were performed. All experimental procedures and protocols were approved by the Clinical Research Ethics Board at the University of British Columbia which conforms to the *Declaration of Helsinki*.

Experimental protocol. Subjects remained recumbent throughout testing while ventilatory, cardiovascular, and MSNA measures were continuously recorded. Following instrumentation, the experimental session began with a minimum of 15 min of eupnoea to obtain resting data. As previously described (Lusina et al. 2006), isocapnic hypoxia was then administered by titrating 100% N_2 into the inspired circuit in order to maintain oxyhemoglobin saturation (SpO_2) at 80% for 20 min. Isocapnia was maintained by the manual addition of 100% CO_2 to the inspirate as needed. Following isocapnic hypoxia, we continued to record ventilatory, cardiovascular, and sympathetic measures while subjects breathed normoxic air.

To investigate the contribution of the CC on MSNA, hyperoxia for 1 min was administered before (2 interventions) and after (2-3 interventions) the 20 min isocapnic hypoxia exposure. Following termination of the isocapnic hypoxia exposure, subjects breathed room air for 5 min

prior to the first hyperoxia intervention. Subsequent hyperoxia interventions were separated by 5 min in order for inspired oxygen levels to normalize. Hyperoxia was accomplished by switching the inspired circuit to a reservoir containing 100% O_2. Hyperoxia was selected given that it has been shown to transiently inhibit the CC (Watt et al. 1943; Hornbein et al. 1961; Nye et al. 1981; Stickland et al. 2008).

Physiological measures. A face mask was connected to a calibrated pneumotachograph (Hans Rudolph 3813, Kansas City, MO, USA) to determine inspiratory flow. V_T and breathing frequency (F_b) were determined from the flow signal, and $\dot{V}_I$ was calculated from the product of F_b and V_T. Oxygen and carbon dioxide were measured at the mouth with calibrated gas analyzers (S-3A/I and CD-A, Applied Electrochemistry, Pittsburgh, PA, USA). SpO_2 was measured with a finger pulse oximeter (Ohmeda 3740, Lousiville, CO, USA). A single lead electrocardiogram was continuously recorded to determine heart rate. Beat-by-beat blood pressure was monitored at the finger with photoplethysmography (Finometer, Finapres Medical System, Arnhem, The Netherlands).

Muscle sympathetic nerve activity. Direct postganglionic MSNA was recorded from the peroneal nerve using the microneurography technique (Vallbo et al. 1979; Lusina et al. 2006). Briefly, the technique involved placement of a recording microelectrode into the common peroneal nerve for recording of electrical activity. Following localization of the common peroneal nerve via surface and subcutaneous stimulation, a recording tungsten microelectrode (tip diameter 10 μm, 35 mm, Frederick Haer, Bowdoinham, ME, USA) was advanced posterior to the fibular head until sufficient electrical activity was obtained. Nerve electrical signals were rectified and amplified (total gain 50 000, custom-built microneurography preamplifier and amplifier, Yale University, New haven, CT, USA), band-pass filtered (300-5000 Hz), and integrated (time constant 100 ms, Integrator model B937C, Bioengineering, University of Iowa, Iowa City, IA, USA). Correct recording placement was confirmed by: (i) pulse-synchronous of nerve electrical activity; (ii) activation with a breath-hold or during Phase 2 of a Valsalva manoeuvre; (iii) activity in response to tapping or stretching of the muscle; (iv) no activation upon gentle stroking of the skin or startle stimuli. Once a sufficient recording was obtained

(signal-to-noise ratio > 3:1) the subjects were instructed to remain relaxed and still in order to avoid displacement of the recording microelectrode. In addition, the leg was placed in a custom-fit brace to ensure leg immobility. In order to time-match the sympathetic bursts with the corresponding systolic pressure that terminated the burst (baroreflex-mediated response), the integrated neurogram was time shifted to an R-wave from the electrocardiogram and high pass filtered to set the noise threshold to zero. Following burst identification in the integrated neurogram from computer software (Chart v7.0.1, ADInstruments), bursts were confirmed by visual inspection. For quantification, MSNA was expressed as burst frequency (bursts/min), burst incidence (burst/100 heart beats) and total MSNA (the product of MSNA frequency and MSNA amplitude; arbitrary units).

Data and statistical analyses. All data were acquired using an analog-to-digital converter and sampled at 1 kHz. Data were stored on a personal computer for subsequent offline analysis. To investigate the effect of hypoxia, measured variables were averaged over 5 min sections during baseline, hypoxia, and recovery. Differences between these conditions were determined with repeated measures ANOVA. To characterize MSNA responses to hyperoxia following hypoxia, one minute steady-state values were compared using repeated measures ANOVA. In the case of a significant F ratio, differences were further investigated with Tukey's post hoc analysis. The nadir 1 min MSNA burst frequency value during hyperoxia interventions was compared to the 1 min steady state MSNA burst frequency prior to hyperoxia with a paired t test. There were no differences in MSNA responses to hyperoxia prior to hypoxia, therefore the mean of the two trials was used. The level of significance was set at $P < 0.05$ for all statistical calculations. Group data are presented as means ± SEM.

2.3 Results

Effects of isocapnic hypoxia. Figure 2.1 shows a MSNA trace and corresponding measurements for one subject. Group mean data from the effects of isocapnic hypoxia on cardiorespiratory and MSNA measurements are shown in Table 2.1. HR increased during hypoxia, but returned to baseline levels once room air breathing was resumed. There was no consistent effect of hypoxia

on MAP. Exposure to hypoxia resulted in significant increases in $\dot{V}_I$ and V_T, but both returned to baseline upon termination of the hypoxia exposure. There was a small (1 mmHg) decrease in end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) during the first 5 min of hypoxia, but overall isocapnia was well maintained throughout the hypoxia exposure. A significant decrease in SpO_2 was achieved within the first 5 min of hypoxia, and the target SpO_2 (80%) was maintained for the remainder of hypoxia. MSNA burst frequency and total MSNA increased in hypoxia relative to baseline, and remained elevated into normoxic recovery.

Table 2.1 – Effects of 20 min of isocapnic hypoxia on cardiorespiratory and MSNA measures.

	Baseline	Hypoxia				Post-hypoxia
		5 min	10 min	15 min	20 min	
SBP (mmHg)	129 ± 4	133 ± 4	133 ± 4	133 ± 5	135 ± 5 *	135 ± 5 *
DBP (mmHg)	76 ± 2	77 ± 2	79 ± 2	78 ± 3	78 ± 2	79 ± 2
MAP (mmHg)	96 ± 2	99 ± 3	100 ± 3	100 ± 4	100 ± 3	100 ± 3
HR (beats/min)	57 ± 3	68 ± 5 *	73 ± 5 *	72 ± 4 *	70 ± 5 *	61 ± 5
Fb (breaths/min)	11.5 ± 1.1	12.9 ± 0.8	13.6 ± 1.0	14.0 ± 1.3	14.1 ± 1.6	12.1 ± 1.7
V_T (l)	0.45 ± 0.08	0.57 ± 0.09 *	0.60 ± 0.11 *	0.60 ± 0.12 *	0.57 ± 0.10 *	0.52 ± 0.10
V_I (l/min)	5.0 ± 1.1	7.2 ± 1.2 *	7.5 ± 1.4 *	7.5 ± 1.4 *	7.3 ± 1.3 *	5.8 ± 1.1
$P_{ET}CO_2$ (mmHg)	38.9 ± 0.9	37.9 ± 0.9 *	38.1 ± 0.9	38.3 ± 0.9	38.5 ± 0.8	39.2 ± 0.9
SpO_2 (%)	98.3 ± 0.14	88.4 ± 1.47 *	80.3 ± 0.69 *	78.8 ± 0.36 *	79.9 ± 0.48 *	96.9 ± 0.36
MSNA (bursts/min)	24 ± 1.6	31 ± 1.7 *	32 ± 1.7 *	30 ± 2.2 *	30 ± 2.2 *	31 ± 0.6 *
MSNA (bursts/100 beats)	43 ± 3.4	48 ± 4.8	46 ± 4.8	42 ± 4.4	44 ± 4.9	53 ± 4 *
Total MSNA	4.2 ± 1.1	5.2 ± 1.3 *	5.3 ± 1.3 *	5.4 ± 1.3 *	5.4 ± 1.3 *	5.5 ± 1.4 *

Values are means ± SEM (n = 7). *significantly different from baseline.

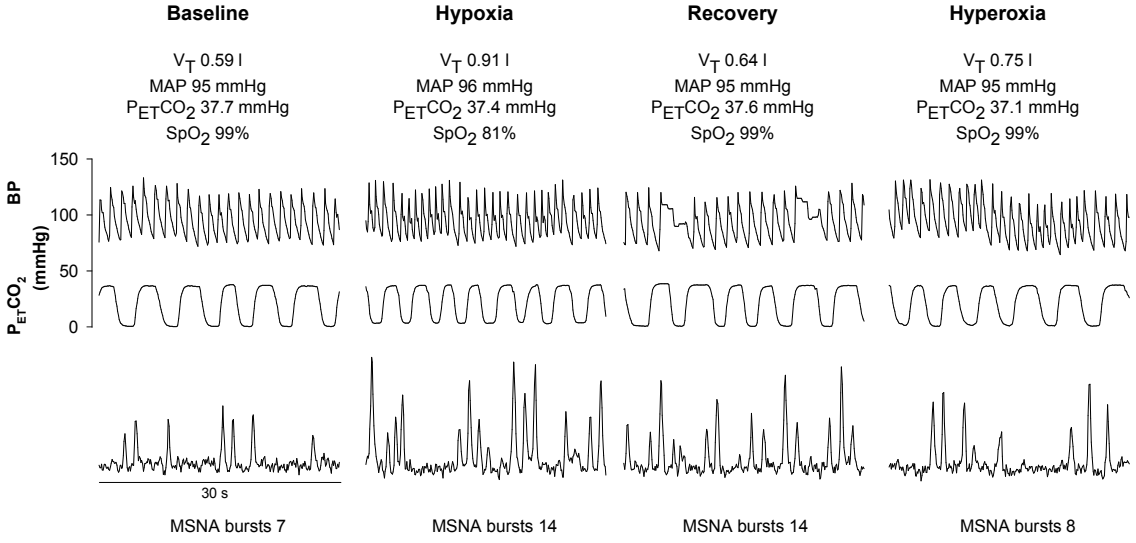

Figure 2.1 – Effect of isocapnic hypoxia and hyperoxia on muscle sympathetic nerve activity MSNA.

Representative traces represent 30 s of data obtained from one subject at baseline, during exposure to isocapnic hypoxia, at recovery, and during exposure to hyperoxia.

Effects of hyperoxia. Hyperoxia interventions performed at baseline resulted in a small increase in F_b and SpO_2 which reached statistical significance (Table 2.2). In contrast, there was no effect of hyperoxia on other cardiorespiratory measures. Similarly, MSNA measures were not affected by hyperoxia ($P > 0.05$).

Table 2.2 – Effects of hyperoxia on cardiorespiratory and MSNA measure before and after 20 min of isocapnic hypoxia.

	Pre-hypoxia		Post-hypoxia					
			Intervention 1		Intervention 2		Intervention 3	
	Normoxia	Hyperoxia	Normoxia	Hyperoxia	Normoxia	Hyperoxia	Normoxia	Hyperoxia
SBP (mmHg)	129 ± 4	130 ± 3	134 ± 6	135 ± 6	135 ± 7	133 ± 7	139 ± 8	141 ± 8
DBP (mmHg)	76 ± 2	76 ± 2	78 ± 2	79 ± 3	80 ± 3	78 ± 2	82 ± 2	84 ± 2
MAP (mmHg)	96 ± 2	97 ± 4	100 ± 4	101 ± 4	102 ± 5	100 ± 4	105 ± 4	108 ± 4
HR (beats/min)	57 ± 3	57 ± 4	58 ± 4	58 ± 4	60 ± 6	59 ± 6	59 ± 5	55 ± 4
F_b (breaths/min)	11.5 ± 1.1	13.7 ± 1.2 *	11.9 ± 1.6	12.7 ± 1.4	11.9 ± 2.0	12.3 ± 1.6	13.7 ± 1.6	14.6 ± 2.1
V_T (l)	0.45 ± 0.08	0.47 ± 0.11	0.53 ± 0.12	0.58 ± 0.14	0.65 ± 0.16	0.63 ± 0.14	0.68 ± 0.16	0.63 ± 0.15
V_I (l/min)	5.0 ± 1.1	6.1 ± 1.4	5.9 ± 1.3	6.9 ± 1.6	6.6 ± 1.4	6.9 ± 1.4	8.3 ± 1.6	8.1 ± 1.8
$P_{ET}CO_2$ (mmHg)	38.9 ± 0.9	38.6 ± 1.0	38.9 ± 1.0	38.7 ± 0.9	38.0 ± 0.9	38.1 ± 0.8	38.2 ± 1.0	38.0 ± 1.2
SpO_2 (%)	98.3 ± 0.14	98.8 ± 0.10 *	98.2 ± 0.26	98.9 ± 0.20 *	98.5 ± 0.21	99.0 ± 0.03	98.5 ± 0.25	98.9 ± 0.01
MSNA (bursts/min)	24 ± 1.6	23 ± 1.9	34 ± 1.0	25 ± 0.8 *	31 ± 1.5	26 ± 0.4 *	33 ± 1.8	29 ± 0.4
MSNA (bursts/100 beats)	43 ± 3.4	42 ± 3.9	61 ± 4.4	44 ± 3.4 *	54 ± 6.3	46 ± 4.6 *	57 ± 5.3	54 ± 4.2

Values are means ± SEM (n = 7). *Significantly different from previous normoxia period.

Post-hypoxia, there was no effect of hyperoxia on cardiorespiratory measures. As a group, MSNA burst frequency decreased during hyperoxia interventions 1 and 2 post-hypoxia (Figure 2.2). The nadir MSNA burst frequency occurred in the minute following hyperoxia; however, the decrease was transient. That is, MSNA burst frequency returned to an elevated level relative to baseline within 5 min after hyperoxia. The nadir reduction in MSNA burst frequency was significantly greater in the first intervention (-28 ± 3% compared to post-hypoxia) relative to the second (-15 ± 4% compared to post-hypoxia) and third (-9 ± 6% compared to post-hypoxia) interventions (Figure 2.2). A total of 18 hyperoxia trials were completed post-hypoxia and there was a decrease in MSNA burst frequency in 16 of these trials (Figure 2.3). The two hyperoxia trials where MSNA burst frequency did not decrease were observed in the same subject (in interventions 2 and 3). In addition, the MSNA burst frequency response to hyperoxia was significantly related to the normoxic MSNA levels (immediately preceding hyperoxia) in the first ($r^2 = 0.77$) and second ($r^2 = 0.89$) hyperoxic interventions. There was no effect of hyperoxia on total MSNA.

2.4 Discussion

Main findings. This study measured the cardiorespiratory and MSNA responses to 20 min of isocapnic hypoxia, and CC inhibition with hyperoxia. Previous studies (Xie et al. 2001; Lusina et al. 2006) have shown that acute exposure to hypoxia results in increases in MSNA which persist following termination of the hypoxic exposure. In this study, a transient decrease in the sympathoexcitation from isocapnic hypoxia by inhibition of the CC with hyperoxia was demonstrated. These results support the concept of facilitation of sympathetic outflow which is dependent upon input from the CC.

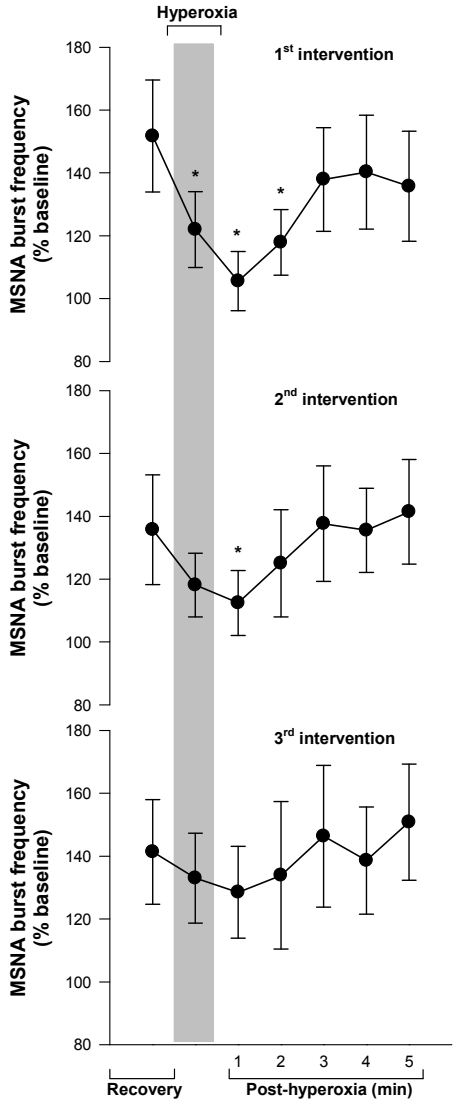

Figure 2.2 – Effect of hyperoxia on MSNA burst frequency following 20 min of isocapnic hypoxia.

Sympathoexcitation with isocapnic hypoxia. This study demonstrated that hypoxia resulted in sympathoexcitation which is thought to be initiated by the carotid chemoreflex (Guyenet 2000). The current study protocol activated the chemoreflex by increasing activity in hypoxia-mediated afferents originating from the CC. Subsequently, an increase in sympathetic vasomotor outflow was observed. This response requires CC stimulation, since sympathoexcitation with hypoxia is abolished following denervation of the carotid sinus nerve in rats (Koshiya and Guyenet 1996). Although rodent data has shown severe hypoxia directly stimulates central neurons and increases sympathetic outflow (Sun and Reis 1994), this response requires the level of hypoxia to be greater (10% - 5% O_2) than that used in the current study (~ 12% O_2). Therefore, it is unlikely that direct stimulation of brainstem neurons contributed to the observed sympathoexcitation during our moderate hypoxia protocol.

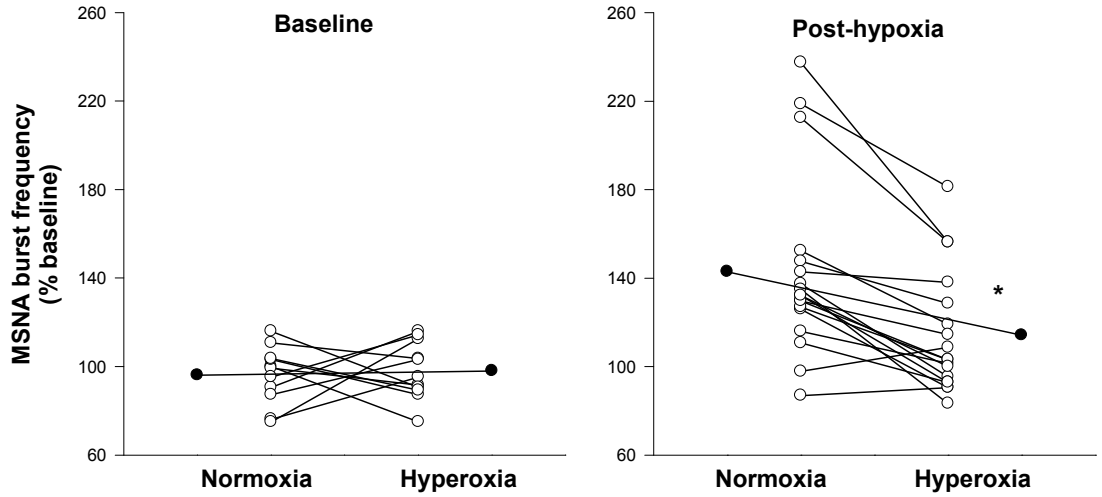

Figure 2.3 – Effect of hyperoxia on MSNA burst frequency before (Baseline) and after (Post-hypoxia) isocapnic hypoxia.

Data represent nadir 1-min value from individual trials (○) from all subjects as a percentage from baseline. ●, Mean responses. *Significantly different from normoxia.

Persistent sympathoexcitation following isocapnic hypoxia and inhibition with hyperoxia. In this study, hypoxia-mediated sympathoexcitation outlasted the hypoxic stimulus. Although this finding is consistent with previous literature (Xie et al. 2001; Lusina et al. 2006), the mechanisms underlying this response are unclear. It is unlikely that a persistent chemical stimulus was responsible for the sustained sympathoexcitation because end-tidal gases and SpO_2 quickly returned to baseline values. The possibility remains for a persistent change in the

intracellular milieu of the CC; however, this would be expected to be accompanied with a sustained increase in $\dot{V}_I$, which was not demonstrated in the data.

It is possible that the persistent sympathoexcitation was an effect of long-term potentiation of postganglionic nerves (Minota et al. 1991). Although the data cannot directly address this, it is doubtful that long-term potentiation explains our results given that exposure to stimuli other than hypoxia do not produce sympathoexcitation that outlasts the stimulus. For example, Xie and colleagues (2001) demonstrated a sustained elevation in sympathetic outflow following isocapnic hypoxia. However, there was no persistent sympathoexcitation following normoxic hypercapnia, even though the magnitude of the MSNA response during the exposure was matched between trials.

The role of the CC in the sympathetic component of the chemoreflex has been well-described elsewhere (Guyenet 2000). However, the role of the CC in sustained sympathoexcitation following hypoxia is less clear. Data from an investigation using goats demonstrated a gradual increase in chemoafferent discharge in hypoxia (Nielsen et al. 1988). This suggests that hypoxia increases hypoxic sensitivity of the CC. Data from humans suggests that CC sensitivity to hypoxia increases, which is demonstrated by a trend for increases in the ventilatory and sympathetic responses to hypoxia following 20 min of hypercapnic hypoxia (Morgan et al. 1995). There is also an increase in CC sensitivity following intermittent hypoxia, which is coupled with sympathoexcitation (Lusina et al. 2006). Lastly, patients with OSA have high levels of MSNA throughout the day which is associated with sensitized peripheral chemoreceptors, and attenuation of peripheral chemoreceptors with hyperoxia decreases MSNA in these patients (Narkiewicz et al. 1998; Narkiewicz et al. 1999). In the current study, hyperoxia resulted in a transient decrease in the persistent sympathoexcitation following exposure to hypoxia. This effect is attributed to a direct consequence of CC inhibition with hyperoxia.

Others have considered the long-lasting sympathoexcitation to be an integrated response between the CC and rostral ventrolateral medullary (VLM) neurons. Morgan and colleagues (1995) found that hypercapnic hypoxia produced sympathoexcitation which outlasted the

stimulus, and hyperoxia attenuated the sympathoexcitation in three subjects. They hypothesized that the persistent sympathoexcitation was due to a memory-like effect in rostral VLM neurons that required peripheral chemoreceptor input. It is possible that the transient decrease in sympathetic outflow with hyperoxia in the current study was due to interplay between CC and central integration of afferent signals. However, short bouts of hyperoxia were used, which inhibit the CC (Dejours 1962). It is possible that longer exposures to hyperoxia could have influenced rostral VLM neurons, although the response would most likely be excitatory (Dean et al. 2004). Furthermore, if the transient hyperoxia protocol had a direct effect on the central medullary neurons, we would have expected to observe an effect on MSNA during baseline (pre-hypoxia) trials. It is also important to note that the study by Morgan and colleagues (Morgan et al. 1995) utilized a hypercapnic hypoxia intervention which could have had a direct stimulatory effect on central medullary chemoreceptors. Further work that incorporates the role of central chemoreceptors would be useful.

Recovery of cardiorespiratory measures following isocapnic hypoxia. If the sustained sympathoexcitation is due to an increase in CC sensitivity, why did $\dot{V}_I$ return to resting levels soon after termination of hypoxia? Indeed, this is not the first investigation to show this disconnect between respiratory and sympathetic systems (Xie et al. 2001; Lusina et al. 2006). Although data from the present study cannot directly address this, the uncoupling between respiratory and sympathetic responses may lie in the central integration of afferent nerve signals. Hypoxic stimulation of the CC produces afferent signals transmitted to the rostral VLM via the nucleus tractus solitarius (NTS). Respiratory synchronization of the rostral VLM neurons suggests mediation by the central respiratory rhythm generator. However, in aenesthetized cats, CC stimulation excited certain neurons in the NTS which sent axonal projections directly to the rostral VLM (Koshiya and Guyenet 1996). Due to the direct projections from the NTS to the rostral VLM, they may provide CC input to presympathetic cells which is respiratory independent (Koshiya and Guyenet 1996). Input from these neurons may explain the quick return of respiration to baseline values once hypoxia was terminated while sympathoexcitation continued (Koshiya and Guyenet 1996; Koshiya and Guyenet 1996).

The MAP response to hypoxia is a multifaceted process, including influence from the CC (Lugliani et al. 1973; Heistad and Abboud 1980). Hypoxia had a variable response on MAP in our subjects, which is consistent with other investigations (Van Mil et al. 2002; Imadojemu et al. 2007). Although the CC can at least partly mediate MAP during hypoxia, the vascular response is selective and can depend on the vascular bed being investigated (Daugherty et al. 1967; Heistad and Abboud 1980). Additionally, hypoxia produces local vasodilation, which may counteract the hypoxia-induced sympathoexcitation (Heistad and Abboud 1980; Schneider et al. 2000). In the current study, when the hypoxic stimulus was removed, and likely any local vasodilatory substances, MAP was not changed from baseline. Evidence from both animals (Fletcher et al. 1992; Marcus et al. 2009), and humans (Arabi et al. 1999; Hansen and Sander 2003) demonstrate that long-term hypoxia, possibly in an intermittent fashion, is a requirement for a hypertensive effect that outlasts the hypoxic stimulus.

Methodological considerations. Hyperoxia is a safe, non-invasive intervention that attenuates CC activity (Watt et al. 1943; Hornbein et al. 1961; Nye et al. 1981). Other investigations have utilized dopamine infusion to 'turn off' input from the CC (Welsh et al. 1978). Hyperoxia was chosen over pharmacological blockade for two main reasons. First, although dopamine infusion inhibits chemoreceptor discharge, there is an accompanying alteration in other cardiorespiratory measures. Specifically, dopamine causes a dose-dependent increase or decrease in $\dot{V}_I$, decrease in PaO_2, and an increase in $PaCO_2$, which would have had a direct neurostimulatory effect (Welsh et al. 1978). Second, in order to characterize the MSNA response to CC inhibition, repeated hyperoxia trials were performed. Repeated infusion of dopamine in the time-course of the present study design would have had a variable effect on CC discharge; from inhibitory to excitatory (Zapata 1975).

The microneurography technique was used to obtain a measure of sympathetic outflow. Although the discharge of the peroneal nerve is similar to that demonstrated in the arm (Rea and Wallin 1989) and is correlated to noradrenaline spillover in other vascular beds (Wallin et al. 1996), it cannot be directly presumed that the current findings apply to other organs. The microneurography technique is particular sensitive to movement, and any change in electrode

position would have confounded our results. This possibility was minimized by immobilizing the leg throughout the experiment and excluding any data with a shift in the baseline signal of the mean voltage neurogram.

Conclusion. An inhibitory effect of hyperoxia on elevated vasomotor outflow following exposure to isocapnic hypoxia was demonstrated. The data in conjunction with previous studies, suggests hypoxia modulates the chemoreflex, in that there is sensitization of the peripheral chemoreceptors. This implies that continued elevated afferent signals from the CC are required in the persistent sympathoexcitation following exposure to hypoxia. These data provide further explanation for the chronic daytime sympathoexcitation in patients with OSA, which may contribute to the elevated incidence of cardiovascular disease in this population.

CHAPTER 3: BAROREFLEX CONTROL OF MUSCLE SYMPATHETIC NERVE ACTIVITY AS A MECHANISM FOR PERSISTENT SYMPATHOEXCITATION FOLLOWING ACUTE HYPOXIA

3.1 Introduction

The autonomic response to acute hypoxia results in predictable changes in various cardiorespiratory measures. For example, chemoreflex activation with acute hypoxia in healthy humans leads to increased ventilation and MSNA (Saito et al. 1988; Somers et al. 1989; Somers et al. 1989; Tamisier et al. 2004). However, upon cessation of breathing a hypoxic inspirate MSNA remains elevated in the normoxic post-hypoxia period for more than 20 min, whereas ventilation and arterial blood pressure quickly return to baseline levels (Xie et al. 2001; Lusina et al. 2006; Querido et al. 2010). Persistent sympathoexcitation in normoxia has also been reported in pathological conditions where hypoxemia is a common feature, such as OSA, and may contribute to hypertension common to the syndrome (Lesske et al. 1997; Narkiewicz et al. 1998; Dempsey et al. 2010). However, the mechanistic basis for the increased sympathetic vasomotor outflow following exposure to hypoxia is unclear.

Given that the chemoreflex is a strong modulator of sympathetic activity, studies have considered the persistent elevation in MSNA following acute hypoxia to be chemoreceptor-mediated. Specifically, acute hypoxia might cause sensitization of the peripheral chemoreceptors which outlasts the hypoxic stimulus (Morgan et al. 1995). In the previous chapter, peripheral chemoreceptor inhibition with hyperoxia resulted in a transient reduction in MSNA following exposure to acute hypoxia. Taken together, the results suggest that hypoxia initiates a persistent increase in chemoafferent activity to the rostral VLM via the NTS, which results in long-lasting sympathoexcitation (Guyenet 2000; Prabhakar and Kumar 2010).

However, it is important to recognize that MSNA is also under strong baroreflex control, with sympathetic bursts occurring during periods of low arterial pressure (diastole) and less bursts during high pressures (systole). Furthermore, many of the neural pathways of the baroreflex

coincide with those of the chemoreflex, resulting in an interdependent relationship (Somers et al. 1991; Vasquez et al. 1997; Guyenet 2000; Guyenet 2006). Peripheral chemoreceptor activation with hypoxia resets the baroreflex to higher pressures, where sympathetic outflow is increased for a given diastolic blood pressure (DBP); although this appears to occur without any change in baroreflex sensitivity (Halliwill and Minson 2002; Halliwill et al. 2003; Cooper et al. 2004). The baroreflex is reset to higher pressures in hypoxia and likely contributes to the elevated sympathetic vasoconstrictor drive in hypoxia. However, it is not known if resetting of the baroreflex persists following acute isocapnic hypoxia.

One previous study has demonstrated a persistent resetting of the baroreflex following 30 min of hypoxic apneas (Monahan et al. 2006). However, interpretation of these results is complicated by the apnea-induced hypercapnia, which has been shown to have a long-lasting effect on sympathetic outflow and baroreflex function (Tamisier et al. 2004; Cooper et al. 2005). Accordingly, the purpose of this study was to test the hypothesis that acute exposure to isocapnic hypoxia results in a resetting of the baroreflex which outlasts the hypoxic stimulus. To test this hypothesis, MSNA was continuously recorded in healthy humans at baseline, during and following exposure to acute isocapnic hypoxia. The primary assessment of baroreflex function was the spontaneous baroreflex threshold analysis which relates the probability of a MSNA burst occurring to spontaneous changes in blood pressure (Sundlof and Wallin 1978; Kienbaum et al. 2001; Hart et al. 2010; Wehrwein et al. 2010). The hypothesis was also tested in a subset of subjects by infusing vasoactive drugs as per the modified Oxford technique to obtain a measure of baroreflex function. A long-lasting resetting of the baroreflex to higher pressures following acute isocapnic hypoxia could provide further explanation for the hypoxia-induced persistent sympathoexcitation.

3.2 Materials and methods

Subject characteristics. Fourteen healthy young subjects (26 ± 1 yrs, 3 female) participated in this study after providing written informed consent. In a preliminary visit, subjects were instrumented for measurement of cardiorespiratory measures (excluding MSNA) and were

exposed to isocapnic hypoxia (SpO_2 = 80%) for familiarization purposes. All subjects were of normal height (178 ± 2 cm) and weight (75 ± 3 kg), and were free from any known cardiovascular, pulmonary, or neurological diseases. Subjects were recreationally active and abstained from eating within 2 h, or caffeine within 24 h prior to the study. All experimental procedures and protocols were approved by the Clinical Research Ethics Board at the University of British Columbia, which conforms to the standards set by the *Declaration of Helsinki*.

Experimental protocol. Subjects remained recumbent throughout testing. Following instrumentation of the subject for all physiological measures, 15 min of resting data was recorded which served as a baseline measure. Subjects were then exposed to 20 min of isocapnic hypoxia where SpO_2 was maintained at 80% by the titration of N_2 to the inspired circuit. Isocapnia was maintained by the addition of 100% CO_2 as needed. All physiological measures were continuously recorded for 5 min following the termination of the hypoxic stimulus. In 5 of the 14 subjects, 20 min of post-hypoxia data were collected.

In order to confirm the findings from the spontaneous baroreflex threshold analysis, additional experiments were performed in a subset of subjects (n = 4 out of 14), by assessing baroreflex function during bolus injections of vasoactive drugs as per the modified Oxford technique (Rudas et al. 1999; Halliwill and Minson 2002). The technique involves an intravenous bolus injection of 100 μg sodium nitroprusside followed 60 s later by 150 μg phenylephrine HCl in order to evoke a ~15 mmHg decrease and an increase in arterial pressure from baseline over ~3 min. Throughout drug infusions, subjects' breathing was matched to a metronome set at the subjects' baseline (eupnoea) breathing rate. The modified Oxford trials were performed following the baseline and post-hypoxia periods.

Physiological measures. Subjects wore a facemask connected to a pneumotachograph (model 3813, Hans Rudolph, Kansas City, MO) for determination of inspiratory flow. V_T and F_b were determined from the flow signal, and $\dot{V}_I$ was calculated from the product of F_b and V_T. Ventilated O_2 and CO_2 were sampled at the mouth with calibrated gas analyzers (models S-3A/I and CD-A, Applied Electrochemistry, Pittsburgh, PA). HR was determined with a single-lead

electrocardiogram. Beat-by-beat blood pressure (which was calibrated against automated blood pressure measurements) and SpO_2 were determined at the finger with photoplethysmography (Finometer, Finapres Medical System, Arnhem, The Netherlands) and pulse oximetry (model 3740, Ohmeda, Louisville, CO), respectively.

Direct multiunit MSNA was recorded from the peroneal nerve at the fibular head with the microneurography technique as we have previously described (Lusina et al. 2006; Querido et al. 2010) and originally developed by Vallbo et al (Vallbo et al. 1979). Following localization of the nerve with surface and subcutaneous stimulation, a recording tungsten microelectrode was advanced and manipulated until a satisfactory electrical signal was obtained. Nerve electrical signals were rectified and amplified (total gain 50,000, custom-built microneurography preamplifier; Yale University, New Haven, CT), band-pass filtered (300-5,000 Hz), and integrated (100 ms time constant; integrator model B937C, Bioengineering, University of Iowa, Iowa City, IA). MSNA recordings (>3:1 signal-to-noise ratio) were confirmed by pulse-synchronous activity, sympathoexcitation during a breath-hold and in response light tapping or stretching of the muscle, and no activation in response to gentle touching of the skin or startle stimuli.

***Data and statistical analyses*.** All data were acquired using an analog-to-digital converter (PowerLab/16SP ML 795, ADIstruments, Colorado Springs, CO, USA) and sampled at 1 kHz. A baroreflex threshold curve was calculated for each individual using the spontaneous baroreflex threshold technique, similar to that described by Sundlöf and Wallin (1978). This non-invasive method determines the occurrence of sympathetic bursts at the corresponding DBP throughout a 4 min steady-state period of data. Briefly, the method produces a linear regression line between burst occurrence and DBP, and provides a valid measure of resting baroreflex sympathetic function (Hart et al. 2010). After applying a time-shift in the MSNA integrated neurogram in order to align each MSNA burst to a corresponding R wave, DBP values were grouped into 1 mmHg bins and the percentage of cardiac cycles with a MSNA burst was calculated for each DBP bin. This analysis provides the likelihood of a burst occurring at each DBP within the section of data. A T50 DBP value, or midpoint value, was then calculated which represents the

DBP at which there is a 50% likelihood of a MSNA burst occurring. Due to the strong baroreflex influence on sympathetic outflow, lower DBP is associated with more MSNA bursts, whereas high DBP is less likely to be associated with a burst (Figure 3.1). In order to quantify and compare the likelihood of a sympathetic burst occurring between conditions (normoxia vs. hypoxia), the DBP error signal was calculated. The calculation of the DBP error signal involves subtracting the average steady-state DBP from the calculated T50 value. At rest, the error signal tends to be negative; that is, the average DBP is higher than the T50 value, and a sympathetic burst is less likely to occur (Wehrwein et al. 2010). Spontaneous baroreflex threshold curves were determined immediately prior to, during, and 5 min following the isocapnic hypoxia exposure.

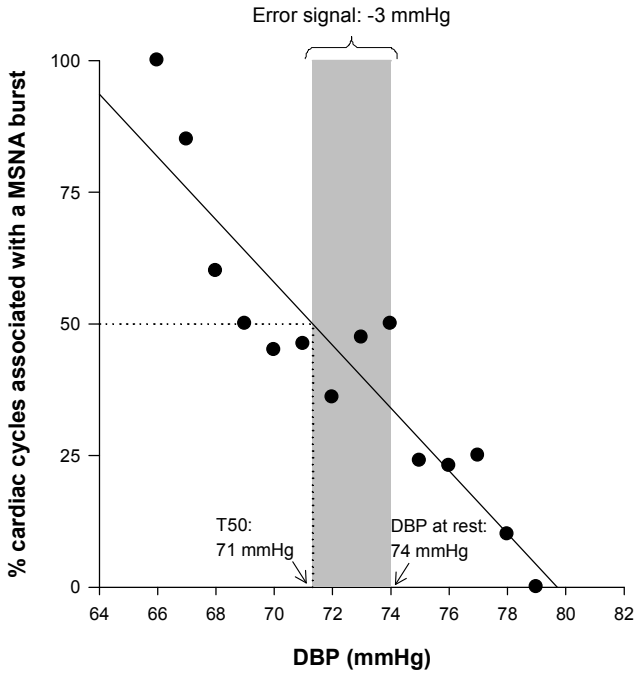

Figure 3.1 – Example of a spontaneous baroreflex threshold curve and error signal in one representative subject at baseline.

The linear relationship between burst occurrence and diastolic blood pressure (DBP) illustrates the greater likelihood of sympathetic bursts occurring at lower pressures (r = 0.92). baseline DBP for this individual was 74 mmHg. From the threshold curve, the T50 (DBP with a 50% likelihood of a burst occurring) was determined to be 71 mmHg. The calculated error signal (T50 – DBP) was -3 mmHg (shaded area). MSNA, muscle sympathetic nerve activity.

Baroreflex sensitivity curves from the modified Oxford technique were determined by the linear regressions between average MSNA (total activity of neurogram) and the means of the 1 mmHg

DBP bins. Analysis of the slope occurred from the start of the decrease in DBP from sodium nitroprusside to the peak DBP from phenylephrine HCl (Ebert and Cowley 1992; Rudas et al. 1999; Monahan et al. 2006). To quantify the magnitude of resetting of the baroreflex curves, we compared MSNA during baseline, hypoxia, and post-hypoxia during identical levels of DBP (Halliwill et al. 2003; Monahan et al. 2006). The DBP chosen for comparison was the average resting pressure obtained from the transient fluctuations at baseline for each individual.

The effect of hypoxia on cardiorespiratory measures, T50 and error signal were compared with repeated-measures analysis of variance procedures. In the case of a significant F-ratio, differences were further investigated with Dunnett's post-hoc test. The relationships of DBP and error signals as well as MSNA were examined with linear regression analysis and the Pearson correlation coefficient. The level of significance was set at $P < 0.05$ for all statistical comparisons. Group data are presented as means ± SEM.

3.3 Results

Cardiorespiratory and neural variables. Spontaneous increases and decreases in DBP from baseline were +9.2 ± 1.0 mmHg and -8.2 ± 0.7 mmHg, respectively (Table 3.1). Group mean cardiorespiratory measures are shown in Table 3.2. There was a significant decrease in SpO_2 within the first 4 min of hypoxia, and it was clamped at ~80% for the remainder of the hypoxic exposure (Table 3.2). Isocapnia was maintained throughout the experiment. Baseline MSNA burst frequency was 20 ± 1 bursts/min, which increased in hypoxia (20 min: +44 ± 9%; $P < 0.05$) and remained elevated in the post-hypoxia period (+33 ± 4%; $P < 0.05$ vs. baseline). There was a small (~3-4 mmHg) yet statistically significant increase in MAP in hypoxia, which persisted in post-hypoxia ($P < 0.05$). In contrast, there were increases in $\dot{V}_I$ and HR in hypoxia ($P < 0.05$), but both returned to baseline upon termination of hypoxia. Among individuals, MSNA (burst incidence; bursts/100 beats) was related to DBP at baseline ($r = 0.55$; $P < 0.05$) and post-hypoxia ($r = 0.62$; $P < 0.05$), but not in hypoxia ($r = 0.47$; $P > 0.05$).

Table 3.1 – Maximum change in DBP from baseline during the spontaneous threshold analysis and modified Oxford assessment

		Baseline	Hypoxia					Post-hypoxia
Method	**DBP (mmHg)**		**4 min**	**8 min**	**12 min**	**16 min**	**20 min**	
Spontaneous	High	9 ± 1	10 ± 1	11 ± 1	12 ± 1	10 ± 1	11 ± 1	12 ± 1
	Low	-8 ± 1	-9 ± 1	-9 ± 1	-8 ± 1	-10 ± 1	-10 ± 1	-9 ± 1
Modified Oxford	High	14 ± 3						12 ± 4
	Low	-17 ± 3						-15 ± 2

Values are means ± SEM in mmHg.

Table 3.2 – Effect of 20 min of isocapnic hypoxia on cardiorespiratory measures.

	BASELINE	HYPOXIA					POST-HYPOXIA
		4 min	**8 min**	**12 min**	**16 min**	**20 min**	
SpO_2 (%)	97.4 ± 0.3	87.2 ± 1.7*	81.6 ± 0.9*	80.8 ± 0.8*	80.2 ± 0.8*	80.5 ± 0.8*	96.2 ± 0.4
F_b (breaths/min)	11.1 ± 1.1	12.1 ± 1.1	12.9 ± 1.0*	13.7 ± 1.0*	13.5 ± 0.9*	13.1 ± 1.0*	11.8 ± 1.1
V_T (l)	0.5 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
V_I(l/min)	4.9 ± 0.6	6.6 ± 0.8*	7.2 ± 0.8*	6.5 ± 0.8*	6.7 ± 0.7*	6.8 ± 0.8*	5.1 ± 0.7
$P_{ET}CO_2$ (mmHg)	41 ± 1	41 ± 1	42 ± 2	41 ± 1	41 ± 1	41 ± 1	41 ± 1
HR (beats/min)	58 ± 2	65 ± 2*	70 ± 3*	68 ± 3*	68 ± 3*	68 ± 3*	58 ± 3
MAP (mmHg)	91 ± 3	94 ± 3*	95 ± 3*	94 ± 4*	94 ± 4	94 ± 4*	95 ± 4*
DBP (mmHg)	74 ± 3	76 ± 3*	76 ± 3*	76 ± 4*	75 ± 4	76 ± 4*	77 ± 4*
SBP (mmHg)	124 ± 4	129 ± 4*	129 ± 4*	128 ± 4	128 ± 4*	129 ± 4*	128 ± 5*
MSNA (bursts/min)	20 ± 1	26 ± 1*	27 ± 2*	28 ± 2*	28 ± 2*	28 ± 2*	26 ± 1*
MSNA (bursts/100 beats)	35 ± 3	40 ± 2*	40 ± 3	42 ± 3*	42 ± 3*	41 ± 3*	46 ± 3*
T50 (mmHg)	70 ± 4	74 ± 4*	74 ± 4*	75 ± 4*	74 ± 4*	74 ± 4*	77 ± 4*
Error (mmHg)	-3.9 ± 0.8	-2.2 ± 0.7*	-2.0 ± 0.8*	-1.1 ± 1.1*	-1.6 ± 0.9*	-1.4 ± 0.6*	0.2 ± 0.6*
Threshold slope	-4.2 ± 0.5	-3.4 ± 0.2	-3.5 ± 0.3	-3.4 ± 0.3	-3.7 ± 0.3	-3.7 ± 0.3	-3.9 ± 0.4

Values are means ± SEM. *significantly different from baseline.

Spontaneous baroreflex threshold slope analysis. Individual baroreflex threshold curves were determined for each subject (Figure 3.1), and the data from the group mean threshold curves were used to construct Figure 3.2A. The group mean T50 value at baseline was 70 ± 4 mmHg with a slope of -4.2 ± 0.5 bursts/100 heart beats/mmHg (Table 3.2). In hypoxia, the T50 value increased (75 ± 4 mmHg) and remained elevated relative to baseline into the post-hypoxia period (post-hypoxia 5 min: 77 ± 4 mmHg, $P < 0.05$). There was an upward and rightward shift in the threshold curve in hypoxia and post-hypoxia ($P < 0.05$); however, there was no change in the slope of the threshold analyses among conditions (Table 3.2; Figure 3.2A).

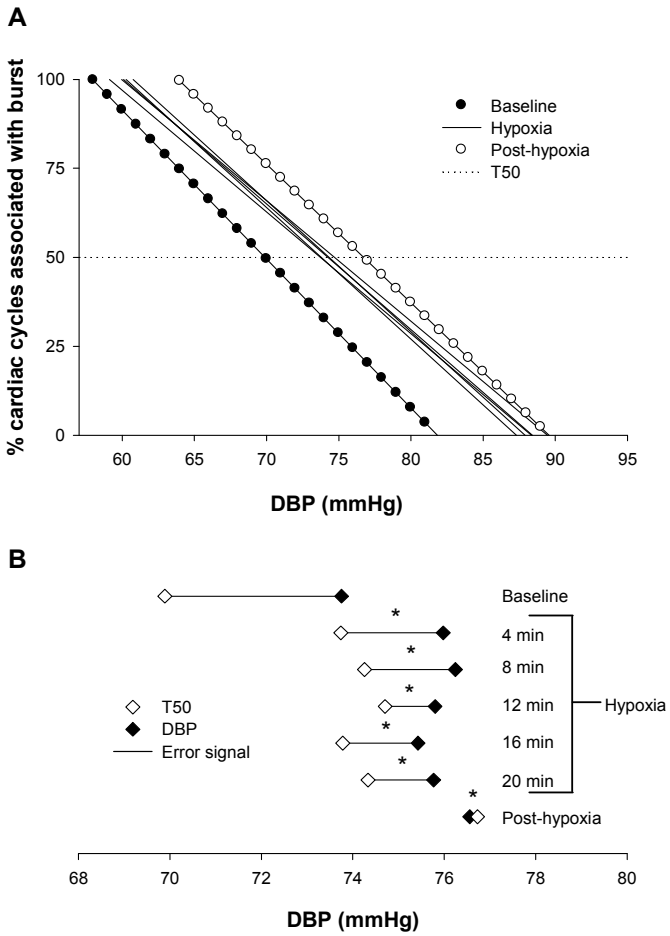

Figure 3.2 – Group mean data demonstrating the effect of isocapnic hypoxia on the threshold curves (A) and DBP error signal (B).

A: the figure was generated from the group average values for T50, DBP, and slope. Note the shift in the curves to higher DBP in hypoxia (solid line; 4-min bins) and post-hypoxia (○) periods relative to baseline (●).

B: DBP (♦), T50 (◊), and DBP error signal (solid lines). *Significant difference in the DBP error signal from baseline.

DBP error signal. Figure 3.1 demonstrates the calculation of the DBP error signal in a representative subject. Sympathetic bursts occurred more frequently at lower pressures, and the T50 (DBP at which 50% of the cardiac cycles had a sympathetic burst) was determined from the linear regression of burst occurrence and DBP (1 mmHg bins). The DBP error signal was then calculated by subtracting resting DBP from the T50 (-3 mmHg in Figure 1). At baseline, all subjects operated at a DBP that was higher than their T50, resulting in a negative error signal (-3.9 ± 0.8 mmHg; Table 3.2). Throughout the hypoxia exposure, the DBP error signal became progressively less negative. In the post-hypoxia period, the mean DBP was lower than the T50 which resulted in a positive error signal of 0.2 ± 0.6 mmHg (Table 3.2; Figure 3.2B). Regression

analysis of the DBP error signal and MSNA burst incidence demonstrated a significant positive relationship during baseline (slope = 2.1; r = 0.62; $P < 0.05$), hypoxia (20 min; slope = 4.2; r = 0.97; $P < 0.05$) and post-hypoxia (slope = 4.2; r = 0.88; $P < 0.05$).

Modified Oxford technique. Bolus injections of sodium nitroprusside and phenylephrine HCl resulted in similar magnitude of increases (~13 mmHg increase) and decreases (~16 mmHg decrease) in DBP from baseline (Table 3.1). There was no difference in the T50 value between the spontaneous baroreflex threshold analysis and the modified Oxford analysis (Baseline: spontaneous = 57.5 ± 3.9 mmHg, Oxford = 57.8 ± 2.9 mmHg, $P > 0.05$; Post-hypoxia: spontaneous = 64.5 ± 3.5 mmHg, Oxford = 61.7 ± 1.3 mmHg, $P > 0.05$; Figure 3.3 shows data from one subject). Similar to the spontaneous baroreflex threshold curve analysis, there was no change in the slope (baseline = -0.003 ± 0.002, post-hypoxia = -0.002 ± 0.001 arbitrary units/mmHg, $P > 0.05$) and 3 of 4 subjects demonstrated an upward and rightward shift in the curve, although the group mean baroreflex setpoint did not reach statistical significance ($P = 0.7$).

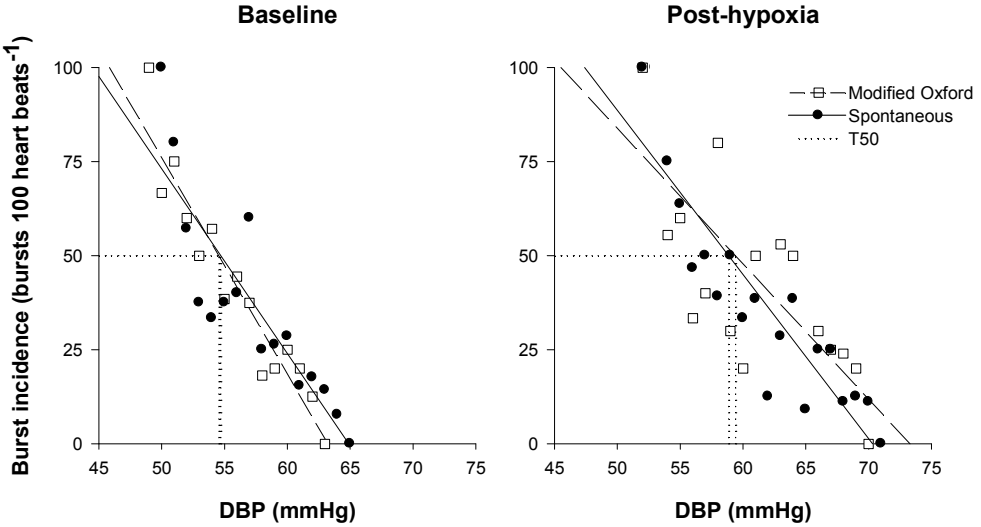

Figure 3.3 – Example threshold curves from the spontaneous baroreflex analysis (solid lines, ●) and during the modified Oxford (dashed lines, □) in one representative subject.

Continued recordings of MSNA during post-hypoxia. All physiological measures were continuously recorded for 20 min following hypoxia in 5 subjects. In those subjects, hypoxia increased MSNA burst frequency and the T50 by 9.5 ± 0.9 bursts/min and 5.5 ± 3.8 mmHg ($P <$

0.05), respectively. There was also an increase in the DBP error signal of 4.6 ± 1.5 mmHg (P <0.05) from baseline; although there was no change in DBP (1 ± 3 mmHg increase, $P > 0.05$). The MSNA burst frequency, T50, and DBP error signal remained elevated relative to baseline for 20 min post-hypoxia (MSNA burst frequency: 8.2 ± 1.9 bursts/min increase; T50: 8.8 ± 2.2 mmHg increase; DBP error signal: 4.3 ± 1.5 mmHg increase; $P < 0.05$; Figure 3.4); however, there was no significant change in DBP from baseline levels (5 ± 3 mmHg increase, $P > 0.05$).

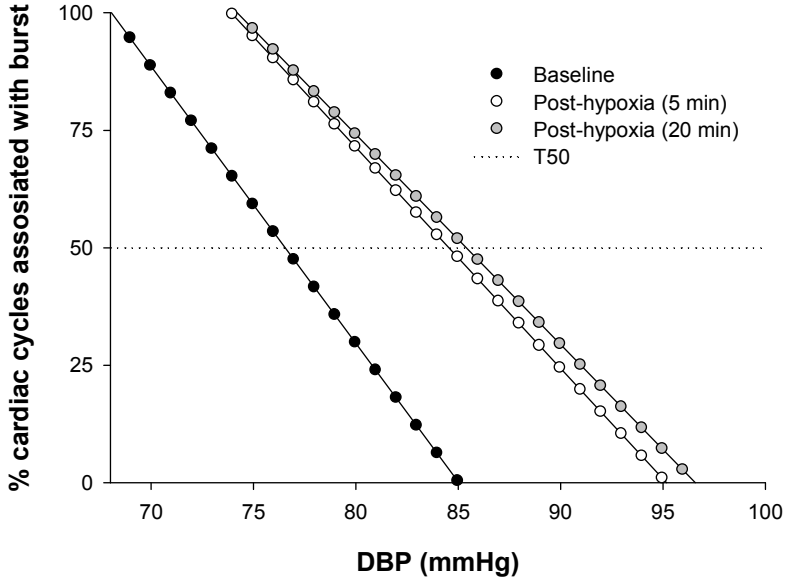

Figure 3.4 – Group mean threshold curves at 5 min (○) and 20 min (shaded circles) post-hypoxia compared with baseline (●).

The figure was generated from the group average values for T50, DBP, and slope. Relative to baseline, there was an increase in the post-hypoxia T50 (5 min: 8 mmHg increase), which persisted for 20 min post-hypoxia (20 min: 9 mmHg increase).

3.4 Discussion

Main findings. The purpose of this study was to test the hypothesis that the baroreflex contributes to persistent sympathoexcitation following acute isocapnic hypoxia in humans. The hypothesis was tested using the spontaneous baroreflex threshold analysis technique (Sundlof and Wallin 1978; Hart et al. 2010; Wehrwein et al. 2010) as well as with the infusion of vasoactive drugs (modified Oxford). The findings demonstrate a persistent resetting of the baroreflex stimulus-response curve upward and rightward (to higher MSNA and pressures) in acute isocapnic hypoxia, without a change in slope. The primary new finding from this study is

that the resetting of the baroreflex that occurs in acute isocapnic hypoxia persists for at least 20 min following the termination of the hypoxic stimulus. This suggests that the baroreflex and chemoreflex are related, in that resetting of the baroreflex occurs in parallel to persistent sympathoexcitation following exposure to hypoxia, likely from the peripheral chemoreceptors (Querido et al. 2010). Our findings provide further insight into the importance of hypoxia for the elevated levels of sympathetic outflow expressed in patients with OSA (Narkiewicz et al. 1998).

Sympathetic control of blood pressure. The sympathetic nervous system plays an important role in short- and long-term control of arterial pressure (Osborn et al. 2005; Lohmeier et al. 2010). An inverse relationship was found between variations in blood pressure and MSNA because of the baroreflex. However, in most young normotensive subjects, *inter*-individual analysis shows that chronic levels of resting sympathetic nerve activity are not related to resting blood pressure levels (Sundlof and Wallin 1978; Kienbaum et al. 2001; Charkoudian et al. 2005; Charkoudian et al. 2006). This somewhat paradoxical phenomenon is thought to be due to the fact that the pressor effects of sympathetically-mediated vasoconstriction are balanced by other factors such as low cardiac output ($\dot{Q}$) and possibly high nitric oxide (Skarphedinsson et al. 1997; Charkoudian et al. 2005). In the present study, there was a modest relationship ($r = 0.55$; $P < 0.05$) between MSNA and DBP. Although the reason for the association is not clear, certainly the heterogeneity in sympathetic control mechanisms noted in previous work might contribute to some subsets of subjects exhibiting this relationship when others do not (Guyenet 2006).

At baseline, all subjects in the present study had a negative DBP error signal (T50 minus DBP); that is, less than 50% of the cardiac cycles at rest were associated with a sympathetic burst. Also, the DBP error signal was related to MSNA burst incidence ($r = 0.62$; $P < 0.05$), where those subjects with the most negative DBP error signal (DBP much higher than T50) also had the lowest MSNA burst incidence. In hypoxia and post-hypoxia periods, the DBP error signal became less negative with a greater likelihood of sympathetic bursts. This occurred even though there was a concurrent increase in DBP, which would normally lower the likelihood of a sympathetic burst (Sundlof and Wallin 1978). The error signal provides an indication of where along the threshold curve the baroreflex tends to operate. The reduction in the error signal in the

current study reflects a hypoxia-induced shift in the baroreflex curve to higher levels of MSNA, and indicates an upward and rightward shift in the setpoint of the baroreflex.

Interaction of the baro- and chemoreflexes. Hypoxia activates peripheral chemoreceptors located in the carotid sinus which leads to an increase in activity of the chemoafferents in the carotid sinus nerve. The carotid sinus nerve projects to the lower brainstem and is integrated in the NTS. Following integration of chemoafferent signals, there are subsequent increases in ventilation and sympathetic outflow. Sustained hypoxia causes a progressive increase in chemosensory discharge and sensitizes the peripheral chemoreceptors to subsequent hypoxia exposures (Nielsen et al. 1988; Morgan et al. 1995), due to a reactive oxygen species-dependent upregulation of endothelin-1 (ET-1) and endothelin receptor A (Prabhakar and Kumar 2010). Accordingly, previous studies have implicated an elevation in chemoafferents from the peripheral chemoreceptors for the persistent sympathoexcitation following hypoxia (Narkiewicz et al. 1998; Querido et al. 2010).

The present study, along with others (Halliwill and Minson 2002; Cooper et al. 2005; Monahan et al. 2006), demonstrates modulation of the baroreflex with acute isocapnic hypoxia; however, the mechanisms are unclear. Carotid artery ultrasound imaging in humans shows that baroreflex impairment (~40-50%) during and following poikilocapnic hypoxia is attributed to the neural component of the baroreflex (i.e. neural properties in the reflex arc), rather than the mechanical component (i.e. transduction arterial pressure into baroreceptors stretch) (Hunt et al. 2008). Furthermore, studies in rodents show that hypoxia modulates the integration of sensory information in the central nervous system (Kline 2010). The current study was not designed to directly address where along the baroreflex arc hypoxia-induced modulation occurs, however medullary integration of baroafferents is likely an important contributor.

It is important to emphasize that the peripheral chemoreflex and baroreflex do not operate in isolation. Rather, in conscious and neurally intact humans, sensory information regarding blood gas homeostasis and arterial blood pressure regulation converge in an integrative fashion. The persistent sympathoexcitation following acute hypoxia may be the result of a coordinated

response from both the baroreflex and chemoreflex. Specifically, neural pathways of the baroreflex and chemoreflex arcs coincide which permits interaction (Loewy and Spyer 1990; Vasquez et al. 1997). In humans, there is a negative relationship between the baro- and chemoreflexes; that is, baroreflex activation inhibits the chemoreflex and vice versa (Somers et al. 1991; Cooper et al. 2005). Therefore, the persistent sympathoexcitation following acute hypoxia may be due to an upregulation of the chemoreflex (i.e. sustained elevation in chemoreceptor activity) (Morgan et al. 1995; Querido et al. 2010) and concurrent resetting of the baroreflex (reduced baroreceptors activity or altered medullary processing from baroafferents) (Halliwill and Minson 2002; Monahan et al. 2006; Kline 2010). Stated differently, the inhibitory influence of the baroreflex on sympathetic tone may be attenuated through central barosensitive neurons, thereby 'allowing' increased sympathetic outflow from elevated chemoreceptor afferent activity.

Persistent effect of hypoxia on baroreflex control. A hypoxia-induced adjustment of the baroreflex could partly explain alterations in blood pressure control inherent to certain pathological conditions. Individuals with OSA have an impaired baroreflex during sleep as well as throughout the day (Carlson et al. 1996; Bonsignore et al. 2002), which suggests that there is a long-lasting effect of night-time exposure to repeated apneas on cardiovascular control. Monahan *et al*. (2006) removed the confounding factors of OSA (e.g. obesity, night-time arousal) and determined the effect of repeated hypoxic apnoeas (20 s apnea, 1 apnea/min, 30 min) on baroreflex function in otherwise healthy humans (comparable to the subjects in the present study). They found a resetting of the baroreflex to higher pressures and MSNA that persisted for 50 min following the hypoxic apneas. It was concluded that repeated apnoeas, specifically the exposure to hypoxia, are responsible for persistent resetting of the baroreflex. However, it is important to note that hypoxic apnoeas are also accompanied with periods of hypercapnia (~6 mmHg increase) which could explain the change in baroreflex set point (Cooper et al. 2005). In the present study, an isocapnic hypoxia protocol was used and also demonstrated a persistent resetting of the baroreflex. The current new findings extend those from Monahan *et al*. (2006) and show a requisite role of hypoxia for a long-lasting resetting of the baroreflex, rather than an effect of concurrent hypercapnia.

Methodological considerations. Assessing baroreflex function in anaesthetized animals permits larger changes in blood pressure, thereby enabling identification of baroreflex threshold and saturation (Persson and Kirchheim 1991). The two measures of baroreflex function used (i.e. spontaneous baroreflex threshold analysis and modified Oxford technique) allow characterization of the linear portion of the curve, and excludes reflex threshold and saturation (Sundlof and Wallin 1978; Rudas et al. 1999; Halliwill and Minson 2002; Hart et al. 2010). Although this limitation prevents characterizing the full baroreflex curve, the changes in blood pressure in our study (~10-15 mmHg change in DBP from resting levels) are similar to the spontaneous changes in blood pressure experienced by individuals during the activities of daily living.

Conclusion. The control of blood pressure and sympathetic outflow is multifactorial; however, the baroreflex plays an important role in regulating both systems. The current study was designed to address whether the baroreflex was involved in the persistent elevation in sympathetic outflow following exposure to acute hypoxia in humans. A resetting of the baroreflex to higher blood pressures and levels of MSNA was found which persisted following termination of the hypoxic stimulus. It is concluded that baroreflex resetting occurs in parallel with a hypoxia-induced overactivity of the peripheral chemoreceptors. The convergence of inputs and areas of integration result in a coordinated relationship between the baro- and chemoreflexes, and provides a complex mechanism for the long-lasting sympathoexcitation following hypoxia exposure. This has implications for explaining, at least in part, the sympathoexcitation and altered blood pressure control in patients with OSA.

CHAPTER 4: CEREBROVASCULAR RESPONSES TO TRANSIENT HYPO- AND HYPERTENSION FOLLOWING ISOCAPNIC HYPOXIA

4.1 Introduction

Autonomic control of CBF with cerebral autoregulation (CA) is a tightly regulated mechanism reported to maintain CBF constant during gradual changes in blood pressure (static CA), and buffer sudden fluctuations in perfusion pressure (dynamic CA) (Aaslid et al. 1989). Although recently challenged (Lucas et al, Hypertension, 2010), the traditional model of CA proposes that CBF is independent of changes in mean arterial pressure (MAP) between ~50 to 160 mmHg (Lassen 1959). The mechanisms of CA are complex and incompletely understood, but likely rely on a combination and interaction of myogenic, neural, endothelial, and metabolic factors (Aaslid et al. 1989; Tsivgoulis and Alexandrov 2009). Early methods of dynamic CA assessment in humans often included an abrupt decrease in blood pressure with rapid thigh-cuff deflation (Aaslid et al. 1989); whereas later assessment focused on transfer function analysis of spontaneous oscillations in blood pressure and CBF over a period of time (Zhang et al. 1998). Although these methods have the advantage of being non-invasive and easily administered, they do not fully characterize CA. Specifically, these methods do not differentiate the CA response in the hypotensive and hypertensive ranges. A recent study which assessed dynamic CA with bolus injections of vasoactive drugs demonstrated a system that is not as effective as originally proposed, in that CBF is more blood pressure-dependent than originally considered (Lucas et al. 2010). Furthermore, one study found dynamic CA to be nonlinear, and more effective at buffering increases in blood pressure, whereas CBF decreases in hypotension (Tzeng et al. 2010).

During exposure to hypoxia, an impairment in CA has been reported (Iwasaki et al. 2007). Although the mechanisms responsible for the impairment are unclear, the hypoxia-induced impairment in dynamic CA may persist in normoxia. For example, pathological models of hypoxia (e.g. OSA) demonstrate impairment in CA in normoxia (Urbano et al. 2008; Nasr et al. 2009). The nightly hypoxia exposure inherent of obstructive sleep apnoea has been implicated

for many of the daytime cardiovascular and cerebrovascular impairments commonly associated with the syndrome. However, it is important to note that data from high-altitude studies or in patients with OSA are complicated by the concurrent hypocapnia and hypercapnia, respectively (Aaslid et al. 1989). Studies in patients with OSA are also confounded by extraneous factors such as night-time arousal and hypertension. Furthermore, to my knowledge, no study has separately assessed the asymmetry of the cerebrovascular response against transient hypertension and hypotension following exposure to hypoxia. Accordingly, the purpose of this study was to test the hypothesis that acute isocapnic hypoxia results in a persistent impairment in CA following a hypoxic exposure. To test the hypothesis, blood pressure was perturbed with intravenous bolus injections of vasoactive drugs as per the modified Oxford technique (Rudas et al. 1999; Tzeng et al. 2010), which enabled separate characterization of the hypertensive and hypotensive function of CA.

4.2 Materials and methods

Subject characteristics. Eight subjects (age = 29 ± 6 yrs; 4 female) of normal height (174 ± 9 cm) and weight (70 ± 9 kg) participated in this study, and were free from any known respiratory, cardiovascular or neurological diseases. Subjects were also excluded from participation if they had a history of sleep apnoea or had been to high-altitude within 12 months of testing, and all abstained from caffeine and alcohol for at least 12 hrs before the study. On an initial visit, subjects provided written consent and were familiarized with the experimental protocol and measurements, excluding drug infusions and microneurography. All experimental procedures were approved by the Clinical Research Ethics Board at the University of British of Columbia, which conforms to the standards set by the *Declaration of Helsinki*.

Experimental protocol. Subjects remained semi-supine throughout experimentation. Following instrumentation, a minimum of 15 min of data was collected which served as a baseline measurement. Blood pressure manipulation was then performed with sequential intravenous bolus injections of sodium nitroprusside (SNP; 100 μg) followed by phenylephrine HCl (150 μg) 60 s later, as per the modified Oxford technique (Rudas et al. 1999; Halliwill and Minson 2002).

Subjects were then exposed to isocapnic hypoxia, where SpO_2 was maintained at 80% for 20 min by titrating 100% N_2 and CO_2 to the inspirate as needed. The modified Oxford technique was then completed again ~7 min following the hypoxic exposure while the subject breathed room air. Throughout drug infusions breathing was coordinated to a metronome that was set at the subject's resting F_b (11 ± 4 breaths/min).

Physiological measures. Inspiratory flow, V_T and F_b were determined from a facemask connected to a pneumotachograph (model 3813, Hans Rudolph, Kansas City, MO). $\dot{V}_I$ was calculated from the product of V_T and F_b. Beat-by-beat blood pressure was determined non-invasively at the finger with photoplethysmography (Finometer, Finapres Medical System, Arnhem, The Netherlands), which was calibrated to an automated blood pressure cuff (BPM-100, VSM Medtech Ltd, Vancouver, BC). Transcranial Doppler ultrasonography (Neurovision 500M, Multigon Industries, Yonkers, NY) was used to measure cerebral blood flow velocity in the middle cerebral artery (MCAV). HR and SpO_2 were determined with electrocardiography and finger pulse oximetry (7500FO, Nonin Medical Inc., Plymouth, MN), respectively. The middle cerebral artery was insonated through the temporal window, and the Doppler probe was held securely in place with a head strap (Marc 600, Spencer Technologies, Seattle, WA, USA).

In two of the subjects, direct recordings of muscle sympathetic nerve activity (MSNA) were obtained with microneurography (Vallbo et al. 1979) (see Chapter 2 for a more comprehensive description). Briefly, multiunit postganglionic MSNA from the peroneal nerve was continuously measured with a tungsten microelectrode (10-μm tip diameter, 35 mm; Frederick Haer, Bowdoinham, ME). MSNA signals were rectified and amplified (total gain 50,000, custom-built microneurography preamplifier; Yale University, New Haven, CT), band-pass filtered (300-5,000 Hz), and integrated (100 ms time constant; integrator model B937C, Bioengineering, University of Iowa, Iowa City, IA).

Data and statistical analyses. All data were acquired continuously (sampled at 1 KHz) using an analog-to digital converter (Powerlab/16SP ML 795; ADInstruments, Colorado Springs, CO, USA) interfaced with a computer for subsequent offline analyses. The effect of hypoxia on

cardiorespiratory measures was determined with repeated measures ANOVA. In the case of a significant *F* ratio, differences were investigated with Tukey's post hoc analysis. CBF values were grouped into 1 mmHg blood pressure bins; the method used to assess CA was by using Pearson product-moment correlation between MAP and MCAV separately during periods of falling and rising blood pressure. Cerebrovascular resistance (CVR; MAP/MCAV) was calculated from MCAV and MAP. Statistical analyses were not performed on the MSNA data (n = 2). The level of significance was set at $P < 0.05$ for all statistical comparisons. Group data are presented as means ± SD.

4.3 Results

Cardiorespiratory response to isocapnic hypoxia. Within 5 min from the start of the hypoxic exposure SpO_2 was significantly decreased, which was maintained at ~80% for the remainder of the exposure. Throughout the protocol, $P_{ET}CO_2$ was held constant relative to baseline levels ($P > 0.05$; Table 4.1). Group mean data for the effect of isocapnic hypoxia are shown in Table 4.1. $\dot{V}_I$ and HR increased in hypoxia (~28% and ~17% increase, respectively) but returned to pre-hypoxia values once the hypoxic exposure was terminated. Similarly, there was an increase in MCAV in hypoxia which returned to pre-hypoxia levels in post-hypoxia.

Table 4.1 – Effect of 20 min of isocapnic hypoxia on cardiorespiratory and MSNA measures.

	Baseline	Hypoxia				Post-hypoxia
		5 min	10 min	15 min	20 min	
SpO_2 (%)	97 ± 1	94 ± 1*	84 ± 2*	82 ± 3*	82 ± 3*	96 ± 1
F_b (breaths/min)	11 ± 4	12 ± 4	13 ± 3	12 ± 3	12 ± 3	12 ± 3
V_T (l)	0.74 ± 0.27	0.84 ± 0.35	0.82 ± 0.29	0.82 ± 0.34	0.80 ± 0.27	0.68 ± 0.15
V_I (l/min)	7.4 ± 1.7	8.7 ± 1.5*	9.5 ± 2.0*	8.9 ± 1.8*	8.7 ± 1.5*	7.3 ± 1.2
$P_{ET}CO_2$ (mmHg)	38 ± 4	37 ± 3	36 ± 3	36 ± 4	36 ± 4	36 ± 4
HR (beats/min)	54 ± 8	57 ± 8	64 ± 10*	64 ± 11*	63 ± 9*	53 ± 9
SV (ml)	94 ± 25	103 ± 27*	99 ± 24	98 ± 21	99 ± 21	101 ± 21*
Q (l)	5.0 ± 1.2	5.8 ± 1.5*	6.3 ± 1.6*	6.2 ± 1.7*	6.2 ± 1.5*	5.3 ± 1.2
MAP (mmHg)	81 ± 5	85 ± 6*	84 ± 7*	83 ± 7	83 ± 8	84 ± 6*
SBP (mmHg)	110 ± 8	114 ± 10	115 ± 11	113 ± 10	115 ± 12	115 ± 7
DBP (mmHg)	67 ± 6	69 ± 8	68 ± 9	68 ± 9	67 ± 9	67 ± 8
MCAV (cm/s)	58 ± 9	56 ± 9	59 ± 9	62 ± 9*	62 ± 10*	54 ± 11
CVR (mmHg/cm/s)	1.43 ± 0.24	1.53 ± 0.23	1.44 ± 0.23	1.36 ± 0.19	1.36 ± 0.21	1.59 ± 0.26*
MSNA (bursts/min)	21.6 ± 0.2	27.4 ± 1.2	27.9 ± 3.7	31.6 ± 1.6	30.0 ± 1.8	29.0 ± 1.4

Values are means ± SD (n = 8; MSNA: n = 2). *significantly different from baseline.

In contrast, post-hypoxia, there was an increase CVR (12 ± 9% increase) ($P < 0.05$). Relative to baseline, MSNA burst frequency increased in hypoxia (maximum increase of 10 bursts min^{-1}), and remained elevated in the post-hypoxia period (7.4 bursts min^{-1} increase).

Cerebrovascular regulation during drugs infusions. Relative to baseline, there was no change in any ventilatory measure during the infusion of vasoactive drugs ($P > 0.05$; Table 4.2). The magnitude of the drug-induced changes in MAP did not change post-hypoxia. There were concurrent increases and decreases in HR during SNP and PE infusions, respectively, and were not affected by hypoxia (Table 4.2). Figure 4.1 shows the change in MCAV during blood pressure perturbations for the group. At baseline, MAP and MCAV demonstrated a significant positive relationship during hypotension (r = 0.75, $P < 0.05$; Figure 1); this relationship was not different in post-hypoxia (r = 0.71, $P < 0.05$). Similarly, MCAV increased linearly with MAP with hypertension (r = 0.73, $P < 0.05$); however, there was no relationship post-hypoxia (r = 0.35, $P > 0.05$). There was no effect of isocapnic hypoxia on CVR during drug infusions in the post-hypoxia period (Table 4.2).

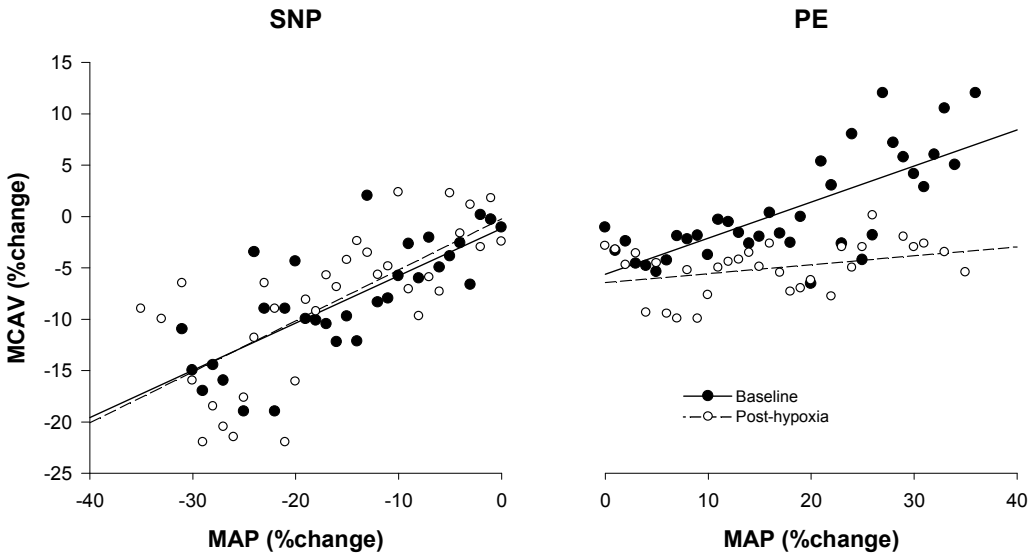

Figure 4.1 – Group mean data of relative changes in middle cerebral artery flow velocity (MCAV) with changes in mean arterial blood pressure (MAP) during bolus injections of sodium nitroprusside (SNP) and phenylephrine (PE).

MCAV was significantly related to MAP in hypotension at baseline and in post-hypoxia ($P < 0.05$). Similarly, MCAV was also related to MAP in hypertension at baseline; however, the two were not correlated post-hypoxia ($r = 0.35$; $P > 0.05$)

Table 4.2 – Cardiorespiratory measures during bolus injections of sodium nitroprusside (SNP) and phenylephrine (PE)

	Pre-hypoxia			Post-hypoxia		
	Baseline	**SNP**	**PE**	**Recovery**	**SNP**	**PE**
SpO_2 (%)	97 ± 1	97 ± 1	97 ± 1	96 ± 1	97 ± 1	97 ± 1
F_b (breaths/min)	11 ± 4	10 ± 3	11 ± 4	12 ± 3	12 ± 4	12 ± 4
V_T (l)	0.74 ± 0.27	0.89 ± 0.41	0.89 ± 0.37	0.68 ± 0.15	0.77 ± 0.31	0.82 ± 0.32
V_I (l/min)	7.40 ± 1.70	8.21 ± 1.88	8.79 ± 2.23	7.25 ± 1.23	8.07 ± 1.51	8.63 ± 1.97
$P_{ET}CO_2$ (mmHg)	38 ± 4	39 ± 4	36 ± 3	36 ± 4	38 ± 3	36 ± 2
MAP (mmHg)	81 ± 5	65 ± 7*	94 ± 10*	84 ± 6	64 ± 7 '	95 ± 9 '
SBP (mmHg)	110 ± 8	95 ± 12	129 ± 14	115 ± 7	97 ± 12	129 ± 9
DBP (mmHg)	67 ± 6	55 ± 7	77 ± 8	67 ± 8	53 ± 8	78 ± 10
HR (beats/min)	54 ± 8	78 ± 15*	48 ± 9*	53 ± 9	76 ± 12 '	47 ± 6 '
SV (ml)	93.9 ± 25.2	85.9 ± 21.7	100.8 ± 34.4	100.6 ± 21.1	91.8 ± 20.2	97.8 ± 25.3
Q (l)	5.0 ± 1.2	6.5 ± 1.3*	4.8 ± 1.7	5.3 ± 1.2	6.8 ± 1.3 '	4.6 ± 1.2 '
MCAV (cm/s)	58 ± 9	53 ± 9	55 ± 13	54 ± 11	50 ± 8	53 ± 12
CVR (mmHg/cm/s)	1.43 ± 0.24	1.24 ± 0.14*	1.77 ± 0.30*	1.59 ± 0.26*	1.31 ± 0.16 '	1.88 ± 0.36 '

Values are means ± SD. *significantly different from baseline; ‘significantly different from recovery.

4.4 Discussion

Main findings. This study was designed to test the hypothesis that acute isocapnic hypoxia results in long-lasting modification in cerebrovascular regulation during transient changes in blood pressure. In contrast to the traditional view of a completely effective cerebral autoregulatory system, this study found that cerebral blood velocity is pressure-dependent. Furthermore, this relationship was evident following exposure to hypoxia only during hypotension, but not during hypertension. Taken together, this study suggests that cerebral blood flow is more 'pressure-passive' than originally thought during these typical fluctuations in blood pressure. However, the dissociation in hypo- and hypertensive responses in cerebral blood velocity following hypoxia suggests the stimulation of a reactive mechanism, leading to the apparent hysteresis.

Cerebral autoregulation and hypoxia. Cerebral autoregulation matches cellular blood flow needs to blood flow by adjusting vessel tone of small cerebral vessels. This protective mechanism is important in maintaining CBF relatively constant over a wide range of pressures. However, in hypoxia, cerebral autoregulation has been shown to be impaired. Iwasaki et al. (2007) used transfer function analyses as a measure of CA in humans breathing 15% oxygen, and found the gain between blood pressure and CBF was increased compared to normoxic baseline. Thus, the fluctuations in CBF were coupled to blood pressure fluctuations to a greater degree. It was suggested that cerebrovascular regulation is dependent on balance between the direct vasodilatory effect of hypoxia on cerebral vessels, and the vasoconstriction from the hypoxia-induced sympathoexcitation. Overall, the balance favoured cerebral vasodilation, leading to the impaired buffering capacity. The same group also demonstrated that acclimatization to high-altitude did not improve CA (Iwasaki et al. 2010). Furthermore, pathological models of hypoxia suggest the hypoxia-induced impairment in CA outlasts the hypoxia stimulus. In particular, patients with OSA demonstrate a coupling of MCAV and arterial blood pressure during hypoxic apnoeic events while sleeping (Balfors and Franklin 1994). Interestingly, the coupling between MCAV and MAP persists during the day, while the patient is eupnoeic and normoxic (Nasr et al. 2009). Accordingly, it was anticipated that CA would be

impaired following the isocapnic hypoxia exposure in the current study. However, the opposite was found: a pressure-passive system at baseline (*impairment*), and *improvement* post-hypoxia. The reason for the inconsistency in the current results from those of previous studies is not immediately apparent; however, this is not the first study to show a lack of CA impairment in hypoxia (Ainslie et al. 2007). Van Osta et al. (2005) found no change in CA following 20 h at ~4500 m (arterial oxygen saturation ~79%); although they used the thigh cuff release technique which only measures the cerebral response to hypotension. This makes comparison to the current study difficult. In another study (Ainslie et al. 2008), exposure to 10% oxygen (arterial oxygen saturation ~78%) lead to a decrease in gain (spectral and transfer function analysis) compared to normoxia, indicating an improvement in cerebral autoregulation. Unfortunately, the study was not designed to investigate potential mechanisms, so the physiological rationale for the improvement is unknown.

Cerebral autoregulation and sympathetic outflow. In the current study, the change in CA only occurred during hypertension, but not hypotension. This emphasizes the importance of considering cerebrovascular tone as being regulated by distinct mechanisms depending on whether perfusion pressure is increased or decreased. Our understanding of the mechanisms of cerebral autoregulation is incomplete, mainly due to the logistical complications of such investigations in humans. However, some have implicated neural control to play an important role in acute fluctuations in perfusion. For instance, Zhang et al. (2002) found impairment of CA following removal of any neural control with ganglion blockade (trimethaphan). It was suggested that autonomic neural control of the cerebral circulation is tonically active and plays an important role in buffering sudden change in perfusion. In the current study, MSNA was obtained in two subjects and demonstrated a long-lasting sympathoexcitation following hypoxia (which was also shown in Chapters 2 and 3). Not only is MSNA elevated post-hypoxia, but the sensitivity of vasomotor outflow is also increased (albeit to chemical stimuli) (Morgan et al. 1995). The increase in CVR post-hypoxia also suggests a persistent increase in sympathetic outflow to the cerebral vasculature. Taken together, the hypoxia-induced increase in sympathetic outflow could provide a protective mechanism for cerebral vessels in order to more effectively buffer sudden increases in perfusion. Although elevated sympathetic outflow is also experienced

during a hypoxia exposure, the impairment reported from other investigators may be due to a local hypoxia-induced vasodilation that 'outweighs' the sympathoexcitation. Thus, when the hypoxia exposure is terminated, along with the local vasodilatory influence, sympathoexcitation persists and could represent a mechanism for the improvement in CA demonstrated in the current study.

Methodological considerations. Due to its temporal resolution, the transcranial Doppler technique is an excellent tool to measure CA; however, the technique provides a measure of blood velocity, not flow. Therefore, the validity of changes in blood velocity reflecting changes in blood flow are based on the assumption that the diameter of the artery being insonated (i.e. the middle cerebral artery) remains constant. With the infusion of vasoactive drugs, it is possible for the measured changes in velocity were due to changes in middle cerebral vessel diameter, rather than flow. However, previous studies have demonstrated that intra-arterial infusion of nitroprusside or phenylephrine does not cause any relevant change in middle cerebral artery diameter (Giller et al. 1993; Johnston et al. 1994). It has been suggested that the integrity of the blood-brain barrier eliminates a possible influence of these drugs on cerebral vessels of different sizes (Giller et al. 1993; Johnston et al. 1994). Therefore, it is likely that the observed changes (or lack thereof) in MCAV measured via the transcranial Doppler represented real changes in cerebral blood flow. Nonetheless, the current experimental design did not include direct measures of cerebral vessel diameter, and a direct effect of drug infusion on the cerebral vessels remains a possibility.

The transcranial Doppler was used to insonate one vessel: the middle cerebral artery. Although this artery supplies blood to a large proportion of the brain, the proportion is variable; this means the $\dot{Q}$ through the anterior and posterior cerebral arteries is also variable. Since this study was limited to measuring one vessel, I cannot predict any possible changes in flow in the other arteries. For instance, although there was no increase in MCAV post-hypoxia with phenylephrine, it is possible that the flow through the anterior and posterior cerebral arteries actually increased (Willie et al. 2011). Further investigations which measure changes in flow

from all three major feeder arteries would be beneficial to explore possible localized alterations in perfusion.

Conclusion. To my knowledge, this is the first study to measure CA after an acute exposure to isocapnic hypoxia. By using the modified Oxford technique to manipulate blood pressure, it was possible to separate the hypotensive and hypertensive cerebral autoregulation responses. At baseline, MCAV was pressure-passive, where CBF would follow transient increases and decreases in blood pressure. The lack of an increase in MCAV with drug-induced hypertension post-hypoxia suggests that the hypoxia-induced persistent sympathoexcitation acts as a protective mechanism to buffer sudden increases in pressure. A principal observation from this study is the dissociation in the cerebral blood velocity response to increases and decreases in blood pressure. This has important implications when interpreting previous studies which have employed protocols that only measure the cerebrovascular response to hypotension (e.g. thigh cuff removal), or analysis techniques (e.g. spectral and transfer function analysis) that assume CA is equally efficient to both hypotensive and hypertensive challenges.

CHAPTER 5: EFFECT OF CO_2 ON HYPOXIC CEREBRAL AUTOREGULATION

5.1 Introduction

The cerebral autoregulatory curve established by Lassen (1959) suggests that fluctuations in MAP between ~60 – 150 mmHg do not result in a measurable change in CBF. For many years, this concept of a cerebral vascular system that is independent of perfusion pressure had been widely accepted. Given the importance of adequate and continuous blood flow to cerebral tissue, the reasoning behind an effective autoregulatory curve seems logical. However, recent investigations (Lucas et al. 2010; Tzeng et al. 2010) have re-assessed the pioneering work by Lassen (1959) and have challenged the traditional view of a completely effective system. For instance, bolus injections of vasoactive drugs to induce transient fluctuations in MAP have been associated with similar fluctuations in MCAV (Tzeng et al. 2010). Furthermore, the results from Chapter 4 of the current thesis show that cerebral blood flow is passive to changes in pressure; data which is not consistent with the originally proposed autoregulatory curve.

One of the major new findings from Chapter 4 was the blunted gain in MCAV for a given PE-induced increase in MAP following 20 min of isocapnic hypoxia. The attenuation in MCAV was attributed to persistent sympathoexcitation which prevented a large increase in cerebral perfusion during hypertension. However, it is important to note that the cerebral vasculature is mediated by many distinct and coordinated (sometimes opposing) autoregulatory systems. In particular, while CA is the typical term associated with the system responsible for changes in perfusion pressure, cerebral chemoregulation regulates cerebral vessel tone during alterations in partial pressures of oxygen and carbon dioxide. On acute exposure to hypoxia, cerebral vessels dilate in order to increase CBF in order to maintain oxygen delivery. Hypoxia-induced dilation could compromise CA by eliminating any possible constriction of cerebral vessels when MAP (perfusion) is increased. Accordingly, previous studies have examined the effect of the hypoxia-induced dilation on cerebral autoregulation; however, the findings have been inconsistent. Studies have demonstrated that CA is either maintained (Ainslie et al. 2007) or impaired (Iwasaki et al. 2007) in hypoxia. Whereas hypoxia is a central component to these studies, the inconsistent findings

between studies may lie in the simultaneous control (or lack thereof) in CO_2, a potent regulator of CBF. Unfortunately, neither of the previously mentioned studies separated the effect of hypoxia from that of hypocapnia/isocapnic. In particular, $P_{ET}CO_2$ was allowed to drop due to hyperventilation in the study by Ainslie et al. (2007); whereas Iwasaki et al. (2007) maintained isocapnia during the hypoxia exposure. Aaslid et al. (1989) observed that it takes less time for MCAV to return to baseline levels following an abrupt decrease in MAP under hyperventilation-induced hypocapnia. Therefore, the hyperventilation-induced hypocapnia in poikilocapnic hypoxia may also counteract the impairment in CA induced by hypoxia. The interpretation of the previous literature on the effects of hypoxia on CA must be accompanied with the consideration of whether hypocapnia resulted from the hypoxia-induced hyperventilation, or whether isocapnia was maintained.

Given this brief summary, the purpose of this study was to test the hypothesis that hypocapnia acts as a protective mechanism in hypoxia, and blunts the impairment in CA that has been reported in isocapnic hypoxia (Ogoh et al. 2010). Chapter 4 demonstrated the importance of separating the CA response to increases and decreases in pressure. In order to further investigate the possible role of hyperventilation-induced hypocapnia on CA in hypoxia, the current study used the squat-stand manoeuvre developed by Claassen et al. (2009). This non-invasive technique induces physiologically-relevant oscillations in blood pressure, and allows characterization of the MCAV response to both hypertension and hypotension.

5.2 Materials and methods

Subject characteristics. Eight healthy young subjects (29 ± 4 yr, 3 female) participated in this study after providing written informed consent. All subjects were of normal height (175 ± 8 cm) and weight (69 ± 11 kg) and were free from any known respiratory, cardiovascular, or neural disease, and all were non-smokers. For familiarization purposes, subjects attended a preliminary session in which they were accustomed with the experimental protocol. Subjects refrained from eating for a minimum of 2 hr, and caffeine and strenuous exercise for 24 hr before testing. All

experimental procedures and protocols were approved by the Clinical Research Ethics Board at the University of British Columbia, which conforms to the *Declaration of Helsinki*.

Experimental protocol. Subjects underwent two experimental days in random order which were separated by a minimum of 48 hours and maximum of 7 days. Testing on the two experimental days was performed at the same time of day. On Day 1, following instrumentation, the subject rested in a sitting position for a minimum of 15 min to obtain baseline measurements. In order to evaluate CA, oscillations in blood pressure were induced by having subjects perform the squat-stand manoeuvre as developed by Claassen et al. (2009). From the original sitting position, subjects stood up, and held this position for 5 s. Subjects then assumed a squatting position (knee angle ~45°) which was also held for 5 s. The 5 s stand followed by 5 s squat was repeated for 5 min, resulting in 30 full cycles (oscillations) in blood pressure. During the manoeuvre, the subject was instructed to breathe normally and avoid any strenuous breathing pattern (e.g. Valsalva manoeuvre). The respiratory trace was continually monitored by an experimenter to ensure this was adhered to. In the event of abnormal breathing, the subject was quickly notified and coached to normalize the breathing pattern. Following the 5 min squat-stand manoeuvre, subjects were exposed to 20 min of either isocapnic or poikilocapnic hypoxia. The fraction of inspired oxygen was adjusted by a computer-controlled system (see '*Physiological measures*') in order to clamp the end-tidal pressure of oxygen at 50 mmHg. The squat-stand manoeuvre was repeated ~15 min into the hypoxia exposure. All physiological measures were continuously recorded following the hypoxia exposure, and the squat-stand manoeuvre was repeated 5 min following the termination of hypoxia. Day 2 was identical to Day 1, except the hypoxia exposure differed in whether it was poikilocapnic or isocapnic (i.e. Day 1 isocapnic hypoxia, making Day 2 poikilocapnic hypoxia).

Physiological measures. Ventilatory flow was continuously recorded by a pneumotachograph (model 3813, Hans Rudolph, Kansas City, MO, USA) connected to a facemask worn by the subjects. From the flow signal, V_T and F_b were determined; $\dot{V}_I$ was established from the product of V_T and F_b. Ventilated O_2 and CO_2 were measured at the mouth with calibrated gas analyzers (models S-3A/I and CD-A, Applied Electrochemistry, Pittsburgh, PA, USA). Beat-by-beat blood

pressure and SpO_2 were determined noninvasively with finger photoplethysmography (which was calibrated against automated blood pressure measurements from the arm) (Finometer, FMS, Arnhem, Netherlands) and finger pulse oximetry (Nonin Model 7500FO, Nonin Medical Inc., Plymouth, MN, USA), respectively. A 2 MHz pulsed-wave Doppler ultrasound (Neurovision 500 M, Multigon Industries, Yonkers, NY, USA) was used to measure CBF velocity in the middle cerebral artery. The Doppler probe was secured in place with a headband device (Marc 600, Spencer Technologies, Seattle, WA, USA). Placement of the Doppler probe was performed by the same investigator, and an outline of the subjects' ear, eye, mouth and probe placement were traced on a transparency film to be used as a guide on the second experimental session. Heart rate was determined with standard 3-lead electrocardiography. Isocapnic and poikilocapnic hypoxia exposures were accomplished with a custom-built, computer-controlled end-tidal forcing system. The system analyzes the expirate of each breath in order to generate the necessary inspirate for the subsequent breath. This was accomplished with solenoid valves which provided appropriate volumes of 100% O_2, CO_2, and N_2 to the inspiratory circuit in order to maintain $P_{ET}O_2$ at 50 mmHg, and $P_{ET}CO_2$ at resting levels (for isocapnic hypoxia) on a breath-by-breath basis. For isocapnic hypoxia, resting $P_{ET}CO_2$ levels were determined from a resting 10 min average. For poikilocapnic hypoxia, no CO_2 was added to the inspirate.

Data and statistical analyses. All data was acquired with an analog to digital converter (Powerlab/16SP ML 795, ADInstruments, Colorado Springs, CO, USA). Data was sampled at 1 KHz, and stored on a computer for offline analyses. Beat-to-beat changes in blood pressure were aligned to the corresponding MCAV trace (~1 s delay). MAP data were split into bins that spanned every oscillation in blood pressure from baseline (~30 oscillations per squat-stand trial). A signal average of each bin was then constructed to obtain a single average trace of all cycles during the manoeuvre. From this signal average, the peak (squat) and nadir (stand) response from baseline was determined for all cardiorespiratory measures. A measure of CA was taken by plotting MCAV as a function of MAP during the squat-stand manoeuvres, and the slope of the linear regression was taken to represent cerebral autoregulation gain (i.e. change in MCAV for a given change in MAP). The linear regressions were determined separately for the increases and decreases in MAP. Any difference in resting cardiorespiratory measure between test days was

determined with a paired *t* test. The effect of hypoxia on all cardiorespiratory measures was compared with repeated measures analysis of variance procedures. When a significant *F* ratio was detected, differences were further investigated with Tukey's post-hoc analyses. The level of significance for all statistical comparisons was set at $P < 0.05$. All data are presented as means ± SD.

5.3 Results

Exposure to hypoxia. Baseline measures are presented in Table 5.1. By design, the use of an end-tidal forcing system effectively controlled $P_{ET}O_2$ and $P_{ET}CO_2$ in hypoxia (Table 5.1). Specifically, $P_{ET}O_2$ was 50 ± 1 mmHg and 52 ± 4 mmHg in isocapnic hypoxia and poikilocapnic hypoxia, respectively. Isocapnia was maintained during the isocapnic hypoxia exposure ($P_{ET}CO_2$: 40 ± 2 mmHg), whereas $P_{ET}CO_2$ was reduced in poikilocapnic hypoxia for all subjects (group mean $P_{ET}CO_2$: 36 ± 4 mmHg). In both hypoxia exposures, SpO_2 significantly decreased to ~83-84%, and this was accompanied with an increase in $\dot{V}_I$ of almost 30%. There was a significant increase in HR in both hypoxic exposures, whereas there was no change in MAP. In isocapnic hypoxia, MCAV significantly increased; however, there was no effect of poikilocapnic hypoxia on MCAV. There was a significant reduction in CVR in isocapnic hypoxia ($P < 0.05$), which was followed by an increase post-hypoxia, however the difference post-hypoxia was not statistically significant compared to baseline ($P > 0.05$); however, there was no change in CVR during the poikilocapnic hypoxia trial. In the post-hypoxia period, all cardiorespiratory measures quickly returned to baseline levels; however, stroke volume (SV) and $\dot{Q}$ returned to levels lower than baseline following poikilocapnic hypoxia.

Table 5.1 – Effect of hypoxia ($P_{ET}O_2$ ~50 mmHg) on cardiorespiratory measures.

	Isocapnic			Poikilocapnic		
	Baseline	Hypoxia	Post-hypoxia	Baseline	Hypoxia	Post-hypoxia
V_T (l)	1.1 ± 0.3	1.5 ± 1.0	1.0 ± 0.3 '	1.0 ± 0.3	1.3 ± 0.5*	1.0 ± 0.2 '
Fb (breaths/min)	14.0 ± 4.0	13.6 ± 4.4	14.5 ± 4.9	12.9 ± 3.9	13.1 ± 4.3	14.1 ± 5.5
V_I (l/min)	13.7 ± 2.4	17.7 ± 2.3*	13.3 ± 3.7 '	12.7 ± 3.1	16.2 ± 4.6*	12.9 ± 3.7 '
$P_{ET}O_2$ (mmHg)	101 ± 3	50 ± 1*	98 ± 4 '	101 ± 4	52 ± 4*	100 ± 5 '
$P_{ET}CO_2$ (mmHg)	41 ± 3	40 ± 2	40 ± 4	40 ± 3	36 ± 4*	39 ± 5 '
SpO_2 (%)	96 ± 1	83 ± 2*	95 ± 1 '	96 ± 1	84 ± 3*	95 ± 1 '
HR (beats/min)	65 ± 12	76 ± 10*	64 ± 12	64 ± 7	71 ± 9*	63 ± 9 '
SV (ml)	86 ± 11	85 ± 9	83 ± 4	93 ± 14	84 ± 14	80 ± 13*
Q (l/min)	5.6 ± 1.2	6.6 ± 1.3*	5.4 ± 1 '	5.9 ± 1.1	6.0 ± 1.5	5.0 ± 1.3* '
MAP (mmHg)	89 ± 10	86 ± 10	91 ± 13	91 ± 5	91 ± 12	87 ± 12
MCAV (cm/s)	64 ± 7	70 ± 10*	63 ± 8 '	61 ± 9	60 ± 7	58 ± 10
CVR (mmHg/cm/s)	1.40 ± 0.17	1.26 ± 0.18*	1.47 ± 0.24 '	1.50 ± 0.19	1.51 ± 0.13	1.50 ± 0.15

Hypoxia data was determined approximately 15 mins into the exposure. *significantly different from baseline; ‘significantly different from hypoxia.

Squat-stand manoeuvres. During the repeated squat-stand manoeuvre, the maximum increase and decrease in MAP was ~15mmHg (during the squat) and ~13mmHg (during the stand), respectively. The mean cardiorespiratory measures during the squat-stand manoeuvres are presented in Table 5.2. Figure 5.1 demonstrates the oscillations in certain cardiorespiratory measures during the squat-stand manoeuvre in one representative subject at baseline. As a group, the repeated squat-stand manoeuvres caused an increase in $\dot{V}_I$ and HR at baseline, in isocapnic and poikilocapnic hypoxia, and in the post-hypoxia periods ($P < 0.05$). In contrast, there was no effect on MAP during the manoeuvres, despite the increased oscillation amplitude. There was a significant increase in MCAV during the squat-stands in isocapnic hypoxia (~7 cm/s increase from baseline); although, the MCAV increase in poikilocapnic hypoxia was small and did not reach statistical significance ($P > 0.05$).

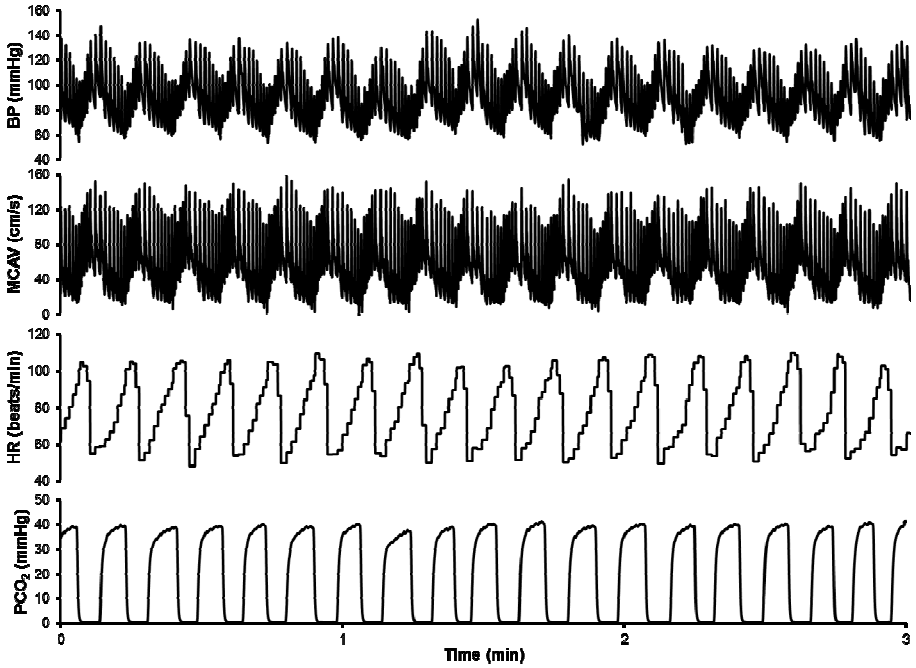

Figure 5.1 – Example recording from one representative subject during the squat-stand manoeuvre at baseline.

Compared to the squat-stand manoeuvre performed at baseline, CVR decreased and increased during the squat-stands in isocapnic hypoxia and the post-hypoxia periods, respectively (Table 5.2). In contrast, there was no significant change in CVR from baseline during the squat-stands in poikilocapnic hypoxia, or in the post-hypoxia period ($P > 0.05$).

Table 5.2 – Cardiorespiratory measures during the squat-stand manoeuvre

	Isocapnic			**Poikilocapnic**		
	Baseline	Hypoxia	Post-hypoxia	Baseline	Hypoxia	Post-hypoxia
V_T (l)	1.5 ± 0.7	1.9 ± 1.0	1.2 ± 0.3 '	1.2 ± 0.3	1.7 ± 0.7	1.1 ± 0.1 '
Fb (breaths/min)	15.1 ± 4.2	15.3 ± 5.0	15.5 ± 4.2	15.7 ± 4.2	13.9 ± 4.4	15.9 ± 4.7
V_I (l/min)	20.1 ± 2.2	25.4 ± 4.5*	17.6 ± 3.5 '	17.5 ± 3.2	20.2 ± 3.9	17.8 ± 4.5
$P_{ET}O_2$ (mmHg)	99 ± 5	50 ± 1*	100 ± 5 '	100 ± 5	49 ± 1*	99 ± 6 '
$P_{ET}CO_2$ (mmHg)	41 ± 3	42 ± 3	39 ± 5	40 ± 5	38 ± 5*	39 ± 5
SpO_2 (%)	96 ± 1	81 ± 2*	96 ± 1 '	96 ± 1	81 ± 3*	96 ± 1 '
HR (beats/min)	79 ± 11	85 ± 11*	78 ± 9 '	73 ± 8	82 ± 10*	77 ± 8
SV (ml)	95 ± 10	92 ± 11	88 ± 9* '	101 ± 20	91 ± 20	88 ± 17*
Q (l/min)	7.5 ± 1.3	7.8 ± 1.6	6.8 ± 1.2	7.3 ± 1.6	7.4 ± 1.9	6.7 ± 1.3
MAP (mmHg)	89 ± 14	91 ± 15	91 ± 13	91 ± 10	90 ± 13	91 ± 11
MCAV (cm/s)	62 ± 8	69 ± 6*	59 ± 8 '	60 ± 11	62 ± 10	60 ± 11
CVR (mmHg/cm/s)	1.44 ± 0.14	1.32 ± 0.16*	1.54 ± 0.16* '	1.54 ± 0.21	1.47 ± 0.20	1.55 ± 0.21

*significantly different from baseline; ‘significantly different from hypoxia.

Cerebral autoregulation gain (i.e. the change in MCAV for a given change in MAP) to hypotension at baseline was 0.76 ± 0.25 cm/s/mmHg, and this was not different between test days (gain at baseline on poikilocapnic hypoxia day: 0.73 ± 0.20 cm/s/mmHg) (Figure 5.2). In hypertension, the baseline gain was also similar between days (isocapnic hypoxia day: 0.74 ± 0.15 cm/s/mmHg; poikilocapnic hypoxia day: 0.81 ± 0.19 cm/s/mmHg; $P > 0.05$). In isocapnic hypoxia, cerebral autoregulation gain in hypertension and hypotension increased; however, only the hypertension gain was significantly reduced from baseline in the post-hypoxia period (0.51 ± 0.14 cm/s/mmHg). In contrast, hypertension and hypotension CA gain did not change from baseline in poikilocapnic hypoxia, or following the exposure.

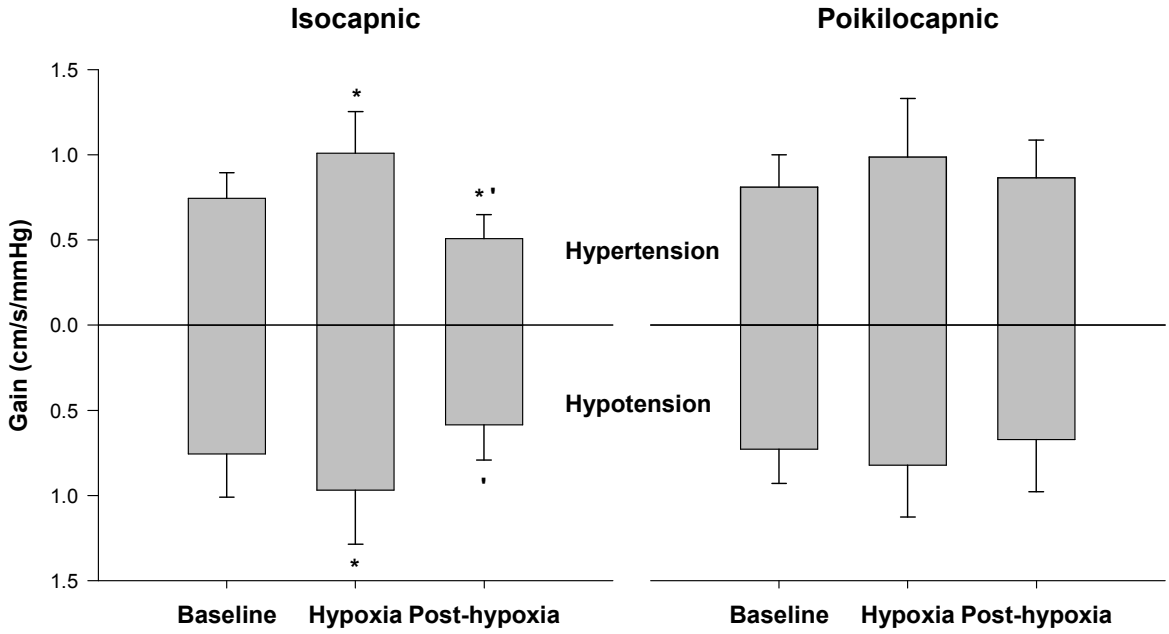

Figure 5.2 – The effect of isocapnic and poikilocapnic hypoxia on the cerebral autoregulation gain.

Note that the CA gain to hypotension is positive. The bars in hypotension are extended below the zero line on the x-axis (CA gain) simply for visual purposes. *significantly different from baseline; ' significantly different from hypoxia.

5.4 Discussion

Main findings. The purpose of this study was to investigate the effect of CO_2 on cerebral autoregulation during, and following an acute hypoxia exposure. The study was designed such that the autoregulatory response could be determined in response to physiologically-relevant

hypertensive and hypotensive challenges. The primary new finding of the current study is that CA is impaired in isocapnic hypoxia. However, the impairment in hypoxic CA is absent when hyperventilation-induced hypocapnia is permitted. Furthermore, consistent with the data from Chapter 4, there was a reduction in the MCAV response to hypertension following isocapnic hypoxia (i.e. the increase in MCAV for a given increase in MAP was attenuated), which did not occur following poikilocapnic hypoxia (Figure 5.2). These findings reinforce the integrated nature of cerebrovascular control to chemical stimuli, and highlight the strong influence of CO_2 on CA.

Cerebral autoregulation in hypoxia. In Chapter 4, a blunting of the CA gain to hypertension was observed following an acute isocapnic hypoxia exposure. However, in that study, there was no measure of CA made during the hypoxic exposure. In the current study, the squat-stand manoeuvre performed in hypoxia provided a measure of CA to conditions of both hypotension and hypertension. Compared to baseline, CA gain increased in isocapnic hypoxia, leading to cerebral blood velocity that was much more pressure-passive. Acute exposure to isocapnic hypoxia leads to dilation of small pial cerebral vessels (Heistad and Kontos 1983). Whether this hypoxia exposure leads to a measurable increase in MCAV depends on the severity of hypoxia, CO_2 control, and neural activity (Ainslie and Ogoh 2010). As demonstrated in Chapters 2 and 3, acute isocapnic hypoxia exposure is accompanied with an increase in vasoconstrictor sympathetic outflow. Furthermore, eliminating neural control of cerebral vessels with a ganglion blockade impairs CA (Zhang et al. 2002). Also, reducing cerebral vessel tone with hypercapnia reduces the effectiveness of CA (Aaslid et al. 1989). Thus, although the hypoxia-induced sympathoexcitation in isocapnic hypoxia may work to improve CA in the current study (as suggested in Chapter 4), the concurrent hypoxia-induced dilation of cerebral arteriols may have counteracted the sympathetically-mediated constriction. Overall, this would lead to an inability to buffer sudden changes in cerebral perfusion, resulting in an increase in CA gain. Exposure to hypoxia activates the peripheral chemoreceptors, leading to increased ventilation. However, the hyperventilation that accompanies poikilocapnic hypoxia leads to hypocapnia, a chemical stimuli that causes cerebral vessel constriction. The hypocapnia-induced arteriole constriction in poikilocapnic hypoxia could have played a role in maintaining cerebral vessel tone (along with

the hypoxia-induced sympathoexcitation), and prevented the impairment in CA observed in isocapnic hypoxia.

Consistent with the current study, Ogoh et al. (2010) found CA impairment in isocapnic hypoxia, but not in poikilocapnic hypoxia. However, certain methodological considerations from the study by Ogoh et al. (2010) makes interpretation difficult. First, hyperventilation in poikilocapnic hypoxia lead to an increase in $P_{ET}O_2$ which was significantly different from isocapnic hypoxia. Consequently, SpO_2 was also significantly elevated in poikilocapnic hypoxia compared to isocapnic hypoxia. Previous research has shown the impairment in hypoxic CA is dependent on the severity of the hypoxic exposure (Iwasaki et al. 2007); therefore, comparing CA between different levels of hypoxia ($P_{ET}O_2$ and SpO_2) is problematic. In the current study, $P_{ET}O_2$ was tightly controlled in both hypoxic exposures with the use of a computer-controlled end-tidal forcing system that adjusted gas concentrations of the inspirate from breath-by-breath feedback. As a result, subjects were exposed to similar levels of hypoxia between the two testing sessions. Second, Ogoh et al. (2010) assessed CA with the rapid thigh cuff deflation method, an uncomfortable technique that produces a sudden reduction in MAP. Although this technique has been used in previous studies (Aaslid et al. 1989), assessing CA based on only hypotension (or hypertension) does not fully characterize CA (see Chapter 4) (Tzeng et al. 2010). In contrast, the current study used a protocol that includes both hypertensive and hypotensive challenges, and permits separating the two responses. Another methodological benefit in the current study was the assessment of CA following the hypoxia exposures; which has demonstrated a 'rebound' of CA to hypertension that exceeds baseline following isocapnic hypoxia, but no effect following poikilocapnic hypoxia. For these reasons, the current study complements and extends the findings from Ogoh et al. (2010).

Cerebral autoregulation following acute hypoxia. Many descriptions of the cardiorespiratory responses to hypoxia have been limited to poikilocapnic hypoxia exposures, and do not focus on the post-hypoxia period. Chapters 2 – 5 have presented studies that have all isolated the effect of hypoxia by maintaining isocapnia, and have included continuous measures post-hypoxia. Findings from the current study are consistent with the results from Chapter 4, and demonstrate a

reduction in the CA gain in hypertension following acute isocapnic hypoxia. One of the new findings of the current study highlights the importance of CO_2 control in acute hypoxia studies. That is, there was no change in CA following poikilocapnic hypoxia, which is in contrast to the reduced gain following isocapnic hypoxia. To my knowledge, this is the first study to focus on CA following an acute hypoxia exposure of different CO_2 backgrounds. Although this study was not designed to specifically investigate the mechanism for the effect of different CO_2 backgrounds, the balance between hypoxia-induced vasodilators and neural influences may play a role in CA. Specifically, following isocapnic hypoxia, circulating levels of vasodilators normalize (e.g. nitric oxide, prostaglandins, etc), whereas sympathetic activity remains elevated (as discussed in Chapter 4) (Lusina et al. 2006; Querido et al. 2010); which could be responsible for the reduction in CA gain.

Given the role of sympathetic outflow on CA following isocapnic hypoxia, could the CA results following poikilocapnic hypoxia also be neurally mediated? Although not measured in the current study, one other study has demonstrated that poikilocapnic hypoxia is not accompanied with persistent sympathoexcitation following the hypoxic exposure. Tamisier et al. (2004) found the increase in MSNA during 20 min of poikilocapnic hypoxia ($P_{ET}CO_2$ ~37 mmHg; SpO_2 ~84%) was not sustained when the hypoxia exposure was terminated. Furthermore, there was no difference in forearm vascular resistance in the post-hypoxia period. Thus hypocapnia, as a sympathoinhibitor (Fukuda et al. 1989), may counteract the long-lasting sympathoexcitation from hypoxia. It is important to note that the measures of vascular function in the study by Tamisier et al. (2004) were taken from the periphery, and the regulatory mechanisms in the periphery may differ from cerebrovascular regulation; however, the results do provide insight into the possible mechanisms responsible for the findings in the current study.

Methodological considerations. A transcranial Doppler was used to measure beat-by-beat changes in cerebral blood flow velocity in the middle cerebral artery. A limitation of this method in estimating CBF is that any change in MCA diameter may lead to a change in velocity but not necessarily in flow. However, the technique has been validated during exposures to a range of O_2 and CO_2 pressures (Giller et al. 1993; Poulin and Robbins 1996; Valdueza et al. 1997; Serrador

et al. 2000). The use of the squat-stand manoeuvre to assess CA exposed subjects to an orthostatic challenge, possibly leading to an alteration in MCA diameter. With the use of MRI measures, simulated orthostasis reduces CBF but does not affect MCA diameter (Serrador et al. 2000). Therefore, it is likely that MCAV measures with the transcranial Doppler represent a valid surrogate of CBF.

Previous studies have suggested that CA is more effective buffering gradual changes in pressure than rapid changes (Zhang et al. 1998). Accordingly, the current data may only be relevant to blood pressure changes in the high-frequency range. However, Lucas et al. (Lucas et al. 2010) used low-frequency changes in MAP (sustained 90 s injections of vasoactive drugs) and also demonstrated cerebral blood velocity changes that were highly influenced by MAP. Further work investigating the effect of hypoxia on CA at different frequencies would be useful.

Conclusion. Previous studies investigating the effect of hypoxia on CA have produced inconsistent results. In the current study, CA in isocapnic hypoxia was impaired, whereas there was no change in poikilocapnic hypoxia compared to baseline. The maintenance of CA in poikilocapnic hypoxia is likely due, at least in part, to the hyperventilation-induced hypocapnia which increases cerebral vessel tone and counteracts the hypoxia-induced dilation. Furthermore, while CA gain to hypertension is reduced following isocapnic hypoxia and is likely neurally mediated, there is no effect following poikilocapnic hypoxia. This study highlights the importance in considering the hypoxic exposure (i.e. isocapnic vs. poikilocapnic) when interpreting CA data, given the differences in the cerebral autonomic response.

CHAPTER 6: CONCLUSION

6.1 Summary of major research findings

The autonomic response to hypoxia is complex and not fully understood. Many studies have sought to understand the underlying mechanisms of respiratory, cardiovascular and neural autonomic systems by studying each system is isolation. Although the findings of these studies have provided important insights into the mechanisms of autonomic control in hypoxia, the integrated fashion in which autonomic systems interact in humans limits our ability to extend these findings to humans. Alternate approaches have included pathological models of hypoxia (e.g. OSA), which have been integral in understanding the pathophysiology of the syndrome; however, interpretation is complicated by comorbidities which can have a direct effect on autonomic function. Accordingly, the current series of studies used a healthy human model of acute hypoxia, in order to investigate the integrated autonomic response to hypoxia alone. Furthermore, findings from studies conducted at high-altitude and in pathological models suggest that the autonomic response to acute hypoxia is not limited to the exposure. As such, a particular focus of the current thesis was to continue physiological measurements into the post-hypoxia period.

The first study (Chapter 2) focused on the role of the CC in the sustained elevation in MSNA following acute isocapnic hypoxia. Brief inhibition of the CC with hyperoxia lead to transient reductions in MSNA following the hypoxia exposure; however, there was no effect of hyperoxia at baseline. These findings suggest that input from the CC is obligatory for the sustained elevation in MSNA initiated by chemoreflex stimulation. However, MSNA is also highly mediated by the arterial baroreflex, which has been demonstrated to be reset in hypoxia. Thus, the second study (Chapter 3) measured baroreflex function throughout and following acute isocapnic hypoxia. Hypoxia caused no change in the slope of the baroreflex stimulus-response curve; however, there was a shift towards higher pressures that favoured elevations in MSNA, which persisted post-hypoxia. These results indicate that there is a resetting of the baroreflex in hypoxia that outlasts the stimulus, and provides further explanation for the complex control of

MSNA following acute hypoxia. The third study (Chapter 4) concentrated on cerebrovascular regulation. Specifically, the study was designed to measure MCAV during bolus injections of hypo- and hypertensive drugs. Contrary to expectations, MCAV increased and decreased with blood pressure at baseline; however, the increase in MCAV during hypertension was blunted post-hypoxia. Cerebrovascular blood flow that is dependent on blood pressure contradicts the traditional view of independent cerebral and peripheral vascular regulation. Furthermore, the findings of this study demonstrate hypoxia-induced alterations in CA and illustrate the importance of separating hypertensive and hypotensive CA responses. Lastly, the final study (Chapter 5) focused on the interaction between chemoregulation and CA. The importance of CO_2 in hypoxic cerebral autoregulation was demonstrated, where the impairment in cerebral autoregulation in acute isocapnic hypoxia was absent in acute poikilocapnic hypoxia. Together, the studies of this dissertation provide basic physiological insights into the integrated nature of the human autonomic response to hypoxia, and demonstrate that the autonomic response is not limited to the hypoxia exposure.

6.2 Perspectives and limitations

An overarching goal of the current thesis was to investigate the interplay of respiratory, cardiovascular and neural autonomic systems in hypoxia in a healthy human model. The findings demonstrated the complexity and integrated nature of the response to hypoxia. These adjustments in autonomic function (i.e. sympathoexcitation, baroreflex resetting, impaired cerebral autoregulation) after short-term hypoxia (20 min) are similar to that observed in the pathophysiology of OSA (Narkiewicz and Somers 1997; Nasr et al. 2009). Our laboratory has demonstrated that these findings which mimic pathology are particularly evident following intermittent hypoxia paradigms (Foster et al. 2005; Lusina et al. 2006; Querido et al. 2008). Given these results, it is important to fully respect the possible physiological consequences of hypoxia exposure. In particular, caution should be practiced by athletes who adhere to ‘live high, train low’ exercise regimes. Aside from performance outcomes, more studies focusing on the health consequences of ‘live high, train low’ are required. Of additional concern is the marketing of personal hypoxia machines for use by the general public. Use of these machines by

uninformed customers with the absence of appropriate monitoring amplifies the possible danger. In particular, the series of studies in this dissertation used a single, short duration hypoxia exposure. Longer, repeated exposures over weeks or months may lead to larger physiological adjustments (Marcus et al. 2009). Rather than obtaining the claimed health benefits, personal hypoxia machine use may be a form of self-induced pathology.

The current series of studies used experimental protocols which are logistically difficult, resulting in small sample sizes (n = 7 – 14). This caveat is acknowledged, and larger sample sizes would have been ideal. However, for the majority of the data, the magnitude of the physiological response was large, and was consistent in all subjects. This provides greater confidence that measured responses are 'real', and not simply due to chance. Nonetheless, interpretation of the results should be accompanied with consideration to the sample sizes.

Although many investigations have warned of the negative physiological consequences of hypoxia, others have disagreed with this assertion. It has been suggested that the autonomic adjustments are not impairments, but rather demonstrate the ability for humans to acclimate to their environment. For instance, one laboratory has performed a series of studies with an 8 hr poikilocapnic hypoxia protocol (SpO_2 ~84%) and has shown an increase in MSNA after 1 hr of exposure; however, MSNA significantly decreased to or below baseline levels after 7 hr of exposure and decreased further in the post-hypoxia period (Tamisier et al. 2007; Hunt et al. 2008). Therefore, although the current thesis has implied the autonomic adjustments in hypoxia as potentially negative, an alternative interpretation is that the observed changes in autonomic function during and following 20 min of isocapnic hypoxia are primary responses which lead to subsequent positive changes in autonomic function. Interestingly, the same group has also used an intermittent hypoxia protocol (fraction inspired oxygen = 0.13 every 3 min; 9 hr/night) over 28 days. Although there was an initial improvement in endothelial function (via reactive hyperaemia) following 14 days, it was reduced compared to baseline at the 28-day mark. This reduction in endothelial function was accompanied with increased MSNA, diastolic blood pressure and forearm vascular resistance. The differences in the length (20 min vs. 9 hr), paradigm (number of hypoxic iterations), and CO_2 control (isocapnic vs. poikilocapnic) in these

studies and in the current thesis (one 20 min isocapnic hypoxia exposure) are important considerations when integrating these findings. However, the autonomic consequences to hypoxia appear to be time domain-dependent of the hypoxic paradigm, which warrants further investigation.

The current thesis used an acute isocapnic hypoxia protocol which has previously been demonstrated to cause alterations in respiratory, cardiovascular, and neural control in healthy humans (Xie et al. 2001; Tamisier et al. 2004; Lusina et al. 2006). However, the 20 min isocapnic hypoxia paradigm is different from that typically experienced by humans. Extending the findings of the current studies to other circumstances with alternate hypoxia profiles must be done with caution. For example, hypoxia at high-altitude typically lasts from days to years, which can lead to alternate physiological changes not experienced in acute hypoxia (e.g. haematopoiesis). Alternatively, OSA is characterized by multiple hypoxic iterations (over and above 60/hr) during sleep, which is accompanied with hypercapnia and arousal from sleep. The particular hypoxic profile can alter the autonomic response in humans (Foster et al. 2005; Tamisier et al. 2005), which should be considered when interpreting the results of the current studies. Nonetheless, the current findings demonstrate hypoxia-induced changes in autonomic function that, in many respects, parallel those of OSA, and provide further insight into the pathophysiology of OSA in the absence of comorbidities.

An underlying goal of the current experimental design was to investigate certain autonomic systems in intact humans, thereby allowing interaction between various autonomic responses. Using an integrated approach to understand the whole-body autonomic response is important, given the interdependence of many autonomic systems. The strength of this experimental design unfortunately suffers when attempting to understand the mechanistic rationale for the physiological observations. Often, interpretation for the responses in intact humans is limited by assumptions and inference. Incorporating the findings of the current studies into reductionist experimental designs could provide further insight into the mechanisms responsible for the hypoxia-induced alterations in autonomic function. Unfortunately, reductionist approaches can suffer in their ability to fully consider the integrated nature of autonomic control. Ultimately,

both the reductionist and integrated models are imperfect, but a greater understanding of the autonomic response to hypoxia is dependent on our ability to integrate the findings from both approaches.

6.3 Future directions and overall conclusion

This thesis provides insight into the integrated autonomic response during and following acute hypoxia; however, there are still unanswered questions that warrant further investigation. For instance, what are the practical or clinical repercussions of these alterations in autonomic function? Although hypoxia results in persistent sympathoexcitation, a comorbidity of OSA, elevated vasomotor outflow does not always lead to longer-term hypertension (Joyner et al. 2010). Furthermore, CA impairment at high-altitude does not predict susceptibility to acute mountain sickness (Subudhi et al. 2011). Additional studies focusing on bridging the connection between alterations in autonomic function and clinical measures that predict pathology would be a useful future direction.

As highlighted above, the current thesis used an acute isocapnic hypoxia protocol (20 min), and although the findings are consistent with other models of hypoxia, generalizing these findings must be done with caution. Acute isocapnic hypoxia was used in order to isolate the effect of hypoxia and compare the results to previously published studies. Although up to 20 min of post-hypoxia data was collected, future research is required to expand these findings to understand how long the hypoxia-induced changes last. Previous research has demonstrated persistent sympathoexcitation for 180 min following 20 min of hypoxia (Cutler et al. 2004), however, differences in the hypoxia paradigm (continuous vs. intermittent hypoxia) makes comparison to the current studies difficult. Future studies which employ longer hypoxia protocols that also include continuous and intermittent hypoxia exposures would provide insight into the role of the hypoxic paradigm on the magnitude of the long-lasting alterations in autonomic function.

The purpose of this thesis was to understand the integrated human response to hypoxia by concentrating on certain autonomic reflexes, with particular focus on the post-hypoxia period.

Through a series of four studies, it was demonstrated that the persistent sympathoexcitation following acute hypoxia was mediated by the CC. The follow-up study implicated a long-lasting resetting of the baroreflex permitted the elevated vasomotor outflow. Furthermore, the third study demonstrated that cerebral autoregulation following acute hypoxia is more effective in buffering increases in blood pressure. Finally, the fourth study found an impairment in cerebral autoregulation in isocapnic hypoxia, but not in poikilocapnic hypoxia. Together, these studies extend our understanding of the integrated autonomic human response to hypoxia; future work is needed to integrate these findings into alternative forms of hypoxia exposure and patient populations.

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