CEREBRAL BLOOD FLOW REGULATION IN INTERMITTENT HYPOXIA

DISSERTATION

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Manuscripts

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ABP	Arterial Blood Pressure
CA	Cerebral Autoregulation
CBF	Cerebral Blood Flow
CBFV	Cerebral Blood Flow Velocity
CPAP	Continuous Positive Airway Pressure
CVCi	Cerebral Vascular Conductance Index
ECG	Electrocardiogram
$ETCO_2$	End-Tidal Carbon Dioxide
FRC	Functional Residual Capacity
HEA	Hypoxic Apnea Event
HR	Heart Rate
IHA	Intermittent Hypoxic Apneas
MAP	Mean Arterial Pressure
MCA *Vmean*	Mean Middle Cerebral Artery Blood Velocity
MSNA	Muscle Sympathetic Nerve Activity
NE	Norepinephrine
OSA	Obstructive Sleep Apnea
RoR	Rate of Regulation
SaO_2	Arterial Oxygen Saturation

LIST OF ABBREVIATIONS (CONTINUED)

Sec	Seconds
TCD	Transcranial Doppler
TFg	Transfer Function Gain
$tcPCO_2$	Transcutaneous Pressure of Carbon Dioxide

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CHAPTER I

INTRODUCTION

Sleep disordered breathing is a broad classification of clinical conditions that produce regular respiratory disturbances during sleep, the most common of which is obstructive sleep apnea (OSA). In the United States, approximately 1 in 5 adults has at least mild OSA and 1 in 15 adults has OSA of moderate or worse severity (133). The presence of OSA is diagnosed when a patient demonstrates an apnea-hypopnea index of greater than 5 per hour (1). Pathophysiological factors with the potential to cause OSA include excess weight, age, hypertension, a narrowed airway, chronic nasal congestion, diabetes, use of alcohol and smoking (133) There is a graded increase in OSA prevalence with increasing body mass index (133). Fat deposits around the upper airway may obstruct breathing. In addition, OSA occurs two to three times more often in adults over 65 yrs (16, 17, 132). Moreover, men are much more likely to have sleep apnea than women (131). Hormonal differences are believed to account for the sex difference in OSA prevalence (129). However, the exact cause of OSA remains unclear, because not everyone who has OSA is overweight, elderly or male.

Importantly, OSA is associated with diabetes, hypertension, coronary artery disease, myocardial infarction, congestive heart failure, and stroke (66, 107, 131). These associations may be due in part to risk factors common to all these conditions and may also reflect the role of OSA in the etiology of these conditions. In patients with hypertension OSA is common and a causal role of OSA in hypertension has been suggested by several studies (66, 95, 107). The correlates of OSA, including excess body weight and hypertension, overlap

with those of diabetes mellitus (98). A longitudinal population study indicated that patients with moderate to severe OSA had three times the chance of developing a new hypertension compared to people without OSA (95). Furthermore, Young, et al. (130) found that the apnea-hypopnea index was predictive of both nocturnal and daytime elevations in blood pressure. These data suggest that as OSA worsens, there is a concomitant increase in both nocturnal and daytime blood pressure. In studies of congestive heart failure, the incidence of OSA ranges from 11% to 37% (59, 112). Interestingly, a recent study has identified that congestive heart failure patients that have received treatment of OSA for a period of one month had an increase in ejection fraction and decreases in systolic blood pressure and heart rate (62).

Furthermore, patients with OSA have more than a three-fold increase in risk for experiencing a stroke or cardiac related death, independent of known risk factors for stroke (10, 126). Both ischemic and hemorrhagic strokes occur in patients with OSA (13). Stroke is the third leading cause of death in the United States and the leading cause of serious long-term physical and cognitive disability. The brain is absolutely dependent on adequate blood flow for normal function. When blood flow falls below a critical flow, brain cells die resulting in stroke. Impairment in cerebral blood flow (CBF) regulation is a major risk factor for stroke (8, 40). However, not all of the factors affecting CBF regulation have been clearly elucidated. Cerebral blood flow (CBF) is maintained relatively constant by regulatory mechanisms during fluctuations in cerebral perfusion pressure (3). These CBF control mechanisms are mainly cerebral autoregulation (CA) and the reactivity of the cerebral vasculature to changes in $PaCO_2$ (2, 73, 86). The apneic episodes that occur during sleep of

the OSA patient and voluntary breath holding during wakefulness in normal subjects perturb the cerebral circulatory control (11, 44, 109, 110), indicating that CBF regulation is attenuated in patients with OSA. Indeed, in a recent study by Urbano, et al., (117) patients with moderate to severe OSA were found to have impaired cerebral autoregulation. This impairment may contribute to the increased risk of cerebral ischemia and stroke (117). However, the exact underlying mechanisms for the attenuation of CBF regulation in patients with OSA are unknown. This is, due to the many alterations in these patient's physiological factors associated with stroke as well as other cardiovascular diseases in both chronic and acute situations (114). In other words, it remains unclear whether it is the apnea event itself, or the increases in physiological risk factors of cardiovascular disease, that the apnea event induces that changes the regulation of CBF.

Recent research has demonstrated altered chemoreflex control of MSNA in untreated OSA patients (80, 81). OSA is associated with chronically elevated sympathetic nerve activity (19, 48, 113). Furthermore, it has been established that an increase in sympathetic activity occurs with prolonged exposure to hypoxia (70). For example, in both healthy human and animal models, experimental paradigms designed to mimic the intermittent hypoxic apneas of OSA have identified a rapid onset and sustained (>3hrs) sympathoexcitation (24, 115, 123) and an increased respiratory chemoreflex (82). These findings suggest that the apnea event itself changes autonomic nervous system function in healthy humans. In addition, it has been demonstrated in healthy subjects exposed to long-term intermittent hypoxia over a 10 day period increases sympathetic activity and chemosensitivity to acute hypoxia (70). Therefore, adaptations to sustained periods of intermittent apneas induced

modifications of the autonomic nervous system's function are different from the adaptations that result following exposure to an acute period of intermittent apneas. However, the autonomic neural control of CBF remains controversial. The traditional thinking is that sympathetic activation has a limited effect on CBF and its regulation at normocapnic $PaCO_2$s (218).

In contrast to the traditional thinking previous studies (12, 14) have reported on the functional importance of the sympathetic nervous system in the regulation of CBF during several physiological conditions. These studies have identified that increased sympathetic activity protects the blood brain barrier from the increases in perfusion pressure. In addition, sympathoexcitation influences cerebral autoregulation ((85, 135). Collectively, this leads us to **hypothesize that a brief period (min) of "intermittent apneas" impairs CBF regulation. In addition, the impaired CBF regulation is augmented by a chronic period (days) of intermittent apneas.**

These hypotheses will be tested by a series of experiments with the following specific aims:

Specific aim 1: To test the hypothesis that an acute series of intermittent apneic events produces sympathoexcitation and causes an impairment of the dynamic regulation of CBF during hypoxia and during hypotension; and

Specific aim 2: To test the hypothesis that when the acute series of intermittent apneic events are continued once per day for 10 days the impairment of CBF regulation during hypoxia and hypotension will be augmented.

CHAPTER II

REVIEW OF LITERATURE

Sleep disordered breathing is a broad classification of clinical conditions that produce regular respiratory disturbances during sleep, the most common of which is obstructive sleep apnea (OSA). Approximately 1 in 5 adults have at least mild OSA and 1 in 15 adults have OSA of moderate or severe intensity (133). The early pathophysiology of OSA is not yet well defined in the human model. However, OSA is characterized by repetitive obstruction of the upper airway during sleep in which ineffective respiratory efforts occur. In addition, OSA is associated with a transient increase in muscle sympathetic nerve activity (MSNA) during each apneic episode, which leads to chronic elevated daytime MSNA in untreated OSA patients via mechanisms that remain unclear.

Left untreated, the chronically elevated MSNA of OSA patients leads to an increased risk of developing hypertension, congestive heart failure, cardiac arrhythmia, cardiac ischemia, and cerebrovascular disease (18, 33, 48, 89, 90, 111, 120). Moderate to severe OSA increases the risk of stroke and or death by more than three-fold independent of other known risk factors for stroke (10, 126). The underlying mechanisms by which OSA increases the risk of stroke, independent of traditional risk factors have not been established (124). Although circulating risk factors, including hypercoagulability and increased platelet aggregation in OSA patients, which occur during sleep, can contribute to the risk of stroke, they do not explain total risk (99, 101).

Pathology of OSA

The exact cause of OSA remains unclear. The site of obstruction in most patients is the soft palate extending to the region at the base of the tongue. The primary abnormality in patients with OSA is an anatomically small pharyngeal airway as a result of obesity or bone and soft tissue structures (105). There are no rigid structures, such as cartilage or bone, in this area to hold the airway open. During daytime wakefulness, muscles in the region keep the passage open. However, this anatomically small airway leads to increased airflow resistance and greater intrapharyngeal negative pressure on inspiration during daytime wakefulness. Mechanoreceptors located in the larynx respond reflexively to the negative pressure produced by inspiration and increase the activity of pharyngeal dilator muscles, thereby maintaining airway patency while awake (105). However, as the OSA patient falls asleep, the reflex pharyngeal muscle activity that drives the neuromuscular compensation is reduced or lost allowing these muscles to relax intermittently to the point that the airway collapses and becomes obstructed. Thus the upper airway that requires reflex-driven muscle activation to maintain patency during wakefulness may be vulnerable to collapse during sleep (114).

Physiological Risk Factors for OSA

Physiological factors with the potential to cause obstructive sleep apnea include excess weight, age, hypertension, a narrowed airway, chronic nasal congestion, diabetes, being male, use of alcohol, and smoking. More than half of patients with OSA are overweight. There is a graded increase in OSA prevalence with increasing body mass index

(124). Fat deposits around the upper airway may obstruct breathing. However, not everyone who has OSA is overweight. Age is another prominent risk factor. Sleep apnea occurs two to three times more often in adults over 65 yrs (16, 17, 128). Loss of muscle mass is a common consequence of the aging process. If muscle mass decreases in the airway it may be replaced with fat, leaving the airway narrow and soft.

Men have a greater risk for OSA. In general, men are twice as likely to have sleep apnea as women. Hormonal differences are believed to account for the sex difference in OSA prevalence. There are few epidemiological studies that address the effect of female hormonal changes on OSA. Of these studies, most focus on menopause. In the population based Wisconsin Sleep Cohort study, postmenopausal women were three times more likely to have moderate or severe OSA compared with premenopausal women independent of age, body mass index, and other potential confounding factors (129). Findings from a study involving women 50 years or older showed hormone therapy users computed with non users had half the probability of OSA (106). However, in a blinded, randomized trial involving postmenopausal women, Polo-Kantola, et al.(2003) found only a weak effect of hormone therapy in reducing apnea and hypopnea (97).

Other predisposing factors associated with OSA include anatomic abnormalities, such as a receding chin. Craniofacial and upper-airway structures have an important role in OSA occurrence. Studies show an acute effect of alcohol use on apnea/hypopnea frequency perhaps due to a relaxation of the musculature in the surrounding upper airway. Smoking is a risk factor for OSA. Smokers are three times as likely to have obstructive sleep apnea. Hypothesized mechanisms for a role of smoking in OSA include inflammation, swelling, and

narrowing of the upper airway. Hypothyroidism, acromegaly, amyloidosis, vocal cord paralysis, post-polio syndrome, neuromuscular disorders, Marfan's syndrome, and Down syndrome and, finally, nasal congestion have also been implicated as possible risk factors for obstructive sleep apnea.

Diseases Associated with OSA

Obstructive sleep apnea is associated with diabetes, hypertension, coronary artery disease, myocardial infarction, congestive heart failure, and stroke (66, 107, 131). The associations may be due in part to risk factors common to all these conditions and may also reflect the role of OSA in the etiology of these conditions. As OSA is common in patients with hypertension and a causal role of OSA in hypertension has been suggested by several studies (66, 94, 107). The correlates of OSA, including excess body weight and hypertension, overlap with those of diabetes mellitus (98). A longitudinal population study indicated that patients with moderate to severe obstructive sleep apnea had three times the chance of developing a new hypertension compared to people without OSA (94). Furthermore, Young, et al. found that the apnea-hypopnea index was predictive of both nocturnal and daytime elevations in blood pressure (130). These data suggest that as OSA worsens, there is a concomitant increase in both nocturnal and daytime blood pressure. Several other studies have reported a similar dose-response relation between OSA and blood pressure (15, 20, 41, 75).

The relationship between the severity of OSA and increase in blood pressure is most likely multifactorial. During OSA, there are both acute and chronic increases in sympathetic nerve activity which has been implicated in the sleep and daytime elevation in blood

pressure. It has been recognized that OSA is associated with chronically elevated activity of the sympathetic nervous system (19, 113). In addition, increased peripheral vasoconstriction in OSA patients has been described (125), while persistent decreases in vagal tone have also been reported in sleep apnea (51). Furthermore, acute and chronic hypoxia stimulate the peripheral chemoreceptors causing sympathetic outflow to resistance vessels (23, 34, 36, 37, 69). Fletcher (32) suggests that the over-activity of the adrenergic and renin-angiotensin systems contribute to early chronic hypertension in OSA patients. Bao and Fletcher (12) used prazosin and yohimbine to block sympathetic responses to repeated episodic hypoxia. Prazosin, but not yohimbine, blunted the blood pressure response. Moreover, AT1 blockade using losartan effectively blocked the blood pressure response to repeated intermittent hypoxia (35). These findings suggest that chronic hypertension in OSA is in part initiated via increased sympathetic nervous activation of the kidney's renin-angiotensin (RAS) system. Therefore, adrenergic and RAS over-activity may contribute to impaired cerebral blood flow (CBF) regulation in OSA patients.

A number of investigations using animal models have identified that sympathetic nerves innervate the cerebral arteries (28, 83, 84). Furthermore, increases in sympathetic nerve activity during hypertension appear to protect the blood brain barrier (14), while direct stimulation of the stellate ganglion and superior cervical ganglion produced marked vasoconstriction of the cerebral arteries in the normotensive primate (74). In the presence of normocapnia, increases in sympathetic activity have little effect on CBF (46, 50, 68). However, others have reported that sympathoexcitation associated with pathology has a direct effect on CBF (49, 55, 60, 93). Prazosin, the α-1 adrenoreceptor blocker, does not influence CBF under resting conditions in normotensives (91). However, in hypertensive

patients, a small but significant increase in CBF along with a significant decrease in blood pressure was observed following Prazosin therapy (103). Previous animal studies (91, 118) have also demonstrated that cervical spinal cord stimulation augments CBF with decreases in sympathetic nerve activity. Interestingly, Prazosin attenuates the cervical spinal cord stimulation-induced increases in CBF (91). These findings suggest that Prazosin induced α-1 adrenoreceptor blockade interferes with autonomic neural control of the cerebral circulation, especially during changes in sympathetic activity.

Urbano, et al. (117) recently reported that patients with moderate to severe OSA were shown to have an impaired cerebral autoregulation in response to changes in blood pressure. This impairment may contribute to an increased risk of cerebral ischemia and stroke. Cerebral autoregulation (CA) is a tightly controlled mechanism to maintain a relatively constant blood flow during fluctuations in perfusion pressure (3). The exact underlying mechanisms for CA are unknown. The endothelium plays an important role in the regulation of blood flow by setting the resting tone in cerebral vessels through the basal release of nitric oxide. This vascular tone acts as a background for other dilator and constrictor processes. Neurons and astrocytes release a number of vasoactive substances that act in concert on smooth muscle cells to increase or decrease cerebral blood flow in accordance with metabolic needs of the brain (53, 54). In addition, the myogenic response to changes in perfusion pressure is thought to be a contributor to the vascular resistance changes involved in CA (64). Accumulating evidence that vascular oxidative stress leads to profound alterations in cerebral autoregulation (30). However, OSA is associated with increased oxidative stress due to hypoxemia-reoxygenation (39).

In a recent study (117), Urbano, et al. employed orthostatic hypotension and CO_2 inhalation separately to assess cerebral autoregulatory control in OSA subjects and control subjects by measuring MCA V, arterial blood pressure and end-tidal CO_2. Recoveries of mean arterial pressure (MAP) and cerebral blood flow velocity (CBFV) after the hypotension / CO_2 challenge were was significantly slower in OSA subjects compared to control subjects (Figure 1). When CA is expressed as the rate of change of vascular conductance (the slope of CBFV/MAP as a function of time) the OSA group had significantly slower compensatory rate than the control group during orthostatic hypotension with no change in end-tidal CO_2 during and after the hypotensive stimulus (Figure 2). This study observed a delayed compensation in both arterial blood pressure and CBFV in the OSA group indicating impairment in their vasoregulatory control (117). These findings provide evidence for one of the underlying reasons for high risk of stroke during sleep and after rising (72).

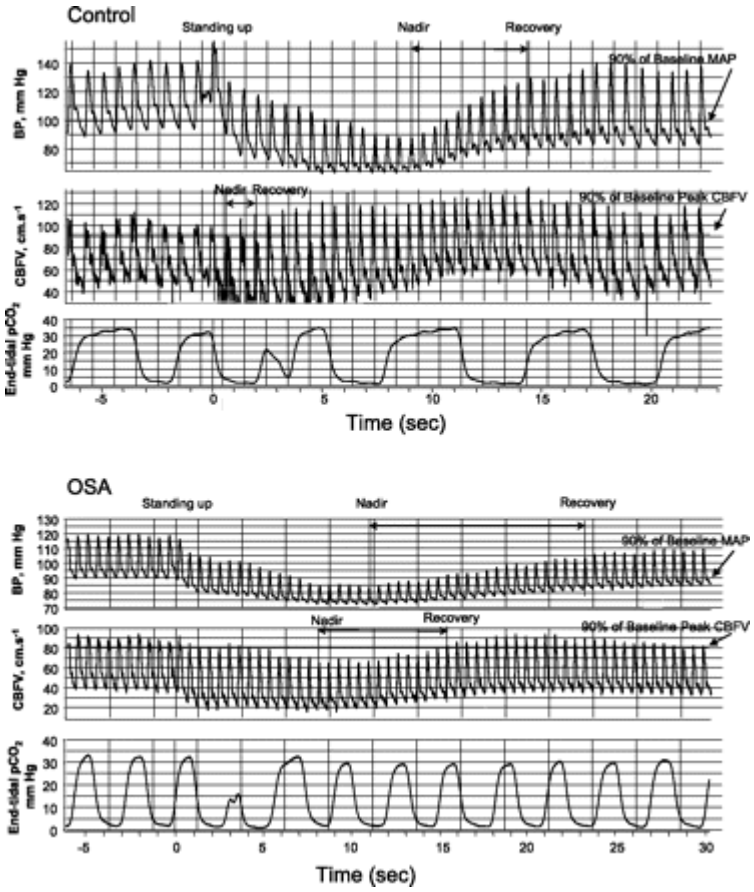


Fig. 1. Original tracings of orthostatic challenge in control and obstructive sleep apnea (OSA) subjects showing the magnitude of the drop of blood pressure (BP) and cerebral blood flow velocity (CBFV) and the time points for calculation of recovery time to 90% of baseline values of mean arterial blood pressure (MAP) and peak CBFV. Adapted from Ubano, et al. (117).

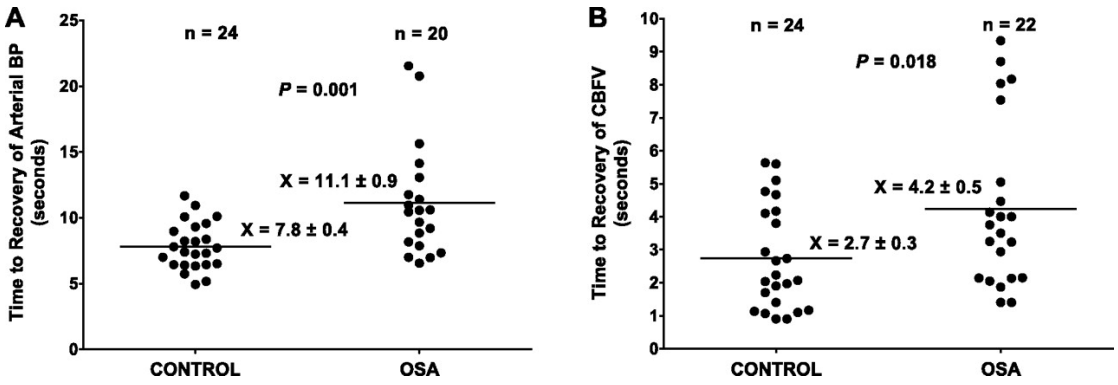


Fig. 2. Time from nadirs of MAP (*A*) and peak CBFV (*B*) to reach 90% of baseline values as a measure of the efficiency of the vasoregulatory capacity. Values are means ± SE; *n*, no. of subjects. Adapted from Urbano, et al. (117).

Cerebral autoregulation has been demonstrated to be primarily mediated by myogenic and metabolic mechanisms (3, 92). However, Aaslid et al. (3) have reported that CA is also affected by the basal vascular tone. This group demonstrated that there was a highly significant relationship between arterial carbon dioxide tension ($PaCO_2$) and CA and confirmed a previous report in which $PaCO_2$ was found to strongly influence the vascular tone of cerebral vascular smooth muscle and resultant changes in CBF. The sympathetic nervous system has recently been demonstrated to have a direct controlling influence on cerebral vascular tone (25, 61). D'Alecy et al. (25) demonstrated that direct electrical stimulation of the stellate ganglion in dogs reduced CBF even during profound hypercapnic mediated vasodilatation. In addition, Jordan et al. (61) demonstrated that using medications which induce vasodilatation tend to cause mild changes in cerebrovascular tone. These findings suggest that the sympathetic nervous system regulates the cerebral circulation by altering the vascular tone of the cerebral smooth muscle. In addition, even though baseline CBF was not altered after removal of autonomic neural activity in humans, CA was impaired (135) providing further support that the sympathetic nervous system has a modulatory effect on CBF regulation.

In studies of congestive heart failure, the incidence of OSA ranges from 11% to 37% (59, 62). Interestingly, a recent study has shown that in patients with congestive heart failure, continuous positive airway pressure (CPAP) treatment of OSA for 1 month was associated with an increase in ejection fraction and decrease in systolic blood pressure and

heart rate (62). In addition, the relationship between cardiac arrhythmias and sleep apnea has been assessed by several studies(42, 107, 108). Some authors have found an increase in both bradyarrhythmias and tachyarrhythmias (42, 65, 136); while others reported a low incidence in patients without serious cardiac or pulmonary disease. However, most experts agree that cardiac arrhythmias occur more frequently in patients who have sleep apnea and that the incidence increases with increases in severity of the sleep apnea (22). Atrioventricular block and asystoles have been reported in up to 10% of patients with sleep apnea (42). These arrhythmias were observed only when oxygen saturation was below 72% (42). Furthermore, almost 90% of these events occur during rapid eye movement sleep (65). Ventricular ectopy has been reported in up to 66% of patients with sleep apnea syndrome (31). These patients experience ectopy mostly during sleep suggesting a direct relationship between arrhythmias and sleep apnea. Ventricular tachycardia, although less prevalent, is still more common in patients with sleep apnea (0-15%) than in the general population (0-4%). In most studies, ventricular tachycardia is almost exclusively limited to apneic events (27, 65).

Recent research has demonstrated altered chemoreflex control of muscle sympathetic nerve activity (MSNA) in untreated OSA patients (80, 81). It has been recognized that obstructive sleep apnea is associated with chronically elevated activity of the sympathetic nervous system (19, 48, 113). Mechanisms for hypoxia-mediated sympathetic overactivity are complex and not fully understood. In general OSA patients exhibit a higher burst frequency of MSNA verses age matched controls during daytime periods of normoxia (81). Thus, increased MSNA is associated with prolonged exposure to hypoxia.

Increases in sympathetic activity have been implicated as a major culprit in the hypertensive condition often accompanying OSA. Furthermore, hypertension is a major risk

factor for the development of stroke and heart disease (63). In hypertensive patients, reductions in cerebral blood flow and increases in cerebrovascular resistance have been reported (63). Cerebral autoregulation is notably impaired in patients with malignant hypertension ((57).

Strokes are the third leading cause of death in the United States and are the leading cause of serious long-term physical and cognitive disability. Since the brain is absolutely dependent on adequate blood flow for normal function, when blood flow falls below a critical flow, brain cells die resulting in a stroke. Impairment in CBF regulation is a major risk factor for stroke (8, 40). Not all of the factors affecting CBF regulation have been clearly elucidated. Although cerebral arteries are richly innervated with sympathetic nerve fibers, (28, 83, 84), it is widely accepted that sympathetic activation has a limited effect on CBF and its regulation at normal arterial PCO_2 values. However, we have recently demonstrated that at rest, changes in sympathetic activity during hypotension modulate cerebral vascular tone and affect dynamic cerebral autoregulation and CBF regulation (87).

Stroke has a circadian pattern of occurrence with increasing incidence during sleep and shortly after rising (72). Cerebrovascular reactivity is an index of the ability of the cerebral vessels to adapt to the metabolic demands of the brain. Any attenuation of this reactivity is indicative of an increased risk of cerebral ischemia and stroke (7, 38, 96). Episodes of obstructive sleep apnea and in normal subjects, voluntary breath holding perturbs the cerebral circulatory control (11, 44, 109, 110). There is a progressive rise in arterial blood pressure and cerebral blood flow velocity (CBFV) during the apneas followed by an abrupt decrease in both pressure and CBFV in the post-apnea hyperventilation period.

The changes in CBFV parallel the changes in systemic blood pressure suggesting impaired cerebral autoregulation.

Acute exposure to hypoxia stimulates oxygen sensitive peripheral chemoreceptors located in the carotid body and central chemoreceptors located in the brain stem. The physiological ramification of stimulation of these excitatory regions includes abrupt increases in ventilation and increased sympathetic nerve activity. When chemoreceptors are stimulated by an increase in CO_2 or a decrease in O_2, sympathetic outflow is increased in a dose-response fashion (79, 113, 121). Acute exposure to 20 min of combined hypoxia and hypercapnia causes increases in ventilation that are paralleled by increases in MSNA (79). After cessation of hypoxia and hypercapnia, ventilation and arterial oxygen saturation return to baseline values, whereas the increases in sympathetic nerve activity persist. Hypoxia rather than hypercapnia has been reported to be the primary stimulus for this prolonged increase in sympathetic tone (24, 115, 123). Interpretation and comparison of hypoxia in physiological and pathological models of hypoxia is difficult and dependent on the duration, severity, and pattern of the exposure as well as the presence or absence of CO_2. However, it appears that repeated or sustained exposure to hypoxia results in sympathetic overactivity. Lusina et al. (2006) tested the effects of 10 daily exposures to hypoxia on MSNA before, during and after an acute 20 minute isocapnic hypoxic exposure. Hypoxia mediated sympathoexcitation is commonly expressed as an increase in burst frequency of MSNA. Similarly, others (79, 123), found an increase in burst frequency of MSNA (70). Specifically, 20 minutes per day of hypoxic intervention for ten days produced a sustained increase in MSNA (70). The increases in ventilatory responses and MSNA were sustained above baseline suggesting that responses may have common central control.

In animal models, electrical stimulation of the sympathetic nerves to the brain prevents forced dilatation of the cerebral arterioles during hypertension (14). In addition, during hypertension when the sympathetic nerves to the brain are sectioned, the increase in perfusion pressure results in a breakdown of the blood brain barrier (14). In contrast to this sympathetically mediated protection of the cerebral vasculature, the hyper-adrenergic disease, OSA, is associated with an impairment in CA and CBF regulation (76, 77) and a propensity for early onset vascular dementia (9, 52, 58, 102). Indeed, other hyper-adrenergic conditions such as exhaustive dynamic exercise, congestive heart failure, idiopathic hypotension and hypertension exhibit similar impairments in CA and CBF regulation (56, 60, 86, 93). In a study by Ogoh, et al, the alpha1 blocker Prazosin caused a significant decrease in the rate of regulation (RoR) of the cerebral vascular conductance (CVC) by cerebral autoregulation. Furthermore, Prazosin blockade accentuated the decrease in middle cerebral artery mean velocity (MCAv mean) during the acute hypotensive stimuli of bilateral thigh cuff release. These results suggest that cerebral autoregulation is attenuated by α-1 receptor blockade and that sympathetic vasoconstriction effects cerebral autoregulation (85) We have recently identified that sympathetically-mediated increases in vascular tone play a major role in both CA and CBF regulation (5). It is, therefore, likely that the increased vascular tone associated with the hyper-adrenergic condition induced by OSA is a factor involved in the impairment of CA and CBF regulation..

In both healthy human and animal models, experimental paradigms designed to mimic the intermittent hypoxic apneas of OSA have identified a rapid onset and sustained (>3hrs) sympathoexcitation (24). In addition, it has been demonstrated in healthy subjects that long-term intermittent hypoxia over a 10 day period increases sympathetic activity and

chemosensitivity to acute hypoxia (70). Therefore, it is important to determine whether the dynamic CA and CBF regulation is further attenuated following a period of chronic intermittent hypoxic apnea as a simulation of OSA. It is also necessary to determine whether dynamic CA is compromised during the conditions of hypoxia and hypotension such as faced by the OSA patient during sleep and upon rising from bed. This review of literature provides sufficient evidence that the increase in sympathetic activity associated with hyper-adrenergic diseases, such as OSA, appears to be a contributing factor in the impairment of cerebral blood flow regulation. The work designed to address these questions is reported in this dissertation.

CHAPTER III

PROCEDURES AND METHODS

The aim of this project was to investigate the effects of acute and chronic exposure to intermittent hypoxic apneas on the control of brain blood flow. The duration of the intermittent hypoxic apnea protocol was chosen in order to cause sustained increases in sympathetic nerve activity similar to that seen in the obstructive sleep apnea patient. Twelve people were selected to participate in the acute protocol and seven people participated in the chronic study. None of these subjects had participated in a hypoxic apnea protocol before this project. Prior to the initiation of the intermittent hypoxic apnea protocols, descriptive cardiovascular data was obtained on each subject, and additionally, each subject was familiarized with the procedures used in the study by experiencing a 'dry run' exposure to the techniques.

Subjects

Volunteers were recruited from the students and staff at the University of North Texas Health Science Center as well as the Greater Fort Worth area. Three prospective subjects were excluded due to various complications which were diagnosed during the initial screening procedures. Twelve healthy volunteers (7 women and 5 men, ages 32 ± 8 yrs, body mass index 24 ± 4), volunteered to participate in the acute investigation and seven healthy volunteers (4 women and 3 men, ages 32 ± 6 yrs, body mass index 24 ± 4), volunteered to participate in the chronic (10 days) investigation. The exclusion criteria required these subjects to be free from cardiovascular, renal, respiratory, metabolic and

neuromuscular diseases. The subjects were also not on any medications, prescription or over the counter that would alter cardiopulmonary function or blood pressure regulation.

Each subject was thoroughly informed of the techniques to be used, as well as the purpose of these techniques. Each subject gave written informed consent and completed a medical history questionnaire before participation in the study and was screened for sleep apnea via overnight pulse oximetry recordings. All experimental procedures were approved by the University of North Texas Health Science Center Institutional Review Board (IRB# 055-2008) and were in accordance with the guidelines of the *Declaration of Helsinki.*

All subjects were nonsmokers, reported no history of cardiovascular, pulmonary, and neurological disease and were not currently using any prescription or over the counter medications. Female subjects all tested negative for pregnancy and were not tested during menses to eliminate potential confounding effects of menses on fluid metabolism, blood volume, and cardiovascular function. Prior to the study, all subjects were familiarized with testing protocols and asked to abstain from vigorous exercise and alcohol for 24h and caffeine for 12h before the start of the experimental day.

Experimental Procedures

Test Days

These studies were performed with subjects in the semi-recumbent position in a laboratory with an ambient temperature of 23-24°C. Before the experimental day, subjects were brought into the laboratory for a familiarization session. During this visit, subjects practiced breathing gas through the facemask and holding their breath. Subjects also completed all necessary paperwork (consent form and medical questionnaire) at the

familiarization session. On the day of the experiment, subjects came to the laboratory in the morning approximately two hours after a light breakfast. Upon arrival, subjects were instrumented for measurement of HR, ABP, respiratory function, Sa_{O2}, MCA *V*, and plasma catecholamine concentrations. Subjects remained in a semi-recumbent position (~45 degrees) in a modified car seat throughout the experimental procedures.

After instrumentation, five minutes of baseline data were recorded as participants breathed room air while wearing the face mask. Before starting treatment, participants performed two to three individual hypoxic apnea events (HAE) consisting of two breaths of 100% nitrogen followed by a 20s end expiratory breath hold. These individual hypoxic apneas produced a range of oxygen saturations (Sa_{O2} 80-85%). Following completion of the individual control apneas, abrupt decreases in ABP were induced using a bilateral leg cuff release protocol.

After control apnea values were obtained and control cuff release protocols were completed, an intermittent hypoxic apnea (IHA) protocol was performed. This protocol entailed 20 min of repetitive hypoxic apneas. In the IHA protocol participants performed one 30-s hypoxic apnea every one min (simulating an apnea/hypopnea index of 60/h) for 20 min. During the first 10 s of the hypoxic apnea, participants were primed with two breaths of 95-100% nitrogen, followed by a 20-s end expiratory voluntary apnea (lung volume equal to FRC) such that Sa_{O2} reached 80-85%. After the 20-min intermittent hypoxic exposure, subjects recovered while breathing room air for five minutes with the face mask while a blood sample was taken for measurement of plasma catecholamines. Then participants performed another HAE. During this one minute period, HR, BP, respiratory function, Sa_{O2}, and MCA *V* were measured continuously as subjects performed a HAE (as described above), sufficient to

produce an Sa_{O2} between 80-85%. Another cuff release protocol was performed to evaluate cerebral autoregulation by producing abrupt decreases in ABP. Then participants performed another single hypoxic apnea followed by a 15 minute break and then the final HAE. Finally, another blood sample was drawn for the last evaluation of plasma catecholamine levels (Figure 3).

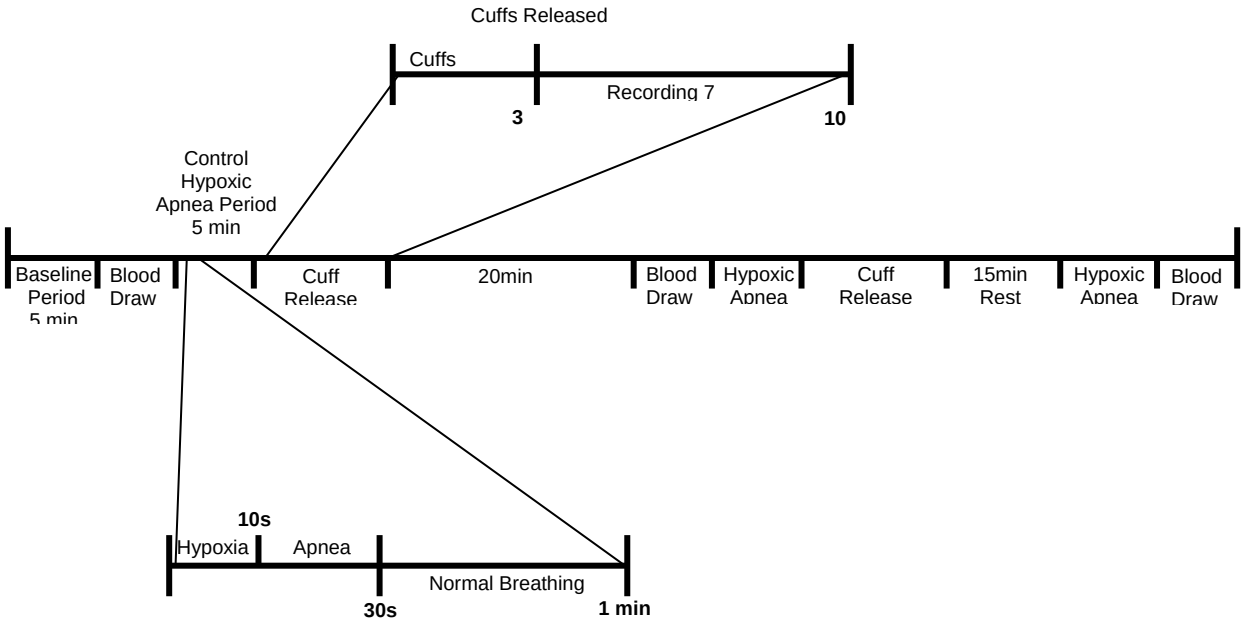


Figure 3. Diagram of the experimental protocol

Protocols

Hypoxic Apnea Events (HAE)

Participants performed one 30 sec HEA. During the first 10s, of hypoxic apnea participants were primed with two to three breaths of 95-100% nitrogen, followed by a 20 sec

end expiratory voluntary apnea (lung volume equal to FRC) such that Sa_{O2} reached 80-85% (24).

Cuff Release Protocol

In order to evaluate cerebral autoregulation, bilateral thigh cuff inflation with rapid deflation was performed (71) before and after the 20 min IHA protocol. Blood pressure cuffs placed at the top of the thigh of both legs were rapidly inflated to a preset suprasystolic pressure (~220 mmHg) and maintained for three minutes. Immediately after the three minutes of cuff inflation, both cuffs were rapidly deflated to produce transient systemic hypotension without changes in central venous pressure (29) as a result of regional vasodilation within the limbs and measurements were continued for an additional seven min. The rate of regulation (RoR) as an index of CA was analyzed from the response data of continuously recorded MAP and MCA V_{mean} in response to acute hypotension produced by the cuff release technique (3). In addition to the cuff-release techniques, we used transfer function analysis between MCA V_{mean} and MAP to identify dynamic CA as described previously (86, 87).

Intermittent Hypoxic Apnea (IHA) Protocol

During the intermittent hypoxic apnea protocol, subjects performed one 30s hypoxic apnea every 1 minute (simulating an apnea/hypopnea index of 60/h) for 20min. During the first 10 sec of each hypoxic apnea, participants were primed with two breaths of 95-100%

nitrogen, followed by a 20 sec voluntary apnea (lung volume equal to FRC) such that Sa_{O2} reached 80-85% (24).

Techniques

Cardiovascular Measurements

Heart rate (HR) was measured using standard three limb-leads ECG. Arterial blood pressure (ABP) was measured noninvasively using photoplethysmography at the finger (Finapres, Finometer blood pressure monitor, Amsterdam, Netherlands). This method has been shown to be a reliable and valid measure of ABP (104).

Cerebral Blood Flow Velocity Measurements

Middle cerebral artery blood velocity (MCA *V*) was obtained by transcranial Doppler ultrasonography (Multidop X, DWL, Sipplingen, Germany). A 2-MHz Doppler probe was placed over the left temporal window and fixed with an adjustable headband and adhesive ultrasonic gel (Tensive, Parker Laboratories, Orange, NJ).

Respiratory Measurements

Respiration was monitored with a low-resistance turbine volume transducer (model VMM, Alpha Technologies; Laguna Hills, CA) attached to a leak-free mask (connected to a breathing circuit). Respiration was monitored to assure that apneas were performed at end expiration. All apneas were performed at functional residual capacity (FRC) because apneas during OSA occur at end expiration. The breathing circuit consisted of a face mask, three-

way Rudolph valve, and a Douglas bag. End-tidal oxygen (P_{ETO2}) and carbon dioxide (P_{ETCO2}) were measured with mass spectrometry (model MGA1100B, Perkin-Elmer, St. Louis, MO).

Arterial Oxygen Saturation Measurements

Arterial oxygen saturation (Sa_{O2}) and transcutaneous pressure of carbon dioxide ($PtcCO_2$) were assessed at the ear using pulse oximetry (TOSCA, Radiometer, Copenhagen, Denmark). Pulse oximetry is a continuous and non-invasive method of measuring the level of arterial oxygen saturation in blood. The measurement is taken by attaching a sensor at the ear lobe of the subject. The sensor collects signal data from the patient and sends it to the monitor. The monitor displays the percent value of arterial oxygen saturation (SpO_2) and as a pulse rate. Pulse oximetry is governed by the following two principles. First, oxyhemoglobin and deoxyhemoglobin differ in their absorption of red and infrared light (spectrophotometry). Second, the amount of arterial blood in tissue changes with the pulse (photoplethysmography) and therefore, the amount of light absorbed by the varying quantities of arterial blood changes as well.

Pulse oximetry uses a two-wavelength pulsatile system to distinguish between oxygenated and deoxygenated blood. Signal data is obtained by passing red and infrared light through a capillary bed and measuring changes in light absorption during the pulsatile cycle. The sensor uses red and infrared light-emitting diodes (LEDs) that pass light through the site to a photodiode. The photodiode receives the light, converts it to an electronic signal and sends it to the TOSCA monitor for calculation. Once the monitor receives the signal from the sensor, it utilizes signal extraction technology to calculate the subject's function

oxygen saturation or the amount of oxygenated hemoglobin expressed as a percentage of the hemoglobin that can transport oxygen and pulse rate.

Transcutaneous Pressure of Carbon Dioxide ($tcPCO_2$)

Transcutaneous measurement of PCO_2 makes use of the fact that carbon dioxide gas is able to diffuse through body tissue and skin and can be detected by a sensor at the skin surface. By warming up the sensor, a local hyperemia is induced, which increases the supply of arterial blood to the dermal capillary below the sensor. The $tcPCO_2$ value has to be interpreted primarily as the PCO_2 partial pressure prevailing at the level of the arterialized skin tissue. In general, this value correlates well with the corresponding arterial PCO_2 partial pressure.

The PCO_2 part of the oximeter sensor consists of a Stow-Severinghaus type electrode. PCO_2 is measured by determining the pH of an electrolyte solution. A change in pH is proportional to the logarithm of the PCO_2 change. The pH is determined by measuring the potential between a miniature glass pH electrode and a Ag/AgCl reference electrode. The electrolyte is encased within a thin hydrophilic spacer, which is placed over the sensor surface and is coupled to the skin via a highly gas permeable hydrophobic membrane. The membrane is protected with a thin golden plate to eliminate any mechanical damage. The sensor is calibrated in a gas with a known CO_2 concentration. The slope (change of potential with PCO_2) is preset in the sensor memory. A high correlation between $tcPCO_2$ and arterial PCO_2 is found in subjects of all ages. However, due to the elevated temperature of the sensor, the transcutaneous PCO_2 is higher than the arterial value. It has therefore become a

common practice to apply a correction to the transcutaneous value to provide a monitor readout which corresponds as close as possible to arterial PCO_2.

The shift of $tcPCO_2$ towards higher values is attributed to two main factors. First, the elevated temperature raises local blood and tissue PCO_2 by approximately 4.5%/C° (anaerobic constant). Secondly, the living epidermal cells produce carbon dioxide, which contributes to the capillary CO_2 level by a constant amount (metabolic constant). This metabolic contribution may change with age, skin thickness, and other variables. A generally accepted estimation is that skin metabolism raises the transcutaneous PCO_2 by approximately 5mmHg (47).

Taking into account both effects, the relationship between $tcPCO_2$ and $PaCO_2$ can be expressed by the following equation:

$$tcPCO_2 = 10\exp [0.019 (T-37)] * PaCO_2 + 5mmHg$$

or

$$tcPCO_2 = F_T * PaCO_2 + C_M$$

whereby:

F_T = temperature correction factor

C_M = metabolic constant

Catecholamine Measurements

A venous catheter (1.1in x 18 gauge) was inserted into the median anticubital vein of the contralateral arm from that which the blood pressure measurement was made. Plasma norepinephrine was evaluated from venous blood samples taken at the antecubital vein pre and post 20-min intermittent hypoxic apnea protocol. Two milliliter venous blood samples

were collected into iced tubes containing 50 μL of glutathione (30.5 mg of molecular weight 307) dissolved in .5ml double distilled water and 2 μL of 10,000 unit Heparin . The plasma was separated by centrifugation within 15 minutes of the blood draw. Plasma samples were then spiked with 40 μL metabisulfite per milliliter of blood and, stored at -90°C, and assayed within 30 days. The plasma catecholamines and an internal standard, dihydroxybenzylamine (Sigma Aldrich, St. Louis, MO) were absorbed onto alumina (Bioanalytical System, West Lafayette, IN) at a pH of 8.6, eluted with 0.1 M perchloric acid (Fischer Scientific, Fair Lawn, NJ), and separated by HPLC on a reverse-phase C18 analytical column (80mm x 4.6mm, 3 μm, ESA, Chelmsford, MA). Mobile phase eluent consisted of 50mM sodium phosphate/ sodium acetate, 0.6 mM SDS, 0.5 mM EDTA, 8% acetonitrile, 5% methanol (Sigma-Aldrich) at a final pH of 3.25. A three electrode ESA Coulochem III Detection System (ESA, Chelmsford, MA) was used for detection. Samples were sequentially oxidized at +300, +100 and reduced at -250mV with quantification at the reducing electrode. .

Data analysis

All data were sampled continuously at 1k Hz using an analog-to-digital converter interfaced with a computer (Windaq, DATAQ Intstruments, Akron, Ohio). Beat-to-beat mean arterial pressure (MAP) and mean MCA *V* (MCA V_{mean}) were obtained from each waveform. The cerebrovascular conductance index (CVCi) was calculated by dividing MCA V_{mean} by MAP and was used as an estimate of changes in cerebrovascular conductance. Control values of MAP, MCA V_{mean} and CVCi were calculated as a four second average of the arterial pulse pressure immediately before the thigh cuffs were released. Changes in

MAP, MCA V_{mean} and CVCi during cuff release and recovery from cuff release were determined relative to their concomitant control values.

Cerebral Autoregulation (CA)

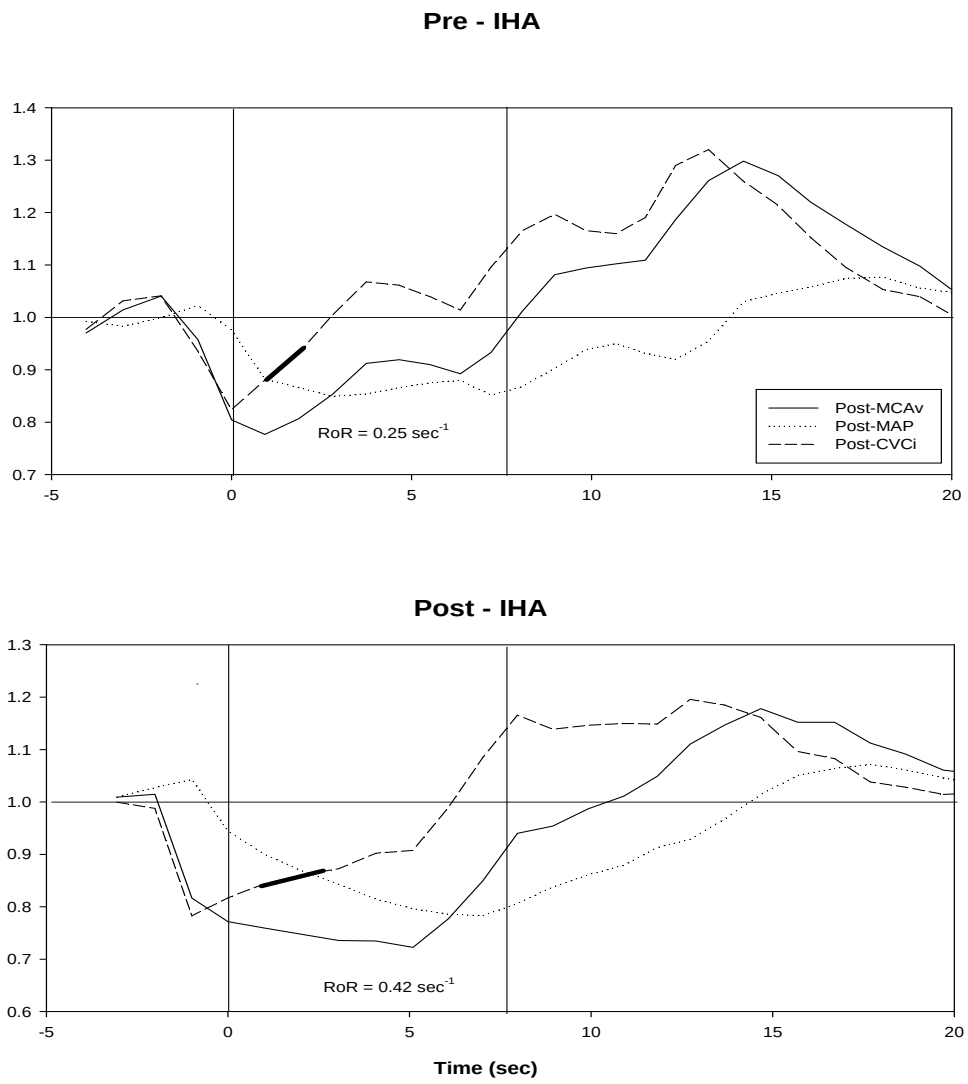
Changes in cerebral autoregulation were determined using two types of analysis.

1. Rate of Regulation:

During the cuff release protocol the ABP decreased abruptly and remained at a low nadir for a limited time (6-8 sec). At the end of the nadir of the ABP the arterial baroreflex gradually restored ABP towards normal. During the time period from 1 to 3.5s post cuff release the rate of change in CVCi is directly related to cerebral autoregulation without arterial baroreflex regulation (3). The rate of regulation (RoR) is calculated as an index of cerebral autoregulation (3).

$$RoR = (\Delta CVCi/ \Delta T)/ \Delta MAP;$$

where $\Delta CVCi / \Delta T$ is the slope of the linear regression between CVCi and time in seconds (T); and ΔMAP was calculated by subtracting control MAP from averaged MAP during the 1 to 3.5s post cuff release (3). See figure 4.



2. Transfer function analysis:

Transfer function gain is an accepted index of dynamic cerebral autoregulation (134). Transfer function gain analysis was employed to further determine changes in cerebral autoregulation (CA) before and after the 20 min IHA protocol. Three-minute steady-state data segments before and after the 20 min IHA protocol were used for transfer function analysis to identify an index of dynamic cerebral autoregulation. The beat-to-beat data of MAP and MCA V_{mean} were then linearly interpolated and resampled at 2 Hz for spectral analysis. The transfer gain and phase shift reflect the relative amplitude and time relationship between the changes in MAP and MCA V_{mean} over a specified frequency range. From the temporal sequences, the frequency-domain transforms were computed with a fast Fourier transformation algorithm. The transfer function H(*f*)

between the MAP and MCA*V* signals was calculated as:

$H(f) = S_{xy}(f) / S_{xx}(f)$

where $S_{xx}(f)$ is the autospectrum of input signal (MAP) and $S_{xy}(f)$ is the cross-spectrum between the two signals. The transfer function magnitude $H(f)$ and phase spectrum $\Phi(f)$ were obtained from the real part $H_R(f)$ and imaginary part $H_I(f)$ of the complex transfer function:

$$H(f) = \{[H_R(f)]^2 + [H_I(f)]^2\}$$

$$\Phi(f) = \tan^{-1} [H_I(f) / H_R(f)]$$

The squared coherence function $MSC(f)$ was estimated as:

$$MSC(f) = [Sxy(f)]^2 / [S_{xx}(f)S_{yy}(f)]$$

where $S_{yy}(f)$ is the autospectrum of changes in MCA V_{mean}.

The squared coherence function reflects the fraction of output power that can be linearly related to the input power at each frequency. Mean value of transfer function gain, phase, and coherence function were calculated in the very-low-frequency (VLF; 0.02–0.07 Hz), low-frequency (LF; 0.07–0.20 Hz), and high-frequency (HF; 0.20–0.30 Hz) ranges to reflect different patterns of the dynamic pressure-flow relationship (134). We used the LF range of each variable for the spectral analysis to identify dynamic cardiovascular and CBF regulation because BP fluctuations in the LF (0.07–0.20 Hz) range are independent of the respiratory frequency and dampened by autoregulatory mechanisms (26). Thus we used the LF spectral power of the mean value of transfer gain, phase, and coherence function to identify dynamic cerebral autoregulation during both supine and standing conditions.

Statistics

The number of subjects required to obtain statistically conclusive results (i.e. sample size) was calculated by using a two-sided significant level α of 0.05 and a power requirement of 90% at target value. For Specific Aims 1 and 2, the primary outcome was CCP.

In Specific Aim 1, we compared measurements of CCP obtained prior to and following experimentally induced sympathoexcitation (20 min of intermittent hypoxia) using a paired t-test as the primary analysis. Based on a preliminary study of three subjects, the sample mean and standard deviation are 10.20 and 5.05, respectively. Therefore, we targeted at a difference of 10.2, and used a standard deviation of 5.1 for the sample size calculation. To achieve a power of 90% at the target of 10.2 for the paired t-test, we needed to recruit five (5) subjects for completion of specific aim 1. This calculation was performed using NCSS/PASS and the actual calculated power was 90.9%.

In Specific Aim 2 and using the same subjects recruited for Specific Aim 1, we compared the CCP measures obtained following 10 days of experimentally induced (20 min intermittent hypoxia each day) symapthoexcitation with the first day of experimentally induced symapthoexcitation obtained in Specific Aim 1. This comparison was made using a paired t-test as the primary analysis. We suggested that the standard deviation for the difference will be similar (5.05) and that it was reasonable to use the same difference target value (10.2). Therefore, the required sample size is the same as in Specific Aim 1. Since the same subjects were used for both aims 1 and 2 the total number of subjects for both aims will be five (5).

Variables are presented as means and standard errors. The primary analysis for the acute protocols was a paired t-test. The data from acute and chronic protocols were then combined, and a repeated measures analysis of variance (ANOVA) was performed as a secondary analysis. For the chronic protocol, one-way analysis of variance (ANOVA) was the primary analysis. If the ANOVA F-test is significant at the 0.05 level further post-hoc analysis (e.g., Tukey's HSD test) were performed. In addition, descriptive statistics, point estimates and confidence are provided. Statistical graphics are also displayed.

CHAPTER IV

RESULTS

Baseline Hemodynamic Variables

Prior to execution of the experimental protocols descriptive cardiovascular data was obtained on each subject. There were no significant differences in tested hemodynamic variables between baseline time periods for the acute protocol. Norepinephrine was significantly increased from pre to post IHA (Table 1).

Baseline Hemodynamic Values	Pre IHA	Post IHA
HR (bpm)	68 ± 2	64 ± 1
SV (ml/beat)	94 ± 6	91 ± 5
CO (ml/min)	6319 ± 399	5806 ± 354
MAP (mmHg)	85 ± 3	87 ± 3
$MCAv_{mean}$ (cm/s)	64 ± 4	65 ± 5
CVCi (cm/s/mmHg)	1 ± 0	1 ± 0
$tcPCO_2$ (mmHg)	39 ± 1	39 ± 1
SaO_2	98 ± 0	98 ± 0
NE (pmol/mol)	0.93 ± 0.15	1.35 ± 0.21 *
Epi (pmol/mol)	0.26 ± 0.09	0.43 ± 0.16

Table 1. Values are means ± SE. HR, heart rate; SV, stroke volume; CO, cardiac output; MAP, mean arterial pressure; MCA V_{mean} middle cerebral artery blood velocity mean; CVCi, cerebral vascular conductance index; $tcPCO_2$, trans cutaneous pressure of carbon dioxide; SaO_2, arterial oxygen saturation; NE, norepinephrine; Epi, epinephrine . * $P < 0.05$.

20 Minute Intermittent Hypoxic Apnea Protocol

During the intermittent hypoxic apneas the MCA *V*m was increased approximately 25% by the hypoxic hypercapnia and decreased 20% by the hyperventilation response to each apnea, while MAP remained elevated. A concomitant decrease in SaO_2 occurred with each hypoxic apnea and trended downward through the 20 apneas, see Figure 5.

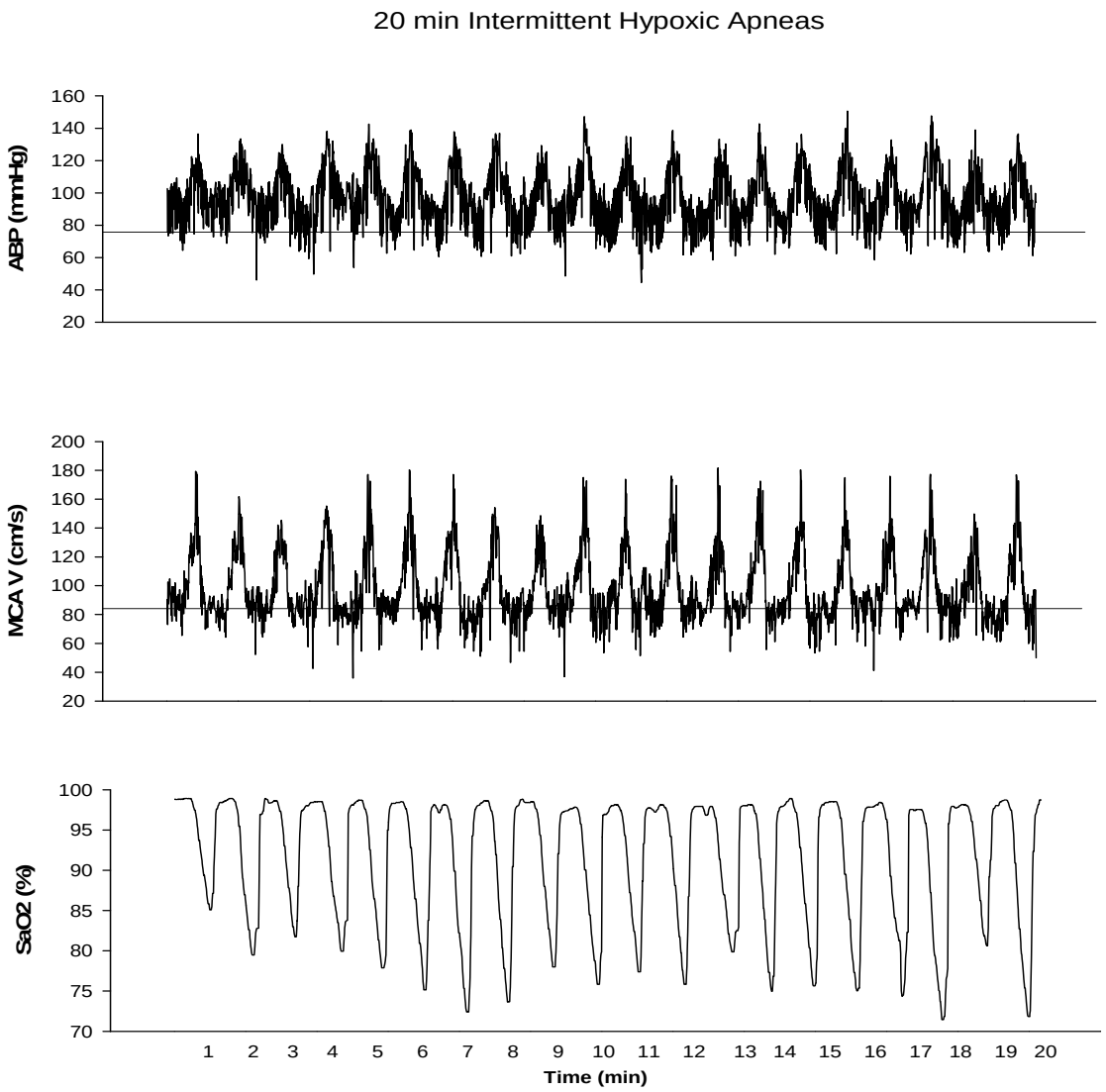


Figure 5. Representative beat-to-beat response data of continuous recordings of mean arterial pressure (MAP, Top) and middle cerebral artery mean blood velocity (MCA *V*m, middle) and arterial oxygen saturation (SaO_2) during the 20 min intermittent voluntary hypoxic apnea protocol. The solid lines show control baseline values.

Norepinephrine Levels Detected in Response to Sympathoexcitatory Stimulus

Plasma norepinephrine was significantly increased in response to the 20 min IHA protocol (Figure 6). During the acute protocol, the increases in norepinephrine due to the 20 min IHA protocol were identified on the before and after IHA.

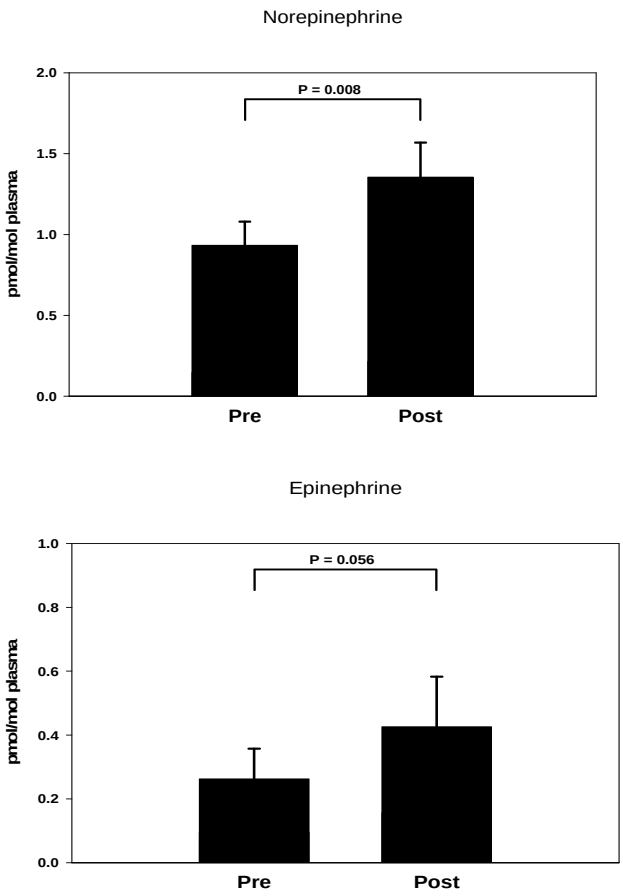


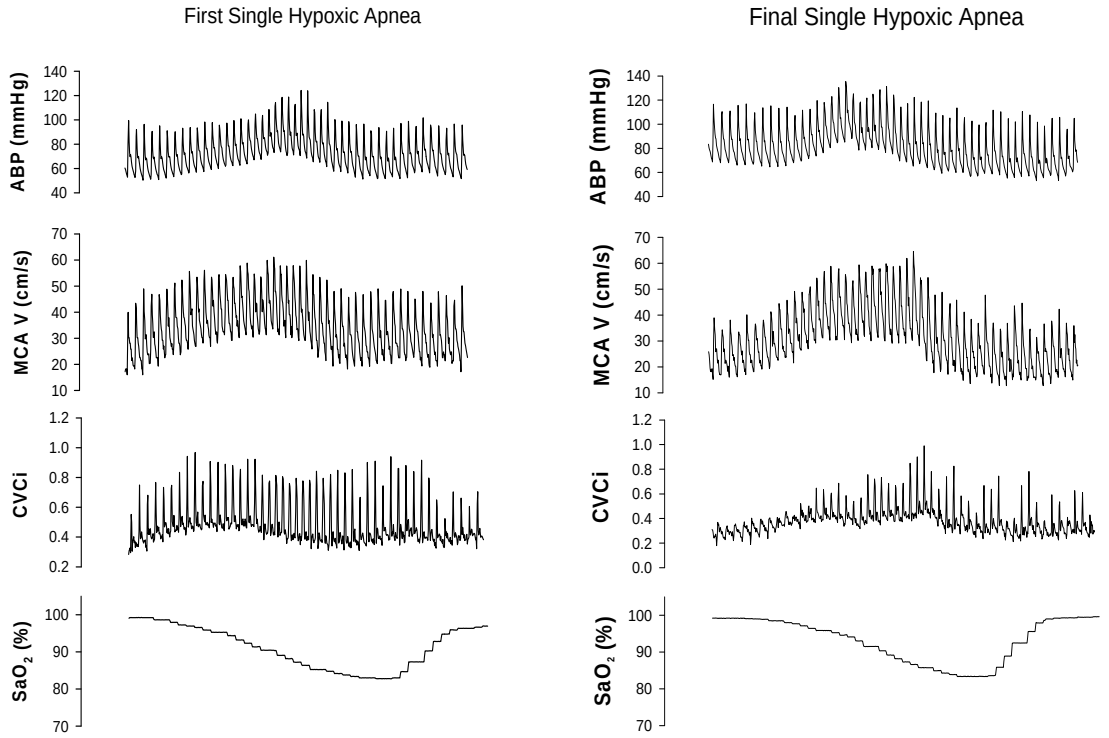
Figure 6. Bar graphs of changes in venous plasma norepinephrine and epinephrine levels across the acute protocol. Norepinephrine was significantly increased from pre to post IHA values on day one of both the acute (n=9).

Hypoxic Apnea Events

Individual control hypoxic apneas were performed in order to ensure a decrease in arterial oxygen saturation (SaO_2) to 80-85%. Figure 4a represents changes in arterial blood pressure (ABP), middle cerebral artery blood velocity (MCA V), cerebrovascular conductance index (CVCi) and arterial oxygen saturation SaO_2 prior to the 20 min intermittent hypoxic apnea protocol. While Figure 4b represents changes in ABP, MCA V, CVCi and SaO_2 after the 20 min intermittent hypoxic apnea protocol. Figure 7a and figure

7b are representative raw data tracings demonstrating a decrease in SaO_2 with concomitant fluctuations in arterial blood pressure (ABP), MCA V and CVCi, while transcutaneous carbon dioxide pressures ($tcPCO_2$) remained relatively constant throughout the 30s hypoxic apnea. Therefore, a hypoxic event was established without significantly altering $PaCO_2$.

a. b.



Figures 7a and 7b. Representative raw data tracings of a single hypoxic apnea event Pre 20 min IHA (4a) and Post 20 min IHA (4b), respectively. Arterial blood pressure (ABP), middle cerebral artery blood velocity (MCA *V*), arterial oxygen saturation (SaO_2), partial pressure of arterial carbon dioxide ($PaCO_2$).

The average changes in MAP and MCA V from baseline during individual hypoxic apnea events employed in the acute and chronic protocols are represented in Figure 8. There were no discernable differences between the increases in MAP and MCA V pre- and post-20 min intermittent hypoxic apnea protocols. The data indicated a greater increase in MCA V above baseline values during individual hypoxic apnea events pre- and post-20 min

intermittent hypoxic apneas compared to the increase in MAP, respectively. This data suggests that the increase in MAP is not the only factor influencing changes in MCA V in these protocols. Hypoxia mediated changes in cerebrovascular tone may also contribute to the greater increase in MCA V.

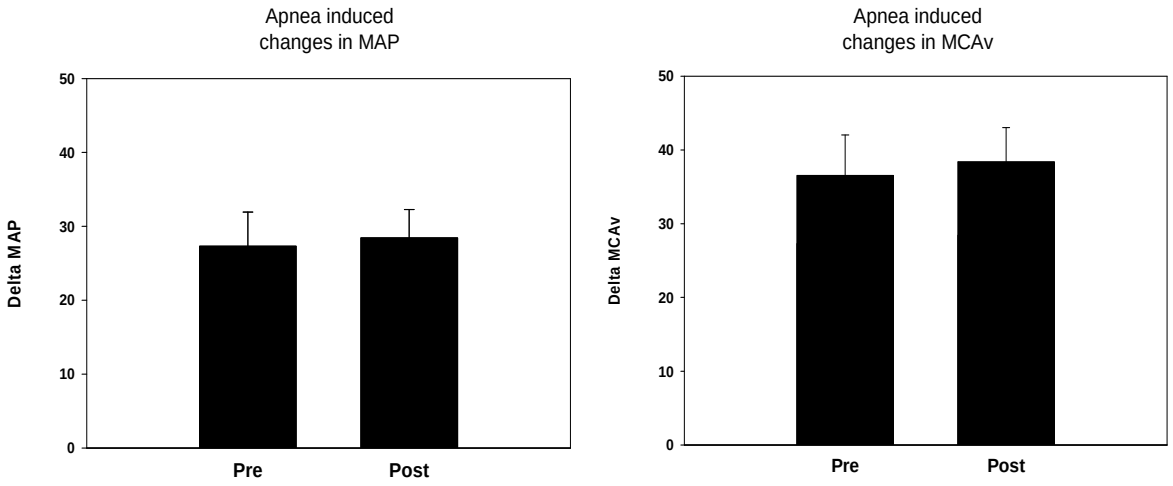


Figure 8.

Left panel: The changes in mean arterial pressure (MAP) induced by a single hypoxic apnea event before and after 20 min IHA protocol for the acute study.

Right panel: The changes in the middle cerebral artery blood velocity (MCA *V*) induced by a single hypoxic apnea event before and after 20 min IHA protocol for the acute study.

Rate of Regulation

The rate of regulation (RoR) was calculated as an index of cerebral autoregulation (CA) from the response data of MAP and MCA V_{mean} to acute hypotension produced by the cuff release technique (3). The RoR was significantly attenuated following 20 minutes of

intermittent hypoxic apneas (Figure 9). This data indicates a loss of dynamic cerebral autoregulation under the condition of orthostatic hypotension.

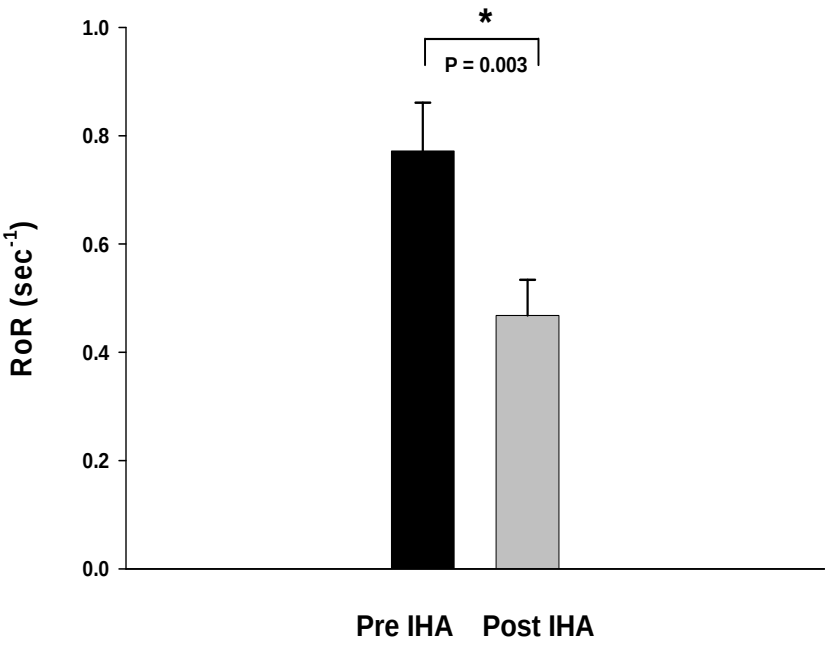


Figure 9. Average data for 12 subjects Pre and Post 20 min IHA protocol for the acute study. All data are shown in normalized units relative to control prerelease values from -4 to 0 seconds.

Low Frequency Transfer Function Gain

In addition to the cuff-release techniques, we used transfer function analysis between MCA V_{mean} and MAP to identify dynamic CA as described previously (86, 87). Transfer function gain is an accepted index of dynamic cerebral autoregulation (134). Transfer function gain analysis was employed to further determine changes in cerebral autoregulation (CA) before and after the 20 min IHA protocol. Low frequency (LF) gain was not significantly changed by the 20 min IHA protocol (Figure 10).

Figure 10.

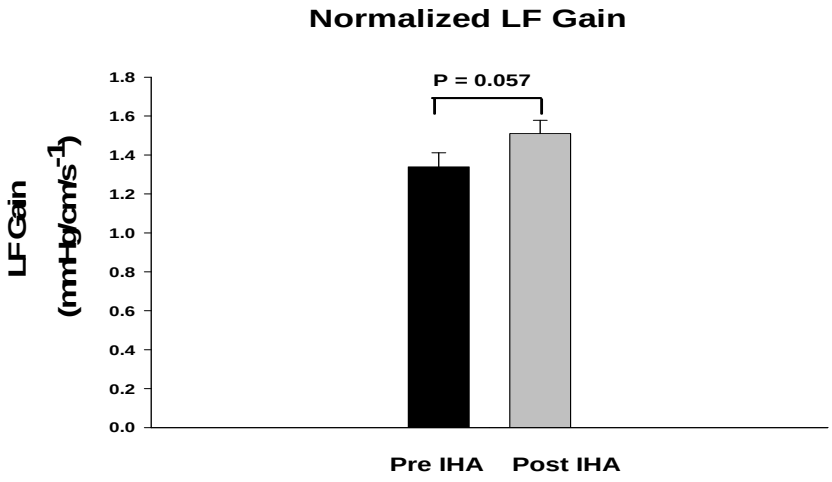


Figure 10. Normalized low frequency gain across the acute protocol. LF gain trended to increase from pre to post 20 min IHAs in the acute protocol but was not significantly increased.

Chronic Protocol

Baseline Hemodynamic Variables

Prior to execution of the experimental protocols descriptive cardiovascular data was obtained on each subject. There were no significant differences in tested hemodynamic variables between baseline time periods for the acute and protocol (Table 2).

Baseline Hemodynamic Values	Day 1 IHA			Day 10 Pre IHA			Day 10 Post IHA		
HR (bpm)	65	±	2	65	±	4	63	±	4
SV (ml/beat)	92	±	5	86	±	7	87	±	7
CO (ml/min)	5896	±	418	5464	±	267	5400	±	369
MAP (mmHg)	84	±	5	79	±	5	89	±	4
$MCAv_{mean}$ (cm/s)	59	±	5	58	±	5	57	±	5
CVCi (cm/s/mmHg)	0.7	±	0.1	0.8	±	0.1	0.7	±	0.1
$tcPCO_2$ (mmHg)	39	±	2	39	±	1	38	±	2
SaO_2	99	±	0	99	±	0	99	±	0
NE (pmol/mol)	1.21	±	0.13	1.26	±	0.03	1.7	±	0.19
Epi (pmol/mol)	0.91	±	0.26	0.83	±	0.17	1.2	±	0.2

Table 2. Values are means ± SE. HR, heart rate; SV, stroke volume; CO, cardiac output; MAP, mean arterial pressure; MCA V_{mean} middle cerebral artery blood velocity mean; CVCi, cerebral vascular conductance index; $tcPCO_2$, trans cutaneous pressure of carbon dioxide; SaO_2, arterial oxygen saturation; NE, norepinephrine; Epi, epinephrine .

Norepinephrine Levels Detected in Response to Sympathoexcitatory Stimulus

Plasma norepinephrine but not plasma epinephrine was significantly increased in response to the 20 min IHA protocol (Figure 11). During the chronic protocol, the increases in norepinephrine due to the 20 min IHA protocol were identified on the first and last day.

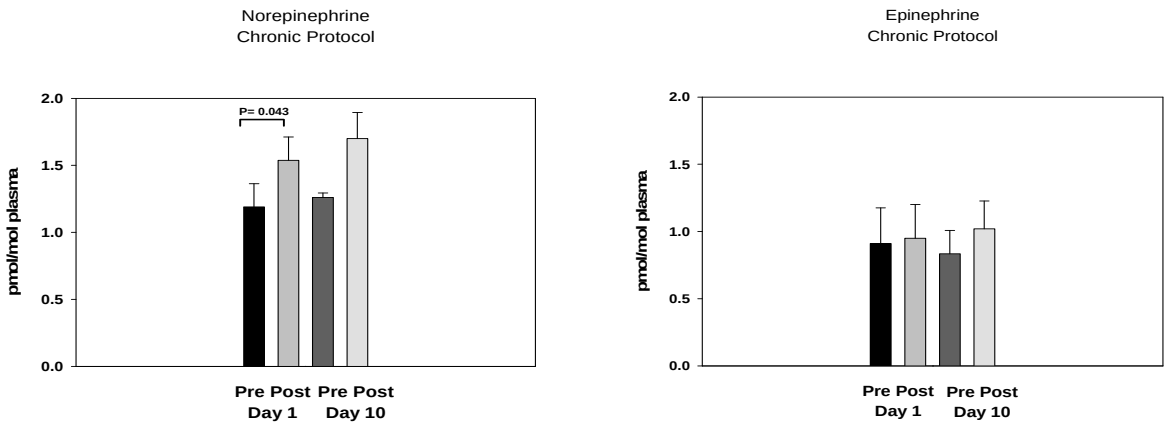


Figure 11. Bar graphs of changes in venous plasma norepinephrine levels across the acute (a) and chronic (b) protocols. Norepinephrine was significantly increased from pre to post IHA values on day one of the chronic (n=3) protocol.

Rate of Regulation

The rate of regulation (RoR) was calculated as an index of cerebral autoregulation (CA) from the response data of MAP and MCA V_{mean} to acute hypotension produced by the cuff release technique (3). The RoR was significantly attenuated following 20 minutes of intermittent hypoxic apneas (Figure 12). This data indicates a loss of dynamic cerebral autoregulation under the condition of orthostatic hypotension. Although the attenuation in CA was not maintained across the 10 day chronic protocol, 20 min IHA did cause a significant attenuation of CA at the end of the chronic protocol (Figure 12).

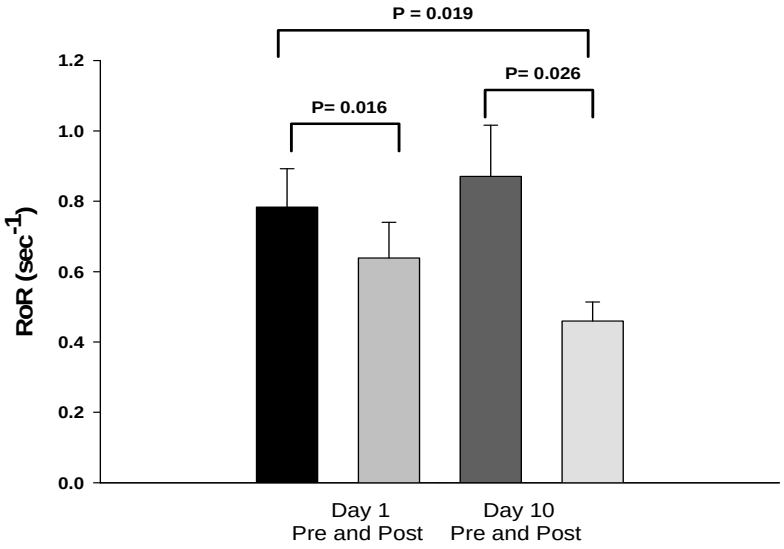


Figure 12. The rate of regulation (RoR) average for 7 subjects on days 1 and 10 of the chronic protocol Pre and Post 20 min IHAs. All data are shown in normalized units relative to control prerelease values from -4 to 0 seconds. Significance is $P< 0.05$.

Low Frequency Transfer Function Gain

In addition to the cuff-release techniques, we used transfer function analysis between MCA V_{mean} and MAP to identify CA as described previously (86, 87) for the chronic protocol as well. Transfer function gain analysis was employed on days 1 and 10 of the chronic protocol before and after the 20 min IHA protocol. Low frequency (LF) gain was not significantly changed by the 20 min IHA protocol (Figure 10). No significant differences were identified in LF gain between days.

However, similar to the acute protocol, there was a trend for an increase in LF gain on days 1 and 10. (Figure 13).

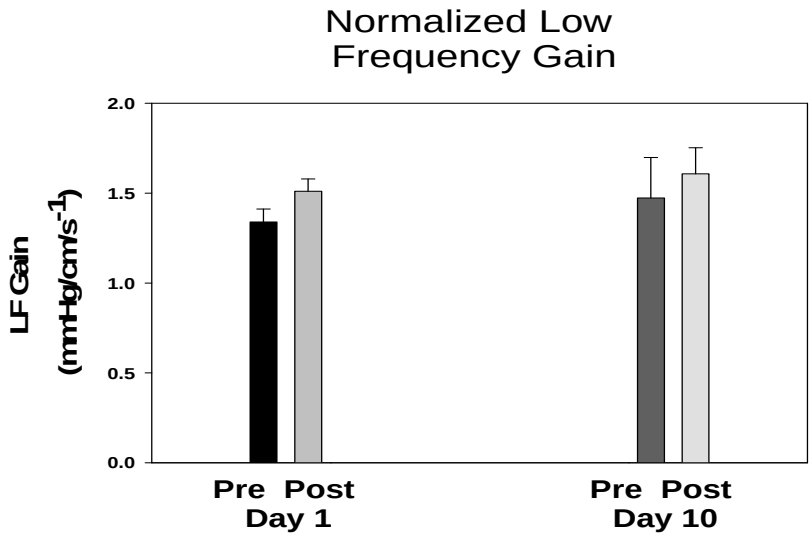


Figure 13. Normalized low frequency gain across the chronic protocols. LF gain trended to increase from pre to post 20 min IHAs in both the acute situations.

Hypoxic Apnea Events

The average changes in MAP and MCA V from baseline during individual hypoxic apnea events employed in the chronic protocol are shown in Figure 14. There were no discernable differences between the increases in MAP and MCA V pre- and post-20 min intermittent hypoxic apnea protocols. The trend for increases in MAP and MCA V were maintained through the 10 day chronic protocol (Figure 14). This data suggests that the increase in MAP is not the only factor influencing changes in MCA V in these protocols. Hypoxia mediated changes in cerebrovascular tone may also contribute to the slightly greater increase in MCA V.

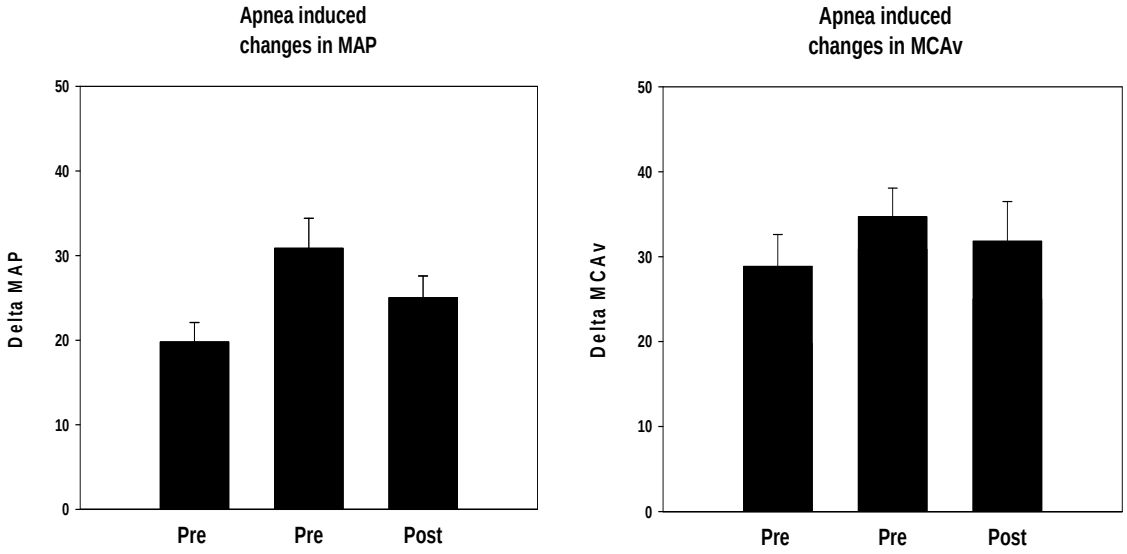


Figure 14.
Left panel: Changes in mean arterial pressure (MAP) induced by a single hypoxic apnea event before and after 20 min IHA protocol for the chronic protocol.

Right panel: Changes in middle cerebral artery blood velocity (MCA V) induced by a single hypoxic apnea before and after 20 min IHA protocol for the chronic protocol.

CHAPTER V

DISCUSSION and CONCLUSIONS

The investigations described in this dissertation used a novel experimental model in humans to address two important questions regarding cerebral autoregulation (CA) and cerebral blood flow (CBF) regulation: Experiment 1.) Do intermittent hypoxic apneas (IHA), which result in a higher sympathetic neural activity (SNA), impair CA and CBF regulation?; Experiment 2.) Are there any differences in CA and CBF regulation between acute (minutes) and chronic (days) exposures to IHAs?

I. Acute (20 min) exposure to intermittent hypoxic apneas (IHA): Experiment 1.

The data of experiment 1, which employed a twenty minute IHA protocol, resulted in significant increases in plasma norepinephrine (fig.3 chapter III). Previously, this brief IHA protocol had resulted in a sustained elevation (200 %) of total muscle sympathetic nerve activity during three hours of recovery (24). Furthermore, these data are consistent with Morgan, et al (79) and Xie, et al. (122) who demonstrated a prolonged (1hr) elevation (200%) of sympathetic nerve activity after sustained and intermittent asphyxia, respectively. In both cases it is likely that this increase in sympathetic tone is due mainly to hypoxic stimulation sensitizing the peripheral chemoreceptors, as shortly into recovery both ventilation and arterial oxygen saturation (SaO2) had returned to normal values (79, 123). The sustained increase in sympathetic activity during recovery enabled us to compare measures of CA and CBF regulation to the pre-IHA control measures in the recovery period without confounding changes in cardiovascular hemodynamics or blood gas status, see Table 1 in Chapter III.

Lassen (67) established that human CBF is maintained relatively constant despite changes in MAP between 60-150 mmHg. This relationship is known as cerebral autoregulation. However, during the development of the definition of the relationship between CBF and MAP all information was based on differences between steady-state CBF between discretely different physiological conditions. Therefore, the traditional and accepted concept of CA only defines static CA. However, with the advent of new technologies, such as transcranial Doppler (TCD) and functional magnetic resonance (fMRI) imaging, the beat-to-beat fluctuations in middle cerebral artery (MCA) blood velocity (TCD) and blood flow can be determined in parallel with the beat-to-beat fluctuations in arterial blood pressure. Consequently, with the use of linear dynamic analyses techniques of correlating the fluctuations in cerebral blood velocity and pressure in the frequency domain and the subsequent calculation of a transfer function gain (TFg) an assessment of dynamic CA can be obtained. More recently, dynamic CA has been evaluated by measuring changes in CBF in response to rapid changes in blood pressure. During orthostatic stress dynamic CA was preserved despite a reduction in CBF (116). If static CA was evaluated under the same circumstance an impaired CA would be concluded. However, static and dynamic CA have a functional role in maintaining CBF constant and both forms of CA are important for the regulation of CBF.

During the cuff release protocol the ABP decreased abruptly and remained at a low nadir for a limited time (6-8 sec). At the end of the nadir of the ABP the arterial baroreflex gradually restored ABP towards normal. During the time period from 1 to 3.5s post cuff release, the rate of change in the cerebral vascular conductance index (CVCi), or the Rate of

Regulation (RoR) was, by definition, directly related to dynamic cerebral autoregulation without the confound of arterial baroreflex regulation (3)

It has recently been identified that increased sympathetic activity influenced dynamic CA (using RoR as the measure) and arterial baroreflex regulation of CBF during hypotension (85), while during a hypertensive condition sympathoexcitation directly causes vasoconstriction (14, 50). The data obtained from the present investigation indicated that one 20 minute bout of IHA significantly increased sympathetic neural activity (SNA) as indicated by significant elevations in plasma norepinephrine levels, which resulted in a decreased RoR, i.e. an impaired dynamic CA, see fig.6 in Chapter IV. In addition, the normalized low frequency TFg of the resting MAP and MCA V appeared to increase, although not significantly, p= 0.057 (see Fig. 7 in Chapter IV). Whether the physiological interpretation of these findings is confounded by a Type 1 or Type 2 error needs to be evaluated by recalculating the power of the sample size and further testing, if appropriate. For example, if TFg is confirmed not to be statistically significant yet, as already identified, the RoR indicates that CA is impaired during hypotension following IHA, it would be concluded that CA during hypertension was not impaired. Conversely, if TFg was significantly increased indicating a loss of CA damping of the MAP effect on MCA V, then CA would be identified as being impaired. The clinical significance of the findings is discussed in a subsequent section.

It is well known that $PaCO_2$ serves as both an important controlled variable and mediator of CBF (56) and because the blood brain barrier is permeable to CO_2 and relatively impermeable to $[H^+]$ and $[HCO3^-]$ ions, it is CO_2 that stimulates the central chemoreceptors. Moreover, CBF is highly sensitive to direct changes in $PaCO_2$ (4) and as a consequence

hypocapnia causes cerebral vasoconstriction, thereby reducing CBF and because of a reduced 'washout' of CO_2 attenuates the fall of brain tissue $PaCO_2$. In contrast, hypercapnia increases CBF by cerebral vascular vasodilation and increases 'washout' limits increases in brain tissue $PaCO_2$. In addition to the fact that hypercapnic cerebral CO_2 reactivity is greater than hypocapnic reactivity an increase in $PaCO_2$ exponentially elevates CBF when a wide range of CO_2 challenge is applied (88, 100) suggesting that cerebrovascular reactivity and the ventilatory response to CO_2 are tightly linked. The respiratory chemoreflex is an important feedback control system which keeps the $PaCO_2$ constant via tight ventilatory regulation. Therefore, changes in CBF play an important role stabilizing the breathing pattern during fluctuating levels of chemical stimuli, especially $PaCO_2$.

Hypoxia indicated as a fall in the partial pressure of arterial oxygen (PaO_2) below 40 mmHg produces cerebrovascular vasodilation (43). However, while hypoxia is a cerebral vasodilator, reflected in a rise in CBF in proportion to the severity of isocapnic hypoxia (21, 127), under normal conditions the hypoxic-induced activation of peripheral chemoreceptor activity leads to a hyperventilation induced lowering of $PaCO_2$ and subsequent cerebral vasoconstriction. Therefore, the cerebral vasculature receives conflicting signals during exposure to acute hypoxia. In addition to the chemoreflex sensitivity to hypoxia the individual sensitivity of the cerebrovascular bed to hypoxia and hypocapnia determines the extent of CBF change upon exposure to hypoxia (78).

During the 20 min IHA protocol it was evident that during each hypoxic apnea the rise in MCAV was greater than the rise in blood pressure. The single hypoxic apnea events employed through out our protocol demonstrate the fluctuations in both ABP and MCA V due to the hypoxic apnea, see Fig 4 in Chapter IV. However, the changes in the MCA V and

MAP response to a single hypoxic apnea were not significantly increased after the IHA protocol, however, we did observe a greater increase in MCA V than MAP, see Figures 5 in Chapter IV. This suggests that the elevation in MCA V is not only due to the increase in blood pressure, but is also influenced by the hypoxia induced vasodilation regardless of the IHA induced increases in SNA.

In summary, one acute (20 min) exposure of healthy subjects to intermittent hypoxic apneas stimulates peripheral chemoreceptors to increase sympathetic activity. This increased sympathetic activity results in an impairment of cerebral autoregulation during hypotension. However, hypoxic stimulation of ventilation following the acute exposure to intermittent hypoxia does not appear to impair cerebral blood flow regulation. Accordingly, this is the first demonstration of altered cerebral autoregulatory control following short term exposure to intermittent hypoxic apneas in the healthy human.

II. Sequential exposure acute intermittent hypoxic apneas (IHA) over 10 days: Experiment 2.

The physiological changes in response to hypoxia are dependent on the duration, severity and pattern of the hypoxic stimulus. For example, SNA of healthy humans was increased three-fold during normoxia following four weeks of sustained exposure to hypobaric hypoxia. This increased SNA persisted for many weeks (45) In patients with obstructive sleep apnea (OSA) daytime SNA was chronically elevated compared to healthy control subjects (82). Recently, chronic elevation in sympathetic activity in response to a similar protocol of intermittent hypoxia used in experiment 1 was observed (70). In this investigation it was reported that after 10 days of acute IHA exposure, the human's MSNA burst frequency was increased compared to pre intermittent hypoxic exposure values. The

increases in MSNA progressively increased from day 1 through day 10. Increases in HR, systolic blood pressure and MAP which occurred during the daily 20 min IHA exposure returned rapidly to baseline values at the termination of each IHA exposure. Furthermore, following each daily IHA the rise in MSNA burst frequency was strongly related to the change in the hypoxic ventilatory response.

In experiment 2 we attempted to more accurately mimic the pathologic situation of the chronic exposure to the repetitive intermittent hypoxic apneas prevalent in obstructive sleep apnea (OSA) patients. Similar to the recent report of Lusina et al., in a separate group of subjects, we employed a repetitive daily exposure to the acute (20 min) IHA protocol of experiment 1 for 10 days. The findings for day 1 of the 10 day protocol were similar to those of experiment 1. Plasma norepinephrine (NE) was significantly increased (see Fig 8) indicating sympathoexcitation and RoR was significantly decreased during hypotension (see Fig 9) indicating impaired dynamic CA. In addition, the TFg at rest for MAP to MCA V trended towards significance but was statistically unchanged (see Fig.10) and there was no difference in the apnea induced changes in MAP or MCA V (see Fig 11) indicating no changes in the ventilation interaction with CBF regulation.

However, at the end of the 10 days of sequential exposure to acute (20 min) IHA no further changes were identified in NE, RoR, TFg, or the ventilation interaction with CBF regulation (see Figs. 8-10). Furthermore, dynamic CA was restored between each day as there was no difference in pre IHA RoR values on day 1 and day 10. This suggests that the daytime dynamic CA remained intact for the 10 days of IHA. These unexpected findings indicate that by using healthy subjects as surrogates for OSA patients and only a brief intermittent IHA protocol as a simulation of the sleep apnea stimulus was limited. However,

the findings of experiment 1 and their confirmation in experiment 2 indicate that increased sympathetic activity impairs CA during hypotension. This finding may explain the greater incidence of orthostatic hypotension and stroke when transferring from the supine to upright position on awakening (6).

Conclusions and Clinical Significance:

We therefore conclude that following an acute stimulus of intermittent hypoxic apneas, CA is attenuated in healthy subjects. Furthermore, that this attenuation of CA may be due to an increase in sympathetic nerve activity produced by the intermittent hypoxia activation of peripheral chemoreceptors. However, this decrease in CA does not persist beyond the immediate cessation of the hypoxic protocol in the healthy subject. This finding is in contrast to what is observed in the OSA patient, whose CA is attenuated throughout daytime wakefulness (117). This difference may be due to the many confounding factors with the condition of OSA such as hypertension, greater duration of hypoxia and higher elevations in daytime SNA.

The fluctuations of MAP and MCAV in response to a single hypoxic apnea were not different before and after intermittent apneas indicating that there was not a loss of regulation under a hypertensive stimulus. Even though the amount of fluctuation of MAP and MCAV in response to a single hypoxic apnea was not different before and after intermittent apneas, there were larger fluctuations in MCAV than MAP. These repetitive bouts of hyperperfusion with ensuing repetitive bouts of low flow increase the risk of damage to the cerebral vasculature and loss of autoregulation. This further suggests the importance of the circadian rhythm for the risk of stroke, in that immediately following a bout of intermittent hypoxia

and upon facing an orthostatic challenge, such as rising from the supine position, the ability to regulate brain blood flow is decreased and the brain becomes susceptible to ischemia leading to a cerebrovascular event.

Clinical Significance

In the past twenty years the idea "that sympathetic control of the cerebral vasculature has minimal functional importance" is being questioned. Although the classic Kety-Schmidt method of measurement of CBF has played a part in our lack of acceptance of the role of sympathetic control, the ability to only identify its involvement as a part of the pathophysiology of hyper-adrenergic disease states has also confounded physiological interpretation. Because hyper-adrenergic disease states such as hypertension, OSA and congestive heart failure are associated with an earlier onset of dementia, it seems reasonable to postulate that chronic sympathetic cerebral vasoconstriction reduces oxygen delivery to the brain during activities of daily living. This reduction in oxygen delivery would result in a chronic relative ischemia, which could be the initiator of the cellular signaling cascade associated with the onset of vascular dementia (119). This investigation has identified a possible the effect of high sympathetic nerve activity associated with OSA patients on CBF regulation.

Conclusions and Clinical Significance:

We conclude that following an acute stimulus of intermittent hypoxic apneas, CA is attenuated in healthy subjects. Furthermore, the attenuation of CA appears to be due to an increase in sympathetic nerve activity produced by the intermittent hypoxic activation of the peripheral chemoreceptors. However, the data from this investigation does not identify as to the length of time that the decrease in CA persists. This finding is in contrast to what is

observed in the OSA patient, whose CA is attenuated throughout daytime wakefulness (117). The differences in the effects of increased sympathetic activity between healthy subjects and OSA patients may be due to the many confounding factors associated with OSA, such as, hypertension, impaired endothelial function of the cerebral vessels and greater duration of hypoxia and/or asphyxia along with the higher elevations in daytime SNA (114).

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