The Effect of Acute Intermittent Hypoxia on Sympathetic Neurovascular Transduction

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Abstract

Obstructive sleep apnea (OSA) patients suffer from intermittent hypoxia (IH) due to repetitive airway obstructions during sleep. IH increases chemoreflex sensitivity, sympathetic vasomotor outflow, and increases blood pressure. The purpose of this study was to determine if acute IH augments sympathetic neurovascular transduction in young healthy men, which could contribute to an increase in blood pressure. It was hypothesized that IH would augment sympathetic neurovascular transduction. Ten healthy males without OSA were exposed to 40 seconds of hypercapnic hypoxia ($P_{ET}O_2$ = 48.1 ± 1 mmHg, $P_{ET}CO_2$: + 4 ± 1 mmHg above baseline) and 20 seconds of normoxia for 40-minutes, mimicking severe OSA. Before and after IH we measured muscle sympathetic nerve activity burst frequency (MSNA BF; peroneal microneurography) and forearm vascular resistance (FVR; brachial artery duplex ultrasound) during rest and three levels of lower body negative pressure (15, 30, and 45 mmHg) while clamping end-tidal gases at baseline values. Sympathetic neurovascular transduction, defined as the slope of the relationship between MSNA BF and FVR, was increased 3-fold immediately following IH (P = 0.015). IH increased minute ventilation (+5 ± 2 l/min, P = 0.025), suggesting long term facilitation of ventilation. Additionally, MSNA BF (+2 ± 1 /min, P = 0.032) and mean arterial pressure (+5 ± 2 mmHg, P = 0.002) were increased following IH. Our results suggest that acute IH increases sympathetic neurovascular transduction and may be an important early mechanism involved in the development of hypertension in OSA.

Lay Summary

Obstructive sleep apnea (OSA) is a disorder where people momentarily stop breathing during sleep, which lowers their blood oxygen levels repeatedly and increases the risk of developing high blood pressure (hypertension). Healthy people also develop high blood pressure when their blood oxygen levels are lowered repeatedly. The changing oxygen levels may make blood vessels more sensitive to the nerve signals that control them, causing blood vessels to narrow. As a result, blood pressure must increase to maintain blood oxygen delivery. In our study, we had healthy people repeatedly breathe low oxygen gas to simulate OSA. We found the changes in oxygen levels made blood vessels become more responsive to nerve signals and increased blood pressure. Therefore, high blood pressure may be caused by an increase in the responsiveness of our blood vessels to the nerve signals that control them. This provides an explanation for how people with OSA develop hypertension.

Preface

This thesis contains original data collected in the Cardiopulmonary Laboratory for Experimental and Applied Physiology by Troy Stuckless and Dr. Glen Foster, who both were responsible for the design of the experiment. Dr. Najib Ayas, Dr. John Floras, and Dr. Michael Smith assisted with aspects of clinical translation, idea conception, and experimental design. Courtney Brown operated our end-tidal forcing system. Tyler Vermeulen operated our lower body negative pressure chamber and data recording software. Silo software was created by Lindsey Boulet and used for data analysis. Dr. Craig Steinback and Dr. Glen Foster completed the microneurography measurements. All data analysis and writing were completed by Troy Stuckless with guidance and editing provided by Dr. Glen Foster. In addition, Troy Stuckless was responsible for participant recruitment and screening, instrumentation, performing ultrasound measurements, and assisting with microneurography. Ethical approval for this project was provided by the University of British Columbia Clinical Research Ethics Board, CREB # H16-02525.

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List of Symbols

AHI	Apnea-hypopnea index
AT_1R	Angiotensin-II type-I receptor
BA	Brachial artery
BF	Burst frequency
BI	Burst Incidence
BL	Burst Latency
BMI	Body mass index
CO_2	Carbon dioxide
CPAP	Continuous positive airway pressure
CVD	Cardiovascular Disease
DBP	Diastolic blood pressure
D_LCO	Diffusing capacity of the lung for carbon monoxide
eNOS	Endothelial cell nitric oxide synthase
FEV_1	Forced expiratory volume in 1 second
F_IO_2	Fraction of inspired O_2
F_ICO_2	Fraction of inspired CO_2
fMRI	Functional magnetic resonance imaging
FVC	Forced vital capacity
FVR	Forearm vascular resistance
f_B	Breathing frequency
HIF	Hypoxia inducible factor
HR	Heart rate
IH	Intermittent hypoxia
LBNP	Lower body negative pressure
LTF	Long term facilitation
MAP	Mean arterial pressure
MBV	Mean blood velocity
MSNA	Muscle sympathetic nerve activity
NADPH	Nicotinamide adenine dinucleotide phosphate

NO	Nitric oxide
O_2	Oxygen
O_2^-	Superoxide anion
ODI	Oxygen desaturation index
OSA	Obstructive sleep apnea
P_ACO_2	Partial pressure of alveolar CO_2
P_AO_2	Partial pressure of alveolar O_2
P_aCO_2	Partial pressure of arterial CO_2
P_aO_2	Partial pressure of arterial O_2
PCO_2	Partial pressure of CO_2
$P_{ET}CO_2$	Partial pressure of end-tidal CO_2
$P_{ET}O_2$	Partial pressure of end-tidal O_2
PO_2	Partial pressure of O_2
PVN	Paraventricular nucleus
$\dot{Q}_{BA}$	Forearm blood flow
RAS	Renin-angiotensin system
ROS	Reactive oxygen species
RVLM	Rostral ventrolateral medulla
SBP	Systolic blood pressure
S_PO_2	Peripheral oxyhemoglobin saturation
SEM	Standard error of the mean
LTF	Long-term facilitation
SV	Stroke volume
TLC	Total lung capacity
$\dot{V}_A$	Alveolar ventilation
$\dot{V}_E$	Minute ventilation
$\dot{V}_I$	Inspired ventilation
V_T	Tidal volume

1 Literature Review

1.1 Introduction to Literature Review

Obstructive sleep apnea (OSA) is a respiratory disease characterized by repetitive airway obstructions during sleep, which leads to intermittent hypoxia and hypercapnia (IH) (Dempsey et al. 2010). OSA affects 13% of middle age adults (Franklin and Lindberg 2015), and prevalence is increasing due to the obesity epidemic (Peppard et al. 2012). This is a concern because OSA is a major cause of secondary hypertension (Pedrosa et al. 2011) and cardiovascular disease (CVD) (Somers et al. 2008). OSA patients have greater daytime muscle sympathetic nerve activity (MSNA) (Narkiewicz, van de Borne et al. 1998), which is involved in the etiology of OSA related hypertension (Fletcher 2003). IH also persistently increases MSNA (Cutler, Swift, Keller, Wasmund, Smith 2004; Jouett et al. 2017; Leuenberger et al. 2005; Xie et al. 2000) and blood pressure (Foster et al. 2010; Jouett et al. 2017; Leuenberger et al. 2005) in healthy humans.

Sympathetic vasomotor outflow produces a large global vasoconstrictive effect on peripheral arteries, the significance of which is demonstrated by the large decrease in peripheral vascular resistance and increased blood flow during blockade of the α_1 and α_2 receptors (Dinenno, Dietz, Joyner 2002) and sympathetic denervation (Puvi-Rajasingham et al. 1997) in humans. Sympathetic nerve activity and local mechanisms balance to regulate vascular resistance (Segal 2005) and the transduction of sympathetic vasomotor outflow into vascular resistance is essential for blood pressure control (Wallin and Nerhed 1982). Sympathetic neurovascular transduction is defined as the relationship between sympathetic vasomotor outflow and vascular resistance, which expresses how effectively a given sympathetic input leads to changes in vascular resistance. Sympathetic neurovascular transduction can be altered by several mediators including angiotensin-II, nitric oxide, and changes in vascular smooth muscle receptor expression (Macarthur et al. 2011). IH could augment sympathetic neurovascular transduction since previous reports show transduction is increased with acute hypoxia exposure (Tan et al. 2013), IH upregulates renin-angiotensin system activity (Nap et al. 2003; Purdy and Weber 1988; Story and Ziogas 1987), and may downregulate nitric oxide bioavailability (Foster et al. 2009; Pialoux et al. 2011).

Animal models demonstrate how chronic IH increases blood pressure that can be prevented by renal artery denervation or blockade of angiotensin-II type-1 receptors (AT_1R) (Fletcher, Bao, Li 1999), suggesting that renin release from the kidney (Davis and Freeman 1976) and increases in circulating angiotensin-II (Shell, Faulk, Cunningham 2016) are important in the blood pressure response to IH. Angiotensin-II has been shown to enhance norepinephrine neurovascular transmission by increasing the rate of norepinephrine synthesis, enhancing its release from sympathetic nerves, inhibiting reuptake from the neurovascular junction, and enhancing vascular smooth muscle responsiveness to norepinephrine (Story and Ziogas 1987). Neurovascular transduction could also be enhanced through vascular endothelial cell dysfunction, as a reduction in nitric oxide bioavailability is associated with an increase in neurotransmitter release and effectiveness (Macarthur et al. 2011). Additionally, a recent study in humans found that 6 hours of IH exposure impairs vascular control when a hypertensive stimulus is applied to the carotid baroreceptor, suggesting that either sympathetic withdrawal is impaired or neurovascular transduction is enhanced by IH (Tremblay et al. 2016).

The only study measuring neurovascular transduction in normotensive OSA patients found no difference from controls (Tamisier et al. 2015), although previous studies have shown that normotensive patients with OSA have a reduced forearm vasoconstrictive response to norepinephrine due to functional downregulation of vascular α and β_2 receptors (Grote, Kraiczi, Hedner 2000), which should act to reduce neurovascular transduction. In normotensive OSA patients, evidence of lower adrenergic receptor density combined with similar blood pressure suggests that neurovascular transduction may be maintained in OSA patients by presynaptic facilitation at the sympathetic nerve terminal. Reduced adrenergic receptor density could act to normalize sympathetic neurovascular transduction under conditions of increased sympathetic vasomotor outflow and blood pressure typical in OSA. To determine if augmented neurovascular transduction could be an early mechanism for developing high blood pressure in OSA, it is important to study the effects of IH on transduction in a healthy human model without previous IH exposure and comorbid disease.

This review will include a brief introduction to the epidemiology and potential mechanisms of CVD in OSA including persistently augmented MSNA after IH-induced chemoreflex sensitization. The importance of sympathetic vasomotor outflow for blood

pressure control and the role of sympathetic neurovascular transduction will be considered. In addition, the influence of IH on vascular sensitivity to neurotransmitters, and other factors that may alter neurovascular transduction including endothelial dysfunction and increased production of vasoconstrictive peptides including angiotensin-II will be examined. Finally, evidence related to potential mechanisms through which IH could alter neurovascular transduction will be summarized and its potential for contributing to hypertension in OSA will be discussed.

1.2 Epidemiology of Obstructive Sleep Apnea

OSA is a respiratory disease characterized by repeated obstructions of the upper airway during sleep. It is currently estimated that moderate-to-severe OSA affects 13% of middle-aged adults (Franklin and Lindberg 2015) and the prevalence of OSA has increased by 14% and 55% in middle-aged women and men respectively over the past two decades (Peppard et al. 2012). This is attributed to the obesity epidemic, as it is estimated that 89% of obese Canadians suffer from sleep apnea (Public Health Agency of Canada 2009). Furthermore, visceral adipose tissue mass is strongly correlated with the apnea-hypopnea index (AHI), a measure of OSA severity, even after accounting for other OSA risk factors including age, total adipose tissue mass, and lean body mass (Shinohara et al. 1997).

In large community based cross-sectional studies, the prevalence of hypertension increases significantly with severity of OSA, as measured by AHI and time spent below 90% oxygen (O_2) saturation, regardless of age, sex, ethnicity, or body mass index (BMI) (Nieto et al. 2000; Peppard et al. 2000). High blood pressure is the leading preventable risk factor causing death and disability in the world (Forouzanfar et al. 2015). Although hypertension has many unique etiologies, it is typically divided into two categories including primary (essential) and secondary hypertension, which are distinguished based on whether or not the underlying condition responsible for raising arterial pressure can be identified. In patients with treatment resistant secondary hypertension, OSA is the most commonly associated cause of hypertension (Pedrosa et al. 2011). Furthermore, a large prospective study demonstrated that normotensive patients with mild OSA had 42% increased odds of developing hypertension over four years (Peppard et al. 2000). Target organ damage, preeclampsia, or acute renal failure can occur when arterial pressure is poorly controlled, and patients require emergency treatment when these hypertensive events occur (Krousel-Wood,

Materson, Whelton 2007). Even in the absence of these acute cardiovascular events, hypertension significantly increases the risk for stroke, coronary artery disease, heart and kidney failure, peripheral vascular disease, and other chronic diseases (Duron and Hanon 2008; Jafar et al. 2003; MacMahon et al. 1990; Tozawa et al. 2003; World Health Organization 2009).

OSA could contribute to CVD development and progression independent of hypertension. A large cross-sectional study found that even mild to moderate levels of sleep apnea significantly increased the odds of coronary heart disease, heart failure, and stroke (Shahar et al. 2001), and the association between CVD and OSA remains after controlling for hypertension (Young, Peppard, Gottlieb 2002). Potential mechanisms that contribute to CVD development in OSA patients will be further discussed.

1.3 Mechanisms of Cardiovascular Disease in OSA

There is strong evidence linking OSA and sleep disordered breathing patterns to the development of CVD. Several mechanisms have been suggested, including increased reactive oxygen and nitrogen species (Lavie 2015), sympathetic activity (Somers et al. 1995), clotting factors (C. L. Phillips et al. 2012), insulin resistance (Punjabi et al. 2004), pulmonary pressure (Bradley 1992), effects of recurrent nightly Mueller maneuvers on the heart (Hohl et al. 2014) and aorta (Gaisl, Bratton, Kohler 2015), systemic inflammation (Gras et al. 2016; Ryan, Taylor, McNicholas 2005; Wu et al. 2016), and angiotensin-II production (Møller et al. 2003). OSA patients have increased intima-media thickness and systemic inflammation compared to controls (Minoguchi et al. 2005), indicating OSA patients are at a higher risk for future coronary events (Hodis et al. 1998). Reactive oxygen species (ROS) are greater in patients with OSA, causing oxidative stress and contributing to the development of CVD (Dhalla, Temsah, Netticadan 2000; Lavie 2003). Circulating angiotensin-II is significantly higher in patients with OSA and is implicated in the pathophysiology of hypertension (Kim and Iwao 2000; Møller et al. 2003). Finally, OSA patients demonstrate higher MSNA that persists into the daytime (Carlson et al. 1993; Narkiewicz, Montano et al. 1998; Somers et al. 1995; Taylor et al. 2016) and is compounded by comorbidities (Spaak et al. 2005). Increased sympathetic activity is associated with CVD (Malpas 2010), and is the suspected key mechanism through which OSA causes hypertension (Abboud and Kumar 2014; Narkiewicz and Somers 1999).

Continuous positive airway pressure (CPAP) therapy, considered the gold standard treatment for OSA, is a device that creates positive pressure at the mouth and/or nose and reduces the frequency of obstructive apneas. CPAP reduces sympathetic activity (Imadojemu et al. 2007; Narkiewicz et al. 1999; Waradekar et al. 1996), improves endothelial function (Schwarz et al. 2015), reduces markers of systemic inflammation (Ryan, Taylor, McNicholas 2005), and reduces insulin resistance in diabetic and non-diabetic OSA patients (Martínez-Cerón et al. 2016; Yang et al. 2013). CPAP also improves OSA symptoms such as daytime sleepiness, quality of life, mood, and attendance at work (McEvoy et al. 2016). However, there is conflicting evidence as to whether CPAP is effective at reducing high blood pressure or reversing CVD. Randomized placebo controlled trials have shown a modest blood pressure reduction with CPAP (Engleman et al. 1996; Hu et al. 2015; Martínez-García et al. 2013; Schwarz et al. 2016) that was more effective in those with the greatest hypoxemia (Faccenda et al. 2001), while others have shown no effect of CPAP on blood pressure (Barbé F, Durán-Cantolla J, Sánchez-de-la-Torre M,et al 2012). In OSA patients that have already experienced a cardiovascular event, CPAP use does not reduce the chances of having a second event (McEvoy et al. 2016). Furthermore, in patients without daytime sleepiness, CPAP has been shown to have no effect on quality of life, cognitive function, or arterial blood pressure (Barbé et al. 2001). This may be due to poor adherence as nearly 50% of people prescribed CPAP abandon therapy (Wolkove et al. 2008).

Secondary hypertension is considered the most important link between OSA and CVD, so it is important to consider what aspects of OSA contribute to increasing sympathetic activity. There is evidence that hypertension develops due to frequent arousals that fragmented sleep, intrathoracic pressure swings from inspiratory efforts against a closed airway and, most importantly, IH due to periodic alveolar hypoventilation. Below, evidence is presented with respect to how IH increases sympathetic nerve activity and contributes to hypertension in OSA patients, and evidence is detailed suggesting that arousals and intrathoracic pressure swings do not account for long-lasting changes in sympathetic activity.

1.3.1 Intermittent Hypoxia and Hypercapnia

Hypercapnia and hypoxia in isolation increase sympathetic activity, and work synergistically when applied together (Somers et al. 1989). Although both hypoxia and hypercapnia increase sympathetic activity, the persistent increase in sympathetic activity that outlasts exposure

occurs after IH alone or when IH and hypercapnia are combined, but not intermittent hypercapnia in isolation (Cutler, Swift, Keller, Wasmund, Burk et al. 2004; Xie et al. 2000; Xie et al. 2001). IH is linked to persistent sympathetic activation through its direct effect of sensitizing the carotid body chemoreflex (Peng et al. 2003) and, to a lesser extent, on brainstem neurons (Guyenet 2000; Prabhakar 2016). The effect of IH on the carotid body chemoreflex is referred to as sensory long-term facilitation (LTF) as demonstrated in animal models of OSA (Peng et al. 2003; Roy et al. 2017).

Sensory LTF is characterized by a tonic increase in carotid body afferent activity, as well as increased sensitivity of the chemoreflex to acute hypoxia, which increases daytime sympathetic activity after IH but not after continuous hypoxia or intermittent hypercapnia alone in animals (Peng et al. 2003). However, hypercapnia has recently been shown have a more important role in sensory LTF than previously thought. Using a rat model where chemoreceptors were perfused independently and blood gases could be tightly controlled, Roy *et al.* (2017) demonstrated that 10 1-minute intermittent hypoxic exposures did not produce sensory LTF in the carotid body unless hypoxia was combined with hypercapnia or a constant hypercapnic background was applied. Also, intermittent hypercapnia alone did not induce sensory LTF, as found previously (Peng et al. 2003; Roy et al. 2017). It seems that IH can cause carotid body sensory LTF much more effectively when hypercapnia is applied concurrently.

Sensory LTF provides a mechanism for the greater baseline sympathetic activity of OSA patients that persists throughout the day, and the increased magnitude of sympathetic response to apneas during sleep. The persistent increase in sympathetic activity has been shown to occur in healthy humans exposed to intermittent hypoxic apneas for as little as 20 minutes, with and without the addition of hypercapnia (Cutler, Swift, Keller, Wasmund, Burk et al. 2004; Jouett et al. 2017; Morgan et al. 1995; Xie et al. 2000). Exposing OSA patients to 15-minutes of hyperoxia to deactivate the chemoreflex caused a significant reduction in MSNA and blood pressure, an effect that did not occur in controls, suggesting chemoreflex sensitization causes increased daytime blood pressure in OSA (Narkiewicz et al. 1998).

The mechanism of carotid body sensory LTF involves the renin-angiotensin system (RAS) and generation of ROS. The carotid bodies primary oxygen sensors are type-I glomus cells, which release neurotransmitters and increase afferent activity in response to low P_aO_2

sensed through proteins including nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidases, and other oxygen sensitive enzymes and ion channels (Prabhakar 2000). In animals, IH has been shown to upregulate NADPH oxidase, angiotensin-II type-I receptors (AT_1R), and ROS production in the carotid body, which increases both chemoreflex sensitivity and sympathetic activity, while treatment with losartan, an AT_1R antagonist, prevents all of these effects (Y. Li et al. 2007; Marcus et al. 2010). Inhibiting xanthine oxidase or NADPH oxidase, which are sources of ROS in type-I glomus cells, prevents chemoreflex sensitization, supporting ROS as necessary for sensory LTF (Morgan et al. 2016). In humans, oxidative stress produced during IH is involved in sensitizing the hypoxic ventilatory response, a measure of chemoreflex sensitivity (Pialoux et al. 2009).

Increased activity of the RAS, specifically the AT_1R, is necessary for sensory LTF and increased blood pressure to occur. Animal models have shown that the IH-induced increase in blood pressure can be abolished with renal artery denervation or treatment with Losartan (Fletcher, Bao, Li 1999), and blocking the AT_1R in humans prevents increases in sympathetic vasomotor outflow and blood pressure (Foster et al. 2010; Jouett et al. 2017). In addition to its role in sensory LTF, vascular AT_1R and ROS also likely participates in the blood pressure response to IH. In OSA patients, AT_1R expression in *ex-vivo* microcirculatory vessels is greater than controls and is reduced by 12 weeks of CPAP therapy (Khayat et al. 2017). In the same study, pre-CPAP treatment of vessels with Losartan reduced vascular production of superoxide (O_2^-) but had no effect on post-CPAP O_2^- (Khayat et al. 2017). This suggests a role for ROS and the AT_1R in regulating blood pressure changes at the vessel itself, independent of sympathetic vasomotor outflow. Interestingly, the carotid body, vasculature, and many other organs have their own intracellular RAS (Paul, Poyan Mehr, Kreutz 2006) and the carotid bodies intracellular RAS is upregulated in animals after exposure to chronic IH (Lam et al. 2014). Therefore, even in the absence of increased plasma angiotensin-II, sensory LTF and increased sympathetic vasomotor outflow could still occur via a RAS related mechanism. However, renal nerve ablation sufficient to prevent the increase in plasma renin activity abolishes the increase in blood pressure following chronic IH in rats (Fletcher, Bao, Li 1999), suggesting renin release from the kidney is important for blood pressure responses to IH.

Changes in the central nervous system could also occur with IH exposure and may contribute to changes in blood pressure and sympathetic vasomotor outflow. Carotid body afferents terminate on the nucleus tractus solitarius in the brainstem. Chronic IH exposure increases neurotransmitter release from carotid body afferent nerves and increases nucleus tractus solitarius neuron activity even though these neurons become less excitable (Kline, Ramirez-Navarro, Kunze 2007). An increase in nucleus tractus solitarius neuron activity increases sympathetic activity because they terminate on the rostral ventrolateral medulla (RVLM), an important site for sympathetic regulation. Additionally, RVLM neurons have been demonstrated to depolarize during central nervous system hypoxia and may also be directly sensitized by hypoxia (Guyenet 2000), although a more recent study has shown that carotid body ablation prevents IH induced changes in the RVLM and nucleus tractus solitarius (Peng et al. 2014). Therefore, it is likely that plasticity in brainstem regions related to chemoreception and sympathetic activity regulation results from carotid body sensory input.

The RAS also may have central nervous system effects on sympathetic activity after IH through the paraventricular nucleus, which is another region influencing sympathetic activity. Input from the median preoptic nucleus to the paraventricular nucleus is increased by chronic IH, which does not occur when angiotensin converting enzyme transcription is blocked (Faulk et al. 2017). The circumventricular organs have projections to the paraventricular nucleus and are stimulated by elevated circulating angiotensin-II after IH exposure (Shell, Faulk, Cunningham 2016). Also, blocking AT_1R transcription in the rat subfornical organ, which projects to the paraventricular nucleus, prevents mean arterial pressure increases after chronic IH (Saxena et al. 2015) similar to blocking the AT_1R at the paraventricular nucleus directly (da Silva, Ana Quenia Gomes, Fontes, Kanagy 2011). Changes in central nervous system structure and function have also been found in OSA patients. Functional magnetic resonance imaging (fMRI) has shown that these changes can occur in regions of the brain upstream of chemoreceptor afferent input (Fatouleh et al. 2014). OSA patients have grey matter reduction in cortical and subcortical regions, which has been attributed to IH exposure (Joo et al. 2010). However, it is unclear if the brain changes result in the persistent increase in sympathetic activity. Concurrent MSNA and fMRI measurements show that functional differences associated with MSNA bursts do not occur in areas where

grey matter reduction has been observed (Fatouleh et al. 2014). If the increase in daytime MSNA are due to IH-induced structural changes in the brain, it would be expected that these regions would show increased activity during bursts of MSNA. It is likely that the regions of the brain that are changed structurally in OSA patients upstream of chemoreceptor input are not associated with MSNA regulation and are not a cause of persistent daytime sympathetic over activity.

1.3.2 Sleep Arousal and Intrathoracic Pressure Swings

Sleep arousal alone can increase MSNA and blood pressure nocturnally. MSNA bursts take place directly after applying an auditory stimulus, usually alongside K-complexes in the electroencephalogram signal (Morgan et al. 1996; Shimizu et al. 1992; Takeuchi et al. 1994), although some studies have found that an arousal response to the stimulus is not required for MSNA activity to increase (Morgan et al. 1996). The blood pressure response to sleep arousal has been shown in healthy people (Morgan et al. 1996; Shimizu et al. 1992; Takeuchi et al. 1994), sub-clinical populations with sleep disordered breathing (Morgan et al. 1998), OSA patients (Ringler et al. 1990), and in animal models using various types of stimuli to induce arousal (Launois et al. 1998). The sympathetic response to arousal is prolonged and could have compounding effects on blood pressure (Blasi et al. 2003). However, as demonstrated in dogs, chronic arousal from sleep without airway occlusion or hypoxia can raise blood pressure acutely but does not cause daytime hypertension (Brooks et al. 1997).

There is little evidence to support that sympathetic activity from arousals could persist into the daytime. When measured with heart rate (HR) variability, sympathetic activation after arousal is sustained beyond the transient increase in MSNA (Blasi et al. 2003), suggesting that arousals could have a cumulative effect. Also, the arousal index of OSA patients significantly predicts daytime sympathetic activation beyond that of minimum S_pO_2, AHI, age, and BMI (Taylor et al. 2016), although minimum S_pO_2 and AHI are significant predictors on their own and were co-related with the arousal index. These studies provide some evidence that arousal contributes to increasing daytime MSNA in OSA patients (Floras 2016). However, no studies have shown that chronic sleep arousal without IH causes a persistent daytime increase in sympathetic activity, and since IH and arousal typically occur concurrently they may simply be co-related in OSA. The HR variability of people with

insomnia suggests that the sympathetic activity is higher with consistent sleep arousal (Bonnet and Arand 1998), although it is unknown if sympathetic activation is a symptom of insomnia or the cause.

Intrathoracic pressure swings likely do not contribute to the acute sympathetic response to apneas or daytime sympathetic overactivity. MSNA increases markedly at the end of a 20-second breath hold, which is prevented by supplemental oxygen and performing a Mueller maneuver concurrently with the breath hold does not change MSNA (Morgan, Denahan, Ebert 1993). MSNA is reduced with supplemental oxygen because chemoreflex stimulation is diminished, suggesting IH is the main stimulus for MSNA. In both primates and dogs, regardless of concurrent negative intrathoracic pressure or arousal, the same blood pressure response to obstructive apneas is observed (O'Donnell et al. 1996; S. G. White, Fletcher, Miller 1995).

Although negative intrathoracic pressure swings do not affect sympathetic activity directly, they do have direct effects on the heart and large vessels. OSA is associated with aortic aneurysms and dissections, likely through mechanical stretching of the vessel (Gaisl, Bratton, Kohler 2015). Pressure swings contribute to left and right atrial arrhythmogenesis, dysfunction, and remodeling over the long term in OSA patients (Hohl et al. 2014). Pressure swings are also implicated in increasing pulmonary pressure and both right and left ventricular impairment (Bradley 1992). Negative intrathoracic pressures may contribute to CVD, but likely do not have an effect on sympathetic nerve activity.

1.4 Sympathetic Activity and Blood Pressure Regulation

Vascular resistance is influenced by sympathetic nerve activity and local mechanisms (Segal 2005), which balance to regulate blood flow and control blood pressure (Wallin and Nerhed 1982). Sympathetic activity produces a large global vasoconstrictive effect on peripheral arteries, the significance of which is demonstrated by the large decrease in peripheral vascular resistance and increased blood flow during blockade of the α_1 and α_2 receptors (Dinenno, Dietz, Joyner 2002) and sympathetic denervation (Puvi-Rajasingham et al. 1997) in humans.

MSNA is crucial in the regulation of systemic arterial pressure because of its ability to regulate peripheral vascular resistance and blood flow. In humans with hypertension a

chronically high peripheral vascular resistance, not a greater cardiac output, is the major driver of increased arterial pressure (London et al. 1984). MSNA regulates beat-by-beat vascular resistance in the forearm and leg as each MSNA burst is related to an increase in vascular resistance that peaks within six to eight beats, and blockade of forearm α-receptors with phentolamine abolishes this response (Fairfax et al. 2013a; Fairfax et al. 2013b). Peripheral vascular resistance is increased in hypertensive patients partially by the growth and remodeling of small resistance arteries (Heagerty et al. 1993), and partially through an increase in vascular reactivity. Hypertensive patients have greater vascular reactivity to norepinephrine when infused with or without sympathetic ganglionic blockade (Doyle and Black 1955; Jie et al. 1985), although other studies have found differences in reactivity between hypertensives and normotensives exist only at high norepinephrine levels (Egan et al. 1987).

A high resting MSNA is prevalent in humans with hypertension, heart failure, obesity, and sleep apnea, and may play a causal role in the development of CVD (Malpas 2010). This being said, both resting MSNA and its influence on blood pressure are highly variable when compared between people. Individuals with similar blood pressure may have differences in resting MSNA burst incidence over 10-fold in magnitude (Sundlöf and Wallin 1977). High between-individual variability is also present in the blood pressure response to MSNA, demonstrated by the lack of relationship between resting MSNA and blood pressure in healthy individuals (Sundlöf and Wallin 1978b). The lack of relationship is also exemplified by comparing hypertensive individuals to matched control subjects, noting that some hypertensive individuals have resting MSNA levels lower than matched controls, and vice versa with some normotensive individuals having greater resting MSNA than those with hypertension (Yamada et al. 1989). The variability of individual responses to MSNA could be due to the large differences in vascular adrenergic receptor density between individuals (Rudner et al. 1999), which could alter the transduction of MSNA into blood pressure. In support of this, the responsiveness of the vasculature to adrenergic stimulation in young healthy men is inversely related to sympathetic vasomotor outflow (Charkoudian et al. 2006).

Despite the large variability in MSNA between individuals, there is little variability in resting MSNA within the same individuals in different limbs, or successive MSNA measurements made weeks apart (Sundlöf and Wallin 1977) or a decade apart (Fagius and

Wallin 1993). Within individuals there is a strong relationship between changes in MSNA and blood pressure (Sundlöf and Wallin 1978a). Together, this evidence suggests that the transmission of MSNA at the neurovascular junction and the sensitivity of vascular smooth muscle tone to MSNA are important for the regulation of blood pressure within an individual and could account for the large differences in resting MSNA between individuals with similar arterial pressures. Also, if the vascular smooth muscle's sensitivity to SNA is augmented, the peripheral vascular resistance and arterial pressure at any absolute level of SNA would be augmented as well. For these reasons, it is important to understand any factors that could influence the transduction of muscle sympathetic nerve activity to smooth muscle contraction and the resulting increase in peripheral vascular resistance.

1.5 Sympathetic Neurovascular Transduction

Sympathetic neurovascular transduction describes the ability of the sympathetic nerve terminal to elicit a response from vascular smooth muscle cell. This can be measured as the relationship between MSNA and vessel responses such as vascular resistance or blood pressure. Greater transduction will be observed as a given sympathetic input leading to larger changes in the vessel response, indicating greater vascular sensitivity or enhanced neurotransmission. This process is essential to regulate blood pressure and distribute blood flow throughout the body.

Whole body neurovascular transduction has been measured in groups of individuals by correlating resting MSNA burst incidence with total peripheral resistance (Hart et al. 2011). Within individuals, transduction has been assessed by creating a range of MSNA with the use of lower body negative pressure (Notarius et al. 2012; Ray and Monahan 2002) or handgrip exercise, with and without post exercise cuff occlusion (Halliwill, Taylor, Eckberg 1996; Minson et al. 2000) and correlating MSNA burst frequency with vascular resistance. To address the dynamic changes in blood pressure and vascular resistance that occur with each MSNA burst, more complex assessments of transduction have been developed including measuring the effect of individual MSNA bursts on beat-by-beat changes in vascular conductance (Fairfax et al. 2013a; Fairfax et al. 2013b; Vranish et al. 2018), using a complex transfer function analyses to estimate neurovascular transduction independent of blood pressure changes (Tan et al. 2012), and correlating MSNA burst size with subsequent beat-by-beat diastolic pressure changes (Briant et al. 2016). All of these methods to assess

sympathetic neurovascular transduction indicate the sensitivity of the peripheral vasculature to MSNA.

Sympathetic neurovascular transduction was once thought to be relatively stable, and changes in blood pressure and vascular resistance were dependent upon changes in MSNA and local vasodilatory mechanisms. However, differences in transduction have been shown to occur with acute stimuli such as hypoxia (Tan et al. 2013) and aerobic exercise (Halliwill, Taylor, Eckberg 1996) but not small muscle mass exercise (Buck et al. 2015). There are also differences in transduction due to fitness level (Notarius et al. 2012), age (Hart et al. 2009), sex (Briant et al. 2016; Minson et al. 2000), race (Vranish et al. 2018), and differences based on the limb in which transduction is measured (Fairfax et al. 2013a; Fairfax et al. 2013b). With this in mind, it is important to look at the mechanisms of sympathetic neurovascular transduction and how these mechanisms can be altered.

1.6 Sympathetic Neurotransmitters and Vascular Smooth Muscle Sensitivity

Action potentials arriving at the sympathetic nerve terminal cause the release of adenosine triphosphate (ATP), neuropeptide Y (NPY), and norepinephrine, the latter being the most well-known and studied neurotransmitter. Sympathetic neurovascular transduction is a dynamic and changing process as there is a great deal of cross-talk occurring between neurotransmitters, mediators that fine tune neurotransmitter release, and the response of the vascular smooth muscle cell (Macarthur et al. 2011).

ATP, the fastest acting neurotransmitter released from sympathetic nerves, stimulates P_{2X} receptors on vascular smooth muscle cells causing contraction and P_{2Y} receptors on sympathetic nerves to reduce norepinephrine release (Macarthur et al. 2011). Norepinephrine stimulates α_1 receptors on vascular smooth muscle cells causing contraction, but also inhibits the release of ATP, NPY, and itself through stimulating the α_2 receptor located on the sympathetic nerve (Macarthur et al. 2011). α_2 and β_2 receptors are also located on the vascular smooth muscle and, paradoxically, cause relaxation when norepinephrine is released (Kneale et al. 2000). Therefore, the balance between adrenergic receptors may determine the vascular response to norepinephrine more so than the density of any one receptor alone. NPY, the longest acting neurotransmitter, stimulates Y_1 receptors on vascular smooth muscle cells causing a relatively small contraction on its own, but greatly enhances norepinephrine

transmission (Ekblad et al. 1984) and, similar to the relationship between norepinephrine and α_2 receptors, NPY inhibits release of all three neurotransmitters through Y_2 receptors on sympathetic nerves (Wahlestedt and Reis 1993).

The complex relationship between these neurotransmitters and their effects on vascular smooth muscle is further muddied by a host of mediators from non-neuronal sources. One of these mediators, angiotensin-II, alters neurovascular transduction prejunctionally and the responsiveness of the vascular smooth muscle cell. At the neurovascular junction angiotensin-II facilitates an increase in norepinephrine release through AT_1R activation and upregulates α_1 and α_2 adrenoreceptors (Nap et al. 2003; Purdy and Weber 1988), enhancing norepinephrine neurovascular transmission and increasing the responsiveness of vascular smooth muscle. Angiotensin-II has been previously shown to increase the rate of norepinephrine synthesis, enhance norepinephrine release from sympathetic nerves, inhibit norepinephrine reuptake from the neurovascular junction, and enhance vascular smooth muscle responsiveness (Story and Ziogas 1987). In addition to angiotensin-II effects on norepinephrine transmission, AT_1R stimulation also increases NPY release (Byku, Macarthur, Westfall 2008b). Epinephrine released during IH also could enhance neurovascular transduction by increasing presynaptic norepinephrine release (Bao et al. 1997).

The endothelium produces several mediators that regulate sympathetic neurovascular transduction including prostanoids, endothelin-1, and nitric oxide. Prostaglandin and prostacyclin reduce the release of norepinephrine and NPY from sympathetic nerves (Hoang et al. 2003), while endothelin-1 has no effect on norepinephrine transmission but reduces NPY release (Hoang et al. 2002). Nitric oxide reduces neurovascular transmission by reducing norepinephrine release and interacting directly with norepinephrine in the neurovascular junction, and enhancing NPY release, therefore a reduction in NO availability may promote hypertension partially through increases in sympathetic neurovascular transduction (Macarthur et al. 2011).

The transduction of sympathetic nerve activity into a vascular response is dynamic and can be influenced by a variety of factors from the neuron, vascular smooth muscle cell, endothelium, and circulating modulators. Since the transduction of action potentials into

vascular smooth muscle contraction is essential for sympathetic regulation of blood pressure and distributing of blood flow, it is important to consider how endothelial dysfunction and IH could alter this process.

1.7 Vascular Endothelial Dysfunction and Remodelling

OSA is associated with endothelial dysfunction in humans, which is reversable with the use of CPAP therapy (Ip et al. 2004). Also, 12 weeks of CPAP causes a 4-fold increase in microcirculatory vessel wall NO expression and improves flow mediated dilation OSA, which shows the absence of IH can improve endothelial function (Khayat et al. 2017). NO release from endothelial cells contributes to vasodilation in humans exposed to continuous hypoxia (Blitzer, Lee, Creager 1996), however, is unclear if IH induces endothelial dysfunction and has an opposing effect. 6 hours of IH for 1-4 consecutive days reduces nitric oxide derivatives (Foster et al. 2009; Pialoux et al. 2011), promotes retrograde shear stress associated with endothelial dysfunction (Tremblay et al. 2016), and 28 days of IH exposure decreases peak forearm reactive hyperemia after occlusion (Gilmartin et al. 2010). However, healthy people after short IH exposures do not show changes in flow mediated dilation compared to controls (Tremblay et al. 2016). It is likely that chronic IH exposure is necessary for endothelial dysfunction to develop.

Endothelial dysfunction induced by chronic IH is likely mediated by ROS overproduction during the rapid and repeated swings in blood oxygenation, analogous to the increase in endogenous ROS production observed during the reperfusion phase of an ischemia-reperfusion injury (Lavie 2003). ROS are produced through multiple pathways including NADPH oxidase production of superoxide anion (O_2^-) and xanthine oxidase production of peroxides and hydroxyl radicals. An increase in ROS has been shown to promote inflammation via activation of endothelial cells and immune cells to express a pro-inflammatory phenotype, which causes greater expression of adhesion molecules, inflammatory cytokines, additional ROS production, and an overall reduction in NO bioavailability via endothelial dysfunction (Lavie 2015). Acutely, IH stimulation of the RAS could stimulate endothelial NO synthase and NO production (Saito et al. 1996), however O_2^- and other ROS molecules can also react directly with NO, converting it to inactive peroxynitrates (Pueyo et al. 1998) and reducing overall NO bioavailability.

Vascular remodeling in OSA related hypertension is dependent upon ROS the RAS. NADPH oxidases are the primary producers of ROS, specifically O_2^-, in vascular smooth muscle cells (Griendling et al. 2000). The AT_1R activates NADPH oxidase (Griendling et al. 1994) via the p47phox subunit (Lavigne et al. 2001), which is required for the vascular smooth muscle and endothelial cell to produce O_2^- in response to angiotensin-II stimulation (J. M. Li and Shah 2003). O_2^- production leads to vascular inflammation (Cai, Griendling, Harrison 2003), and mediates the growth, hypertrophy, and apoptosis of vascular smooth muscle that is cause by AT_1R activation (Griendling et al. 2000). ROS produced by NADPH oxidase also contributes to vascular smooth muscle cell growth and angiogenesis by increasing vascular endothelial growth factor and hypoxia inducible factor-1 (HIF-1) (Gorlach et al. 2001; Ushio-Fukai et al. 2001). HIF-1 contributes to vascular inflammatory remodeling through endothelin-1 production (Gras et al. 2016), and HIF-1 has been shown to increase expression of NADPH oxidase in response to IH, creating a feed forward mechanism for ROS generation (Yuan et al. 2011).

An issue with detecting neurovascular transduction changes after IH is the confounding influence of endothelial dysfunction. Since vasoconstricting and vasodilating forces balance to regulate vascular resistance, endothelial dysfunction could mask changes in sympathetic neurovascular transduction. Studies looking at beat-by-beat resting neurovascular transduction demonstrate that vascular resistance is reduced during periods between MSNA bursts (Fairfax et al. 2013a; Fairfax et al. 2013b), presumably due to vasodilating forces such as NO. The reduction in vascular resistance during periods of low MSNA may have a potentiating effect, enhancing vasoconstrictor responses to subsequent MSNA bursts. Therefore, endothelial dysfunction and a reduction in vasodilation could result in greater vasoconstriction and vascular resistance independent of changes to sympathetic neurovascular transduction. However, although endothelial dysfunction would cause vasoconstriction, a reduction in NO could enhance norepinephrine neurovascular transmission by presynaptic enhancement of norepinephrine release, reducing NO-norepinephrine reactions occurring in the neurovascular junction, as well as through enhancing NPY release (Macarthur et al. 2011). Therefore, endothelial dysfunction and reduced NO bioavailability may augment sympathetic neurovascular transduction.

1.8 Sympathetic Neurovascular Transduction and Intermittent Hypoxia

It is unknown if IH influences sympathetic neurovascular transduction. A study in OSA patients found neurovascular transduction is similar to controls at baseline and is unchanged by six months of CPAP therapy (Tamisier et al. 2015). However, there are limitations to this study: 1) Blood pressure and vascular resistance were similar between OSA and control groups at baseline, even though MSNA was significantly higher in OSA, suggesting their sample of OSA patients actually had lower baseline sympathetic neurovascular transduction compared to the control group, 2) Hypertensive OSA patients were screened out of this study, which may have removed OSA patients with the greatest baseline transduction; 3) OSA patients were still exposed to relatively large amounts of IH during CPAP treatment as adherence averaged only 4.5 hr/night. Also, it is possible that neurovascular transduction is an acute adaptation to IH exposure, which normalizes over time as vascular remodeling occurs. Therefore, this study in patients with established OSA may not reflect the acute effect of IH on sympathetic neurovascular transduction. However, IH mediated changes in sympathetic neurovascular transduction could be one of the earliest stimuli contributing to high blood pressure in patients beginning to develop OSA. It is also possible that a relatively small exposure to IH is required for changes in sympathetic neurovascular transduction, and without complete CPAP adherence OSA patients may experience augmented transduction. Therefore, it is important to study the effect of IH in a healthy human model to determine if acute IH, independent of long term changes to vascular structure or comorbid disease, can influence sympathetic neurovascular transduction.

Although there are no studies looking at IH on neurovascular transduction, augmented neurovascular transduction has been demonstrated during continuous hypoxia (Tan et al. 2013). Hypoxia may influence vascular sensitivity by increasing norepinephrine receptor expression. Rat aortic smooth muscle cells exposed to 8 hours of continuous hypoxia show increased expression of α_1-adrenergic receptors by upregulating both transcription and translation (Eckhart et al. 1996). On the other hand, OSA patients experience significantly less peripheral resistance during norepinephrine infusion and significantly less dilation with isoproterenol infusion compared to controls (Grote, Kraiczi, Hedner 2000), which suggests both α and β adrenergic receptors are functionally down-regulated. Similarly, chronic IH exposed animals have reduced norepinephrine sensitivity

(Phillips et al. 2006). Temporal differences in when neurovascular transduction is measured could account for the conflicting results. Adrenergic receptors may be upregulated acutely with continuous hypoxia or IH exposure, while longer term exposure may result in a down-regulation of adrenergic receptors as vascular remodeling occurs.

The RAS, specifically the AT_1R, is essential for blood pressure increases after IH (Fletcher, Bao, Li 1999; Foster et al. 2010; Marcus et al. 2010). Blocking the AT_1R in healthy humans before exposure to IH prevents an increase in resting blood pressure and MSNA (Foster et al. 2010; Jouett et al. 2017), however since neurovascular transduction has not been studied in the context of IH, it is unknown if the AT_1R is involved in this process. During IH exposure, circulating angiotensin-II is increased due to chemoreflex activation, which increases renal sympathetic nerve activity, reduces renal artery blood flow, and upregulates renin release and angiotensin-II (Davis and Freeman 1976). In addition to angiotensin-II circulating in plasma, endothelial cells also produce and secrete angiotensin-II (Kifor and Dzau 1987), which may have a paracrine function on vascular smooth muscle cells. Local renin-angiotensin systems have been discovered in tissues throughout the body including vascular smooth muscle and the endothelium (Paul, Poyan Mehr, Kreutz 2006). It is possible that the AT_1R activation observed in IH would augment sympathetic neurovascular transduction because AT_1R activation upregulates α_1 and α_2 adrenoreceptors and increases norepinephrine release (Nap et al. 2003; Purdy and Weber 1988),which may enhance norepinephrine neurovascular transmission and increasing the responsiveness of vascular smooth muscle. Epinephrine released during IH also could enhance neurovascular transduction by increasing presynaptic norepinephrine release (Bao et al. 1997). Further to this, NPY and ATP are other neurotransmitters released from sympathetic nerves and play a role in sympathetic neurovascular transduction (Macarthur et al. 2011). In rats, angiotensin-II weakly enhances neuropeptide Y transmission, which vasoconstricts over a longer time course than norepinephrine, and this effect is blocked by AT_1R antagonists or neuropeptide Y_1 receptor antagonists (Byku, Macarthur, Westfall 2008b). Sympathetic neurovascular transduction may be enhanced after IH through a mechanism dependent upon the AT_1R at the neurovascular junction.

In conclusion, persistent sympathetic activation and high blood pressure occur after IH exposure. It is possible that enhanced neurovascular transduction also contributes to the

blood pressure response after IH. This could occur due to hypoxia exposure directly (Tan et al. 2013), increased renin-angiotensin system activity (Nap et al. 2003; Purdy and Weber 1988; Story and Ziogas 1987), and reduced nitric oxide bioavailability (Macarthur et al. 2011). If sympathetic neurovascular transduction is enhanced after IH, it could be an early mechanism that contributes to high blood pressure development in OSA.

2 Introduction

Obstructive sleep apnea (OSA) is a respiratory disease characterized by repeated obstructions of the upper airway during sleep (Dempsey et al. 2010) and is currently estimated to affect 13% of middle-aged adults (Franklin and Lindberg 2015). OSA is associated with the development of hypertension (Nieto et al. 2000; Peppard et al. 2000) that is likely neurogenic (Abboud and Kumar 2014; Narkiewicz and Somers 1999) as OSA patients show increased sympathetic vasomotor outflow during sleep and when awake (Carlson et al. 1993; Narkiewicz et al. 1998) that can be reduced with CPAP therapy (Imadojemu et al. 2007; Narkiewicz et al. 1999; Waradekar et al. 1996). Sympathetic vasomotor outflow and local mechanisms influence vascular resistance (Segal 2005) and balance to regulate blood pressure (Wallin and Nerhed 1982). However, mechanisms underlying the transduction of sympathetic vasomotor outflow to vascular resistance in OSA are not well understood.

Normotensive OSA patients are less sensitive to norepinephrine due to functional downregulation of α and β_2 receptors (Grote, Kraiczi, Hedner 2000). Reduced adrenergic receptor density would lower sympathetic neurovascular transduction and blood pressure under conditions of increased sympathetic vasomotor outflow typical in OSA. In support of this, normotensive OSA patients have similar sympathetic neurovascular transduction and vascular resistance compared to controls (Tamisier et al. 2015), even though sympathetic vasomotor outflow was greater in the OSA group. Similar input (sympathetic vasomotor outflow), output (blood pressure and limb blood flow), and neurovascular transduction, combined with evidence of reduced adrenergic receptor density in OSA patients, suggests that presynaptic facilitation of sympathetic nerves may be present.

Exposure to intermittent hypoxia (IH) is the most likely underlying mechanism for the development of hypertension and cardiovascular disease in OSA (Somers et al. 1995). IH increases sympathetic vasomotor outflow and blood pressure in humans (Gilmartin et al. 2010; Jouett et al. 2017; Leuenberger et al. 2005; Tamisier et al. 2011) and animal models (Brooks et al. 1997; Fletcher, Bao, Li 1999; Fletcher 2000; Leke et al. 1997), which are dependent upon increased renin-angiotensin system activity (Foster et al. 2010; Jouett et al. 2017). IH can also reduce nitric oxide availability (Foster et al. 2009; Pialoux et al. 2011) contributing to endothelial dysfunction (Gilmartin et al. 2010), and chronic IH reduces

norepinephrine sensitivity in animals (Phillips et al. 2006). IH could also increase blood pressure by augmenting sympathetic neurovascular transduction, which has been reported with acute exposure to hypoxia (Tan et al. 2013), increased renin-angiotensin system activity (Nap et al. 2003; Purdy and Weber 1988; Story and Ziogas 1987), and reduced nitric oxide bioavailability (Macarthur et al. 2011).

The primary objective of this study was to determine if an acute exposure to IH could augment sympathetic neurovascular transduction in healthy men free from comorbid CVD. The IH paradigm was designed to simulate the blood gas changes that patients with severe OSA experience each night. We hypothesized that sympathetic neurovascular transduction, measured as the slope of the relationship between forearm vascular resistance and muscle sympathetic nerve activity would be augmented by 40-minutes of IH.

3 Methods

3.1 Participants

Ethical approval was obtained from the Clinical Research Ethics Board at the University of British Columbia. Written informed consent was obtained from all participants. 22 male volunteers were recruited for participation in this study. Participants were excluded if they had systolic blood pressure ≥135 mmHg or diastolic pressure ≥85 mmHg (Nerenberg et al. 2018), were obese (BMI ≥ 30 kg/m^2), were taking any medications (prescribed or over-the-counter), smoked within the past year, or had a history of hypertension, impaired renal function, liver disease, heart failure, myocardial infarction, coronary artery disease, stroke, chronic obstructive pulmonary disease, asthma, diabetes, or sleep disordered breathing. Females were excluded from this preliminary investigation because blood pressure (Boukari et al. 2017) and neurovascular control (Briant et al., 2016) differ between the sexes, and females are less likely to develop OSA (Peppard et al. 2012).

3.2 Screening Procedures

Participants arrived at the lab for a screening visit where they completed a health history questionnaire (*see* Appendix A: Forms: Inclusion/Exclusion Assessment). Participants then underwent a battery of pulmonary function tests to acquire flow volume loops, diffusion capacity, and lung volumes. Participants sat inside a whole-body plethysmograph box (V62J, Sensormedics, Yorba Linda, CA) and breathed through an apparatus including a spirometer and filter. Predicted norms for spirometry (Knudson et al. 1983), lung volumes (Crapo et al. 1982), and diffusing capacity (Crapo and Morris 1981) were based on height and age. Participants were excluded if they displayed an abnormal forced expiratory volume in 1 second (FEV_1) / forced vital capacity (FVC) ratio < 0.70.

Participants received a take-home pulse oximeter (WristOx2, Model 3150, Nonin) to be worn during sleep. Nocturnal oximetry data was analyzed with nVision Software (Nonin, Plymouth, MN, USA) to determine the oxygen desaturation index, mean and mean minimum S_PO_2, and percent time < 95% and < 90% S_PO_2. Participants were excluded if they had undiagnosed sleep apnea, classified as an oxygen desaturation index ≥ 5 events/hr (desaturation ≥ 4%). Participants who met all inclusion criteria returned to complete the experimental day.

3.3 General Procedures

Participants visited the lab on two occasions; one screening day and one experimental day (Figure 1). Prior to the experimental day, participants were instructed to fast for ≥ 4 hours and abstain from exercise, caffeine, and alcohol for ≥ 12 hours prior to arrival due to their effects on the peripheral vasculature (Thijssen et al. 2011). Upon arrival, participants were instructed to void their bladder and were instrumented for measurements of HR, systolic and diastolic blood pressure (SBP, DBP), brachial artery (BA) mean blood velocity (MBV) and diameter, peroneal MSNA, peripheral oxyhemoglobin saturation (S_pO_2), and a facemask to control end-tidal gases, while laying supine with legs and hips inside of a lower body negative pressure (LBNP) chamber (Figure 2). Obtaining an acceptable MSNA signal took as long as 1-hour in some subjects. BP, HR, S_PO_2, ventilation, MSNA, and end-tidal gases were measured continuously throughout the experiment.

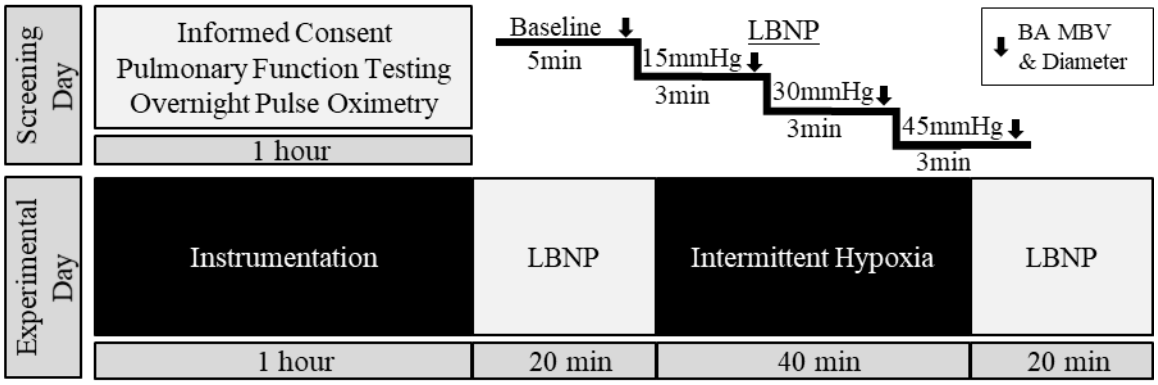

Figure 1. Diagram of the experimental protocol. Abbreviations: BA, brachial artery; LBNP, lower body negative pressure; MBV, mean blood velocity.

Baseline measurements were taken for 5-minutes to determine normal resting $P_{ET}O_2$ and $P_{ET}CO_2$ in each participant. After the baseline period participants began LBNP which consisted of three stages, each held for three minutes, in the following order: 15 mmHg, 30 mmHg, and 45 mmHg. End-tidal gases were clamped at baseline values throughout LBNP. Measurements of BA MBV and diameter were performed on the left arm during the last minute of baseline and each LBNP stage. Participants then endured 40-minutes of IH before repeating the baseline and LBNP stages.

Participants were exposed to 40-minutes of IH. Dynamic end-tidal forcing was used to control end-tidal and arterial blood gases and has been previously validated in our laboratory (Tymko et al. 2016). Using dynamic end-tidal forcing, volunteers were subjected to 20 seconds of normoxic room air, followed by 40 seconds of hypercapnic hypoxia (end-tidal partial pressure of O_2 ($P_{ET}O_2$) = 45 mmHg, $P_{ET}CO_2$ +6 mmHg above baseline). Each normoxia-hypoxia cycle lasted 1-minute, and IH continued for 40-minutes. This protocol mimicked the rate and magnitude of arterial oxyhemoglobin desaturations observed in severe OSA patients during sleep (AHI $\geq$ 60 /hr).

After IH, end-tidal gases were clamped at pre-IH baseline levels and resting measurements were taken for 5-minutes. Participants then repeated the LBNP protocol identical to that conducted prior to IH.

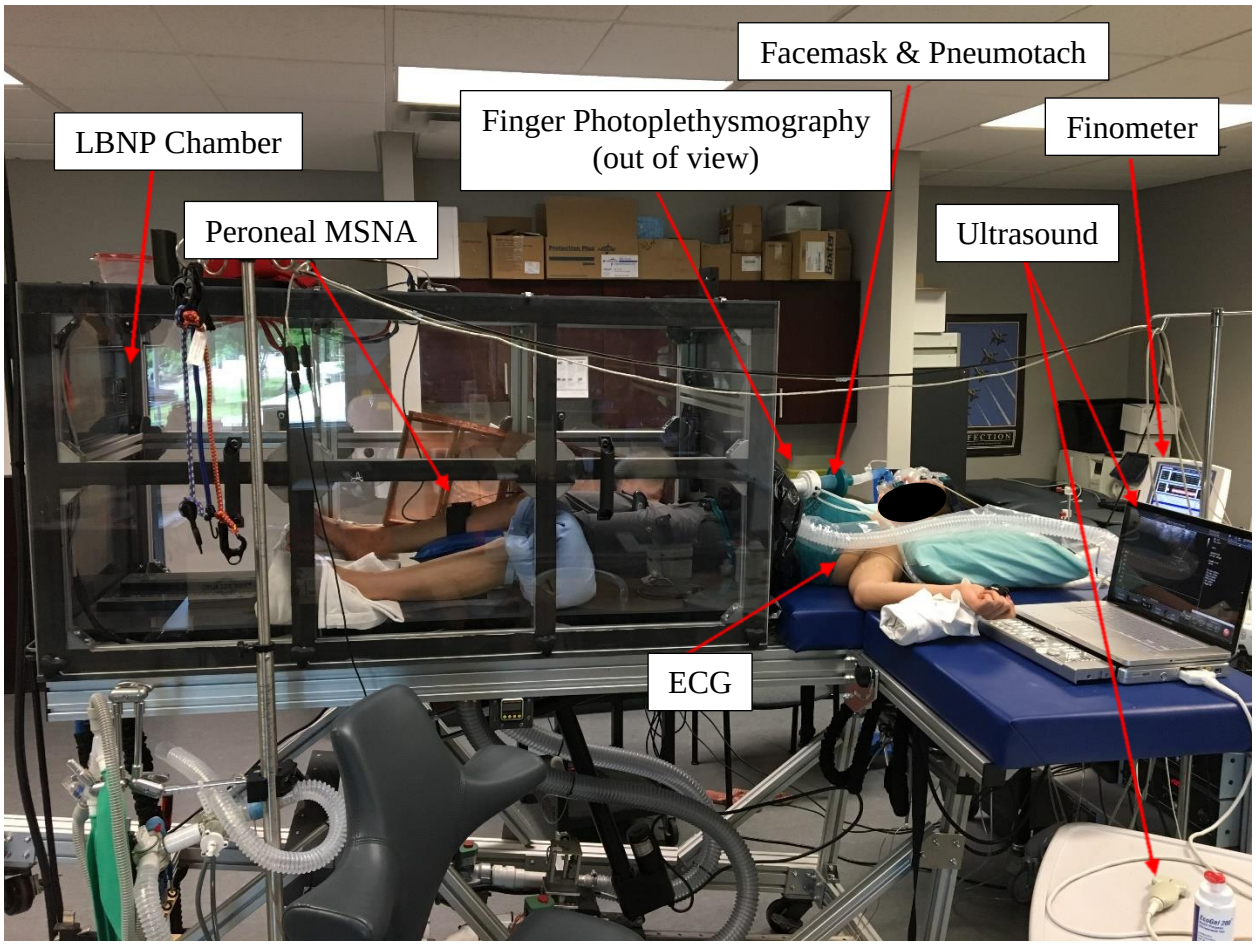

Figure 2. Photo of the experimental setup with equipment labelled.

3.4 Specific Methodologies

3.4.1 Cardiorespiratory Measurements

All cardiopulmonary measurements were acquired at 200Hz using an analog-to-digital converter (Powerlab/16SP ML 880, ADInstruments, Colorado Springs, CO) interfaced with a personal computer, and analyzed using commercially available software (LabChart V8, ADInstruments). Beat-by-beat BP was measured using finger pulse photoplethysmography (Finometer Pro, Finapres Medical Systems, Amsterdam, Netherlands), HR was measured from a lead-II electrocardiogram, and S_pO_2 was measured using a finger pulse oximeter (7500FO, Nonin Medical Inc., Plymouth, MN).

Throughout all procedures participants breathed through a facemask and a two-way non-rebreathing valve (7900 series, Hans Rudolph, Shawnee, KS). Respired gases were

sampled at the mouth, dried with nafion tubing, and analyzed for $P_{ET}O_2$ and $P_{ET}CO_2$. Gas analyzers (ML206; ADinstruments) were calibrated before each experiment with gases of known concentration using the same sample line used in the experiment. Measured partial pressure of O_2 (PO_2) and PCO_2 were time corrected for gas analyzer sample delay, and the values corresponding to the end of expiration were classified as $P_{ET}O_2$ and $P_{ET}CO_2$. Respiratory flow was measured near the mouth using a pneumotachograph (HR 800L, Hans Rudolph) and a differential pressure amplifier (Series 1110, Hans Rudolph), which was calibrated with a 3-liter syringe at a variety of expected flow rates.

3.4.2 End-Tidal Forcing

$P_{ET}O_2$ and $P_{ET}CO_2$ was controlled by a dynamic end tidal forcing system (Tymko et al. 2016). This fast gas mixing system uses independent gas solenoid valves for O_2, CO_2, and N_2 and controls the volume of each gas being delivered to the inspiratory reservoir through a mixing and humidification chamber. $P_{ET}O_2$, $P_{ET}CO_2$, tidal volume (V_T), f_B, and inspired ventilation ($\dot{V}_I$) are determined for each breath online using specifically designed software (Labview 13.0, National Instruments, Austin, TX). Using feedback information regarding $P_{ET}O_2$, $P_{ET}CO_2$, inspired V_T, and expired V_T, the system controls the inspirate to bring end-tidal gas partial pressures to the desired target. Feed-forward control of the inspirate is based on estimates of metabolic O_2 consumption and CO_2 production and employs the alveolar gas equation to determine the required fraction of inspired O_2 (F_IO_2) and F_ICO_2. Feedback control is accomplished using a proportional and integral error reduction control system. This system has been used previously to effectively control end-tidal gases during physiological stressors (Bain et al. 2013; Tremblay et al. 2016; Tymko et al. 2015).

3.4.3 Lower Body Negative Pressure

LBNP (LBNP Chamber, Physiology Research Instruments L.C., Austin, TX) was applied at the beginning and end of the experiment and consisted of consecutive 3-minute stages of LBNP at 15-, 30-, and 45-mmHg. Participants were supine with their lower body in a vacuum-sealed chamber for the duration of the experiment. The pressure within the chamber was decreased relative to atmospheric pressure using an industrial vacuum and was measured using a differential pressure amplifier (Series 1110, Hans Rudolph). The magnitude of negative pressure was manipulated using a 120V input/140V output variable transformer

(Powerstat transformer, The Superior Electric Co, Bristol, CT) enabling pressure to be slowly brought on to minimize disruption of the MSNA microelectrode.

3.4.4 Duplex Ultrasound

BA MBV and diameter were measured in the center of the upper arm using a high-frequency linear array transducer (ML6-15, GE Medical Systems, Mississauga, Canada) operating simultaneously in Doppler and B-mode, connected to a high-resolution ultrasound machine (Vivid E9, GE Medical Systems). The image was captured using a VGA to USB frame grabber (Epiphan Systems Inc., Ottawa, Canada) and the two quadrature Doppler audio signals were converted into a real-time calibrated analog MBV using a Doppler audio translator (Pennsylvania State University Hershey Medical Center, Hershey, PA), which has been previously validated (Herr et al. 2010). The ultrasound image and MBV were recorded using commercially available software (LabChart V8, ADInstruments).

3.4.5 Microneurography

Microneurography was used to assess sympathetic vasomotor outflow throughout baseline and all stages of LBNP. MSNA is a direct measurement of multi-unit postganglionic activity and was obtained from fascicles of the peroneal nerve posterior to the fibular head using a common microneurographic technique, which has been used since the 1960's (Hagbarth and Vallbo 1968). Internationally accepted guidelines for the acquisition and assessment of MSNA were followed (D. W. White, Shoemaker, Raven 2015). Foam padding was placed under the arms, back, and buttocks, and air-filled splints were used to secure the leg. Participants were advised to remain stationary for the duration of the experiment. A reference electrode was placed on the skin of the kneecap before palpation and surface stimulation were used to locate the peroneal nerve (S48-K, Grass Technologies, Warwick, RI). Upon finalizing the location of the nerve, a sterile tungsten microelectrode (tip diameter 5-10 um, 35mm long, Fredrick Haer, Bowdoinham, ME) was inserted. Nerve signals were amplified (100,000 ×), band pass filtered (0.7-2 kHz), integrated (time constant = 100 ms), and processed by a Nerve Traffic Analysis System (Model 662C-4, University of Iowa Bioengineering, Iowa City, IA).

An acceptable MSNA signal was confirmed by visual inspection of the mean voltage neurogram. An acceptable neurogram had the following characteristics: pulse synchronous

bursts occurring 1.2-1.4 seconds after a QRS complex, reproducible activation during end-expiratory breath-holds, a signal to noise ratio of >3:1, and no bursting occurring with stroking of the skin or startle stimuli. If a stable MSNA signal was not acquired within 60-minutes the experiment was terminated.

3.5 Data Analysis

Using LabChart software (LabChart V8, ADInstruments) respiratory variables including $\dot{V}_E$, V_T, and f_B were calculated from measured respiratory flow. As previously mentioned, PO_2 and PCO_2 were time corrected for gas analyzer sample delay, and the values corresponding to the end of expiration were classified as $P_{ET}O_2$ and $P_{ET}CO_2$. All respiratory data was analyzed and extracted on a breath-by-breath basis.

SBP and DBP were extracted from the peak and nadir of the blood pressure waveform, and MAP was calculated as:

$$MAP = \frac{1}{3}SBP + \frac{2}{3}DBP$$

HR and S_pO_2 were calculated from the R-wave time intervals of the lead-II ECG and the continuous S_PO_2 signals respectively. BA MBV was calculated by averaging the mean velocity signal throughout each heartbeat. Ultrasound video images stored in audio video interleave format were analyzed using automated edge detection software (Woodman et al. 2001) to obtain BA diameter. Forearm blood flow ($\dot{Q}_{BA}$) in ml/min was calculated by multiplying the BA MBV and vessel cross-sectional area using the equation:

$$\dot{Q}_{BA} = BA\ MBV \times \pi(\frac{1}{2}BA\ Diameter)^2 \times 60$$

Forearm vascular resistance (FVR) in mmHg/ml/min was calculated as:

$$FVR = \frac{MAP}{\dot{Q}_{BA}}$$

All cardiovascular variables were analyzed and extracted on a beat-by-beat basis.

MSNA burst frequency (BF) and burst incidence (BI) were calculated using peak analysis software to identify bursts of sympathetic vasomotor outflow in the mean voltage integrated neurogram (LabChart V8, ADInstruments). MSNA bursts were identified if they were pulse synchronous, occurring 1.2-1.4 seconds after a QRS complex, had a signal to noise ratio of >3:1, and exhibited a spiked morphology with similar rise and fall. Once bursts

were identified, MSNA BF was calculated in bursts/min, and incidence was calculated using the following equation:

$$MSNA\ BI = \frac{\#\ of\ MSNA\ bursts}{\#\ of\ heart\ beats} \times 100$$

All respiratory, cardiovascular, hemodynamic, and MSNA variables from the last minute of baseline and the last minute of each LBNP stage were time-aligned and averaged at 1-minute intervals with custom made software available free online (Silo, https://github.com/lindseyboulet/silo), built using the shiny web application and R programming (Chang et al. 2015; R Core Team 2018). This data was analyzed statistically to determine baseline changes after IH, responses to LBNP, and changes in sympathetic neurovascular transduction (*see* 3.6 Statistical Analysis).

Data collected during IH was analyzed to determine the mean, mean minimum, and mean maximum S_PO_2, and time spent below 90%, 85%, and 80% S_pO_2. These measures were reported to characterize the severity of IH delivered. S_PO_2 was calculated from $P_{ET}O_2$ using the Severinghaus transformation (Severinghaus 1979) to account for circulatory time delays:

$$S_PO_2 = \left(\left(\left(P_{ET}O_2{}^3 + 150 \times P_{ET}O_2\right)^{-1} \times 23400\right) + 1\right)^{-1} \times 100\%$$

In addition, breath-by-breath and beat-by-beat cardiorespiratory data from the first and last 6-cycles of IH were linearly interpolated at 1s intervals and signal averaged for each subject, as previously conducted by Foster *et al* (2009). Data from all subjects were then combined to create a mean IH profile for the first and last-6 cycles of IH. The peak and nadir values from the average profiles were extracted and compared statistically in order to assess responses to individual bouts of hypoxia at the beginning and end of IH.

3.6 Statistical Analysis

All data were compared within participant, and significance was set at $P < 0.05$. A linear mixed effects model was used to determine differences in the relationship between FVR and MSNA before and following IH. This analysis was conducted using R (R Core Team 2018) and lme4 (Bates et al. 2015) and lmerTest (Kuznetsova, Brockhoff, Christensen 2017) statistical packages. The difference in slope between conditions (i.e. Pre versus Post IH) represents the change in neurovascular transduction. Within the linear mixed effect model, main effects and interaction terms were included for condition as a fixed effect and MSNA

BF as a continuous predictor. Random effects included by-subject intercepts and by-condition slopes. These random effects were entered to account for correlation due to subject (random intercept component) as well as differences in the FVR response to IH (random slope component). Using a likelihood ratio test, the final model was compared to (1) a null model without the fixed effect of MSNA BF, (2) a null model without the interaction term, and (3) a model including random slope by subjects. The effect size was reported as both R^2 and ω^2 values.

All additional analyses were performed using SPSS statistical software (SPSS Statistics 23, IBM Corp. Armonk, NY). The time of peak and nadir $P_{ET}O_2$ values were extracted from the signal averaged traces of the first and last-6 cycles of IH. These peak and nadir respiratory and hemodynamic measurements were compared using a two (pre vs post)-by-two (peak normoxia vs nadir hypoxia) repeated measures ANOVA. Baseline cardiopulmonary, hemodynamic, and sympathetic vasomotor outflow measures were compared before and after IH using a two-tailed paired Students t-test except for MSNA BF and BI, which were tested using a one-tailed paired Students t-test based on our *a priori* hypothesis that MSNA BF and BI would be augmented after IH in line with previous observations (Cutler, Swift, Keller, Wasmund, Smith 2004; Jouett et al. 2017; Leuenberger et al. 2005; Xie et al. 2000). Data from baseline and the last minute of each LBNP stage was compared using a two (pre vs post)-by-four (LBNP levels: 0, 15, 30, 45) repeated measures ANOVA. All post-hoc analyses were conducted using Bonferroni corrected t-tests.

4 Results

4.1 Participants

We recruited 22 healthy male volunteers that fit the inclusion and exclusion criteria for this study. Of the 22 volunteers, 3 participants withdrew consent for reasons unrelated to the research study, 8 participants were excluded because an acceptable MSNA signal could not be obtained, and 1 participant was excluded because the MSNA signal was lost partway through experimentation. Ten healthy male volunteers completed the entire study and were included in the primary analysis. Participant characteristics including measurements of pulmonary function and nocturnal hypoxemia are presented in Table 1. All participants were normotensive (SBP/DBP < 135/85 mmHg), did not suffer from sleep apnea or nocturnal hypoxemia (ODI < 5), and had normal spirometry measurements ($FEV_1/FVC > 0.70$).

Table 1. Participant characteristics and pulmonary function data.

Variable	Mean ± SEM	Variable	Mean ± SEM (% predicted)
Age (years)	23 ± 2	**FVC (l)**	5.5 ± 0.3 (103 ± 4)
Height (cm)	175 ± 3	**FEV_1 (l)**	4.2 ± 0.2 (95 ± 3)
Body Mass (kg)	80 ± 5	**FEV_1/FVC (%)**	78 ± 2 (93 ± 3)
BMI (kg/m^2)	26 ± 1	**TLC (l)**	7.0 ± 0.4 (105 ± 5)
ODI (/hr)	2.3 ± 0.3	**VC (l)**	5.6 ± 0.2 (105 ± 4)
Mean Nocturnal S_PO_2 (%)	95.5 ± 0.2	**FRC (l)**	3.0 ± 0.2 (94 ± 7)
Mean Minimum Nocturnal S_PO_2 (%)	92.3 ± 0.3	**D_LCO (ml/min/mmHg)**	36 ± 2 (99 ± 4)
% of Time <95% S_PO_2	18 ± 8	**V_A (l)**	6.4 ± 0.3 (95 ± 4)
% of Time <90% S_PO_2	0 ± 0	**D_LCO/V_A (ml/min/mmHg/l)**	5.7 ± 0.3 (104 ± 5)

Abbreviations: BMI, body mass index; D_LCO, diffusion capacity of the lung for carbon monoxide transfer; D_LCO/V_A, D_LCO corrected for alveolar volume; FEV_1, forced expired volume in one second; FRC, functional residual capacity; FVC, forced vital capacity; ODI, oxygen desaturation index; S_PO_2, peripheral oxyhemoglobin saturation; TLC, total lung capacity; V_A, alveolar volume; VC, vital capacity.

4.2 Intermittent Hypoxia

Data from a single representative subject throughout 40-minutes of IH is shown in Figure 3 and demonstrates the cyclic changes in $\dot{V}_I$, $P_{ET}O_2$, $P_{ET}CO_2$, and S_PO_2. During IH in this subject, $P_{ET}O_2$ fluctuated between 90 and 45 mmHg, S_PO_2 dropped below 85% with each hypoxic bout and $P_{ET}CO_2$ increased by ~3-5 mmHg.

The IH protocol was designed to mimic severe sleep apnea with an ODI fixed at 60 events/hour. Hypoxia stimulated a mean minimum S_PO_2 of 82.7 ± 1.1 % while normoxia led to a mean maximum S_PO_2 of 97.2 ± 0.3 %, demonstrating that participants desaturated to a clinically relevant level during hypoxia and baseline S_PO_2 levels were restored during normoxia. The mean S_PO_2 during IH was 91.4 ± 0.5 %, and participants spent 36.9 ± 3.0 % of time with S_PO_2 < 90 %, 17.2 ± 3.8 % of time with S_PO_2 < 85 %, and 3.4 ± 2.7 % of time with S_PO_2 < 80 %.

The mean signal averaged data from the first and last 6-cycles of IH are shown in Figure 4, along with mean ± SEM values from time points 0 and 35, representing the peak

and nadir $P_{ET}O_2$ responses to normoxia and hypoxia, respectively. $P_{ET}O_2$ was significantly reduced from 94 ± 3 mmHg to 48 ± 1 mmHg during hypoxia ($P < 0.001$), and $P_{ET}CO_2$ was significantly increased by 4 ± 1 mmHg ($P = 0.002$) despite the accompanying 10 ± 2 l/min increase in $\dot{V}_I$ ($P = 0.001$). Table 2 shows the increase in $\dot{V}_I$ during hypoxia is mainly due to an increase in V_T (0.5 ± 0.1 l, $P < 0.001$) and a small insignificant increase in f_B ($P = 0.079$).

Table 2 displays relevant respiratory and cardiovascular changes during the first and last 6-cycles of IH. Compared to the first 6-cycles, the last 6-cycles of IH resulted in a greater reduction in $P_{ET}CO_2$ (-0.4 ± 0.1 mmHg, $P = 0.028$) and elevation in $\dot{V}_I$ (+5 ± 2 l/min, $P = 0.049$). There was a significant interaction effect for MAP ($P = 0.048$), suggesting that the change in MAP induced by hypoxia was greater during the first 6-cycles (11 ± 2 mmHg, $P < 0.001$) compared with the last 6-cycles (9 ± 2 mmHg, $P = 0.001$). During hypoxia, there were increases in SBP (9 ± 2 mmHg, $P = 0.001$) and DBP (8 ± 1 mmHg, $P < 0.001$), but no differences between the first and last 6-cycles ($P \geq 0.481$). Finally, there was no change in HR during hypoxia ($P = 0.170$) or comparing the first and last 6-cycles ($P = 0.115$).

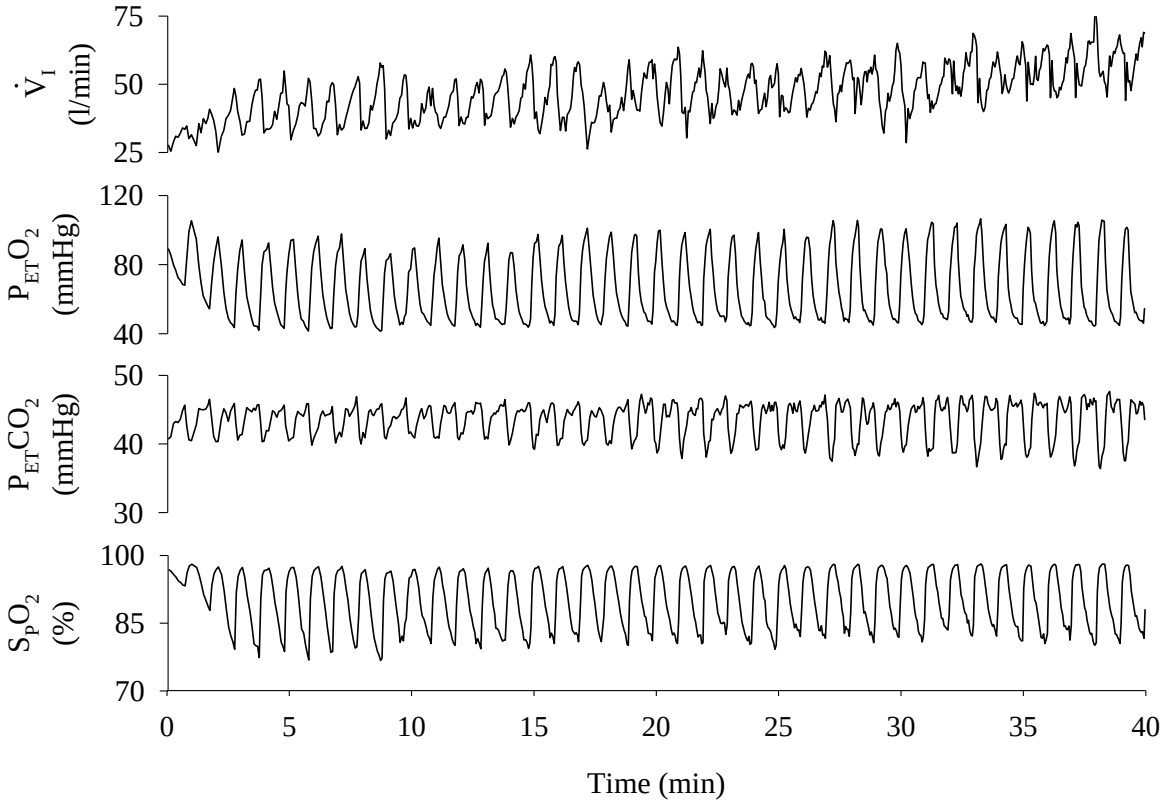

Figure 3. Raw data from a representative subject throughout 40-minutes of intermittent hypoxia demonstrating changes in ventilation, end-tidal gases, and oxyhemoglobin saturation. Abbreviations: $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; $P_{ET}O_2$, partial pressure of end-tidal oxygen; S_PO_2, peripheral oxyhemoglobin saturation; $\dot{V}_I$, inspired ventilation.

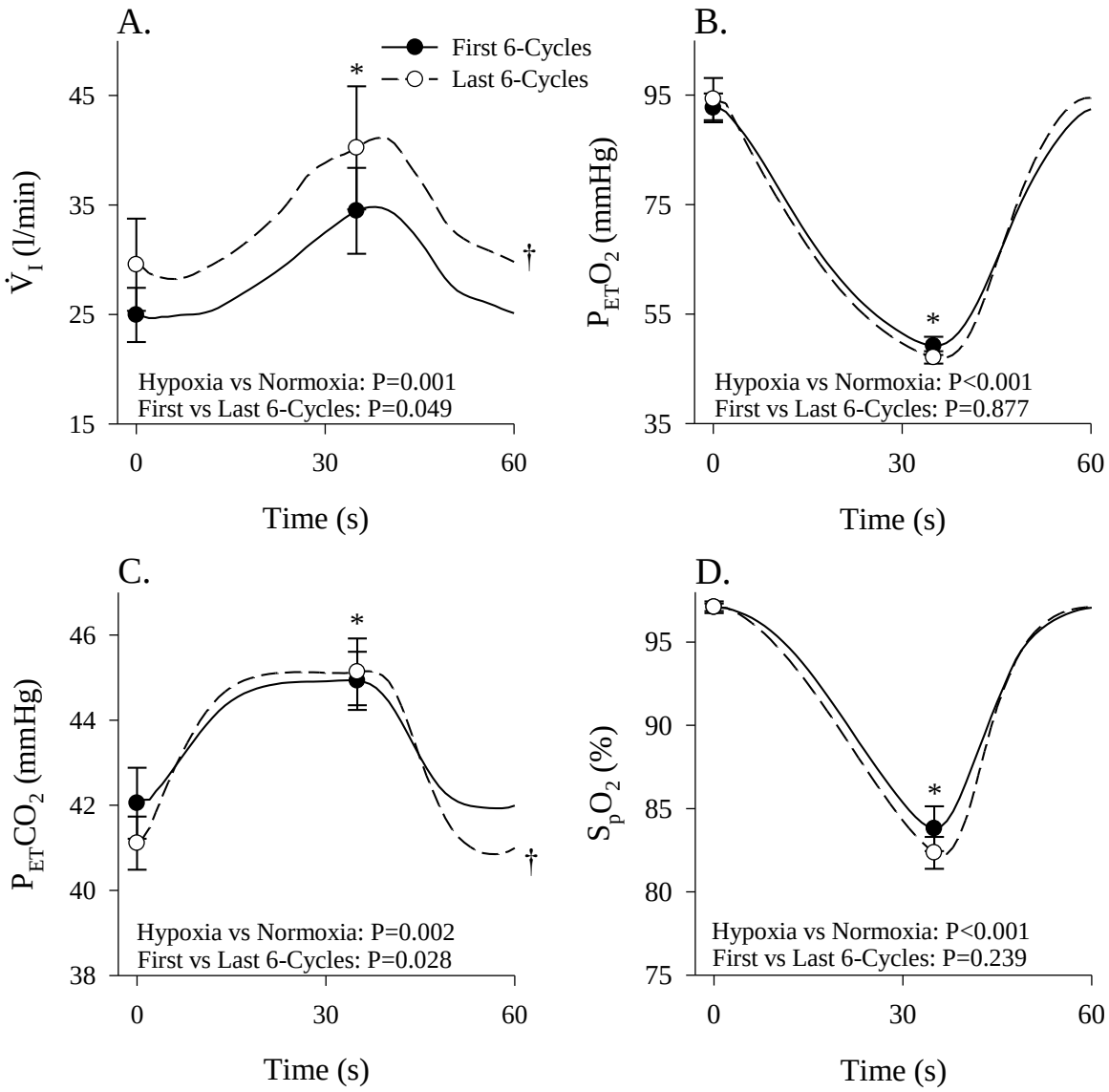

*Figure 4. Ventilatory, end-tidal gas, and oxyhemoglobin saturation profile during the first and last 6 minutes of intermittent hypoxia. Data points are mean ± SEM at 0 (normoxia) and 35 (hypoxia) seconds which represent the peak and nadir $P_{ET}O_2$ values. *P<0.05 compared with normoxia, †P<0.05 compared with first 6-cycles of intermittent hypoxia. Abbreviations: $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; $P_{ET}O_2$, partial pressure of end-tidal oxygen; S_PO_2, peripheral oxyhemoglobin saturation; $\dot{V}_I$, inspired ventilation.*

Table 2. Comparison of ventilatory and cardiovascular responses during peak normoxia and nadir hypoxia across 40-minutes of intermittent hypoxia (i.e. first vs last 6-cycles).

		Normoxia	Hypoxia	First vs Last 6-Cycles	Normoxia vs Hypoxia	Interaction
V_T (l)	First 6-cycles	1.8 ± 0.2	2.2 ± 0.2	P = 0.297	**P < 0.001**	P = 0.177
	Last 6-cycles	1.8 ± 0.2	2.4 ± 0.2			
f_B (/min)	First 6-cycles	13 ± 1	15 ± 2	P = 0.115	P = 0.079	P = 0.058
	Last 6-cycles	15 ± 2	15 ± 2			
HR (/min)	First 6-cycles	69 ± 3	70 ± 3	P = 0.115	P = 0.170	P = 0.647
	Last 6-cycles	73 ± 5	74 ± 4			
MAP (mmHg)	First 6-cycles	91 ± 3	101 ± 4*	P = 0.691	**P < 0.001**	**P = 0.048**
	Last 6-cycles	93 ± 3	101 ± 4*			
SBP (mmHg)	First 6-cycles	131 ± 3	141 ± 4	P = 0.481	**P = 0.001**	P = 0.200
	Last 6-cycles	134 ± 3	142 ± 3			
DBP (mmHg)	First 6-cycles	70 ± 3	79 ± 4	P = 0.732	**P < 0.001**	P = 0.104
	Last 6-cycles	71 ± 3	79 ± 3			

Values represent mean ± SEM. *P<0.05 compared with Normoxia. Abbreviations: DBP, diastolic blood pressure, f_b, breathing frequency; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure; V_T, tidal volume.

4.3 Effects of IH on Baseline Cardiorespiratory Measures and Sympathetic Vasomotor Outflow

Figure 5 shows the effect of IH on baseline hemodynamic and sympathetic vasomotor outflow measurements. IH increased MSNA BF (2 ± 1 /min, P = 0.032), and MAP (5 ± 2 mmHg, P = 0.002). Additional baseline cardiorespiratory and sympathetic vasomotor outflow variables, before and after IH, are shown in Table 3. The increase in MAP was driven by increases in both SBP (7 ± 2 mmHg, P = 0.014) and DBP (4 ± 1 mmHg, P < 0.001). BA MBV was reduced after IH (P = 0.005), contributing to a 26 ± 8% reduction in $\dot{Q}_{BA}$ (P = 0.014). Overall, the increase in MAP and reduction in $\dot{Q}_{BA}$ caused a 39 ± 10% increase in baseline FVR post-IH (P = 0.004).

$\dot{V}_I$ was increased following IH by 5 ± 2 l/min (P = 0.025), largely due to a small non-significant increase in f_b (P = 0.053), and there was a small reduction in $P_{ET}CO_2$ (0.5 ± 0.2 mmHg, P = 0.048). Both $P_{ET}O_2$ (P = 0.752) and HR (P = 0.584) were similar before and after IH. MSNA BI (P = 0.044) was increased after IH, while MSNA BL tended to be greater following IH (P = 0.058).

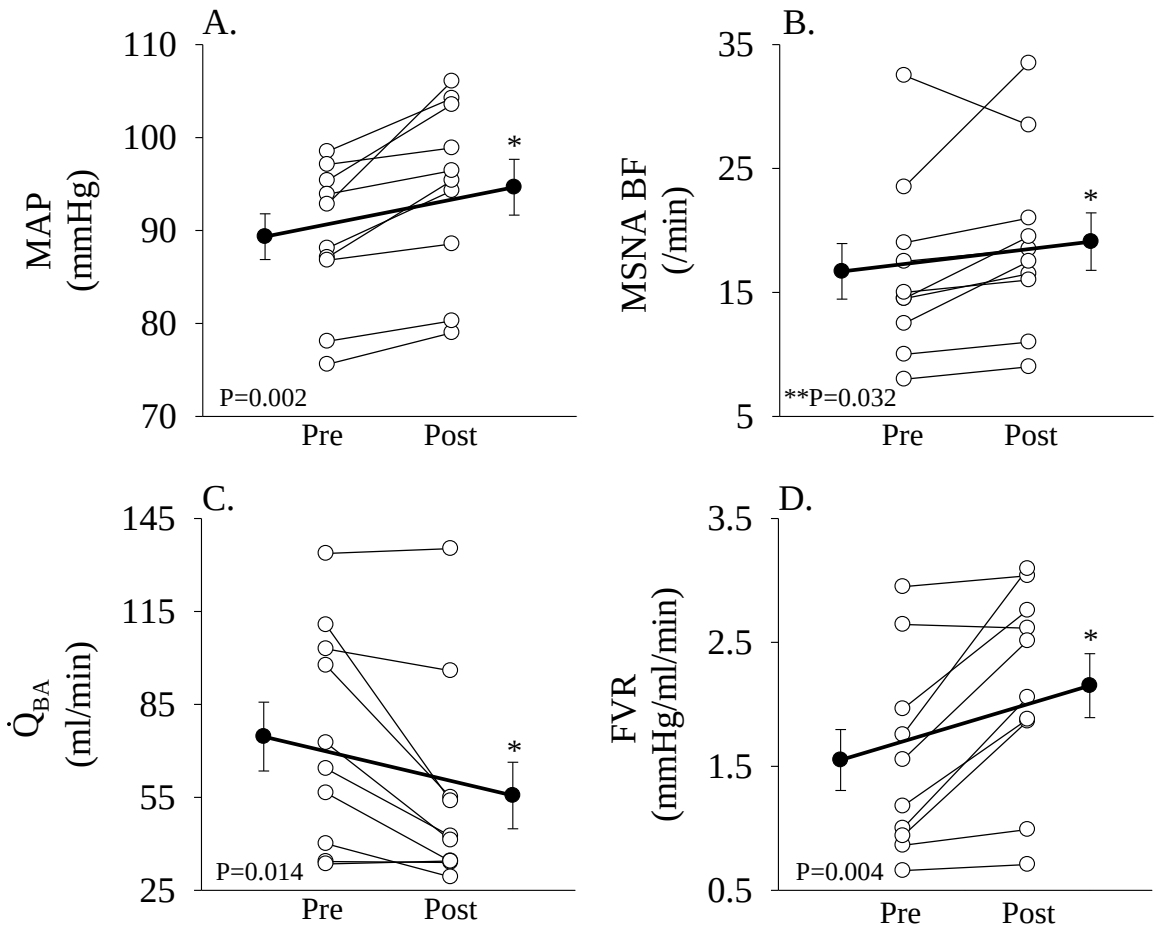

*Figure 5. Baseline changes before (Pre) and following (Post) intermittent hypoxia in mean arterial pressure (A), muscle sympathetic nerve activity (B), brachial artery blood flow (C), and forearm vascular resistance (D). Individual subjects (open circles) and mean ± SEM (filled circles). ** indicates one-tailed paired t-test p-value provided based on a priori hypotheses. *P<0.05 compared with Pre. Abbreviations: BF, burst frequency; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; $\dot{Q}_{BA}$, brachial artery blood flow; FVR, forearm vascular resistance.*

Table 3. Resting cardiorespiratory and sympathetic vasomotor outflow before (pre) and following (post) intermittent hypoxia.

	Pre	**Post**	**P-value**
$\dot{V}_I$ (l/min)	12 ± 1	17 ± 2	**P = 0.025**
V_T (l)	1.3 ± 0.1	1.2 ± 0.1	P = 0.717
f_b (/min)	10 ± 1	13 ± 3	P = 0.053
$P_{ET}O_2$ (mmHg)	90 ± 1	90 ± 2	P = 0.752
$P_{ET}CO_2$ (mmHg)	41 ± 1	40 ± 1	**P = 0.048**
HR (/min)	63 ± 3	64 ± 4	P = 0.584
SBP (mmHg)	128 ± 3	134 ± 3	**P = 0.014**
DBP (mmHg)	69 ± 3	74 ± 3	**P < 0.001**
MSNA BI (/100hb)	27 ± 3	31 ± 3	****P = 0.044**
MSNA BL (s)	1.31 ± 0.03	1.34 ± 0.03	P = 0.058
BA MBV (cm/s)	10 ± 1	7 ± 1	**P = 0.005**
BA Diameter (cm)	0.40 ± 0.01	0.40 ± 0.01	P = 0.189

Values are mean ± SEM. ** indicates one-tailed paired t-test p-value provided based on a priori hypotheses. Abbreviations: 100hb, 100 heart beats; BA, brachial artery; BI, burst incidence; BL, burst latency; DBP, diastolic blood pressure; f_b, breathing frequency; HR, heart rate; IH, intermittent hypoxia; MBV, mean blood velocity; MSNA, muscle sympathetic nerve activity; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; $P_{ET}O_2$, partial pressure of end-tidal oxygen; SBP, systolic blood pressure; $\dot{V}_I$, inspired ventilation; V_T, tidal volume.

4.4 Responses to Lower Body Negative Pressure

Figure 6 shows data from the last minute of each LBNP stage in a single representative subject demonstrating the expected changes in MAP, $\dot{Q}_{BA}$ and sympathetic vasomotor outflow. LBNP was well controlled at each stage of LBNP, and as LBNP was increased this subject had a large increase in sympathetic vasomotor outflow, small reduction in $\dot{Q}_{BA}$, with little change in MAP.

The pressure generated during each stage of LBNP was comparable before and following IH ($P = 0.087$, Table 4). Figure 7 shows selected cardiorespiratory and sympathetic vasomotor outflow responses to LBNP. MSNA BF was increased at all stages of LBNP (significant interaction, $P < 0.01$ for all comparisons). Before IH, FVR increased from baseline at only 15 mmHg of LBNP ($P = 0.011$), whereas after IH FVR was significantly greater than baseline at all stages of LBNP (significant interaction, $P < 0.05$ for all comparisons). In addition, when comparing within each stage of LBNP, FVR was greater after IH compared to before IH ($P < 0.01$ for all comparisons). Heart rate increased in response to rising levels of LBNP ($P < 0.001$) but was not affected by IH ($P = 0.167$). MAP was elevated after IH ($P = 0.001$) but remained similar throughout LBNP. There was a significant interaction effect for SBP ($P = 0.016$) and DBP ($P = 0.044$) shown in Table *5*. SBP was reduced at 30 and 45mmHg before-IH ($P \leq 0.04$ for both comparisons) but was not affected by LBNP after IH ($P \geq 0.30$ for all comparisons). DBP was greater after IH compared to before IH at all times ($P \leq 0.04$ for all comparisons) but was not affected by LBNP before or after IH ($P \geq 0.28$ for all comparisons).

Figure 7 also shows $\dot{V}_I$ was significantly greater following IH ($P = 0.014$) and increased at the last stage of LBNP relative to baseline ($P = 0.001$). Additional respiratory responses to LBNP are shown in Table 4. End-tidal gases were well-controlled throughout LBNP. Although there was a small baseline reduction in $P_{ET}CO_2$ of 0.5 ± 0.2 mmHg ($P = 0.024$), there were no pre-to-post IH differences in end-tidal gases during LBNP.

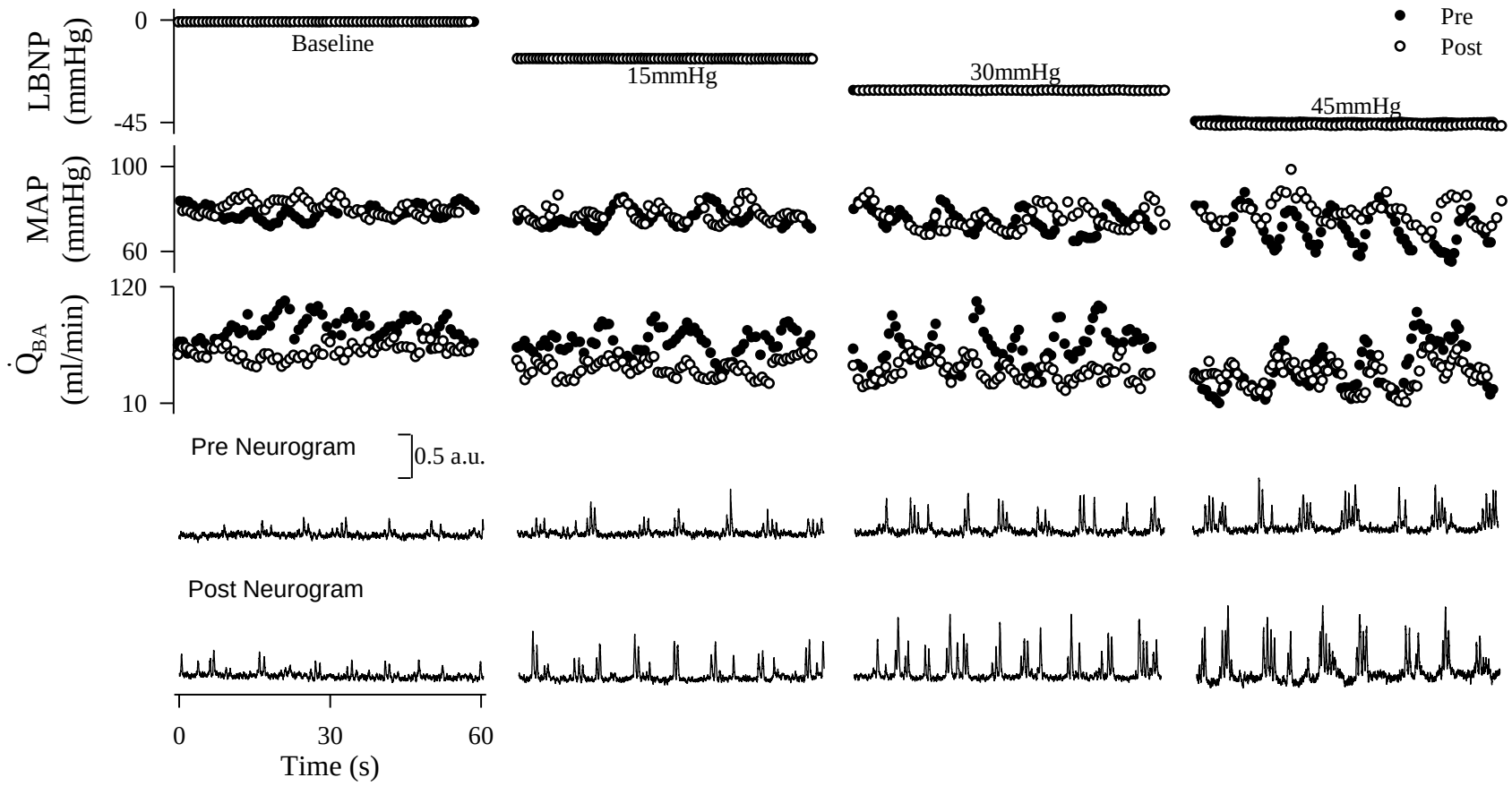

Figure 6. Representative hemodynamic and sympathetic vasomotor outflow data from a single subject (ID: 20) during LBNP before (Pre) and following (Post) intermittent hypoxia. Presented data includes lower body negative pressure (LBNP), beat-by-beat mean arterial pressure (MAP) and brachial artery blood flow ($\dot{Q}_{BA}$), and raw sympathetic integrated neurograms. Calibration beats were removed from MAP data. Abbreviations: a.u., arbitrary units.

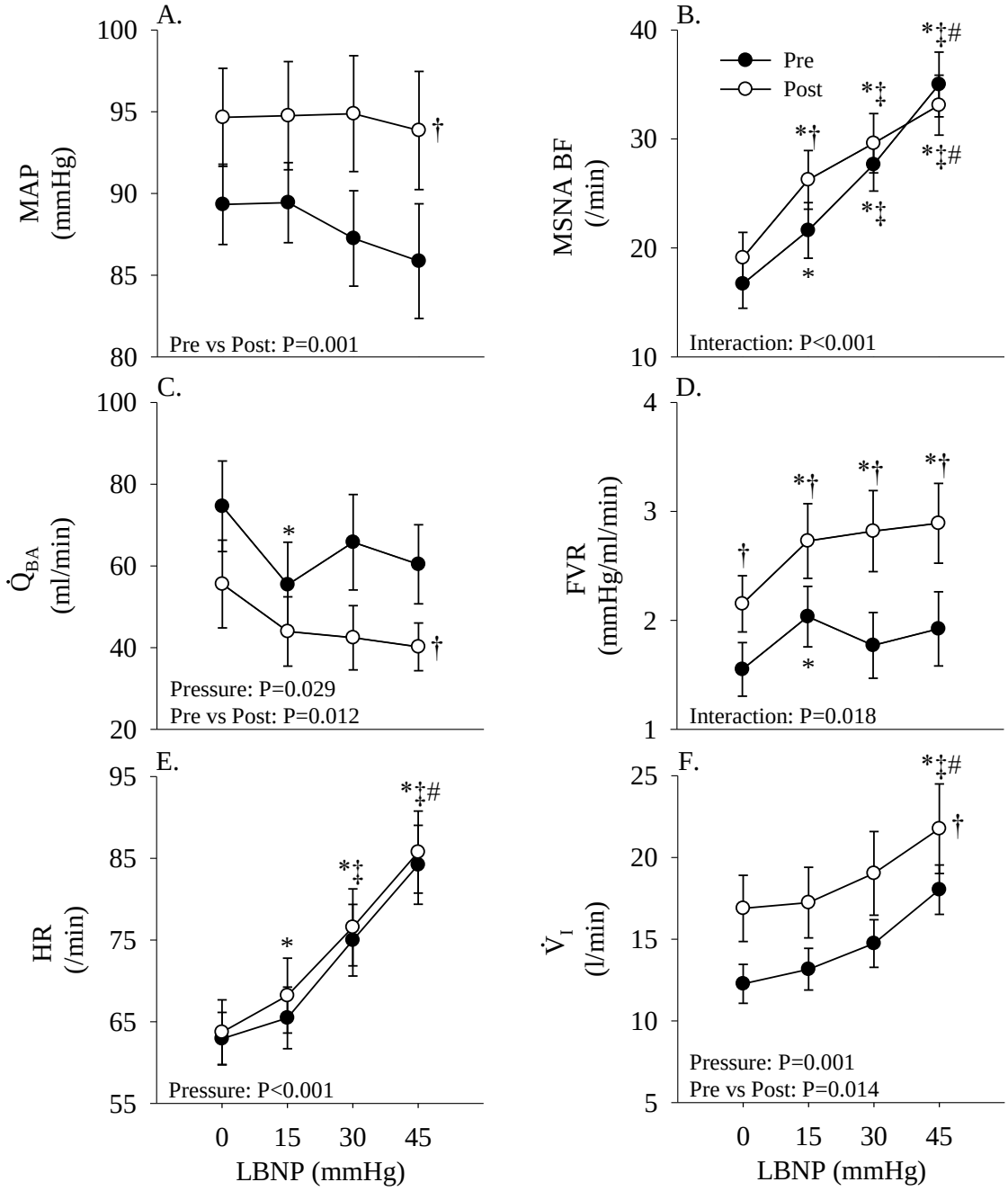

*Figure 7. The effect intermittent hypoxia and lower body negative pressure on MAP (A), MSNA BF (B), $\dot{Q}_{BA}$ (C), FVR (D), HR (E), and $\dot{V}_I$ (F). *P<0.05 compared with baseline, †P<0.05, Pre vs Post-IH. ‡P<0.05, compared with 15 mmHg, #P<0.05, compared with 30 mmHg. Values are mean ± SEM. Abbreviations: BF, burst frequency; FVR, forearm vascular resistance; HR, heart rate; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; $\dot{Q}_{BA}$ brachial artery blood flow; $\dot{V}_I$, inspired ventilation.*

Table 4. The effect of intermittent hypoxia on respiratory measurements and lower body negative pressure (LBNP) magnitude during LBNP.

		Baseline	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Pre vs Post**	**Pressure**	**Interaction**
V_T (l)	Pre	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.5 ± 0.1*‡#	P = 0.794	**P < 0.001**	P = 0.241
	Post	1.2 ± 0.1	1.3 ± 0.2	1.4 ± 0.2	1.5 ± 0.2*‡#			
f_b (/min)	Pre	10 ± 1	11 ± 1	11 ± 1	11 ± 1	P = 0.080	P = 0.380	P = 0.313
	Post	13 ± 1	13 ± 1	13 ± 1	14 ± 1			
$P_{ET}O_2$ (mmHg)	Pre	90 ± 1	91 ± 2	92 ± 2	93 ± 2	P = 0.793	P = 0.261	P = 0.440
	Post	91 ± 2	90 ± 2	94 ± 3	93 ± 3			
$P_{ET}CO_2$ (mmHg)	Pre	41 ± 1	41 ± 1	40 ± 1	40 ± 1	P = 0.687	P = 0.338	P = 0.093
	Post	40 ± 1	41 ± 1	40 ± 1	40 ± 1			
LBNP (mmHg)	Pre	0.6 ± 0.1	16.2 ± 0.2*	30.7 ± 0.1*‡	45.4 ± 0.2*‡#	P = 0.087	**P < 0.001**	P = 0.692
	Post	0.7 ± 0.1	16.3 ± 0.2*	31.0 ± 0.1*‡	45.7 ± 0.2*‡#			

Values represent mean ± SEM. †P<0.05 compared to pre; *P<0.05 compared to baseline; ‡P<0.05 compared to 15 mmHg, #P<0.05 compared to 30 mmHg. Abbreviations: f_b, breathing frequency; LBNP, lower body negative pressure; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; $P_{ET}O_2$, partial pressure of end-tidal oxygen; V_T, tidal volume.

Table 5. *The effect of intermittent hypoxia on cardiovascular, hemodynamic, and sympathetic vasomotor outflow during lower body negative pressure.*

		Baseline	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Pre vs Post**	**Pressure**	**Interaction**
SBP (mmHg)	Pre	128 ± 3	126 ± 3	120 ± 3*‡	117 ± 5*‡	**P = 0.002**	**P = 0.005**	**P = 0.016**
	Post	134 ± 3†	133 ± 4†	130 ± 4†	128 ± 4†			
DBP (mmHg)	Pre	69 ± 3	70 ± 3	70 ± 3	70 ± 4	**P < 0.001**	P = 0.250	**P = 0.044**
	Post	74 ± 3†	75 ± 3†	76 ± 3*†	76 ± 3†			
BA MBV (cm/s)	Pre	10 ± 1	7 ± 1*	9 ± 2	8 ± 1	**P = 0.003**	**P = 0.047**	P = 0.344
	Post	7 ± 2	6 ± 1*	6 ± 1	6 ± 1			
BA Diameter (cm)	Pre	0.40 ± 0.01	0.40 ± 0.02	0.39 ± 0.02*‡	0.39 ± 0.02*‡	P = 0.152	**P < 0.001**	P = 0.810
	Post	0.40 ± 0.01	0.40 ± 0.02	0.40 ± 0.02*‡	0.39 ± 0.02*‡			
MSNA BI (/100hb)	Pre	27 ± 3	33 ± 3*	37 ± 3*	42 ± 3*‡#	P = 0.145	**P < 0.001**	**P = 0.001**
	Post	31 ± 3	39 ± 3*†	40 ± 3*	39 ± 3			

Values represent mean ± SEM. †P<0.05 compared to pre-IH; *P<0.05 compared to baseline; ‡P<0.05 compared to 15 mmHg, #P<0.05 compared to 30 mmHg. Abbreviations: 100hb, 100 heart beats; BA, brachial artery; BI, burst incidence; DBP, diastolic blood pressure; MBV, mean blood velocity; MSNA, muscle sympathetic nerve activity; SBP, systolic blood pressure.

4.5 Neurovascular Transduction

A linear mixed effect model was constructed for the relationship between FVR and MSNA BF to determine the influence of IH on sympathetic neurovascular transduction. Using a likelihood ratio test, the best statistical model was found to include an interaction term for MSNA BF and condition (i.e. pre- vs. post-IH) as a fixed effect, while including subject as a random intercept and condition as a random slope component. The final model was a significantly better fit compared to a null model without the fixed effect of MSNA BF ($\chi^2(1) = 12.85$, $P < 0.001$) and a null model without the interaction term ($\chi^2(1) = 5.50$, $P = 0.019$), demonstrating the interaction between condition and MSNA burst frequency is a significant predictor of FVR (Figure 8). The fit of the model did not improve when including a random slope by subjects component ($\chi^2(7) = 10.29$, $P = 0.173$). The final model had a strong effect size ($R^2 = 0.903$, $\omega^2 = 0.902$).

The slope of the relationship between FVR and MSNA BF is representative of sympathetic neurovascular transduction. IH led to a greater than 3-fold change in slope ($P = 0.015$) suggesting greater sympathetic neurovascular transduction following IH (Figure 8). Interestingly, the intercepts between the two relationships did not differ ($P=0.970$).

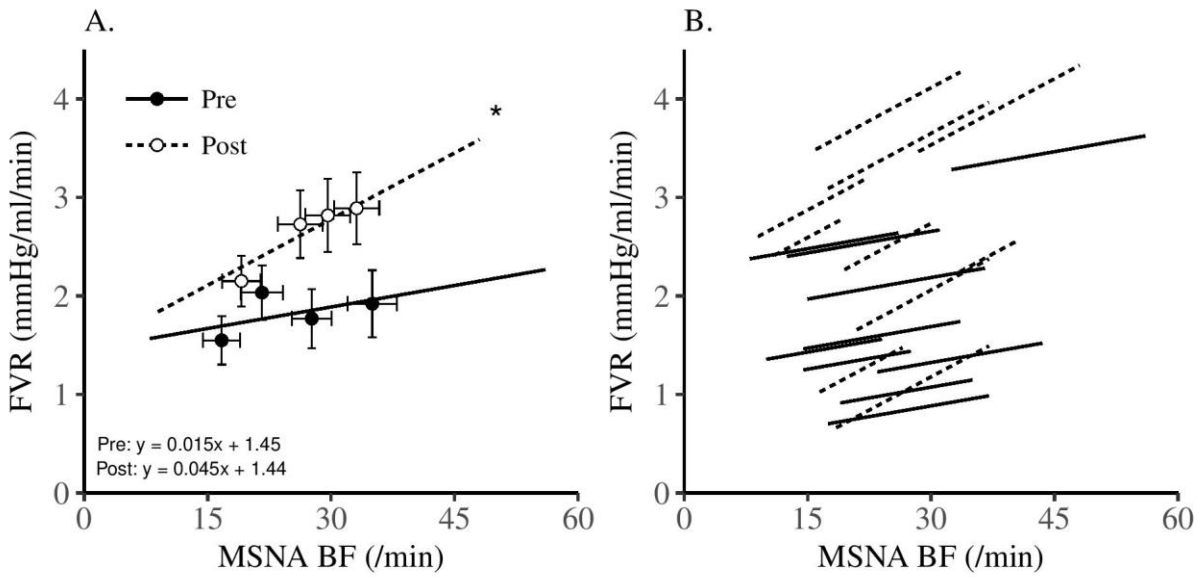

*Figure 8. Effect of intermittent hypoxia on the relationship between MSNA BF and FVR demonstrating augmented sympathetic neurovascular transduction. Panel A: The mixed effect linear model for all subjects and the mean ± SEM data from each stage of LBNP. Panel B: The mixed effect linear model fit to each subject. *P<0.05 comparing the slope vs pre. Abbreviations: FVR, forearm vascular resistance; MSNA, muscle sympathetic nerve activity; BF: burst frequency.*

5 Discussion

5.1 Main Findings of this Study

The purpose of this study was to determine if acute exposure to the IH associated with OSA augments sympathetic neurovascular transduction and may be a contributing factor to the heightened blood pressure previously observed following IH. Our data show, for the first time, that sympathetic neurovascular transduction is augmented immediately after acute exposure to IH, as measured by an increase in the sensitivity of FVR to sympathetic vasomotor outflow during LBNP. Additionally, in contrast to other reports (Deacon et al. 2017; Diep et al. 2007; Jordan et al. 2002), we observed a progressive increase in $\dot{V}_I$ throughout and following IH suggesting ventilatory long term facilitation (LTF). Finally, we confirmed previous reports (Cutler, Swift, Keller, Wasmund, Smith 2004; Jouett et al. 2017; Leuenberger et al. 2005; Xie et al. 2000) illustrating augmented sympathetic vasomotor outflow and blood pressure following IH. Our data suggest that changes in blood pressure following acute IH are not only related to increased central sympathetic vasomotor outflow but also to greater sympathetic neurovascular transduction. Increases in sympathetic neurovascular transduction may be an important early mechanism involved in the development of hypertension in OSA.

5.2 Intermittent Hypoxia and Sympathetic Neurovascular Transduction

This is the first study to report augmented sympathetic neurovascular transduction after acute exposure to IH. We observed a 3-fold increase in the slope of the relationship between FVR and sympathetic vasomotor outflow during LBNP after 40-minutes of IH, as shown in Figure 8. The greater forearm vascular resistance response to sympathetic vasomotor outflow suggests that exposure to IH facilitated sympathetic neurotransmitter release or sensitized the forearm vasculature to sympathetic neurotransmitters. Although we are the first to report augmented sympathetic neurovascular transduction after an IH protocol similar to OSA, an augmented sympathetic neurovascular transduction effect has also been reported during continuous isocapnic hypoxia (Tan et al. 2013).

It is likely that the RAS and increased AT_1R expression played a role in augmenting sympathetic neurovascular transduction after IH. Circulating measures of RAS activity do not increase in humans exposed to acute IH (Foster et al. 2010), however, blocking the AT_1R

with Losartan before IH exposure prevents the known increase in resting blood pressure (Foster et al. 2010), sympathetic vasomotor outflow (Jouett et al. 2017), and oxidative stress (Pialoux et al. 2011), and also reduces endothelial dysfunction in rats exposed to chronic IH (Marcus et al. 2012). Local infusion of angiotensin-II insufficient to change resting blood pressure exacerbates the FVR response to mild LBNP while having no effect on the FVR response to local noradrenaline infusion (Seidelin et al. 1991), which supports that RAS mediated changes in sympathetic neurovascular transduction may occur via presynaptic facilitation. Evidence from animal models provides further insight into the mechanisms that could be involved in RAS and AT_1R induced changes in sympathetic neurovascular transduction. The RAS plays a critical role in both pre- and post-junctional facilitation through the AT_1R (Nap et al. 2003). Inhibition of the AT_1R causes a dose-dependent decrease in norepinephrine release from sympathetic nerves, reduces vascular resistance in rat arteries exposed to angiotensin-II (Cox et al. 1995; Dendorfer et al. 2002), and reduces NPY release (Byku, Macarthur, Westfall 2008a). Angiotensin-II also increases α_1 and α_2 adrenoreceptor expression in the rabbit femoral artery (Purdy and Weber 1988), however, most evidence supports that the majority of angiotensin-II effects on neurotransmission occur via presynaptic facilitation (Nap et al. 2003).

An alternative explanation for our observed change in neurovascular transduction is that IH caused vascular endothelial dysfunction and decreased nitric oxide bioavailability, increasing FVR independently of sympathetic vasomotor outflow. Although no studies have measured endothelial function after acute IH, 6 hours of IH for 1-4 consecutive days reduces nitric oxide derivatives (Foster et al. 2009; Pialoux et al. 2011), promotes retrograde shear stress associated with endothelial dysfunction (Tremblay et al. 2016), and 28 days of IH exposure decreases peak forearm reactive hyperemia after occlusion, baseline forearm blood flow, and increases baseline forearm vascular resistance (Gilmartin et al. 2010). Also, OSA patients with no comorbidities show 4-fold greater NO expression in microcirculatory vessel walls and improvement in flow mediated dilation after 12 weeks of CPAP, supporting how the absence of IH can improve endothelial function (Khayat et al. 2017). However, it is unlikely endothelial dysfunction was responsible for our results, as Tremblay (2016) found 6-hours of IH did not reduce flow mediated dilation compared to controls. Also, the y-axis intercept of the relationship between FVR and MSNA BF was similar pre- and post-IH, as

shown in Figure 8. If endothelial dysfunction was present it would be expected that the predicted FVR with no sympathetic vasomotor outflow would be greater after IH, whereas we did not observe a difference. Alternatively, a reduction in nitric oxide bioavailability could also augment sympathetic neurovascular transduction. Animal studies have demonstrated that nitric oxide reduces the release of norepinephrine from sympathetic nerves and reacts with norepinephrine directly to convert it to an inactive form (Macarthur et al. 2011). Therefore, if a reduction in nitric oxide occurred in this study, it may have played a role in augmenting sympathetic neurovascular transduction.

5.3 Intermittent Hypoxia and Ventilatory Long Term Facilitation

We observed a progressive increase in $\dot{V}_I$ throughout and following IH suggesting ventilatory LTF. $\dot{V}_I$ was greater during the last 6-cycles of IH compared to the first 6 cycles (Figure 4), as well as after the IH exposure at rest (Table 3) and during LBNP (Figure 7). The increase in $\dot{V}_I$ during the last 6-cycles of IH was present throughout both the normoxic and hypoxic portions of the IH cycle. The increase in $\dot{V}_I$ after IH was independent of blood gases, which were controlled with end tidal forcing at baseline levels. Although we did not measure $\dot{V}_I$ beyond 20-minutes after the IH exposure was completed, we did observe $\dot{V}_I$ as elevated at all time points after IH, suggesting that our IH exposure caused ventilatory long term facilitation (LTF). Ventilatory LTF describes the plasticity in the neural control of respiration such that $\dot{V}_I$ remains elevated after removal of the stimulus (Mitchell and Johnson 2003). Ventilatory LTF has been demonstrated in several animal preparations after IH, although human studies have been inconsistent (Mateika and Sandhu 2011).

Initial human studies assessing $\dot{V}_I$ after exposure to IH alone did not find ventilatory LTF (Jordan et al. 2002), however, ventilatory LTF has been found in studies combining IH with a constant hypercapnic background of $P_{ET}CO_2 \approx 3$ - 5 mmHg above baseline (Griffin et al. 2012; Harris et al. 2006; Syed, Lin, Mateika 2012), but not with IH protocols where IH and hypercapnia were cycled together (Diep et al. 2007). Our study is in opposition to this, as we are the first to report ventilatory LTF after an acute bout of IH and cycling hypercapnia in humans. The intensity of IH and hypercapnia exposure might account for the differences between our results and previous studies. The IH protocol used by Diep *et al.* (2007) included 15-bouts, 30-seconds each, with 90-second normoxic periods, which achieved a $P_{ET}O_2 \approx 50$ mmHg and $P_{ET}CO_2 \approx 5$ mmHg above baseline. An example of our IH protocol is

shown in Figure 3. We delivered 40-bouts of IH and hypercapnia, extended each bout to 40-seconds duration, reduced the normoxic periods to 20 seconds between bouts, and achieved similar end-tidal gases. The increase in duration of each bout, increased number of bouts, reduced normoxic duration, and the increase in overall length of the IH period all act to increase the hypoxic and hypercapnic stimulus applied to the carotid body, which may be responsible for the ventilatory LTF observed. A study in sleeping humans also could not induce ventilatory LTF with IH and cycled hypercapnia (Deacon et al. 2017), however, the IH exposure was similar to Diep et al. (2007) who did not show ventilatory LTF in awake humans. Sleep may have a role in ventilatory LTF with a more intense IH and hypercapnic stimulus, however this would be difficult to determine as the authors note that they applied the maximal hypercapnia achievable without waking participants during each bout, which was $P_{ET}CO_2 \approx 2$ mmHg above baseline (Deacon et al. 2017).

In humans, respiratory plasticity including ventilatory LTF and respiratory muscle LTF are elicited after IH with concurrent hypercapnia (Mahamed and Mitchell 2007; Mateika and Syed 2013). Carotid body sensory LTF is another form of respiratory plasticity characterized by tonic increases in carotid body afferent activity, which was initially demonstrated in rodent models exposed to 10-days of chronic IH (Peng et al. 2003). Sensory and ventilatory LTF may be closely related as both sensory and ventilatory LTF are dependent upon serotonin signaling and ventilatory LTF is induced in rats by stimulation of the carotid sinus nerve (Mitchell and Johnson 2003; Peng et al. 2006). Sensory LTF is not elicited with hypercapnia alone (Peng et al. 2003) however, as with other forms of respiratory plasticity, hypercapnia has been shown to have a key role in sensory LTF. In naïve carotid bodies with no IH conditioning, a 10-cycle acute-IH exposure can stimulate sensory LTF, but only when hypercapnia is applied concurrently with hypoxia or constantly in the background throughout IH (Roy et al. 2017). Hypercapnia has an important role in respiratory plasticity and may be the reason we were able to induce ventilatory LTF in this study.

5.4 Intermittent Hypoxia, Blood Pressure, and Sympathetic Vasomotor Outflow

Similar to previous reports, IH caused an increase in resting sympathetic vasomotor outflow and blood pressure, as shown in Figure 5. MSNA BF and BI increased by 14 and 15%, respectively. SBP and DBP were both greater after IH, contributing to a 5 ± 2 mmHg increase in MAP. Greater FVR likely contributed to the increase in MAP as HR was not

changed after IH. Blood gases were controlled at baseline levels by end-tidal forcing so changes in blood pressure and sympathetic outflow were not a result of altered chemoreceptor stimulation. Previous studies using a short bout of IH have also reported an increase in sympathetic vasomotor outflow that outlasted the IH stimulus (Cutler, Swift, Keller, Wasmund, Smith 2004; Jouett et al. 2017; Leuenberger et al. 2005; Xie et al. 2000), which occur regardless of whether IH was hypercapnic (Cutler, Swift, Keller, Wasmund, Smith 2004; Leuenberger et al. 2005; Xie et al. 2000), isocapnic, or poikilocapnic (Cutler, Swift, Keller, Wasmund, Smith 2004; Jouett et al. 2017; Leuenberger et al. 2005). Also, the increase in MSNA occurs independently of whether IH involves apnea (Jouett et al. 2017; Leuenberger et al. 2005), a free breathing protocol (Xie et al. 2000), or a combination of both (Cutler, Swift, Keller, Wasmund, Smith 2004). The IH protocols ranged in length from 20-30 minutes, time in hypoxia ranged from 20-40 seconds each minute, a nadir S_PO_2 between 83-88% was achieved during hypoxia, and in studies that controlled CO_2 there was a 3-5 mmHg increase in $P_{ET}CO_2$ achieved (Cutler, Swift, Keller, Wasmund, Smith 2004; Jouett et al. 2017; Leuenberger et al. 2005; Xie et al. 2000). However, our 14% increase in MSNA BF is low compared to these studies, which have reported between 31-45% increases in MSNA BF. Nonetheless, we did see an increase in MSNA BF in 9 out of 10 subjects studied, and the subject who did not show an increase in MSNA BF had the highest baseline to begin with.

It is likely that both the changes in sympathetic vasomotor outflow and MAP after IH occur via an RAS related mechanism specifically involving the AT_1R, and oxidative stress. Blocking the AT_1R before IH exposure in humans has been shown to prevent increases increase in MSNA and MAP (Foster et al. 2010; Jouett et al. 2017). AT_1R blockade also reduces markers of oxidative stress after IH in humans (Pialoux et al. 2011) and in rodents reduces chemoreflex sensitization and sympathetic vasomotor outflow via a mechanism dependent upon NADPH oxidase superoxide production in the carotid body (Marcus et al. 2010; Morgan et al. 2016).

An increase in blood pressure occurs in some human studies using an acute IH exposure (Jouett et al. 2017; Leuenberger et al. 2005) but not all (Cutler, Swift, Keller, Wasmund, Smith 2004; Xie et al. 2000). The 5 ± 2 mmHg increase in MAP reported in the current study is the largest reported increase after an IH exposure of this length. Furthermore, we have observed that the blood pressure response is driven by both SBP and DBP, while

other studies of similar length have found it driven by SBP (Jouett et al. 2017) or only reported MAP (Leuenberger et al. 2005). The greater blood pressure response observed in the current study could be a result of hypercapnia added throughout the exposure, which has been associated with greater sympathetic activation and MAP compared with poikilocapnic hypoxia (Tamisier et al. 2004). Studies in humans with longer IH exposures are more reliably able induce larger increases in blood pressure outlasting the stimulus (Beaudin et al. 2014; Foster et al. 2010; Gilmartin et al. 2010; Tamisier et al. 2009; Tremblay et al. 2016).

5.5 Clinical Relevance

We designed our experimental IH exposure to mimic the pattern and magnitude of blood gas changes that occur in patients with severe OSA. Severe OSA is defined as an apnea/hypopnea index of > 30 events/hr during sleep (Fleetham et al. 2006). During each minute of IH, participants experienced a simultaneous 15% reduction in S_PO_2 and 4 ± 1 mmHg increase in $P_{ET}CO_2$ before baseline values were reestablished, simulating the blood gas changes of an OSA patient with an oxygen desaturation index of 60 events/hr. However, our protocol has distinct differences from OSA as our exposure was 40-minutes in duration, significantly shorter than nightly exposures in OSA, and we did not account for sleep state, arousal from sleep, apnea, and mueller maneuvers inducing negative intrathoracic pressures. Nonetheless, the present study and others have shown changes in sympathetic activity, blood pressure, ventilatory LTF, and, in animals, carotid body sensory LTF occur after as little as 30 minutes of IH exposure (Harris et al. 2006; Leuenberger et al. 2005; Roy et al. 2017; Xie et al. 2000). Also, studies in humans and animal models have identified IH as the primary OSA component responsible for cardiovascular consequences including hypertension (Dematteis et al. 2009). The acute 40-minute IH exposure was chosen for this investigation because microneurography is a sensitive procedure, requiring participants to remain completely still for the duration of the study.

OSA can progress for many years prior to diagnosis as people commonly do not report daytime sleepiness or other signs of sleep apnea until they become severe (Franklin and Lindberg 2015; Peppard et al. 2012). The amount of IH that someone with OSA experiences before diagnosis is unknown, and neurovascular transduction may be affected differently by chronic and acute IH. Therefore, our study focuses on the early adaptations that take place following acute IH. Although there are distinct differences between acute IH

and OSA, there is value in studying the direct effects of acute IH on sympathetic neurovascular transduction. The only study to measure sympathetic neurovascular transduction in OSA patients found it similar to controls, and 6-months of CPAP therapy had no effect on transduction (Tamisier et al. 2015). However, the results of our study indicate that just 40-minutes of IH is enough to stimulate a greater than 3-fold increase in transduction. Tamisier *et al. (2015)* had an average CPAP compliance of 4.5 hr/night, leaving OSA patients exposed to 2 or more hours of IH throughout an average night of sleep, which may have prevented changes in sympathetic neurovascular transduction. Furthermore, baseline transduction and blood pressure were similar in OSA and control groups, despite greater sympathetic vasomotor outflow. Normotensive patients with established OSA have a reduced forearm vasoconstrictive response to norepinephrine due to functional downregulation of vascular α and β_2 receptors (Grote, Kraiczi, Hedner 2000), and animals exposed to chronic IH also show reduced norepinephrine sensitivity (Phillips et al. 2006). Reduced adrenergic receptor density could act to reduce sympathetic neurovascular transduction and blood pressure under conditions of increased sympathetic vasomotor outflow typical in OSA.

It is also possible that mechanisms inducing hypertension in OSA patients are not the same as those responsible for maintenance. Animal models have demonstrated that chronic IH induces vascular inflammation and remodeling through hypoxia inducible factor-1 dependent increases in endothelin-1 expression and activation of nuclear factor-κB cytokine release (Gras et al. 2016). Augmented neurovascular transduction and sympathetic vasomotor outflow occur initially, while structural changes may maintain high blood pressure later in disease and may be more treatment resistant, as shown by the SAVE trial where CPAP was ineffective at preventing secondary cardiovascular events and mortality in OSA patients with established cardiovascular disease, even while most of these patients were on blood pressure medications including AT_1R blockers (McEvoy et al. 2016). Therefore, in order to prevent the development of hypertension, there is value in studying the acute effects of IH to determine mechanisms that could contribute to increased blood pressure.

5.6 Methodological Considerations

We measured sympathetic neurovascular transduction by increasing sympathetic vasomotor outflow with LBNP and observing the forearm vascular response. Specifically, we observed

the strength of the relationship between MSNA BF and FVR before and immediately after acute IH. This method has been used previously to determine the effect of race and fitness on sympathetic neurovascular transduction (Notarius et al. 2012; Ray and Monahan 2002), but we improved upon this method by using end-tidal forcing to control blood gases at baseline levels. This is essential as we demonstrated a graded ventilatory response to increasing LBNP that could have induced hypocapnia, which lowers sympathetic vasomotor outflow via reduced chemoreceptor stimulation and reduces peripheral vascular resistance through direct effects on the vessel (Burnum, Hickam, McIntosh 1954). Handgrip exercise, which also increases ventilation, has similarly been used instead of LBNP to increase MSNA BF for the measurement of sympathetic transduction (Halliwill, Taylor, Eckberg 1996; Minson et al. 2000; Tamisier et al. 2015) but these studies also did not control for blood gas changes during neurovascular transduction measurement. Compared to previous studies measuring sympathetic neurovascular transduction, clamping blood gases at baseline levels prevented distortion of the relationship between MSNA BF and FVR, allowing for more precise measurement that is chemoreflex independent.

Our sympathetic neurovascular transduction technique is limited in that we are unable to measure transduction in a purely resting state, and it does not consider the dynamic changes in transduction that occur in response to each burst of MSNA. Studies have investigated these burst-by-burst effects on subsequent changes in forearm and leg vascular conductance (Fairfax et al. 2013a; Fairfax et al. 2013b; Vranish et al. 2018), diastolic blood pressure (Briant et al. 2016), and a complex autoregressive model of transduction independent of blood pressure during handgrip exercise (Tan et al. 2012). The effect of individual MSNA bursts on vascular responses is a valid and accurate way to describe sympathetic neurovascular transduction and provides insight into the dynamic regulation of resting blood pressure. However, our measure of neurovascular transduction informs us of vascular responses over a wide range of sympathetic vasomotor activity. Using LBNP to achieve graded increases in sympathetic vasomotor outflow allow us to measure the sensitivity of the vasculature beyond the resting state.

Another limitation of our experimental design is that we are unable to account for changes in the contractile properties of vascular smooth muscle cells or endothelial cell function, as we did not include a control group that was not exposed to IH. In animal models,

chronic IH has been show to alter the mechanics of resistance arteries by reducing vessel wall distensibility and increasing stiffness (Phillips et al. 2006). In humans exposed to 6 hours of IH circumferential strain, a measure of arterial stiffness, is reduced in the common carotid artery but not the femoral artery, potentially indicating greater vessel stiffness (Tremblay et al. 2016). Also, endothelial cell function can be altered after a period of prolonged inactivity. Flow mediated dilation is reduced after seven days of dry water immersion (Navasiolava et al. 2010) and bed rest for 5 days reduces microvascular endothelial cell function (Hamburg et al. 2007). Prolonged sitting for 6 hours causes a reduction in forearm microvascular function (Restaino et al. 2015) and a reduction in brachial artery flow mediated dilation (Tremblay et al. 2016). Changes in vessel stiffness, contractility, or endothelial cell dysfunction could change the relationship of sympathetic vasomotor outflow and FVR independently of changes to sympathetic neurovascular transduction. Although this is a possibility, it is unlikely because the y-axis intercept of the relationship between FVR and MSNA BF was similar before and after IH indicating a similar predicted FVR with no sympathetic input (Figure 8).

A strength of our experimental exposure to IH is the addition of hypercapnia to better simulate the blood gas changes in OSA patients, to which we credit the large blood pressure response in comparison to studies of IH similar in length (Jouett et al. 2017; Leuenberger et al. 2005), as hypercapnic hypoxia stimulates a greater MAP response compared with poikilocapnic hypoxia (Tamisier et al. 2004). We are unable to conclude whether IH, intermittent hypercapnia, or the combination is responsible for augmenting sympathetic neurovascular transduction. However, hypercapnia has been demonstrated to have a role in ventilatory LTF, respiratory muscle and phrenic nerve LTF, and carotid body sensory LTF (Peng et al. 2003; Roy et al. 2017), all of which may have a role in controlling breathing stability and upper airway patency in OSA (Mahamed and Mitchell 2007; Mateika and Syed 2013). Therefore, it was important to include hypercapnia in our IH paradigm to better replicate changes in sympathetic neurovascular transduction that OSA patients may experience.

6 Conclusion

We found sympathetic neurovascular transduction was augmented immediately after acute exposure to IH, as measured by an increase in the sensitivity of FVR to sympathetic vasomotor outflow during LBNP. Additionally, we observed a progressive increase in $\dot{V}_I$ throughout and following IH suggesting ventilatory LTF. Finally, we confirmed previous reports where acute IH exposure augmented sympathetic vasomotor outflow, blood pressure, and FVR following IH. Importantly, our data suggest that changes in blood pressure following acute IH are not only related to increased central sympathetic vasomotor outflow but also to greater sympathetic neurovascular transduction. An early mechanism for the development of hypertension in OSA patients may be augmented sympathetic neurovascular transduction.

References

Abboud F, Kumar R. 2014. Obstructive sleep apnea and insight into mechanisms of sympathetic overactivity. J Clin Invest. 124(4):1454-7.

Bain AR, Smith KJ, Lewis NC, Foster GE, Wildfong KW, Willie CK, Hartley GL, Cheung SS, Ainslie PN. 2013. Regional changes in brain blood flow during severe passive hyperthermia: Effects of PaCO2 and extracranial blood flow. J Appl Physiol (1985). 115(5):653-9.

Bao G, Metreveli N, Li R, Taylor A, Fletcher EC. 1997. Blood pressure response to chronic episodic hypoxia: Role of the sympathetic nervous system. J Appl Physiol (1985). 83(1):95-101.

Barbé F, Durán-Cantolla J, Sánchez-de-la-Torre M,et al. 2012. Effect of continuous positive airway pressure on the incidence of hypertension and cardiovascular events in nonsleepy patients with obstructive sleep apnea: A randomized controlled trial. Jama. 307(20):2161-8.

Barbé F, Mayoralas LR, Duran J, Masa JF, Maimó A, Montserrat JM, Monasterio C, Bosch M, Ladaria A, Rubio M. 2001. Treatment with continuous positive airway pressure is not effective in patients with sleep apnea but no daytime sleepiness: A randomized, controlled trial. Ann Intern Med. 134(11):1015-23.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software. 67(1):1-48.

Beaudin AE, Pun M, Yang C, Nicholl DD, Steinback CD, Slater DM, Wynne-Edwards KE, Hanly PJ, Ahmed SB, Poulin MJ. 2014. Cyclooxygenases 1 and 2 differentially regulate blood pressure and cerebrovascular responses to acute and chronic intermittent hypoxia: Implications for sleep apnea. J Am Heart Assoc. 3(3):e000875.

Blasi A, Jo J, Valladares E, Morgan BJ, Skatrud JB, Khoo MC. 2003. Cardiovascular variability after arousal from sleep: Time-varying spectral analysis. J Appl Physiol (1985). 95(4):1394-404.

Blitzer ML, Lee SD, Creager MA. 1996. Endothelium-derived nitric oxide mediates hypoxic vasodilation of resistance vessels in humans. Am J Physiol. 271(3):H1182-5.

Bonnet M, Arand D. 1998. Heart rate variability in insomniacs and matched normal sleepers. Psychosom Med. 60(5):610-5.

Boukari R, Laouafa S, Ribon-Demars A, Bairam A, Joseph V. 2017. Ovarian steroids act as respiratory stimulant and antioxidant against the causes and consequences of sleep-apnea in women. Respiratory Physiology & Neurobiology. 239:46-8.

Bradley TD. 1992. Right and left ventricular functional impairment and sleep apnea. Clin Chest Med. 13(3):459-79.

Briant LJB, Burchell AE, Ratcliffe LEK, Charkoudian N, Nightingale AK, Paton JFR, Joyner MJ, Hart EC. 2016. Quantifying sympathetic neuro-haemodynamic transduction at rest in humans: Insights into sex, ageing and blood pressure control. J Physiol. 594(17):4753-68.

Brooks D, Horner RL, Kozar LF, Render-Teixeira CL, Phillipson EA. 1997. Obstructive sleep apnea as a cause of systemic hypertension. evidence from a canine model. J Clin Invest. 99(1):106-9.

Buck TM, Romero SA, Ely MR, Sieck DC, Abdala PM, Halliwill JR. 2015. Neurovascular control following small muscle-mass exercise in humans. Physiological Reports. 3(2):e12289.

Burnum JF, Hickam JB, McIntosh HD. 1954. The effect of hypocapnia on arterial blood pressure. Circulation. 9(1):89-95.

Byku M, Macarthur H, Westfall TC. 2008a. Angiotensin 1–7 decreases nerve stimulated norepinephrine (NE) and neuropeptide Y (NPY) overflow from the mesenteric arterial beds of SHR. The FASEB Journal. 22(1 Supplement):912.19,912.19.

Byku M, Macarthur H, Westfall TC. 2008b. Nerve stimulation induced overflow of neuropeptide Y and modulation by angiotensin II in spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol. 295(5):H2188-97.

Cai H, Griendling KK, Harrison DG. 2003. The vascular NAD (P) H oxidases as therapeutic targets in cardiovascular diseases. Trends Pharmacol Sci. 24(9):471-8.

Carlson JT, Hedner J, Elam M, Ejnell H, Sellgren J, Wallin BG. 1993. Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. Chest. 103(6):1763-8.

Chang W, Cheng J, Allaire J, Xie Y, McPherson J. 2015. Shiny: Web application framework for R. R Package Version 0.11. 1(4):106.

Charkoudian N, Joyner MJ, Sokolnicki L, Johnson C, Eisenach J, Dietz N, Curry TB, Wallin B. 2006. Vascular adrenergic responsiveness is inversely related to tonic activity of sympathetic vasoconstrictor nerves in humans. J Physiol (Lond). 572(3):821-7.

Cox S, Ben A, Story D, Ziogas J. 1995. Evidence for the involvement of different receptor subtypes in the pre-and postjunctional actions of angiotensin II at rat sympathetic neuroeffector sites. Br J Pharmacol. 114(5):1057-63.

Crapo RO, Morris AH. 1981. Standardized single breath normal values for carbon monoxide diffusing capacity. Am Rev Respir Dis. 123(2):185-9.

Crapo RO, Morris AH, Clayton PD, Nixon CR. 1982. Lung volumes in healthy nonsmoking adults. Bull Eur Physiopathol Respir. 18(3):419-25.

Cutler MJ, Swift NM, Keller DM, Wasmund WL, Smith ML. 2004. Hypoxia-mediated prolonged elevation of sympathetic nerve activity after periods of intermittent hypoxic apnea. J Appl Physiol. 96(2):754-61.

Cutler MJ, Swift NM, Keller DM, Wasmund WL, Burk JR, Smith ML. 2004. Periods of intermittent hypoxic apnea can alter chemoreflex control of sympathetic nerve activity in humans. American Journal of Physiology-Heart and Circulatory Physiology. 56(5):H2054.

da Silva, Ana Quenia Gomes, Fontes MAP, Kanagy NL. 2011. Chronic infusion of angiotensin receptor antagonists in the hypothalamic paraventricular nucleus prevents hypertension in a rat model of sleep apnea. Brain Res. 1368:231-8.

Davis JO, Freeman RH. 1976. Mechanisms regulating renin release. Physiol Rev. 56(1):1-56.

Deacon NL, McEvoy RD, Stadler DL, Catcheside PG. 2017. Intermittent hypercapnic hypoxia during sleep does not induce ventilatory long-term facilitation in healthy males. J Appl Physiol. 123(3):534-43.

Dematteis M, Godin-Ribuot D, Arnaud C, Ribuot C, Stanke-Labesque F, Pépin J, Lévy P. 2009. Cardiovascular consequences of sleep-disordered breathing: Contribution of animal models to understanding of the human disease. ILAR Journal. 50(3):262-81.

Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP. 2010. Pathophysiology of sleep apnea. Physiol Rev. 90(1):47-112.

Dendorfer A, Raasch W, Tempel K, Dominiak P. 2002. Comparison of the vascular and antiadrenergic activities of four angiotensin II type 1 antagonists in the pithed rat. J Hypertens. 20(6):1151-6.

Dhalla NS, Temsah RM, Netticadan T. 2000. Role of oxidative stress in cardiovascular diseases. J Hypertens. 18(6):655-73.

Diep TT, Khan TR, Zhang R, Duffin J. 2007. Long-term facilitation of breathing is absent after episodes of hypercapnic hypoxia in awake humans. Respiratory Physiology & Neurobiology. 156(2):132-6.

Dinenno FA, Dietz NM, Joyner MJ. 2002. Aging and forearm postjunctional alpha-adrenergic vasoconstriction in healthy men. Circulation. 106(11):1349-54.

Doyle AE, Black H. 1955. Reactivity to pressor agents in hypertension. Circulation. 12(6):974-80.

Duron E, Hanon O. 2008. Hypertension, cognitive decline and dementia. Archives of Cardiovascular Diseases. 101(3):181-9.

Eckhart AD, Zhu Z, Arendshorst WJ, Faber JE. 1996. Oxygen modulates alpha 1B-adrenergic receptor gene expression by arterial but not venous vascular smooth muscle. Am J Physiol. 271(4 Pt 2):H1599-608.

Egan B, Panis R, Hinderliter A, Schork N, Julius S. 1987. Mechanism of increased alpha adrenergic vasoconstriction in human essential hypertension. J Clin Invest. 80(3):812-7.

Ekblad E, Edvinsson L, Wahlestedt C, Uddman R, Håkanson R, Sundler F. 1984. Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibers. Regul Pept. 8(3):225-35.

Engleman HM, Gough K, Martin SE, Kingshott RN, Padfield PL, Douglas NJ. 1996. Ambulatory blood pressure on and off continuous positive airway pressure therapy for the sleep apnea/hypopnea syndrome: Effects in “non-dippers”. Sleep. 19(5):378-81.

Faccenda JF, Mackay TW, Boon NA, Douglas NJ. 2001. Randomized placebo-controlled trial of continuous positive airway pressure on blood pressure in the sleep apnea–hypopnea syndrome. American Journal of Respiratory and Critical Care Medicine. 163(2):344-8.

Fagius J, Wallin BG. 1993. Long-term variability and reproducibility of resting human muscle nerve sympathetic activity at rest, as reassessed after a decade. Clinical Autonomic Research. 3(3):201-5.

Fairfax ST, Padilla J, Vianna LC, Davis MJ, Fadel PJ. 2013a. Spontaneous bursts of muscle sympathetic nerve activity decrease leg vascular conductance in resting humans. Am J Physiol Heart Circ Physiol. 304(5):H759-66.

Fairfax ST, Holwerda SW, Credeur DP, Zuidema MY, Medley JH, Dyke II PC, Wray DW, Davis MJ, Fadel PJ. 2013b. The role of α-adrenergic receptors in mediating beat-by-beat sympathetic vascular transduction in the forearm of resting man. J Physiol (Lond). 591(14):3637-49.

Fatouleh RH, Hammam E, Lundblad LC, Macey PM, McKenzie DK, Henderson LA, Macefield VG. 2014. Functional and structural changes in the brain associated with the increase in muscle sympathetic nerve activity in obstructive sleep apnoea. NeuroImage: Clinical. 6:275-83.

Faulk K, Shell B, Nedungadi TP, Cunningham JT. 2017. Role of angiotensin-converting enzyme 1 within the median preoptic nucleus following chronic intermittent hypoxia. Am J Physiol Regul Integr Comp Physiol. 312(2):R245-52.

Fleetham J, Ayas N, Bradley D, Ferguson K, Fitzpatrick M, George C, Hanly P, Hill F, Kimoff J, Kryger M. 2006. Canadian thoracic society guidelines: Diagnosis and treatment of sleep disordered breathing in adults. Canadian Respiratory Journal. 13(7):387-92.

Fletcher EC. 2003. Sympathetic over activity in the etiology of hypertension of obstructive sleep apnea. Sleep. 26(1):15-9.

Fletcher EC, Bao G, Li R. 1999. Renin activity and blood pressure in response to chronic episodic hypoxia. Hypertension. 34(2):309-14.

Fletcher EC. 2000. Effect of episodic hypoxia on sympathetic activity and blood pressure. Respir Physiol. 119(2–3):189-97.

Floras J. 2016. Sympathetic nervous system in patients with sleep related breathing disorders. Current Hypertension Reviews. 12(1):18-26.

Forouzanfar MH, Alexander L, Anderson HR, Bachman VF, Biryukov S, Brauer M, Burnett R, Casey D, Coates MM, Cohen A. 2015. Global, regional, and national comparative

risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990–2013: A systematic analysis for the global burden of disease study 2013. The Lancet. 386(10010):2287-323.

Foster GE, Hanly PJ, Ahmed SB, Beaudin AE, Pialoux V, Poulin MJ. 2010. Intermittent hypoxia increases arterial blood pressure in humans through a renin-angiotensin system-dependent mechanism. Hypertension. 56(3):369-77.

Foster GE, Brugniaux JV, Pialoux V, Duggan CTC, Hanly PJ, Ahmed SB, Poulin MJ. 2009. Cardiovascular and cerebrovascular responses to acute hypoxia following exposure to intermittent hypoxia in healthy humans. J Physiol (Lond). 587:3287-99.

Franklin KA, Lindberg E. 2015. Obstructive sleep apnea is a common disorder in the population—a review on the epidemiology of sleep apnea. Journal of Thoracic Disease. 7(8):1311-22.

Gaisl T, Bratton DJ, Kohler M. 2015. The impact of obstructive sleep apnoea on the aorta. Eur Respir J. 46(2):532-44.

Gilmartin GS, Lynch M, Tamisier R, Weiss JW. 2010. Chronic intermittent hypoxia in humans during 28 nights results in blood pressure elevation and increased muscle sympathetic nerve activity. American Journal of Physiology-Heart and Circulatory Physiology. 299(3):H925-31.

Gorlach A, Diebold I, Schini-Kerth VB, Berchner-Pfannschmidt U, Roth U, Brandes RP, Kietzmann T, Busse R. 2001. Thrombin activates the hypoxia-inducible factor-1 signaling pathway in vascular smooth muscle cells: Role of the p22(phox)-containing NADPH oxidase. Circ Res. 89(1):47-54.

Gras E, Belaidi E, Briancon-Marjollet A, Pepin JL, Arnaud C, Godin-Ribuot D. 2016. Endothelin-1 mediates intermittent hypoxia-induced inflammatory vascular remodeling through HIF-1 activation. J Appl Physiol (1985). 120(4):437-43.

Griendling KK, Sorescu D, Lassegue B, Ushio-Fukai M. 2000. Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. Arterioscler Thromb Vasc Biol. 20(10):2175-83.

Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. 1994. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res. 74(6):1141-8.

Griffin HS, Pugh K, Kumar P, Balanos GM. 2012. Long-term facilitation of ventilation following acute continuous hypoxia in awake humans during sustained hypercapnia. J Physiol (Lond). 590(20):5151-65.

Grote L, Kraiczi H, Hedner J. 2000. Reduced α-and β2-adrenergic vascular response in patients with obstructive sleep apnea. American Journal of Respiratory and Critical Care Medicine. 162(4):1480-7.

Guyenet PG. 2000. Neural structures that mediate sympathoexcitation during hypoxia. Respir Physiol. 121(2):147-62.

Hagbarth K, Vallbo Å. 1968. Pulse and respiratory grouping of sympathetic impulses in human muscle nerves. Acta Physiologica. 74(1-2):96-108.

Halliwill J, Taylor JA, Eckberg DL. 1996. Impaired sympathetic vascular regulation in humans after acute dynamic exercise. J Physiol (Lond). 495(1):279-88.

Hamburg NM, McMackin CJ, Huang AL, Shenouda SM, Widlansky ME, Schulz E, Gokce N, Ruderman NB, Keaney JF,Jr, Vita JA. 2007. Physical inactivity rapidly induces insulin resistance and microvascular dysfunction in healthy volunteers. Arterioscler Thromb Vasc Biol. 27(12):2650-6.

Harris DP, Balasubramaniam A, Badr MS, Mateika JH. 2006. Long-term facilitation of ventilation and genioglossus muscle activity is evident in the presence of elevated levels of carbon dioxide in awake humans. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 291(4):R1111-9.

Hart EC, Charkoudian N, Wallin BG, Curry TB, Eisenach J, Joyner MJ. 2011. Sex and ageing differences in resting arterial pressure regulation: The role of the β-adrenergic receptors. J Physiol (Lond). 589(21):5285-97.

Hart EC, Joyner MJ, Wallin BG, Johnson CP, Curry TB, Eisenach JH, Charkoudian N. 2009. Age-related differences in the sympathetic-hemodynamic balance in men. Hypertension. 54(1):127-33.

Heagerty AM, Aalkjaer C, Bund SJ, Korsgaard N, Mulvany MJ. 1993. Small artery structure in hypertension. dual processes of remodeling and growth. Hypertension. 21(4):391-7.

Herr MD, Hogeman CS, Koch DW, Krishnan A, Momen A, Leuenberger UA. 2010. A real-time device for converting doppler ultrasound audio signals into fluid flow velocity. American Journal of Physiology-Heart and Circulatory Physiology. 298(5):H1626-32.

Hoang D, Macarthur H, Gardner A, Yang C, Westfall T. 2003. Prostanoid-induced modulation of neuropeptide Y and noradrenaline release from the rat mesenteric bed. Autonomic and Autacoid Pharmacology. 23(2):141-7.

Hoang D, Macarthur H, Gardner A, Westfall TC. 2002. Endothelin-induced modulation of neuropeptide Y and norepinephrine release from the rat mesenteric bed. American Journal of Physiology-Heart and Circulatory Physiology. 283(4):H1523-30.

Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu C, Liu C, Azen SP. 1998. The role of carotid arterial intima-media thickness in predicting clinical coronary events. Ann Intern Med. 128(4):262-9.

Hohl M, Linz B, Bohm M, Linz D. 2014. Obstructive sleep apnea and atrial arrhythmogenesis. Current Cardiology Reviews. 10(4):362-8.

Hu X, Fan J, Chen S, Yin Y, Zrenner B. 2015. The role of continuous positive airway pressure in blood pressure control for patients with obstructive sleep apnea and hypertension: A Meta-Analysis of randomized controlled trials. The Journal of Clinical Hypertension. 17(3):215-22.

Imadojemu VA, Mawji Z, Kunselman A, Gray KS, Hogeman CS, Leuenberger UA. 2007. Sympathetic chemoreflex responses in obstructive sleep apnea and effects of continuous positive airway pressure therapy. Chest. 131(5):1406-13.

Ip MS, Tse H, Lam B, Tsang KW, Lam W. 2004. Endothelial function in obstructive sleep apnea and response to treatment. American Journal of Respiratory and Critical Care Medicine. 169(3):348-53.

Jafar TH, Stark PC, Schmid CH, Landa M, Maschio G, de Jong PE, de Zeeuw D, Shahinfar S, Toto R, Levey AS. 2003. Progression of chronic kidney disease: The role of blood pressure control, proteinuria, and angiotensin-converting enzyme inhibition: A patient-level meta-analysis. Ann Intern Med. 139(4):244-52.

Jie K, van Brummelen P, Vermey P, Timmermans PB, van Zwieten PA. 1985. Alpha 1- and alpha 2-adrenoceptor mediated vasoconstriction in the forearm: Differences between normotensive and hypertensive subjects. J Hypertens Suppl. 3(3):S89-91.

Joo EY, Tae WS, Lee MJ, Kang JW, Park HS, Lee JY, Suh M, Hong SB. 2010. Reduced brain gray matter concentration in patients with obstructive sleep apnea syndrome. Sleep. 33(2):235-41.

Jordan AS, Catcheside PG, O'Donoghue FJ, McEvoy R. 2002. Long-term facilitation of ventilation is not present during wakefulness in healthy men or women. J Appl Physiol. 93(6):2129-36.

Jouett NP, Moralez G, Raven PB, Smith ML. 2017. Losartan reduces the immediate and sustained increases in muscle sympathetic nerve activity after hyper-acute intermittent hypoxia. J Appl Physiol. 122(4):884-8.

Khayat RN, Varadharaj S, Porter K, Sow A, Jarjoura D, Gavrilin MA, Zweier JL. 2017. Angiotensin receptor expression and vascular endothelial dysfunction in obstructive sleep apnea. American Journal of Hypertension. 31(3):355-6.

Kifor I, Dzau VJ. 1987. Endothelial renin-angiotensin pathway: Evidence for intracellular synthesis and secretion of angiotensins. Circ Res. 60(3):422-8.

Kim S, Iwao H. 2000. Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. Pharmacol Rev. 52(1):11-34.

Kline DD, Ramirez-Navarro A, Kunze DL. 2007. Adaptive depression in synaptic transmission in the nucleus of the solitary tract after in vivo chronic intermittent hypoxia: Evidence for homeostatic plasticity. J Neurosci. 27(17):4663-73.

Kneale BJ, Chowienczyk PJ, Brett SE, Coltart DJ, Ritter JM. 2000. Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature. J Am Coll Cardiol. 36(4):1233-8.

Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. 1983. Changes in the normal maximal expiratory flow-volume curve with growth and aging. Am Rev Respir Dis. 127(6):725-34.

Krousel-Wood M, Materson BJ, Whelton PK. 2007. Initial evaluation and approach to the patient with hypertension. In: Cardiovascular therapeutics: A companion to braunwald's heart disease. Elsevier Inc.

Kuznetsova A, Brockhoff PB, Christensen RH. 2017. lmerTest package: Tests in linear mixed effects models. Journal of Statistical Software. 82(13):1-26.

Lam S, Liu Y, Ng K, Liong EC, Tipoe GL, Leung PS, Fung M. 2014. Upregulation of a local renin–angiotensin system in the rat carotid body during chronic intermittent hypoxia. Exp Physiol. 99(1):220-31.

Launois SH, Abraham JH, Weiss JW, Kirby DA. 1998. Patterned cardiovascular responses to sleep and nonrespiratory arousals in a porcine model. J Appl Physiol (1985). 85(4):1285-91.

Lavie L. 2015. Oxidative stress in obstructive sleep apnea and intermittent hypoxia–revisited–the bad ugly and good: Implications to the heart and brain. Sleep Medicine Reviews. 20:27-45.

Lavie L. 2003. Obstructive sleep apnoea syndrome – an oxidative stress disorder. Sleep Medicine Reviews. 7(1):35-51.

Lavigne MC, Malech HL, Holland SM, Leto TL. 2001. Genetic demonstration of p47phox-dependent superoxide anion production in murine vascular smooth muscle cells. Circulation. 104(1):79-84.

Leke J, Fletcher EC, Bao G, Unger T. 1997. Hypertension caused by chronic intermittent hypoxia–influence of chemoreceptors and sympathetic nervous system. J Hypertens. 15(12):1593-603.

Leuenberger UA, Brubaker D, Quraishi S, Hogeman CS, Imadojemu VA, Gray KS. 2005. Effects of intermittent hypoxia on sympathetic activity and blood pressure in humans. Autonomic Neuroscience. 121(1):87-93.

Li JM, Shah AM. 2003. Mechanism of endothelial cell NADPH oxidase activation by angiotensin II. role of the p47phox subunit. J Biol Chem. 278(14):12094-100.

Li Y, Gao L, Zucker IH, Schultz HD. 2007. NADPH oxidase-derived superoxide anion mediates angiotensin II-enhanced carotid body chemoreceptor sensitivity in heart failure rabbits. Cardiovasc Res. 75(3):546-54.

London GM, Safar ME, Sassard JE, Levenson JA, Simon AC. 1984. Renal and systemic hemodynamics in sustained essential hypertension. Hypertension. 6(5):743-54.

Macarthur H, Wilken GH, Westfall TC, Kolo LL. 2011. Neuronal and non-neuronal modulation of sympathetic neurovascular transmission. Acta Physiologica. 203(1):37-45.

MacMahon S, Peto R, Collins R, Godwin J, Cutler J, Sorlie P, Abbott R, Neaton J, Dyer A, Stamler J. 1990. Blood pressure, stroke, and coronary heart disease: Part 1, prolonged differences in blood pressure: Prospective observational studies corrected for the regression dilution bias. The Lancet. 335(8692):765-74.

Mahamed S, Mitchell GS. 2007. Is there a link between intermittent hypoxia-induced respiratory plasticity and obstructive sleep apnoea? Exp Physiol. 92(1):27-37.

Malpas SC. 2010. Sympathetic nervous system overactivity and its role in the development of cardiovascular disease. Physiol Rev. 90(2):513-57.

Marcus NJ, Li Y, Bird CE, Schultz HD, Morgan BJ. 2010. Chronic intermittent hypoxia augments chemoreflex control of sympathetic activity: Role of the angiotensin II type 1 receptor. Respiratory Physiology & Neurobiology. 171(1):36-45.

Marcus NJ, Philippi NR, Bird CE, Li Y, Schultz HD, Morgan BJ. 2012. Effect of at(1) receptor blockade on intermittent hypoxia-induced endothelial dysfunction. Respiratory Physiology & Neurobiology. 183(2):67-74.

Martínez-Cerón E, Barquiel B, Bezos A, Casitas R, Galera R, García-Benito C, Hernanz A, Alonso-Fernández A, Garcia-Rio F. 2016. Effect of continuous positive airway pressure on glycemic control in patients with obstructive sleep apnea and type 2 diabetes. A randomized clinical trial. American Journal of Respiratory and Critical Care Medicine. 194(4):476-85.

Martínez-García M, Capote F, Campos-Rodríguez F, Lloberes P, de Atauri, María Josefa Díaz, Somoza M, Masa JF, González M, Sacristán L, Barbé F. 2013. Effect of CPAP on blood pressure in patients with obstructive sleep apnea and resistant hypertension: The HIPARCO randomized clinical trial. Jama. 310(22):2407-15.

Mateika JH, Syed Z. 2013. Intermittent hypoxia, respiratory plasticity and sleep apnea in humans: Present knowledge and future investigations. Respiratory Physiology & Neurobiology. 188(3):289-300.

Mateika JH, Sandhu KS. 2011. Experimental protocols and preparations to study respiratory long term facilitation. Respiratory Physiology & Neurobiology. 176(1-2):1-11.

McEvoy RD, Antic NA, Heeley E, Luo Y, Ou Q, Zhang X, Mediano O, Chen R, Drager LF, Liu Z, et al. 2016. CPAP for prevention of cardiovascular events in obstructive sleep apnea. N Engl J Med. 375(10):919-31.

Minoguchi K, Yokoe T, Tazaki T, Minoguchi H, Tanaka A, Oda N, Okada S, Ohta S, Naito H, Adachi M. 2005. Increased carotid intima-media thickness and serum inflammatory markers in obstructive sleep apnea. American Journal of Respiratory and Critical Care Medicine. 172(5):625-30.

Minson CT, Halliwill JR, Young TM, Joyner MJ. 2000. Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. Circulation. 101(8):862-8.

Mitchell GS, Johnson SM. 2003. Invited review: Neuroplasticity in respiratory motor control. J Appl Physiol. 94(1):358-74.

Møller DS, Lind P, Strunge B, Pedersen EB. 2003. Abnormal vasoactive hormones and 24-hour blood pressure in obstructive sleep apnea. American Journal of Hypertension. 16(4):274-80.

Morgan BJ, Denahan T, Ebert TJ. 1993. Neurocirculatory consequences of negative intrathoracic pressure vs. asphyxia during voluntary apnea. J Appl Physiol (1985). 74(6):2969-75.

Morgan BJ, Crabtree DC, Palta M, Skatrud JB. 1995. Combined hypoxia and hypercapnia evokes long-lasting sympathetic activation in humans. J Appl Physiol (1985). 79(1):205-13.

Morgan BJ, Crabtree DC, Puleo DS, Badr MS, Toiber F, Skatrud JB. 1996. Neurocirculatory consequences of abrupt change in sleep state in humans. J Appl Physiol (1985). 80(5):1627-36.

Morgan BJ, Dempsey JA, Pegelow DF, Jacques A, Finn L, Palta M, Skatrud JB, Young TB. 1998. Blood pressure perturbations caused by subclinical sleep-disordered breathing. Sleep. 21(7):737-46.

Morgan BJ, Bates ML, Rio RD, Wang Z, Dopp JM. 2016. Oxidative stress augments chemoreflex sensitivity in rats exposed to chronic intermittent hypoxia. Respiratory Physiology & Neurobiology. 234:47-59.

Nap A, Balt J, Mathy M, Van Zwieten P. 2003. AT1-receptor blockade and sympathetic neurotransmission in cardiovascular disease. Autonomic and Autacoid Pharmacology. 23(5-6):285-96.

Narkiewicz K, Somers VK. 1999. Obstructive sleep apnea as a cause of neurogenic hypertension. Curr Hypertens Rep. 1(3):268-73.

Narkiewicz K, van de Borne PJ, Cooley RL, Dyken ME, Somers VK. 1998. Sympathetic activity in obese subjects with and without obstructive sleep apnea. Circulation. 98(8):772-6.

Narkiewicz K, Kato M, Phillips BG, Pesek CA, Davison DE, Somers VK. 1999. Nocturnal continuous positive airway pressure decreases daytime sympathetic traffic in obstructive sleep apnea. Circulation. 100(23):2332-5.

Narkiewicz K, Montano N, Cogliati C, van de Borne PJ, Dyken ME, Somers VK. 1998. Altered cardiovascular variability in obstructive sleep apnea. Circulation. 98(11):1071-7.

Narkiewicz K, van dB, Montano N, Dyken ME, Phillips BG, Somers VK. 1998. Contribution of tonic chemoreflex activation to sympathetic activity and blood pressure in patients with obstructive sleep apnea. Circulation. 97(10):943.

Navasiolava NM, Dignat-George F, Sabatier F, Larina IM, Demiot C, Fortrat J, Gauquelin-Koch G, Kozlovskaya IB, Custaud M. 2010. Enforced physical inactivity increases endothelial microparticle levels in healthy volunteers. American Journal of Physiology-Heart and Circulatory Physiology. 299(2):H248-56.

Nerenberg KA, Zarnke KB, Leung AA, Dasgupta K, Butalia S, McBrien K, Harris KC, Nakhla M, Cloutier L, Gelfer M. 2018. Hypertension canada's 2018 guidelines for diagnosis, risk assessment, prevention, and treatment of hypertension in adults and children. Can J Cardiol. 34(5):506-19.

Nieto FJ, Young TB, Lind BK, Shahar E, Samet JM, Redline S, D'agostino RB, Newman AB, Lebowitz MD, Pickering TG. 2000. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. Jama. 283(14):1829-36.

Notarius CF, Murai H, Morris BL, Floras JS. 2012. Effect of fitness on reflex sympathetic neurovascular transduction in middle-age men. Med Sci Sports Exerc. 44(2):232-7.

O'Donnell CP, Ayuse T, King ED, Schwartz AR, Smith PL, Robotham JL. 1996. Airway obstruction during sleep increases blood pressure without arousal. J Appl Physiol (1985). 80(3):773-81.

Paul M, Poyan Mehr A, Kreutz R. 2006. Physiology of local renin-angiotensin systems. Physiol Rev. 86(3):747-803.

Pedrosa RP, Drager LF, Gonzaga CC, Sousa MG, de Paula LK, Amaro AC, Amodeo C, Bortolotto LA, Krieger EM, Bradley TD, et al. 2011. Obstructive sleep apnea: The most common secondary cause of hypertension associated with resistant hypertension. Hypertension. 58(5):811-7.

Peng Y, Yuan G, Jacono FJ, Kumar GK, Prabhakar NR. 2006. 5-HT evokes sensory long-term facilitation of rodent carotid body via activation of NADPH oxidase. J Physiol (Lond). 576(1):289-95.

Peng Y, Yuan G, Khan S, Nanduri J, Makarenko VV, Reddy VD, Vasavda C, Kumar GK, Semenza GL, Prabhakar NR. 2014. Regulation of hypoxia-inducible factor-α isoforms and redox state by carotid body neural activity in rats. J Physiol (Lond). 592(17):3841-58.

Peng Y, Overholt JL, Kline D, Kumar GK, Prabhakar NR. 2003. Induction of sensory long-term facilitation in the carotid body by intermittent hypoxia: Implications for recurrent apneas. Proc Natl Acad Sci U S A. 100(17):10073-8.

Peppard PE, Young T, Palta M, Skatrud J. 2000. Prospective study of the association between sleep-disordered breathing and hypertension. N Engl J Med. 342(19):1378-84.

Peppard PE, Young T, Barnet JH, Palta M, Hagen EW, Hla KM. 2012. Increased prevalence of sleep-disordered breathing in adults. Am J Epidemiol. 177(9):1006-14.

Phillips CL, McEwen BJ, Morel-Kopp MC, Yee BJ, Sullivan DR, Ward CM, Tofler GH, Grunstein RR. 2012. Effects of continuous positive airway pressure on coagulability in obstructive sleep apnoea: A randomised, placebo-controlled crossover study. Thorax. 67(7):639-44.

Phillips SA, Olson EB, Lombard JH, Morgan BJ. 2006. Chronic intermittent hypoxia alters NE reactivity and mechanics of skeletal muscle resistance arteries. J Appl Physiol (1985). 100(4):1117-23.

Pialoux V, Foster GE, Ahmed SB, Beaudin AE, Hanly PJ, Poulin MJ. 2011. Losartan abolishes oxidative stress induced by intermittent hypoxia in humans. J Physiol (Lond). 589:5529-37.

Pialoux V, Hanly PJ, Foster GE, Brugniaux JV, Beaudin AE, Hartmann SE, Pun M, Duggan CT, Poulin MJ. 2009. Effects of exposure to intermittent hypoxia on oxidative stress and acute hypoxic ventilatory response in humans. Am J Respir Crit Care Med. 180(10):1002-9.

Prabhakar NR. 2016. Carotid body chemoreflex: A driver of autonomic abnormalities in sleep apnoea. Exp Physiol. 101(8):975-85.

Prabhakar NR. 2000. Oxygen sensing by the carotid body chemoreceptors. J Appl Physiol (1985). 88(6):2287-95.

Public Health Agency of Canada. 2009. What is the impact of sleep apnea on canadians? Canada: Public Health Agency of Canada.

Pueyo ME, Arnal JF, Rami J, Michel JB. 1998. Angiotensin II stimulates the production of NO and peroxynitrite in endothelial cells. Am J Physiol. 274(1 Pt 1):C214-20.

Punjabi NM, Shahar E, Redline S, Gottlieb DJ, Givelber R, Resnick HE. 2004. Sleep-disordered breathing, glucose intolerance, and insulin resistance: The sleep heart health study. Am J Epidemiol. 160(6):521-30.

Purdy RE, Weber MA. 1988. Angiotensin II amplification of alpha-adrenergic vasoconstriction: Role of receptor reserve. Circ Res. 63(4):748-57.

Puvi-Rajasingham S, Smith GD, Akinola A, Mathias CJ. 1997. Abnormal regional blood flow responses during and after exercise in human sympathetic denervation. J Physiol (Lond). 505(3):841-9.

R Core Team. 2018. R: A language and environment for statistical computing. .

Ray CA, Monahan KD. 2002. Sympathetic vascular transduction is augmented in young normotensive blacks. J Appl Physiol. 92(2):651-6.

Restaino RM, Holwerda SW, Credeur DP, Fadel PJ, Padilla J. 2015. Impact of prolonged sitting on lower and upper limb micro-and macrovascular dilator function. Exp Physiol. 100(7):829-38.

Ringler J, Basner RC, Shannon R, Schwartzstein R, Manning H, Weinberger SE, Weiss JW. 1990. Hypoxemia alone does not explain blood pressure elevations after obstructive apneas. J Appl Physiol (1985). 69(6):2143-8.

Roy A, Farnham MM, Derakhshan F, Pilowsky PM, Wilson RJ. 2017. Acute intermittent hypoxia with concurrent hypercapnia evokes P2X and TRPV1 receptor dependent sensory long-term facilitation in naïve carotid bodies. J Physiol (Lond). .

Rudner XL, Berkowitz DE, Booth JV, Funk BL, Cozart KL, D'Amico EB, El-Moalem H, Page SO, Richardson CD, Winters B, et al. 1999. Subtype specific regulation of human vascular alpha(1)-adrenergic receptors by vessel bed and age. Circulation. 100(23):2336-43.

Ryan S, Taylor CT, McNicholas WT. 2005. Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. Circulation. 112(17):2660-7.

Saito S, Hirata Y, Emori T, Imai T, Marumo F. 1996. Angiotensin II activates endothelial constitutive nitric oxide synthase via AT1 receptors. Hypertension Research. 19(3):201-6.

Saxena A, Little JT, Nedungadi TP, Cunningham JT. 2015. Angiotensin II type 1a receptors in subfornical organ contribute towards chronic intermittent hypoxia-associated sustained increase in mean arterial pressure. Am J Physiol Heart Circ Physiol. 308(5):H435-46.

Schwarz EI, Puhan MA, Schlatzer C, Stradling JR, Kohler M. 2015. Effect of CPAP therapy on endothelial function in obstructive sleep apnoea: A systematic review and meta-analysis. Respirology. 20(6):889-95.

Schwarz EI, Schlatzer C, Rossi VA, Stradling JR, Kohler M. 2016. Effect of CPAP withdrawal on BP in OSA: Data from three randomized controlled trials. Chest. 150(6):1202-10.

Segal SS. 2005. Regulation of blood flow in the microcirculation. Microcirculation. 12(1):33-45.

Seidelin PH, Collier JG, Struthers AD, Webb DJ. 1991. Angiotensin II augments sympathetically mediated arteriolar constriction in man. Clin Sci (Lond). 81(2):261-6.

Severinghaus JW. 1979. Simple, accurate equations for human blood O2 dissociation computations. J Appl Physiol Respir Environ Exerc Physiol. 46(3):599-602.

Shahar E, Whitney CW, REdline S, Lee ET, Newman AB, Javier Nieto F, O'CONNOR GT, Boland LL, Schwartz JE, Samet JM. 2001. Sleep-disordered breathing and cardiovascular disease: Cross-sectional results of the sleep heart health study. American Journal of Respiratory and Critical Care Medicine. 163(1):19-25.

Shell B, Faulk K, Cunningham JT. 2016. Neural control of blood pressure in chronic intermittent hypoxia. Curr Hypertens Rep. 18(3):19.

Shimizu T, Takahashi Y, Suzuki K, Kogawa S, Tashiro T, Takahasi K, Hishikawa Y. 1992. Muscle nerve sympathetic activity during sleep and its change with arousal response. J Sleep Res. 1(3):178-85.

Shinohara E, Kihara S, Yamashita S, Yamane M, Nishida M, Arai T, Kotani K, Nakamura T, Takemura K, Matsuzawa Y. 1997. Visceral fat accumulation as an important risk factor for obstructive sleep apnoea syndrome in obese subjects. J Intern Med. 241(1):11-8.

Somers VK, Dyken ME, Clary MP, Abboud FM. 1995. Sympathetic neural mechanisms in obstructive sleep apnea. J Clin Invest. 96(4):1897-904.

Somers VK, Mark AL, Zavala DC, Abboud FM. 1989. Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans. J Appl Physiol (1985). 67(5):2101-6.

Somers VK, White DP, Amin R, Abraham WT, Costa F, Culebras A, Daniels S, Floras JS, Hunt CE, Olson LJ, et al. 2008. Sleep apnea and cardiovascular disease: An american heart association/american college of cardiology foundation scientific statement from the american heart association council for high blood pressure research professional education committee, council on clinical cardiology, stroke council, and council on cardiovascular nursing. in collaboration with the national heart, lung, and blood institute national center on sleep disorders research (national institutes of health). Circulation. 118(10):1080-111.

Spaak J, Egri ZJ, Kubo T, Yu E, Ando S, Kaneko Y, Usui K, Bradley TD, Floras JS. 2005. Muscle sympathetic nerve activity during wakefulness in heart failure patients with and without sleep apnea. Hypertension. 46(6):1327-32.

Story D, Ziogas J. 1987. Interaction of angiotensin with noradrenergic neuroeffector transmission. Trends Pharmacol Sci. 8(7):269-71.

Sundlöf G, Wallin B. 1978a. Human muscle nerve sympathetic activity at rest. relationship to blood pressure and age. J Physiol (Lond). 274(1):621-37.

Sundlöf G, Wallin BG. 1977. The variability of muscle nerve sympathetic activity in resting recumbent man. J Physiol (Lond). 272(2):383-97.

Sundlöf G, Wallin BG. 1978b. Muscle–Nerve sympathetic activity in man. relationship to blood pressure in resting normo- and hyper-tensive subjects. Clin Sci. 55(s4):387s-9s.

Syed Z, Lin H, Mateika JH. 2012. The impact of arousal state, sex, and sleep apnea on the magnitude of progressive augmentation and ventilatory long-term facilitation. J Appl Physiol. 114(1):52-65.

Takeuchi S, Iwase S, Mano T, Okada H, Sugiyama Y, Watanabe T. 1994. Sleep-related changes in human muscle and skin sympathetic nerve activities. J Auton Nerv Syst. 47(1-2):121-9.

Tamisier R, Nieto L, Anand A, Cunnington D, Weiss JW. 2004. Sustained muscle sympathetic activity after hypercapnic but not hypocapnic hypoxia in normal humans. Respiratory Physiology & Neurobiology. 141(2):145-55.

Tamisier R, Gilmartin GS, Launois SH, Pépin J, Nespoulet H, Thomas R, Levy P, Weiss JW. 2009. A new model of chronic intermittent hypoxia in humans: Effect on ventilation, sleep, and blood pressure. J Appl Physiol. 107(1):17-24.

Tamisier R, Pepin JL, Remy J, Baguet JP, Taylor JA, Weiss JW, Levy P. 2011. 14 nights of intermittent hypoxia elevate daytime blood pressure and sympathetic activity in healthy humans. Eur Respir J. 37(1):119-28.

Tamisier R, Tan CO, Pepin J, Levy P, Taylor JA. 2015. Blood pressure increases in OSA due to maintained neurovascular sympathetic transduction: Impact of CPAP. Sleep. 38(12):1973-80.

Tan CO, Tamisier R, Hamner JW, Taylor JA. 2012. Characterizing sympathetic neurovascular transduction in humans. PLoS ONE. 8(1):e53769.

Tan CO, Tzeng Y, Hamner JW, Tamisier R, Taylor JA. 2013. Alterations in sympathetic neurovascular transduction during acute hypoxia in humans. Am J Physiol Regul Integr Comp Physiol. 304(11):R959.

Taylor KS, Murai H, Millar PJ, Haruki N, Kimmerly DS, Morris BL, Tomlinson G, Bradley TD, Floras JS. 2016. Arousal from sleep and sympathetic excitation during wakefulness. Hypertension. 68(6):1467-74.

Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, Green DJ. 2011. Assessment of flow-mediated dilation in humans: A methodological and physiological guideline. Am J Physiol Heart Circ Physiol. 300(1):H2-12.

Tozawa M, Iseki K, Iseki C, Kinjo K, Ikemiya Y, Takishita S. 2003. Blood pressure predicts risk of developing end-stage renal disease in men and women. Hypertension. 41(6):1341-5.

Tremblay JC, Boulet LM, Tymko MM, Foster GE. 2016. Intermittent hypoxia and arterial blood pressure control in humans: Role of the peripheral vasculature and carotid baroreflex. Am J Physiol Heart Circ Physiol. 311(3):H699-706.

Tymko MM, Ainslie PN, MacLeod DB, Willie CK, Foster GE. 2015. End tidal-to-arterial CO2 and O2 gas gradients at low- and high-altitude during dynamic end-tidal forcing. Am J Physiol Regul Integr Comp Physiol. 308(11):R895-906.

Tymko MM, Hoiland RL, Kuca T, Boulet LM, Tremblay JC, Pinske BK, Williams AM, Foster GE. 2016. Measuring the human ventilatory and cerebral blood flow response to CO_2: A technical consideration for the end-tidal-to-arterial gas gradient. J Appl Physiol. 120(2):282-96.

Ushio-Fukai M, Griendling KK, Becker PL, Hilenski L, Halleran S, Alexander RW. 2001. Epidermal growth factor receptor transactivation by angiotensin II requires reactive oxygen species in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol. 21(4):489-95.

Vranish JR, Holwerda SW, Young BE, Credeur DP, Patik JC, Barbosa TC, Keller DM, Fadel PJ. 2018. Exaggerated vasoconstriction to spontaneous bursts of muscle sympathetic nerve activity in healthy young black men. Hypertension. 71(1):192-8.

Wahlestedt C, Reis DJ. 1993. Neuropeptide Y-related peptides and their receptors--are the receptors potential therapeutic drug targets? Annu Rev Pharmacol Toxicol. 33(1):309-52.

Wallin BG, Nerhed C. 1982. Relationship between spontaneous variations of muscle sympathetic activity and succeeding changes of blood pressure in man. J Auton Nerv Syst. 6(3):293-302.

Waradekar NV, Sinoway LI, Zwillich CW, Leuenberger UA. 1996. Influence of treatment on muscle sympathetic nerve activity in sleep apnea. Am J Respir Crit Care Med. 153(4 Pt 1):1333-8.

White DW, Shoemaker JK, Raven PB. 2015. Methods and considerations for the analysis and standardization of assessing muscle sympathetic nerve activity in humans. Autonomic Neuroscience: Basic and Clinical. 193:12-21.

White SG, Fletcher EC, Miller CC,3rd. 1995. Acute systemic blood pressure elevation in obstructive and nonobstructive breath hold in primates. J Appl Physiol (1985). 79(1):324-30.

Wolkove N, Baltzan M, Kamel H, Dabrusin R, Palayew M. 2008. Long-term compliance with continuous positive airway pressure in patients with obstructive sleep apnea. Canadian Respiratory Journal. 15(7):365-9.

Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Mori TA, et al. 2001. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. J Appl Physiol (1985). 91(2):929-37.

World Health Organization. 2009. Global health risks: Mortality and burden of disease attributable to selected major risks. World Health Organization.

Wu J, Stefaniak J, Hafner C, Schramel JP, Kaun C, Wojta J, Ullrich R, Tretter VE, Markstaller K, Klein KU. 2016. Intermittent hypoxia causes inflammation and injury to human adult cardiac myocytes. Anesth Analg. 122(2):373-80.

Xie A, Skatrud JB, Crabtree DC, Puleo DS, Goodman BM, Morgan BJ. 2000. Neurocirculatory consequences of intermittent asphyxia in humans. J Appl Physiol (1985). 89(4):1333-9.

Xie A, Skatrud JB, Puleo DS, Morgan BJ. 2001. Exposure to hypoxia produces long-lasting sympathetic activation in humans. J Appl Physiol. 91(4):1555-62.

Yamada Y, Miyajima E, Tochikubo O, Matsukawa T, Ishii M. 1989. Age-related changes in muscle sympathetic nerve activity in essential hypertension. Hypertension. 13(6 Pt 2):870-7.

Yang D, Liu Z, Yang H, Luo Q. 2013. Effects of continuous positive airway pressure on glycemic control and insulin resistance in patients with obstructive sleep apnea: A meta-analysis. Sleep and Breathing. 17(1):33-8.

Young T, Peppard PE, Gottlieb DJ. 2002. Epidemiology of obstructive sleep apnea: A population health perspective. American Journal of Respiratory and Critical Care Medicine. 165(9):1217-39.

Yuan G, Khan SA, Luo W, Nanduri J, Semenza GL, Prabhakar NR. 2011. Hypoxia-inducible factor 1 mediates increased expression of NADPH oxidase-2 in response to intermittent hypoxia. J Cell Physiol. 226(11):2925-33.

Appendices

Appendix A: Forms

Inclusion/Exclusion Assessment

THIS INDIVIDUAL MAY BE A CANDIDATE FOR THE INTERMITTENT HYPOXIA AND NEUROVASCULAR TRANSDUCTION STUDY

PLEASE DISCUSS WITH VOLUNTEER

INCLUSION CRITERIA (all must be yes):	**YES**	**NO**
AGE 18-45 years	☐	☐
Body mass index < 30 kg/m^2	☐	☐
Normotensive (<140/90, >90/60)	☐	☐

EXCLUSION CRITERIA (all must be no)	**YES**	**NO**
≥5 hypopnea/hour during sleep	☐	☐
Known hypertension	☐	☐
Known impaired renal function	☐	☐
Known liver disease	☐	☐
Known heart failure	☐	☐
Known myocardial infarct	☐	☐
Known coronary artery disease	☐	☐
Known history of stroke	☐	☐
Known COPD or Asthma	☐	☐
Known central sleep apnea	☐	☐
Known obstructive sleep apnea	☐	☐
Taking any medications (over the counter or prescribed)	☐	☐
Smoker (within last year)	☐	☐

NOT INTERESTED ☐ **INTERESTED IN PARTICIPATION** ☐

For more information contact Troy Stuckless:
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250.807.8083

Subject Demographics and Prescreening Questionnaire

OKANAGAN

Subject Demographics and Prescreening Questionnaire:

The effect of intermittent hypoxia on neurovascular transduction

Subject Identification Code: _____ Race:_____ or circle: Prefer not to disclose

Weight (Kg): _____ Height (cm): _____ BMI: _____

Age (years): _____ Time of last meal: _____

Please answer **Yes/No** for each question (if yes, please explain):

Have you consumed caffeine, alcohol or completed vigorous exercise within the 12 hours prior to the experimental visit?
YES NO

Do you have a history of fainting or have ever experienced a syncopal episode?
YES NO

Do you have a previous history of or a current liver disease or abnormality?
YES NO

Do you have a previous history of or a current respiratory disease or abnormality (e.g., asthma, chronic bronchitis, cystic fibrosis, chronic obstructive pulmonary disease)?
YES NO

Do you have a previous history of or a current cardiovascular disease or abnormality (e.g., cardiac arrhythmia, hypertension, myocardial infarction)?
YES NO

Do you have a previous history of or a current neurological disease or abnormality (e.g., epilepsy, chronic migraines, stroke)?
YES NO

Are you currently on any kind of medication, over the counter or prescribed?
YES NO

Do you have type I or II diabetes?
YES NO

Do you partake in regular physical activity (e.g., moderate physical activity 3-5 days/ week)?
YES NO

Do you smoke?
YES NO

Do you have any drug allergies?
YES NO

Have you had all of your questions or concerns addressed?
YES NO

Appendix B: Individual Raw Data Tables and Figures

Subject Characteristics

Table 6. Individual subject demographics.

Subject ID	Race	Height (cm)	Weight (kg)	Age (yrs)	BMI (kg/m^2)
6	Caucasian	170	72	23	24.9
7	Caucasian	183	102	22	30.5
13	Asian	169	78	19	27.3
14	Caucasian	188	96	37	27.0
15	Caucasian	167	73	22	26.2
16	Caucasian	177	98	22	31.1
17	Hispanic	161	63	21	24.3
20	Caucasian	173	69	21	23.1
21	Caucasian	182	86	21	26.0
22	First Nations	179	64	20	20.0
Mean	-	**174.9**	**80.0**	**22.8**	**26.0**
SEM	-	2.7	4.5	1.6	1.0

Abbreviations: SEM, standard error of the mean.

Table 7. Individual subject sleep study data.

Subject ID	**Sleep Duration (hr)**	**ODI (/hr)**	**Basal S_PO_2 (%)**	**Mean Min S_PO_2 (%)**	**% of Time <95%S_PO_2**	**% of Time <90%S_PO_2**	**% of Time <85%S_PO_2**
6	4.8	2.7	96.0	93.2	7.6	0.0	0.0
7	7.4	4.0	95.6	91.9	6.5	0.2	0.0
13	7.7	1.6	95.6	91.4	6.3	0.1	0.1
14	9.2	3.3	93.7	90.3	80.4	0.4	0.0
15	8.0	2.2	94.8	92.3	38.3	0.0	0.0
16	6.4	0.6	96.0	92.0	5.2	0.0	0.0
17	7.6	2.6	95.1	92.4	20.2	0.0	0.0
20	9.1	3.2	96.2	92.4	7.9	0.0	0.0
21	9.2	1.8	96.1	93.5	3.0	0.1	0.0
22	14.5	1.2	95.9	93.5	4.0	0.0	0.0
Mean	**8.4**	**2.3**	**95.5**	**92.3**	**17.9**	**0.08**	**0.0**
SEM	0.8	0.3	0.2	0.3	7.7	0.04	0.0

Abbreviations: ODI, oxygen desaturation index; S_PO_2, peripheral oxyhemoglobin saturation; SEM, standard error of the mean.

Table 8. Individual pulmonary function test and lung volume data in absolute values and % of predicted reference value.

Subject ID	**FVC (l) (%)**	**FEV_1 (l) (%)**	**FEV_1/FVC (%)**	**TLC (l) (%)**	**VC (l) (%)**	**FRC (l) (%)**	**DLCO (ml/min/mmHg) (%)**	**V_A (l) (%)**	**DLCO/V_A (ml/min/mmHg/l) (%)**
6	5.6 (109.0)	4.5 (103.0)	80.0 (94.0)	6.5 (102.0)	5.6 (110.0)	3.0 (100.0)	28.0 (78.0)	6.1 (95.0)	4.6 (83.0)
7	5.9 (101.0)	4.2 (87.0)	71.0 (86.0)	6.5 (89.0)	5.9 (101.0)	2.7 (77.0)	36.2 (96.0)	6.4 (90.0)	5.6 (107.0)
13	5.6 (121.0)	4.0 (102.0)	74.0 (84.0)	6.4 (120.0)	5.6 (122.0)	2.9 (109.0)	32.5 (113.0)	6.1 (92.0)	5.3 (99.0)
14	5.4 (93.0)	4.3 (91.0)	79.0 (98.0)	7.3 (94.0)	5.7 (97.0)	2.9 (73.0)	32.1 (91.0)	7.0 (94.0)	4.6 (97.0)
15	3.7 (73.0)	3.1 (73.0)	86.0 (101.0)	4.5 (75.0)	3.9 (79.0)	1.9 (66.0)	33.0 (93.0)	4.3 (69.0)	7.7 (136.0)
16	5.5 (101.0)	3.9 (84.0)	70.0 (83.0)	7.0 (103.0)	5.6 (101.0)	2.5 (76.0)	39.6 (107.0)	6.3 (92.0)	6.3 (116.0)
17	4.6 (100.0)	4.2 (107.0)	91.0 (107.0)	6.8 (116.0)	4.8 (104.0)	2.6 (94.0)	30.2 (84.0)	5.6 (95.0)	5.4 (91.0)
20	6.1 (116.0)	4.4 (99.0)	72.0 (85.0)	7.6 (117.0)	6.5 (122.0)	3.3 (109.0)	38.1 (104.0)	7.1 (108.0)	5.4 (96.0)
21	6.3 (109.0)	5.0 (104.0)	79.0 (94.0)	8.1 (112.0)	6.4 (110.0)	4.7 (135.0)	47.2 (124.0)	7.9 (111.0)	6.0 (112.0)
22	5.9 (103.0)	4.7 (96.0)	79.0 (93.0)	9.3 (123.0)	6.0 (105.0)	3.8 (104.0)	43.2 (104.0)	7.6 (101.0)	5.7 (102.0)
Mean	**5.5 (102.6)**	**4.2 (94.6)**	**78.1 (92.5)**	**7.0 (105.1)**	**5.6 (105.1)**	**3.0 (94.3)**	**36.0 (99.4)**	**6.4 (94.7)**	**5.7 (103.9)**
SEM	0.3 (4.2)	0.2 (3.4)	2.1 (2.5)	0.4 (4.9)	0.2 (3.9)	0.2 (6.8)	1.9 (4.4)	0.3 (3.6)	0.3 (4.7)

Abbreviations: FVC, forced vital capacity; FEV_1, forced expired volume in one second; TLC, total lung capacity; VC, vital capacity; FRC, functional residual capacity; D_LCO, diffusion capacity of the lung for carbon monoxide transfer; V_A, alveolar volume; D_LCO/V_A, D_LCO corrected for alveolar volume; SEM, standard error of the mean.

Lower Body Negative Pressure Testing

Table 9. Individual tidal volume at each target level of lower body negative pressure, before and after intermittent hypoxia.

	Pre V_T (l)				Post V_T (l)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	0.47	0.49	0.57	0.70	0.30	0.31	0.33	0.33
7	1.24	1.39	1.57	1.76	1.27	1.49	1.51	1.39
13	0.91	0.94	0.57	1.36	1.70	2.34	2.26	2.02
14	1.83	1.88	1.97	1.98	1.87	1.73	1.93	2.03
15	1.25	1.03	1.03	1.39	1.20	1.20	1.26	1.49
16	1.65	1.29	1.26	1.74	1.03	0.97	0.92	1.11
17	1.38	1.52	1.46	1.50	1.34	1.42	1.44	1.63
20	1.31	1.28	1.63	2.04	1.19	1.39	1.44	1.77
21	1.49	1.24	1.28	1.54	1.20	1.07	1.36	1.72
22	1.04	1.13	1.27	1.20	1.03	1.21	1.27	1.48
Mean	**1.26**	**1.22**	**1.26**	**1.52**	**1.21**	**1.32**	**1.37**	**1.50**
SEM	0.12	0.12	0.14	0.12	0.13	0.17	0.16	0.16

Abbreviations: IH, intermittent hypoxia; V_T, tidal volume; SEM, standard error of the mean.

Table 10. Individual breathing frequency during lower body negative pressure, before and after intermittent hypoxia.

	Pre f_B (/min)				Post f_B (/min)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	17.2	15.5	17.4	16.7	16.6	15.3	14.7	17.5
7	15.9	13.9	12.5	11.5	17.6	15.4	17.8	19.0
13	14.9	16.2	13.8	11.0	9.8	6.9	7.5	8.1
14	4.9	7.6	9.5	12.6	12.4	14.1	14.6	16.9
15	8.7	11.7	12.9	12.5	13.5	15.3	16.3	16.2
16	6.3	8.3	9.5	10.4	14.1	14.2	15.8	16.0
17	8.4	9.1	11.8	11.6	16.3	15.0	16.0	14.6
20	7.4	7.4	7.9	6.5	12.0	10.4	9.5	11.3
21	10.0	11.5	11.4	10.4	11.8	12.6	10.2	10.4
22	8.9	7.3	6.9	8.6	8.7	7.6	9.3	8.9
Mean	**10.3**	**10.8**	**11.4**	**11.2**	**13.3**	**12.7**	**13.2**	**13.9**
SEM	1.3	1.1	1.0	0.8	0.9	1.0	1.2	1.2

Abbreviations: IH, intermittent hypoxia; f_B, breathing frequency; SEM, standard error of the mean.

Table 11. Individual inspired ventilation during lower body negative pressure, before and after intermittent hypoxia.

	Pre $\dot{V}_I$ (l/min)				Post $\dot{V}_I$ (l/min)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	9.0	8.5	10.3	12.7	5.6	5.2	5.5	6.4
7	21.7	21.2	21.8	22.1	24.3	25.1	29.7	29.3
13	14.7	16.4	8.6	16.5	17.8	17.8	18.6	18.1
14	9.8	15.8	20.7	27.5	25.4	26.7	31.1	37.8
15	11.9	13.3	14.7	19.3	17.1	20.3	22.4	26.6
16	9.9	11.6	13.2	20.2	15.9	15.3	15.9	19.5
17	12.7	15.1	19.0	19.2	24.0	23.6	25.5	26.2
20	10.5	9.9	14.2	14.6	14.4	14.0	13.7	19.8
21	12.9	11.2	15.1	17.1	14.6	14.3	15.2	19.2
22	9.6	8.6	9.7	11.1	9.7	10.2	12.6	14.7
Mean	**12.3**	**13.2**	**14.7**	**18.0**	**16.9**	**17.2**	**19.0**	**21.8**
SEM	1.2	1.3	1.5	1.5	2.0	2.2	2.6	2.7

Abbreviations: IH, intermittent hypoxia; $\dot{V}_I$, inspired ventilation; SEM, standard error of the mean.

Table 12. Individual partial pressure of end-tidal oxygen during lower body negative pressure, before and after intermittent hypoxia.

	Pre $P_{ET}O_2$ (mmHg)				Post $P_{ET}O_2$ (mmHg)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	92.1	94.9	91.3	91.2	94.4	92.6	92.9	92.4
7	89.5	89.1	89.8	89.0	91.3	89.8	90.0	89.4
13	91.3	88.1	103.0	110.7	88.0	90.8	114.7	112.6
14	85.8	86.2	87.6	85.6	85.8	86.3	86.3	85.7
15	91.2	99.5	95.2	100.2	101.2	101.5	98.1	101.4
16	99.2	97.2	95.5	95.3	93.1	90.4	93.7	96.3
17	85.5	84.5	86.4	90.5	86.1	86.1	86.5	86.4
20	90.3	100.3	92.3	94.5	91.1	90.1	101.5	90.7
21	85.8	85.8	85.9	85.9	88.2	81.7	89.8	85.9
22	89.7	84.7	94.0	87.1	85.6	87.6	85.5	87.7
Mean	**90.0**	**91.0**	**92.1**	**93.0**	**90.5**	**89.7**	**93.9**	**92.8**
SEM	1.3	2.0	1.6	2.4	1.5	1.6	2.8	2.7

Abbreviations: IH, intermittent hypoxia; $P_{ET}O_2$, partial pressure of end tidal oxygen; SEM, standard error of the mean.

Table 13. Individual partial pressure of end-tidal carbon dioxide during lower body negative pressure before and after intermittent hypoxia.

	Pre $P_{ET}CO_2$ (mmHg)				Post $P_{ET}CO_2$ (mmHg)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	39.3	39.1	38.8	39.2	39.1	38.5	38.6	38.7
7	39.0	39.2	39.0	39.3	39.0	39.3	39.4	39.6
13	43.4	42.4	37.8	34.3	43.0	45.0	41.0	38.6
14	40.5	41.3	41.0	40.7	40.7	40.5	40.8	40.7
15	41.4	39.8	38.0	41.3	39.6	39.9	39.7	40.3
16	38.4	36.8	36.2	37.6	36.9	37.8	36.6	37.2
17	42.9	43.5	43.6	43.4	43.1	43.3	43.1	42.9
20	38.6	38.7	38.9	38.1	37.8	38.6	38.0	38.6
21	43.7	41.8	42.5	41.9	42.7	42.7	43.1	42.9
22	40.9	41.9	40.8	41.1	40.7	40.5	40.8	41.3
Mean	**40.8**	**40.5**	**39.7**	**39.7**	**40.3**	**40.6**	**40.1**	**40.1**
SEM	0.6	0.6	0.7	0.8	0.7	0.7	0.7	0.6

Abbreviations: IH, intermittent hypoxia; $P_{ET}CO_2$, partial pressure of end tidal carbon dioxide; SEM, standard error of the mean.

Table 14. Individual peripheral oxyhemoglobin saturation during lower body negative pressure, before and after intermittent hypoxia.

	Pre S_pO_2 (%)				Post S_pO_2 (%)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	96.2	96.5	96.5	95.8	97.2	97.4	96.7	97.0
7	96.4	96.4	97.1	97.1	97.4	97.4	97.4	97.3
13	96.1	96.1	97.4	97.5	96.7	97.4	97.5	97.4
14	97.4	97.4	97.4	97.4	97.4	97.4	97.6	97.4
15	95.4	96.4	95.8	95.9	96.3	96.7	96.6	96.7
16	96.6	97.0	96.3	96.7	96.6	97.5	97.4	97.4
17	96.4	95.1	95.8	96.8	97.1	96.7	97.0	96.3
20	98.4	98.4	98.1	98.4	98.4	98.2	97.8	97.4
21	96.3	95.6	95.8	95.2	96.9	97.2	97.3	96.7
22	95.6	95.9	97.0	95.4	96.2	97.4	97.4	96.9
Mean	**96.5**	**96.5**	**96.7**	**96.6**	**97.0**	**97.3**	**97.3**	**97.1**
SEM	0.3	0.3	0.3	0.3	0.2	0.1	0.1	0.1

Abbreviations: IH, intermittent hypoxia; S_pO_2, peripheral oxyhemoglobin saturation; SEM, standard error of the mean.

Table 15. Individual systolic blood pressure during lower body negative pressure, before and after intermittent hypoxia.

	Pre SBP (mmHg)				Post SBP (mmHg)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	123.6	127.9	128.1	123.1	138.3	135.5	134.4	135.9
7	117.1	120.8	115.9	122.0	132.1	133.4	139.5	145.8
13	129.7	124.6	113.8	103.8	127.3	113.9	114.6	107.9
14	138.8	132.1	135.8	131.3	144.7	142.6	143.6	136.4
15	127.4	129.8	119.2	119.9	135.6	135.6	130.4	128.6
16	134.8	134.8	126.7	123.9	135.8	141.7	136.9	134.8
17	108.8	111.6	101.6	93.7	117.8	124.0	116.9	108.1
20	122.2	116.6	111.9	104.2	122.8	115.1	110.1	111.9
21	140.5	133.3	127.7	129.2	139.3	141.0	136.3	135.4
22	134.6	132.4	115.8	115.2	149.3	145.8	141.5	130.9
Mean	**127.7**	**126.4**	**119.7**	**116.6**	**134.3**	**132.9**	**130.4**	**127.6**
SEM	3.2	2.5	3.2	3.9	3.0	3.6	3.8	4.2

Abbreviations: IH, intermittent hypoxia; SBP, systolic blood pressure; SEM, standard error of the mean.

Table 16. Individual diastolic blood pressure during lower body negative pressure, before and after intermittent hypoxia.

	Pre DBP (mmHg)				Post DBP (mmHg)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	67.8	71.7	76.2	75.5	72.2	74.6	78.5	81.7
7	70.0	72.5	72.5	73.7	74.3	75.1	82.6	84.5
13	63.8	63.9	62.4	58.0	67.0	63.0	64.2	62.2
14	77.6	78.1	81.2	80.4	83.4	85.8	87.5	84.4
15	78.0	80.0	78.4	78.9	84.6	86.7	86.3	85.4
16	78.2	81.2	81.3	80.5	79.8	82.7	84.6	85.5
17	55.8	59.4	56.6	51.6	59.5	64.7	66.2	60.8
20	58.0	57.0	56.8	54.1	60.4	59.6	57.0	59.9
21	72.4	68.7	69.4	73.0	74.3	74.9	75.6	79.5
22	70.2	68.8	67.1	71.0	80.5	79.6	81.8	80.3
Mean	**69.2**	**70.1**	**70.2**	**69.7**	**73.6**	**74.7**	**76.4**	**76.4**
SEM	2.5	2.6	3.0	3.5	2.8	3.0	3.3	3.4

Abbreviations: IH, intermittent hypoxia; DBP, diastolic blood pressure; SEM, standard error of the mean.

Table 17. Individual mean arterial pressure during lower body negative pressure, before and after intermittent hypoxia.

	Pre MAP (mmHg)				Post MAP (mmHg)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	88.1	91.8	95.2	93.0	94.3	95.3	97.8	100.1
7	87.1	89.7	87.5	88.6	95.4	95.1	103.0	104.9
13	86.8	84.8	81.2	74.7	88.5	80.7	81.5	78.4
14	98.5	95.7	98.6	96.7	104.2	103.7	105.2	101.1
15	95.4	97.6	92.9	92.6	103.5	105.0	102.1	100.5
16	97.1	99.5	96.7	95.9	98.9	103.2	102.5	101.8
17	75.6	78.2	71.9	66.5	79.0	84.0	82.2	76.1
20	78.1	76.0	74.2	70.5	80.3	77.5	74.6	77.9
21	93.9	90.1	89.7	92.7	96.4	97.9	95.8	99.2
22	92.8	90.8	84.5	87.4	106.1	105.1	104.1	98.5
Mean	**89.3**	**89.4**	**87.2**	**85.9**	**94.7**	**94.8**	**94.9**	**93.9**
SEM	2.5	2.5	2.9	3.5	3.0	3.3	3.5	3.6

Abbreviations: IH, intermittent hypoxia; MAP, mean arterial pressure; SEM, standard error of the mean.

Table 18. Individual heart rate during lower body negative pressure, before and after intermittent hypoxia.

	Pre HR (/min)				Post HR (/min)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	47.5	50.0	59.5	69.4	49.6	56.7	65.6	71.3
7	59.1	61.6	69.6	76.2	60.0	64.5	69.2	73.8
13	81.7	85.0	94.6	108.2	85.8	93.6	102.3	110.2
14	70.3	80.1	91.8	103.4	77.1	84.2	92.4	102.8
15	59.5	60.1	70.8	76.6	61.2	64.6	77.0	87.3
16	60.7	62.8	70.1	74.0	54.3	57.0	65.4	71.3
17	68.6	69.8	84.5	93.1	65.8	68.4	83.1	94.5
20	71.5	76.1	86.8	96.6	74.3	84.1	88.9	102.2
21	51.7	50.8	53.0	62.4	45.8	48.6	54.1	65.2
22	58.5	58.3	69.3	82.1	63.3	60.1	67.4	78.8
Mean	**62.9**	**65.5**	**75.0**	**84.2**	**63.7**	**68.2**	**76.5**	**85.7**
SEM	3.2	3.8	4.4	4.8	4.0	4.6	4.7	5.0

Abbreviations: IH, intermittent hypoxia; HR, heart rate; SEM, standard error of the mean.

Table 19. Individual brachial artery diameter during lower body negative pressure, before and after intermittent hypoxia.

	Pre BA Diameter (cm)				Post BA Diameter (cm)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	0.395	0.389	0.389	0.386	0.402	0.402	0.399	0.395
7	0.384	0.380	0.374	0.373	0.390	0.383	0.379	0.376
13	0.358	0.353	0.351	0.342	0.363	0.357	0.354	0.351
14	0.376	0.371	0.373	0.368	0.378	0.368	0.362	0.368
15	0.404	0.402	0.399	0.387	0.415	0.403	0.392	0.383
16	0.502	0.503	0.500	0.495	0.510	0.528	0.513	0.507
17	0.440	0.434	0.431	0.429	0.430	0.424	0.423	0.425
20	0.342	0.337	0.335	0.323	0.344	0.339	0.338	0.324
21	0.430	0.427	0.421	0.415	0.430	0.434	0.426	0.418
22	0.374	0.366	0.357	0.360	0.370	0.367	0.365	0.365
Mean	**0.401**	**0.396**	**0.393**	**0.388**	**0.403**	**0.400**	**0.395**	**0.391**
SEM	0.015	0.015	0.015	0.016	0.015	0.017	0.016	0.016

Abbreviations: IH, intermittent hypoxia; BA, brachial artery; SEM, standard error of the mean.

Table 20. Individual brachial artery mean blood velocity during lower body negative pressure, before and after intermittent hypoxia.

	Pre BA MBV (cm/s)				Post BA MBV (cm/s)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	14.0	8.0	7.2	12.4	12.6	10.7	11.4	9.5
7	19.2	19.8	20.3	12.5	18.9	14.7	13.1	9.9
13	5.7	5.2	6.0	6.0	5.5	4.3	5.3	5.1
14	5.0	4.0	3.9	4.0	5.1	4.3	3.9	3.7
15	12.7	6.9	9.8	6.7	6.8	6.0	5.5	4.8
16	9.3	7.1	9.4	10.0	4.4	3.6	3.9	4.8
17	7.1	5.0	6.2	7.7	4.9	3.5	3.5	4.4
20	7.3	6.3	5.9	4.2	5.3	4.0	3.7	3.7
21	8.4	6.6	11.7	9.7	4.7	4.0	4.0	5.0
22	8.6	5.4	7.5	7.7	5.3	3.9	3.7	3.8
Mean	**9.7**	**7.4**	**8.8**	**8.1**	**7.3**	**5.9**	**5.8**	**5.5**
SEM	1.4	1.4	1.5	1.0	1.5	1.2	1.1	0.7

Abbreviations: IH, intermittent hypoxia; BA, brachial artery; MBV, mean blood velocity; SEM, standard error of the mean.

Table 21. Individual forearm blood flow during lower body negative pressure, before and after intermittent hypoxia.

	Pre $\dot{Q}_{BA}$ (ml/min)				Post $\dot{Q}_{BA}$ (ml/min)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	102.9	57.0	51.2	87.4	95.8	81.5	85.3	70.0
7	133.7	134.7	133.5	81.9	135.2	101.5	88.4	66.1
13	34.2	30.4	34.7	33.4	34.0	25.9	31.3	29.6
14	33.5	25.8	25.7	25.7	34.4	27.3	24.0	23.5
15	97.6	52.2	73.4	47.0	55.0	45.4	39.5	33.2
16	110.7	84.9	110.7	115.2	53.8	46.6	48.4	58.2
17	64.2	44.7	54.4	66.7	42.5	29.6	29.5	37.9
20	40.0	33.7	31.0	20.9	29.3	21.5	19.8	18.5
21	72.6	56.3	98.0	78.5	41.2	35.6	34.3	40.7
22	56.4	34.0	45.2	47.0	34.3	24.6	23.5	23.9
Mean	**74.6**	**55.4**	**65.8**	**60.4**	**55.6**	**43.9**	**42.4**	**40.2**
SEM	11.1	10.4	11.7	9.7	10.8	8.5	7.9	5.8

Abbreviations: IH, intermittent hypoxia; $\dot{Q}_{BA}$, forearm blood flow; SEM, standard error of the mean.

Table 22. Individual forearm vascular resistance during lower body negative pressure, before and after intermittent hypoxia.

	Pre FVR (mmHg/ml/min)				Post FVR (mmHg/ml/min)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	0.86	1.64	1.89	1.08	0.99	1.17	1.15	1.45
7	0.66	0.67	0.66	1.27	0.71	0.94	1.18	1.62
13	2.64	2.86	2.44	2.33	2.61	3.13	2.62	3.48
14	2.95	3.72	3.84	3.78	3.04	3.80	4.50	4.32
15	1.00	1.91	1.37	1.98	2.06	2.43	2.67	3.07
16	0.94	1.21	0.92	0.84	1.86	2.23	2.18	1.80
17	1.18	1.77	1.33	1.04	1.88	2.87	2.80	2.10
20	1.96	2.26	2.43	3.73	2.76	3.62	3.78	4.44
21	1.55	1.63	0.96	1.26	2.51	2.78	2.82	2.48
22	1.75	2.68	1.88	1.90	3.09	4.30	4.48	4.13
Mean	**1.55**	**2.03**	**1.77**	**1.92**	**2.15**	**2.73**	**2.82**	**2.89**
SEM	0.25	0.28	0.30	0.34	0.26	0.34	0.37	0.37

Abbreviations: IH, intermittent hypoxia; FVR, forearm vascular resistance; SEM, standard error of the mean.

Table 23. Individual forearm vascular conductance during lower body negative pressure, before and after intermittent hypoxia.

	Pre FVC (ml/min/mmHg)				Post FVC (ml/min/mmHg)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	1.17	0.62	0.54	0.94	1.02	0.86	0.87	0.70
7	1.54	1.50	1.52	0.93	1.42	1.07	0.86	0.63
13	0.40	0.36	0.43	0.45	0.38	0.32	0.38	0.37
14	0.34	0.27	0.26	0.27	0.33	0.26	0.23	0.23
15	1.02	0.54	0.80	0.51	0.53	0.44	0.39	0.33
16	1.14	0.85	1.15	1.21	0.55	0.45	0.47	0.57
17	0.85	0.57	0.76	1.01	0.54	0.35	0.36	0.50
20	0.51	0.44	0.42	0.30	0.37	0.28	0.27	0.24
21	0.80	0.63	1.10	0.85	0.43	0.36	0.36	0.41
22	0.61	0.37	0.53	0.54	0.32	0.23	0.23	0.24
Mean	**0.84**	**0.62**	**0.75**	**0.70**	**0.59**	**0.46**	**0.44**	**0.42**
SEM	0.12	0.11	0.13	0.10	0.11	0.09	0.07	0.05

Abbreviations: IH, intermittent hypoxia; FVC, forearm vascular conductance; SEM, standard error of the mean.

Table 24. Individual muscle sympathetic nerve burst frequency during lower body negative pressure, before and after intermittent hypoxia.

	Pre MSNA BF (/min)				Post MSNA BF (/min)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	14.5	16.0	19.0	27.5	16.5	20.0	24.5	26.5
7	17.5	22.5	23.5	37.0	18.5	30.0	34.0	37.0
13	8.0	11.5	23.5	26.0	9.0	18.0	19.0	22.0
14	32.5	40.0	41.5	56.0	28.5	42.0	41.0	48.0
15	14.5	19.5	26.5	33.5	19.5	25.0	28.0	30.0
16	19.0	23.0	32.5	35.0	21.0	24.5	33.0	37.0
17	23.5	26.5	33.5	43.5	33.5	36.0	40.0	40.5
20	12.5	22.0	28.5	31.0	17.5	30.0	34.5	37.0
21	10.0	12.5	15.5	24.0	11.0	13.5	14.5	19.5
22	15.0	22.5	32.5	36.5	16.0	23.5	27.5	33.5
Mean	**16.7**	**21.6**	**27.7**	**35.0**	**19.1**	**26.3**	**29.6**	**33.1**
SEM	2.2	2.6	2.4	3.0	2.3	2.7	2.7	2.8

Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; BF, burst frequency; SEM, standard error of the mean.

Table 25. Individual muscle sympathetic nerve burst incidence during lower body negative pressure, before and after intermittent hypoxia.

	Pre MSNA BI (/100hb)				Post MSNA BI (/100hb)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	30.5	31.9	32.2	39.3	34.1	35.4	37.8	36.8
7	29.0	36.1	32.9	47.3	29.9	47.1	49.0	49.5
13	10.0	13.4	24.1	23.8	10.6	19.5	18.6	20.1
14	45.8	50.8	46.0	54.6	37.2	49.9	44.2	46.5
15	24.1	33.4	38.6	44.6	31.9	38.0	37.8	34.5
16	31.2	36.3	46.3	45.9	38.6	43.7	51.0	51.9
17	34.0	38.7	39.6	47.0	50.5	53.0	49.2	43.1
20	17.2	28.9	32.3	33.0	23.3	36.6	38.8	36.2
21	19.5	24.8	28.6	38.6	23.9	28.5	26.8	29.3
22	25.4	38.6	46.9	46.1	26.0	38.2	41.3	43.0
Mean	**26.7**	**33.3**	**36.7**	**42.0**	**30.6**	**39.0**	**39.5**	**39.1**
SEM	3.1	3.1	2.5	2.8	3.4	3.2	3.2	3.1

Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; BI, burst incidence; SEM, standard error of the mean.

Table 26. Individual muscle sympathetic nerve burst latency during lower body negative pressure, *before and after intermittent hypoxia.*

	Pre MSNA BL (s)				Post MSNA BL (s)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	1.40	-	-	-	1.40	-	-	-
7	1.31	-	-	-	1.44	-	-	-
13	1.34	-	-	-	1.36	-	-	-
14	1.49	-	-	-	1.53	-	-	-
15	1.11	-	-	-	1.19	-	-	-
16	1.22	-	-	-	1.26	-	-	-
17	1.26	-	-	-	1.29	-	-	-
20	1.28	-	-	-	1.31	-	-	-
21	1.37	-	-	-	1.30	-	-	-
22	1.33	-	-	-	1.37	-	-	-
Mean	**1.31**	**-**	**-**	**-**	**1.34**	**-**	**-**	**-**
SEM	0.03	-	-	-	0.03	-	-	-

Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; BL; burst latency; SEM, standard error of the mean.

Table 27. Individual lower body negative pressures (LBNP) during LBNP, before and after intermittent hypoxia.

	Pre LBNP (mmHg)				Post LBNP (mmHg)			
Subject ID	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**	**Baseline**	**15 mmHg**	**30 mmHg**	**45 mmHg**
6	-	-	-	-	-	-	-	-
7	-0.3	-15.9	-30.4	-45.5	-0.4	-16.0	-31.6	-46.2
13	-0.1	-15.9	-30.5	-45.3	-0.2	-16.1	-30.8	-45.3
14	-0.9	-16.9	-31.3	-45.0	-1.0	-16.3	-31.1	-45.3
15	-	-	-	-	-	-	-	-
16	-0.4	-15.9	-30.3	-44.4	-0.5	-15.9	-30.9	-45.5
17	-0.7	-16.0	-31.1	-45.5	-0.8	-16.7	-30.8	-46.2
20	-1.0	-17.1	-30.8	-45.0	-1.0	-17.4	-31.0	-46.3
21	-0.7	-16.0	-30.7	-45.4	-0.8	-16.3	-30.8	-45.2
22	-0.9	-16.1	-30.8	-46.8	-1.0	-16.2	-31.3	-45.2
Mean	**-0.6**	**-16.2**	**-30.7**	**-45.4**	**-0.7**	**-16.3**	**-31.0**	**-45.7**
SEM	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2

Dashes indicate values that were not measured. Abbreviations: IH, intermittent hypoxia; LBNP, lower body negative pressure; SEM, standard error of the mean.

Intermittent Hypoxia

Table 28. Individual oxyhemoglobin desaturation characteristics during 40-minutes of intermittent hypoxia.

Subject ID	% of Time <95% S_PO_2	% of Time <90% S_PO_2	% of Time <85% S_PO_2	% of Time <80% S_PO_2	Mean Minimum S_PO_2 (%)	Mean Maximum S_PO_2 (%)	Mean S_PO_2 (%)
6	44.2	20.0	0.0	0.0	86.7	97.4	94.1
7	48.3	28.3	13.3	0.0	82.8	97.6	92.9
13	71.7	35.0	10.0	0.0	85.1	96.6	91.7
14	65.8	48.3	30.0	0.0	81.1	97.4	90.2
15	68.3	50.8	38.3	26.7	75.6	97.8	88.2
16	58.3	40.0	25.8	7.5	79.0	98.2	91.1
17	60.8	42.5	24.2	0.0	81.5	98.2	91.2
20	68.3	32.5	5.0	0.0	85.9	96.7	92.1
21	82.5	41.7	11.7	0.0	83.9	95.9	90.8
22	73.3	30.0	13.3	0.0	85.0	96.1	91.5
Mean	**64.2**	**36.9**	**17.2**	**3.4**	**82.7**	**97.2**	**91.4**
SEM	3.7	3.0	3.8	2.7	1.1	0.3	0.5

Abbreviations: S_PO_2, peripheral oxyhemoglobin saturation; SEM, standard error of the mean.

Table 29. Individual tidal volumes during the first and last 6-cycles of intermittent hypoxia.

	First 6-Cycles V_T (l)		Last 6-Cycles V_T (l)	
Subject ID	**Normoxia**	**Hypoxia**	**Normoxia**	**Hypoxia**
6	0.77	1.08	0.58	0.73
7	2.03	2.60	2.03	3.13
13	2.27	2.33	2.29	2.24
14	2.47	2.90	2.36	2.76
15	1.79	2.08	1.75	2.29
16	1.78	2.39	1.67	2.52
17	1.51	1.69	1.86	2.16
20	1.93	2.72	1.79	2.47
21	1.92	2.22	1.77	3.06
22	1.76	2.32	2.16	2.71
Mean	**1.82**	**2.23**	**1.83**	**2.41**
SEM	0.15	0.17	0.16	0.21

Abbreviations: V_T, tidal volume; SEM, standard error of the mean.

Table 30. Individual breathing frequencies during the first and last 6-cycles of intermittent hypoxia.

	First 6-Cycles f_B (/min)		Last 6-Cycles f_B (/min)	
Subject ID	**Normoxia**	**Hypoxia**	**Normoxia**	**Hypoxia**
6	15.7	18.9	13.4	15.6
7	15.4	16.2	18.8	17.3
13	8.2	8.1	9.7	10.3
14	12.4	15.7	19.5	20.5
15	14.2	16.7	13.9	15.9
16	16.0	19.9	16.0	21.1
17	19.2	20.3	23.3	23.7
20	9.0	9.4	13.3	11.4
21	10.6	10.3	11.0	8.8
22	8.4	9.1	8.6	9.7
Mean	**12.9**	**14.5**	**14.7**	**15.4**
SEM	1.2	1.5	1.5	1.7

Abbreviations: f_B, breathing frequency; SEM, standard error of the mean.

Table 31. Individual partial pressures of end-tidal carbon dioxide during the first and last 6-cycles of intermittent hypoxia.

	First 6-Cycles $P_{ET}CO_2$ (mmHg)		**Last 6-Cycles $P_{ET}CO_2$ (mmHg)**	
Subject ID	**Normoxia**	**Hypoxia**	**Normoxia**	**Hypoxia**
6	41.2	42.9	40.3	43.1
7	40.8	43.6	39.9	44.2
13	44.7	46.9	44.8	47.4
14	42.0	45.5	40.5	46.0
15	43.1	43.6	41.0	44.5
16	39.1	43.1	39.4	43.6
17	39.3	49.4	39.0	48.9
20	40.0	43.1	39.7	41.9
21	47.6	46.7	43.9	48.6
22	42.7	44.4	42.6	43.0
Mean	**42.0**	**44.9**	**41.1**	**45.1**
SEM	0.8	0.7	0.6	0.8

Abbreviations: $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; SEM, standard error of the mean.

Table 32. Individual partial pressures of end tidal oxygen during the first and last 6- of intermittent hypoxia.

	First 6-Cycles $P_{ET}O_2$ (mmHg)		Last 6-Cycles $P_{ET}O_2$ (mmHg)	
Subject ID	**Normoxia**	**Hypoxia**	**Normoxia**	**Hypoxia**
6	91.2	51.1	96.5	55.0
7	94.8	47.1	99.6	48.3
13	88.4	53.6	84.5	47.7
14	87.1	44.8	98.7	46.4
15	98.7	40.7	102.2	41.9
16	106.7	44.0	106.1	42.7
17	104.4	46.8	113.8	45.8
20	89.7	54.0	85.3	49.3
21	82.3	52.6	76.1	46.7
22	83.6	57.0	80.2	46.9
Mean	**92.7**	**49.2**	**94.3**	**47.1**
SEM	2.6	1.7	3.9	1.1

Abbreviations: $P_{ET}O_2$, partial pressure of end-tidal oxygen; SEM, standard error of the mean.

Table 33. Individual inspired ventilations during the first and last 6-cycles of intermittent hypoxia.

	First 6-Cycles V_I (l/min)		Last 6-Cycles V_I (l/min)	
Subject ID	**Normoxia**	**Hypoxia**	**Normoxia**	**Hypoxia**
6	13.2	22.1	8.2	12.5
7	34.4	46.7	41.8	60.0
13	20.4	20.7	24.1	24.8
14	33.7	50.3	50.6	62.3
15	28.1	38.6	26.6	39.9
16	31.4	53.1	29.6	59.0
17	31.9	37.9	47.8	56.1
20	18.6	26.8	25.7	29.1
21	21.7	24.9	20.4	29.3
22	16.2	23.6	20.6	29.2
Mean	**25.0**	**34.5**	**29.5**	**40.2**
SEM	2.5	3.9	4.2	5.6

Abbreviations: V_I, inspired ventilation; SEM, standard error of the mean.

Table 34. Individual peripheral oxyhemoglobin saturations during the first and last 6-cycles of intermittent hypoxia.

	First 6-Cycles S_PO_2 (%)		Last 6-Cycles S_PO_2 (%)	
Subject ID	**Normoxia**	**Hypoxia**	**Normoxia**	**Hypoxia**
6	97.1	85.8	97.5	88.2
7	97.4	82.7	97.7	83.7
13	96.8	87.4	96.3	83.2
14	96.6	80.5	97.7	82.0
15	97.7	75.8	97.9	77.4
16	98.1	79.7	98.1	78.3
17	98.0	82.4	98.5	81.5
20	96.9	87.6	96.4	84.5
21	96.0	86.8	95.1	82.3
22	96.2	89.2	95.8	82.5
Mean	**97.1**	**83.8**	**97.1**	**82.3**
SEM	0.2	1.4	0.4	1.0

Abbreviations: S_PO_2, peripheral oxyhemoglobin saturation; SEM, standard error of the mean.

Table 35. Individual heart rates during the first and last 6-cycles of intermittent hypoxia.

	First 6-Cycles HR (/min)		Last 6-Cycles HR (/min)	
Subject ID	**Normoxia**	**Hypoxia**	**Normoxia**	**Hypoxia**
6	54.3	56.3	55.3	56.9
7	69.5	63.8	73.8	74.1
13	85.9	85.5	88.3	88.4
14	78.1	84.6	88.1	90.5
15	62.6	61.9	62.7	63.4
16	73.0	71.6	64.3	64.6
17	70.9	73.6	84.5	84.5
20	77.4	79.1	90.5	86.8
21	53.0	57.7	48.9	51.2
22	62.7	69.5	70.0	77.2
Mean	**68.7**	**70.4**	**72.6**	**73.8**
SEM	3.4	3.3	4.7	4.4

Abbreviations: HR, heart rate; SEM, standard error of the mean.

Table 36. Individual mean arterial pressures during the first and last 6-cycles of intermittent hypoxia.

	First 6-Cycles MAP (mmHg)		Last 6-Cycles MAP (mmHg)	
Subject ID	**Normoxia**	**Hypoxia**	**Normoxia**	**Hypoxia**
6	90.6	104.9	95.4	99.9
7	92.0	100.4	97.8	107.9
13	84.9	88.8	86.1	87.6
14	99.4	108.4	103.6	111.1
15	106.0	120.9	98.2	114.1
16	97.8	118.8	89.9	106.8
17	72.5	82.5	85.2	93.9
20	80.0	84.6	76.3	79.3
21	92.7	103.8	92.6	102.9
22	90.3	100.4	102.6	107.0
Mean	**90.6**	**101.3**	**92.8**	**101.1**
SEM	3.1	4.2	2.7	3.5

Abbreviations: MAP, mean arterial pressure; SEM, standard error of the mean.

Table 37. Individual systolic blood pressures during the first and last 6-cycles of intermittent hypoxia.

	First 6-Cycles SBP (mmHg)		Last 6-Cycles SBP (mmHg)	
Subject ID	**Normoxia**	**Hypoxia**	**Normoxia**	**Hypoxia**
6	130.8	144.1	140.5	142.9
7	126.8	135.0	133.3	147.2
13	123.1	126.8	125.7	126.2
14	142.4	150.6	146.9	153.8
15	148.0	157.3	135.9	150.1
16	135.5	157.9	126.6	144.9
17	109.6	120.7	128.3	136.8
20	126.5	129.2	118.1	121.3
21	135.7	145.2	140.3	146.5
22	130.9	139.9	146.8	147.6
Mean	**130.9**	**140.7**	**134.2**	**141.7**
SEM	3.4	4.0	3.0	3.3

Abbreviations: SBP, systolic blood pressure; SEM, standard error of the mean.

Table 38. Individual diastolic blood pressures during the first and last 6-cycles of intermittent hypoxia.

	First 6-Cycles DBP (mmHg)		Last 6-Cycles DBP (mmHg)	
Subject ID	**Normoxia**	**Hypoxia**	**Normoxia**	**Hypoxia**
6	70.7	82.9	74.2	79.4
7	72.8	79.1	77.1	83.7
13	63.3	67.0	64.9	65.9
14	77.2	85.1	81.0	86.7
15	86.5	98.2	77.1	91.5
16	76.4	94.9	70.8	86.8
17	52.5	61.4	62.5	71.1
20	60.0	64.0	56.2	59.5
21	71.5	80.8	71.2	81.4
22	67.9	76.4	78.6	82.2
Mean	**69.9**	**79.0**	**71.4**	**78.8**
SEM	3.0	3.9	2.5	3.2

Abbreviations: DBP, diastolic blood pressure; SEM, standard error of the mean.

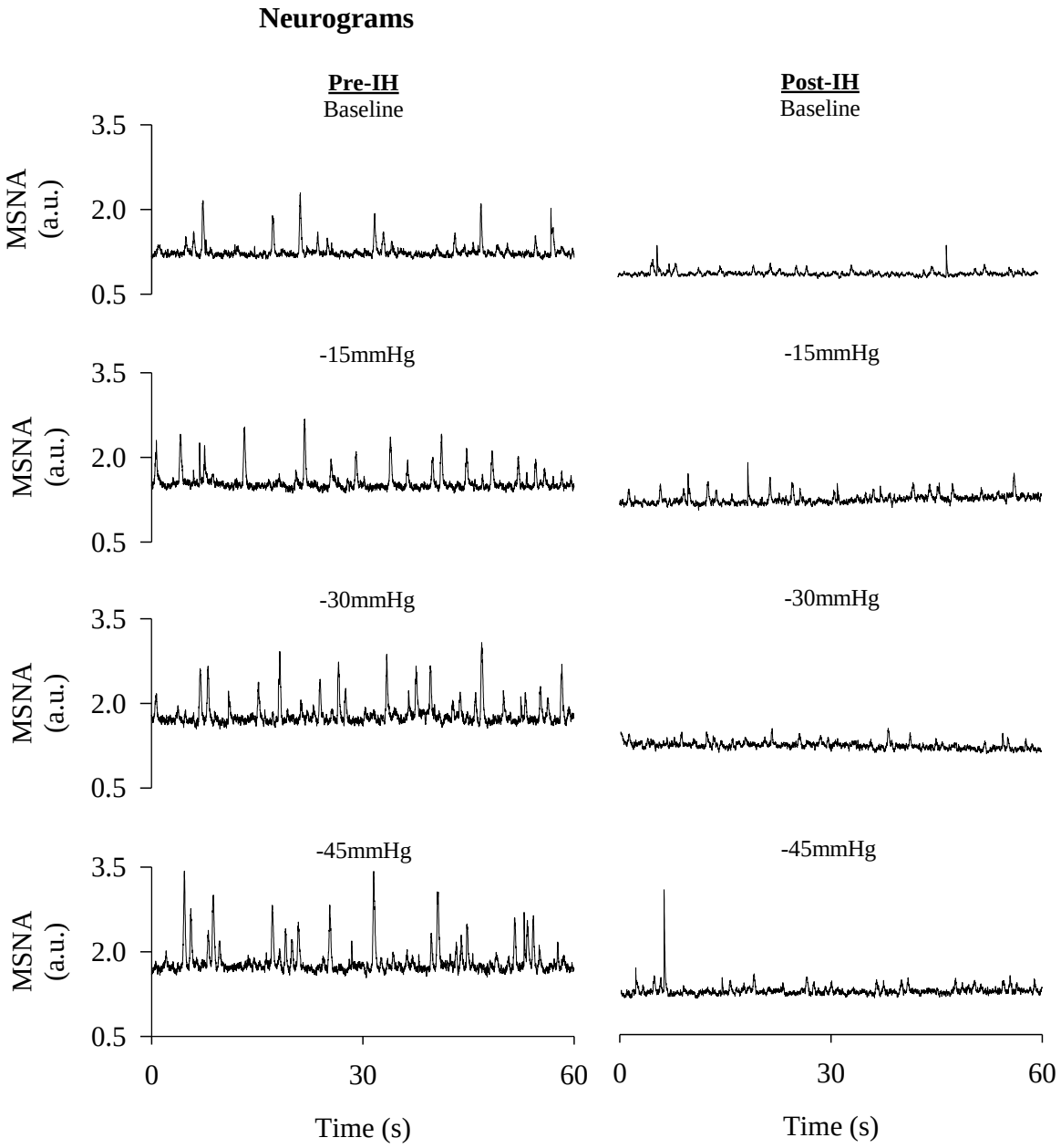

Figure 9. Individual subject neurograms (subject 6) from each stage of lower body negative pressure, before and after the intermittent hypoxia exposure. Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; a.u., arbitrary units.

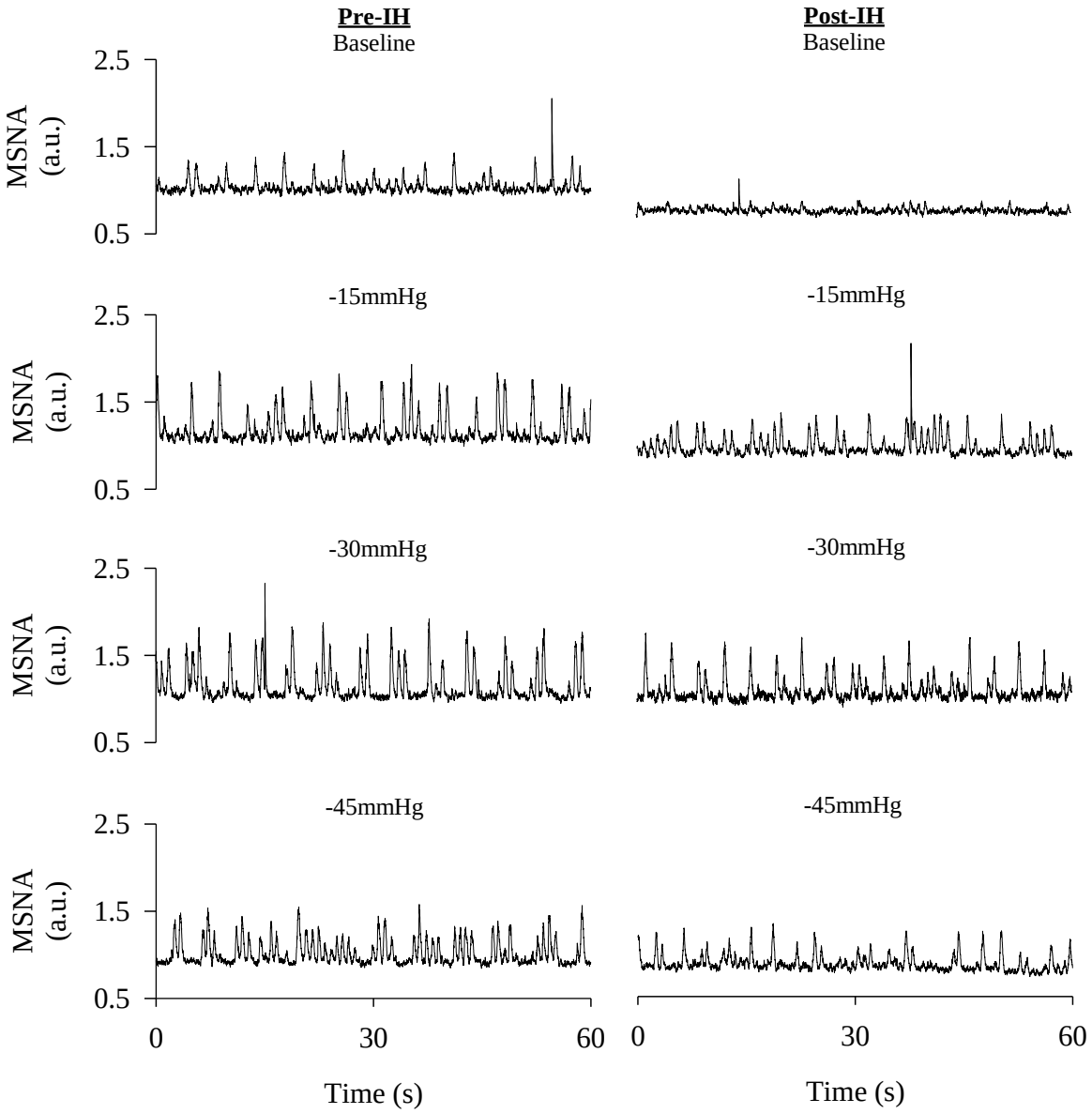

Figure 10. Individual subject neurograms (subject 7) from each stage of lower body negative pressure, before and after the intermittent hypoxia exposure. Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; a.u., arbitrary units.

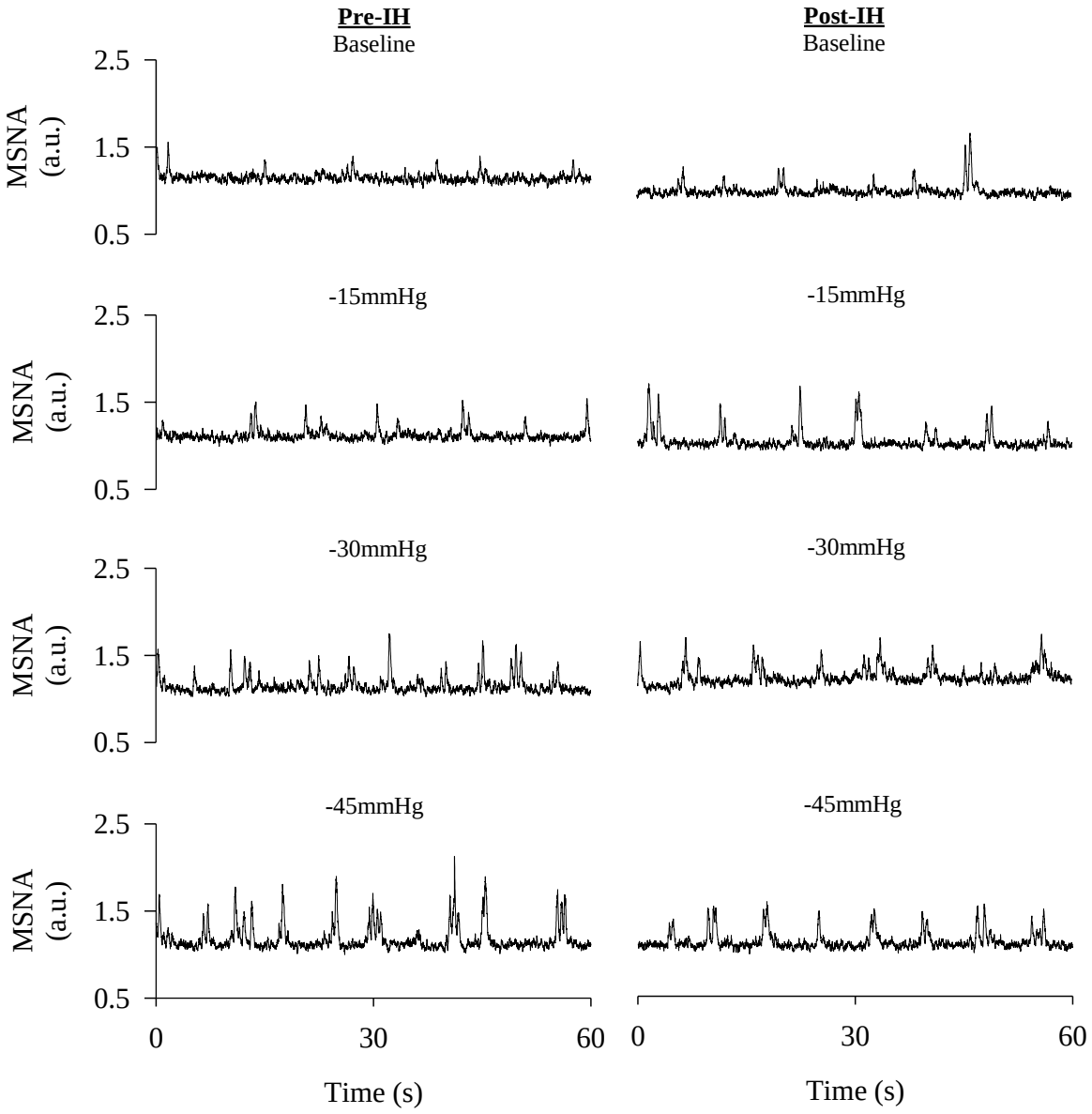

Figure 11. Individual subject neurograms (subject 13) from each stage of lower body negative pressure, before and after the intermittent hypoxia exposure. Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; a.u., arbitrary units.

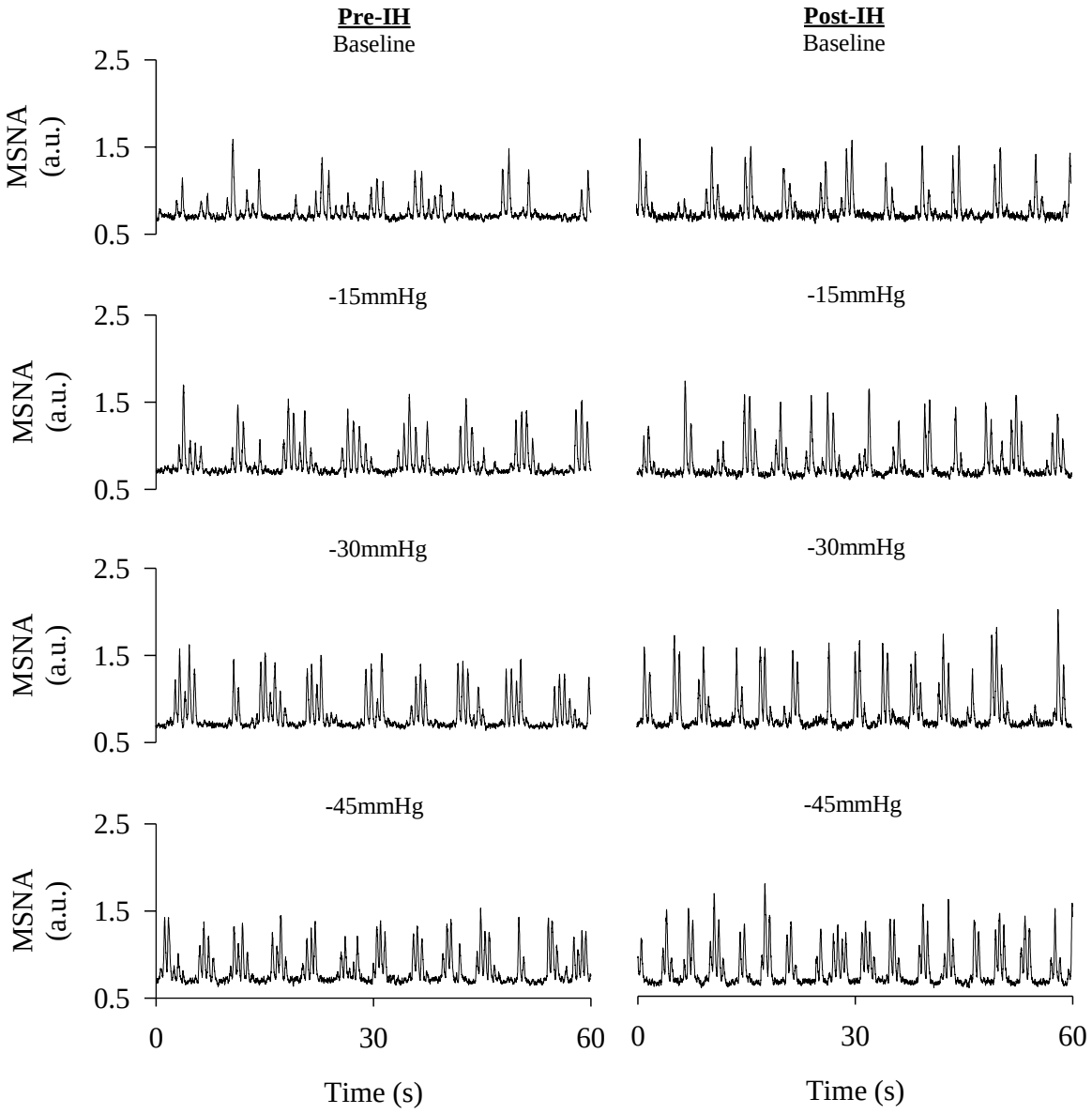

Figure 12. Individual subject neurograms (subject 14) from each stage of lower body negative pressure, before and after the intermittent hypoxia exposure. Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; a.u., arbitrary units.

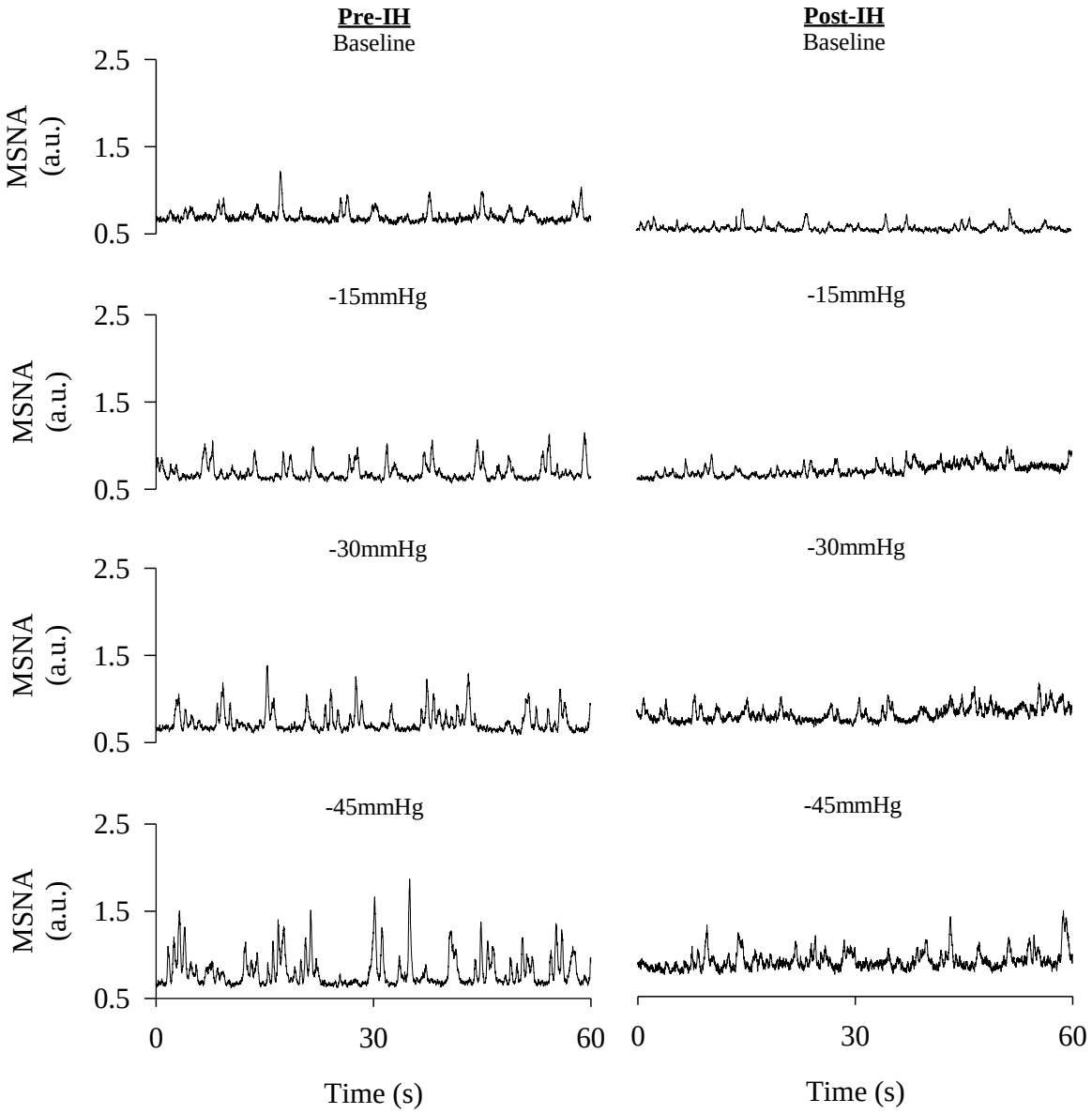

Figure 13. Individual subject neurograms (subject 15) from each stage of lower body negative pressure, before and after the intermittent hypoxia exposure. Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; a.u., arbitrary units.

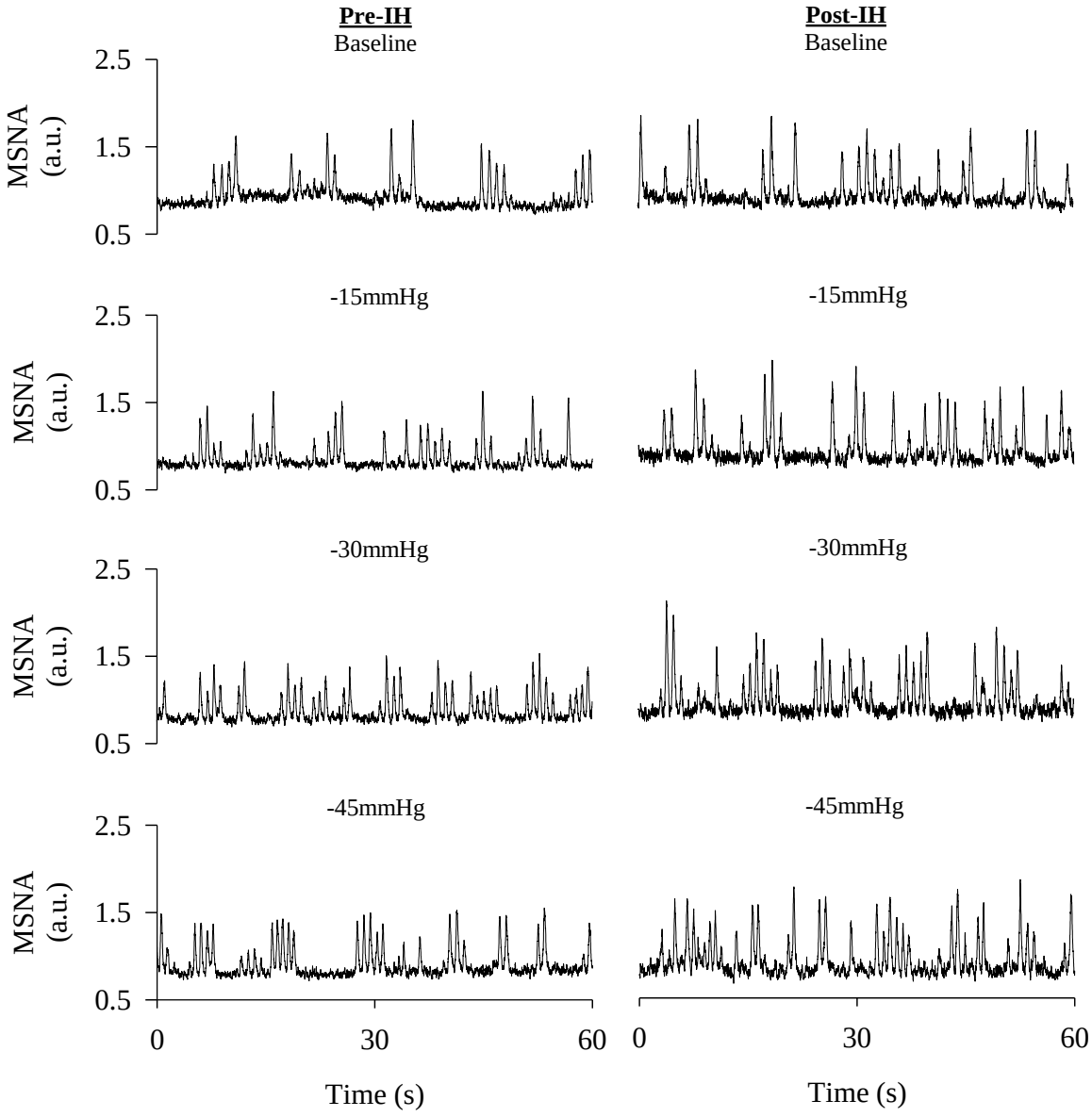

Figure 14. Individual subject neurograms (subject 16) from each stage of lower body negative pressure, before and after the intermittent hypoxia exposure. Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; a.u., arbitrary units.

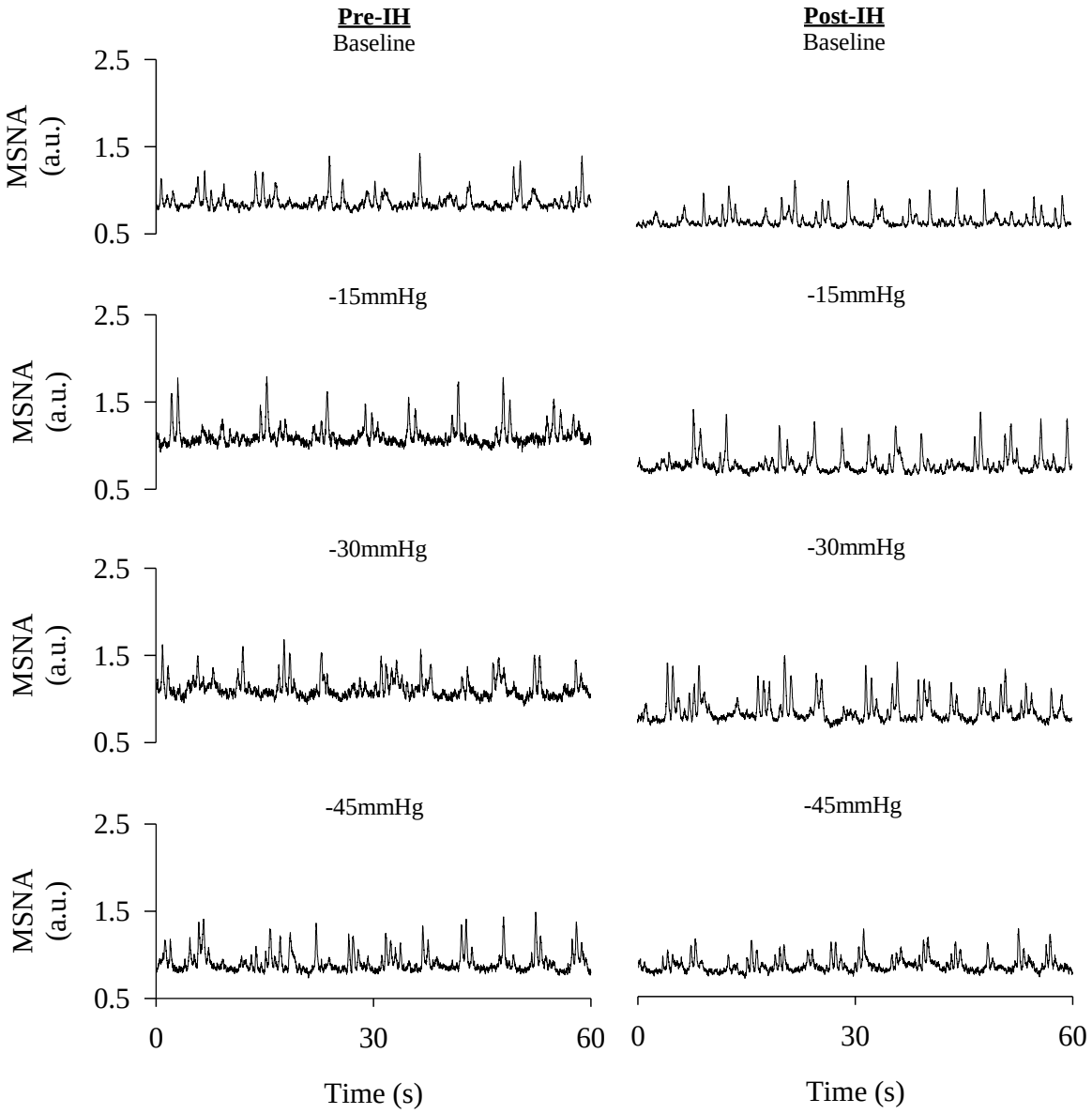

Figure 15. Individual subject neurograms (subject 17) from each stage of lower body negative pressure, before and after the intermittent hypoxia exposure. Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; a.u., arbitrary units.

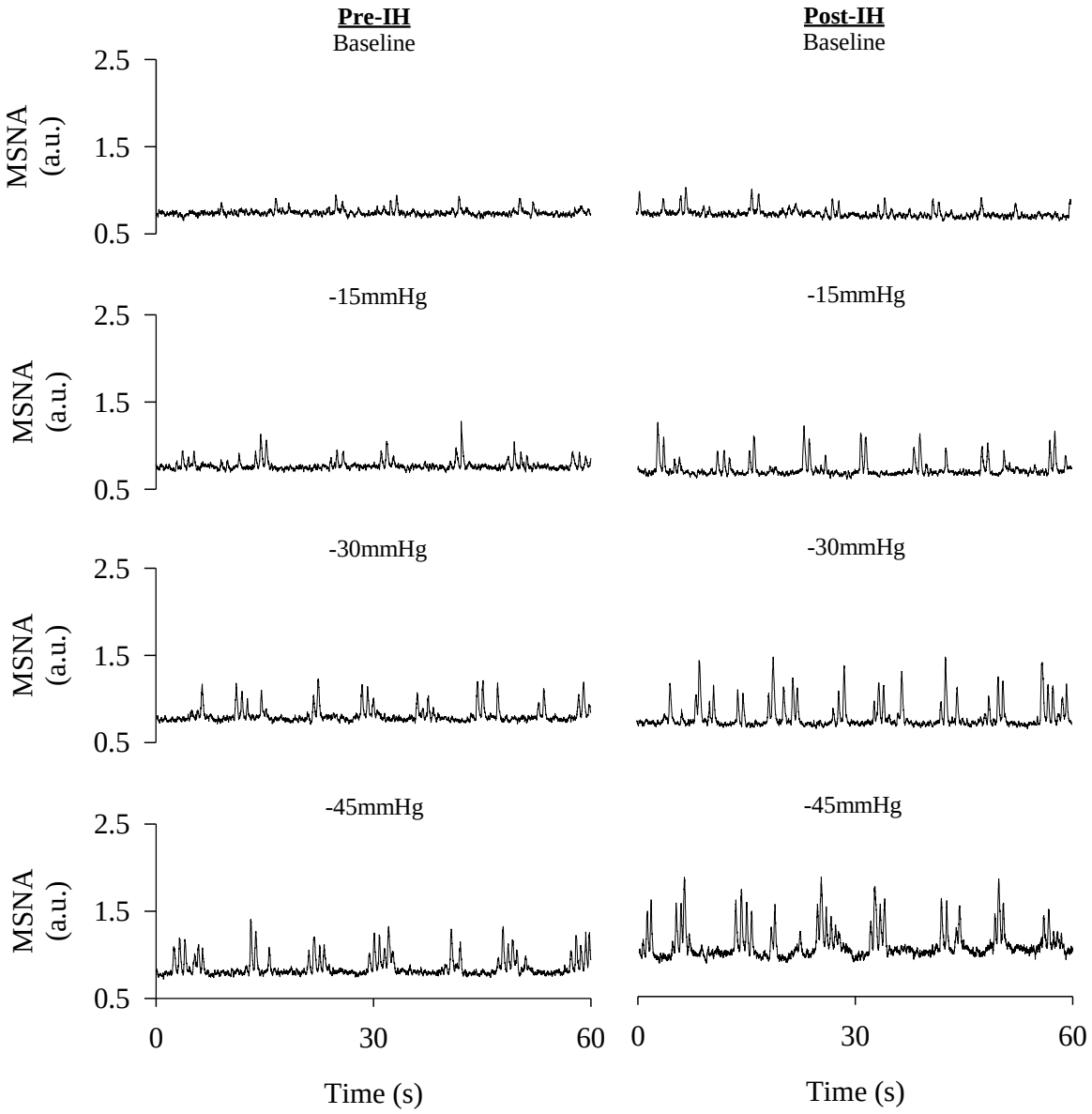

Figure 16. Individual subject neurograms (subject 20) from each stage of lower body negative pressure, before and after the intermittent hypoxia exposure. Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; a.u., arbitrary units.

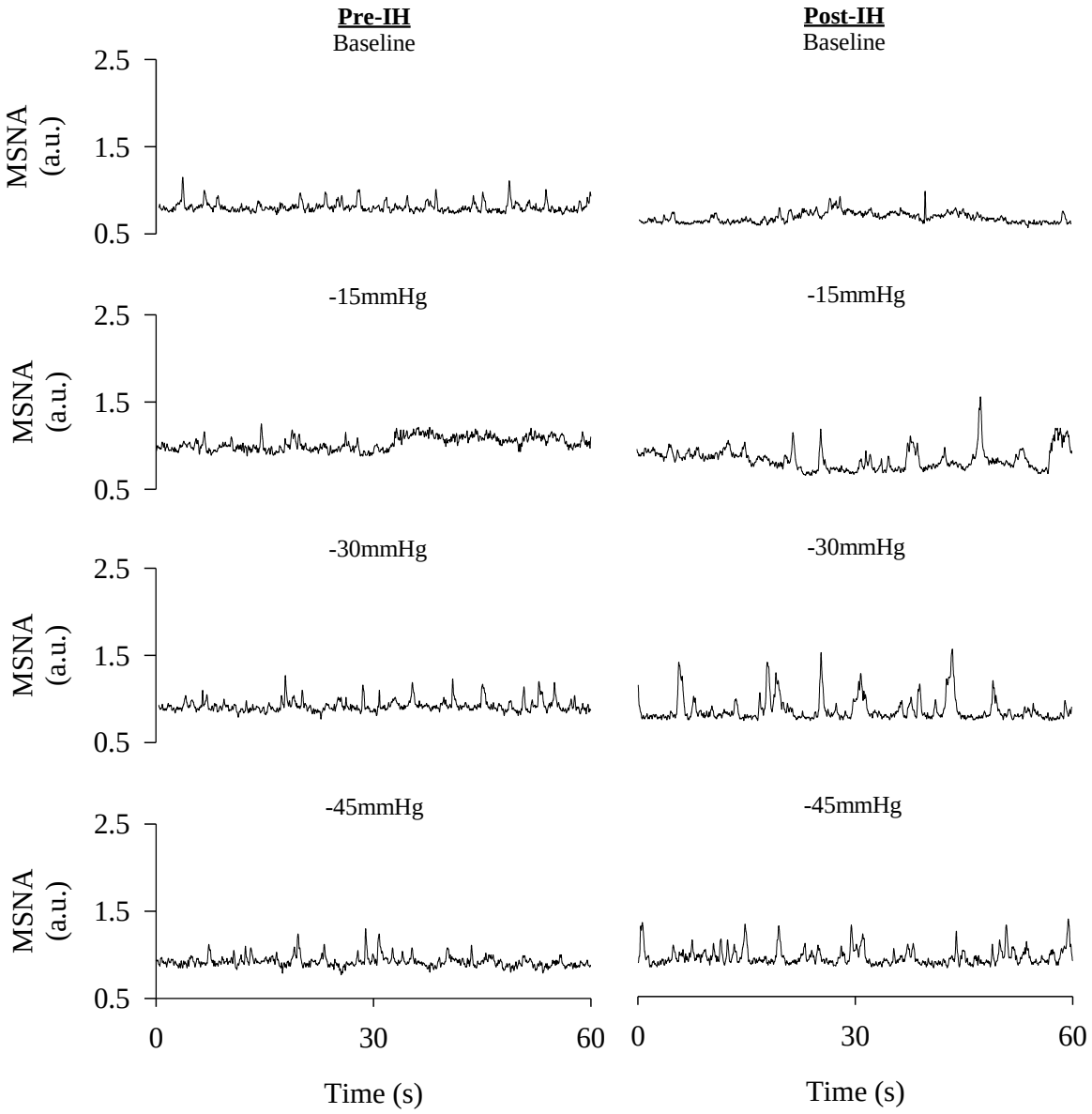

Figure 17. Individual subject neurograms (subject 21) from each stage of lower body negative pressure, before and after the intermittent hypoxia exposure. Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; a.u., arbitrary units.

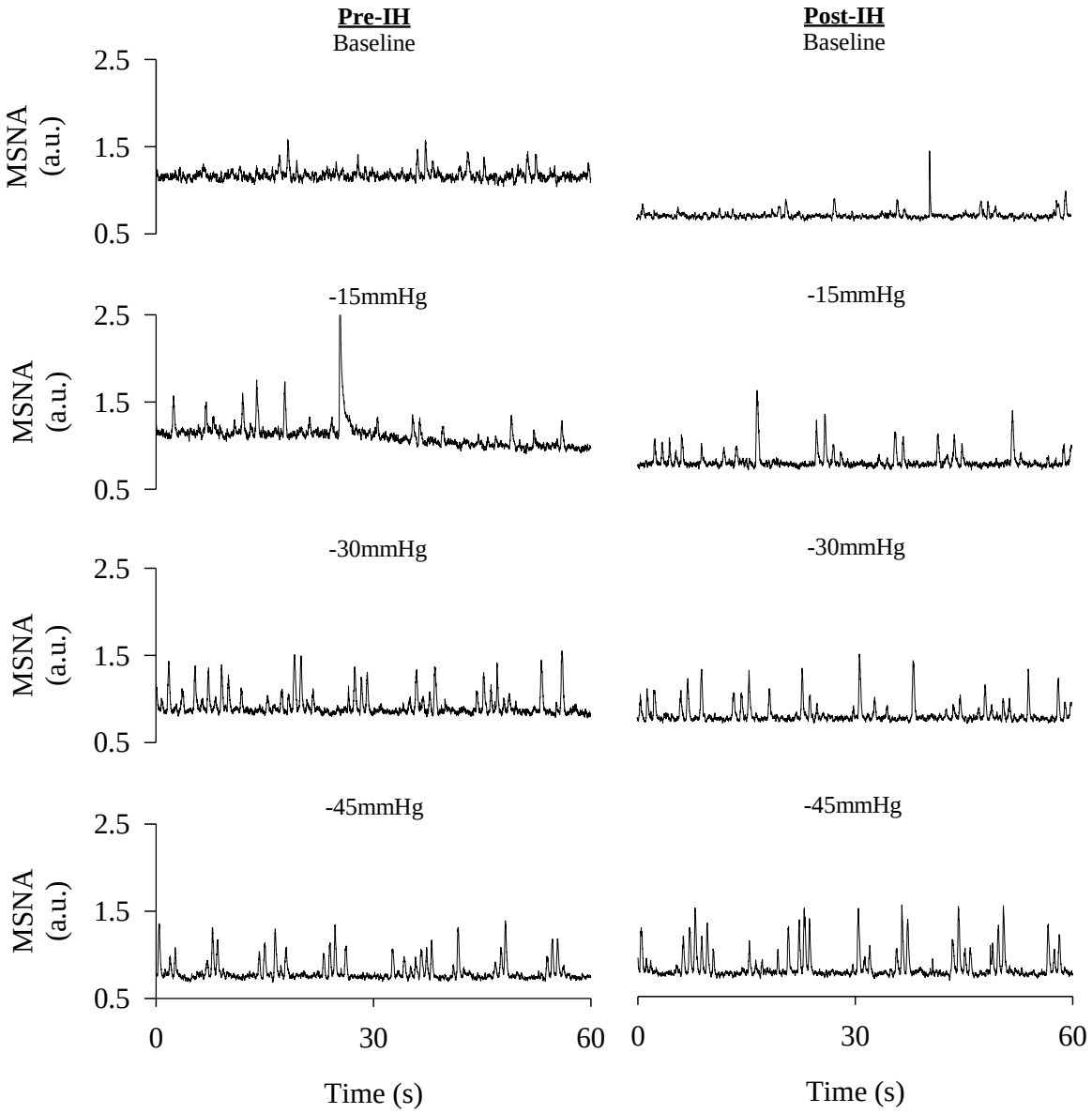

Figure 18. Individual subject neurograms (subject 22) from each stage of lower body negative pressure, before and after the intermittent hypoxia exposure. Abbreviations: IH, intermittent hypoxia; MSNA, muscle sympathetic nerve activity; a.u., arbitrary units.