INTERMITTENT HYPOXIA:

ACTIVATION OF THE SYMPATHETIC NERVOUS SYSTEM

by

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ABSTRACT

BACKGROUND: Individuals with obstructive sleep apnea (OSA) are reported to have elevated muscle sympathetic nerve activity (MSNA). In this complicated pathological condition numerous factors can be implicated in the elevated sympathetic activity; however periodic exposures to hypoxia appear to be the primary cause. In laboratory interventions with healthy humans, it is well documented that acute hypoxia increases MSNA, which persists after removal of the hypoxic stimulus. The effect of long term exposure of to intermittent hypoxia (IH) on MSNA is unknown.

PURPOSE: The present study was undertaken to address the effect of long term IH on MSNA during an acute hypoxic exposure and during the following normoxic recovery period. Concurrent vastus lateralis oxygenation, cerebral oxygenation, ventilatory and cardiovascular measurements were acquired to examine the relationship between the various physiological systems and how they are altered in response to IH.

HYPOTHESIS: Ten days of IH will augment the rise in MSNA during hypoxia and recovery.

METHODS: Eleven healthy males underwent two experimental sessions, consisting of 4 stages: 10 minutes baseline, 5-7 minutes hypoxic ventilatory response (HVR), 20 minutes isocapnic hypoxia (80% arterial oxygen saturation; SaO_2), and 20 minutes normoxic recovery. Experimental days were separated by 10 days of IH where 1 hour of isocapnic hypoxia (SaO_2 = 80%) was administered. During both experimental sessions the following parameters were collected: 1) MSNA was acquired from peroneal nerve recordings. Total MSNA was calculated as the product of burst frequency and burst amplitude; 2) blood pressure (BP); 3) heart rate (HR) was acquired from electrocardiogram; 4) vastus lateralis and cerebral tissue oxygenation were monitored with near infrared spectroscopy (NIRS); 5) ventilatory measures; and 6) isocapnic and hypoxic stimuli was assessed by end-tidal carbon dioxide ($P_{et}CO_2$) and SaO_2, respectively.

RESULTS: Total MSNA, burst frequency and burst amplitude increased during hypoxia ($p<0.01$). Post IH, burst frequency was higher ($p<0.01$), total MSNA trended towards higher values ($p=0.06$), there was no effect on burst amplitude ($p=0.82$), and the HVR increased significantly from 0.30 ± 0.03 to 0.61 ± 0.12 L min^{-1} $\%SaO_2^{-1}$ (means $\pm$ SE, $p<0.01$). Those subjects with the greatest rise in burst frequency during the hypoxic exposure demonstrated the greatest increase in HVR post IH ($r = 0.91$, $p<0.05$); this relationship did not exist pre IH ($r = 0.39$, $p>0.05$). During the hypoxic exposure HR, BP, and minute ventilation all increased ($p<0.05$) and returned to baseline during recovery; however, there was no effect of IH. For both the vastus lateralis and cerebral tissue, indices of tissue oxygenation significantly decreased ($p<0.05$) during the hypoxic exposure and returned to baseline values during recovery. Cerebral tissue oxygenation was unaffected by 10 days of IH. Vastus lateralis total haemoglobin (tHb) increase from baseline post IH ($p=0.03$), where as pre IH values for tHb did not (. However, tHb values were not statistically different between trials ($p=0.49$). There was no difference between $P_{et}CO_2$ or SaO_2 values in each experimental session ($p>0.05$).

CONCLUSION: Exposure to 10 days of IH significantly increases MSNA and augments the ventilatory response to hypoxia. The enhanced MSNA is mediated primarily through an increase in burst frequency, which shows a strong relationship to HVR post IH. This suggests that concurrent adaptations to the ventilatory and neurovascular control systems may occur with IH. Although our subjects only experienced brief IH, our data support the hypothesis that repeated exposures to IH in OSA could contribute to the sustained increases in MSNA observed in the absence of hypoxic stimuli.

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INTRODUCTION

Understanding the physiological consequences of hypoxia on the autonomic, cardiovascular, hemodynamic, and ventilatory systems has implications for many individuals, including: persons with diseases, such as obstructive sleep apnoea (OSA) and congestive heart failure, those looking to improve athletic performance, and those ascending to high altitude. Hypoxia can be defined as a reduction in oxygen availability, either in the inspired air or arterial blood. With exposure to hypoxia biological homeostasis is disturbed. In an effort to protect vital cellular and physiological system functioning oxygen sensitive peripheral (94, 127, 129), and when severe enough, central chemoreceptors (137, 150) are activated. Consequently, a brisk increase in ventilation is the initial and most observable physiological response to hypoxia and functions to preserve oxygen delivery.

Apart from ventilation, increases in the activity of the sympathetic nervous system (SNS) activity are evident. Both chemoreceptor (43, 60, 113, 114, 153, 159) and baroreceptor (55, 56, 72, 157, 159) activation have been implicated in mediating the sympathoexcitation observed in response to hypoxia. Both these inputs have neural connections with sympathetic control centers in the central nervous system (CNS). Direct measurements of sympathetic nerve activity can be obtained using a technique called microneurography. In humans microneurography has been used to measure multi unit recordings of muscle sympathetic nerve activity (MSNA) in peripheral nerves using microelectodes. This technique has proved insightful for testing the contribution of different stimuli, such as hypoxia on the SNS. Both human and animal models implicate hypoxia, and not changes in carbon dioxide (CO_2) or cessation of breathing as the primary cause of the enhanced autonomic activity. This statement is supported by evidence of persistent MSNA only after the removal of a hypoxic stimulus. Morgan et al. (103) were the first to demonstrate this using a 20 minute exposure to either sustained hypoxia or hyperoxia in a

background of hypercapnia. Both experimental conditions caused an increase in MSNA and ventilation. During recovery, increased MSNA only persisted in the hypoxic trial, when ventilation and chemical stimuli had normalized.

What could cause the persistent MSNA after the removal of the hypoxic stimulus? Animal and human studies present conflicting results with regards to the ventilatory and sympathetic responses to hypoxia. This can be attributed to either species-specific differences or limitations due to the non-invasive nature of human work. In rodents, long term facilitation (LTF) of the carotid body afferent activity has been observed and it has been suggested that this is the mechanism mediating the maintained increases in sympathetic activity in humans. This theory is supported by data showing continued afferent activity from the carotid sinus nerve during normoxic recovery. In humans, LTF of the carotid body is not observed and therefore it is not a likely explanation for the persistent MSNA. To support this, the hypoxia induced increases in ventilation return to baseline within minutes into recovery. To address the persistent MSNA in humans, two explanations have been presented. First, the gain in chemoreflex control of sympathetic output could be higher than that of ventilation. Therefore, during recovery, low levels of afferent activity from the carotid body may cause large increases in sympathetic outflow with no effect on ventilation. In other words, sensitization of the carotid body may occur where chemoreceptor drive may continue despite a normalization of arterial oxygen saturation (SaO_2) (121) and may selectively augment sympathetic activity based on central neural structures. Secondly, central regions in charge of sympathetic activity, such as the medulla may undergo a type of LTF, analogous to that seen in the carotid bodies of rodents resulting in maintenance of neurotransmitter release and/or modulation of synaptic pathways controlling sympathetic signal transduction. Central LTF could be achieved directly by stimulation of central chemoreceptor or peripheral chemoreceptor stimulation that leads to centrally mediated increases in sympathetic output.

It is well established that local peripheral vasodilatation occurs response to hypoxia and it has been reasoned that this occurs to offset the sympathetic vasoconstriction. The study of sympathoexcitation and hypoxia has traditionally assumed that stimulation of the carotid body occurs first, causing an increase in sympathetic activity and that vasodilatation is a secondary response initiated with the purpose of maintaining vascular tone. An alternative view has been postulated, where peripheral vasodilatation is initiated first, causing a reduction in blood pressure and consequently unloading pressure sensitive baroreceptors (156, 157). In an effort to maintain blood pressure, baroreceptors will decrease sensory afferent output which will lead to a cascade of central neural events that will increase sympathetic activity. In the context of this hypothesis, the persistent increases in MSNA during normoxic recovery could be explained by a persistent production of vasodilatory elements causing reductions in blood pressure and therefore leading to baroreceptor disengagement. The mechanisms controlling sympathetic outflow are not clear – and strong evidence supporting baroreflex or chemoreflex processes have been presented. Regardless, it is not likely that one step along the oxygen sensing pathway is exclusively responsible for the initial rise in sympathetic activity or its persistence.

Indirect measurements of sympathetic activity with exposure to hypoxia have been made in humans, but due to the nature of indirect techniques, results have been equivocal. Few investigations have looked at the long term effects of hypoxia on MSNA in humans. Individuals with OSA, a pathological model of chronic intermittent hypoxia (IH) has been useful in understanding the potential physiological effects of long term repeated nocturnal exposures to hypoxia. These individuals show marked increases in MSNA during normoxia (106, 108) and hypoxia (107). The continuous bouts of hypoxia have been implicated as the primary cause of the elevated MSNA (68), although other conditions intrinsic to this disease my also contribute (106, 108). When treated for the repeated nocturnal exposures to hypoxia, MSNA is reduced in OSA patients. In healthy humans, Hansen and Sander (57) showed that after chronic sustained

hypoxia (4 weeks at 5360 m), MSNA was higher compared to that at sea level. The elevated MSNA persisted despite either disengagement of the carotid body via the administration of supplemental oxygen while at altitude or with normoxic breathing with the return to sea level. The persistent MSNA with administration of supplemental oxygen and the return to sea level could be due to a type of LTF of central mechanisms.

With long term exposure to hypoxia increases in the ventilatory sensitivity to hypoxia are observed. Peripheral chemoreceptors, specifically the carotid bodies, are essential in detecting systemic hypoxia and are required for the hypoxic ventilatory response (HVR) (43, 60, 127, 128). These cells are neuronal in nature and send sensory afferent signals to the CNS, where they are processed by the respiratory control centers (53). The ventilatory response to hypoxia is dependent on the carotid body as bilateral carotid body resection completely eliminates ventilatory response to hypoxia (60-62). The HVR has been shown to be dependent on the pattern in which hypoxia is administered in animals (131, 133) but not in humans (44). For example, in rodents (121) chronic IH and not chronic sustained hypoxia enhances hypoxic ventilatory sensitivity. Typically, any form of hypoxia delivered over the long term increases HVR in humans. At altitude (12 days at 3810 m), Sato et al. (143) showed chronic sustained hypoxia increased the HVR by approximately 50%. With exposure to IH in a laboratory setting Garcia et al. (49) showed that the maximal increases in the HVR were similar when humans were exposed to IH and chronic hypoxia; however, the time course of the increase in HVR was earlier with IH. Based on these data, it appears that IH can have an enhanced effect in the ventilatory response compared to other paradigms of long term hypoxic exposure. The enhanced HVR with IH can be attributed to the unique modulation of protein and ion pathways occurring within the carotid body (133), but is not exempt from alterations in the CNS and efferent signal transduction.

The cardiovascular system is also altered with acute hypoxia and this is in part mediated by the SNS. Cardiovascular alterations function to increase cardiac output, redistribute blood flow to essential regions, and counteract hypoxia induced peripheral vasodilatation. In humans, 20 minutes of acute hypoxia results in increases in heart rate and blood pressure with both variables returning to normal values upon resumption of normal room air breathing (20, 103, 157, 171, 172). Like the ventilatory response to hypoxia, the heart rate and blood pressure response is dependent on intact carotid bodies (61). With long term exposure to hypoxia in healthy humans, heart rate and blood pressure is unaltered during normoxia (57, 70, 71); however, the sensitivity of these cardiovascular measures to hypoxia is increased (44, 70). Sympathetically mediated vasoconstriction has been suggested to play an important role in the increased blood pressure response to hypoxia (54, 157). However, it can be reasoned that with the enhanced production of peripheral vasodilators thereby offsetting vasoconstriction, it is possible to observe no changes in blood pressure (157). In patients with OSA, high blood pressure is observed during non-apnoeic periods and is attributed to sympathoexcitation caused by long term IH (108, 168). Furthermore, compared to healthy controls, patients with OSA show increased blood pressure sensitivity to hypoxia (106, 107). In an experimental animal set up modeled to simulate some of the conditions of OSA, Fletcher and colleagues (41) showed that chronic IH caused a 13.7 torr increase in mean arterial pressure (MAP). In subsequent studies, by the same group also showed that the rise in MAP was dependent on intact carotid bodies (82) and was terminated with chemical sympathectomy (40) suggesting the important role of the peripheral chemoreceptors and the SNS in the blood pressure response to chronic hypoxia (5).

The skeletal muscle haemodynamic response to acute hypoxia is determined by the balance between vasoconstriction and vasodilatation, mediated by sympathetic activation and production of local dilatory elements, respectively. Human studies have examined hypoxic induced vasodilatation by indirect means. In humans exposed to acute hypoxia (SaO_2 = 74%)

Leuenberger et al. (83) measured skin and forearm blood flow and vascular resistance via plethysmography and showed that skin measurements did not change while there was a significant rise in forearm measurements. It was concluded that if vasodilatation occurs with hypoxia, it must be manifested locally within the vasculature of skeletal muscle. In a separate study by the same group femoral blood flow velocity acquired using Doppler ultrasound was significantly decreased during apnoeas coupled with hypoxia versus hyperoxia or normoxia (85, 157, 170). The authors suggested that sympathetic vasoconstriction induced by hypoxia caused the reduction in blood flow. In line with these results, reductions in forearm vascular resistance were observed in skeletal muscle during hypoxia when unmasked by local alpha-adrenergic blockade, thereby blunting the sympathetic mediated vasoconstriction and permitting vasodilatation.

Tissue oxygenation can be assessed using near infrared spectroscopy (NIRS), another indirect technique that is useful in examining the haemodynamic responses to hypoxia (135, 142) and will be determined by blood flow and tissue metabolism. Using the NIRS technique, the total haemoglobin (tHb) can be calculated as the sum of the deoxygenated haemoglobin (HHb) and oxygenated haemoglobin (HbO_2) signal and represents blood volume, which is a surrogate measurement for blood flow. Based on the skeletal muscle vascular responses obtained from blood flow and vascular resistance measurements during acute hypoxia, one might expect that skeletal muscle tissue oxygenation would be compromised during acute hypoxia. However, this is not the case in animal preparations. In rabbit hind limb muscles exposed to graded hypoxia large increases in HHb with small relative decreases in HbO_2 were observed resulting in increases in tHb (142). Long term exposure to hypoxia will have additional affects on skeletal muscle tissue oxygenation as dilatory and constriction functions in the vessel can be altered. After chronic exposure to IH in rats, isolated vessel preparations show dilatory sensitivity to adenosine is increased and vasoconstrictor response to norepinephrine is reduced (122). Others

suggest that chronic exposure IH blunts vessel dilatorily responsiveness to hypoxia by reducing nitric oxide release from the endothelium of skeletal muscle (15). No investigation has looked at the effect of IH on skeletal muscle oxygenation in humans.

Like the skeletal muscle, cerebral haemodynamics are affected by acute hypoxia. Additional factors must be considered when examining the cerebral haemodynamic response to hypoxia. Cerebral blood flow is tightly regulated by a phenomenon known as autoregulation which functions to protect the brain from high perfusion pressures through the cerebral vessels. Cerebral blood flow is increased from baseline values with exposure to acute hypoxia and this response is highly dependent on CO_2 tension (3, 78, 124). After long term IH, the sensitivity of cerebral blood flow to hypoxia is reported to increase (77). Alternatively, the sensitivity of cerebral oxygenation to hypoxia was reduced which was interpreted as an impairment of the cerebral vessels to regulate cerebral blood flow and therefore tissue oxygenation (44). Direct evidence from rodent models extends this hypothesis. During acute systemic hypoxia adenosine from endothelial cells produce the major part of the hypoxia-induced dilatation in the cerebral tissue (16). These processes may be compromised with long term hypoxia as Philips et al. (122) showed that in isolated cerebral vessel preparations a reduced vasodilatory response to hypoxia was evident after long term IH. Implications of reduced vasodilatory response can be seen in humans. Patients with OSA show reductions in cerebral oxygenation during periods of hypoxia (58, 160). Instead of a reduced vasodilatory response, reductions in cerebral oxygenation with hypoxia might be the due to a decrease in the metabolic activity of the CNS neurons (109, 113). Additionally, with long term and/or severe hypoxia, neuronal injury, neuron cell death (173), and brain atrophy (46) have been reported and may lead to decrements in cognitive function (46). Conversely, the metabolic activity of medullary regions may actually increase during hypoxia, such as the pre-Boetzinger region in charge of respiratory control, or the rostral ventral lateral medulla (RVLM) in charge of sympathetic tone (53).

The study of hypoxia on cellular and physiological responses has used a wide range of paradigms and techniques, making comparison among investigations difficult. Both acute and chronic models have been employed, which have varied in severity (assessed either by the fraction of inspired oxygen or as the percentage of arterial oxygen saturation), pattern (sustained or intermittent), and exposure duration (minutes, hours, days). Additionally, hypoxia has been examined in combination with differing levels of other stimuli, namely carbon dioxide (CO_2), atmospheric pressure, and breathing pattern. For our purposes, hypoxia was defined as an arterial oxygen saturation (SaO_2) of 80%. To minimize the contribution of other stimuli, end tidal carbon dioxide (CO_2) was held at eucapnic levels and spontaneous breathing was permitted. This current protocol was chosen as similar conditions have been used by others (20, 103, 157, 171, 172) making comparisons between studies possible. Intermittent hypoxia was defined as 1 hour daily exposures of hypoxia where SaO_2 = ~80%.

The present study was undertaken to address the effects of IH on direct measurements of SNA in humans. Specifically, we sought to characterize the increases in MSNA during an acute exposure to hypoxia and how this response is modulated after 10 days of IH. Furthermore, we wanted to test the effects of IH on the persistent MNSA during the normoxic recovery period. Concurrent ventilatory, cardiovascular, vastus lateralis oxygenation and cerebral tissue oxygenation measurements were acquired to examine how their response is altered with IH.

HYPOTHESES

The goal of the current study was to document the augmentation of sympathoexcitation, measured as MSNA following 10 days of IH in healthy males. The primary aim of the present study was to test the hypothesis that 10 days of IH would augment the rise in MSNA during an acute 20 minute isocapnic hypoxic exposure. Furthermore, IH was expected to elevate the persistent MSNA during the normoxic recovery period. A secondary purpose was to examine the effect of 10 days of IH on the ventilatory, cardiovascular, vastus lateralis oxygenation, and cerebral tissue oxygenation parameters. Lastly, we further hypothesized that the changes in the hypoxic ventilatory response after IH would show a strong relationship with the changes in MSNA since both the ventilatory and sympathetic systems have overlapping neural structures in the CNS and therefore may undergo concurrent modulation with IH.

METHODS

All methods and procedures were approved by the Clinical Research Ethics Board at the University of British Columbia. All data was collected at the Health and Integrative Physiology Laboratory on the University of British Columbia campus.

SUBJECTS

Eleven healthy male subjects gave their informed written consent to participate in the present study. Of the eleven subjects, MSNA was obtained from six subjects. Inclusion criteria were as follows: recreationally active males, between the ages of 19-35, and a body mass index (BMI) of ~24 kg m^2. The rationale for these inclusion criteria is based on evidence that sympathetic activity is influenced by gender, training status, age, and BMI (67, 98).

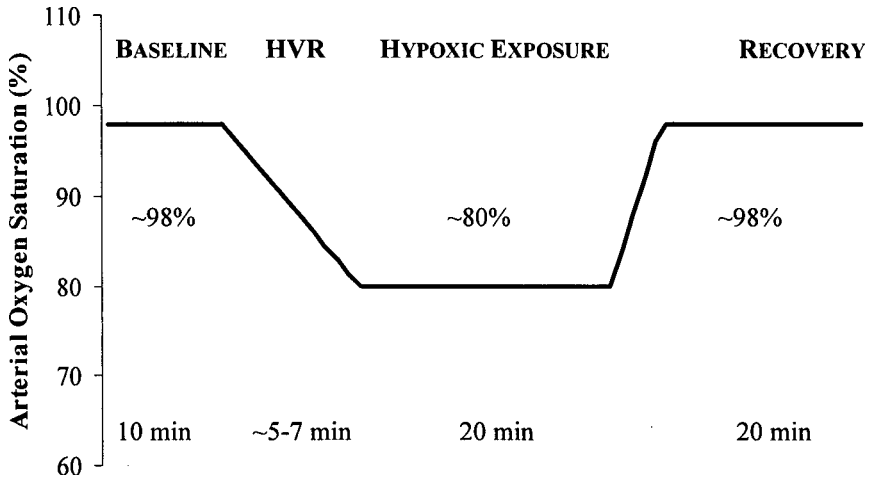
Subjects were excluded if they had ascended to altitude (>3,000 m) three months prior to testing, had history of smoking, were taking regular medication, or had been diagnosed with sleep apnoea, asthma, or neurological disease. No subjects had participated in competitive swimming, breath-hold diving, or any other form of apnoeic training activity (31). Subjects were asked to refrain from caffeine, alcohol, and exercise for 24 hr prior to all testing procedures.

EXPERIMENTAL PROCEDURES

Refer to Table 1 for a summary of the experimental time line. See Figure 1 for a diagram of the experimental protocol.

FAMILIARIZATION: On DAY 1 subjects signed consent forms and anthropometric data was recorded and pulmonary function testing was conducted. Additionally, a HVR was performed to familiarize subjects with masked and hypoxic breathing.

Figure 1. Overview of the experimental protocol



During baseline subjects breathed room air for 10 minutes. The HVR lasted approximately 5 to 7 minutes. The hypoxic exposure was 20 minutes in duration where the SaO_2 was reduced to 80% and held constant. Recovery was 20 minutes where subjects were returned to room air breathing.

Abbreviations: hypoxic ventilatory response (HVR)

PRE INTERMITTENT HYPOXIA (PRE IH): On DAY 2, a four-phased experimental protocol was conducted, consisting of a 10 minute baseline, a ~5-7 minute HVR, 20 minute isocapnic hypoxic exposure, and a 20 minute normoxic recovery period (see MEASUREMENTS section for details about each phase). Ventilatory, cardiovascular, oximetry, MSNA, and NIRS measurements were collected. Subjects from which MNSA data was collected were studied in the semi-recumbent position, while the other subjects were studied in the supine position.

INTERMITTENT HYPOXIA (IH): Subjects returned to the laboratory daily for 10 consecutive days (DAYS 3-13). Isocapnic-hypoxia was administered, such that SaO_2 was maintained at ~80%. On these days only, ventilatory measurements were recorded and subjects were allowed to sit upright to read, watch videos, and listen to music.

POST INTERMITTENT HYPOXIA (POST IH): On DAY 14 the identical experimental protocol was conducted and the same measurements were recorded as on DAY 2.

Table 1: Time line of study

	DAY 1 FAMILIARIZATION	EXPERIMENTAL DAY 2 PRE IH	TRAINING DAY 3-13 INTERMITTENT HYPOXIA	EXPERIMENTAL DAY 14 POST IH
DESCRIPTION	Consent Forms ⬇ Pulmonary Function Testing and Anthropometrics Data ⬇ Familiarization with Mask and HVR procedure	Experimental Protocol ⬇ Baseline HVR Hypoxic Exposure Recovery	Daily Hypoxia ⬇ Exposures 1-10	Experimental Protocol ⬇ Baseline HVR Hypoxic Exposure Recovery
MEASUREMENTS	Height, weight, age, BMI, FVC, FEV_1, FEV_1/FVC, HVR, VENT	HVR, VENT, HR, BP, SaO_2, MSNA, NIRS	VENT, SaO_2	HVR, VENT, HR, BP, SaO_2, MSNA, NIRS

The days included in the study are indicated along the horizontal bars and the descriptions and measurements taken each day are indicated along the vertical bars.

Abbreviations: body mass index (BMI), forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), forced expiratory volume in one second as a percentage of predicted (FEV_1/FVC), hypoxic ventilatory response (HVR), ventilatory measurements (VENT), arterial oxygen saturation (SaO_2), heart rate (HR) blood pressure (BP), muscle sympathetic nerve activity (MSNA), near infrared spectroscopy (NIRS)

MEASUREMENTS

All variables were acquired online, in real time using an analog-to-digital converter (Powerlab/16SP ML795 ADInstruments, Colorado Springs, CO, USA) interfaced with a personal computer (Satellite, Toshiba, Irvine, CA, USA). All variables were sampled at 1kHz. Data were stored on a personal computer for offline analysis using commercially available software (Chart 5 for Powerlab, ADInstruments, Colorado Springs, CO, USA).

ANTHROPOMETRIC MEASURES: Height, weight, BMI, and age were documented.

PULMONARY FUNCTION TESTING: Using a calibrated spirometer (Spirolab II, Medical International Research, Via del Maggiolino, Roma, Italy) and abiding by the recommendations of the American Thoracic Society, pulmonary function was assessed. Three forced vital capacity (FVC) manoeuvers were performed and FVC, forced expiratory volume in one second (FEV_1), and forced expiratory volume in one second as a percentage of FVC (FEV_1/FVC) were documented. Those subjects unable to achieve 80% of their predicted capacity as determined by the European Respiratory Society prediction equations for adult men were excluded from the study (1).

VENTILATORY MEASUREMENTS: Inspiratory flow was obtained using a heated pneumotach connected to the inspired side of a face mask (8930 Series, Hans Rudolph Inc., Kansas City, MO, USA). Prior to each test, flow calibration was carried out with a 3 L syringe (MedTech, Model #2030-2, Hans Rudolph Inc, Kansas City, MO, USA). Using the integral of the inspiratory flow signal, inspiratory volume of each breath was determined. During offline analysis, tidal volume (V_T; L) and breathing frequency (f_B; breaths min^{-1}) values were calculated from inspiratory volume and minute ventilation (V_I; L min^{-1}) was calculated as the product of f_B and V_T.

Dedicated analyzers were used to monitor end-tidal oxygen ($P_{et}O_2$) and carbon dioxide ($P_{et}CO_2$) (S-3A/I and CD-3A, Applied Electrochemistry, Pittsburg, PA, USA). Analyzers were calibrated prior to each test using medical grade gases of known concentrations.

HYPOXIC VENTILATORY RESPONSE (HVR): The HVR procedure was conducted in a similar manner to that described previously (14) and used in our laboratory (44, 52, 75). Subjects breathed room air from a mixing chamber (13.5 L) as 100% nitrogen (N_2) was progressively

added to the inspiratory circuit (~2 L every 30 seconds), thereby reducing the concentration of oxygen until SaO_2 reached 80%. Isocapnia was maintained during the procedure by monitoring end tidal CO_2 ($P_{et}CO_2$) and adding 100% CO_2, as required, to the inspired tubing via a needle inserted ~30 cm from the mouth piece. The total time for each HVR procedure was ~ 5-7 minutes. The HVR was calculated using a linear regression model for V_I versus SaO_2. The slope represents the HVR, or the ventilatory sensitivity to hypoxemia. Values are expressed as L min^{-1} $\%SaO_2^{-1}$.

ISOCAPNIC HYPOXIC EXPOSURE: Immediately following the HVR procedure, subjects continued to breathe a hypoxic inspirate from the mixing chamber for 20 minutes, such that the SaO_2 was maintained at a mean of 80% and isocapnia was maintained. These procedures are similar to those used by others (20, 103, 171, 172).

NORMOXIC RECOVERY: Following the 20 minute hypoxic exposure, the flow of N_2 ceased and subjects were returned to room air breathing for 20 minute. The $P_{et}CO_2$ trace was continually monitored and CO_2 was regulated at baseline values if needed.

MICRONEUROGRAPHY. Direct intraneural recordings of multiunit post ganglionic MSNA were acquired using a common microneurographic technique that has been employed previously by others (19, 20, 103, 156, 161, 171, 172). Recordings were made from the peroneal nerve. An appreciation for the location of the nerve was obtained through surface stimulation (Stimulator, model S48, Astromed Inc, Grass Product Group, W.Warwick, RI, USA). Upon location, two sterile microelectrodes (tip diameter 5-10 μm, 30-35 mm long, Fredrick Haer, Bowdoinham, ME) were inserted. Stimulating pulses were administered via the reference microelectrode (UNR32FRS) and once a clean dorsiflexion movement was evoked using a low stimulating

voltage (~5-10 Hz), the active tungsten microelectrode (UNA32F2S) was inserted for recording of MSNA.

Nerve signals were amplified (custom-built microneurography preamplifier and amplifier, Yale University, New Haven, CT, USA) with a total gain of 10,000. Processed signals were band pass filtered (700-2000 Hz), rectified and discriminated. The nerve signal was integrated at a time constant of 100 ms (Integrator model B937C, Bioengineering, University of Iowa, Iowa City, IA, USA). The nerve signal was assessed via audio representation of burst activity (Audio Monitor, model AMIO, Astromed Inc, Grass Product Group, W. Warwick, RI, USA) and visual inspection of the mean voltage neurogram (ADInstruments, Colorado Springs, CO, USA). The following criteria were used to validate the recording site: 1) demonstration of pulse synchronous bursts occurring 1.2-1.4 sec after a QRS complex (89, 92, 167); 2) reproducible activation upon apnoea or phase II and III of a Valsalva manoeuvre; 3) no activation upon pinch, stroking of the skin, or startle stimuli (indicating skin sympathetic activation); and 4) a signal to noise ratio of >3:1(161).

The integrated neurogram was high pass filtered at 0.5 Hz to set the mean baseline to zero and low pass filtered at 10 Hz to smooth the signal for analysis. The integrated and filtered neurogram was then time shifted to align each burst with the preceding R-wave. Burst labelling was automated and then manually inspected and edited according to the validation criteria described above and yielded burst frequency (burst min^{-1}) values. To normalize the neurogram, commercially available software (Chart 5, ADInstruments, Colorado Springs, CO, USA) was used to establish baseline and threshold settings for each individual data file and permitted calculation of peak amplitude (arbitrary units). Total MSNA (arbitrary units) was calculated as the product of burst frequency and peak amplitude.

CARDIOVASCULAR MEASUREMENTS: Electrocardiogram (ECG; ADInstruments, ML110, 5303) tracing was continuously collected from a standard bipolar limb lead. Heart rate (HR) was determined via the peak detection of the R-wave. Beat-by-beat blood pressure was obtained using non-invasive photoplethysmography at the finger (Finometer, Finapres Medical System, Arnhem, Netherlands). To ensure accurate estimates of blood pressure, the hydrostatic height sensor was zeroed prior to each test and subject height, weight, and age were entered into the device. Similar methods have been validated as reliable measures of blood pressure (63, 64). Additional blood pressure measurements were acquired from an automated cuff (BPM-100, VSM Medical Technologies Ltd, Vancouver, Canada) every two minutes throughout the experimental protocol and statistical analyses were preformed on these values. The on-line blood pressure trace was calibrated from the cuff measurements collected during baseline. The maximum and minimum values of the beat-by-beat blood pressure trace were determined and represented systolic (SBP) and diastolic (DBP) blood pressure, respectively. Mean arterial blood pressure (MAP) was calculated as the sum of DBP and 1/3 (SBP-DBP).

PULSE OXIMETERY: Finger oximetery was used to monitor SaO_2 (3740, Ohmeda, Louisville, CO, USA). This is a non-invasive tool to determine the delivery of oxygen using an absorbtion spectrum from a two-wavelength pulsatile system. Calculations are made based on the relative percentages of HbO_2 and HHb chromophores and values are displayed as SaO_2 (%).

NEAR-INFRARED SPECTROSCOPY (NIRS). Oxyhaemoglobin (HbO_2; μMol), deoxyhaemoglobin (HHb; μMol) and tissue oxygenation index (TOI; %) were assessed in the right vastus lateralis muscle and right cerebral cortex using the NIRO-300 (Hamamatsu Photonics K.K., Hamamatsu, Japan). Total haemoglobin (tHb) was calculated as the sum of HbO_2 and HHb. The NIRO-300

uses four wavelengths (775, 810, 847, and 913nm) and employs two theoretical models: spatially resolved spectroscopy to calculate TOI and the modified Beer-Lambert Law to assess the oxygenation status of the haemoglobin molecule. The details of these models are described elsewhere (23, 24, 87, 97). For both the brain and muscle, a tissue specific differential pathlength factor (DPF) was multiplied by probe spacing (4.0 –5.0 cm) and was internally set within the NIRO-300 unit.

The optodes, containing a light source and detector were placed in a black vinyl holder that shields light and were fixed against the skin with double sided tape and loosely covered with a dark cloth. One optode was applied to the right side of the head with detection probe was placed closest to the midline of the frontal lobe at the level of the hairline. Care was taken to avoid placement over the temporal muscles and sinuses. These methods are in compliance with those used previously (44, 74, 115). For the brain, a DPF of 5.93 was used (87, 164); therefore the pathlength set within the NIRO unit was 23.7 – 29.7 cm. A separate optode was applied to the right vastus lateralis muscle in accordance with criteria used in other investigations (21, 22). The detector was placed proximally, midway between the greater trochanter and the lateral condyle of the knee. Care was taken to avoid placement over the dense fascia of the IT band. For skeletal muscle, the DPF used was 4.0 (23, 28); therefore the pathlength set within the NIRO unit was 16.0 – 20.0 cm.

DATA AND STATISTICAL ANALYSIS. Ventilatory, cardiovascular, and NIRS data were averaged over 5 minute sections during baseline, hypoxic exposure, and recovery and are expressed as absolute values. Additionally, these measurements are expressed as changes from baseline and for each 5 minute period during the hypoxic exposure and recovery period. MSNA is reported as absolute normalized units and as percent change from baseline for within subject comparison

(pre and post IH). Statistical analysis was performed on both absolute values and percent change values, however emphasis was placed on the percent change in MSNA only. The rationale for MSNA data to be presented in this manner is three-fold: 1) MSNA is not reported as absolute values as it is dependent on the proximity of the microelectrode to the nerve (161); 2) MSNA is highly variable between subjects (19, 98, 161); and 3) to allow for comparison between previously conducted investigations.

All variables are expressed as means ± SE. Statistical analyses were performed at a significance level of $\alpha = 0.05$ using commercially available software (Statistica software V.6.1, Statsoft Inc, Tulsa, OK, USA). For each variable a repeated measures ANOVA was performed to 1) determine the difference between baseline, the isocapnic-hypoxic exposure, and the recovery; and 2) the differences between pre and post IH values. When a significant F-ratio was detected, Tukey's test was applied poc-hoc to ascertain where the differences resided. Linear correlation analysis was used to examine the relationship between HVR and maximum burst frequency for both pre and post IH. A paired t-test was preformed to analyze the difference between pre and post IH values of HVR.

RESULTS

SUBJECT CHARACTERISTICS. All subjects completed each of the 14 days of the study. Cardiovascular, ventilatory, and NIRS measurements were acquired from all subjects (n=11). MSNA measurements were obtained from only six subjects. Mean descriptive data for anthropometric and pulmonary function testing can be found in Table 2 and were collected on the Familiarization day (Day 1). The pulmonary function values for each subject are the mean of three manoeuvers.

Table 2. Subject characteristics

	ANTHROPOMETRIC DATA				PULMONARY FUNCTION DATA		
Subject Number	**Age (yrs)**	**Height (m)**	**Mass (kg)**	**BMI ($kg \cdot m^2$)**	**FVC (L)**	**FEV_1 (L)**	**FEV_1/FVC (%)**
1*	20	1.71	72	24.6	4.9	4.2	85.2
2*	33	1.78	79	24.9	5.2	4.4	85.3
3*	27	1.85	70	20.5	6.2	4.8	85.6
4*	27	1.84	77	22.7	6.1	4.6	86.1
5*	23	1.89	94	26.2	5.4	4.7	86.5
6*	24	1.85	89	26.0	6.0	4.7	77.5
7	25	1.87	75	21.5	6.6	5.3	80.5
8	24	1.91	106	29.1	5.3	4.5	83.6
9	21	1.86	81	23.4	6.2	4.9	82.1
10	26	1.92	109	29.6	6.0	5.2	86.0
11	24	1.80	86	26.5	6.2	5.4	87.2
Mean (±SE)	24.9 (1.0)	1.8 (0.02)	85.3 (4.0)	25.0 (0.87)	5.8 (0.16)	4.8 (0.11)	84.1 (0.90)

Individual data was collected on the Familiarization day (Day 1). Pulmonary function data is reported as the mean of three FVC manoeuvres for each subject. Values are expressed as means (±SE). Cardiovascular, ventilatory, and NIRS measurements were acquired from all subjects (n=11). MSNA measurements were obtained from only six subjects and are indicated (*).

Abbreviations: body mass index (BMI), forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), forced expiratory volume in one second as a percentage of predicted (FEV_1/FVC), muscle sympathetic nervous activity (MSNA).

EFFECTS OF IH ON MSNA. Individual and mean data for absolute (normalized) burst frequency, amplitude, and total MNSA can be found in Tables 3, 4, and 5 respectively. Individual and mean data for percent change in burst frequency, amplitude, and total MNSA can be found in Tables 6,

7, and 8 respectively. Figure 2 displays the percent change in burst frequency (A), amplitude (B), and total MNSA (C). A mean value for baseline and for each 5 minute period during the hypoxic exposure and recovery period was calculated. Burst frequency increased from baseline immediately with exposure to hypoxia and remained significantly elevated for the duration of the exposure and recovery period ($p<0.001$). Burst frequency was significantly higher post IH compared to pre IH during the hypoxic exposure and recovery ($p=0.01$). Burst amplitude increased from baseline immediately with exposure to hypoxia and remained significantly elevated for the duration of the exposure and recovery period ($p<0.01$); however, there was no difference between pre and post IH burst amplitude ($p=0.82$). For both pre and post IH, total MSNA increased significantly during the hypoxic exposure ($p<0.001$) mediated primarily by the increases in burst frequency. Post IH, total MSNA values trended to be higher than pre IH values ($p=0.06$).

Figure 3 is a representative trace of MSNA and corresponding cardiovascular and ventilatory variables and can be found in Appendix A. These data represent 30 seconds of baseline, hypoxia exposure, and recovery collected during the post IH trial from subject 5. Although ventilatory, cardiovascular, and SaO_2 all return to baseline values during recovery, an appreciation of a persistence in the MSNA can be acquired from the mean voltage neurogram.

Table 3. Effect of IH on absolute values (normalized) for MSNA burst frequency

	BASELINE		HYPOXIC EXPOSURE				RECOVERY			
	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	50 min
PRE IH										
Subject										
1	21.6	19.9	21.9	21.8	22.2	20.5	21.1	19.1	20.0	21.3
2	20.7	18.7	24.3	26.0	24.0	27.5	28.2	30.5	30.1	27.3
3	16.4	16.7	19.2	17.4	18.0	19.1	16.9	20.5	16.0	16.4
4	15.1	13.2	16.4	16.6	16.3	16.3	15.5	16.3	14.2	14.4
5	8.6	9.7	11.7	12.3	11.1	13.5	14.0	10.1	11.9	12.1
6	22.8	21.8	24.1	26.5	25.8	26.6	27.4	24.7	24.2	25.3
Mean (±SE)	17.5 (2.2)	16.7 (1.8)	19.6 (2.0)*+	20.1 (2.3)*+	19.6 (2.3)*+	20.6 (2.3)*+	20.5 (2.5)*+	20.2 (2.9)*+	19.4 (2.8)*+	19.5 (2.5)*+
POST IH										
Subject										
1	8.6	6.2	11.0	12.4	12.0	13.4	12.3	10.5	9.5	9.4
2	19.0	19.0	23.6	24.4	26.8	27.8	28.6	28.2	27.8	28.6
3	17.7	16.0	25.0	20.8	22.4	24.4	24.6	23.8	26.2	26.0
4	11.5	10.4	18.5	19.5	25.5	27.5	21.3	25.5	22.8	20.5
5	14.0	7.8	12.0	16.2	13.0	13.2	14.2	16.3	12.7	11.6
6	15.4	15.2	19.4	25.8	26.2	25.8	29.8	24.6	25.8	26.8
Mean (±SE)	14.4 (1.6)	12.4 (2.1)	18.3 (2.4)*+	19.9 (2.1)*+	21.0 (2.8)*+	22.0 (2.8)*+	21.8 (3.0)*+	21.5 (2.7)*+	20.8 (3.2)*+	20.5 (3.4)*+

The MSNA trace was high pass filtered at 10 Hz for normalization purposes, setting the mean voltage to zero. Burst frequency values are reported as absolute bursts per minute for each 5 min period during baseline, the hypoxic exposure, and recovery (n=6). Values are expressed as means (±SE). Significance was set at $p<0.05$ and is indicated as change from baseline (*) and difference between pre and post IH (+)

Abbreviations: intermittent hypoxia (IH), muscle sympathetic nerve activity (MSNA)

Table 4. Effect of IH on absolute values (normalized) for MSNA burst amplitude

	BASELINE		HYPOXIC EXPOSURE				RECOVERY			
	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	50 min
PRE IH										
Subject										
1	18.4	17.5	22.8	21.0	21.0	22.6	25.2	25.8	27.0	23.6
2	17.6	18.4	21.4	21.0	20.8	25.0	23.8	23.4	23.4	23.4
3	16.4	16.7	19.2	17.4	18.0	19.1	16.9	20.5	16.0	16.4
4	12.0	13.6	19.4	23.8	22.6	24.9	18.2	25.8	21.2	22.0
5	15.4	20.0	20.8	18.2	21.7	19.4	20.6	18.5	17.9	19.7
6	20.2	23.2	29.0	24.2	26.4	27.2	25.8	28.4	28.0	29.0
Mean	16.7	18.2	22.1	20.9	21.8	23.0	21.8	23.7	22.2	22.3
(±SE)	(1.2)	(1.3)	(1.5)*	(1.1)*	(1.1)*	(1.3)*	(1.5)*	(1.5)*	(2.0)*	(1.7)*
POST IH										
Subject										
1	8.6	6.2	11.0	12.4	12.0	13.4	12.3	10.5	9.5	9.4
2	19.0	19.0	23.6	24.4	26.8	27.8	28.6	28.2	27.8	28.6
3	17.7	16.0	25.0	20.8	22.4	24.4	24.6	23.8	26.2	26.0
4	11.5	10.4	18.5	19.5	25.5	27.5	21.3	25.5	22.8	20.5
5	14.0	7.8	12.0	16.2	13.0	13.2	14.2	16.3	12.7	11.6
6	15.4	15.2	19.4	25.8	26.2	25.8	29.8	24.6	25.8	26.8
Mean	14.4	12.4	18.3	19.9	21.0	22.0	21.8	21.5	20.8	20.5
(±SE)	(1.6)	(2.1)	(2.4)*	(2.1)*	(2.8)*	(2.8)*	(3.0)*	(2.7)*	(3.2)*	(3.4)*

The MSNA trace was high pass filtered at 10 Hz for normalization purposes, setting the mean voltage to zero. Burst amplitude values are reported as absolute normalized mVunits for each 5 min period during baseline, the hypoxic exposure, and recovery (n=6). Values are expressed as means (±SE). Significance was set at $p<0.05$ and is indicated as change from baseline (*)

Abbreviations: intermittent hypoxia (IH), muscle sympathetic nerve activity (MSNA)

Table 5. Effect of IH on absolute values (normalized) for total MSNA

	BASELINE		HYPOXIC EXPOSURE					RECOVERY		
	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	50 min
PRE IH										
Subject										
1	397.8	348.5	498.5	457.1	466.8	463.3	532.9	493.4	540.9	502.9
2	364.0	344.0	519.1	546.0	500.2	688.3	672.3	714.2	704.2	639.1
3	269.5	278.5	369.1	303.4	324.8	365.1	286.8	420.8	255.4	268.8
4	181.6	179.5	317.8	394.6	367.6	404.9	281.7	420.5	302.0	315.9
5	132.4	194.4	243.4	223.6	239.9	262.0	289.1	186.0	213.1	238.7
6	350.7	331.1	621.7	693.9	666.5	793.2	673.4	638.0	649.2	649.0
Mean (±SE)	282.7 (43.9)	279.3 (31.1)	428.3 (58.1)*	436.4 (69.4)*	427.6 (61.7)*	496.1 (83.3)*	456.0 (79.2)*	478.8 (76.3)*	444.1 (87.6)*	435.7 (76.1)*
POST IH										
Subject										
1	105.9	73.2	130.4	156.3	149.5	170.0	151.1	136.3	114.1	139.7
2	290.4	282.5	435.1	448.0	506.9	512.0	462.1	493.6	448.5	476.5
3	236.3	197.1	361.8	330.8	391.2	450.5	443.1	391.2	382.0	415.9
4	137.4	139.7	323.9	322.1	444.9	534.8	362.1	394.1	375.8	338.5
5	196.3	89.3	185.7	283.1	201.2	174.5	189.2	236.0	168.8	155.8
6	580.0	555.4	836.0	1078.8	1005.2	988.8	882.7	797.5	828.2	863.0
Mean (±SE)	257.7 (70.2)	222.9 (73.8)	378.8 (102.8)*	436.5 (134.6)*	449.8 (125.3)*	471.8 (123.3)*	415.1 (107.7)*	408.1 (94.0)*	386.2 (103.8)*	398.2 (108.7)*

The MSNA trace was high pass filtered at 10 Hz for normalization purposes, setting the mean voltage to zero. Total MSNA values are reported absolute normalized arbitrary units and are the product of burst frequency and burst amplitude. Total MSNA values are reported for each 5 min period during baseline, the hypoxic exposure, and recovery (n=6). Values are expressed as means (±SE). Significance was set at $p<0.05$ and is indicated as change from baseline (*)

Abbreviations: intermittent hypoxia (IH), muscle sympathetic nerve activity (MSNA)

Table 6. Effect of IH on percent change in MNSA burst frequency

	BASELINE	HYPOXIC EXPOSURE					RECOVERY		
	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	50 min
PRE IH									
Subject									
1	97.5	130.3	120.0	120.0	129.1	144.0	147.4	154.3	134.9
2	97.8	116.3	114.1	113.0	135.9	129.3	127.2	127.2	127.2
3	100.8	115.1	104.4	108.0	114.5	101.5	122.9	95.7	98.2
4	106.3	142.6	175.0	166.2	183.1	133.8	189.7	155.9	161.8
5	113.0	117.5	102.8	122.5	109.3	116.7	104.3	101.1	111.2
6	106.9	133.6	104.3	113.8	117.2	111.2	122.4	120.7	129.0
Mean (±SE)	103.7 (2.5)	125.9 (4.6)*+	120.1 (11.4)*+	123.9 (8.7)*+	131.5 (11.1)*+	122.7 (6.5)*+	135.7 (12.2)*+	125.8 (10.5)*+	127.0 (8.9)*+
POST IH									
Subject									
1	83.8	177.4	200.6	193.3	216.8	198.6	168.9	152.8	151.6
2	100.0	124.2	128.6	141.1	146.3	150.4	148.4	146.3	150.5
3	95.1	156.3	130.0	140.0	152.5	154.1	148.8	163.8	162.5
4	95.0	178.9	187.7	245.5	265.0	205.8	245.6	219.9	198.1
5	71.6	153.8	207.7	166.7	169.2	182.1	209.0	162.4	149.1
6	99.3	168.6	171.2	168.6	194.8	160.8	168.6	175.2	175.2
Mean (±SE)	90.8 (11.1)	159.9 (20.3)*+	171.0 (34.6)*+	175.9 (39.5)*+	190.8 (45.0)*+	175.3 (23.7)*+	181.6 (38.4)*+	170.1 (26.4)*+	164.5 (19.2)*+

Burst frequency values are reported bursts per minute and are represented as change from baseline. A mean value for baseline was calculated and for each 5 min period during the hypoxic exposure and recovery period (n=6). Values are expressed as means (±SE). Significance was set at $p<0.05$ and is indicated as change from baseline (*) and difference between pre and post IH (+).

Abbreviations: intermittent hypoxia (IH), muscle sympathetic nerve activity (MSNA)

Table 7. Effect of IH on percent change in MSNA burst amplitude

	BASELINE	HYPOXIC EXPOSURE					RECOVERY		
	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	50 min
PRE IH									
Subject									
1	95.9	109.8	109.3	111.6	103.0	106.2	96.0	100.6	107.0
2	95.0	129.7	139.0	128.6	147.2	151.1	163.2	160.9	146.0
3	100.8	115.1	104.4	108.0	114.5	101.5	122.9	95.7	98.2
4	93.2	124.1	125.6	123.2	123.2	117.2	123.5	107.9	108.8
5	106.1	120.4	126.4	113.8	139.3	144.1	103.7	122.5	124.8
6	97.8	110.6	121.5	118.5	122.1	125.6	113.5	111.2	116.2
Mean	98.1	118.3	121.1	117.3	124.9	124.3	120.5	116.5	116.9
(±SE)	(1.9)	(3.2)*	(5.2)*	(3.2)*	(6.6)*	(8.2)*	(9.7)*	(9.7)*	(6.9)*
POST IH									
Subject									
1	97.9	100.5	106.5	105.7	107.2	104.0	110.3	102.0	125.9
2	98.6	124.0	123.3	127.2	123.9	108.8	117.7	108.5	112.0
3	95.9	117.5	129.1	141.8	149.9	145.9	133.4	118.3	129.8
4	105.8	129.6	122.8	129.8	144.5	125.9	114.9	122.3	122.3
5	89.9	135.2	152.6	135.1	115.5	116.4	126.4	116.4	117.0
6	98.5	117.9	114.4	116.2	124.3	124.4	116.3	110.9	111.5
Mean	97.8	120.8	124.8	126.0	127.5	120.9	119.8	113.1	119.8
(±SE)	(2.1)	(4.9)*	(6.5)*	(5.4)*	(6.8)*	(6.1)*	(3.5)*	(3.0)*	(3.1)*

Burst amplitude values are reported as normalized mVunits and are represented as change from baseline. A mean value for baseline was calculated and for each 5 min period during the hypoxic exposure and recovery period (n=6). Values are expressed as means (±SE). Significance was set at $p<0.05$ and is indicated as change from baseline (*)

Abbreviations: intermittent hypoxia (IH), muscle sympathetic nerve activity (MSNA)

Table 8. Effect of IH on percent change in total MSNA

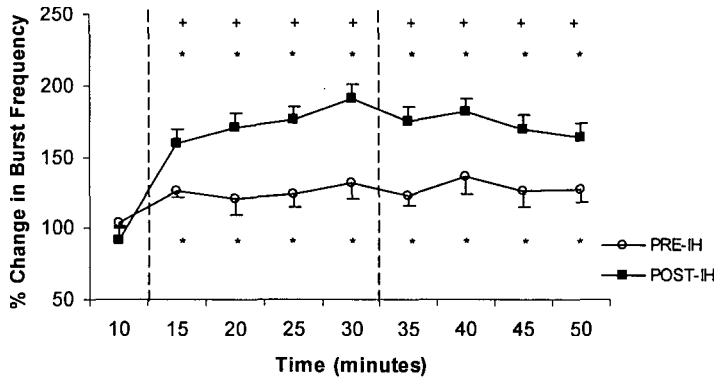
	BASELINE	HYPOXIC EXPOSURE					RECOVERY		
	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	50 min
PRE IH									
Subject									
1	93.4	143.1	131.2	133.9	132.9	152.9	141.6	155.2	144.3
2	100.0	150.9	158.7	145.4	200.1	195.4	207.6	204.7	185.8
3	101.7	132.5	108.9	116.6	131.1	103.0	151.1	91.7	96.5
4	99.4	177.0	219.8	204.8	225.6	156.9	234.3	168.3	176.0
5	58.7	73.5	67.5	72.5	79.1	87.3	56.2	64.3	72.1
6	100.0	187.7	209.5	201.3	239.5	203.3	192.7	196.0	196.0
Mean	92.2	144.1	149.3	145.7	168.1	149.8	163.9	146.7	145.1
(±SE)	(6.8)	(16.6)*	(24.1)*	(20.8)*	(25.9)*	(19.3)*	(25.9)*	(23.3)*	(20.8)*
POST IH									
Subject									
1	81.7	178.2	213.6	204.3	232.3	206.6	186.3	155.9	190.9
2	98.6	154.1	158.6	179.5	181.3	163.6	174.7	158.8	168.7
3	91.0	183.5	167.8	198.4	228.5	224.8	198.5	193.7	210.9
4	100.8	231.9	230.6	318.5	382.9	259.2	282.1	269.0	242.3
5	62.6	207.9	316.9	225.1	195.3	211.8	264.1	189.0	174.3
6	97.8	150.5	194.2	181.0	178.0	158.9	143.6	149.1	155.4
Mean	88.8	184.4	213.6	217.8	233.1	204.1	208.2	185.9	190.4
(±SE)	(6.0)	(12.8)*	(23.5)*	(21.4)*	(31.5)*	(15.6)*	(22.0)*	(18.3)*	(13.1)*

Total MSNA values are reported normalized arbitrary units and are represented as change from baseline. A mean value for baseline was calculated and for each 5 min period during the hypoxic exposure and recovery period (n=6). Values are expressed as means (±SE). Significance was set at $p<0.05$ and is indicated as change from baseline (*)

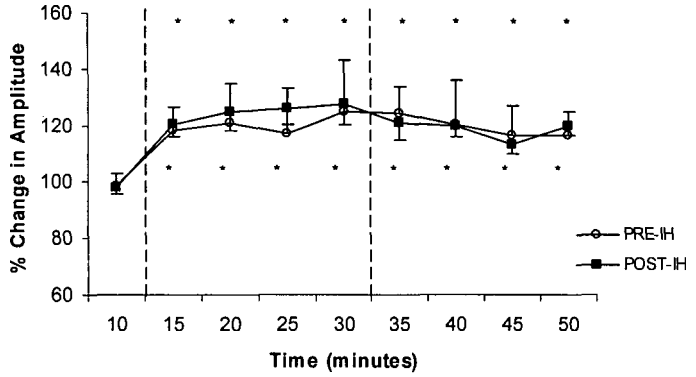
Abbreviations: intermittent hypoxia (IH), muscle sympathetic nerve activity (MSNA)

Figure 2: The effect of IH on the percent change in muscle sympathetic nervous activity (MSNA). A mean value for baseline and for each 5 min period during the hypoxic exposure and recovery was calculated (n=6). Values are expressed as means ± SE and symbolized as pre (○) and post (■) IH. Significance was set at $p<0.05$ and is represented as change from baseline (*) and difference between pre and post IH (+). The area between the dashed lines indicates the hypoxic exposure. A: Burst frequency increased significantly over time ($p<0.001$), as a function of protocol ($p<0.001$), and as a result of time vs. protocol ($p<0.001$). B: Amplitude increased significantly over time ($p<0.001$); however, protocol and time vs. protocol were not significantly different between pre and post IH ($p=0.82$ and $p=0.89$, respectively). C: Total MSNA increased significantly over time ($p<0.001$), approached significance as a function of protocol ($p=0.08$), and approached significance as a result of time vs. protocol ($p=0.06$). Note the difference in scales of the y-axis.

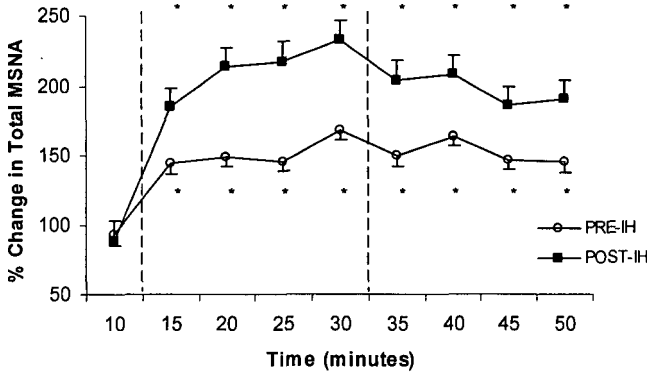
A



B



C



EFFECTS OF IH ON THE VENTILATORY RESPONSE TO HYPOXIA. Table 9 displays the absolute values for ventilatory measures during the entire period of baseline and for each 5 minute period during the hypoxic exposure and recovery. During pre IH, f_B increased during the hypoxic exposure when compare to baseline ($p<0.001$) and remained elevated for 15 minute into recovery

Table 9. Effects of IH on ventilatory measures

	BASELINE	HYPOXIC EXPOSURE				RECOVERY			
	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	50 min
PRE IH									
f_B (breaths min^{-1})	12.6	14.6	14.9	14.7	15.2	14.6	14.8	14.4	13.5
	(1.2)	(1.1)*	(1.1)*	(0.90)*	(1.0)*	(1.1)*	(1.1)*	(1.1)*	(1.2)
V_T (litres)	0.81	1.1	0.99	0.92	0.92	0.79	0.75	0.74	0.80
	(0.12)	(0.08)*	(0.11)*	(0.90)*	(0.13)*	(0.12)	(0.13)	(0.09)	(0.15)
V_I (l min^{-1})	7.3	10.9	10.6	10.3	9.8	7.4	7.4	7.5	7.5
	(0.98)	(1.4)*	(1.5)*	(1.4)*	(1.4)*	(1.1)	(1.1)	(1.2)	(1.2)
SaO_2 (%)	97.3	80.9	80.8	80.5	80.4	94.4	97.4	97.4	97.4
	(0.28)	(0.99)*	(0.94)*	(0.79)*	(1.0)*	(0.40)	(0.22)	(0.23)	(0.22)
$P_{et}CO_2$ (mmHg)	41.4	42.0	41.2	41.1	41.3	41.5	41.2	41.3	41.6
	(0.82)	(1.1)	(1.2)	(1.4)	(1.2)	(0.80)	(0.98)	(1.1)	(1.3)
POST IH									
f_B (breaths min^{-1})	12.9	14.2	14.0	13.8	13.2	12.7	12.7	12.6	12.2
	(1.0)	(1.1)	(1.2)	(1.1)	(1.1)	(1.0)	(1.1)	(0.9)	(0.90)
V_T (litres)	0.75	1.0	1.0	1.0	0.96	0.71	0.72	0.75	0.75
	(0.07)	(0.06)*	(0.07)*	(0.07)*	(0.07)*	(0.04)	(0.03)	(0.03)	(0.05)
V_I (l min^{-1})	6.4	9.8	9.7	9.3	8.9	6.31	6.4	6.5	6.6
	(1.2)	(1.7)*	(1.8)*	(1.7)*	(1.6)*	(1.1)	(1.2)	(1.2)	(1.2)
SaO_2 (%)	97.4	79.7	80.5	80.2	79.9	94.6	97.6	97.7	97.7
	(0.14)	(0.48)*	(0.46)*	(0.71)*	(0.32)*	(0.33)	(0.36)	(0.26)	(0.10)
$P_{et}CO_2$ (mmHg)	41.2	41.7	41.6	41.2	41.3	41.7	40.9	41.0	41.4
	(0.93)	(1.2)	(1.4)	(1.5)	(1.7)	(1.2)	(1.2)	(1.3)	(1.2)

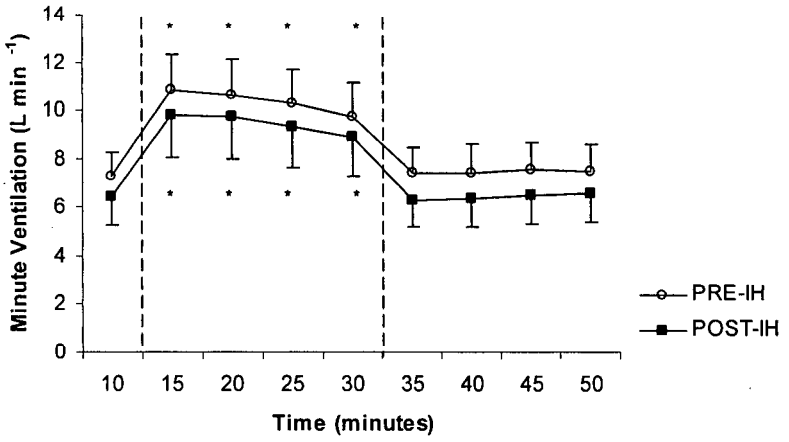
Absolute ventilation values were calculated as the mean values for the entire period of baseline and for each 5 min period during the hypoxic exposure and recovery (n=11). Values are expressed as means (±SE). Significance was set at $p<0.05$ and is indicated as change from baseline (*).

Abbreviations: intermittent hypoxia (IH), respiratory rate (f_B), tidal volume (V_T), minute ventilation (V_I), arterial oxygen saturation (SaO_2), end tidal carbon dioxide ($P_{et}CO_2$)

($p=0.04$), eventually returning to baseline during the last 5 minute of recovery. Post IH, increases in f_B were observed ($p=0.01$) and during recovery f_B returned to baseline. For both pre and post IH, V_T increased from baseline and remained significantly elevated during the hypoxic exposure

($p<0.001$), returning to baseline values during recovery. There was no significant differences in V_T between pre and post IH ($p=0.69$). Significant increases in V_I were observed from baseline during the hypoxic exposure for both pre and post IH ($p<0.001$), mediated primarily through f_B. During recovery, V_I returned to baseline values. There was no significant difference between pre and post IH for V_I ($p=0.78$). Figure 4 displays V_I during baseline, the hypoxic exposure, and recovery for pre and post IH.

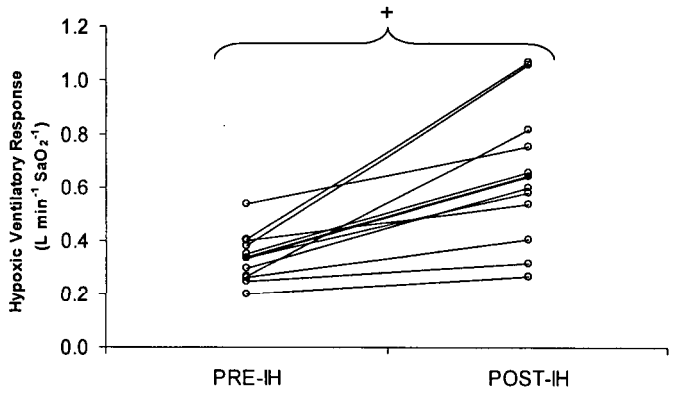
Figure 4. The effect of IH on minute ventilation (V_I). A mean value for baseline and for each 5 min period during the hypoxic exposure and recovery was calculated (n=11). Data is represented in absolute terms and as pre (○) and post (■) IH. The area between the dashed lines indicates the hypoxic exposure. Significance was set at $p<0.05$ and is indicated as change from baseline (*). Values are expressed as means ± SE. Significant increases in V_I were observed from baseline during the hypoxic exposure for both pre and post IH ($p<0.001$). During recovery, V_I returned to baseline values. There was no significant difference between pre and post IH for V_I ($p=0.78$).



Mean values for HVR increased from 0.30 ± 0.07 to 0.61 ± 0.29 L min^{-1} $\%SaO_2^{-1}$ ($p=0.01$), for pre and post IH, respectively. All subjects showed an increase in HVR with the magnitude of increases ranging from 0.06 to 0.68 L min^{-1} $\%SaO_2^{-1}$. The HVR data is displayed in Figure 5 for all subjects (A) and for subjects from whom MSNA data was collected (B).

Figure 5. The effect of IH on the hypoxic ventilatory response (HVR). Data is symbolized as individual (○) and the group mean (●) and is expressed as l min^{-1} $\%SaO_2^{-1}$ for both pre and post IH. The HVR value was constructed from the slope of the line when arterial oxygen saturation (SaO_2) and minute ventilation (V_I) were plotted against each other. Significance was set at $p<0.05$ and is represented as different between pre and post IH (+). A: HVR data for all subjects (n=11). B: HVR data for subjects from whom MSNA data was collected (n=6).

A



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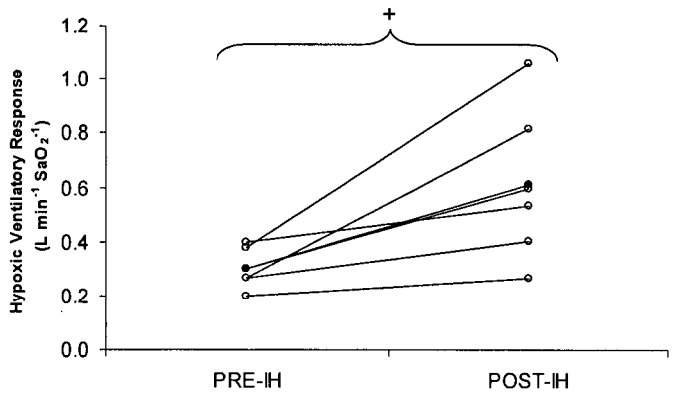
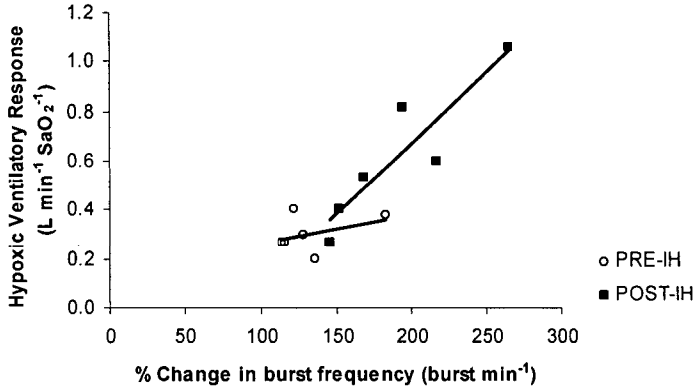


EFFECTS OF IH ON THE INTERACTION BETWEEN THE SNA AND HVR. When individual data points were plotted, the maximum percent change in burst frequency during the last 5 minutes of the hypoxic exposure and the corresponding HVR showed a significant correlation post IH ($r=0.91$ $p<0.001$)), but not pre IH ($r=0.39$, $p>0.05$). Figure 6 shows the relationship between HVR and the maximum percent change in burst frequency.

Figure 6. The relationship between hypoxic ventilatory response (HVR) and burst frequency during pre (○) and post (■) IH. Data is represented for each individual (n=6). The HVR value was constructed from the slope of the line when arterial oxygen saturation (SaO_2) and minute ventilation (V_I) were plotted against each other. The burst frequency value was obtained from the 5 min of the hypoxic exposure and is the percent change from baseline in burst per minute. Burst frequency and HVR showed a significant correlation post IH ($r=0.91$, $p<0.001$), but not pre IH ($r=0.39$, $p>0.05$).



EFFECTS OF IH ON THE CARDIOVASCULAR RESPONSE TO HYPOXIA. Table 10 displays the mean absolute values for SBP, DBP, MAP, and HR for each 5 minute period during baseline, the hypoxic exposure and recovery. Systolic blood pressure increased significantly over time ($p<0.001$) with no differences between pre and post IH ($p=0.09$) (Figure 7A). Diastolic blood pressure did not change as a result of hypoxia ($p=0.99$) or IH ($p=0.35$) (Figure 7B). Mean arterial blood pressure increased significantly during the hypoxic exposure ($p<0.001$), but no difference was detected between pre and post IH ($p=0.08$) (Figure 7C).

Table 10. Effects of IH on cardiovascular measures

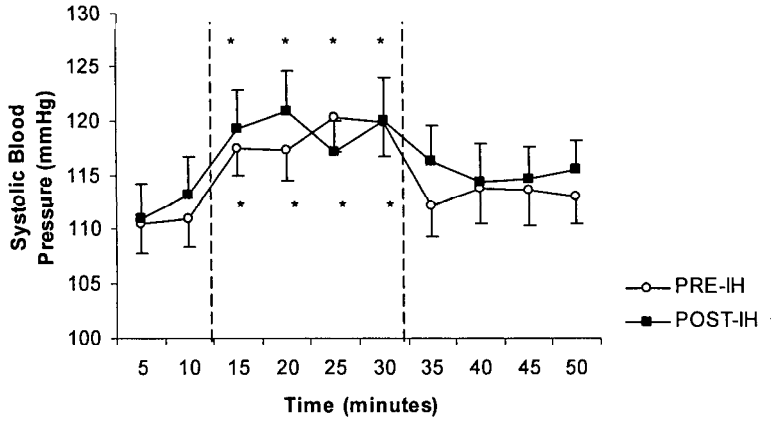
	BASELINE		HYPOXIC EXPOSURE				RECOVERY			
	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	50 min
PRE IH										
SBP (mmHg)	110.5	111.0	117.5	117.4	120.3	119.8	112.2	113.7	113.5	113.0
	(2.7)	(2.6)	(2.6)*	(2.9)*	(3.1)*	(3.1)*	(2.9)	(3.2)	(3.2)	(2.5)
DBP (mmHg)	66.6	66.8	65.9	66.4	65.3	68.5	67.3	68.6	69.0	67.4
	(3.0)	(3.1)	(3.6)	(1.9)	(2.9)	(2.4)	(2.8)	(3.9)	(3.4)	(2.8)
MAP (mmHg)	88.6	89.0	97.7	97.4	100.7	99.3	90.0	91.1	90.8	90.8
	(2.0)	(1.8)	(3.6)*	(4.0)*	(3.8)*	(4.3)*	(2.5)	(2.2)	(2.4)	(1.9)
HR (beats min^{-1})	56.2	57.3	67.7	67.6	66.5	66.6	56.0	57.4	57.3	59.7
	(2.1)	(2.9)	(3.5)*	(4.3)*	(4.2)*	(3.7)*	(2.9)	(3.0)	(2.7)	(3.3)
POST IH										
SBP (mmHg)	110.9	113.1	119.2	120.9	117.1	119.9	116.3	114.3	114.6	115.5
	(3.3)	(3.6)	(3.6)*	(3.6)*	(2.9)*	(4.1)*	(3.2)	(3.6)	(2.9)	(2.8)
DBP (mmHg)	71.3	70.5	70.5	70.0	69.8	68.5	68.4	68.1	68.8	70.4
	(2.5)	(2.4)	(2.9)	(2.7)	(3.2)	(2.4)	(2.3)	(2.8)	(2.3)	(2.7)
MAP (mmHg)	87.4	89.8	95.9	97.8	94.1	97.3	93.7	91.8	91.9	92.2
	(2.9)	(3.2)	(3.2)*	(3.0)*	(2.0)*	(3.7)*	(2.9)	(3.2)	(2.7)	(2.2)
HR (beats min^{-1})	57.4	58.6	69.4	67.4	68.0	67.3	56.7	57.6	58.0	58.3
	(3.1)	(3.7)	(4.6)*	(4.4)*	(3.9)*	(4.2)*	(3.4)	(3.6)	(3.4)	(3.4)

Absolute cardiovascular values were calculated as the mean values for each 5 min period during the baseline, hypoxic exposure, and recovery (n=11). Data was acquired from cuff measurements. Values are expressed as means (±SE). Significance was set at $p<0.05$ and is indicated as change from baseline (*).

Abbreviations: intermittent hypoxia (IH), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP), heart rate (HR)

Figure 7. The effect of IH on blood pressure. A mean value for each 5 min period during baseline, the hypoxic exposure and recovery was calculated (n=11). Values are expressed as means ± SE and are symbolized as pre (○) and post (■) IH. Data was obtained from cuff measurements. Significance was set at $p<0.05$ and is represented as change from baseline (*). The area between the dashed lines indicates the hypoxic exposure. A: Systolic blood pressure increased significantly over time ($p<0.001$). There was no difference between pre and post IH ($p=0.09$) B: Diastolic blood pressure did not change as a result of hypoxia ($p=0.99$) or pre post IH trial ($p=0.35$) C: Mean arterial blood pressure increased significantly over time ($p<0.001$) but no difference was detected between pre and post IH ($p=0.08$). Note the difference in scales of the y-axis.

A



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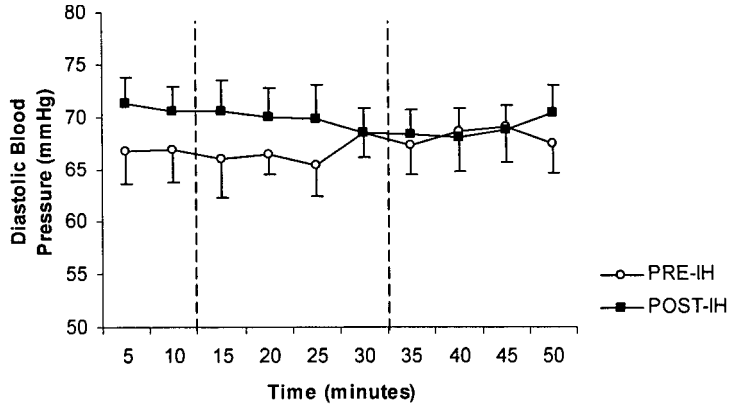

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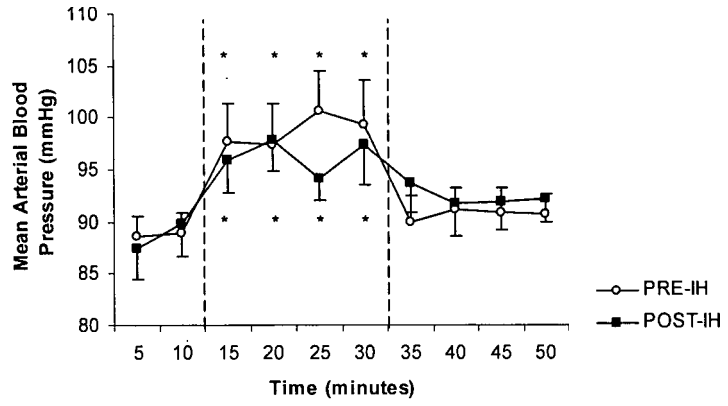
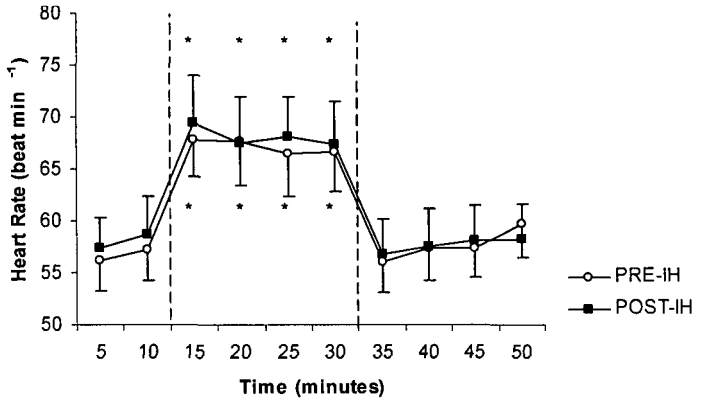


Figure 8 shows HR for pre and post IH during baseline, the hypoxic exposure, and recovery. There was no difference between pre and post IH values for HR ($p=0.94$). Compared to baseline, HR increased significantly during the hypoxic exposure ($p<0.001$). For both pre and post IH, HR returned to baseline values during recovery.

Figure 8. The effect of IH on heart rate (HR). A mean value for each 5 min period during baseline, the hypoxic exposure and recovery was calculated (n=11). Values are expressed as means ± SE and symbolized as pre (○) and post (■) IH. Significance was set at $p<0.05$ and is represented as change from baseline (*). The area between the dashed lines indicates the hypoxic exposure. There was no difference between pre and post IH values for HR ($p=0.94$). Compared to baseline, HR increased significantly during the hypoxic exposure ($p<0.001$) and returned to baseline values during recovery.



EFFECTS OF IH ON MUSCLE OXYGENATION. Table 11 displays the absolute mean values for NIRS derived variables (HbO_2, HHb, tHb) for the vastus lateralis during the entire period of baseline and for each 5 minute period during the hypoxic exposure and recovery. When represented in absolute terms, no difference were observed between pre and post IH for any of the Beer-Lambert derived variables including HbO_2 ($p=0.80$), HHb ($p=0.64$), tHb ($p=0.51$). Figure 9 represents percent change from baseline for the NIRS derived variables. The hypoxic exposure caused significant reductions in HbO_2 ($p=0.01$) with values increasing significantly above baseline during recovery ($p=0.02$) (Figure 9A). No differences between pre and post IH were observed HbO_2 ($p=0.30$).

Table 11. Effects of IH on vastus lateralis muscle oxygenation

	BASELINE	HYPOXIC EXPOSURE					RECOVERY		
	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	50 min
PRE IH									
TOI (%)	66.3	63.2	63.2	63.1	63.0	66.0	65.9	65.4	65.7
	(2.3)	(2.2)*	(2.1)*	(1.7)*	(1.8)*	(1.8)	(1.8)	(2.1)	(2.1)
HbO_2 (μMol)	3.9	2.0	2.4	3.1	3.1	6.6	6.6	5.9	6.6
	(1.2)	(0.62)*	(0.72)*	(1.2)*	(1.0)*	(1.5)*	(1.7)*	(1.6)*	(1.5)*
HHb (μMol)	1.1	5.5	5.5	5.6	5.8	0.6	1.7	1.1	1.6
	(1.03)	(0.98)*	(0.88)*	(1.1)*	(0.92)*	(0.94)	(1.27)	(0.73)	(0.82)
tHb (μMol)	4.8	7.4	7.9	8.7	8.9	8.8	8.8	9.0	8.2
	(1.8)	(1.1)*	(1.1)*	(1.3)*	(1.2)*	(1.3)*	(1.3)*	(1.2)*	(1.5)*
POST IH									
TOI (%)	65.7	63.1	63.2	63.1	63.1	66.2	66.6	66.7	66.3
	(2.3)	(2.2)*	(2.2)*	(2.3)*	(2.3)*	(2.5)	(2.5)	(2.5)	(2.5)
HbO_2 (μMol)	3.3	1.8	2.3	2.6	2.8	6.0	6.5	7.1	7.1
	(1.1)	(0.72)*	(0.82)*	(0.77)*	(0.81)*	(1.2)*	(1.4)*	(1.3)*	(1.5)*
HHb (μMol)	1.0	5.7	5.2	6.0	5.9	1.0	0.5	0.9	0.5
	(0.98)	(0.70)	(0.67)	(0.94)	(0.94)	(0.72)	(0.73)	(0.76)	(0.89)
tHb (μMol)	3.3	6.0	6.3	7.2	7.3	5.8	5.7	5.4	5.4
	(1.1)	(0.81)*	(0.90)*	(0.91)*	(1.1)*	(1.6)*	(1.5)*	(1.8)*	(1.9)*

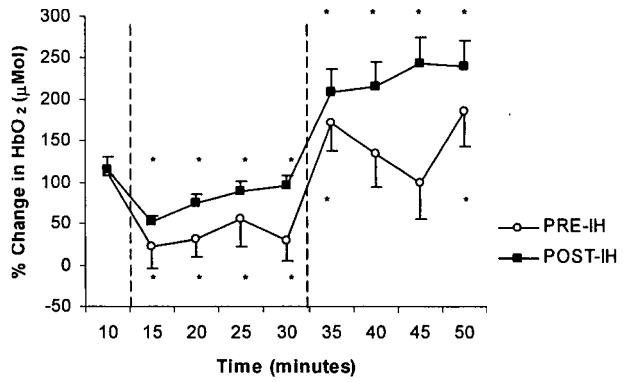
Values are expressed as means (SE). Vastus lateralis muscle oxygenation values are reported as the mean values for the entire period of baseline and for each 5 min period during the hypoxic exposure and recovery (n=6). Significance was set at $p<0.05$ and is indicated as change from baseline (*).

Abbreviations: intermittent hypoxia (IH), oxyhaemoglobin (HbO_2), deoxyhaemoglobin (HHb), total haemoglobin (tHb), tissue oxygenation index (TOI)

Post IH, but not pre IH the hypoxic exposure caused significant increases in HHb ($p<0.001$) with values returning to baseline during recovery (Figure 9B). No differences between pre and post IH values were observed for HHb ($p=0.63$). Post IH, but not pre IH increases in tHb were observed during the hypoxic exposure ($p=0.03$), compared to baseline, which were maintained during the recovery period ($p=0.05$). The increases in tHb were mediated primarily by the increases in HHb (Figure 9C). No difference was observed between pre and post IH ($p=0.49$).

Figure 9. The effect of IH on the Beer Lambert derived measures of haemoglobin saturation for the vastus lateralis muscle. A mean value for baseline and for each 5 min period during the hypoxic exposure and recovery was calculated (n=11). Values are expressed as means ± SE and symbolized as pre (○) and post (■) IH. Significance was set at $p<0.05$ and is represented as change from baseline (*). The area between the dashed lines indicates the hypoxic exposure. A: No difference between pre and post IH were observed for HbO_2 ($p=0.30$). Compared to baseline, the hypoxic exposure caused significant reductions in HbO_2 ($p=0.01$). During recovery HbO_2 increased significantly above baseline ($p=0.02$). B: No difference between pre and post IH were observed for HHb ($p=0.63$). During the hypoxic exposure significant increases in HHb ($p<0.001$) were observed. Values for HHb returned to baseline during recovery. C: No difference in tHb were observed between pre and post IH ($p=0.49$). For the post IH trial tHb increased during the hypoxic exposure compared to baseline ($p=0.03$) and was maintained during recovery ($p=0.05$). This was not observed for the pre IH trial. Note the difference in scales of the y-axis.

A



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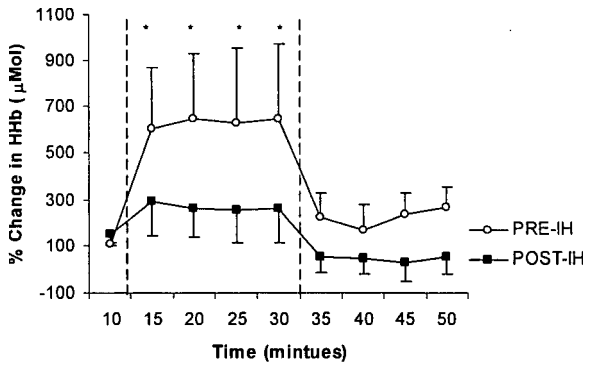

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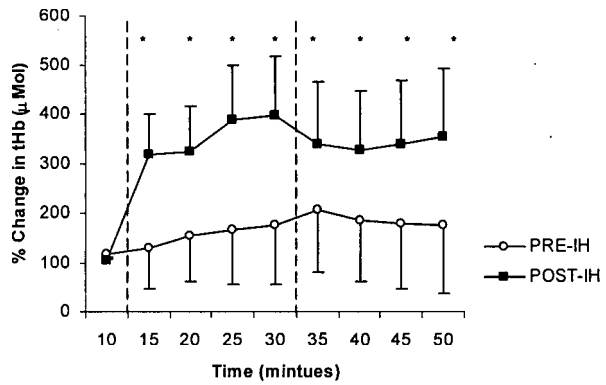
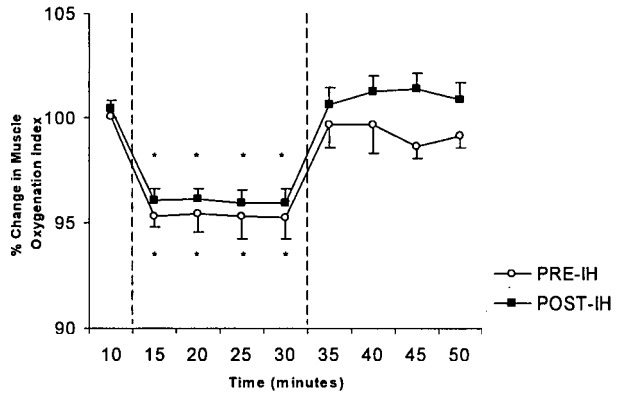


Figure 10 displays mTOI. The percent change in mTOI from baseline during the hypoxic exposure showed no statistical difference between the pre and post IH ($p=0.55$). Compared to baseline, the mTOI was significantly reduced ($p<0.001$) during the hypoxic exposure and returned to baseline values during recovery

Figure 10. The effect of IH on the percent change in vastus lateralis muscle tissue oxygenation (mTOI). A mean value for baseline and for each 5 min period during the hypoxic exposure and recovery was calculated (n=11). Values are expressed as means ± SE and symbolized as pre (○) and post (■) IH. The area between the dashed lines indicates the hypoxic exposure. Significance was set at $p<0.05$ and is indicated as change from baseline (*). Compared to baseline, mTOI was significantly reduced ($p<0.001$) during the hypoxic exposure and returned to baseline values during recovery. No statistical difference between the pre and post IH ($p=0.55$) was observed.



EFFECTS OF IH ON CEREBRAL OXYGENATION. Table 12 displays the absolute mean values for NIRS derived variables for the cerebral tissue during the entire period of baseline and for each 5 minute period during the hypoxic exposure and recovery. When represented as absolute values no difference between pre and post IH were observed for any of the Beer-Lambert derived variables including HbO_2 ($p=0.80$), HHb ($p=0.64$), tHb ($p=0.78$)

When represented as a percent change from baseline no difference between pre and post IH were observed for any of the Beer Lambert derived variables including HbO_2 ($p=0.61$), HHb ($p=0.83$), tHb ($p=0.49$). Compared to baseline, the hypoxic exposure caused significant reductions ($p<0.001$) in cerebral HbO_2 with values returning to baseline values during recovery (Figure 11A). During the hypoxic exposure significant increases in cerebral HHb ($p<0.001$) were

observed and values returned to baseline during recovery (Figure 9B). Cerebral tHb increases significantly ($p<0.001$) during the hypoxic exposure and returned to baseline values during the recovery (Figure 9C). For cTOI (Figure 12), the percent change from baseline during the hypoxic exposure showed no statistical difference ($p=0.64$) between the pre and post IH trials. Compared to baseline, the cTOI was significantly reduced ($p<0.001$) during the hypoxic exposure and returned to baseline values during recovery.

Table 12. Effects of IH on cerebral tissue oxygenation

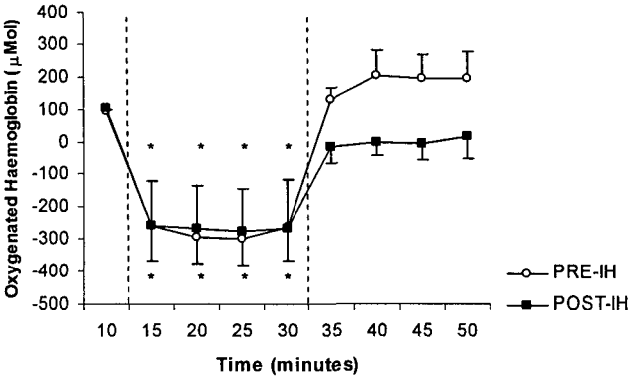
	BASELINE	HYPOXIC EXPOSURE					RECOVERY		
	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	50 min
PRE IH									
TOI (%)	74.3	66.4	64.6	64.7	65.1	74.0	74.3	74.2	74.4
	(2.3)	(2.2)*	(2.5)*	(2.6)*	(2.5)*	(2.3)	(2.4)	(2.7)	(2.4)
HbO_2 (μMol)	2.7	-3.6	-3.7	-4.1	-3.7	0.59	1.3	1.3	1.1
	(1.1)	(0.38)*	(0.47)*	(0.71)*	(0.74)*	(0.53)	(0.59)	(0.57)	(0.62)
HHb (μMol)	-0.33	6.4	7.2	8.2	7.5	1.4	0.60	0.34	0.02
	(0.26)	(0.71)*	(0.75)*	(0.73)*	(0.74)*	(0.20)	(0.20)	(0.21)	(0.20)
tHb (μMol)	2.3	2.8	3.4	4.2	3.7	1.9	1.9	1.6	1.1
	(0.81)	(0.91)*	(0.99)*	(1.1)*	(1.1)*	(0.47)	(0.50)	(0.49)	(0.51)
POST IH									
TOI (%)	75.2	66.7	67.3	66.7	67.2	75.9	76.1	76.1	75.5
	(2.0)	(1.5)*	(1.6)*	(1.6)*	(1.6)*	(2.5)	(2.5)	(2.4)	(2.4)
HbO_2 (μMol)	0.20	-5.4	-5.0	-5.1	-5.2	-0.26	0.13	0.28	0.02
	(1.4)	(0.69)*	(0.66)*	(0.69)*	(0.79)*	(0.66)	(0.70)	(0.98)	(0.85)
HHb (μMol)	0.23	6.7	6.6	7.5	6.6	0.56	-0.11	-0.20	-0.36
	(0.65)	(0.88)*	(0.81)*	(0.77)*	(0.95)*	(0.21)	(0.12)	(0.14)	(0.15)
tHb (μMol)	0.43	1.3	1.7	2.4	1.4	0.30	0.01	0.09	-0.34
	(1.3)	(1.2)*	(1.1)*	(1.1)*	(1.4)*	(0.54)	(0.47)	(0.63)	(0.58)

Values are expressed as means (SE). Cerebral tissue oxygenation values are reported as the mean values for the entire period of baseline and for each 5 min period during the hypoxic exposure and recovery (n=6). Significance was set at $p<0.05$ and is indicated as change from baseline (*).

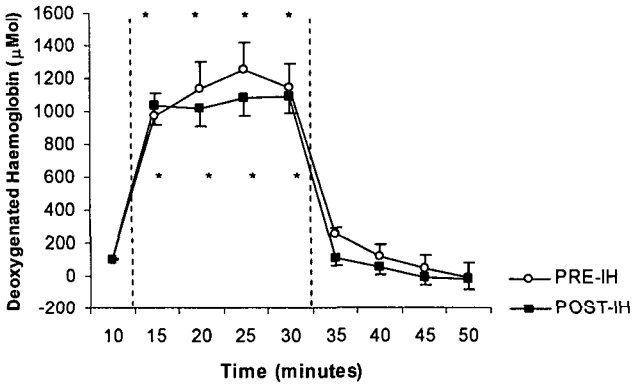
Abbreviations: intermittent hypoxia (IH), oxyhaemoglobin (HbO_2), deoxyhaemoglobin (HHb), total haemoglobin (tHb), tissue oxygenation index (TOI)

Figure 11. The effect of IH on the Beer Lambert derived measures of haemoglobin saturation for the cerebral tissue. A mean value for baseline and for each 5 min period during the hypoxic exposure and recovery was calculated (n=11). Values are expressed as means ± SE and symbolized as pre (○) and post (■) IH. Significance was set at $p<0.05$ and is represented as change from baseline (*). The area between the dashed lines indicates the hypoxic exposure. A: No difference between pre and post IH were observed for HbO_2 ($p=0.61$). Compared to baseline, the hypoxic exposure caused significant reductions ($p<0.001$) in HbO_2 with values returning to baseline during recovery. B: No difference between pre and post IH were observed for HHb ($p=0.83$). During the hypoxic exposure significant increases in cerebral HHb ($p<0.001$) were observed and values returned to baseline during recovery. C: No difference between pre and post IH ($p=0.49$) were observed for tHb. Increases in tHb were observed during the hypoxic exposure ($p<0.001$) and tHb returned to baseline values during the recovery. Note the difference in scales of the y-axis.

A



B



C

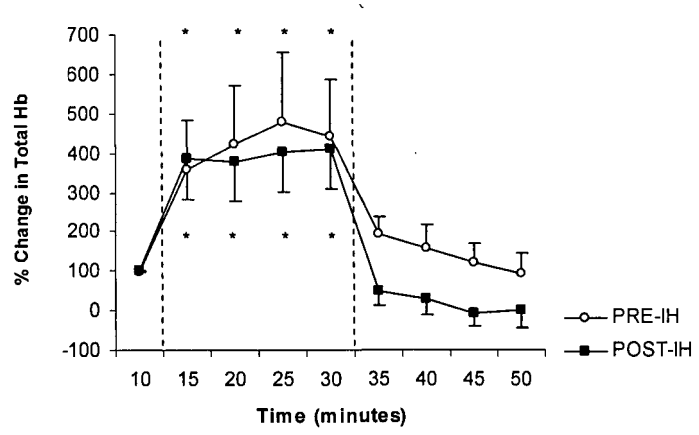
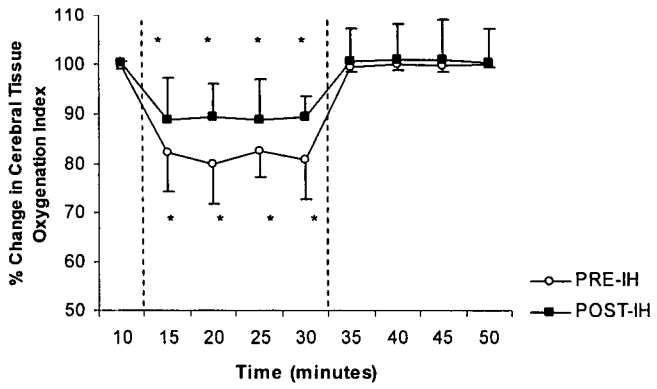


Figure 12. The effect of IH on the percent change in cerebral tissue oxygenation (cTOI). A mean value for baseline and for each 5 min period during the hypoxic exposure and recovery was calculated (n=11). Values are expressed as means ± SE and symbolized as pre (○) and post (■) IH. The area between the dashed lines indicates the hypoxic exposure. Significance was set at $p<0.05$ and is indicated as change from baseline (*). Compared to baseline, the cTOI was significantly reduced ($p<0.001$) during the hypoxic exposure and returned to baseline values during recovery. No statistical difference between the pre and post IH ($p=0.44$) was achieved.



CHEMICAL STIMULI DURING PRE AND POST IH. Table 9 displays the absolute values for SaO_2 and $P_{et}CO_2$ during pre and post IH for each 5 minute period of baseline, the hypoxic exposure, and recovery. No differences were observed between the pre and post IH trials for SaO_2 ($p=0.83$) or $P_{et}CO_2$ ($p=0.41$). Compared to baseline, SaO_2 decreased significantly during the hypoxic exposure ($p<0.001$) and returned to baseline values during recovery (Figure 13). End tidal CO_2 was not significantly different when baseline and hypoxic exposure ($p=0.84$) or baseline and recovery ($p=0.72$) were compared.

DISCUSSION

This is the first study to compare the effect of long term IH on MSNA with concurrent cerebral tissue oxygenation, muscle tissue oxygenation, cardiovascular, and ventilatory measurements. There are several new finding from this study. First, after 10 day IH intervention, significant increases in MSNA burst frequency during the 20 minute hypoxic exposure are observed which persist during the 20 minute normoxic recovery. Second, increases in burst amplitude in response to a 20 minute hypoxic exposure were observed which persisted during the normoxic recovery period; however, there was no additional augmentation of burst amplitude with our IH intervention. Post IH caused significant increases in HVR which showed a strong relationship with the maximum percent increases in burst frequency and HVR. Lastly, skeletal muscle and cerebral tissue oxygenation are reduced during the 20 minute hypoxic exposure, but only skeletal muscle tHb was significantly affected by the IH intervention.

THE EFFECTS OF HYPOXIA ON MSNA

The advantage of the microneuroraphic technique is that it measures direct sympathetic activity to the muscle vasculature and requires no inferences or assumptions in the same way as indirect measures (i.e. catecholamine concentration and the dependence on the site of collection, hormone production and/or clearance). Multiunit recordings of MSNA exhibit two distinct components, both a discharge frequency and relative amplitude. The sum of these two components is total MSNA, an indicator of overall sympathetic activity. It has been suggested that each component may be controlled by separate central mechanisms as each are modulated differently in response to various stimuli (90). Therefore, it is important to examine all aspects of the MSNA signal. To discern the effects of IH on burst frequency, amplitude, and total MSNA, the present investigation used a 20 minute isocapnic hypoxic exposure with a hypoxic stimulus of ~80% SaO_2. An SaO_2 of 80% was chosen to ensure equal peripheral chemoreceptor

engagement between subjects and it has been suggested that this is the threshold required to elicit hypoxic induced sympathoexcitation (148). The FiO_2 required to produce an SaO_2 of 80% varied between subjects, ranging from ~10-15%. We intended to examine only the effects of low oxygen on the SNS response, therefore we held $P_{et}CO_2$ at eucapnia levels and spontaneous breathing was permitted. This study specifically addressed how the increases in MSNA observed during a 20 minute hypoxic exposure and normoxic recovery period may be modulated after 10 days of IH.

ACUTE MSNA RESPONSE TO THE HYPOXIA EXPOSURE. During the pre IH trial, the 20 minute isocapnic hypoxia exposure significantly increased burst frequency above baseline by a mean of ~ 130%. This was observable within the first 5 minutes of the hypoxic exposure and remained elevated during the entire 20 minute hypoxic exposure. This observation is consistent with the work of others. Xie et al. (172) reported burst frequencies of ~170% after 20 minutes of acute hypoxia combined with either isocapnia or hypercapnia. Morgan et al. (103) showed burst frequency increases of ~220% after acute asphyxia (combined hypoxia and hypercapnia). Because these two these investigations used the exact same level of hypoxia (SaO_2 = ~80%), the difference in the magnitude of burst frequencies reported in the current investigation and that reported by others can be attributed to the additional effects of CO_2 on sympathetic outflow and some differences in experimental design. Hypercapnia has additive effects on the increases burst frequency during hypoxia. The sympathetic response to CO_2 can be the result of direct activation of central chemoreceptors or the result of increased sensitivity of the peripheral chemoreceptors to hypoxia (156). The effect of CO_2 in our study is not a factor as we were successful in maintaining end tidal concentrations at eucapnic levels. With regards to total MSNA, we observed an increase of ~150% in total MSNA during the 20 minute hypoxic exposure in the pre

IH trial. This is in line with the work of Cutler et al. (20) who showed a ~200% increase in total MSNA following 20 minute hypoxic apnoeas.

During the 20 minute hypoxic exposure we observed a ~120% increase in MSNA burst amplitude compare to baseline. There was no difference between pre and post IH trials. Burst amplitude is not typically reported for various reasons. First, common analysis techniques have been not been established for the computation of burst amplitude (83, 90, 92). Additionally, controversy exists regarding what burst amplitude represents physiologically. It has been suggested that burst amplitude, acquired from multi unit recordings, reflects changes in the population or type of neurons recruited, as different neurons can vary in their sympathetic response characteristics (i.e. conduction velocities, size) (89, 116). It is not likely that the increases in burst amplitude are the result of an altered recruitment of a particular population or type of neuron, given the large variation in amplitudes observed in a multi-unit recording during a sympathetic stress, such as hypoxia and the fact that few neurons are in actual contact with the microelectrode. It is more likely that alterations in burst amplitude are representative of changes in the actual number of activated neurons in contact with the microelectrode, regardless of their type (90, 116). Another reason burst amplitude may not be reported is that there is relatively little information regarding the predictability of burst amplitudes (89), whereas methods of predicting burst frequency are well established (6).

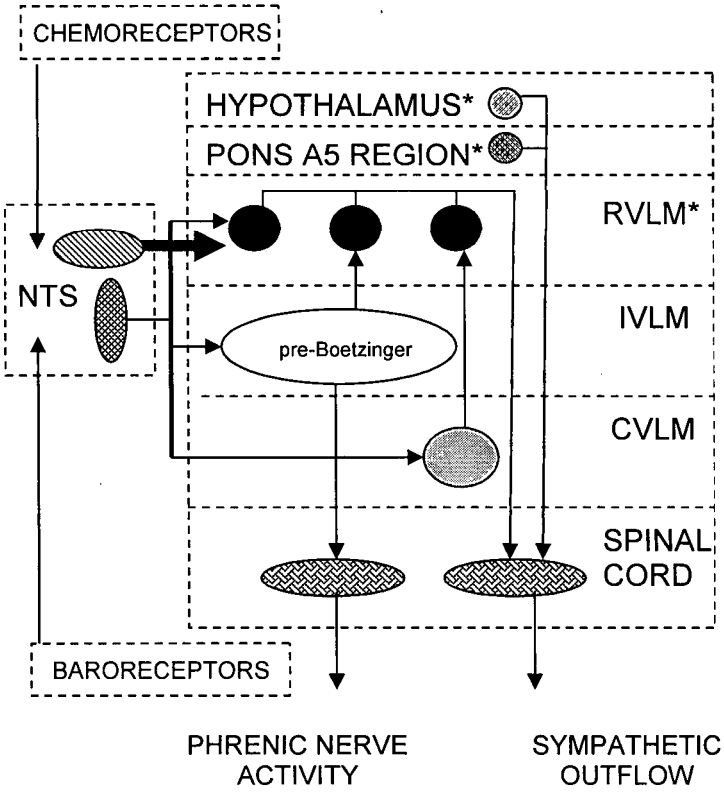
In human studies only burst frequency and total MSNA are typically reported when examining the hypoxia induced sympathoexcitation. Xie's group (172) did mention burst amplitude citing no changes in response to a 20 minute hypoxic exposure, although the data were not shown. Only one other study in humans reported burst amplitude response with hypoxia in humans. Leuenberger et al. (83) reported an increase of ~210% in total amplitude after 25-30 minutes of hypoxia (123 ± 36 to 255 ± 50 mm min^{-1} for baseline and exposure, respectively). An appreciation of the changes in burst amplitude can be constructed from studies that report both

burst frequency and total MSNA (burst amplitude is a factor in the calculation of total MSNA). For example, Cutler et al. (20) reported that during hypoxic apnoeas total MNSA and burst frequency increased ~200% and ~175%, respectively. What could account for the additional 25% increase in total MNSA? It is reasonable to speculate that increases in burst amplitude contributed to the increases in total MSNA Cutler's study. Our data support this speculation. Although the observed rise in burst frequency was greater than the observed rise in burst amplitude, both variables mediated the rise in total MSNA.

It has been shown that hypoxia can augment burst amplitude in animal preparations (90, 91). In anaesthetized cats exposed to systemic hypoxia, direct renal sympathetic nerve activity showed increases burst amplitude (91). In the same preparation, when baroreceptor activity was increased, burst frequency rose whereas burst amplitude did not. These data suggest that burst frequency and amplitude may be controlled for by separate central mechanisms. Therefore, amplitude and frequency may respond differently to acute hypoxia. Furthermore, increases in renal nerve burst amplitude, confirming our findings of increases in MSNA burst amplitude with hypoxia.

MECHANISMS CONTROLLING THE ACUTE MSNA RESPONSE TO THE HYPOXIA EXPOSURE. The cause of the sympathetic activation during hypoxia can be the result of three relatively distinct pathways: activation of peripheral chemoreceptors, central chemoreceptors, or baroreceptors. Figure 13 depicts the three pathways in which sympathetic activation can be generated in response to hypoxia, as well as the simultaneous respiratory activity and is a modified version to that described by Guyenet (53). It is well documented that peripheral chemoreflex activity is related to increases sympathetic outflow and it is likely the primary cause of sympathoexcitation observed in humans exposed to systemic hypoxia (140, 158). We suspect that this pathway is a

Figure 13. Neural pathways in the medulla that control ventilatory and sympathetic activity in response to hypoxia (modified from Guyenet, 2000). This pathway can be initiated by both peripheral and central (indicated by an astrix) chemoreceptor stimulation and baroreceptor disengagement. Consequently, a cascade of events within the medullary cell groups that generate sympathetic and phrenic nerve activity will be triggered (see text for abbreviations and a detailed description).



major contributor to the observed sympathoexcitation during hypoxia in our study, although we do not have direct evidence to support this hypothesis conclusively. The carotid bodies can respond to hypoxia by generating sensory afferent signals which are then transmitted from the carotid sinus nerve to the CNS and terminate in a specific group of medullary cells called the nucleus tractus solitarius (NTS). The neurons within the NTS then relay excitatory signals to other cell groups within the medulla, including the RVLM, the pre-Boetzinger region located in the intermediate ventral lateral medulla (IVLM), and the caudal ventral lateral medulla (CVLM). These areas have neural linkages and are primarily responsible for generation of sympathetic tone, respiratory rhythm, and blood pressure control, respectively. The physiological

consequences of these structural linkages has been observed in both reduced animal preparations and in intact humans.

Direct recordings of cellular activity within the neurons of the RVLM show strong correlation to post ganglionic sympathetic nerve activity (i.e. MSNA) suggesting that there is little modulation within the pre ganglionic neurons or the spinal ganglia. In both humans and animals respiratory rhythmicity has been observed in recordings of direct post ganglionic sympathetic activity (89, 145, 152, 175). Respiratory rhythmicity is possible by various pathways (see Figure 13). First, a common pool of neurons in the NTS that control respiratory and sympathetic activity concurrently in response to peripheral chemoreceptor stimulation can be activated with hypoxia an influence the activity of the sympathetic and respiratory system. Alternatively, respiratory rhythmicity could also be the result of excitatory activity of pre-Boetzinger cells, as there are neural connections between this region and the RVLM. It has also been suggested that the NTS contains a subset of neurons that may be excited by peripheral chemoreceptor stimulation and project directly to the RVLM, without an intervening relay within the respiratory network (depicted in Figure 13 by thick arrow).

The relationships between the neural networks that respond to peripheral chemoreceptor activation have been demonstrated experimentally. In anaesthetized animals selective chemical blockade of cells within the NTS that project to the respiratory networks (IVLM and pre-Boetzinger region) omitted the respiratory oscillations in sympathetic activity, but did not alter the overall magnitude of sympathoexcitation (79). This is evidence that a separate set of neurons exist that project directly from the NTS to the RVLM. Because the activity and patterning of the RVLM is under some influence of central respiratory networks, but is not dependent on these pathways for the generation of sympathetic activity suggests that neural projections from the NTS that control the both the RVLM and pre-Boetzinger, or the connections from the pre-Boetzinger to RVLM are activated concurrently.

In humans, the relationship between central respiratory and sympathetic neural control has been shown. Sympathetic activity is modulated by ventilation as MSNA patterns are unique to each phase of the breathing cycle (25, 26, 151, 152). Compared to the exhalation phase, during inhalation, sympathetic outflow is reduced via the influence of inhibitory signals from pulmonary mechanoreceptors (152); however, alterations in ventilation do not affect the overall magnitude of sympathetic activity (152). Collectively, when the results from animal and human studies are taken together, it is evident that sensory afferents from the carotid body can be relayed from the NTS to the RVLM, with no interaction or dependence on the ventilatory control centers. As a result increased sympathetic outflow may occur without affecting the ventilatory system. Our data support this possibility as MSNA and ventilation increase simultaneously during the initial minutes of the hypoxic exposure bust over time ventilation declined and MSNA remained elevated (for more detail see section: EFFECTS OF IH ON THE VENTILATORY RESPONSE TO HYPOXIA). Our data suggest that the direct pathways from the NTS to the RVLM may be independently facilitated during hypoxia.

Direct activation of central chemoreceptors can generate increases in sympathetic outflow. Cell groups within the medulla that are involved in the control of sympathetic activity, such as the A5 region of the pons, the hypothalamus, and the RVLM (indicated by an asterix in Figure 13) can detect systemic hypoxia. In rodents, systemic hypoxia has been shown to directly activate the presympathetic neurons of the RVLM, regardless of whether or not the carotid sinus nerves are intact. Severe levels of hypoxia ($FiO_2 \leq 10\%$) were required to directly elicit increased activity of these RVLM presympathetic neurons (154). In our study, to achieve an SaO_2 of 80%, FiO_2 ranged between ~10-15%. Consequently, it is unlikely that central chemoreception was a major influence in the sympathoexcitation in our study, but it cannot be excluded for certain.

Lastly, the rise in MSNA observed during hypoxia may be mediated by a cascade of events leading to activation of pressure sensitive cardio-pulmonary baroreceptors. During hypoxia, an initial reduction in blood pressure is caused by abrupt increases in heart rate, decreases in cardiac output (85), and vasodilatation of peripheral vessels (10, 16, 54, 84, 93, 95, 122, 147, 157). Reductions in blood pressure can result in baroreflex disengagement (72, 159) causing increases in afferent signal transmission which synapse in the NTS and CVLM (see Figure 13). The CVLM has neural extensions to cell groups within the RVLM. In an attempt to regulate blood pressure these baroreceptor inputs may play an important role in the reflex increases in MSNA in response to physiological stress (141), including hypoxia. Tamisier et al. (157) tested the role of baroreceptor activation in the hypoxia induced increases in MSNA. By blunting sympathetic vasoconstriction via the blockade of alpha-receptors, distinct local forearm vasodilatation occurred. The authors concluded that under non-pharmacological conditions this vasodilatation was masked by sympathetic vasoconstriction. We did not measure baroreceptor function or take measurements of peripheral vasodilatory production in the current study. Nonetheless, the contribution of baroreceptor activation in the hypoxia induced increases in sympathetic activity cannot be ruled out.

PERSISTENT MSNA DURING NORMOXIC RECOVERY. In both the pre and post IH trials, the increases in burst frequency, burst amplitude, and total MSNA generated during the hypoxic exposure were maintained for the duration of the 20 minute normoxic recovery period. Persistent MSNA following the removal of the chemical stimuli is a well documented phenomenon in humans (20, 103, 156, 157, 171, 172) and it has been reported to remain elevated for as long as 180 minutes (20). Morgan et al. (103) were the first to document the persistent sympathetic activity during a 20 minute normoxic recovery period (60 minutes for two subjects). In this study ~66% of increase in burst frequency generated during the 20 minute asphyxic

exposure was maintained. In a subsequent study Xie et al. (172) showed that the ~170% increase in burst frequency induced by an isocapnic hypoxic exposure remained almost unchanged throughout the entire 20 minutes of normoxic recovery.

The mechanisms responsible for the sustained elevation in MSNA after a brief exposure to hypoxia are not clear. The persistent sympathoexcitation has been attributed to hypoxia exclusively, as hypercapnic hyperoxia (103) and hypercapnic normoxia (172) did not result in persistent MSNA during recovery. It is not likely that residual chemical stimuli (i.e. retention of low oxygen pressures in carotid body and/or chemoreceptive medullary cells) are the cause of the persistent sympathetic activity. We showed that SaO_2 values had returned to baseline almost immediately into normoxic recovery indicating that the pressure of oxygen in the arterial blood had normalized, likely permitting normalization of cellular oxygen pressures throughout the body. If normalization of cellular oxygen pressures were not the case, elevated ventilation would be expected via continued carotid body stimulation and activation of respiratory neural networks. As discussed in the earlier, since severe hypoxia was not experienced, direct central chemoreceptor alterations are not likely to contribute to the increase in MSNA and therefore persistent of MSNA. Instead the mechanisms causing the persistent MSNA observed after hypoxia may be due to long term modulation in the sympathetic control centers in the CNS and may be dependent increased carotid sinus nerve activity during the hypoxic exposure. This modulation could include a combination of continued neurotransmitter release and/or neural transmissions. These are the same mechanisms thought to be involved in the development of sympathetic activity in response to acute to hypoxia.

It has been postulated that the persistent MSNA in humans is the result of continued afferent signal transduction from carotid body activity post hypoxic exposure because persistent increases in ventilation are observed in dogs (13). Conversely, isocapnic hypoxia in anesthetized cats showed no persistent effect on carotid body afferent activity after the removal of the hypoxic

stimulus (7). No persistence in ventilation is observed in any human study of hypoxia and the following normoxic recovery. To explain the observed divergence in ventilatory and sympathetic responses post hypoxia from both animal and human studies it has been suggested that in humans the gain in chemoreflex control of sympathetic activity could be larger than that for ventilatory activity in humans (103). If this were the case, some sort of undetected residual chemical stimuli within the carotid bodies could cause sustained afferent signal transmission

An alternative explanation is that hypoxia may engage the sympathetic and respiratory control centers differently. If hypoxia is sensed directly by the CNS centrally, independent of carotid body inputs, perhaps the effect on the RVLM and ventilatory control centers are different. As discussed above, direct central chemoreception is possible by numerous cell groups within the medulla and these areas could remain active after hypoxic exposures (53, 113). Long term facilitation of a number of central mechanisms, dependent and independent of carotid body inputs, have been postulated in contributing to the persistent MSNA observed after hypoxia (113). As a result of carotid body afferent activity, cellular and neural processes in the RVLM could remain active after the termination of the hypoxic stimulus. Specifically, stimulation of the carotid body during the hypoxic exposure could potentate neurotransmitter release in the CNS maintaining sympathetic output post exposure. Sympathoexcitation may be maintained at the level of the preganglionic neurons, where hypoxic stimulation potentates neurotransmitter release from sympathetic ganglia in a similar way to that seen after electrical stimulation (101). Additionally, evidence suggests the specific mechanisms at the level of the spinal cord are capable of detecting and responding to systemic hypoxia (137). Hypoxia could directly facilitate conduction and synapses in neural pathways in charge of sympathetic transmission in the same way that electrical stimulation has elicited long term sympathetic transmission in the brainstem.

As opposed to the chemoreception theory, enhanced baroreceptor disengagement could be the primary mechanism in the persistent MSNA after hypoxic expsoures (55, 56, 72, 159). It

has been suggested that vasodilatation precedes sympathetic vasoconstriction as it causes a reduction in blood pressure and deactivation of baroreceptor inputs to the NTS. Pharmacological blockade of alpha adrenergic receptors that mediate sympathetic vasoconstriction permitted hypoxia induced vasodilatation (157). In addition, with continued infusion of the alpha adrenergic receptor blocker, vasodilatation persisted for as long as 30 minutes after termination of the hypoxic (157). Therefore, peripheral vasodilatation as a result of hypoxia may continue into recovery without pharmacological intervention, causing baroreceptor disengagement and could be rooted in the cause of the persistent MSNA in an effort to maintain blood pressure. Again, because we did not measure the role of the baroreceptors in the current study we cannot make conclusions regarding this mechanism's contribution to the observed persistent in MSNA.

MSNA POST 10 DAYS OF IH. Post IH, we observed a ~150% increase in burst frequency in response to a 20 minute hypoxic exposure that was maintained through the entire period of recovery and was significantly higher than that observed in the pre IH trial (~120%). Increases in burst frequency after IH indicated that the occurrence of neuronal firing has increased. Burst amplitude increased ~120% in response to the 20 minute hypoxic exposure and was maintained throughout recovery. The rise in burst amplitude post IH was identical to the pre IH trial, indicating that this component of MSNA was not affected with our IH intervention. Based on our results, it appears that the number and/or the population of neurons recruited do not change with long term exposure to IH. Moreover, because we saw different response of burst amplitude and frequency to our IH intervention, leads us to hypothesize that these two components can be modulated differently with IH. With regards to total MSNA during the post IH trial, a mean increase of ~200% was observed within the first 10 minutes of the hypoxic exposure, rising to ~220% during the final 10 minutes. During recovery total MSNA was maintained at ~200% of baseline values. The patterns in total MSNA were mediated primarily through the increases in

burst frequency. A trend towards statistical significance (p=0.06) was observed when pre and post IH were compared and was likely not achieved due to the fact that burst amplitude did not undergo any modulation with IH.

It is likely that the same mechanisms involved in the development of sympathetic activity in response to acute hypoxia and its persistence during recovery are involved in the increased sensitivity of the sympathetic nervous system after IH. Because no other study has sought to describe the effects of IH delivered in a laboratory setting on MNSA in humans, an explanation of our findings requires speculation. To put our results into context of the current literature, we must draw from studies that measured sympathoexcitation at altitude in healthy humans (both directly via MSNA and indirectly via catecholamine production), long-term IH studies in animals, and pathological models of IH in humans.

The only other investigation to acquire direct measurements of sympathetic activity was conducted by Hansen and Sander (57). They recorded MSNA at sea level and after 4 weeks at 5260 m and showed burst frequency increased by 32 burst min^{-1}, or increases of ~300% of baseline. This substantial increase in burst frequency occurred with mean SaO_2 values of ~85%, which is relatively high considering that an SaO_2 of 80% is the reported threshold required to elicit sympathoexcitation (149). In comparison, our intervention increased burst frequency significantly, but only by a mean of 7 burst min^{-1}, or increases of ~166% of baseline . The most obvious explanation for the discrepancy between our study and that of Hansen and Sander is the total amount of time exposed to hypoxia: 10 hours at an SaO_2 of ~80% delivered for one hour for 10 days versus ~ 672 hours at a sustained SaO_2 of ~85%, respectively. Additionally, the larger increases in MSNA in the Hansen and Sander study could be attributable to the additive effects of hypobaria, the physiological effects of hypocapnia (cellular alkalosis), and anxiety associated with travel to altitude (138).

Other investigations have used urine and plasma catecholamines, such as norepinephrine (NE) as an indirect measure of sympathetic activity (18) as sampling is relatively easy compared with the microneurograhic technique. Norepinephrine primarily represents the activity of sympathetic nerves that terminate in muscle vasculature (30, 99). Therefore, measurements of NE and MSNA have shown a strong relationship (83). With long term exposure to hypoxia at altitude increases in NE have been demonstrated (4, 12, 18, 99, 138). However, because it is an indirect assessment, results have been inconsistent (138). Therefore, caution must be taken when interpreting the results. For example, it has been shown that hypoxia can increase both NE spillover and clearance and one must account for both mechanisms to obtain an accurate assessment of hypoxic mediated increases in NE concentration (83). Further studies of the effects of IH sympathoexcitation should include simultaneous collection of both NE and MSNA to discern the relationship between these two measurements and these two techniques.

In animal models of long term hypoxia, it has been postulated that peripheral chemoreflex control is altered after long term exposure to hypoxia; therefore, because peripheral chemoreceptor afferents have excitatory neural linkages in the CNS, one could speculate that this altered control of peripheral chemoreflexes may have downstream implications on the MSNA response after long term IH. In anesthetized goats, carotid body discharge progressively increased during a sustained level of hypoxia indicating an increased sensitivity of the carotid body to hypoxia (114). If this finding is true, it is reasonable to speculate that carotid body discharge can progressively increase with a sustained exposure to hypoxia in humans and since carotid body afferents terminate in the RVLM progressive activation could contribute to the enhanced MSNA. This is a possible mechanism in the persistent MSNA in our study, as we used a sustained hypoxic exposure of ~80% SaO_2, although we cannot conclude conclusively, as we did not obtain measurements of carotid body afferent activity.

Further evidence in humans supports the hypothesis that peripheral chemoreflex and sympathetic control are altered after long term exposure to hypoxia (102, 103, 171). Unique augmentation of carotid body reflexes and control of sympathetic nerve traffic were observed in healthy humans exposed short term intermittent hypoxic apnoeas (19). The same findings were observed in patients with OSA, a pathological model of IH (107). The repetitive apnoeic events during sleep cause repeated bouts of hypoxia and hypercapnia which act via the chemoreflexes to increase sympathetic nerve activity. During non-apnoeic periods burst frequency is elevated in those with OSA compared to healthy controls (43.0 ± 4.0 versus 21.0 ± 3.0 bursts min^{-1}) (107). Long term exposure to IH during sleep is likely the cause of the increased sympathoexcitation leading to other physiological consequences, such as increase normoxic blood pressure; however, it is difficult to demonstrate a clear causal relationship. In rodents, work by Fletcher's group modeled their IH interventions to replicate the hypoxic conditions experienced in OSA. They show that 35 days of IH significantly affected sympathetic outflow to the muscle vasculature (40), kidney (38, 42), and adrenal medulla (5) and that this response is dependent on intact carotid chemoreceptors (32). Furthermore, this group has shown that increases sympathetic outflow has physiological ramifications, such as increased normoxic blood pressure (33-35, 39)(for further details see section: EFFECTS OF IH ON THE CARDIOVASCULAR RESPONSE TO HYPOXIA). Although our subjects only underwent 10 days of IH, our data support the concept that repeated exposure to IH in OSA or in the rat model could elicit a sustained increase in MSNA during non-apnoenic periods and in the absence of the hypoxic stimulus. However, we did not see any alteration in cardiovascular measurements with our model of IH.

Molecular and cellular alterations within the peripheral chemoreceptors and central nervous system could contribute to the enhanced MSNA observed with long term IH. Chronic IH showed increased production of reactive oxygen species causing functional plasticity in carotid body sensory activity in experimental animal (128, 132, 144). The sensory facilitation of

the carotid body may contribute to persistent reflex activation of sympathetic nerve activity due to the overlapping central structures controlling ventilation and sympathetic activity (120). However, this possibility likely has a minor role at best as we did not see any augmentation in ventilation during the hypoxic exposure post IH while we saw enhanced MSNA. More convincing is the suggestion that central mechanisms undergo long term potentation due to IH, leading to enhance neurotransmitter release or a facilitation of synaptic transmission in areas in charge of sympathetic outflow, such as the RVLM (53, 113). Furthermore, within the cells of the CNS, genomic processes that respond to hypoxia could facilitate the sustained MSNA. In the medullary regions expression of a genomic factor, c-fos has been shown to increase after chronic IH in rabbits (59) and rodents (50). The production of these 'first response genes' indicate changes in neuronal genetic transcription which can lead to physiological alterations, such as sympathetic outflow, further down stream.

EFFECTS OF IH ON THE VENTILATORY RESPONSE TO HYPOXIA

Ventilation increased significantly from baseline during the 20 minute isocapnic hypoxic exposure, mediated by an increase in both breathing frequency and tidal volume. The pattern of change in ventilation in response to the 20 minute isocapnic hypoxic exposure was identical for the pre and post IH trials, indicating that the IH intervention did not alter the sensitivity of the carotid body to sustained hypoxia. Alterations in carotid body sensitivity is reasoned to be the mechanism for augmentation of ventilatory response to progressive hypoxia in animal and human models (127, 140). Ventilation trended towards a decline after ~5-8 minutes of the hypoxic exposure. This phenomenon is known as the hypoxic ventilatory decline (HVD), and is extensively documented (47, 124, 143). The occurrence of HVD may be the result of down regulation in the nerves of the CNS in charge of ventilation (i.e., the pre-Boetzinger region) (112). Alternatively, HVD may be due to a reduced phrenic nerve output, which has been is

reported during isocapnic hypoxia in surgically manipulated cat preparations (100). A reduction in tidal volume was the primary cause of HVD in the current study. Ventilation returned to baseline values within the first 5 minutes after the removal of the hypoxic stimulus. Therefore, LTF of the carotid body is unlikely in humans as ventilation returns to baseline during the normoxic recovery (12, 19, 20, 47, 77, 85, 104, 123-125).

During both pre and post IH and prior to the 20 minute hypoxic exposure, an HVR procedure was performed. The HVR value obtained from the post IH day was significantly higher than that obtained from the pre IH trial, nearly doubling. Other studies conducted in humans have produced similar results (44, 69, 88). In a recent study by Katayama et al. (69) 3 hr of daily IH (FiO_2= ~12%) caused HVR to increase from 0.26 ± 0.12 to 0.59 ± 0.21 L min^{-1} $\%SaO_2^{-1}$ after one week and 0.23 ± 0.12 to 0.61 ± 0.37 L min^{-1} $\%SaO_2^{-1}$ after two weeks, resulting in a increase of 38 – 44%. In the present study, 10 days of IH at SaO_2 of 80% provided a similar hypoxic stimulus, which augmented ventilatory sensitivity. The current study is comparable to that of Katayama's as our HVR values increased 49% from the pre IH trial and absolute HVR values were 0.30 ± 0.07 and 0.61 ± 0.29 L min^{-1} $\%SaO_2^{-1}$ for pre and post IH, respectively.

The carotid body plays an obligatory role in the HVR. Bilateral carotid body tumor resection results in abolition of the ventilatory response to hypoxia, due to peripheral chemoreflex failure (60, 158). Increases in HVR following IH in humans can be attributed to an enhanced carotid body chemoreceptor reflex. To support this hypothesis is the observation that continuous hypoxia increases carotid body sensory activity in goats (114). The increases in HVR after IH appear to be greatly affected by the paradigm employed – dependent on factors such as pattern and severity of the hypoxic exposures (119, 121, 128, 134). Peng and Prabhakar (121) showed that in rats the hypoxic sensory response of the carotid body was significantly enhanced only after short durations of IH (hypoxia lasting several seconds cycled with

normoxia). Our daily hypoxic stimulus may have acted in a similar way, increasing the sensory response of the carotid body and causing an enhanced HVR. Short exposures to IH may cause unique engagement of cellular mechanisms, such as oxygen sensing free radicals, heme proteins, and facilitation of ion channels which in turn induce sensitization of the carotid body (132, 133). To account for the observed increases in HVR in humans after long term IH, it has been reasoned that the first few exposures of IH could cause a time limited sensitization of the carotid body, engaging pathways within the CNS. It is possible that these mechanisms may remain elevated with long term IH, facilitating an enhanced ventilatory response.

Recent work in rodents suggest that central mechanism that respond to carotid body inputs are acting to facilitate a change in HVR after chronic hypoxia. Dopamine is an important inhibitory transmitter involved in central neural synapses and has been shown to respond to peripheral chemoreceptor stimulation. Dwinell's group (29) used chronic hypoxia to address the role of central mechanisms in the alteration of HVR by testing the involvement of the dopamine-2 receptor. With chronic hypoxia, time-dependent reductions in the dopamine-2 receptor were observed in ventilatory control centers in the brain and the authors suggest that these changes could modulate the ventilatory response to hypoxia. Although we did not measure the chemical changes in the CNS in our IH intervention, we cannot rule out the possibility that such changes may have occurred and may be the cause of the increases in HVR in our study. It is possible that centrally mediated changes in areas unique to the ventilatory control, such as the IVLM and the pre-Boetzinger region are mediating the changes in HVR in our study.

Effects of IH on the Interaction Between the Sympathetic Activity and Ventilation

After the IH intervention we showed a strong relationship between the maximum change in burst frequency during the last 5 minutes of the hypoxic exposure and HVR. This relationship

was not observed pre IH. Coupled alterations in the ventilatory and sympathetic systems have been previously shown after long term exposure to hypoxia. Asano et al. (4) showed a significant correlation between urine NE and tidal volume at after long term hypoxia at altitude, suggesting that the response of the ventilatory and sympathetic systems are in some way related to each other and controlled for together. In experiments that addressed the relationship between breathing phase and the sympathetic response more directly, opposing data is presented. In humans it has been shown that within breath variations in MSNA exist (25, 26, 145, 151, 152). During inhalation, which is the end phase in the determination of tidal volume, activation of pulmonary stretch receptors cause reflex reductions in sympathetic outflow (152). Therefore, when the lungs are at their largest volumes, sympathetic activity is at its lowest values. Our data support this concept in two ways. First, MSNA remained elevated, or increased during the latter stages of the hypoxic exposure while ventilation experienced a decline mediated primarily by a reduction in tidal volume. Secondly, maximal burst frequency value for correlation analysis with HVR was taken from the last 5 minutes of the hypoxic exposure where HVD is observed (Figure 6). At this point HVD may have permitted this maximal rise in MSNA.

Not all trends in ventilatory and sympathetic activity occurred in parallel in the current investigation. Divergence in the activity of these two systems was observed during both the pre and post IH trials. First, as mentioned previously, during the 20 minute hypoxic exposure, ventilation and MNSA increased concurrently, but within ~5-8 minutes of sustained hypoxia, ventilation declined whereas MSNA continued to rise. Secondly, although MSNA remained elevated after the removal of the hypoxic stimulus, ventilation returned to baseline measures.

Noteworthy, in the present study, the two variables used for correlation were not collected at identical time points. The HVR was collected before the start of the 20 minute exposure and the maximum burst frequency was obtained from the final 5 minutes of the hypoxic exposure. It is not clear how an indicator of hypoxic acclimatization (i.e. increased HVR) and

high sympathetic activity can exist together after IH. This is contradictory given that increased tidal volumes during hypoxia will act to reduce sympathetic activity through inhibitory lung mechanoreceptors. The most likely explanation is that central modulation occurs with IH in areas of the medulla that have common neural extensions in the RVLM and pre-Boetzinger region. Such modulations would include long-term potentiations of synaptic transmission in the brain and cellular/genomic alterations.

EFFECTS OF IH ON THE CARDIOVASCULAR RESPONSE TO HYPOXIA

During the 20 minute exposure to hypoxia, MAP and SBP increased and returned to baseline values within 5 minutes of normoxic breathing. The DBP was not significantly altered during the hypoxic exposure or recovery. There was no effect of the 10 days of IH on blood pressure response during baseline to the 20 minute hypoxic exposure or recovery. Pre and post IH, values for SBP and MAP were nearly identical. Other laboratory interventions using human subjects and employing some paradigm of IH have shown increases in blood pressure. Foster et al. (44) showed that 12 days of short duration IH, and not long duration IH increased the SBP and DBP sensitivity to an acute hypoxic exposure. The change in SBP sensitivity ($r=+0.68$; $p<0.05$) and the change in DBP sensitivity ($r=+0.73$; $p<0.05$) was related to the change in HVR. They attributed this response to a potentially greater stimulation of the carotid body with the short duration protocol. It can be speculated that an enhanced carotid body afferent outflow will lead to augmentation of central mechanisms in charge of sympathetic activity and vascular tone. After 8 hours of severe IH for 35 days in rodents, Fletcher and colleagues (41) showed increases in resting blood pressure of ~13 mmHg which is dependent on the carotid body and sympathetic activity. Taken together, the present study likely did not find significant augmentation of blood pressure because the sustained hour long exposures did not effectively activate the carotid body and/or central mechanisms. The time line of the experiment may have been too brief suggesting

that more than 10 days of IH or a more severe hypoxic stimulus is required to show alterations in cardiovascular responses. Additionally, analysis of cardiovascular measurements during steady state hypoxia may not detect the changes in sensitivity of after IH.

In the present study, heart rate rose significantly during the 20 minute hypoxic exposure and this augmentation was not different between pre and post trials. Our data is supported by the work of Foster et al. (44) who show that heart rate sensitivity was not affected by a 12 day IH intervention. More research is needed to explain why heart rate response to hypoxia do not change with IH, as heart rate variability a measure of autonomic control of heart rate has been shown to be altered. Like blood pressure, heart rate may have required longer or more severe hypoxic exposures. Heart rate follows patterns in respiration and is affected by the interaction of lung mechanoreceptors, baroreceptors, and chemoreceptors. There was no difference in minute ventilation, blood pressure, and chemical stimuli between pre and post IH trial, and therefore it can be reasoned that heart rate is not different.

EFFECTS OF IH ON MUSCLE OXYGENATION

During both pre and post IH, the 20 minute hypoxic exposure resulted in significant reductions in HbO_2 and increases in both HHb and tHb. The mTOI represents vastus lateralis muscle oxygen saturation and during hypoxia, and significant reductions were observed. Considering their dependence on SaO_2, NIRS derived variables are reported to closely match the changes in oxygen pressure in arterial blood during hypoxia (115); our data confirm this observation. With normoxic recovery HHb and mTOI returned to baseline values where as HbO_2 and tHb remained elevated. Patterns in vastus lateralis HHb, HbO_2 and TOI were not different when expressed as either percent change or in absolute terms after the IH intervention. However, tHb showed significant elevations from baseline post IH, but not pre IH.

Values for muscle tissue oxygenation are dependent on blood flow, metabolism,

vasoactive mechanisms, and SaO_2. We anticipated that markers of tissue oxygenation such as mTOI and HbO_2 would be reduced as they are dependent on oxygenation of the arterial blood (58). During normoxic recovery, when SaO_2 returned to baseline values skeletal muscle oxygenation measures also return to baseline values. This occurred despite the persistent increases in MSNA and the potential increase in vasoconstriction drive. To achieve this normalized muscle oxygenation in recovery, it is possible that a continued production of vasodilator elements may occur to balance sympathetic vasoconstriction. However, we did not collect these measures and cannot conclude this for certain.

It has been demonstrated that tHb is strongly correlated with measures of muscle vessel conductance and is a valid method for assessing muscle vasodilatation (169). Therefore, comparisons can be made between studies that use blood flow and NIRS to examine hypoxia induced vasodilatation. Measurements of tHb represent blood volume and are a surrogate for blood flow (87). Based on the current findings of vascular dysfunction in skeletal muscle vessels post IH (122) we expected that our IH intervention would cause vascular dysfunction resulting in a reduced dilatory ability in response to hypoxia and causing reductions tHb. Furthermore, based on our hypothesis that MSNA would be augmented after IH we expected to observe a smaller increase in tHb, mediated by sympathetic vasoconstriction.

The balance between local vasodilatation and sympathetic vasoconstriction will dictate the alterations in blood flow, vascular resistance, and blood volume. The purpose of vasodilatation with exposure to hypoxia is to increases vascular conductance and limit the fall in oxygen delivery to muscle when the oxygen content of the arterial blood is low or restricted due to hypoxia mediated sympathetic vasoconstriction. The primary mechanism of hypoxia induced vasodilatation is unknown, and is likely due to a combination of mechanism. Endothelium derived nitric oxide (10) and adenosine (96, 147), are a means of skeletal muscle vasodilatation observed during hypoxia. The interaction of these two elements is shown to vary along the

vascular tree (95), which may explain the divergent findings between studies using different non-invasive methods of assessing vasodilatation, such as plethysmograph, Doppler ultrasound, and NIRS. The NIRS technique assesses tissue oxygenation, but more specifically venous blood affluent. Only small portions of the NIRS signals are derived from arterial blood oxygenation where as blood flow and resistance measurements are acquired from the arterial side.

The haemodynamic responses of the muscle will be dependent on whether or not hypoxia has been administered acutely or chronically. With acute systemic hypoxia in intact humans increases in blood flow and reductions in vascular resistance have been reported (84, 85, 157). In a study by Leuenberger et al. (83), 25-30 minutes of hypoxia at a mean SaO_2 of 74% caused a significant rise in forearm blood flow and a decrease in forearm vascular resistance. In the same study, skin blood flow and vascular resistance did not change implying that if vasodilatation in the forearm did occur, it must be manifested locally within the vasculature of skeletal muscle. This idea is supported by our data. Both muscle HHb and tHb increase during the 20 minute hypoxic exposure and represent increases in blood volume within the area under the probes. In a separate study Leuenberger et al.(85) showed an alternative trend. Voluntary apnoea was associated with a decrease in leg blood flow and an increase in vascular resistance and these observations were most evident during hypoxic apnoea compared to hyperoxia apnoea. The authors suggested that the increases in MSNA induced during the hypoxic apnoeas stimulated the arterial chemoreceptors, leading to increased vasoconstriction and therefore impeded blood flow and augmented vascular resistance.

Tamisier et al. (157) showed that reductions in forearm blood flow and increases in forearm vascular resistance during hypoxia (mean SaO_2 = ~80%) are in part affected by sympathetic activity. They showed that reductions in forearm blood flow and increases in forearm vascular resistance could be reversed by alpha receptor blockade, thereby eliminating sympathetic constriction within the local vasculature. Interestingly, they showed that during

normoxic recovery, vasodilatation persisted with continued infusion of the alpha receptor blocker permitted after the termination of hypoxia. In our study, it can be reasoned that the continued vasodilatory release during recovery combined with the maintenance of increased MSNA permitted the NIRS measurements to return to baseline or exceed baseline values during recovery. Caution must be taken when assessing blood flow changes during hypoxia via Doppler ultrasound, the method common among the abovementioned studies. Specifically, Doppler reveals velocity measurements, which assumes a constant lumen diameter. Given that sympathetic activity increases with hypoxia, and reports show changes in BP with hypoxia, it is likely that the diameter of the vessel is decreased during hypoxia.

To our knowledge, there have been no studies that have examined the effects of long term IH on skeletal muscle haemodynamics or tissue oxygenation in humans. However, we can look to studies using isolated vessel preparations to provide insight to what may be occurring in vivo after chronic exposure to hypoxia. In rats, isolated vessel preparations of the gracilis muscle show a reduced dilatory sensitivity to hypoxia (122). The authors attribute these finding to molecular changes within the vessel, implicating a reduced bioavailability of nitric oxide (122) and suggest an impairment of the vessel to protected against reductions in tissue oxygenation with acute hypoxic exposures. Others have shown that during acute hypoxia constrictor response to NE is reduced after chronic hypoxia (95), suggesting that vasodilatation, mediated through the SNS may be favoured. Additionally, to preserve tissue oxygenation structural changes such as arteriolar remodeling and capillary angiogenesis can occur with hypoxia (63, 81, 146). The fact that we saw increases in tHb post IH compared to pre IH suggests that mechanisms acting to increase blood volume and therefore preserve tissue oxygenation are enhanced after 10 days of IH. Our results go against the reports cited above where vasodilatory mechanisms are compromised after long term exposures to hypoxia. It must be considered that because the NIRS technique measures oxygenation on the venous side primarily, it may not be able to detect

changes occurring at the arterial side of the vascular tree where much of the vascular changes with IH have been reported.

EFFECTS OF IH ON CEREBRAL OXYGENATION

With acute exposure to 20 minutes of hypoxia, indices of cerebral oxygenation decreased, which returned to baseline values during the 20 minutes of recovery. An appreciation of this reduced cerebral oxygenation can be achieved by examining the specific oxygen associated chromophores. In our study, cerebral HbO_2 was reduced, and HHb and tHb were increased. The large increase in HHb mediated the reduction in cTOI, an indicator of cerebral oxygen saturation. In the current study, no effect of the IH intervention was observed in during exposure to 20 minutes of hypoxia in any measurement acquired by NIRS in the region of tissue examined in the brain. Based on previous examinations of cerebral vessel haemodynamics during hypoxia in vivo and in isolated vessel preparations, we anticipated that the post IH response to the 20 minute hypoxic exposure would demonstrate reductions in cerebral oxygenation measured via NIRS. Specifically, we hypothesized that tHb would be reduced post IH, indicating a reduced blood flow and an impairment of the cerebral vessels to respond to hypoxia. Additionally, we expected that the reduction in tHb would be mediated by a reduction in HHb. Our data show no indication of differences in HHb or tHb between pre and post IH. Nonetheless, our hypotheses were based on convincing data, and it is likely that our intervention failed to show significant differences due to the method of analysis, and equally likely, the short duration and mild severity of the hypoxic intervention.

Cerebral oxygenation during hypoxia will be affected by various mechanisms and stimuli, including alterations in blood flow (77, 123, 166), tissue metabolism (110, 111), and CO_2 tension (2, 65, 73, 124). Like the skeletal muscle, NIRS and blood flow measurements correlate well in the brain (165) and tHb can be used as an indicator for blood volume (58).

Cerebral oxygenation is also directly influenced by changes in SaO_2 (44, 77, 160). Unique to the cerebral vasculature is a phenomenon known as autoregulation, which tightly regulates blood flow to the brain and must be considered when assessing the response of the cerebral vasculature to hypoxia.

In response to acute hypoxia it is documented that vasodilatation of cerebral vessels occurs, mediating increases in blood flow and it can be reasoned that this functions to prevent tissue hypoxia. The mechanisms by which vasodilatation in the cerebral circulation occur is primarily mediated through nitric oxide (122). In the current study we see a marked increase in tHb and HHb, indicating reductions in cerebral oxygenation and increased blood volume. These findings have been confirmed by others (44). With exposure to long term IH (14 days), the bioavailability of nitric oxide in the cerebral circulations is severely blunted. This results in a reduced vasodilator responsiveness to acute hypoxia (122). Using NIRS, Foster and colleagues (44) show that exposure to daily hypoxia reduces in the sensitivity of cerebral tissue oxygen saturation to hypoxia. The differing results between our study, and that of Foster's group can be attributed to the method of analysis. We examined the changes in NIRS parameters during a sustained level of hypoxia at SaO_2 of 80% where as they looked at the changes in the sensitivity of these parameters during a progressive exposure. Opposite to the NIRS assessment of oxygenation sensitivity, increases in the sensitivity of blood flow to acute variations oxygen was observed after IH (5 nights, 8 hours/night, simulated altitude ~4,300 m, SaO_2=~85%) (77). In patients with OSA, hypoxic apoenas is reported to cause increases in cerebral blood flow velocity (CBF) which appears to be modulated by increases in MAP or some other unknown mechanism controlled for by some autonomic mechanism in the cerebral circulation (136). Perhaps we did not observe alterations in our measurements of cerebral tissue oxygenation, as we did not see increases in BP variables as a result of our IH intervention.

Although directionally opposite, it can be argued that reductions in cerebral tissue oxygenation measured via NIRS and increases blood flow measure by transcranial Doppler (TCD) indicate similar physiological changes in response to hypoxia. Depending on the level of the vascular tree, vascular response to vasodilator elements may be different. Using TCD, blood flow is typically measured at the middle cerebral artery, a relatively large vessel that is highly responsive to nitric oxide while NIRS measurements generally represent tissue oxygenation or blood oxygenation on the venous side which may not respond to nitric oxide in the same way (87). Additionally divergent findings between "blood flow" or more specifically blood volume changes with NIRS and other methods can be attributed to certain methodological factors. NIRS uses oxygen, based on its association with haemoglobin to track changes in blood volume (117). Using this method, it must be assumed that changes in tissue metabolism and blood flowdo not occur. It is well documented that reductions in the metabolic rate of the CNS neurons occur with hypoxia (113), as do alterations in blood flow (123) and therefore complicate the applications of this technique. Lastly, measurement of blood flow with Doppler is possible only when the innsonated vessel diameter does not change. This assumption may not be met when alterations in constrictor activity are known to occur with hypoxia.

The rationale for the lack of statistical significance between pre and post IH NIRS measures in the muscle and cerebral tissue is four-fold: 1) the IH intervention was either too short or not severe enough to cause vascular dysfunction; 2) NIRS is not sensitive to the changes in vascular dysfunction; 3) the method of analysis is not an appropriate way to detect changes in vascular dysfunction using NIRS; 4) large inter-subject variability in the response to hypoxia confounded our results and not the lack of physiological alterations caused by the IH intervention.

CRITIQUE OF METHODS/LIMITATIONS

Because pre and post IH hypoxic exposures occurred on different days, it is possible that the significant differences observed in MSNA burst frequency and HVR were the result of day-to-day variability. Using the same techniques employed in the present study and conducted in the same laboratory, HVR values show a day-to-day coefficient of variation of 27% (75). Burst frequency values acquired on separate days are highly repeatable with less than 15% variability (162), but this is not a consistent finding (163). Based on these findings we are confident that the changes observed in MSNA burst frequency and HVR are the result of our 10 day IH intervention.

It is possible that the initial rise in sympathetic activity and its persistence was the result of other factors, such as anxiety induced from masked breathing, long periods of sitting, or the laboratory procedures (161). A familiarization day was included to reduce this possibility and acquaint the subjects with the procedures. Additionally, if the anxiety associated with the unfamiliarity of laboratory procedures were the cause of the increased sympathoexcitation, it would be expected that the MSNA values would be higher during the pre IH trial. In other studies employing a similar protocol, anxiety was ruled out as a possible cause for the increased sympathetic activity, as time control subjects did not show increases in MSNA (20, 103).

In the present study, sympathetic nerve activity was assessed from post ganglionic sympathetic nerves leading to the muscle vasculature in the lower leg. This activity represents that of whole body sympathetic activity to muscle vasculature, and does not necessarily represent the activity directed to other organs systems. Therefore, speculation as to what is occurring in the kidney, heart, and brain cannot be made. Shifts in electrode position, activation of mechanoreceptors, or motor neuron activity may have confounded our results. To eliminate the influence of these factors, data was excluded from analysis if a baseline shift or an increase in the density of the spikes occurred. To avoid the influence of inter-individual variation in MNSA

during rest and hypoxia, strict guidelines were followed pertaining to inclusion/exclusion criteria (see SUBJECTS under the METHODS section). Regardless, to make comparisons between subjects and for statistical purposes MSNA is expressed as percent change from baseline.

Both peripheral and central stimulation of oxygen sensitive chemoreceptors can play a role in the development of sympathoexcitation in response to acute or chronic hypoxia. In an intact human we are unable to assess the relative contribution of these pathways to the increased MSNA. Regardless, we suspect that sympathoexcitation arises primarily from peripheral chemoreception. Although we collected both MSNA and blood pressure data, we are unable to implicate chemoreceptor or baroreceptor engagement as the primary cause for the development and maintenance of the heightened MSNA. To more accurately discern the contribution of these mechanisms in humans, a broad range of blood pressure values, measures of central venous pressure, and collection of endogenous vasodilatory elements would be required.

Arterial oxygen saturation, arterial pressure of CO_2 ($PaCO_2$) and BP were assessed non-invasively by finger pulse oximetry, $P_{et}CO_2$, and by finger pulse photoplethysmography, respectively. Finger pulse oximetry does not account for changes in temperature and pH, which affect the association of oxygen and haemoglobin. It is not likely that changes in temperature or pH occurred in our subjects, as the experiments were conducted during rest and $P_{et}CO_2$ was held constant at baseline levels. Furthermore, it has been shown that pulse oximetery accurately reflects SaO_2 when values obtained from the oximeter are > 70% (51) and our subjects remained at ~80% through out the hypoxic exposure. The $P_{et}CO_2$ has been reported to be accurate at ranges between 30 to 55 mmHg during hypoxia. Considering our subjects were held at isocapnic levels of ~42 mmHg, it can be expected that these values accurately reflect $PaCO_2$. Beat-by-beat BP devices have been reported to be accurate at tracking changes in blood pressure (118); however, the ability of these devices to track changes in blood pressure is dependent on the quality of the pressure wave (51). To ensure accurate readings, beat-by-beat blood pressure was

calibrated to cuff measurements taken during baseline and cuff measurements acquired thought out the protocol were used for statistical analysis.

Near infrared spectroscopy is non-invasive technique used to monitor the oxygenation of haemoglobin in the sampled tissue. Some have suggested that NIRS measurements reflect blood flow and tissue metabolism. Although the NIRS signals will be affected by these factors, it must be recognized that NIRS only tracks oxygen's association with haemoglobin. Furthermore, NIRS cannot differentiate between the location of the haemoglobin molecule, be it in the arterial or venous side. In the brain, it has been demonstrated that 5% of the blood is situated in the capillaries, 20% in the arteries, and with the remainder located in the venous side (87). As a result, NIRS measurements of the cerebral tissue likely reflect venous haemoglobin oxygenation, and not tissue oxygenation. In skeletal muscle at rest, ~70% of the blood is situated in the venous capacitance vessels, suggesting that NIRS measurements taken from the muscle also reflect venous hemoglobin oxygenation and not that of skeletal muscle tissue (11).

The NIRS signals can be affected by insufficient light shielding, optode placement, and optode shifting. To minimize the influence of these factors, optodes were fixed to the skin with double sided tape covered with a dark clothe. We ensured accurately placement over both the vastus lateralis avoiding the iliotibial band and cerebral tissue avoiding the sinuses and temporal muscles. Sections of data were excluded due to subject movement or a loss of signal. It should be noted that the NIRS unit used in the present study cannot distinguish between the haemoglobin and myoglobin, and therefore the reported HbO_2, HHb, and tHb signals are representative of both molecules.

CONCLUSIONS

The primary finding from the current study is that 10 days of IH caused a significant increase in MSNA burst frequency and a trend towards an increase in total MSNA during a 20

minute isocapnic hypoxic exposure. Indices of sympathoexcitation are maintained throughout the 20 minutes of normoxic recovery. It has been previously reported that long term exposure to hypoxia causes increases in the HVR, and this is supported by our work. A new finding from our study is that the increase in HVR is significantly correlated to the maximal increase in burst frequency. Because burst frequency and HVR are modulated concurrently after 10 days of IH, suggests that these physiological responses may have common central controllers. A new finding is that increases in burst amplitude occur with exposure to acute hypoxia which is maintained during recovery. This indicates that the number or population of neurons recruited during the exposure is altered. Furthermore, we are the first to report that 10 days of IH did not elicit a change in burst amplitude. Therefore, and increase in MSNA as a result of long term exposure to IH will be mediated by increases in burst frequency, or how often a signal is transmitted. Cardiovascular, ventilatory, and cerebral tissue oxygenation measurements were not significantly affected by the 10 days of IH, although they were affected by acute exposure to hypoxia and our results are consistent with the current literature. No effect of our IH intervention on these measurements is likely due to the fact that the hypoxic stimulus was either too short, not severe enough, or that steady state analysis is not a sensitive enough method to detect changes.

This is the first study to show that IH in humans can significantly increase on MSNA during hypoxia and recovery. Because OSA is characterized by IH, elevated MSNA in our model may prove useful in examining the mechanisms involved in the development of the increased sympathoexcitation in this population.

LITERATURE REVIEW

With exposure to low oxygen, numerous adaptive responses at the microvascular, organ system, and organismal level are activated to preserve oxygen delivery and transport in an effort to avoid cellular hypoxia. At the organismal level for example, peripheral chemoreceptors can detect reductions in arterial oxygen pressures and through a series of pathways increase ventilation to preserve oxygen uptake and delivery. At the mircovascular level, vasodilatation of terminal arterioles and increased recruitment of capillaries allows tissues to extract more oxygen from a limited supply.

The pattern by which hypoxia is experienced is an important consideration. Both continuous and intermittent exposures to hypoxia cause unique physiological responses, and it is likely that the distinctive cellular and molecular processes intrinsic to each paradigm are responsible. Regardless of the pattern, long term exposures to hypoxia will cause physiological changes with the organism – some appear to be beneficial, while others have harmful consequences. Protein production, ion channel alterations (133), activation of transcriptional enzymes (50, 59), induction of genomic pathways (45), erythropoiesis (48), angiogenesis (144), and increases in the hypoxic ventilatory response (131) are just a few ways to protect against cellular hypoxia. However, as a result of hypoxia increases in sympathetic outflow may have negative cardiovascular implications (32, 82). This is evident in patients with obstructive sleep apnoea (OSA), a pathological condition characterized by long term intermittent hypoxia (IH) during sleep. This population is reported to develop diurnal hypertension, mediated primarily through persistent sympathoexcitation (68, 86, 106, 107, 168).

The purpose of this review is to outline the molecular processes by which a biological system can detect and respond to hypoxia and how these processes function to modulate the activity of the sympathetic nervous system (SNS). The concurrent alterations in the ventilatory,

cardiovascular, and circulatory (or haemodynamic) systems observed with hypoxia will be briefly covered, and put into context as they relate to the SNS modulations. The various measurements of the sympathetic activity to acute and chronic hypoxia will be discussed and data from laboratory interventions, excursions to altitude, and patients with OSA will be presented.

Oxygen Sensing Pathways and The Sympathetic Nervous System

Both peripheral and central chemoreceptors play a key role in detecting and responding to hypoxia (29, 53, 68, 126, 127). It is not likely that these pathways function exclusively to produce the sympathoexcitation observed with hypoxia. Instead, each step along the oxygen sensing pathway may contribute, to a varying degree, to the sympathetic activation and may be uniquely modulated with long term exposures to hypoxia. Numerous other stimuli that often accompany hypoxia, such as changes in atmospheric pressure and CO_2 tension can contribute to the observed sympathetic activation. Details of these stimuli are beyond the scope of this review and the reader is referred elsewhere (53, 80, 94, 125, 138) .

Peripheral Chemoreceptors

Peripheral arterial chemoreceptors, specifically the carotid bodies, are primarily responsible for detecting and responding to systemic hypoxia in most environmental and experimental conditions. In response to acute or chronic hypoxia, early work by Forester et al. (43) demonstrated that intact carotid bodies in goats were essential for the acute ventilatory response and ventilatory acclimatization. More recently, Peng and colleagues (119) have demonstrated that sensory discharge from the carotid body, and the magnitude of such discharge is increased after IH in rodents. Furthermore, this group has shown that the response of the carotid body is dependent on the pattern of hypoxia administered (121). Short, cyclical bouts of

hypoxia, and not long continuous exposures selectively augment the response to low oxygen. It has been proposed that the cyclic exposures elicit unique production of oxygen free radicals, which alter the redox state of various proteins within the cells of the carotid body, affecting signal transduction. In humans, the important role of the carotid body has been demonstrated on a more phenotypic level. Carotid body resection results in the abolishment of a ventilatory response to hypoxia (60, 62, 158). It has been extensively reported that the ventilatory response to hypoxia can be increased with long term exposure to hypoxia of varying degrees and patterns (44, 71, 76). Garcia and colleagues (49) showed that with chronic hypoxia (3,800 m altitude), maximal increases in HVR where achieved after 2 weeks, as there was no additional effect after 8 weeks of exposure. In an earlier study this group showed that an equivalent level of hypoxia (F_IO_2 of 0.13 =~3800 m altitude) delivered intermittently for 12 days elicits similar changes in HVR) (48).

The use of animal preparations has significantly advanced our current understanding of cellular signaling within the carotid body in response to hypoxia, and has allowed hypotheses to be made about what occurs in vivo. Cellular and molecular mechanisms by which the carotid body responds to hypoxia are brought about by the activation of two types of specialized cells: glomus cells, which are neuronal in nature and sustanticular cells which act as a type of support cell (133). Within the glomus cells, chemical stimuli can activate a number of cellular processes to cause membrane depolarization, the release of neurotransmitters, causing stimulation of afferent nerve endings. Thus, the first step in a series of nervous transmissions that contribute to the sympathetic response to hypoxia is initiated. With regards to glomus cell depolarization and transmitter release, two pathways have been suggested as the primary oxygen sensors. One hypothesis supports that reduced oxygen tensions activate heme proteins (and some non-heme proteins). These proteins could lead to the production of reactive oxygen species which can facilitate neural transmission and controls the release of neurotransmitters (132). An alternative

theory implicates the inhibition of the K^+ ion channel which leads to an increase of cytosolic Ca^{2+}(130). Calcium is partly responsible for neurotransmitter release and is an important regulator in gene transcription (128, 130, 132). To respond to hypoxia, it is likely K^+ ion channel and the redox state of heme proteins do not function exclusively. Instead each pathway may dominate during a particular severity of hypoxia, in the presence of other chemical stimuli, and may experience unique alternations with long term hypoxia.

A phenomenon known as long term facilitation (LTF), has been observed in the carotid body after hypoxic exposures, despite the normalization of blood gases and blood pressure and may contribute to the persistent sympathetic nervous activity (120, 134). In animals, LTF of carotid body afferents occurs after hypoxic exposures and is characterized by sustained elevations in breathing (120) and sympathoexcitation (131). Peng et al. (120) showed that the development of LTF in the carotid body is dependent on the pattern in which hypoxia is administered, and implicated a unique activation of ROS in the cyclic paradigm. However, sustained elevations in breathing have not been reported in humans while persistent sympathetic activity is consistently observed (20, 103, 156, 157, 171, 172). To explain the divergent findings with regard to LTF of the carotid body, it has been postulated that in humans, the gain of ventilatory sensitivity is less that the gain of sympathetic sensitivity within the CNS (103).

CENTRAL NERVOUS SYSTEM

In vivo, direct central chemoreception cannot be assessed. It is likely that direct activation of central chemoreceptors plays a minor role in human studies where increased sympathetic activity has been cited, as FiO_2 values are typically above 10% and SaO_2 values are not often below 80%. Therefore, much of the centrally mediated increase in sympathetic outflow during hypoxia is the result of peripheral afferents inputs that terminate within the medulla, including those from the carotid body, phrenic nerve, and arterial baroreceptors.

The interaction of the carotid body synaptic transmissions with the medulla is likely the primary peripheral contributor to centrally derived sympathoexcitation. Alterations in ventilatory activity, which is closely linked to sympathetic activity, confirm the important relationship of central processing of afferent information from peripheral chemoreceptors Dwinell et al. (29) exposed rats to a maximum of 7 days of chronic hypobaric hypoxia (P_IO_2 = 80 torr). As a result of the intervention, ventilation increased and was dependent on the dopamine D-2 receptor, an indicator of peripheral and central interaction. The authors concluded that chronic hypoxia causes changes in dopamine D-2 receptor that could result in changes in the ventilatory response to hypoxia.

Sensory afferent transmission from the carotid body travel via the carotid sinus nerve causing a cascade of synapses within the CNS (refer to Appendix B) (53). The nucleus tractus solaris (NTS), a regulatory center for sympathetic activity, receives afferents from the carotid body. Here, interneurons synapse with a number of other control centers, namely the rostral ventral lateral medulla (RVLM), a region in charge of sympathetic tone. The neurons of the RVLM contain two types of cells that produce elements regulating sympathetic outflow. C1 cells produce catecholamines which regulate vascular tone via the sympathetic nervous system and non-C1 cells can increase sympathetic activity by the production of proteins, such as glutamate. The pre ganglionic neurons from the RVLM extend to the spinal cord and synapse with their respective post ganglionic neurons, whereby efferent sympathetic transmission is directed to the blood vessels and organs. The pre and post ganglionic neurons from the RVLM to the organ systems and vasculature are different (refer to Appendix B). As a result it is likely that regulation and modulation of sympathetic activation to each region will be distinctive.

In animal preparations, it has been demonstrated that during severe hypoxia (FiO_2<10%) central chemoreceptive cells located in the hypothalamus, thalamus, pons, and medulla are capable of responding directly to hypoxia via enhanced neurotransmitter release (53, 137) and

can therefore contribute to sympathoexcitation. This is achieved mainly through the alteration in biochemical pathways within the central chemoreceptive cells leading to the production of c-fos and june-B, genetic markers of neuronal activity. Hirroka et al. (59) showed that in conscious rabbits, 60 minutes of moderate hypoxia (FiO_2 = 10%) increased the expression of Fos in many neurons of the NTS, parts of the medulla, pons, and several regions in the midbrain and forebrain. The activation of these early response genes can trigger a cascade of genomic transcriptions, coding for other mediators, potentially effecting phenotypic outcomes. For example, hypoxia can increase c-fos expression and has been linked to the up regulation of HIF-1, an oxygen sensitive transcriptional complex that drives the expression of downstream genes, resulting in the production of erythropoietin and vascular endothelial growth factor (45).

Interneurons of the RVLM extend to other regions within the medulla that can modulate sympathetic activity and respiration. Noteworthy, is the interaction of the RVLM and the pre-Boetzinger region, the respiratory rhythm generator. The pre-Boetzinger region receives inputs from the phrenic nerve. The activity of the phrenic nerve is increased with hypoxia since ventilation is increased. Therefore, sympathetic transmissions from the RVLM can be influenced by activity in the pre-Boetzinger region. Arterial baroreceptors and phrenic nerve activity can affect the hypoxic induced sympathoexcitation as they both have neural inputs terminating the medulla (94). Both of these inputs were factored in the control of MSNA in a comprehensive study by St Croix et al. (152). They showed that the maximal and minimal activation of the sympathetic nervous system occurred at end expiration and end inspiration, respectively. The changes in MSNA that occurred with each breath were negatively related to diastolic blood pressure (DBP) and had no relationship with changes in intrathoracic pressure. Reductions in DBP demonstrate baroreflex unloading and reflect changes in baroreflex sensitivity and/or threshold. Therefore, the authors concluded that alterations in MSNA derived via respiratory rhythmicity were secondary to alterations in carotid sinus baroreceptors.

Measurements of Sympathetic Responses to Hypoxia

To measure sympathoexcitation in response to hypoxia, quantification of numerous markers have been acquired. Sympathetic nerve activity, collected via the microneurographic technique, is the only direct assessment of autonomic activation and has been applied to renal and splanchnic nerves and various skeletal muscle nerves. The use of microneurography has been limited due to accessibility and technical expertise. The information acquired from microneurography represents whole body sympathetic activation to muscle vasculature and is collected via peripheral nerves in humans, typically the peroneal. Similarly, indirect measures of whole body sympathetic activity have been made via the collection of urine and blood catecholamines. Indirect indices of sympathetic activity to organ systems are possible by collection of heart rate variability and plasma renin activity. In humans, acute hypoxia has been studied in a laboratory setting using masked breathing or hypobaric chambers while chronic hypoxia in humans has been conducted at altitude and in hypobaric chambers.

Muscle Sympathetic Nerve Activity

Morgan and colleagues (103) sought to discern the effects of acute hypoxia on MSNA in humans and whether this activity persisted with the return to normoxia. The motivation for their query came from observations made in experimental animals where increases in ventilation were maintained after the removal of the hypoxic stimuli. To address their question 20 minutes of sustained hypoxia combined with hypercapnia (SaO_2=80% and $P_{et}CO_2$= eucapnia +5torr) was administered. Compared to baseline, the asphyxic stimulus caused a sudden increase of 220 ± 28% in sympathetic burst frequency. The novel aspect of their study was that during the 20 minute normoxic recovery burst frequency remained significantly elevated even with the normalization of cardio-respiratory variables. Hypoxia was implicated as the primary stimulus as a hypercapnic-hyperoxic trial (F_iO_2 = 5% and $P_{et}CO_2$ = eucapnia + 5 torr) saw a smaller rise in

sympathetic minute activity (197 ± 32%), with values returning to baseline upon resumption of room air breathing.

The observation of persistent sympathoexcitation with the return to normoxia prompted subsequent studies, which systematically addressed the effects of various patterns of hypoxia and stimuli. In correspondence with Morgan's findings, Xie et al.(172) showed that 20 minute of sustained isocapnic hypoxia and hypercapnic normoxia evoked increases in MSNA; again, persistent MSNA was only observed in the hypoxic trial. Even when delivered in a discontinuous fashion (20 seconds of asphyxia separated by 40 seconds of normoxia; SaO_2 = 79-85%; $P_{et}CO_2$ = eucapnia +3-5 torr), hypercapnic hypoxia resulted in sympathetic activation rose 175 ± 12% above baseline and was sustained after the removal of the chemical stimuli (171). Culter and colleagues (19) examined the role of apoena and CO_2 combined with discontinuous hypoxia on the prolonged elevation in MSNA. This experimental design was constructed in an effort to replicate the conditions of OSA and potentially unveil the mechanisms involved in the sustained sympathoexcitation common with this disease. However, among the discontinuous hypoxic challenges, no differences were detected between apoenic, isocapnic, or hypercapnic groups but all groups did show that MNSA persisted for 180 minute post exposure. Again hypoxia was implicated as the primary stimulus in the development and maintenance of MSNA.

How could hypoxia cause the sustained increased MSNA in humans post exposure? Because invasive measurements could not be acquired, inferences regarding the control mechanisms were made. These authors supported the idea that peripheral chemoreceptor activation acting through some facilitated central mechanisms was the cause and functioned in a way analogous to LTF in the carotid body. Specifically, changes within the medullary regions that controlled sympathetic outflow and meditate the persistent efferent sympathetic activity could include a maintained excitatory neurotransmitter release, synaptic memory, or enhanced post ganglionic neuronal activity. These hypotheses seem to be reasonable mechanisms for the

persistent MSNA in humans. Within the CNS, the gain of the chemoreflex control of sympathetic outflow may be higher than that for ventilation (103). Ventilation normalized during recovery, and although ventilatory and sympathetic control centers share common structures, ventilation is also controlled for by separated regions in the medulla (pre-Boetzinger). Therefore, perhaps the chemoreflex control of the SNS was selectively facilitated post exposure.

An alternative view has been postulated, suggesting that the arterial baroreceptors are the primary mechanism for the initial rise and the continued maintenance in sympathetic activity (55, 56, 156, 157). Baroreflex engagement can result from reductions in vascular tone and blood pressure, therefore unloading these pressure sensitive fibers. This was originally though to be a secondary response to buffer carotid body vasoconstriction and maintain vascular tone. It is well established that dilatation (54, 83, 122, 157, 169, 170), and therefore reductions in vascular tone, occurs in the muscle vasculature in response to hypoxia and is maintained during normoxic recovery. Endothelium derived nitric oxide and adenosine (10, 84, 93, 95, 122, 147) contribute to the vasodilatation observed in skeletal muscle during hypoxia. The interaction of these to elements varies along the vascular tree, but ultimately their actions lead to an increased vascular conductance to limit the fall in oxygen delivery (95). Consequently, reductions in vascular resistance and blood pressure are observed (55, 72) leading to an unloading of the arterial baroreceptors. Furthermore, local peripheral vascular mechanisms causing vasodilatation and baroreceptor engagement may persist during recovery and could be rooted in the cause of the persistent MSNA (20) with short term exposures to hypoxia.

Only one investigation has looked at the long term effects of hypoxia on MSNA in healthy humans. Hansen and Sander (57) showed that after 4 weeks of chronic hypoxia at altitude (5360 m) MSNA burst frequency was higher compared to that at sea level. The elevated MSNA was maintained at altitude despite either disengagement of the peripheral chemoreceptors or baroreceptors via administration of supplemental oxygen (100%) or saline, respectively. After

descent (3 days at normoxic sea level), burst frequency remained significantly elevated – a comparable phenomenon to the persistent MSNA seen during the normoxic recovery and following an acute exposure to hypoxia (103, 155-157, 171, 172).

In a pathological model of chronic intermittent hypoxia (IH), persons with OSA experience hypoxic apnoeas during sleep which causes marked increases in MSNA that persist during the daytime, even when breathing pattern and blood gases are normal (168). The nocturnal hypoxic apnoeas have been proposed as the primary cause of the sympathoexcitation in this population (107) and this is supported by several lines of evidence. First, normoxic burst frequencies have been reported to be over 50% higher in patients with OSA compared to healthy controls who do not experience apnoeic events. Secondly, the elevated MSNA in response to hypoxia is dependent on the stimulation of the carotid body in OSA patients (108, 149). Lastly, when treated for the hypoxic apnoeas with a continuous positive airway pressure device, normoxic MSNA was significantly reduced (105, 108, 168). These data support the hypothesis that the hypoxic apnoeas are the primary stimulus for the sympathoexcitation observed in OSA.

Numerous other factors could contribute to the sympathoexcitation in OSA, such as spontaneous arousals, airway obstruction, and hypercapnia. Additionally, obesity is a risk factor in the development of OSA and it has been proposed that obesity may contribute to the high sympathetic activity. However, during normoxic breathing subjects of normal weight without OSA, obese without OSA, and obese with OSA showed 41 ± 3, 42 ± 3, and 61 ± 8 bursts per 100 heartbeats, respectively indicating that obesity did not contribute to the elevated sympathetic activity in this investigation (106).

CATECHOLAMINES

Catecholamine production, in particular norepinephrine (NE) has been extensively examined during hypoxia. The brain, heart, sympathetic nerve endings, and the adrenal medulla

can all produce NE in response to hypoxia. As a consequence HR and myocardial contractility, vessel contractility, blood flow distribution and energy substrate mobilization are affected (8). The results from studies that have used NE as a marker of sympathoexcitation have been equivocal. This is likely due to the nature of non-invasive methods as it increases the probability of error. With regards to NE specifically, values will be dependent on thecite of collection (urine, arterial blood, venous blood) and the production and clearance rates which are known to change with hypoxia. Nonetheless, sympathoexcitation using NE as a marker has been shown to increase with hypoxic exposure. Plasma and urine NE has been shown increase in response to hypoxia achieved via high altitude (99), hypobaric chamber (66), and laboratory administration (83).

Leuenberger et al (83), observed a net increase in arterial NE after an acute exposure of 25-30 minutes of hypoxia delivered via a mask (SaO_2 =~74%) despite an increase in NE clearance. Others have demonstrated different results. In an earlier investigation Rowell et al. (139)showed that although acute hypoxia (F_iO_2 of 8-12%) increases MSNA, no rise in venous NE was observed. It was suggested that hypoxia may enhance clearance of NE as a result of an alteration of blood flow or neuronal uptake of NE. Therefore, the dynamics (production and metabolism) of NE during both acute and chronic hypoxic exposures must be considered. The effects of chronic hypoxia on NE have also been addressed. In humans, exposure to 21 days of chronic high altitude (4300 m) increased arterial NE by 84% (99). Nine weeks at altitude saw arterial NE concentrations increase 3.7 fold and were caused by higher whole body NA spillover (12). Alternative to the findings of Leuenberger's group, this study reported similar NE clearance rates pre and post the sojourn to altitude.

HEART RATE VARIABILITY

Unique control of the heart during a sympathetic stress is possible due to the fact that the heat is innervated by a separate series of pre and post ganglionic neurons and is controlled for by a separate set of centrally located neurons in the RVLM (Appendix C). In vivo, direct neural recordings of cardiac sympathetic nerve activity are not possible. However, non-invasive measurements of heart rate variability (HRV) have been used to assess the relative contribution of the sympathetic and parasympathetic nervous systems on cardiac autonomic control. This is possible by calculating the fluctuation in the time intervals between heart beats. Using the ECG waveform, an algorithm is set and the intervals are analyzed via spectral analysis giving high (> 0.15-Hz; PH) and low (0.0- to 0.15-Hz; PL) frequencies. Low frequencies represent sympathetic activity and high frequencies represent parasympathetic activity of the heart. The ratio of these frequency bands gives an indication of what system is dominating and controlling heart rate.

Measurements of HRV have been made at altitude and with hypoxic exposures administered in the laboratory. With brief exposure simulated altitude (3500 m for 2 hrs) HRV is altered (174). Increases in heart rate are observed at altitude, thereby shortening the time interval between heart beats and this indicates increases in the relative contribution of the sympathetic control of heart rate (relative decrease in parasympathetic systems indicator). Chronic exposed to altitude (4350 m for 6 days) also affected HRV over time (17). Low frequency-to-high frequency ratios increased from day 1-2 and day 5-6. With acclimatization, the reductions in parasympathetic and rise in sympathetic control of heart rate tended to be reversed (17). The reduction in sympathetic control of heart rate with acclimatization to altitude is contradictory to that observed with MSNA, as Hanson and Sander saw progressive and persistent increases in MSNA burst frequencies. When taken together, these studies suggest that heart and vasculature autonomics are modulated differently with long term exposure to hypoxia. Also, the limitations in the current non-invasive methods in which to assess heart rate autonomics must be recognized.

Rodent models have provided a unique opportunity to study the effects of hypoxia on renal sympathetics. Again, separate pre ganglionic nerves from the RVLM extend to the kidney and adrenal medulla (Appendix C), indicating that differential recruitment can be anticipated with exposure to sympathetic stimuli, such as hypoxia. In anesthetized artificially ventilated rats, graded levels of severe hypoxia was implicated in the increase in direct measures of adrenal sympathetic nerve activity, measured at the splanchnic nerve and parallel with increases in catecholamine secretion measured in the adrenal venous effluent (9). This response was dependent on intact carotid sinus nerves and splanchnic nerve.

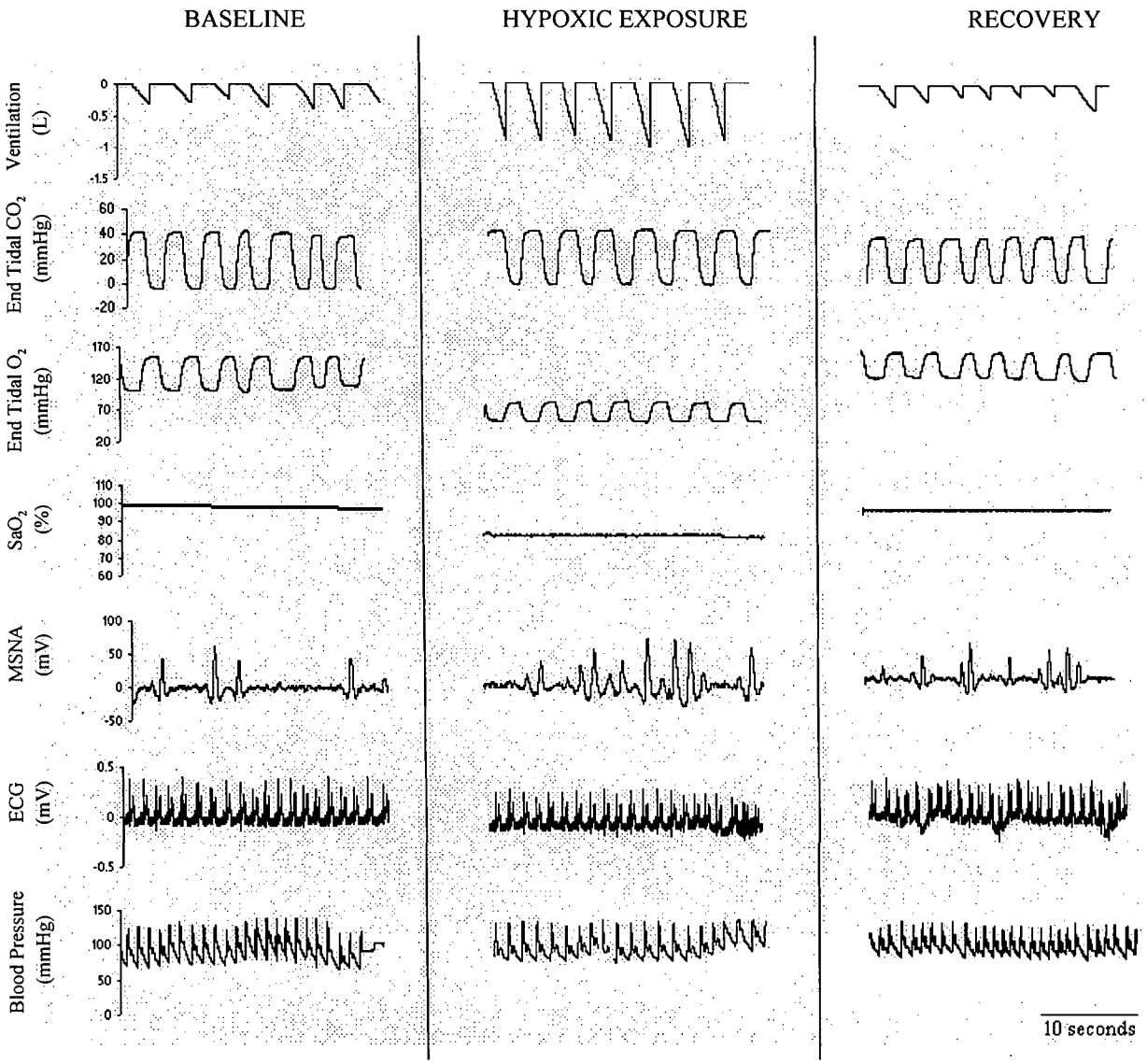
The consequences of hypoxia on renal sympathetic nerve activity has been extensively studied by Fletcher's group using a 35 day recurrent episodic hypoxia protocol (~7 hours a day; F_iO_2 = ~10%) (33, 34, 36-38, 40-42). Two key findings suggest a role of the kidney on the sympathetically mediated increases in blood pressure. First, both renal deinervation and chemical blockade of renal nerve activity showed no blood pressure change or a lowering of blood pressure in response to hypoxia, whereas the sham-operated and unhandled rats showed a progressive, sustained increase in resting room air blood pressure (36). Second, sympathetic stimulation of alpha and beta adrenergic receptors increase the production of renin. Renin, an enzyme produced by the juxtaglomerular complex (located in the renal cortex of the kidney), cleaves angiotensinogen, an alpha-2 globulin produced by the liver. The result is Angiotensin I (AngI) which yields Angiotensin II (AngII) via reaction with angiotensin converting enzyme. The ratio of AngII to AngI represents plasma renin activity (PRA) an indirect measurement of kidney sympathetic activation. In rats, PRA is increases ~4 fold after episodic hypoxic exposures (38).

Elevations in renal and adrenal sympathetics, indicated directly or indirectly have acute and chronic cardiovascular implications. Activation of renal sympathetics may play a role in the

persistent MSNA observed with hypoxia, and therefore constrictor action of vascular tissue. It has been suggested that epinephrine (EPI), released from the adrenal medulla during hypoxia, may be taken up by post ganglionic nerves and released as a co-transmitter with NE (9) (refer to Appendix D). Furthermore, Ang II facilitates NE release through its action on AT_1 receptors located at terminal branches. Consequently, potentiation of peripheral sympathetic neurotransmission and end organ response (vasoconstriction) may result. Additionally, increases in AngII, such as those observed with hypoxia increase heart rate and myocardial contractility. Chronic overproduction of AngII can lead to hypertrophy and/or hyperplasia of vascular smooth muscle and activation of vascular trophic factors (27). Therefore, the renin-angiotensin system is an important contributor in the elevated blood pressure observed in response to chronic hypoxia and may be implicated in those with dirurnal hypertension associated with OSA.

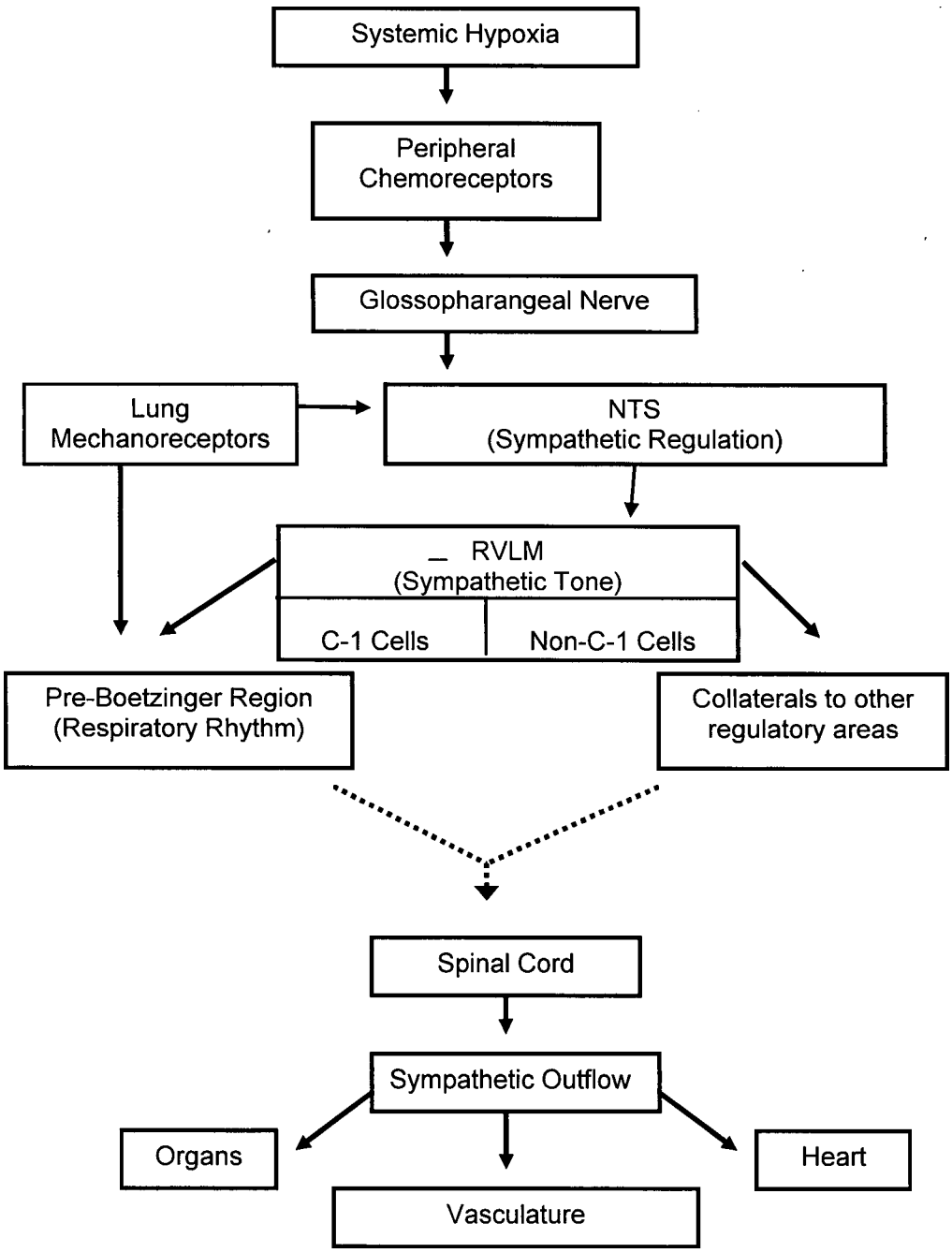
Appendix A

Figure 3. Representative trace for one subject during the post IH trial. Note that although ventilation, SaO_2, and F_iO_2 all return to baseline values during recovery, MSNA remains elevated.



Abbreviations: arterial oxygen saturation (SaO_2), fraction of inspired oxygen (F_iO_2), muscle sympathetic nerve activity (MSNA).

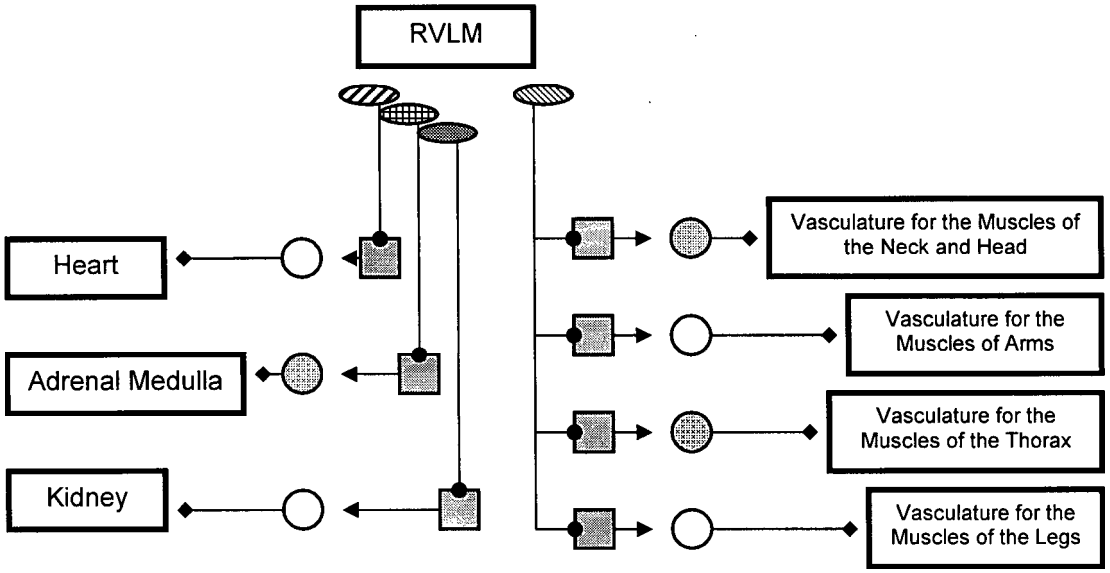
Appendix B



Hypoxia and sympathetic drive Hypoxia is detected in the periphery, primarily via the carotid bodies and sensory afferent transmissions are relayed to the central nervous systems. The central nervous system responds by enhanced sympathetic activity directed to the various organs and vasculature.

Abbreviations: nucleus tractus solitarius (NTS), rostral ventral lateral medulla (RVLM)

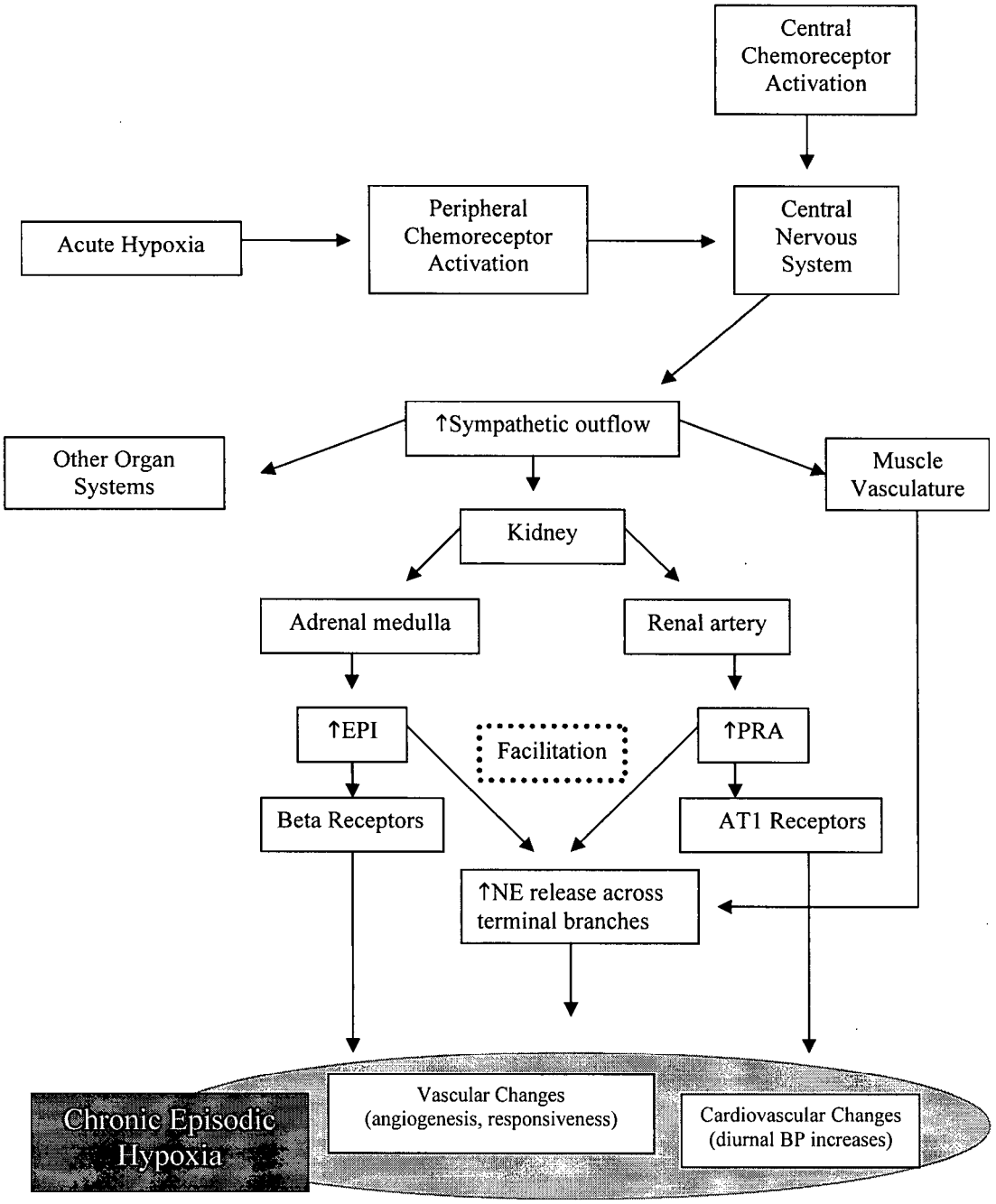
Appendix C



Sympathetic control via the rostral ventral lateral medulla. The RVLM is charged with sympathetic control. The presympathetic neurons extending from the RVLM terminate in separate regions and therefore differential recruitment can be anticipated in response to physiological stimuli, such as hypoxia. Figure adapted from Guyenet, 2000.

Abbreviations: rostral ventral lateral medulla (RVLM)

Appendix D



Hypoxia and renal sympathetics. The kidney and the adrenal medulla may play a role in the persistent activation of the sympathetic nervous system during hypoxia. Chronic activation of such pathways has implications on the cardiovascular system and vascular properties. Concepts adapted from Biesold et al. 1989.

Abbreviations: epinephrine (EPI), plasma renin activity (PRA), norepinephrine (NE)

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