Acute intermittent hypercapnic hypoxia and sympathetic neurovascular transduction in men

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Key Points Summary:

- Intermittent hypoxia leads to long-lasting increases in muscle sympathetic nerve activity and blood pressure contributing to increased risk for hypertension in obstructive sleep apnoea patients.
- We determined whether augmented vascular responses to increasing sympathetic vasomotor outflow, termed sympathetic neurovascular transduction (sNVT), accompanied changes in blood pressure following acute intermittent hypercapnic hypoxia (IH) in men.
- Lower body negative pressure was utilized to induce a range of sympathetic vasoconstrictor firing while measuring beat-by-beat blood pressure and forearm vascular conductance.
- IH reduced vascular shear stress and steepened the relationship between diastolic blood pressure and sympathetic discharge frequency suggesting greater systemic sNVT.
- Our results indicate that recurring cycles of acute IH characteristic of obstructive sleep apnoea could promote hypertension by increasing sNVT.

ABSTRACT

Acute intermittent hypercapnic hypoxia (IH) induces long-lasting elevations in sympathetic vasomotor outflow and blood pressure in healthy humans. It is unknown whether IH alters sympathetic neurovascular transduction (sNVT), measured as the relationship between sympathetic vasomotor outflow and either forearm vascular conductance (FVC; regional sNVT) or diastolic blood pressure (DBP; systemic sNVT). We tested the hypothesis that IH augments sNVT by exposing healthy males to 40 consecutive 1-minute breathing cycles, each comprising 40-seconds of hypercapnic hypoxia ($P_{ET}CO_2$: +4±3 mm Hg above baseline; $P_{ET}O_2$: 48±3 mm Hg) and 20-seconds of normoxia (n=9), or a 40-minute air-breathing control (n=7). Before and after the intervention, lower body negative pressure (LBNP; 3 minutes at -15, -30, and -45 mmHg) was applied to elicit reflex increases in muscle sympathetic nerve activity (MSNA, fibular microneurography) while clamping end-tidal gases at baseline levels. Ventilation, arterial pressure (SBP, DBP, MAP), brachial artery blood flow ($\dot{Q}_{BA}$), FVC ($\dot{Q}_{BA}$/MAP), and MSNA burst frequency were measured continuously. Following IH, but not control, ventilation (5 l/min; 95% CI: 1 - 9), and MAP (5 mmHg; 95% CI: 1 - 9) were increased, while FVC (-0.2 ml/min/mm Hg; 95% CI: -0.0 - -0.4) and mean shear rate (SR; -21.9 /s; 95% CI: -5.8 - -38.0; all $P<0.05$) were reduced. Systemic sNVT was increased following IH (0.25 mm Hg/burst/min; 95% CI: 0.01 – 0.49; $P<0.05$), while changes in regional forearm sNVT were similar between IH and sham. Reductions in vessel wall shear stress and consequently nitric oxide production, may contribute to heightened systemic sNVT and provide a potential neuro-vascular mechanism for elevated blood pressure in obstructive sleep apnoea.

Key Words: sympathetic neurovascular transduction; intermittent hypoxia; hypoxia; muscle sympathetic nerve activity

INTRODUCTION

Obstructive sleep apnoea (OSA) is characterized by repeated obstructions of the upper airway during sleep (Dempsey *et al.*, 2010), leading to periods of intermittent hypercapnic hypoxia (IH). Longitudinal and cross-sectional epidemiological studies have established a link between the presence of OSA and both the development and the presence of hypertension (Peppard *et al.*, 2000; Kasai *et al.*, 2012; Floras, 2018). The observed acute and chronic effects of OSA on both night- and day-time efferent sympathetic vasoconstrictor nerve firing (Somers *et al.*, 1995; Taylor *et al.*, 2017) are consistent with the concept that such hypertension is of neurogenic origin. However, many patients with OSA do not manifest hypertension, which suggests variation between individuals in their susceptibility to pro-hypertensive neural, inflammatory, and vascular stimuli induced by repeated exposure to IH (Floras, 2018).

Following periods of IH, activation of the angiotensin-II type-I receptor (AT_1R) (Fletcher *et al.*, 1999; Marcus *et al.*, 2010; Foster *et al.*, 2010; Jouett *et al.*, 2016) has been shown to contribute to the increased sympathetic vasomotor outflow and blood pressure observed in both healthy humans (Foster *et al.*, 2009, 2010; Gilmartin *et al.*, 2010; Tremblay *et al.*, 2016; Jouett *et al.*, 2017) and animal models (Brooks *et al.*, 1997; Fletcher, 2000; Marcus *et al.*, 2009). This response depends on peripheral chemoreceptor afferent activity (Fletcher *et al.*, 1992) and afferent-efferent translation within brainstem centers, the median pre-optic nucleus and the subfornical organs (Saxena *et al.*, 2015; Shell *et al.*, 2019). IH can also reduce nitric oxide (NO) bioavailability (Foster *et al.*, 2009; Pialoux *et al.*, 2011), contributing to reductions in endothelial function (Gilmartin *et al.*, 2010; Khayat *et al.*, 2017), as well as reduce vascular sensitivity to exogenous norepinephrine (in animals) (Phillips, 2003). The translation of increases in post-ganglionic muscle sympathetic nerve activity (MSNA) to changes in vascular tone (*i.e.*, sympathetic neurovascular transduction [sNVT]) is the final effector of the peripheral chemoreceptor reflex arc and may be augmented by IH-induced increases in renin-angiotensin system activity (Story & Ziogas, 1987), and reduced NO bioavailability (Macarthur *et al.*, 2011). While enhanced sNVT has been observed during acute continuous hypoxia (Tan *et al.*, 2013*b*), whether sNVT changes following periods of IH is unclear. An IH-mediated increase in sNVT could, in part, contribute to the onset of arterial hypertension in some patients with OSA.

The primary objective of this study was to determine if acute IH could augment regional and systemic sNVT in healthy men. The IH paradigm utilized in this study was designed to simulate the blood gas changes observed in patients with severe OSA. We hypothesized that sNVT would be augmented by 40-minutes of IH compared with an air-breathing time control. Blood pressure, forearm blood flow ($\dot{Q}_{BA}$) and MSNA were recorded continuously during baseline and three stages of lower body negative pressure (LBNP) to estimate sNVT. LBNP was applied to induce reflexively greater efferent sympathetic discharge via graded baroreceptor unloading (Floras *et al.*, 2001).

MATERIALS AND METHODS

Ethical Approval

Ethical approval was obtained from the Clinical Research Ethics Board at the University of British Columbia (H16-02525) and conformed to the Declaration of Helsinki with the exception of registration as a clinical trial. Written informed consent was obtained from all participants.

Study Participants

Non-obese (BMI < 30 kg/m^2), normotensive (SBP <135 mm Hg, DBP < 85 mm Hg), men were invited to participate in this study. Participants were excluded if they were taking any medications (prescribed or over-the-counter), smoked tobacco within the past year, or had a history of hypertension, impaired renal function, liver disease, heart failure, myocardial infarction, coronary artery disease, stroke, chronic obstructive pulmonary disease, asthma, diabetes, or sleep disordered breathing. All participants underwent pulmonary function testing (V62J, Sensormedics, Yorba Linda, CA), and values were compared against age and height dependent predictions for normative spirometry (Knudson *et al.*, 1983) and diffusing capacity (Crapo & Morris, 1981) measurements. Participants were excluded if their forced expiratory volume in 1 second (FEV_1) / forced vital capacity ratio was < 0.70. Participants underwent a single night of sleep with nocturnal pulse oximetry (WristOx2, Model 3150, Nonin, Plymouth, MN, USA) and were excluded if they had indications of undiagnosed sleep apnoea, defined as an oxygen desaturation index ≥ 5 events/hr (based on a desaturation ≥ 4%; nVision V6.4, Nonin). Finally, only male participants were enrolled as women are less likely to develop OSA (Peppard *et al.*, 2013), less sensitive to alpha-adrenergic stimulation (both alpha 1 and 2) (Schmitt *et al.*, 2010) and have greater beta-adrenergic sensitivity due to oestrogen-dependent increases in beta-adrenoceptor expression (Riedel *et al.*, 2019).

Experimental Protocol

In preparation for the experimental protocol, participants fasted for ≥ 4 hours and abstained from exercise, caffeine, and alcohol for ≥ 12 hours. Upon arrival at the laboratory, participants voided their bladder and lay supine with legs and hips inside a custom-built lower body negative pressure (LBNP) chamber, which was sealed at the iliac crest. Participants were instrumented

for measures of heart rate (HR, lead-II electrocardiogram, FE 132, AD Instruments, Colorado Springs, CO), systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) (finger pulse photoplethysmography, Finometer Pro, Finapres Medical Systems, Amsterdam, Netherlands), brachial artery mean blood velocity (BA MBV) and diameter (Duplex ultrasound, ML6-15 probe, Vivid E9, GE Medical Systems, Mississauga, Canada), fibular MSNA (Nerve Traffic Analysis System Model 662C-4, University of Iowa Bioengineering, Iowa City, IA), finger S_PO_2 (7500FO, Nonin Medical Inc., Plymouth, MN) and a facemask to measure ventilation ($\dot{V}_I$) and control end-tidal gases. $\dot{V}_I$ was measured using a pneumotachograph (HR 800L, Hans Rudolph, Shawnee, KS) and differential pressure amplifier (PA-1, Hans Rudolph) while respired gas fractions were sampled at the mouth and measured using an oxygen and carbon dioxide analyzer (ML 206, AD Instruments). Blood pressure, HR, S_PO_2, $\dot{V}_I$, MSNA, and end-tidal gases were measured continuously throughout the experiment. Cardiopulmonary measurements were recorded at 200 Hz while MSNA signals were recorded at 2 kHz using an analog-to-digital converter (Powerlab/16SP ML 880, AD Instruments) and commercially available software (LabChart V8, AD Instruments).

The experimental protocol is shown in Figure 1. Following acquisition of a stable, high quality MSNA signal, baseline measurements were obtained over 5-minutes to calculate the resting end-tidal partial pressures of O_2 ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$). Dynamic end-tidal forcing was then used to clamp $P_{ET}O_2$ and $P_{ET}CO_2$ at resting values independent of $\dot{V}_I$ for the remainder of the LBNP protocol (Tymko *et al.*, 2016). LBNP was then applied at -15, -30, and -45 mm Hg with each stage held for three minutes. Negative pressure within the box was continuously measured by a calibrated differential pressure signal amplifier (PA-1 Series 1100, Hans Rudolph). To minimize the potential of dislodging the MSNA microelectrode, LBNP stages were not randomized. BA MBV and diameter were acquired from the left arm during the last minute of baseline and at each LBNP stage. Participants repeated a second LBNP protocol after 40-minutes of either IH (experimental) or air-breathing (time control). Due to logistical limitations, the time control was conducted after the collection of the experimental group and utilized a separate sonographer. Following baseline, dynamic end-tidal forcing was used to administer 40 consecutive 1-minute breathing cycles of IH, each comprising 40-seconds of hypercapnic hypoxia (targeting $P_{ET}CO_2$ +6 mm Hg above baseline, $P_{ET}O_2$ = 45 mm Hg) and 20-seconds of normoxic recovery. During the time control experiments, participants continued

breathing through the mouthpiece but did not undergo dynamic end-tidal forcing. The control of end-tidal and arterial blood gases using dynamic end-tidal forcing has been previously validated in our laboratory (Tymko *et al.*, 2016). This IH protocol mimicked the rate and magnitude of arterial oxyhaemoglobin desaturations observed in severe OSA patients during sleep (apnoea-hypopnoea index ≥ 60 /hr). Following each 40-minute intervention (i.e. IH or control), dynamic end-tidal forcing clamped $P_{ET}O_2$ and $P_{ET}CO_2$ at baseline levels and resting measurements were taken for 5-minutes.

Analyses

BA diameter was calculated from the ultrasound video images using automated edge detection software (Woodman *et al.*, 2001). BA MBV was obtained continuously from a Doppler audio signal translator (qDAT, Pennsylvania State University Hershey Medical Center, Hershey, PA). Forearm blood flow ($\dot{Q}_{BA}$; ml/min) was calculated as $\dot{Q}_{BA}$ = BA MBV×π×BA radius^2×60. Forearm vascular conductance (FVC; ml/min/mm Hg) was calculated as the quotient of $\dot{Q}_{BA}$ and MAP. We selected FVC over forearm vascular resistance as the independent variable because during LBNP, $\dot{Q}_{BA}$ was reduced while MAP was maintained. Under these conditions, the relationship between conductance and blood flow is linear (Lautt, 1989; Joyce *et al.*, 2019). Mean, antegrade, and retrograde shear rate (SR) were calculated from BA MBV as four times the MBV divided by vessel diameter and from these the oscillatory shear index (OSI) was calculated [|retrograde SR|/(|antegrade SR| + |retrograde SR|)] (Tremblay *et al.*, 2016). Sympathetic bursts were determined by inspection of the integrated neurogram based on internationally accepted guidelines (White *et al.*, 2015). Bursts were classified as of muscle sympathetic origin if they occurred 1.2-1.4 seconds after a QRS complex, had a signal to noise ratio of >3:1, and exhibited a spiked morphology. Once bursts were identified, MSNA burst frequency (BF) was calculated and expressed as bursts/min. Further, MSNA burst incidence (BI) was calculated and expressed as bursts/100 heart beats. All respiratory, cardiovascular, and MSNA measures were extracted for each event (breath, beat and burst, respectively). Data from the last minute of baseline and each LBNP stage were averaged at 1-minute intervals.

To characterize the severity of IH delivered, the mean, mean minimum, and mean maximum S_PO_2, and time spent below 90%, 85%, and 80% S_PO_2 was determined. S_PO_2 was calculated from $P_{ET}O_2$ using the Severinghaus transformation (Severinghaus, 1979) to account

for circulatory time delays. Since $P_{ET}O_2$ over estimates arterial PO_2 in normoxia and acute hypoxia (Tymko *et al.*, 2016), and the Severinghaus transformation assumes a standard human blood oxyhaemoglobin dissociation curve, it is likely that the calculated S_PO_2 underestimates the true magnitude of desaturation during IH. Additionally, breath-by-breath and beat-by-beat cardiorespiratory data were linearly interpolated at 1s intervals across IH and signal averaged for each subject [*see* Foster *et al*., (2009)]. To characterize each individual's IH severity, the peak and nadir values from the average profiles for the total 40-minute exposure were extracted and compared by repeated measures ANOVA.

Breath-by-breath, beat-by-beat, and burst-by-burst data were averaged across the last minute of baseline and each LBNP stage and presented in absolute and relative changes from the pre-test baseline to account for any baseline group differences. Subject characteristics were compared between groups by independent samples t-test. Baseline data were compared statistically by linear mixed effect modelling, including fixed factors for group (IH vs Control) and time (Pre vs Post), and group-by-time interactions are reported. Additionally, baseline changes within IH or control were assessed using a paired samples t-test and presented as the mean difference (Post minus Pre) with 95% confidence intervals. Data from all stages of LBNP were compared statistically by linear mixed effect modelling and included fixed factors for group (IH vs Control), time (Pre vs Post) and LBNP stage (0, -15, -30, -45 mm Hg). In all mixed models, subject identifiers were entered as a random effect within each group to control for correlation within subjects across fixed factors. In addition, stage was entered as a random effect permitting slope to vary by subject across varying levels of LBNP. When significant effects were identified, Tukey's post-hoc was used to assess the relevant contrasts.

Whole body sNVT was determined for each subject by determining the slope and intercept of the linear relationship between changes in MSNA BF and changes in DBP across all stages of LBNP (0, -15, -30, & -45 mm Hg) before and after IH or control (Halliwill *et al.*, 1996). Similarly, forearm sNVT was determined for each subject by determining the slope and intercept of the linear relationship between changes in MSNA BF and changes in FVC before and after each treatment across all stages of LBNP. Anticipating shifts in the integrated neurogram, which occur often and unpredictably during the course of graded LBNP, we elected, *a priori*, not to quantify MSNA burst amplitude or total MSNA. The change in sNVT slopes and intercepts (post minus pre) were determined for IH and control and compared by a one-tailed

independent samples t-test to determine the effect of treatment. Additionally, the sNVT slopes and intercepts were compared within each treatment (IH or control) by paired samples t-tests. Data are expressed as means ± SD and mean differences with 95% confidence intervals. All statistical analyses were conducted using R (R Core Team, 2018), lme4 (Bates *et al.*, 2015), lmerTest (Kuznetsova *et al.*, 2017), and emmeans (Lenth, 2019) statistical packages. An alpha value of $P < 0.05$ was considered statistically significant.

RESULTS

Participants

Twenty healthy males were enrolled. One volunteer allocated to the control group was excluded because his nocturnal oxygen desaturation index was ≥ 5 events per hour. Three additional subjects, two from the control and one from the IH cohorts were excluded subsequently from group analyses because their neurograms or ultrasound images acquired during LBNP were of low quality. The present analysis is therefore comprised of data assembled from 9 participants in the IH group and 7 in the control group. Both cohorts were of similar age (IH: 23 ± 6, control: 27 ± 8 years; P = 0.30), height (IH: 1.74 ± 0.09, control: 1.80 ± 0.08 m; P = 0.08), weight (IH: 79 ± 15, control: 80 ± 13 kg; P = 0.78), and BMI (IH: 26 ± 3, control: 25 ± 3 kg/m^2; P = 0.40). Nocturnal oximetry and pulmonary function data are presented in Table 1.

Intermittent Hypercapnia Hypoxia

IH resulted in 40 bouts of hypercapnic hypoxaemia (mean minimum S_PO_2 of 83 ± 3 %) with full restoration of peripheral saturation between bouts (mean maximum S_PO_2 of 97 ± 0 %). Participants spent 37 ± 9 % of the exposure time with S_PO_2 < 90 %, 17 ± 12 % of time with S_PO_2 < 85 %, and 3 ± 9 % of time with S_PO_2 < 80 %. On average within each bout $P_{ET}O_2$ was significantly reduced from 94 ± 9 to 48 ± 3 mm Hg during IH (P < 0.001), and $P_{ET}CO_2$ was significantly increased from 41 ± 3 to 45 ± 3 mm Hg (P = 0.002) despite a concurrent increase in $\dot{V}_I$ by 10 ± 6 l/min (P = 0.001).

Baseline Cardiorespiratory, Sympathetic, and Haemodynamic Parameters following Intermittent Hypercapnic Hypoxia

The influence of IH on baseline respiratory, cardiovascular, sympathetic, and haemodynamic parameters is presented in Table 2. By study design, end-tidal gases were similar before and after IH and control. V_I was increased following IH but not control (group-by-time, P = 0.02). This effect tended to be driven by an increase in breathing frequency rather than tidal volume. Although MAP increased following IH (+5 mm Hg; 95% CI: 3 – 8; P = 0.002) but not control (+3 mm Hg; 95% CI: -3 – 9; P = 0.23), the effect lacked a significant group-by-time effect (P = 0.31). Similar observations were made with respect to SBP and DBP (Table 2). Heart rate was

similar following IH and control, while MSNA BF and BI tended to be increased by IH but lacked significant group-by-time effects (P = 0.24). IH tended to reduce $\dot{Q}_{BA}$ and led to significant reductions in FVC and SR. Antegrade SR tended to be reduced (IH: -22 /s; 95% CI: -38 – -6; P = 0.01; Control: -3 /s; 95% CI: -24 – 17; P = 0.70; group-by-time, P = 0.08) while retrograde SR increased (IH: 2 /s; 95% CI: -0 – 4; P = 0.08; Control: -2 /s; 95% CI: -5 – 1; P = 0.16; group-by-time, P = 0.01) by IH leading to a significant increase in the OSI (IH: 0.03; 95% CI: 0.01 – 0.05; P = 0.01; Control: -0.03; 95% CI: -0.08 – 0.03; P = 0.28; group-by-time, P = 0.02).

Influence of Intermittent Hypercapnic Hypoxia on Cardiorespiratory, Sympathetic, and Haemodynamic Responses to LBNP

Figure 2 illustrates the haemodynamic and sympathetic response to LBNP from a representative subject before and after IH. The pressure generated during each stage of LBNP was similar between pre- and post-tests (time effect; P = 0.66) and did not differ between IH and control (group effect; P = 0.57). Mean LBNP for each stage was 16.1 ± 0.4, 30.3 ± 0.4, and 45.0 ± 0.4 mm Hg.

The influence of IH on respiratory, cardiovascular, sympathetic, and haemodynamic parameters during LBNP are presented in Table 3 and Figure 3. $P_{ET}O_2$ was similar across each stage of LBNP (stage effect, P = 0.46) but was modestly elevated (1.1 mm Hg; 95% CI: 0.1 – 2.1; time effect, P = 0.02) in the post test across both groups. $P_{ET}CO_2$ was similar across each stage of LBNP (stage effect; P = 0.51) and across conditions (time effect; P = 0.45) for both groups. $\dot{V}_I$ increased across stages of LBNP in both IH and control (stage effect, P < 0.001). There was a significant group-by-time effect (P < 0.001; Figure 3A) suggesting that V_I was 5.2 l/min greater across all stages of LBNP following IH (95% CI: 4.2 – 6.2; P < 0.001) but not following control (-0.2 l/min; 95% CI: -1.4 – 1.0; P = 0.99). This effect was driven by breathing frequency, since tidal volume was similar between groups (IH vs control; P = 0.21) and time (pre vs post; P = 0.96) across all levels of LBNP. There was a significant group-by-time interaction (P = 0.01) for breathing frequency; whereby breathing frequency increased by 3 breaths/min following IH (95% CI: 1.0 – 5.0; P < 0.001) but not following control (1 breaths/min; 95% CI: -1 – 3; P = 0.40).

There were significant group-by-time interactions for MAP, SBP, and DBP (each $P \leq 0.001$). Regardless of LBNP stage, MAP was 7 mm Hg higher following IH (95% CI: 5 – 9; $P < 0.001$) but only 3 mm Hg higher following control (95% CI: 1 – 5; $P = 0.01$; Figure 3B). SBP was reduced with increasing LBNP ($P = 0.007$). Across all stages of LBNP, SBP was 9 mm Hg higher following IH (95% CI: 7 – 11; $P < 0.001$) but only 3 mm Hg higher following control (95% CI: 1 – 5; $P = 0.12$). DBP tended to increase with increasing LBNP (stage effect, $P = 0.06$) and was 6 mm Hg higher following IH (95% CI: 4 - 8; $P < 0.001$) but only 3 mm Hg higher following control (95% CI: 1 – 5; $P = 0.01$). HR increased with LBNP in both groups (stage effect, $P < 0.001$) but this response was larger in IH compared with control ($P < 0.001$; stage-by-group interaction). HR was lower in the control group compared with the IH group (group effect, $P = 0.02$) and was higher across all stages of LBNP following IH (2 /min; 95% CI: 1 – 3; $P = 0.01$) but not following control (0 /min; 95% CI: -1 – 1; $P = 1.0$).

With respect to MSNA BF the group-by-time-by-stage interaction ($P = 0.11$) and the group-by-time interaction were not significant ($P = 0.18$; Figure 3C). MSNA BF increased with each stage of LBNP (stage effect, $P < 0.001$), was higher in the post test regardless of group (1.3 /min; 95% CI: 0.3 – 2.3; time effect, $P = 0.007$) and was higher in the IH group (11 /min; 95% CI: 5 – 17; group effect, $P = 0.002$). Similarly, MSNA BI increased with LBNP (stage effect, $P < 0.001$) but the group-by-time interaction was not significant ($P = 0.15$).

$\dot{Q}_{BA}$ was reduced by LBNP (stage effect, $P < 0.001$) and there was a significant group-by-time interaction ($P = 0.02$, Figure 3D). $\dot{Q}_{BA}$ was reduced to a greater extent following IH compared with control (IH: 16 ml/min, 95% CI: 11 – 21; $P < 0.001$; control: 7 ml/min, 95% CI: 1 – 13; $P = 0.06$). Similarly, SR was reduced by LBNP (stage effect, $P < 0.001$) and there was a significant group-by-time interaction ($P = 0.01$, Figure 3E). Specifically, SR was reduced by 21 /s following IH (95% CI: 15 – 27; $P < 0.001$) and to a lesser extent following control (10 /s, 95% CI: 2 – 18; $P = 0.05$; Figure 3E). FVC decreased across LBNP ($P < 0.001$) and there was a significant group-by-time interaction ($P = 0.003$; Figure 3F). IH reduced FVC by 0.23 ml/min/mm Hg (95% CI: 0.17 – 0.29; $P < 0.001$) and to a lesser extent following control (0.10 ml/min/mm Hg, 95% CI: 0.04 – 0.16; $P = 0.02$).

Sympathetic Neurovascular Transduction

sNVT was assessed before and after IH or control using the slope and intercept of the relationship between the change in MSNA BF and the percent change in FVC (Figure 4) or absolute change in DBP (Figure 5) throughout LBNP. The change in FVC transduction slope was similar between IH (-0.84 %/burst/min, 95% CI: -1.82 – 0.14) and control (-1.09 %/burst/min, 95% CI: -3.01 – 0.82, Figure 4C). Specifically, the difference in FVC transduction slope change was similar between groups (-0.25 %/burst/min, 95% CI: -1.88 – ∞; P = 0.61). Likewise, the change in FVC transduction intercept was similar following IH (-16.1 %, 95% CI: -31.9 – -0.3) and control (-6.4 %, 95% CI: -37.5 – 24.8, Figure 4D). The difference in FVC transduction intercept change was similar between groups (9.8 %, 95% CI: -16.6 – ∞; P = 0.26).

The DBP transduction slope was increased by IH (0.25 mm Hg/burst/min, 95% CI: 0.02 – 0.48, P = 0.04) but not following control (0.00 mm Hg/burst/min, 95% CI: -0.18 – 0.18, P = 0.99). The DBP transduction slope change differed between groups (0.25 mmHg/burst/min, 95% CI: -∞ – 0.03; P = 0.03, Figure 5C). The change in DBP transduction intercept was similar between IH (2.96, 95% CI: 0.02 – 5.89) and control (3.05, 95% CI: -2.66 – 8.77). The DBP transduction intercept change was similar between groups (-0.1, 95% CI: -4.9 – ∞, P = 0.51, Figure 5D).

DISCUSSION

The purpose of this study was to determine the impact of acute exposure to IH on regional and systemic sNVT. Our data demonstrate, for the first time, that systemic sNVT is augmented following IH. Yet, changes in forearm sNVT were similar following IH and control. We interpret these results to suggest regional differentiation in the neurovascular response to IH, which support an overall systemic increase in sNVT. Additionally, IH led to a robust increase in $\dot{V}_I$, suggesting ventilatory long term facilitation (LTF), an increase in mean arterial pressure, and a reduction in vascular shear rate.

Sympathetic Neurovascular Transduction

We observed a significant increase in the DBP transduction slope after 40-minutes of IH (Figure 5C), which did not occur in the control group, suggesting that systemic sNVT was increased by IH. This effect differed in the forearm where similar changes in FVC transduction slope and intercept were observed following either IH or control (Figure 4). Augmented sNVT has also been reported during continuous isocapnic hypoxia (Tan *et al.*, 2013*b*). Our results suggest that the systemic sNVT response to IH was more pronounced than the regional sNVT response measured in the forearm. Differences in haemodynamics between upper and lower limbs during IH may explain why systemic sNVT was increased while changes are less evident in the forearm. In line with this hypothesis, leg sNVT measured at the femoral artery is greater than forearm sNVT (Fairfax *et al.*, 2013*a*, 2013*b*), possibly owing to reduced responsiveness to α-adrenergic stimulation in the forearm (Pawelczyk & Levine, 2002). Also, during IH the leg is exposed to a greater reduction in antegrade SR and increased OSI, compared with the forearm (Tremblay *et al.*, 2016). Since the leg receives a greater proportion of circulation compared with the forearm, changes in sNVT in the leg may contribute more to blood pressure control and whole body sNVT.

The AT_1R may have a role in augmenting sNVT after IH. Blocking the AT_1R with Losartan before intermittent hypoxia prevents the known increase in resting blood pressure (Foster *et al.*, 2010), sympathetic vasomotor outflow (Jouett *et al.*, 2017), oxidative stress (Pialoux *et al.*, 2011), and endothelial dysfunction associated with intermittent hypoxia (Marcus *et al.*, 2012). Local infusion of angiotensin-II exacerbates the forearm vascular resistance

response to mild LBNP while having no effect on the response to local norepinephrine infusion (Seidelin *et al.*, 1991), which supports the hypothesis that renin-angiotensin system mediated changes in sNVT may occur via presynaptic facilitation.

An alternative explanation is that IH increases sNVT as a result of decreased NO production due to reductions in $\dot{Q}_{BA}$ (reducing antegrade SR) and sympathetically-mediated increases in resistance vessel tone (increasing retrograde SR). Additionally, as a result of an IH induced alteration in redox homeostasis, free-radical mediated scavenging of NO may further contribute to reductions in NO bioavailability. Our results showed that IH reduced forearm SR and increased the OSI to a greater extent than control (Tables 2 & 3, Figure 3). Nonetheless, there were modest but significant increases in DBP and reductions in FVC, $\dot{Q}_{BA}$, and SR (Table 3) following control which may have contributed to the similar increase in forearm sNVT observed between IH and control. Therefore, the effect on forearm sNVT may have less to do with IH and more to do with prolonged inactivity and consequent reductions in SR and NO production. The greater systemic sNVT observed following IH, may be more related to the greater IH mediated reductions in vascular SR that we have previously observed within the vasculature of the lower limb (Tremblay *et al.*, 2016). In OSA, hypoxia-induced sympathetic activation is also believed to increase retrograde blood flow (Millar *et al.*, 2011). The observed changes in SR may be related to increased MSNA BF or sNVT, as retrograde SR and OSI are increased with sympathetic vasomotor outflow and reduced conductance during LBNP (Padilla *et al.*, 2010) but alternatively could be due to local effects of IH on endothelial cell function. Although no studies have measured endothelial-dependent dilation after 40 minutes of IH, longer duration exposure to IH without hypercapnia reduces NO derivatives (Foster *et al.*, 2009; Pialoux *et al.*, 2011), promotes retrograde shear stress (Tremblay *et al.*, 2016), reduces peak reactive hyperaemia and resting blood flow in the forearm, and increases baseline forearm vascular resistance (Gilmartin *et al.*, 2010). After 12 weeks of CPAP therapy in OSA patients whom are without comorbidities, NO expression in microcirculatory vessel walls increased 4-fold alongside improved flow-mediated dilation, which supports that the absence of IH can improve endothelial function (Khayat *et al.*, 2017). However, in healthy young males, 6-hours of IH did not reduce flow-mediated dilation compared to controls (Tremblay *et al.*, 2016). Animal studies have demonstrated that NO reduces the release of norepinephrine from sympathetic nerves and inactivates norepinephrine directly (Macarthur *et al.*, 2011). Therefore,

sNVT may have been increased in this study, through mechanisms reducing NO production (reduced $\dot{Q}_{BA}$) and bioavailability (alteration in redox homeostasis).

Sympathetic Vasomotor Outflow and Blood Pressure

IH tended to increase resting sympathetic vasomotor outflow (Table 2) and increased blood pressure throughout all stages of LBNP (Table 3, and Figure 3B). Blood pressure and sympathetic responses after IH were independent of chemoreceptor stimulation, as end-tidal gases were clamped at baseline levels. Generally, our results are similar to other studies which reported long-lasting increases in sympathetic vasomotor outflow after a short bout of intermittent or continuous hypoxia (Xie *et al.*, 2000; Cutler, 2004; Leuenberger *et al.*, 2005; Jouett *et al.*, 2017). We observed an increase in MSNA BF in 89% of participants after IH, but only 43% of participants following control. The greater BP observed following IH across all stages of LBNP despite only a modest increase in MSNA BF following IH suggests an important regulatory role for systemic sNVT.

Long Term Facilitation of Ventilation

Ventilatory LTF describes plasticity in the neural control of respiration such that $\dot{V}_I$ remains elevated following IH. Our results show $\dot{V}_I$ was increased following IH, independent from changes in arterial blood gases (Figure 3, Table 2 & 3). In the control group, $\dot{V}_I$ remained similar to baseline following 40-minutes of room air breathing (Table 2). Previous studies in awake humans demonstrate ventilatory LTF following intermittent hypoxia only when combined with hypercapnia ($P_{ET}CO_2 \approx$ 3-5 mm Hg above baseline) throughout both exposure and recovery (Harris *et al.*, 2006; Mateika & Sandhu, 2011; Griffin *et al.*, 2012), but not when $P_{ET}CO_2$ is uncontrolled in recovery (Deacon *et al.*, 2017). The contrasting results with others may be a result of longer duration IH, longer period of hypoxia/hypercapnia, and shorter normoxic recovery periods. The greater intensity of IH may have increased carotid body activation eliciting robust ventilatory LTF. Future studies are needed to confirm our finding of ventilatory LTF following intermittent hypercapnic hypoxia.

Methodological Considerations

We measured sNVT by increasing sympathetic vasomotor activity with LBNP and observing the forearm vascular (FVC) and systemic vasomotor (DBP) responses. Specifically, we observed the strength of the relationship between MSNA BF and FVC or DBP before and immediately after acute IH. Similar methods have been used previously to determine the effect of race and fitness on sNVT (Ray & Monahan, 2002; Notarius *et al.*, 2012). We improved upon these methods by using end-tidal forcing to clamp blood gases at baseline levels. This is essential as the graded ventilatory response which accompanies LBNP would result in hypocapnia, reducing chemoreceptor activation and lowering sympathetic vasomotor outflow and peripheral vascular resistance through direct effects on the vessel (Burnum *et al.*, 1954). Handgrip exercise, which also increases ventilation, has similarly been used instead of LBNP to increase MSNA BF for the measurement of sympathetic transduction (Halliwill *et al.*, 1996; Minson *et al.*, 2000; Tamisier *et al.*, 2015) but these studies also do not control for blood gas changes during sNVT measurement. Clamping blood gases at baseline levels prevents distortion of the relationship between MSNA BF and FVC, allowing for a more precise measurement of sNVT that is independent of secondary chemoreflex effects.

Our sNVT technique is limited in that we are unable to measure transduction in a purely resting state, and it does not consider the dynamic changes in transduction that occur in response to each burst of MSNA. By directly measuring sympathetic vasomotor activity and relating it to the observed strength of the vascular response, we are able to assess sNVT. However, in our current experimental paradigm, we are unable to determine the relative contributions of increased afferent activity from the peripheral chemoreceptors, reduced afferent activity from baroreceptors, or changes in the central gain of afferent-efferent transmission, to the increase in sympathetic outflow. Previous studies have investigated burst-by-burst effects on subsequent changes in forearm and leg vascular conductance (Fairfax *et al.*, 2013*a*, 2013*b*; Vranish *et al.*, 2018), diastolic blood pressure (Briant *et al.*, 2016), and a complex autoregressive model of transduction independent of blood pressure during handgrip exercise (Tan *et al.*, 2013*a*). However, our measure of sNVT informs the vascular responses over a wide range of sympathetic vasomotor activity. Using LBNP to achieve graded increases in sympathetic vasomotor outflow permits the sensitivity of the vasculature beyond the resting state to be measured.

A strength of our experimental exposure to IH is the addition of hypercapnia to better simulate the blood gas changes in OSA. However, we are unable to conclude whether intermittent hypoxia, intermittent hypercapnia, or the combination of the two is responsible for augmenting whole body sNVT. Hypercapnia has been demonstrated to have a role in ventilatory LTF, respiratory muscle and phrenic nerve LTF, and carotid body sensory LTF (Peng *et al.*, 2003; Roy *et al.*, 2018), all of which may have a role in controlling breathing stability and upper airway patency in OSA (Mahamed & Mitchell, 2007; Mateika & Syed, 2013). Therefore, it was important to include hypercapnia in our IH paradigm to better replicate changes in sNVT that may accompany OSA early in its progression.

For logistical reasons, control group data were collected after the IH group was studied. This sequential approach may have contributed to significant between group differences at baseline in heart rate, which may reflect lower fitness, and in heart rate-coupled MSNA BF. Importantly, our analyses focused on intervention (*i.e.*, IH or sham) effects. Whether fitness status influences the sNVT response to IH is unknown. Additionally, the effect of IH on sNVT in females requires study.

CONCLUSION

In these experiments, systemic but not forearm sNVT was augmented after an acute 40-minute bout of IH. Such selectivity may result from the known differential effects of IH on upper and lower limb vasculature (Tremblay *et al.*, 2016). Specifically, reductions in vessel wall shear rate, augmented oscillatory shear rate, and the consequent reductions in NO production may in part increase sNVT. The larger volume of vasculature within the lower legs and greater reduction in shear rate observed in these limbs may explain the effect of IH on whole body sNVT. The greater $\dot{V}_I$ observed following IH is suggestive of ventilatory LTF. Our data adds to the body of evidence suggesting that IH has long-lasting effects on the arterial vasculature and leads to respiratory and neurocirculatory plasticity. The variance between individuals in the sNVT response to IH might explain why some patients with OSA develop hypertension while others do not. Our results provide a potential mechanism for increasing blood pressure in patients with OSA early in disease progression and may contribute to the increased risk of hypertension well documented in OSA patients.

ADDITIONAL INFORMATION

Competing Interests

None

Author Contributions

Conception/design of the work: TJRS, TDV, NTA, JSF, GEF;
Acquisition/analysis/interpretation of data for the work: All authors; Drafting/revising the work for important intellectual content: All Authors; Final approval: All authors;

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TABLES AND FIGURES

Table 1. Participant nocturnal oximetry and pulmonary function data by intermittent hypercapnic hypoxia and control group.

Variable	IH	Control	P
ODI (/hr)	2.4 ± 1.2	1.7 ± 1.1	0.35
Mean Min. S_pO_2 (%)	92.2 ± 0.9	92.9 ± 1.1	0.30
Forced vital capacity (l)	5.37 ± 0.78	5.97 ± 0.93	0.18
Forced vital capacity (% predicted)	102 ± 12	111 ± 16	0.40
FEV_1 (l)	4.14 ± 0.48	4.63 ± 0.61	0.13
FEV_1 (% predicted)	94 ± 12	103 ± 11	0.28
FEV_1/Forced vital capacity (%)	78.0 ± 7.2	77.7 ± 3.7	0.81
D_LCO (% predicted)	97 ± 12	113 ± 8	0.10
V_A (l)	6.29 ± 0.96	7.13 ± 1.19	0.27
D_LCO/V_A (% predicted)	103 ± 15	107 ± 11	0.46

Values are means ± SD. IH, N = 9; Control, N = 7. Abbreviations: ODI, oxygen desaturation index; Mean Min. S_pO_2, mean minimum nocturnal peripheral oxyhaemoglobin saturation; FEV_1, forced expiratory volume in 1 second; D_LCO, diffusing capacity of the lung for carbon monoxide transfer; V_A, alveolar volume; P, probability for difference between IH and Control based on an independent samples t-test.

Table 2. Influence of intermittent hypercapnic hypoxia on baseline respiratory, cardiovascular, sympathetic, and haemodynamic parameters.

	Group	N	Pre	Post	Δ {95% CI}	Within Group (P)	Group by Time (P)
Respiratory							
$\dot{V}_I$, l/min	IH	9	11 ± 3	16 ± 3	5.1 {1.5 – 8.7}	0.01	0.02
	Control	6	12 ± 2	12 ± 2	0.0 {-2.1 – 2.2}	0.99	
V_T, l	IH	9	1.1 ± 0.3	1.1 ± 0.3	-0.0 {-0.3 – 0.3}	0.90	0.67
	Control	6	1.1 ± 0.2	1.0 ± 0.2	-0.1 {-0.2 – 0.0}	0.10	
f_B, /min	IH	9	10 ± 3	14 ± 3	3 {-0 – 7}	0.07	0.20
	Control	6	10 ± 2	11 ± 2	1 {-1 – 3}	0.24	
$P_{ET}O_2$, mm Hg	IH	9	92 ± 3	92 ± 3	0.4 {-3.4 – 4.1}	0.82	0.89
	Control	7	94 ± 3	94 ± 3	0.1 {-1.8 – 2.0}	0.89	
$P_{ET}CO_2$, mm Hg	IH	9	41 ± 3	41 ± 3	-0.4 {-1.1 – 0.2}	0.15	0.39
	Control	7	40 ± 3	40 ± 3	0.2 {-1.8 – 2.3}	0.80	
Cardiovascular							
SBP, mm Hg	IH	9	122 ± 9	130 ± 9	7 {2 – 12}	0.01	0.27
	Control	7	118 ± 11	121 ± 11	3 {-6 – 12}	0.43	
DBP, mm Hg	IH	9	68 ± 9	72 ± 9	5 {3 – 7}	<0.01	0.36
	Control	7	61 ± 3	63 ± 3	3 {-3 – 8}	0.26	
HR, /min	IH	9	64 ± 9	66 ± 9	2 {-2 – 5}	0.27	0.24
	Control	7	55 ± 8	54 ± 8	-1 {-4 – 3}	0.66	
Sympathetic							
MSNA BF, /min	IH	9	17 ± 9	20 ± 9	3 {-0 – 6}	0.05	0.08
	Control	7	12 ± 5	11 ± 5	-0 {-4 – 3}	0.76	
MSNA BI, /100 HB	IH	9	26 ± 12	31 ± 12	4 {-1 – 10}	0.11	0.10
	Control	7	23 ± 16	21 ± 16	-2 {-8 – 5}	0.59	
Haemodynamic							
FVC, ml/min/mm Hg	IH	9	0.86 ± 0.39	0.62 ± 0.39	-0.24 {-0.40 – -0.08}	<0.01	0.05
	Control	7	0.63 ± 0.21	0.59 ± 0.21	-0.04 {-0.22 – 0.14}	0.60	
$\dot{Q}_{BA}$, ml/min	IH	9	75 ± 36	57 ± 36	-17 {-33 – -2}	0.03	0.09
	Control	7	51 ± 19	49 ± 19	-2 {-15 – 11}	0.72	
SR, /s	IH	9	101 ± 48	77 ± 48	-24 {-39 – -8}	<0.01	0.05
	Control	7	72 ± 32	70 ± 32	-1 {-23 – 21}	0.89	

Values are means ± SD or 95% confidence intervals {95% CI}. Definition of abbreviations: $\dot{V}_I$, minute ventilation; V_T, tidal volume; f_B, breathing frequency; $P_{ET}O_2$, partial pressure of end-tidal O_2; $P_{ET}CO_2$, partial pressure of end-tidal CO_2; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; MSNA BF, muscle sympathetic nerve activity burst frequency; MSNA BI, muscle sympathetic nerve activity burst incidence; FVC, forearm vascular conductance; $\dot{Q}_{BA}$, brachial artery blood flow; SR, shear rate. Within Group (P), probability for a difference within IH or Control based on a paired-samples t-test. Group by time (P), probability for a group-by-time interaction based on a linear mixed effect model including fixed factors for group and time.

Table 3. Influence of intermittent hypercapnic hypoxia on respiratory, cardiovascular, and haemodynamic parameters during LBNP.

Variable		LBNP Level			
Group	**Time**	**0 mm Hg**	**-15 mmHg**	**-30 mmHg**	**-45 mm Hg**
Respiratory					
$\dot{V}_I$, l/min		*Group: P = 0.14; Time: P < 0.001; Stage: P < 0.001; Group*Time: P < 0.001*			
IH	PRE	11 ± 3	12 ± 3	13 ± 6	16 ± 6†
	POST	16 ± 6*	18 ± 6*	20 ± 6*	22 ± 6*†
Control	PRE	12 ± 3	13 ± 3	13 ± 3	14 ± 3†
	POST	12 ± 3	12 ± 3	13 ± 5	14 ± 5†
Cardiovascular					
SBP, mm Hg		*Group: P = 0.56; Time: P < 0.001; Stage: P < 0.01; Group*Time: P < 0.001*			
IH	PRE	122 ± 9	122 ± 9	115 ± 12	111 ± 12†
	POST	130 ± 6*	128 ± 9*	126 ± 12*	123 ± 15*†
Control	PRE	118 ± 11	121 ± 8	119 ± 8	115 ± 11†
	POST	121 ± 8	123 ± 8	122 ± 11	119 ± 13†
DBP, mm Hg		*Group: P = 0.06; Time: P < 0.001; Stage: P = 0.06; Group*Time: P < 0.01*			
IH	PRE	68 ± 9	69 ± 9	69 ± 12	68 ± 12
	POST	72 ± 9*	73 ± 9*	75 ± 12*	75 ± 12*
Control	PRE	61 ± 3	63 ± 3	63 ± 3	63 ± 3
	POST	63 ± 5*	64 ± 5*	65 ± 5*	65 ± 8*
HR, /min		*Group: P < 0.01; Time: P < 0.05; Stage: P < 0.001; Group*Time: P < 0.05*			
IH	PRE	64 ± 9	67 ± 12†	78 ± 12†	87 ± 12†
	POST	66 ± 12	70 ± 15†	79 ± 12†	88 ± 15†
Control	PRE	55 ± 8	56 ± 8	58 ± 8	63 ± 8†
	POST	54 ± 5	56 ± 5	58 ± 5	63 ± 8†
Sympathetic					
MSNA BF, /min		*Group: P < 0.01; Time: P < 0.01; Stage: P < 0.001; Group*Time: P = 0.18*			
IH	PRE	17 ± 9	23 ± 9†	29 ± 6†	36 ± 9†
	POST	20 ± 6	27 ± 9†	32 ± 6†	34 ± 9†
Control	PRE	12 ± 5	14 ± 5†	17 ± 5†	21 ± 5†
	POST	11 ± 5	15 ± 5†	19 ± 8†	22 ± 8†
MSNA BI, /100 HB		*Group: P = 0.13; Time: P = 0.06; Stage: P < 0.001; Group*Time: P = 0.15*			
IH	PRE	26 ± 12	35 ± 12†	37 ± 9†	42 ± 9†
	POST	31 ± 12	40 ± 12†	41 ± 9†	39 ± 9†
Control	PRE	23 ± 16	26 ± 13†	31 ± 11†	35 ± 13†
	POST	21 ± 13	27 ± 11†	33 ± 13†	35 ± 11†
Haemodynamic					
FVC, ml/min/mm Hg		*Group: P = 0.47; Time: P < 0.001; Stage: P < 0.001; Group*Time: P < 0.01*			
IH	PRE	0.86 ± 0.39	0.62 ± 0.36†	0.73 ± 0.39†	0.71 ± 0.33
	POST	0.62 ± 0.36*	0.48 ± 0.30*†	0.46 ± 0.24*†	0.44 ± 0.18*
Control	PRE	0.63 ± 0.21	0.50 ± 0.11†	0.58 ± 0.26†	0.60 ± 0.24
	POST	0.59 ± 0.26*	0.42 ± 0.19*†	0.43 ± 0.13*†	0.46 ± 0.21*
$\dot{Q}_{BA}$, ml/min		*Group: P = 0.33; Time: P < 0.001; Stage: P < 0.001; Group*Time: P < 0.05*			
IH	PRE	75 ± 36	55 ± 36†	62 ± 36†	59 ± 33†
	POST	57 ± 36*	45 ± 27*†	43 ± 27*†	41 ± 21*†
Control	PRE	51 ± 19	41 ± 8†	47 ± 21†	48 ± 21†
	POST	49 ± 24	35 ± 16†	36 ± 11†	38 ± 19†
SR, /s		*Group: P = 0.42; Time: P < 0.001; Stage: P < 0.001; Group*Time: P < 0.05*			
IH	PRE	101 ± 48	77 ± 51†	88 ± 51†	82 ± 30
	POST	77 ± 51*	62 ± 42*†	62 ± 39*†	58 ± 27*
Control	PRE	72 ± 32	59 ± 19†	71 ± 32†	70 ± 29
	POST	70 ± 29*	53 ± 16*†	54 ± 16*†	55 ± 19*

*Values are means ± SD. Bold type **IH** or **Control** indicates a main effect of group, with the bold group possessing a higher value. *, P < 0.05 compared to PRE within each group. †, P < 0.05 compared to '0' LBNP. Definition of abbreviations: $\dot{V}_I$, minute ventilation; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; MSNA BF, muscle sympathetic nerve activity burst frequency; MSNA BI, muscle sympathetic nerve activity burst incidence; FVC, forearm vascular conductance; $\dot{Q}_{BA}$, brachial artery blood flow; SR, shear rate. Data were compared with a mixed effects linear model including fixed factors for group, time, and stage of LBNP and random effects for subject ID.*

Figure Legends

Figure 1. Experimental protocol schematic.

Abbreviations: BA MBV, brachial artery mean blood velocity; LBNP, lower body negative pressure.

Figure 2. Haemodynamic and sympathetic vasomotor activity from a single representative subject during LBNP before (Pre; filled circles) and following (Post; open circles) intermittent hypercapnic hypoxia.

Presented data includes lower body negative pressure (LBNP), beat-by-beat mean arterial pressure (MAP), brachial artery blood flow ($\dot{Q}_{BA}$), and the integrated sympathetic neurogram. Shown data is the final minute of each three-minute stage. Abbreviations: a.u., arbitrary units.

Figure 3. Change (Post – Pre) in cardiorespiratory, haemodynamic, and sympathetic vasomotor outflow variables averaged across all stages of LBNP for control and IH.

*Data are means ± SEM illustrating the main effect of condition (IH vs Control) across baseline and all stages of LBNP. Statistical testing by mixed effect linear model with fixed factors for group, time, and stage of LBNP, and random factors for subject ID. *, $P < 0.05$ represents a significant treatment effect between IH and control. Abbreviations: $\dot{V}_I$, minute ventilation; MAP, mean arterial pressure, MSNA BF, muscle sympathetic nerve activity burst frequency; $\dot{Q}_{BA}$, brachial artery blood flow; SR, shear rate; FVC, forearm vascular conductance.*

Figure 4. Sympathetic neurovascular transduction measured by forearm vascular conductance (FVC) before and after control (A) or IH (B), and the change in FVC transduction slope (C) and intercept (D).

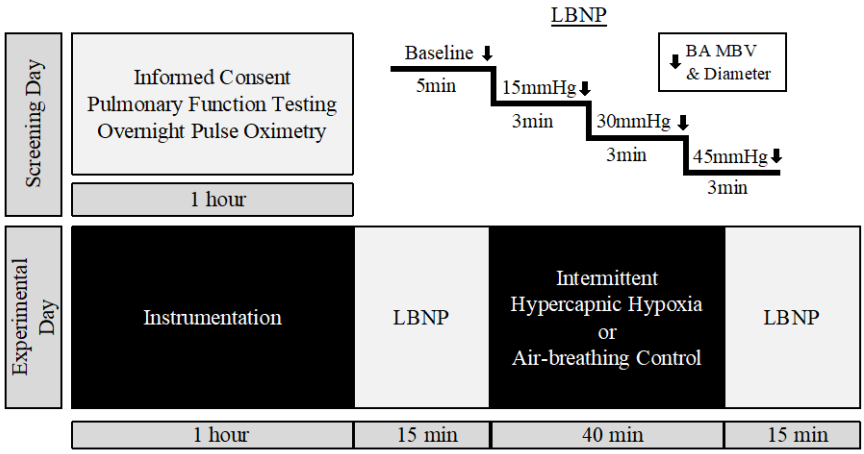
The mean coefficient of determination (r^2) for the relationship between the change in FVC and MSNA BF were 0.41 ± 0.10 and 0.53 ± 0.10 for the IH group before (pre) and following (post) IH, respectively. In the control group they were 0.20 ± 0.06 for pre and 0.37 ± 0.12 for post. In top panels (A and B), data are derived from measurements made during the four stages of LBNP (0, -15, -30, -45), pre is represented in white circles and dashed lines while post is shown in black circles and solid lines. In all panels, data are means ± SEM. In panels, C and D, data points are individual differences in FVC transduction slope and intercept. Statistical comparisons made by independent samples t-test. Control, N = 7; IH, N = 9.

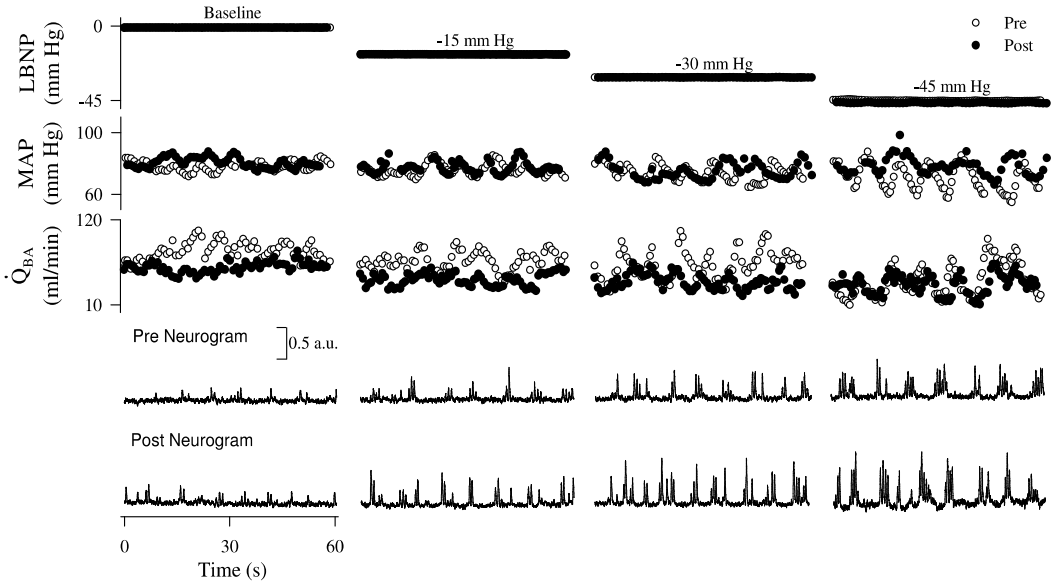
Figure 5. Sympathetic neurovascular transduction measured by diastolic blood pressure (DBP) before and after control (A) or IH (B), and the change in DBP transduction slope (C) and intercept (D).

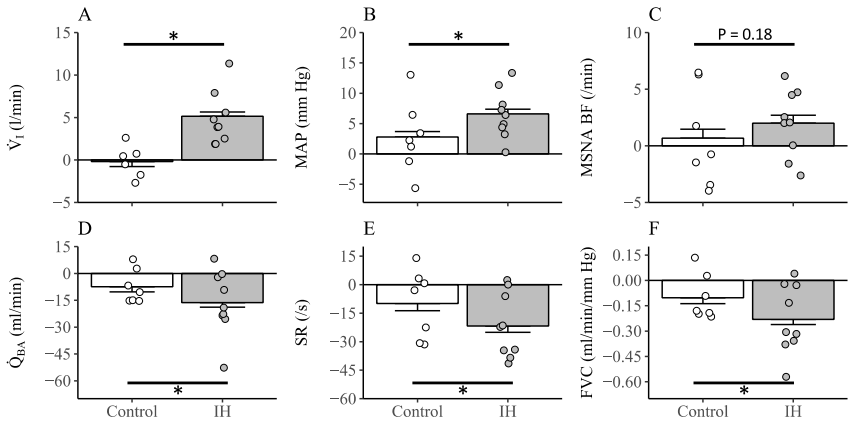
The mean coefficient of determination (r^2) for the relationship between the change in DBP and MSNA BF were 0.48 ± 0.10 and 0.51 ± 0.11 for the IH group before (pre) and following (post) IH, respectively. In the control group they were 0.32 ± 0.10 for pre and 0.59 ± 0.10 for post. In top panels (A and B), data are derived from measurements made during the four stages of LBNP (0, -15, -30, -45), pre is represented in white circles and dashed lines while post is shown in black circles and solid lines. In all panels, data are means ± SEM. In

panels C and D, data points are individual differences in DBP transduction slope and intercept. Statistical comparisons made by independent samples t-test. Control, N = 7; IH, N = 9

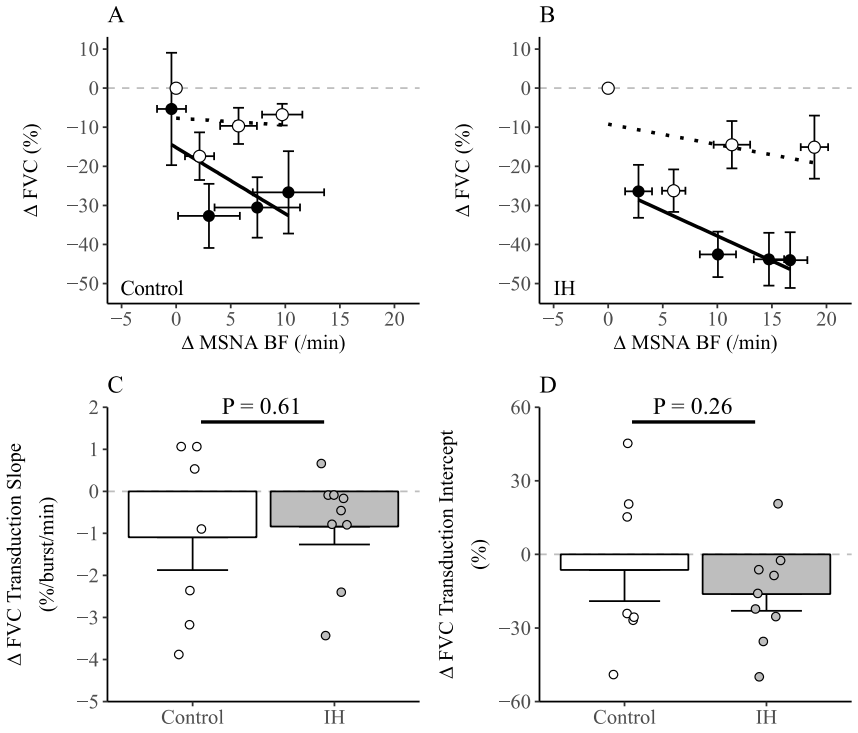
Figure 1.


Screening Day
Informed Consent
Pulmonary Function Testing
Overnight Pulse Oximetry
1 hour
LBNP
Baseline
5min
15mmHg
3min
30mmHg
3min
45mmHg
3min
BA MBV
& Diameter
Experimental Day
Instrumentation
LBNP
Intermittent
Hypercapnic Hypoxia
or
Air-breathing Control
LBNP
1 hour
15 min
40 min
15 min


Baseline
-15 mm Hg
-30 mm Hg
-45 mm Hg
Pre
Post
LBNP (mm Hg)
0
-45
MAP (mm Hg)
100
60
$\dot{Q}_{BA}$ (ml/min)
120
10
Pre Neurogram
0.5 a.u.
Post Neurogram
0
30
60
Time (s)

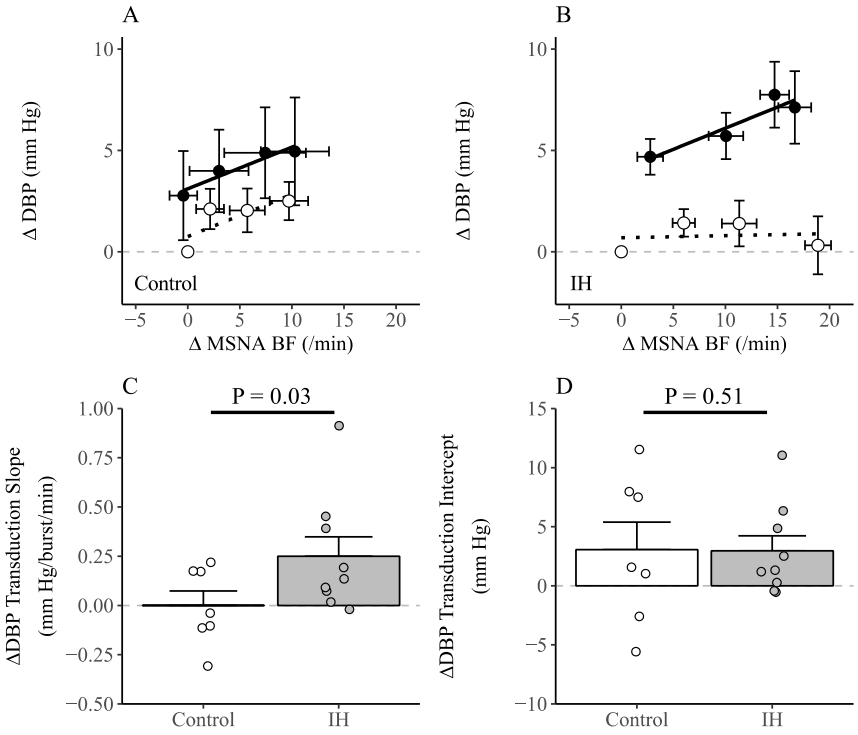


between IH and control. Abbreviations: $\dot{V}_I$, minute ventilation; MAP, mean arterial pressure, MSNA BF, muscle sympathetic nerve activity burst frequency; $\dot{Q}_{BA}$, brachial artery blood flow; SR, shear rate; FVC, forearm vascular conductance.



transduction slope (C) and intercept (D).

The mean coefficient of determination (r^2) for the relationship between the change in FVC and MSNA BF were 0.41 ± 0.10 and 0.53 ± 0.10 for the IH group before (pre) and following (post) IH, respectively. In the control group they were 0.20 ± 0.06 for pre and 0.37 ± 0.12 for post. In top panels (A and B), data are derived from measurements made during the four stages of LBNP (0, -15, -30, -45), pre is represented in white circles and dashed lines while post is shown in black circles and solid lines. In all panels, data are means ± SEM. In panels, C and D, data points are individual differences in FVC transduction slope and intercept. Control, N = 7; IH, N = 9.



(C) and intercept (D).

The mean coefficient of determination (r^2) for the relationship between the change in DBP and MSNA BF were 0.48 ± 0.10 and 0.51 ± 0.11 for the IH group before (pre) and following (post) IH, respectively. In the control group they were 0.32 ± 0.10 for pre and 0.59 ± 0.10 for post. In top panels (A and B), data are derived from measurements made during the four stages of LBNP (0, -15, -30, -45), pre is represented in white circles and dashed lines while post is shown in black circles and solid lines. In all panels, data are means ± SEM. In panels C and D, data points are individual differences in DBP transduction slope and intercept. Control, N = 7; IH, N = 9.