Intermittent Hypoxia Increases Arterial Blood Pressure in Humans Through a Renin-Angiotensin System–Dependent Mechanism

Glen E. Foster, Patrick J. Hanly, Sofia B. Ahmed, Andrew E. Beaudin, Vincent Pialoux, Marc J. Poulin

Abstract—Intermittent hypoxia (IH) is believed to contribute to the pathogenesis of hypertension in obstructive sleep apnea through mechanisms that include activation of the renin-angiotensin system. The objective of this study was to assess the role of the type I angiotensin II receptor in mediating an increase in arterial pressure associated with a single 6-hour IH exposure. Using a double-blind, placebo-controlled, randomized, crossover study design, we exposed 9 healthy male subjects to sham IH, IH with placebo medication, and IH with the type I angiotensin II receptor antagonist losartan. We measured blood pressure, cerebral blood flow, and ventilation at baseline and after exposure to 6 hours of IH. An acute isocapnic hypoxia experimental protocol was conducted immediately before and after exposure to IH. IH with placebo increased resting mean arterial pressure by 7.9±1.6 mm Hg, but mean arterial pressure did not increase with sham IH (1.9±1.5 mm Hg) or with losartan IH (−0.2±2.4 mm Hg; P<0.05). Exposure to IH prevented the diurnal decrease in the cerebral blood flow response to hypoxia, independently of the renin-angiotensin system. Finally, in contrast to other models of IH, the acute hypoxic ventilatory response did not change throughout the protocol. IH increases arterial blood pressure through activation of the type I angiotensin II receptor, without a demonstrable impact on the cerebrovascular or ventilatory response to acute hypoxia. (Hypertension. 2010;56:369-377.)

Key Words: blood pressure ■ cerebrovascular circulation ■ hypoxia ■ renin ■ physiology

Patients with obstructive sleep apnea (OSA) are exposed to chronic intermittent hypoxia (IH), which is thought to be the underlying mechanism that links OSA with an increased risk of cardiovascular disease. The specific pathophysiologic mechanism whereby OSA causes hypertension has not been fully elucidated, but it has been proposed that persistent sympathoexcitation, oxidative stress, and endothelial dysfunction contribute. Central to this concept is the important interaction between the sympathetic nervous system and the renin-angiotensin system (RAS). Renin release from the kidney is tightly controlled by activity in renal sympathetic nerves. In OSA patients, sympathetic nerve activity, the plasma concentration of angiotensin II (ANG-II), and the vasoconstrictor response to ANG-II are elevated. ANG-II has potent vasoconstrictor capabilities through its action on type I ANG-II receptors (AT1Rs) located on vascular smooth muscle cells. ANG-II production can also regulate blood volume by increasing aldosterone production. The combined potential for the RAS to be stimulated by IH and its ability to regulate peripheral resistance and blood volume make it a credible pathway through which OSA can lead to the development of systemic hypertension. The role of the RAS in the IH-dependent increase in arterial blood pressure has yet to be addressed in human experiments.

The primary objective of this study was to assess the role of the AT1 receptor in mediating an increase in arterial blood pressure associated with a single 6-hour IH exposure in healthy men. The IH profile was designed to be similar to that experienced by OSA patients whereas excluding other factors that may also affect blood pressure, such as hypercapnia, sleep fragmentation, and intrathoracic pressure swings, which are commonly associated with OSA. We hypothesized that blockade of the AT1R with losartan prevents the increase in arterial blood pressure induced by IH. Secondary effects of IH may include changes in ventilatory control, cerebral autoregulation, pressor responses to hypoxia, and circulating components of the RAS. For this reason, we assessed the changes in circulating plasma renin activity (PRA) and aldosterone, and the ventilatory, cerebral blood flow, and pressor responses to hypoxia before and after 6 hours of IH. Finally, we assessed endothelin 1 in the plasma to see whether this vasoconstrictor may also be upregulated by our IH exposure.
Methods
The study protocol was approved by the institutional Conjoint Health Research Ethics Board and conformed to the Declaration of Helsinki. Based on a priori sample size calculations conducted on our primary outcome variable, changes in mean arterial pressure (MAP) in response to IH, from previously published data, \( \geq 8 \) subjects were required to provide sufficiently high power (\( \beta = 0.10 \)) for 2-sided tests with \( \alpha = 0.05 \). Allowing for potential dropouts and to account for nonadherence, we chose to study 10 healthy male volunteers who met the inclusion/exclusion criteria for the study and provided written, informed consent. An expanded methods section is available in the online Data Supplement (see http://hyper.ahajournals.org).

Experimental Protocols
Each experimental protocol is displayed in Figure 1. Each protocol was separated by 1 week minimum, occurred at the same time of day, and were randomized. The losartan and placebo protocols occurred in a double-blind, randomized design. Subjects controlled their diet and measured their blood pressure 3 times daily for 3 days before each experiment. This allowed us to monitor diurnal changes in blood pressure that may have been a result of the pharmacokinetic profile of losartan. All of the protocols involved baseline air-breathing cardiovascular, cerebrovascular, and respiratory measurements; a morning and afternoon acute isocapnic hypoxia test; a 6-hour exposure to sham IH or IH; and recovery air breathing measurements. During placebo, all of the subjects took placebo tablets for 4 consecutive days in the morning before IH exposure. In a similar manner, during the losartan protocol, subjects took 25 mg of losartan on day 1, 50 mg on day 2, and 100 mg on days 3 and 4. Twenty-four–hour sodium excretion was calculated each morning before experimentation.\(^9\) Venous blood samples were collected at the beginning and end of each experimental day and analyzed for PRA, aldosterone, and endothelin 1.

Experimental Techniques
End-tidal \( \text{PO}_2 \) (PET\( \text{O}_2 \)) and \( \text{PCO}_2 \) (PET\( \text{CO}_2 \)), blood pressure (MAP, systolic blood pressure [SBP], and diastolic blood pressure [DBP]), heart rate, ventilation, and cerebral blood flow (\( V_p \)) were obtained as the subject rested quietly for 10 minutes while breathing room air through a mouthpiece and during a 5-minute period of isocapnic euoxia followed by 6 square wave cycles of 90 seconds of isocapnic hypoxia (PET\( \text{O}_2 \)=45.0 mm Hg) and 90 seconds of isocapnic euoxia. A 90-second acute hypoxia protocol was chosen because this duration is sufficiently long to measure the ensuing cerebrovascular responses. Accurate control of end-tidal gases was achieved by dynamic end-tidal forcing.\(^9\) Data from each cycle of hypoxia and euoxia were interpolated, overlaid, and averaged together to create a single 3-minute cycle of euoxia and hypoxia.\(^9\) Blood pressure was recorded at the finger by photoplethysmography (Portapress, TPD Biomedical Instrumentation) and calibrated against measurements taken from an automated arm cuff (Dinamap, Johnson and Johnson Medical, Inc), heart rate by 3-lead ECG (Micromon, 7142B monitor, Kontron Medical), arterial oxyhemoglobin saturation (\( \text{SaO}_2 \)) by pulse oximetry (3900 Datex-Ohmeda), and mean peak cerebral blood flow velocity by transcranial Doppler ultrasonography of the middle cerebral artery (PCDOP 842, SciMed). Collection of the above variables has been described previously.\(^8\) IH consisted of 6 hours of continuous cycles of 1 minute of isocapnic hypoxia (nadir PET\( \text{O}_2 \)=45.0 mm Hg) and 1 minute of normoxia (peak PET\( \text{O}_2 \)=88.0 mm Hg). During IH, continuous measurements of PET\( \text{O}_2 \), PET\( \text{CO}_2 \), and \( \text{SaO}_2 \) were made. Sleep was not permitted during the experiments.

Statistical Analysis
All of the data are expressed as mean±SE. A 3 (condition: sham, placebo, or losartan)-by-2 (time of day: AM or PM) repeated-measures ANOVA was used to compare data statistically. When significant effects were found, post hoc comparisons were undertaken using the Sidak correction for multiple comparisons. A 2-tailed \( P \) value of \( P < 0.05 \) was considered statistically significant. Normality was assessed using the Shapiro-Wilk statistic.

Results
Subjects
Ten male subjects completed 3 experimental protocols. One subject did not comply with the medication and diet control protocol and was excluded from the study and all of the analyses. The data presented reflect the remaining 9 subjects. Subjects studied had an age of 29.9±0.6 years (mean±SE) and were free from cardiopulmonary and renal diseases, were not obese (body mass index: 24.7±0.7 kg·\( m^2 \)), and did not experience sleep-disordered breathing (respiratory disturbance index: 2.3±0.6 events·h\(^{-1} \), mean \( \text{SaO}_2 \): 94.3±0.4%). All of the subjects were normotensive (MAP: 88.9±2.5 mm Hg) and nondiabetic (fasting blood glucose level: 5.29±0.18 mmol·L\(^{-1} \)). Estimated

Figure 1. Experimental protocol. Solid arrows indicates capillary and venous blood samples; broken arrows, urine sample; AHR testing, the acute isocapnic hypoxia response testing.
24-hour sodium excretion and MAP in the morning, afternoon, and evening during the 3 days before each experimental day were not different between conditions (see supplemental results in the online Data Supplement, available at http://hyper.ahajournals.org).

**IH and Sham Exposures**

Supplemental Figure S1 (available in the online Data Supplement, at http://hyper.ahajournals.org) illustrates the PETO₂, PETCO₂, and SaO₂ evoked by exposure to isocapnic IH (placebo and losartan) and to sham IH in 3 representative subjects (A through C; see the online Data Supplement). The oxygen desaturation index and other indices of the oxygen saturation profiles are shown in Table S1 (available in the online Data Supplement at http://hyper.ahajournals.org). Placebo and losartan were significantly different compared with sham for each parameter except mean maximum SaO₂. Placebo and losartan elicited similar oxygen saturation profiles.

**Morning and Afternoon Resting Measurements**

Isocapnic euoxia MAP and DBP on placebo were significantly greater than the changes with sham or losartan (Figure 3). Fifteen minutes after the afternoon isocapnic hypoxia measurements, MAP and DBP still remained elevated by 6.6±2.1 and 8.4±2.0 mm Hg, respectively, during placebo, by 4.0±1.4 and 5.3±1.7 mm Hg on SHAM, but only by 0.4±1.2 and 2.2±1.4 mm Hg on losartan (P<0.05 compared with both placebo and sham; data measured 15 minutes after postacute hypoxia not displayed in Table). Diurnal effects for heart rate and cerebrovascular resistance were found (P<0.001) such that heart rate was less and cerebrovascular resistance was greater in the afternoon (Table). There were no differences in isocapnic euoxia PETCO₂, PETO₂, VE, SaO₂, Vp, and index of middle cerebral artery cross-sectional area between protocols (Table). The index of middle cerebral artery cross-sectional area was not different between conditions (P=0.17) or between morning and afternoon protocols (P=0.29), suggesting that Vp reflects underlying changes in middle cerebral artery blood flow.

**Acute Hypoxic Cardiovascular, Cerebrovascular, and Ventilatory Responses**

Figure 4 illustrates the group VE, MAP, and Vp responses to the morning and afternoon isocapnic hypoxic response test for placebo and losartan. Data from each hypoxic and normoxic cycle was overlaid and interpolated to produce a single normoxia and hypoxia cycle (see Figure 5). Each bout of hypoxia elicited an increase in VE, MAP, and Vp.
After 6 hours of exposure to IH with placebo, we observed a 7.9-mm Hg increase in MAP during isocapnic euoxia (Figure 3). MAP was still significantly elevated 15 minutes after the posttesting acute isocapnic hypoxia measurements (6.6 mm Hg), suggesting that the increase in MAP outlasts the IH stimulus in a manner similar to the sustained sympathoexcitation reported previously. Other human studies have found similar results by using different patterns of IH but have found milder increases in arterial blood pressure. We demonstrated recently that 4 daily 6-hour poikilocapnic IH exposures increased MAP by 3.9 mm Hg. Tamisier et al showed a 5.0-mm Hg increase in MAP after 2 weeks of IH. In their study, IH involved nocturnal exposure to 2-minute bouts of poikilocapnic hypoxia separated by 15 seconds of oxygen administration. We believe that the greater blood pressure response observed in our current study is a result of shorter periods of hypoxia (1 minute versus 2 minutes) and maintenance of isocapnia throughout the exposure, which has been associated previously with greater sympathetic activation compared with poikilocapnic IH. The mechanisms responsible for elevation of blood pressure after exposure to acute IH are likely to be different from those responsible for the development of hypertension after chronic IH. However, we propose that the mechanisms are part of a continuum that begins with enhanced sympathoexcitation and ANG-II production. Reactive oxygen species may contribute to these mechanisms and to the reduction in vasodilatory capacity. With continued exposure, inflammatory cytokines may cause remodeling of the arterial wall, thereby making it less likely that correction of hypoxemia will correct hypertension. We have demonstrated previously that exposure to a similar model of IH is capable of increasing reactive oxygen species production and reducing NO bioavailability.

The effect of IH on MAP while on placebo was strong, with all 9 of the subjects displaying an increase in MAP (Figure 3). AT1R blockade with losartan abolished the

Discussion

We have shown that the AT1R plays an important role in modulating the blood pressure response to isocapnic IH. To the extent that our experimental model mimics the nocturnal hypoxemia experienced by patients with OSA, these findings, which have not been demonstrated previously in humans, may have important implications for the pathogenesis of hypertension in OSA. Specifically, we demonstrated that a single 6-hour exposure to isocapnic IH induced a significant increase in SBP, DBP, and MAP that outlasts the IH stimulus. Second, blockade of the AT1R with losartan prevented the increase in blood pressure associated with exposure to IH. Third, we observed no significant changes in the cerebral blood flow and ventilatory response to hypoxia because of AT1R blockade. Finally, we did not observe an increase in endothelin 1 production. The results from this study have substantial implications for understanding an increase in blood pressure after exposure to IH and may be similar to the hypertension experienced by OSA patients.

Growing evidence suggests that IH is responsible for the known association between OSA and hypertension. Our results support this hypothesis by demonstrating that IH can alter blood pressure regulation and add to the current literature by providing a potential mechanistic pathway in humans. After 6 hours of exposure to IH with placebo, we observed a

Table. Resting Hematologic Data and Isocapnic Euoxia Ventilatory, Cerebrovascular, and Cardiovascular Data

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sham</th>
<th>Placebo</th>
<th>Losartan</th>
</tr>
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<tbody>
<tr>
<td>PETO2, mm Hg</td>
<td>38.7±0.7</td>
<td>39.6±0.5</td>
<td>38.6±0.7</td>
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<td>PETCO2, mm Hg</td>
<td>87.8±0.1</td>
<td>88.0±0.1</td>
<td>87.9±0.1</td>
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<td>VE, L min⁻¹</td>
<td>13.1±1.1</td>
<td>15.5±1.0</td>
<td>13.9±0.8</td>
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<td>SaO2, %</td>
<td>95.2±0.3</td>
<td>95.5±0.2</td>
<td>95.2±0.2</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>66.0±3.2</td>
<td>72.0±2.3</td>
<td>71.3±2.7</td>
</tr>
<tr>
<td>(V_{E}p), cm·s⁻¹</td>
<td>51.0±3.0</td>
<td>54.6±3.9</td>
<td>51.3±2.9</td>
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<tr>
<td>P, arbitrary units</td>
<td>3.0±0.3</td>
<td>3.3±0.2</td>
<td>3.3±0.2</td>
</tr>
<tr>
<td>CVR, mm Hg·cm·s⁻¹</td>
<td>1.63±0.09</td>
<td>1.58±0.1</td>
<td>1.66±0.08</td>
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<tr>
<td>Endothelin 1, pg·mL⁻¹</td>
<td>1.08±0.12</td>
<td>1.11±0.16</td>
<td>0.92±0.17</td>
</tr>
<tr>
<td>Glucose, mmol·L⁻¹</td>
<td>6.1±0.4</td>
<td>6.3±0.3</td>
<td>6.4±0.3</td>
</tr>
<tr>
<td>Aldosterone/PRRA, pmol·s⁻¹·ng⁻¹</td>
<td>595±127</td>
<td>550±88</td>
<td>213±43</td>
</tr>
</tbody>
</table>

\(V_{E}\) indicates minute ventilation; SaO2, arterial oxyhemoglobin saturation; \(P_{\text{et}}\), index of middle cerebral artery cross-sectional area; CVR, cerebrovascular resistance.

*P<0.001 vs AM.

†P<0.05 vs AM.

‡P<0.001 vs sham and placebo.
crease in MAP in 6 of 9 subjects and attenuated the response in the remaining 3 subjects, suggesting an important link between hypoxia and increased RAS activity. Interestingly, whereas all of the subjects had the anticipated increase in PRA in response to losartan,16 this effect was blunted in 2 of the 3 subjects who also did not demonstrate complete correction of the IH-mediated increase in blood pressure with losartan. This finding raises the possibility that AT1R blockade was incomplete in these individuals, as has been shown in other studies of healthy humans.17 Involvement of other mechanisms can also be considered, such as direct sympathetic activation of vascular smooth muscle (catecholamine release) or increased production of endothelin 1, another potent vasoconstrictor thought to be involved in the pathogenesis of hypertension in OSA (although we did not observe increases in endothelin 1 production; Table).18 The release of catecholamines into the circulation may contribute to increases in blood pressure in the short term, although its contribution is likely minimal, because the effect of AT1R blockade appeared to negate any blood pressure response to IH. In the long term, the contribution of catecholamines is likely minimal because of the downregulation of adrenergic receptors, as demonstrated previously.19

Animal models have shown that chronic IH can increase resting MAP. Fletcher and et al20 have extensively studied the effects of IH on the development of hypertension and the role
of the sympathetic nervous system, peripheral chemoreceptors, and the RAS in the blood pressure response. They reported a 13-mm Hg increase in resting MAP after 35 days of IH in rats, and subsequently prevented the increase in MAP by carotid body denervation, chemical sympathectomy, adrenal demedullation, and renal artery denervation. They also showed that blockade of the AT1R with losartan prevented the rise in MAP after chronic IH and that RAS suppression prevented the increase in MAP. This work strongly suggests that hypertension induced by IH is a result of stimulation of the RAS through a pathway involving the carotid body and the sympathetic nervous system. Our data support a similar mechanism for the increase in arterial pressure that we observed after a single 6-hour exposure to IH in humans.

Diurnal variations in PRA and aldosterone are well recognized. Typically, aldosterone and PRA reach a peak in the early morning hours (6:00 AM) and reach a nadir in the evening (6:00 to 11:00 PM). We also found that aldosterone and PRA declined throughout the day (Figure 2; AM versus PM). This raises the possibility that the observed increase in blood pressure would have been greater if exposure to IH had occurred during sleep, when aldosterone and PRA are higher. However, exposure of humans to IH during sleep can be accompanied by factors such as sleep fragmentation and central apnea, which may complicate interpretation of the impact of IH on outcome variables, such as blood pressure. Although these factors may be important in the hypertension observed in OSA, there is little evidence to support this. Nonetheless, we elected to use a protocol that was void of these potential complicating factors. Aldosterone escape is unlikely to have occurred in our experimental design, but we were unable to assess this because of the short nature of exposure. If aldosterone escape did occur, it would have been expected to increase salt and water retention, therefore increasing blood pressure. This would have reduced the magnitude of our findings. As a result, we do not believe that aldosterone escape contributed to our results. It may be expected to contribute to resistant hypertension in patients with OSA who are undergoing long-term angiotensin receptor blockade.

AT1Rs are found not only in the vasculature but also in other organs, such as the liver, adrenals, brain, lung, kidney,
and heart. Within the brain, ANG-II is synthesized independently from peripheral sources and acts on AT_1R in the hypothalamus and brainstem. In the brain, AT_1Rs can modulate blood pressure, drinking behavior, sodium intake, natriuresis, and vasopressin release. There are conflicting data regarding whether or not losartan crosses the blood-brain barrier. Consequently, we cannot rule out the possibility that the effect of IH on blood pressure regulation is partly attributed to the central effects of ANG-II. Furthermore, it may be that other antihypertensive medications (ie, \( \beta \)-blockers and angiotensin-converting enzyme inhibitors) may have the same effect. However, previous data suggest that there is a functional downregulation of both \( \alpha \) - and \( \beta \)-adrenergic vascular receptors, making direct sympathetic activation an unlikely pathway. An angiotensin-converting enzyme inhibitor would be expected to prevent the IH-dependent increase in blood pressure, because it is part of the same RAS pathway. Regardless, the AT_1R appears to be an important component of the IH-dependent blood pressure response. Losartan is a specific antagonist of the AT_1R (both the “a” and “b” isoforms) and has no effect on type II ANG-II receptors.

Our experimental model was designed to mimic the pattern of IH experienced by patients with moderately severe OSA. Moderately severe OSA is defined as an apnea-hypopnea index of 15 to 30 events per hour of sleep. In our model of IH, we induced a 15% reduction in \( \text{SaO}_2 \) over 60 seconds, followed by 60 seconds of reoxygenation without concomitant changes in \( \text{PCO}_2 \), which resulted in an oxygen desaturation index of 30 per hour. Although experimental models of IH appear to elicit similar physiological responses to those seen in OSA patients, models in humans and others in rats are limited as facsimiles of OSA by the absence of accompanying apnea, sleep fragmentation, and exposure to hypercapnia, making extrapolation of experimental findings in healthy subjects to patients with OSA less reliable. However, using a model of IH similar to ours is advantageous because it allows us to study the direct effects of IH on the
cardiovascular system. Our model of IH has 2 major advantages compared with other human models that have been described.8,13 First, our model yields an oxygen desaturation index and a mean SaO₂ of similar severity to that seen in humans with moderate-to-severe OSA. Second, we have prevented the fall in PETCO₂ that accompanies most models of IH. Consequently, we believe that our experimental human model of IH replicates the pattern of hypoxemia associated with OSA more effectively than previous models and anticipate that it will be an effective experimental paradigm for future studies.

OSA is a common cause for treatment-resistant arterial hypertension.32 Many OSA patients are on RAS inhibitors, suggesting that RAS activation may not be a crucial mechanism mediating hypertension in this subset of patients. Several potential mechanisms may explain this discrepancy between our results and that of the clinical population. First, the duration of exposure to hypoxia was brief in our model in contrast to OSA patients who often experience hypoxia for years before diagnosis and treatment. Second, we studied healthy, young subjects, whereas OSA is often diagnosed in older patients with comorbidities. These differences provide the opportunity for vascular remodeling in OSA patients who would not occur in our model of IH. Notwithstanding these differences, OSA patients with resistant hypertension are a highly selected group who has several underlying mechanisms for their hypertension that are independent of OSA. It is quite possible that a significant proportion of OSA patients do develop hypertension that is responsive to RAS inhibitors.

**Perspectives**

In summary, we have demonstrated a significant increase in arterial pressure after exposure to isocapnic IH in healthy humans. The increase in blood pressure is abolished by blockade of the AT₁R, suggesting an important role for the RAS in the pathophysiology of hypertension associated with IH. Although the development of hypertension in OSA patients is likely multifactorial, we believe that our results indicate that the RAS is involved, at least initially, and provide strong mechanistic evidence for a causal relationship between exposure to IH and an increase in blood pressure.

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**Disclosures**

None.

**References**


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SUPPLEMENTAL MATERIAL

Intermittent hypoxia increases arterial blood pressure in humans through a renin-angiotensin-system dependent mechanism.

Supplemental Methods

Subjects

We studied ten healthy male volunteers who met the inclusion/exclusion criteria for the study. Subjects were included if they were between 19 and 45 years, were free from cardiovascular and respiratory disease and, at the time of study, had resided in Calgary (elevation 1,100m) for at least one year. Subjects were excluded if they had a prior diagnosis or history of cardio-pulmonary and renal disease (urinary protein excretion > 150mg per 24hrs; estimated glomerular filtration rate < 60 ml min\(^{-1}\) 1.73m\(^2\)), obesity (BMI>35 kg m\(^{-2}\)), sleep apnea (RDI>10 events hr\(^{-1}\), confirmed by home cardio-pulmonary monitoring during sleep), smoking within the past year, hypertension (systolic > 140 mmHg; diastolic > 90 mmHg), and a high fasting blood glucose level (≥7 mmol) during the initial screening visit. These criteria helped to confirm that all subjects were healthy and did not have medical conditions that could alter cardio-respiratory function and RAS activity. Females were not studied because previous research has shown that female rats may be protected from the hypertension and tachycardia induced by IH.\(^1\) Furthermore, fluctuations in estrogen throughout the menstrual cycle may affect the RAS response to IH.\(^2, 3\)

Experimental Protocols

Subjects attended the Laboratory of Human Cerebrovascular Physiology at the University of Calgary on four separate occasions to complete one screening session and three experimental protocols described below (in print Figure 1). Each protocol was separated by a minimum of one week, occurred at the same time of day, and were randomized in a single blind fashion. The LOSARTAN and PLACEBO protocols occurred in a double blind, randomized, crossover design. For each of the three experimental protocols subjects were asked to control their diet. On each testing day and the three days leading up to the experimental session, subjects were instructed to consume the same foods at similar times of day. This information was recorded on a dietary diary. On the morning of each experimental session, subjects provided a urine sample to estimate sodium balance using the method described by Tanaka \textit{et al.}\(^4\) to ensure a similar salt state on each study day. Subjects were also provided with an automatic blood pressure monitoring device (model HEM-775, Omron Healthcare, Burlington, ON, Canada) and instructed to measure their blood pressure three times a day (morning, afternoon, and evening) over the three days leading up to each experimental day. Subjects were asked to make three blood pressure measures at each time of day, and the average of these three values was taken as their resting blood pressure.

Screening Session

During the screening session subjects were able to view the experimental set-up, ask the investigators questions, and provide written informed consent. Subjects completed a short questionnaire to assess their inclusion/exclusion criteria as described above. In addition, subjects provided a fasting venous blood sample taken from the antecubital vein to assess the blood glucose level. Further, 5 mL of venous blood was collected into one serum separator tube, centrifuged and the serum aliquoted into 1.5 mL microcentrifuge tubes and analyzed for serum creatinine for estimation of glomerular filtration rate.\(^5\) Proteinuria was estimated by urinalysis. Subject characteristics were also measured during this time (i.e. age, height, weight). Finally, all subjects were instructed how to set-up and perform an unattended, continuous, overnight, home cardiopulmonary monitoring study (Remmers Sleep Recorder Model 4.2, Saga Tech Electronic, Calgary, AB, Canada).\(^6\) This device consists of an oximeter to record oxyhemoglobin saturation and heart rate variability, a pressure transducer to record nasal airflow, a microphone to record snoring, and a body position sensor. The oximeter provides the data for an automated scoring algorithm, which calculates the respiratory disturbance index (RDI) based on the number of episodes of oxyhemoglobin desaturation greater than 4% divided by the duration of recording. The raw data were reviewed for each subject by an experienced sleep medicine physician
The Remmers recorder has been validated by comparison to attended polysomnography.6,7

**SHAM IH Protocol**

A time control study was completed to ensure that any observed changes were due to exposure to IH and not to diurnal variation. Subjects reported to the laboratory at 8:00am. The protocol began with baseline cardiovascular, cerebrovascular, and respiratory measurements. Following baseline measurements, the cardiovascular, cerebrovascular, and respiratory responses to acute isocapnic hypoxia were conducted before and after exposure to 6 hours of *SHAM IH* or *normoxia*. The experimental set-up was identical to that used to administer *IH*. Fifteen minutes following the afternoon acute hypoxia measurements, a period of recovery measurements was made. A typical experimental day lasted approximately 8 – 8.5 hours.

**PLACEBO IH Protocol**

PLACEBO was identical to SHAM except that placebo tablets were taken for 4 consecutive days with the last placebo tablet being taken approximately one hour prior to the beginning of baseline measurements. In addition, *IH* was administered rather than *SHAM IH*.

**LOSARTAN IH Protocol**

LOSARTAN was identical to PLACEBO except that subjects were pre-treated with once daily doses of the AT1R antagonist, losartan, for 4 consecutive days. The dose of losartan was 25mg on day 1, 50mg on day 2, and 100mg on days 3 and 4. The last tablet was taken approximately one hour prior to baseline measurements on the experimental day.

**Experimental Techniques**

**Baseline and Recovery Measurements**

Resting measurements of PETO2, PETCO2, blood pressure (MAP, SBP, DBP), heart rate, and indices of cerebral blood flow (\(\bar{V_P}, \bar{V_{IWM}}, \bar{P}, \bar{P_{IWM}}\)) were obtained as the subject rested quietly and comfortably for 10 minutes while breathing room air through a mouthpiece. These measures were made prior to the morning acute hypoxia measurements and again 15 minutes following the afternoon acute hypoxia measurements. Respired gases were sampled via a fine catheter at the mouth. The gas was sampled continuously at a rate of 20 ml/min and analyzed for \(\text{PO}_2\) and \(\text{PCO}_2\) by mass spectrometry (AMIS 2000, Innovision, Odense, Denmark). A computer was used to acquire values for \(\text{PO}_2\) and \(\text{PCO}_2\) every 10 ms and the values for PETO2 and PETCO2 were determined and recorded for each breath using dedicated software (Chamber V2.43, University Laboratory of Physiology, Oxford, UK). All cardiovascular parameters were acquired every 10 ms and the values for each determined beat-by-beat by specifically designed computer software (BreatheM V2.40, University Laboratory of Physiology, Oxford, UK). Baseline and recovery values were taken as a 3-minute average of breath-by-breath and beat-by-beat data during the final 3 minutes of the 10-minute baseline period.

**Morning and Afternoon Acute Isocapnic Hypoxia Measurements**

Following baseline measurements, the subject continued breathing through a mouthpiece with the nose occluded by a nose clip. The protocol began with a 5-minute period of isocapnic euoxia (PETO2 = 88.0 mmHg and PETCO2 = +1.5 mmHg above rest) followed by six cycles comprised of 90 seconds of isocapnic hypoxia (PETO2 = 45.0 mmHg and PETCO2 = +1.5 mmHg above rest) and 90 seconds of isocapnic euoxia. Respiratory volumes and flow were obtained by using a pneumotachograph and differential pressure transducer (RSS 100-HR, Hans Rudolf Inc., Kansas City, MO, USA). Respiratory flow direction and timing were measured by using a turbine volume transducer (VMM-400, Interface Associates, CA, USA).
Accurate control of end-tidal gases was achieved by using the technique of dynamic end-tidal forcing (BreatheM V2.40, University Laboratory of Physiology, Oxford, UK) as previously described. All measurements described above were also recorded throughout acute isocapnic hypoxia. Data from each cycle of hypoxia and euoxia were interpolated at a 1s interval, overlaid, and averaged together to create a single 3-minute cycle of euoxia and hypoxia using specifically designed software created in Matlab (V7.4.0.287, MathWorks, Inc., MA, USA).

Intermittent Hypoxia Exposure

IH consisted of 6 hours of continuous cycles of one-minute of hypoxia (nadir PETO$_2$ = 45.0 mmHg) and one-minute of normoxia (peak PETO$_2$ = 88.0 mmHg). IH was delivered by using a purpose-built normobaric chamber. Periods of normoxia were constituted by delivering 100% oxygen to the subject’s inspirate through a facemask (mirage NV Series 2, Resmed, New South Wales, Australia) connected to a two-way non-rebreathing valve (2600 Series, Hans Rudolph, Kansas, USA) and a 25cm long section of wide bore tubing. During periods of normoxia, oxygen flowed through the inspired circuit at a rate that provided a PETO$_2$ = 88.0 mmHg. PETCO$_2$ was also controlled during IH by adding 100% CO$_2$ to the subject’s inspirate at a flow rate that maintained PETCO$_2$ constant. Computer-controlled gas solenoid valves were used to turn the flow of oxygen and carbon dioxide on and off at 60 second intervals. During exposure to IH, respired gas was sampled from a nasal cannula and analyzed by a dual oxygen and carbon dioxide analyzer (NormocapOxy, Datex-Ohmeda, Louisville, CO, USA) for PO$_2$ and PCO$_2$. These values were acquired every 10ms by a computer, and PETO$_2$ and PETCO$_2$ were identified and recorded for each breath using a computer and dedicated software (LabChart V6.13 and Powerlab/16SP, ADInstruments, Colorado Springs, CO, USA). SaO$_2$ was recorded continuously throughout the exposure by means of an ear probe and pulse oximetry (3900, Datex-Ohmeda, Louisville, CO, USA). The same set-up was used for sham IH except that gas in the chamber was kept at a normoxic level (i.e., PETO$_2$ = 88 mmHg), and compressed air, instead of 100% oxygen, was administered through the wide bore tubing at 60-second intervals.

RAS measurements

Blood samples taken for the measurement of plasma renin activity (PRA) and aldosterone were drawn in the morning before the acute isocapnic hypoxia measurements and repeated in the afternoon after the acute isocapnic hypoxia measurements. The details for each collection are outlined below:

**Plasma Renin Activity (PRA):** Venous blood samples were collected into two 6.0 mL EDTA coated tubes. Collected samples were immediately centrifuged and the separated plasma was aliquoted into 1.5 mL flat top microcentrifuge tubes. The separated plasma was stored at -80°C until later assayed. PRA was assayed by radioimmunoassay techniques (RIA) and the PRA assessed by measuring the amounts of angiotensin I generated per hour.

**Aldosterone:** Venous blood was collected into two 5.0 mL serum separator tubes. The sample was centrifuged 20 minutes after its collection, and serum was aliquoted into 1.5 mL flat top microcentrifuge tubes. The separated serum was stored at -80°C until later assayed. Aldosterone was assayed by RIA techniques.

Plasma endothelin-1 measurements

Venous blood samples were collected into two 6.0 mL EDTA coated tubes. Collected samples were immediately centrifuged and the separated plasma was aliquoted into 1.5 mL flat top microcentrifuge tubes. The separated plasma was stored at -80°C until later assayed. Concentrations of plasma endothelin-1 was determined using an ELISA kit from R&D Systems.
(R&D Systems, Inc. Minneapolis, MN, USA) and in following the procedures provided by the manufacturer.

Capillary Blood Samples

Capillary (i.e., arterialized) blood samples (200 µl) were taken in the morning immediately before the acute isocapnic hypoxia measurements and repeated in the afternoon following the acute isocapnic hypoxia measurements. These samples were taken from a small puncture on the finger and analyzed for acid-base status and glucose.
Supplemental Results.
Estimated 24-hour sodium excretion: SHAM = 126 ± 7; PLACEBO = 116 ± 6; LOSARTAN = 123 ± 10 mmol day⁻¹; p=0.49

MAP in the morning, afternoon and evening during the three days prior to each experimental day:
SHAM: Day-3 = 87 ± 2; Day-2 = 88 ± 2; Day-1 = 88 ± 2 mmHg; PLACEBO: Day-3 = 88 ± 2; Day-2 = 87 ± 2; Day-1 = 87 ± 2 mmHg; LOSARTAN: Day-3 = 87 ± 2; Day-2 = 86 ± 2; Day-1 = 85 ± 2 mmHg; p=0.23

Arterialized blood pH: 7.42 ± 0.01 for SHAM and PLACEBO and 7.42 ± 0.00 for LOSARTAN (p=0.27).

Bicarbonate ion concentration: 24.7 ± 0.2 for SHAM, 24.6 ± 0.3 for PLACEBO and 24.7 ± 0.2 mmol L⁻¹ for LOSARTAN (p=0.89).
Supplemental References


**Supplemental Tables**

Table S1. Indices of hypoxemia and reoxygenation.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SHAM</th>
<th>PLACEBO</th>
<th>LOSARTAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODI, desaturations hr$^{-1}$</td>
<td>0.0 ± 0.0</td>
<td>28.8 ± 0.2*</td>
<td>28.7 ± 0.1*</td>
</tr>
<tr>
<td>% time SaO$_2$&lt;90%</td>
<td>0.6 ± 0.1</td>
<td>23.1 ± 1.9*</td>
<td>25.7 ± 1.0*</td>
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<td>% time SaO$_2$&lt;85%</td>
<td>0.0 ± 0.0</td>
<td>2.2 ± 0.7†</td>
<td>3.6 ± 1.2†</td>
</tr>
<tr>
<td>% time SaO$_2$&lt;80%</td>
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<td>0.1 ± 0.1</td>
<td>0.2 ± 0.2†</td>
</tr>
<tr>
<td>Mean Minimum SaO$_2$, %</td>
<td>94.5 ± 0.4</td>
<td>86.0 ± 0.4*</td>
<td>85.2 ± 0.3*</td>
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<tr>
<td>Mean Maximum SaO$_2$, %</td>
<td>96.4 ± 0.1</td>
<td>95.8 ± 0.2</td>
<td>96.0 ± 0.2</td>
</tr>
<tr>
<td>Mean SaO$_2$, %</td>
<td>95.7 ± 0.2</td>
<td>92.3 ± 0.3*</td>
<td>92.2 ± 0.2*</td>
</tr>
</tbody>
</table>

* p<0.001; † p<0.05 compared to SHAM; Definition of abbreviations: ODI = oxygen desaturation index (number of times SaO$_2$ decreases by greater than or equal to 4%); Mean minimum SaO$_2$ = the mean nadir for all SaO$_2$ desaturations; Mean maximum SaO$_2$ = the mean peak for all SaO$_2$ resaturations.
Supplemental Figures.
Figure S1A.
Figure S1B.
Figure S1C. Representative data traces from three different subjects (A-C) during exposure to SHAM and intermittent hypoxia (PLACEBO and LOSARTAN).

Footnote: The first and last 20 minutes of the 6-hour exposure are expanded. Breaks in the data occur during lunch and washroom breaks and if the pulse oximeter signal was lost. Definition of abbreviations: PETO$_2$ = end-tidal partial pressure of oxygen; PETCO$_2$ = end-tidal partial pressure of carbon dioxide; SaO$_2$ = arterial oxyhemoglobin saturation.