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Fourteen nights of intermittent hypoxia elevate daytime blood pressure and sympathetic activity in healthy humans

Tamisier: Sympathetic blood pressure elevation with CIH

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Abstract :

Background Obstructive sleep apnea syndrome (OSAS) causes nocturnal chronic intermittent hypoxia (IH) that contributes to excess cardiovascular morbidity. To explore the consequences of IH, we used our recently developed model of nocturnal IH in healthy humans to characterize the profile of this blood pressure increase, to determine if it is sustained, and to explore potential physiologic mechanisms. **Methods and Results** We performed 24 h ambulatory monitoring of blood pressure in 12 healthy subjects before and after two weeks of IH exposure. We also assessed systemic hemodynamics, muscle sympathetic nerve activity (MSNA), ischemic calf blood flow responses, and baroreflex gain. We obtained blood samples for inflammatory markers before, during, and after exposure. IH significantly increased daytime ambulatory blood pressure after a single night of exposure (3 mmHg for mean and diastolic) and further increased daytime pressures after two weeks of exposure (8 mmHg systolic and 5 mmHg diastolic). MSNA increased across the exposure (17.2 ± 5.1 vs. 21.7±7.3 burst/min $p<0.01$) and baroreflex control of sympathetic outflow declined from -965.3 ± 375.1 to -598.4 ± 162.6 $AIU.min^{-1}.mmHg^{-1}$ ($p<0.01$). There were no evident changes in either vascular reactivity or systemic inflammatory markers. **Conclusion** These data are the first to show that the arterial pressure rise is sustained throughout the waking hours beyond the acute phase immediately after exposure. Moreover, they may suggest that sympathoactivation induced by IH likely contributes to blood pressure elevation and may derive from lesser baroreflex inhibition. These mechanisms may reflect those underlying the blood pressure elevation associated with OSAS.

Keywords: Sleep apnea; Hypertension; Pathophysiology; atherosclerosis

Obstructive sleep apnea syndrome (OSAS) is highly prevalent in western countries with an age-related prevalence ranging from 5 to 15% up to age 60 [1]. The primary health concern for OSAS is increased cardiovascular morbidity. OSAS is independently associated with hypertension [2] and confers an increased risk for fatal and non fatal cardiovascular complications [3-5]. Hence, OSAS is a significant health concern for Western countries [6, 7].

Considering these epidemiologic associations, the pathophysiologic link between OSAS and cardiovascular disease must be defined to consider potential avenues for treatment. Several mechanisms have been proposed that could link OSAS to cardiovascular disease: high vascular sympathetic tone exhibited by OSAS patients may result in elevated systemic resistance and hence elevated pressure [8-11]; impaired arterial vasodilatory capacity [12-14] may contribute to elevation of blood pressure and lead to vascular disease; and sustained inflammation may lead to endothelial damage [15] and contribute to atherosclerosis. Although this is a non exhaustive list we propose to focus on these in the present study. A primary stimulus for these alterations in OSAS is nocturnal exposure to chronic intermittent hypoxia (CIH). Animal models of CIH alone [16] or CIH with the other stimuli that characterize OSAS (i.e., respiratory effort, asphyxia, arousal from sleep) [17] show elevated blood pressure during the non-CIH portion of the day. Data derived from the former model suggest that the blood pressure elevation results from sympathetic activation [18]. This may require an intact chemoreflex loop [19, 20], but data also suggests that following CIH arterial baroreflex gain is decreased [21, 22].

Although animal models have improved our understanding, there are specific aspects of human physiology that may not be adequately represented. Clinical research has been crucial to define the relationships between sleep apnea and cardiovascular morbidity [23, 24]. However, confounders such as obesity, age, and metabolic disorders prevent clear understanding of the pathophysiologic effects of nightly exposure to CIH. Therefore, to

explore whether the blood pressure rise is sustained beyond the acute phase immediately after IH exposure, we used our recently developed model of intermittent hypoxia in healthy humans that induces, unstable ventilation, and sleep fragmentation similar to that observed in OSAS patients [25]. Interestingly our model induced after 7 and 14 days of CIH an increase in both acute isocapnic hypoxic ventilatory response and acute hyperoxic hypercapnic ventilatory response [25]. This was also reported for isocapnic hypoxic ventilatory response after 4 days of awake IH [26]. Moreover, using the same model Foster demonstrated changes in cardiovascular and cerebrovascular responses to acute hypoxia following exposure to intermittent hypoxia [27].

Moreover, we explored three possible contributors to the IH-induced elevation in blood pressure and atherogenesis: greater sympathetic activation, lesser post occlusive-mediated vaso-relaxation, and systemic inflammation. We hypothesized that after two weeks exposure of IH arterial blood pressure would remain elevated and that this would relate to increased sympathetic activity to muscle vasculature, decreased flow-mediated dilation, and increased circulating biomarkers of inflammation.

Methods

Subjects

Twelve healthy, non-smoking, normotensive subjects (two females) aged 23 ± 6 yrs (body mass index 21.7 ± 1.9 kg/m^2) who were free of vasoactive medications completed the study. A screening history and physical exam was performed to assure each subject was free of cardiac, pulmonary, or neurologic diseases. Individuals who had journeyed to or lived at an altitude >2500 meters in the last 6 months were excluded. All women were studied during the first week following menses and tested negative for pregnancy before exposure, after one night of CIH, and at the end of the protocol. This current report encompasses data not previously reported, but acquired from subjects that completed the two week protocol previously described in Tamisier et al [25].

The sample size of 12 subjects was based on the expected changes in sympathetic activity and diastolic blood pressure measured by 24h ambulatory blood pressure measurement. We were powered at 80% to observe a 20% increase in sympathetic activity from a baseline level of 15.6 ± 5.6 burst/min [28] and a 10 mmHg (11%) increase in daytime diastolic blood pressure from a baseline of 82.8 ± 9.1 mmHg [29] with an alpha of 0.05.

All subjects provided written informed consent approved by the ethical committee at the Grenoble University Hospital Center.

Intermittent Hypoxia Exposure

This CIH exposure has been previously reported in Tamisier et al, [25].

Following a two night adaptation to the environment (room air), and a one night adaptation to an intermediate IH level, subjects were exposed to 8 hours of severe IH between the hours of 11PM and 7AM for 14 consecutive nights. The IH stimulus was intermittent poikilocapnic hypoxia, i.e. inspired oxygen fraction (FIO2) was controlled and carbon dioxide was allowed to fluctuate normally. For all nights, subjects slept with a nasal cannula and in a commercially available hypoxia tent (Hypoxico system). The tent exposed subjects to an FIO2 of 0.15 for the intermediate IH level and of 0.13 for the 14 nights of severe IH. The tent was continuously flushed and oxygen fraction in the tent was continuously monitored (Maxtec OM-25MEI) to limit rebreathing. The nasal cannula restored oxygen saturation via a 15 second bolus of oxygen every 120 seconds. Oxygen saturation was monitored continuously (BlueNight, SleepInnov Technology, Moirans, France) and oxygen boluses were adjusted between 1.5 to 2 liters per min to achieve an 85 to 95% range of oxygen desaturation-resaturation. The combination of tent and nasal cannula allowed for 30 oxygen desaturation-resaturation sequences per hour. This level and frequency of desaturation is clinically analogous to severe OSAS (Fig. 1 A).

General procedures

Diurnal blood pressure patterns as well as urine and blood samples were obtained before exposure, after one and 13 nights (i.e., two weeks) of severe CIH, and after 5 days of recovery. Cardiovascular measurements were performed before exposure and following the 14^{th} night (two weeks) of severe IH (See Fig. 1 B).

Ambulatory blood pressure was measured in the dominant arm over 24 hours at 15 min intervals (ABP monitor 90207, Spacelabs Healthcare, Issaquah WA). Blood pressure

acquisition began at 9AM and ended 24 hours later. Measurements before exposure and after recovery represent room air conditions for the entire 24 hours, whereas measurements after one and 13 nights of IH represent daytime room air and nighttime IH conditions.

Cardiovascular measurements were recorded between 8:00 am and 12:00 am after subjects had fasted overnight and with subjects in the supine position during room air breathing. Testing was performed in the following order, with >15 min separating each: resting supine measurements, modified Oxford baroreflex test, reactive hyperemia assessment, and hypoxic challenge. All data were digitized continuously at 500 Hz to computer and analyzed subsequently with signal processing software (Windaq, Dataq instruments, Akron, OH, USA).

Echocardiographic assessment was performed in the afternoon of the same day at the same time for all subjects.

Measurements

Heart period (i.e., heart rate and R-R interval) was obtained from a 3 lead electrocardiogram. Arterial pressures were measured in the right arm at 1-min intervals via an automated arm-cuff sphygmomanometer (Dinamap Model, Critikon, Tampa, FL) and on a beat-by-beat basis via digital photoplethysmography (Finapres®; Ohmeda, Louisville, CO, USA). We obtained peroneal nerve recordings via standard sympathetic microneurographic procedures with tungsten microelectrodes, as described previously [28]. Signals were filtered, amplified and full-wave rectified (Nerve Traffic Analyzer, Model 662c-3, University of Iowa, Bioengineering Dept., Iowa City, IA) and sympathetic bursts (MSNA) were identified using an algorithm developed by Hamner and colleagues [30] using Matlab software (The Mathworks Inc., Natick, MA USA). MSNA was averaged over five minute periods and

expressed as burst frequency (bursts/min), burst frequency normalized by heart beat (burst/100 hb), and burst amplitude (AUI/min).

Calf blood flow (CBF) was measured by venous occlusion plethysmography (EC6 plethysmography, Hokanson, Bellevue, WA, USA), as described previously [28]. An average of 8 to 10 flow measurements were used to compute values before and after exposure. CBF was expressed in ml/100 ml of limb tissue/minute. Calf vascular resistance (CVR) was derived from the ratio of mean arterial pressure (MAP) and CBF. These measures were also used to estimate the reactive hyperemia over 2.5 minutes in response to 5 minutes of local ischemia.

Baroreflex control of sympathetic activity was assessed from concurrent beat-by-beat arterial pressures, MSNA, and R-R intervals acquired during sequential bolus injections of 100 µg of nitroprusside followed in one minute by 150 µg of phenylephrine (modified Oxford technique). Two trials were performed separated by at least 15 minutes.

The sympatho-excitatory response to hypoxia was assessed from concurrent beat-by-beat arterial pressures, MSNA, and R-R intervals during a single hypoxic challenge. The hypoxic challenge was isocapnic to eliminate potential differences in end-tidal CO2 across subjects. The target oxygen saturation for this test was 80-85%. The average achieved was 80 ± 3 and 80 ± 2%, before and after exposure, and all subjects were between 78 and 85%. Responses to hypoxia were measured during a five minute period only after steady state oxygen saturation was reached.

Transthoracic echocardiography (HP Sonos 2500; Hewlett-Packard; Santa Clara, CA) was performed by the same investigator (JPB) before and after exposure. Subjects were studied while in the left lateral decubitus position to obtain 3 standard left ventricular apical views (apical 4-chamber, 2-chamber, and long-axis) using TM, 2D and Doppler modes with a 2.5 MHz probe. Three stable and well-defined consecutive cardiac cycles were acquired for

offline analysis of left ventricular dimensions, ejection fraction, stroke volume, and ascending aorta diameter. This analysis was performed by the same investigator with an intra observer reproducibility of 5%.

Data analysis

When possible, data were acquired on 12 subjects. Ambulatory blood pressure measurements were not obtained on one subject after 5 days of recovery. The difficulty of obtaining and maintaining an adequate sympathetic neurogram resulted in incomplete data on four subjects either before or after exposure (one without pre-exposure, three without post-exposure recordings) and in loss of data during the procedures in three more. Since this latter reduced the number of sympathetic recordings for the sympatho-excitatory response to hypoxia to only five subjects and consequently rendered paired sampling too small, data for these responses are only presented for discussion. In addition, for technical reasons, reactive hyperemia was not obtained in one subject.

Ambulatory blood pressure monitoring over 24 hours at 15 min intervals results in a surfeit of values such that comparisons of blood pressure measurements across the 4 study days on an hour by hour basis would be statistically intractable. Therefore we averaged daytime and nighttime blood pressures. The classic definition for daytime (7AM - 10PM) and nighttime (10PM - 7AM) measurements was applied to characterize diurnal blood pressure patterns.

Plasma markers for sympathetic activation (epinephrine, norepinephrine, dopamine, metanephrine and normetanephrine) were quantified by high-performance liquid chromatography. Nocturnal 8 hours urine samples were collected and acidified with acetic acid, stored at -20°C until analysis. Catecholamines (epinephrine, norepinephrine, dopamine) were measured in one milliliter of urine by high-performance liquid chromatography with

electrochemical detection (Coularray Detector, ESA Dionex, Chelmsford, USA). Plasma markers for systemic inflammatory responses (Interleukin 1 receptor antagonist (Il-1Ra), interleukin 8 (Il-8), Tumor Necrosis Factor α - (TNF-α), Monocyte Chemoattractant Protein-1 (MCP-1), adiponectin, leptin, and RANTES (RANTES is the acronym of Regulated upon Activation, Normal T-cell Expressed and Secreted)) were quantified by a commercially available multiplex beads immunoassay (R&D Systems, Minneapolis, MN) using a Bioplex 200 array reader (Bio-Rad, Hercules, CA) with Luminex xMAP Technology (Luminex, Austin, TX). Serum hs-CRP (high-sensitivity CRP) level was measured using automated immunonephelometry (Behring Nephelometer II Analyzer, Dade Behring, Germany). Soluble Intercellular Adhesion Molecule-1 (sICAM-1) was quantified by ELISA (British Biotechnology, Abingdon, United Kingdom).

Baroreflex function was estimated from the relation of systolic pressure to RR interval as described previously [31]. We assessed data from the pressure rise since it represents baroreflex afferent activation of cardiac vagal outflow. Analysis began at the lowest pressure value after the bolus injection of nitroprusside and ended whit the phenylephrine induced peak. This selection of data points often encompasses threshold and saturation regions of the sigmoid relationship. To make the analysis objective and therefore independent of investigator bias, we analyzed the data via a piecewise linear regression that required at least 5 data points to define the presence of threshold and/or saturation (if any). Arterial baroreflex control of MSNA was derived from the method developed by Studinger et al [32]. Briefly, this technique excludes all data 2 mmHg above the greatest pressure associated with a sympathetic burst and weights all cardiac cycles associated with zero sympathetic activity. Zeros above the highest pressure associated with a burst of activity are assigned a weight of 1 and zeroes between the lowest and highest pressures are assigned a weight progressively increasing from 0 to 1, proportional to the range of pressures observed. The linear gain for

baroreflex-mediated sympathoinhibition is determined by eliminating threshold and/or saturation regions via piecewise linear regression. This approach provides a slope for baroreflex gain in an objective manner.

Statistics

Differences among the multiple means for ambulatory blood pressures and plasma markers were evaluated by ANOVA corrected for multiple measures or a Friedman test when appropriate. When follow-up blood pressure data were missing, we assumed no change occurred to keep conclusions from the statistics conservative. When the ANOVA differences were detected ($p<0.05$), individual means were tested with the Bonferroni test. The Bonferroni correction for these repeated comparisons required p values < 0.008 to be considered statistically significant.

Comparison of cardiovascular measurements before exposure to those following 14 nights of severe CIH was via a paired, two-tailed t-test. For these, p values < 0.05 were considered statistically significant. Data are reported as means ± standard deviation in the text, tables and figures.

Results

Although IH was applied during sleep, increased ambulatory blood pressure occurred during the daytime and not the nighttime (Fig. 2). The 24 hour profile suggested that blood pressure increased prior to waking and then returned to normal until increasing again over the late afternoon and into the evening. This daytime pattern became evident after only one night of exposure and was more sustained after two weeks of exposure. As a result, the average daytime blood pressure was increased for both mean and diastolic pressures after one and thirteen nights of exposure (3 mmHg and 5 mmHg for both, $p<0.05$; Fig. 3). At two weeks of exposure, there was a further significant increase in systolic blood pressure (8 mmHg, $p<0.05$; Fig. 3). Thus, the rise in blood pressure was sustained throughout the daytime, beyond the acute phase of severe IH, but returned toward baseline by 5 days of recovery.

Resting heart rate was unchanged following two weeks of IH exposure (58.6 ± 6.8 [pre] vs. 59.4 ± 7.6 [post]). After exposure, resting MSNA was elevated by ~25% after the 14 day IH exposure ($p<0.01$; (Fig. 4). Moreover, resting leg blood flow was unchanged but there was a significant increase in vascular resistance (44.5 ± 16.4 [pre] vs. 50.9 ± 17.4 units [post], $p<0.05$). In addition, the initial peak leg blood flow following release of 5-minutes of ischemia was lower following two weeks of IH (31.46 ± 1.95 [pre] vs. 24.35 ± 2.07 $ml.min^{-1}.100\ g\ tissue^{-1}$ [post]; $p<0.05$). However, from 30 seconds onward after the peak reactive hyperemia, blood flow values were similar before and after two weeks of IH. Fig. 5 shows a representative sequence of baroreflex testing and the vagal and sympathetic baroreflex gains before and after exposure. Two weeks of IH increased cardiovagal baroreflex gain from 21.7 ± 8.0 to 28.7 ± 7.9 $ms.mmHg^{-1}$ ($p<0.05$), but decreased vascular sympathetic gain from -965.3 ± 375.1 to -598.4 ± 162.6 $AIU.min^{-1}.mmHg^{-1}$ ($p<0.01$).

Echocardiography showed that both left ventricular end-diastolic diameter and end-systolic diameter were reduced ($p<0.05$; Table). As a result, ejection fraction and stroke

volume were unchanged and so cardiac output remained the same from pre to post exposure. Given the increase in daytime blood pressure, this indicates that systemic vascular resistance was elevated after two weeks of severe IH.

Despite the elevation in muscle sympathetic activity under awake, room air conditions, urinary and plasma catecholamines demonstrated no change at any time during the severe IH exposure. Likewise, circulating plasma level of usCRP, Il-1Ra, Il-8, TNF-α, adiponectin, leptin, RANTES and sICAM-1 did not change across the exposure, whereas MCP-1 tended to decrease ($p=0.06$) across the exposure Fig. 6.

To explore possible explanatory relationships for the increase in MSNA, we examined the correlation between the changes in MSNA, left ventricular end-systolic and end-diastolic diameters, and baroreflex sympathetic gain. The increase in MSNA with exposure was positively correlated with the decreases in both left ventricular end-systolic ($r=0.73$ $p<0.05$) and diastolic ($r=0.72$ $p=0.043$) diameters. But, surprisingly, there was no relationship between the increase in MSNA and the decrease in baroreflex sympathetic gain ($r=0.46$, $p=0.294$).

Discussion

Our data clearly demonstrate that repeated exposure to an IH stimulus similar to that observed in severe OSAS patients produces a sustained daytime elevation in blood pressure in healthy humans. Moreover, this exposure increases resting sympathetic outflow and reduces sympathetic baroreflex gain. However, in our young healthy subjects, vasodilatory function was essentially unaltered and systemic markers of inflammation were virtually unchanged by severe IH. However, it is possible that the time course for changes in these parameters requires longer duration exposure. Nonetheless, these data strongly suggest that exposure to severe IH in humans is responsible for a maintained elevation in blood pressure that is secondary, at least in part to increased sympathetic activity and decreased baroreflex function.

Our human model of severe IH [25] produced particularly marked increases in pressure in the morning (8 AM) and again later in the evening (6 and 8 PM). In contrast, there was no nighttime increase in blood pressure when subjects were exposed to IH during sleep. This resulted in an exaggeration of the normal nocturnal blood pressure decline, or 'dip.' This is in contrast to the classic 'non dipper' profile that has been reported in 30 % of OSAS patients [29]. We do not have definite explanation for this unexpected result. One point is that nocturnal catecholamine excretion did not demonstrate a change, and so it does not appear that there was any obvious alteration in sympathetic vasoconstrictor effects, and thus no effect on blood pressure. An alternative explanation is that nocturnal fall in blood pressure mainly relates to sleep duration and sleep architecture. We recently demonstrated that, in type 1 diabetic OSA, shorter sleep duration and not parameters of OSA severity was the main determinant for non dipping pattern of BP [33]. Our healthy subjects even hypoxemic during night continue to exhibit a normal sleep organisation with a significant amount of slow wave sleep (Time spent in stage III/IV sleep in % of TST 17.7±10.9 before to 12.7±5.8 after exposure)[25]. This could lead to persistent physiological changes in the autonomic nervous

system activity resulting in BP fall overnight. Interestingly enough, this was however followed by persistent sympathetic hyperactivity during daytime as evidenced by MSNA recordings. On the other hand, early morning and late afternoon rises in blood pressure have been described in OSAS patients [29], similar to what we observed after only two weeks of exposure to severe IH. In addition, it is important to note that the increased pressures induced by this protocol were resolving by 5 days of recovery. Though these data do not speak to the effect of longer exposures, the changes in blood pressure we observed suggest that increased daytime blood pressures can develop relatively quickly and that eliminating the stimulus for the sustained blood pressure elevation results in rapid resolution.

In tandem with this daytime elevation of pressure after two weeks of severe IH, we observed a significant sympathetic activation. Although sympathetic activation with hypoxic exposure is thought to be mainly driven by increased peripheral chemoreflex sensitivity [8], we also observed a significant decrease in arterial baroreflex control of sympathetic outflow. However, there was not a simple linear relation between the increase in sympathetic activity and decrease in baroreflex gain. This does not exclude a baroreflex mechanism for the sympathoexcitation after IH, but merely suggests the changes in baroreflex control do not necessarily lead to proportional changes in sympathetic activity. Lastly, we did find a greater cardiovagal gain after two weeks of IH. More robust bradycardia responses to pressure rises might offset lesser sympatho inhibition; however, these offsetting effects, if present, were apparently insufficient to prevent elevated ambulatory pressure after severe IH.

As noted above, peripheral chemoreflex sensitivity may also play a role in maintained sympathoexcitation after exposure to hypoxia [8]. In a minority of subjects, we were able to obtain data suggestive of attenuation in the sympathoexcitatory responses to hypoxia. In our study, 14 nights of IH did tend to enhance the blood pressure increase in response to acute hypoxia (11.0 ± 6.9 to 25.7 ± 11.9 mmHg for systolic and 2.3 ± 3.0 to 10.1 ± 6.5 mmHg for

diastolic blood pressure before exposure and after exposure respectively). However, the magnitude of sympathetic response in these five subjects with acute hypoxia appears to be diminished (pre-exposure: 15.9 ± 5.5 to 27.0 ± 11.9 burst/min, post-exposure 21.0 ± 7.5 to 25.0 ± 10.1 burst/min). This does not fit with the fact that prolonged exposure to hypoxia results in ventilatory acclimatization to hypoxia -- greater increases in ventilation [34] due to augmented peripheral chemosensitivity [35]. Moreover, inhalation of 100% FiO2 decreases sympathetic tone in OSAS patients [8], and surgical denervation of the peripheral chemoreceptors in rats prevents the increase in blood pressure induced by CIH [19]. Although these findings might suggest an increase in peripheral chemosensitivity as a mechanism of sympathoactivation, there is no standard approach to assess chemoreflex sensitivity in terms of sympathetic responsiveness. Hence, it remains unclear whether increased chemoreflex sensitivity plays a role in heightened sympathetic outflow after exposure to CIH in humans.

Several studies have proposed impaired vasodilation as a likely contributor to high blood pressure in OSAS patients [13, 36]. This work should be interpreted with caution since many confounding factors (e.g., obesity, diabetes) may alter endothelial function independent of OSAS in these patients. In these young healthy subjects, we found a change only in the peak vasodilation following a hyperemic stimulus. On the other hand, in contrast to previous statements in OSA patients [13, 37], we did not confirm an impairment in endothelial mediated vasodilation. We cannot rule out that a longer exposure to CIH might induce some change in vasodilatory capacity, but our data show that these changes are not necessary to observe the blood pressure increase with CIH. Moreover, the systemic and endothelial inflammation reported in OSAS patients [15] and in rodent models of CIH [38] were not reproduced in our subjects.

Although given the number of desaturations, thirty per hour, the stimulus we applied could be considered as clinically analogous to severe OSAS (Fig. 1 A), our model does have

certain limitations and is not completely analogous to severe OSAS. Several points need to be discussed in order to present how our model differs from the disease obstructive sleep apnea syndrome (Fig. 1A). Although the amount of desaturation is close to a typical sleep apnea patient, the time line of 2 minute for a cycle is longer than what is usually exhibited by a patient (less than one minute). Our exposure approximates IH paradigms applied to the rodent, however is closer to exposures in patients when considering breathing frequency (about 16/min in humans versus 80/min in rats). Moreover, apneas produce asphyxia (i.e., hypoxia plus hypercapnia), whereas our model produces hypoxia plus hypocapnia. This may underestimate the effect since increased CO2 enhances the cardiovascular responses to hypoxia in both healthy individuals [39, 40] and sleep apnea patients [41]. In addition, the absence of crescendo in respiratory effort will not result in the alterations in cardiac preload and afterload observed in sleep apnea patients [42, 43]. However, the goal of this model is to explore a disease component to extricate a particular mechanism. Thus, the present model allows studying the specific effect of intermittent hypoxia during a specific physiological state: sleep. This stimulus in this very young (23 years) and lean (BMI of 22 $kg.m^{-2}$) cohort did not produce increased nocturnal catecholamine excretion, decreased daytime vagal drive, nor surges in nocturnal blood pressure. These responses have been well illustrated previously in obese, middle aged, borderline hypertensive individuals with established sleep apnea for at least 10 years duration [42]. Therefore, it should be kept in mind that this model establishes that IH per se can lead to elevations in both blood pressure and vascular sympathetic activity and over only a very short course. In addition and as noted above, the duration of exposure may not allow full resolution of other, potentially important pathophysiologic changes. Two weeks of IH is likely a much shorter exposure than experienced by individuals presenting with clinically significant OSAS. Finally, neither renin-angiotensin system nor sodium balance were investigated in this study. Indeed, it would be interesting to explore several

other mechanisms that may be involved in blood pressure increased as the renin-angiotensin system, or endothelin pathway. Unfortunately we did not include in our design sampling that would allows us a posteriori to run renin and angiotensin activity assays. This should be considered in the design of future studies.

Perspective.

This is some of the first work to explore whether the blood pressure rise is sustained beyond the acute phase immediately after IH exposure, both throughout the waking hours and after five days of recovery. We found that only two weeks of severe IH exposure produces a sustained daytime blood pressure elevation in the setting of sympathoactivation and blunted vascular sympathic baroreflex gain in healthy individuals. This may provide a foundation from which interventional studies can be designed to explore prevention of the cardiovascular impairments due to CIH.

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Table: Echocardiographic parameters monitored before and after exposure.

	N	Pre exposure	Post exposure	
VTI (cm)	12	20.7 ± 2.2	20.1 ± 4.1	NS
CO ($ml.min^{-1}$)	11	8374.1 ± 1020.3	7855.5 ± 2040.9	NS
LVEF (Teicholz) %	10	67.8 ± 2.7	69.2 ± 5.8	NS
Heart (Rate beat/min)	11	66.9 ± 9.7	65.3 ± 12.3	NS
Stroke Volume (ml)	12	127.1 ± 16.2	124.6 ± 30.1	NS
Aortic diameter (mm)	12	27.6 ± 3.2	28.4 ± 2.9	0.07
LV end-diastolic diameter (mm)	12	50.2 ± 4.5	48.7 ± 4.3	< 0.05
LV end-systolic diameter (mm)	12	33.8 ± 3.2	32.3 ± 4.2	0.06

VTI : Velocity time integral ratio, CO : cardiac output, LVEF : Left Ventricular Ejection Fraction (Teicholz method)

Figure Legends

Fig. 1 A This figure illustrates SpO2 tracings from a typical obstructive sleep apnea patient compared to the exposure obtained with the present model in a representative subject.

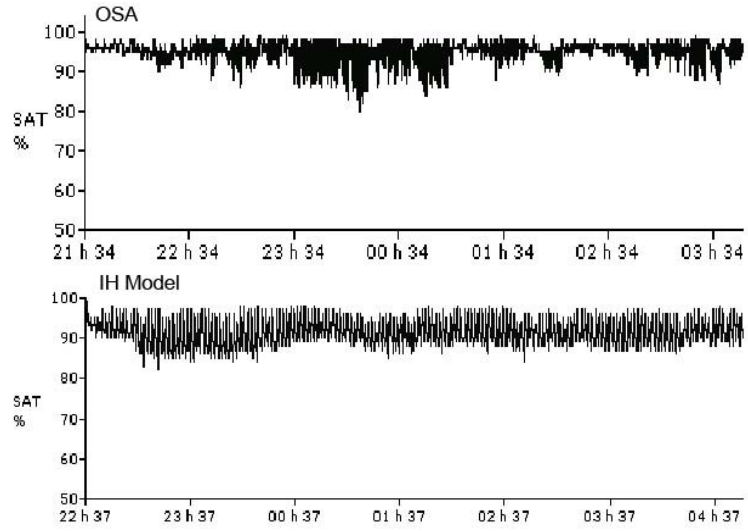


Fig. 1 B Time line of the study. Diurnal blood pressure patterns (Ambulatory Blood Pressure Measurement, ABPM) as well as urine and blood samples were obtained before exposure, after one and 13 nights of severe IH, and after 5 days of recovery. Cardiovascular measurements (Muscle Sympathetic Nerve Activity, MSNA) and reflex assessments were performed before exposure and following the two weeks of severe IH.

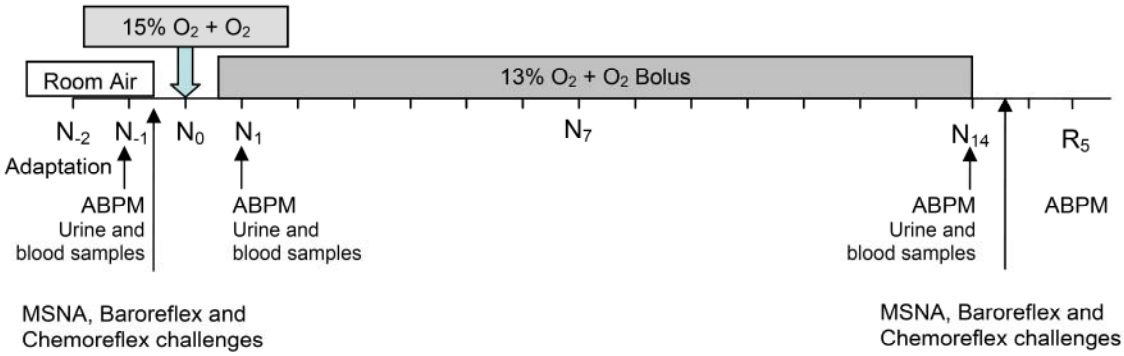


Fig. 2 Average and standard errors for the hour by hour blood pressures during 24 hours of monitoring (N=12). One night, thirteen nights, and recovery from exposure to intermittent hypoxia (IH) are compared to pre-exposure. Pre-exposure is represented by the solid lines;

each time point after exposure is represented by dashed lines. Hourly values were averaged across the daytime and nighttime for statistical analysis.

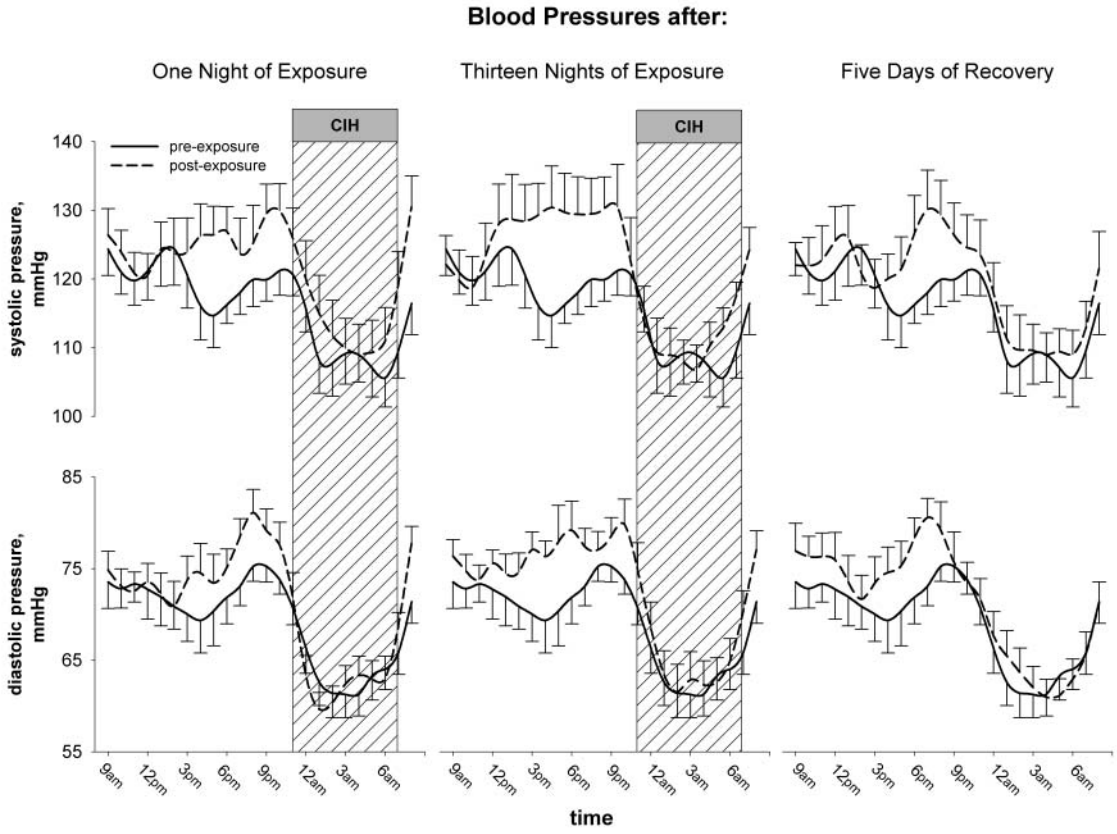


Fig. 3 Mean daytime (7 AM to 10 PM) and nighttime (10 PM to 7 AM) blood pressures across the exposure. Values are mean with standard deviations. Lines with asterisk (*) indicate significant differences at $p<0.008$. (N=12)

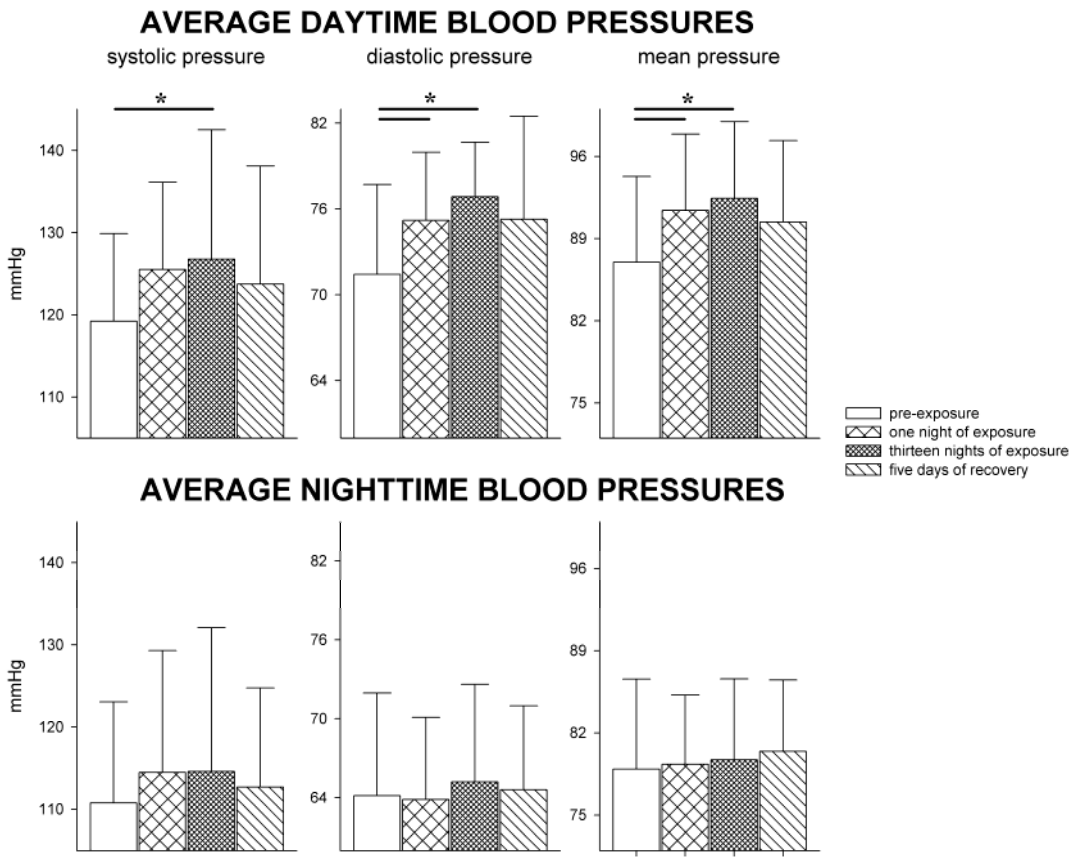


Fig. 4 A representative neurogram of muscle sympathetic nerve activity (MSNA) during supine rest while breathing room air before and after two weeks IH exposure

The average values before and after two weeks IH exposure in burst/min (n=8).

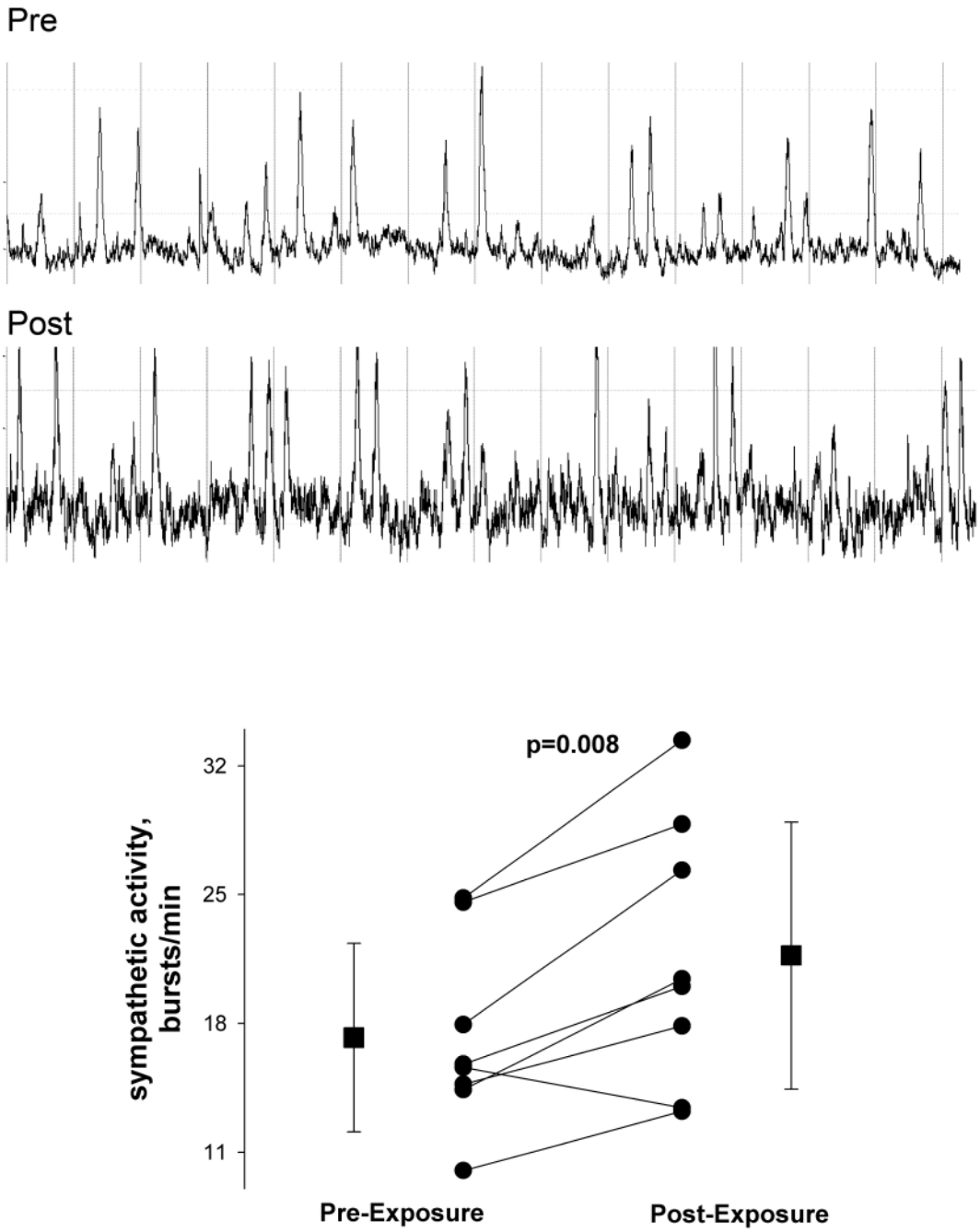


Fig. 5 Representative sequences of arterial baroreflex testing before and after exposure and the relations derived from these time series. Analysis of these relations to obtain baroreflex gains is described in the text.

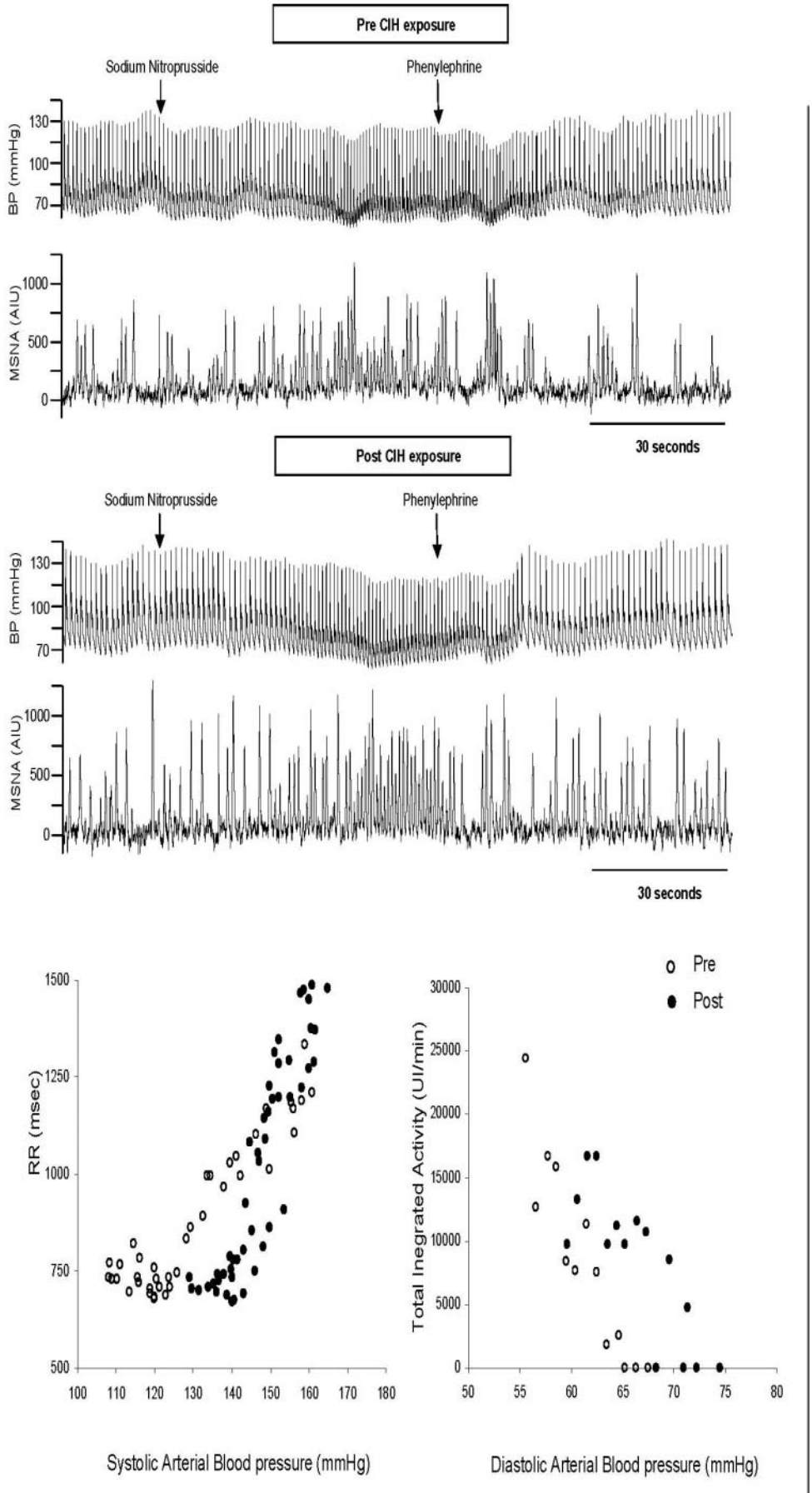
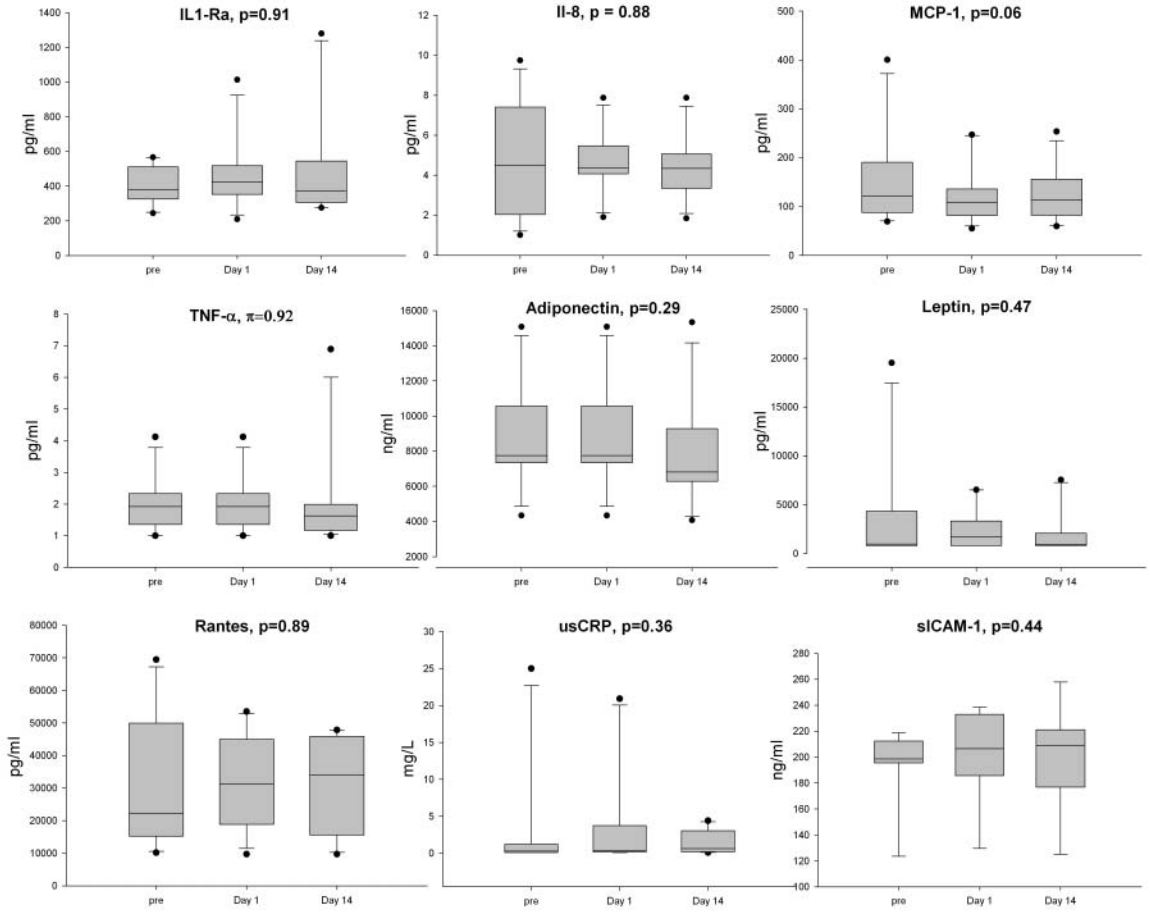

Pre CIH exposure
Sodium Nitroprusside
Phenylephrine
BP (mmHg)
130
100
70
MSNA (AIU)
1000
500
0
30 seconds
Post CIH exposure
Sodium Nitroprusside
Phenylephrine
BP (mmHg)
130
100
70
MSNA (AIU)
1000
500
0
30 seconds
Pre
Post
RR (msec)
1500
1250
1000
750
500
100 110 120 130 140 150 160 170 180
Systolic Arterial Blood pressure (mmHg)
Total Inegrated Activity (UI/min)
30000
25000
20000
15000
10000
5000
0
50 55 60 65 70 75 80
Diastolic Arterial Blood pressure (mmHg)

Fig. 6 Box plot from circulating plasma level of Il-1Ra, Il-8, MCP-1, TNF-α, adiponectin, leptin, RANTES, usCRP and sICAM-1. Although MCP-1 tended to decrease (p=0.06) across the exposure no significant change were found in other markers.



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