Eur Respir J 2011; 37: 119–128
DOI: 10.1183/09031936.00204209

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

R. Tamisier*,#, J.L. Pépin*,#, J. Rémy#, J.P. Baguet¶, J.A. Taylor+, J.W. Weiss§ and P. Lévy*,#

ABSTRACT: Obstructive sleep apnoea 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 characterise the profile of this blood pressure increase, to determine if it is sustained and to explore potential physiological mechanisms.

We performed 24-h ambulatory monitoring of blood pressure in 12 healthy subjects before and after 2 weeks of IH exposure. We also assessed systemic haemodynamics, muscle sympathetic nerve activity (MSNA), ischaemic 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 2 weeks of exposure (8 mmHg systolic and 5 mmHg diastolic). Mean $\pm$SD MSNA increased across the exposure (17.2 $\pm$ 5.1 *versus* 21.7 $\pm$ 7.3 bursts·min^{-1}; $p<0.01$) and baroreflex control of sympathetic outflow declined from -965.3 $\pm$ 375.1 to -598.4 $\pm$ 162.6 AIU·min^{-1}·mmHg^{-1} ($p<0.01$). There were no evident changes in either vascular reactivity or systemic inflammatory markers.

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 reduced baroreflex inhibition. These mechanisms may reflect those underlying the blood pressure elevation associated with OSAS.

KEYWORDS: Atherosclerosis, hypertension, pathophysiology, sleep apnoea

Obstructive sleep apnoea syndrome (OSAS) is highly prevalent in western countries, with an age-related prevalence ranging 5–15% up to 60 yrs of age [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 nonfatal cardiovascular complications [3–5]. Hence, OSAS is a significant health concern in Western countries [6, 7].

Considering these epidemiologic associations, the pathophysiological 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: the 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 not an exhaustive list, we proposed 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 with the other stimuli that characterise OSAS (*i.e.* respiratory effort, asphyxia and 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].

AFFILIATIONS
*Sleep Laboratory and EFCR, Dept of Rehabilitation and Physiology,
#HP2 Laboratory, INSERM U1042, EA 3745 Joseph Fourier University,
¶Dept of Cardiology and Hypertension, University Hospital, Grenoble, France.
+Dept of Physical Medicine and Rehabilitation, Harvard Medical School,
§Pulmonary and Sleep Research Laboratory, Division of Pulmonary, Critical Care and Sleep Medicine, Beth Israel Deaconess Medical Center, Boston, MA, USA.

CORRESPONDENCE
R. Tamisier
Laboratoire d'Exploration Fonctionnelle Respiratoire
Pôle Rééducation et Physiologie
Centre Hospitalier Universitaire de Grenoble BP 217
38043 Grenoble Cedex 9
France
E-mail: rtamisier@chu-grenoble.fr

Received:
Dec 29 2009
Accepted after revision:
May 15 2010
First published online:
June 04 2010

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

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 apnoea 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 intermittent hypoxia (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, after 7 and 14 days of CIH, our model induced 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 waking IH [26]. Moreover, using the same model, FOSTER *et al.* [27] demonstrated changes in cardio- and cerebrovascular responses to acute hypoxia following exposure to intermittent hypoxia.

Moreover, we explored three possible contributors to the IH-induced elevation in blood pressure and atherogenesis: greater sympathetic activation, lesser post-occlusive-mediated vasorelaxation and systemic inflammation. We hypothesised that after 2 weeks of exposure to IH, arterial blood pressure would remain elevated and this would relate to increased sympathetic activity to muscle vasculature, decreased flow-mediated dilation and increased circulating biomarkers of inflammation.

METHODS

Subjects

12 healthy, nonsmoking, normotensive subjects (two of whom were female), with a mean±SD age of 23±6 yrs (body mass index (BMI) 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 that each subject was free of cardiac, pulmonary or neurologic diseases. Individuals who had travelled to or lived at an altitude >2,500 m in the 6 months prior to the study were excluded. All females 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 report encompasses data not previously reported, but acquired from subjects that completed the 2-week protocol described previously [25].

The sample size of 12 subjects was based on the expected changes in sympathetic activity and diastolic blood pressure measured by 24-h ambulatory blood pressure measurement. The study was powered at 80% to observe a 20% increase in sympathetic activity from a baseline level of 15.6±5.6 bursts·min^{-1} [28] and a 10-mmHg (11%) increase in daytime diastolic blood pressure from a baseline of 82.8±9.1 mmHg [29] with an α of 0.05.

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

IH exposure

This CIH exposure has been previously reported [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 h severe IH between the 23:00 and 07:00 h for 14 consecutive nights. The IH stimulus was intermittent poikilocapnic hypoxia, *i.e.* inspiratory oxygen fraction (F_{I,O_2}) was controlled and carbon dioxide was allowed to fluctuate normally. For all nights, subjects slept with a nasal cannula in a commercially available hypoxia tent (Hypoxico Inc., New York, NY, USA). The tent exposed subjects to an F_{I,O_2} of 0.15 for the intermediate IH level and 0.13 for the 14 nights of severe IH. The tent was continuously flushed and the oxygen fraction in the tent was continuously monitored (Maxtec OM-25 MEI; Maxtec Inc., Salt Lake City, UT, USA) to limit rebreathing. The nasal cannula restored oxygen saturation *via* a 15-s bolus of oxygen every 120 s. Oxygen saturation was monitored continuously (BlueNight; SleepInnov Technology, Moirans, France) and oxygen boluses were adjusted between 1.5 and 2 L·min^{-1} to achieve an 85–95% 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. 1a and b).

General procedures

Diurnal blood pressure patterns as well as urine and blood samples were obtained before exposure, after one and 13 nights (*i.e.* 2 weeks) of severe CIH, and after 5 days of recovery. Cardiovascular measurements were performed before exposure and following the 14th night (2 weeks) of severe IH (fig. 1c).

Ambulatory blood pressure was measured in the dominant arm over 24 h at 15-min intervals (ABP monitor 90207; Spacelabs Healthcare, Issaquah, WA, USA). Blood pressure acquisition began at 09:00 h and ended 24 h later. Measurements before exposure and after recovery represent room air conditions for the entire 24 h, whereas measurements after one and 13 nights of IH represent daytime room air and nighttime IH conditions.

Cardiovascular measurements were recorded between 08:00 and 12:00 h 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 hyperaemia assessment and hypoxic challenge. All data were digitalised continuously at 500 Hz to a computer and analysed 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 three-lead electrocardiogram. Arterial pressures were measured in the right arm at 1-min intervals *via* an automated arm-cuff sphygmomanometer (Dinamap; Critikon, Tampa, FL, USA) 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,

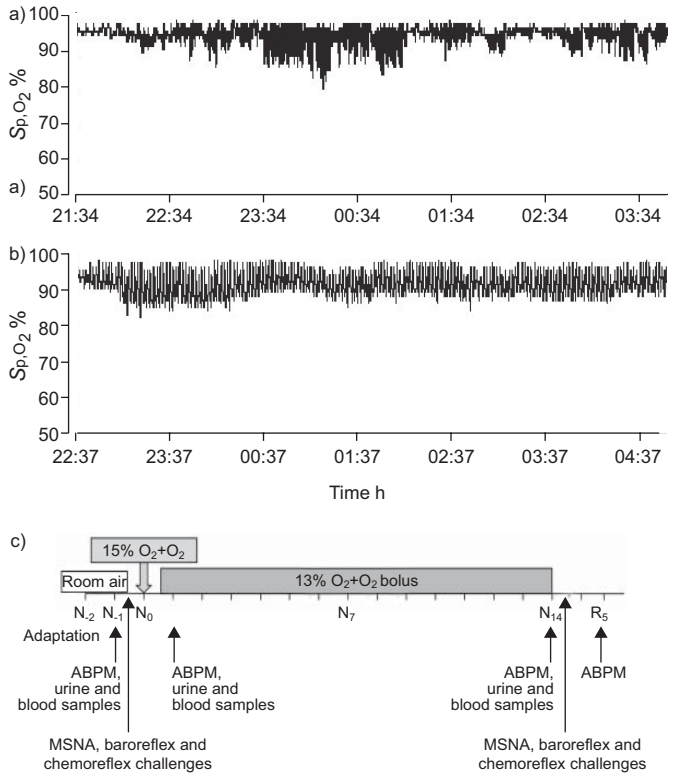

FIGURE 1. Arterial oxygen saturation measured by pulse oximetry (S_{p,O_2}) tracings from a) a typical obstructive sleep apnoea patient compared to b) the exposure obtained with the present model in a representative subject. c) Time line of the study. Diurnal blood pressure patterns (ambulatory blood pressure measurement; ABPM) as well as urine and blood samples were obtained before exposure, after one and 13 nights of severe intermittent hypoxia (IH), and after 5 days of recovery. Cardiovascular measurements (muscle sympathetic nerve activity; MSNA) and reflex assessments were performed before exposure and following the 2 weeks of severe IH. N: night; R: recovery.

as described previously [28]. Signals were filtered, amplified and full-wave rectified (Nerve Traffic Analyser, model 662c-3; Bioengineering Dept, University of Iowa, Iowa City, IA, USA), and muscle sympathetic nerve activity (MSNA) bursts were identified using an algorithm developed by HAMNER and TAYLOR [30], using Matlab software (The Mathworks Inc., Natick, MA, USA). MSNA was averaged over 5-min periods and expressed as burst frequency (bursts·min^{-1}), burst frequency normalised to heart beat (bursts per 100 beats) and burst amplitude (AIU·min^{-1}).

Calf blood flow (CBF) was measured by venous occlusion plethysmography (EC6 Plethysmograph; Hokanson, Bellevue, WA, USA), as described previously [28]. An average of eight to 10 flow measurements were used to compute values before and after exposure. CBF was expressed in millilitres per minute per 100 g tissue. Calf vascular resistance (CVR) was derived from the ratio of mean arterial pressure to CBF. These measures were also used to estimate the reactive hyperaemia over 2.5 min in response to 5 min local ischaemia.

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 nitroprusside followed 1 min later by 150 μg phenylephrine (modified Oxford technique). Two trials were performed separated by $\geqslant$15 min.

The sympathoexcitatory 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 carbon dioxide across subjects. The target oxygen saturation for this test was 80–85%. The mean$\pm$SD achieved was 80$\pm$3 and 80$\pm$2% before and after exposure, respectively, and all subjects were between 78 and 85%. Responses to hypoxia were measured during a 5-min period only after steady state oxygen saturation was reached.

Transthoracic echocardiography (HP Sonos 2500; Hewlett-Packard, Santa Clara, CA, USA) was performed by the same investigator (J.P. Baguet, Dept of Cardiology and Hypertension, University Hospital, Grenoble, France) before and after exposure. Subjects were studied while in the left lateral decubitus position to obtain three standard left ventricular apical views (apical four-chamber, two-chamber and long-axis) using time motion, two-dimensional 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 from 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 loss of data during the procedures in three more. Since the latter reduced the number of sympathetic recordings for the sympathoexcitatory response to hypoxia to only five subjects and, consequently, rendered paired sampling too small, data for these responses are presented only for discussion. In addition, for technical reasons, reactive hyperaemia was not obtained in one subject.

Ambulatory blood pressure monitoring over 24 h at 15-min intervals results in a surfeit of values, such that comparisons of blood pressure measurements across the four study days on an hour-by-hour basis would be statistically intractable. Therefore, we averaged day- and night-time blood pressures. The classical definitions for day- (07:00–22:00 h) and night-time (22:00–07:00 h) measurements were applied to characterise diurnal blood pressure patterns.

Plasma markers for sympathetic activation (adrenaline, noradrenaline, dopamine, metanephrine and normetanephrine) were quantified by high-performance liquid chromatography. Nocturnal 8-h urine samples were collected, acidified with acetic acid and stored at -20°C until analysis. Catecholamines (adrenaline, noradrenaline and dopamine) were measured in 1 mL of urine by high-performance liquid chromatography with electrochemical detection (Coularray Detector; ESA Dionex, Chelmsford, MA, USA). Plasma markers for systemic

inflammatory responses (interleukin (IL)-1 receptor antagonist (IL-1Ra), IL-8, tumour necrosis factor (TNF)-α, monocyte chemoattractant protein (MCP)-1, adiponectin, leptin and RANTES (regulated upon activation, normal T-cell expressed and secreted)) were quantified using a commercially available multiplex-bead immunoassay (R&D Systems, Minneapolis, MN, USA) using a Bioplex 200 array reader (Bio-Rad, Hercules, CA, USA) with Luminex xMAP Technology (Luminex, Austin, TX, USA). Serum high-sensitivity C-reactive protein (hsCRP) level was measured using automated immunonephelometry (Behring Nephelometer II Analyser; Dade Behring, Berlin, Germany). Soluble intercellular adhesion molecule (sICAM)-1 was quantified by ELISA (British Biotechnology, Abingdon, UK).

Baroreflex function was estimated from the relation of systolic pressure to R–R 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 with 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 analysed the data *via* a piecewise linear regression that required at least five 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 between 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 this, p-values <0.05 were considered statistically significant. Data are presented as mean±SD 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-h 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 2 weeks of exposure. As a result, the average daytime blood pressure was increased for both mean and diastolic pressures after one and 13 nights of exposure (3 mmHg and 5 mmHg for both; $p<0.05$; fig. 3). At 2 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 to baseline by 5 days of recovery.

Resting heart rate was unchanged following 2 weeks of IH exposure (58.6±6.8 beats·min^{-1} pre-exposure *versus* 59.4±7.6 beats·min^{-1} post-exposure). After exposure, resting MSNA was elevated by ~25% after the 14 day IH exposure ($p<0.01$; fig. 4). Moreover, resting CBF was unchanged but there was a significant increase in CVR (44.5±16.4 mmHg·min·mL^{-1}·100 mL tissue pre-exposure *versus* 50.9±17.4 mmHg·min·mL^{-1}·100 mL tissue post-exposure; $p<0.05$). In addition, the initial peak CBF following release of 5 min of ischaemia was lower following 2 weeks of IH (31.46±1.95 mL·min^{-1}·100 g tissue^{-1} pre-exposure *versus* 24.35±2.07 mL·min^{-1}·100 g tissue^{-1} post-exposure; $p<0.05$). However, from 30 s following the peak reactive hyperaemia, CBF values were similar before and after

TABLE 1 Echocardiographic parameters monitored before and after exposure

Parameter	Subjects n	Pre-exposure	Post-exposure	p-value
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 %	10	67.8±2.7	69.2±5.8	NS
Heart rate beats·min^{-1}	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

Data are presented as mean±SD, unless otherwise stated. VTI: velocity time integral ratio; CO: cardiac output; LVEF: left ventricular (LV) ejection fraction (Teicholz method); NS: not significant.

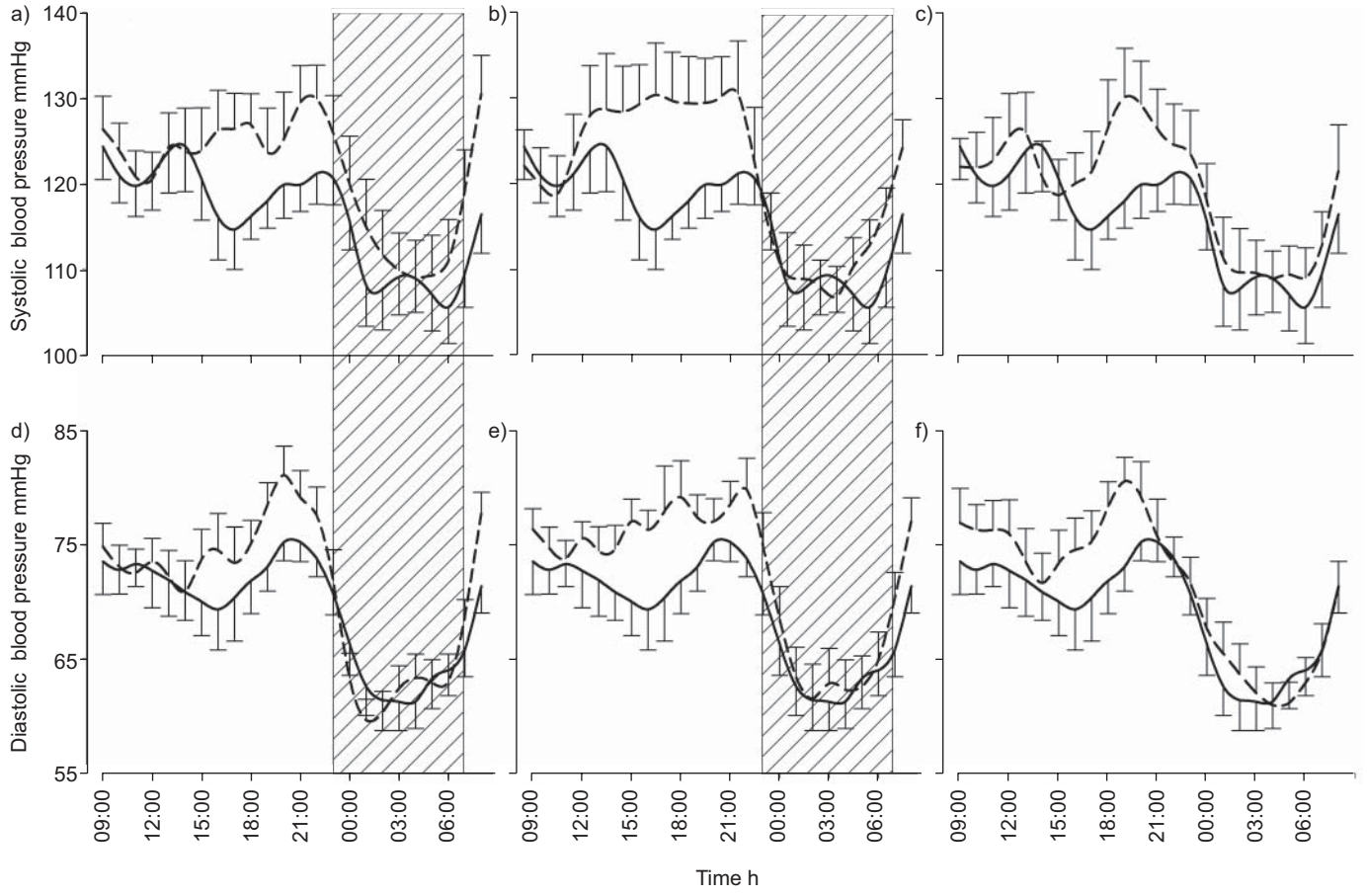

FIGURE 2. Hour-by-hour a, b, c) systolic and d, e, f) diastolic blood pressures during 24 h of monitoring (n=12). Data are presented as mean±SE. a, d) One night, b, e) 13 nights and c, f) recovery from exposure to intermittent hypoxia (▨) are compared to pre-exposure values. —: pre-exposure; ------: post-exposure. Hourly values were averaged across the daytime and night-time for statistical analysis.

2 weeks of IH. Figure 5 shows a representative sequence of baroreflex testing, and the vagal and sympathetic baroreflex gains before and after exposure. 2 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 and -systolic diameters were reduced ($p<0.05$; table 1). As a result, ejection fraction and stroke volume were unchanged and, thus, cardiac output remained the same before and after exposure. Given the increase in daytime blood pressure, this indicates that systemic vascular resistance was elevated after 2 weeks of severe IH.

Despite the elevation in MSNA while awake, room air conditions, and urinary and plasma catecholamines demonstrated no change at any time during the severe IH exposure. Likewise, circulating plasma level of hsCRP, 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 figure 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 -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 a longer duration of 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 (08:00 h) and

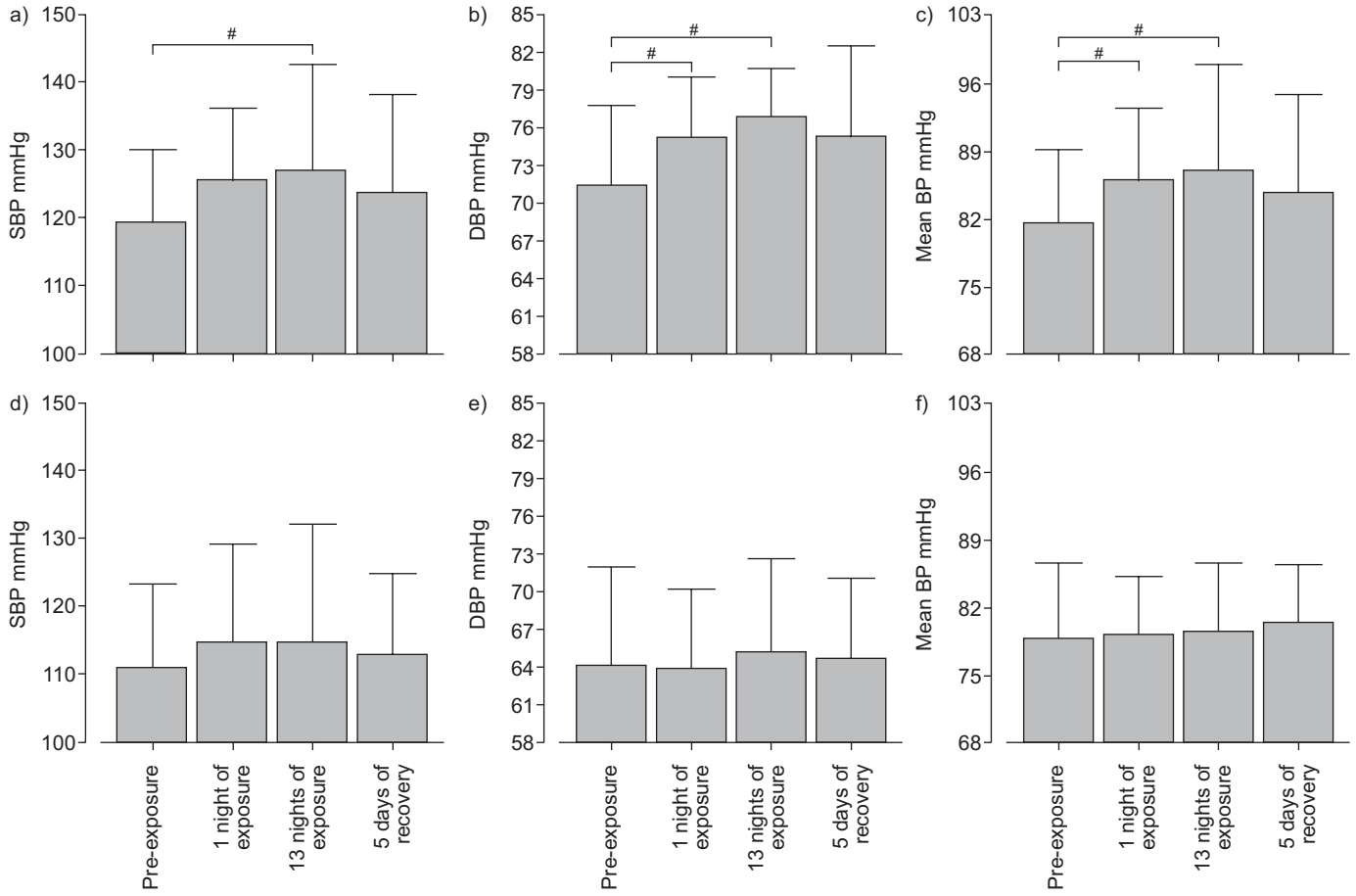

FIGURE 3. a, b, c) daytime (07:00–22:00 h) and d, e, f) night-time (22:00–07:00 h) a, d) systolic blood pressure (SBP) b, e) diastolic blood pressure (DBP) and c, f) mean blood pressures (BP) across the exposure. Data are presented as mean±SD. n=12. #: $p<0.008$.

again later in the evening (18:00 and 20:00 h). In contrast, there was no night-time 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 ''nondipper'' profile that has been reported in 30 % of OSAS patients [29]. We do not have definite explanation for this unexpected result. However, one point of note is that nocturnal catecholamine excretion did not 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 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 blood pressue [33]. Our healthy subjects, even when hypoxaemic during the night, continued to exhibit a normal sleep organisation with a significant amount of slow wave sleep (time spent in stage III–IV sleep in 17.7±10.9% total sleep time (TST) before to 12.7±5.8% TST after exposure) [25]. This could lead to persistent physiological changes in the autonomic nervous system activity, resulting in a fall in blood pressure overnight. Interestingly, this was followed by persistent sympathetic hyperactivity during daytime, as evidenced by MSNA recordings. Conversely, early morning and late afternoon increases in blood pressure have been described in OSAS patients [29], similar to what we observed after only 2 weeks of exposure to severe IH. In addition, it is important to note that the increased pressures induced by this protocol were resolved 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 2 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 2 weeks of IH. More robust bradycardic responses to pressure rises might offset

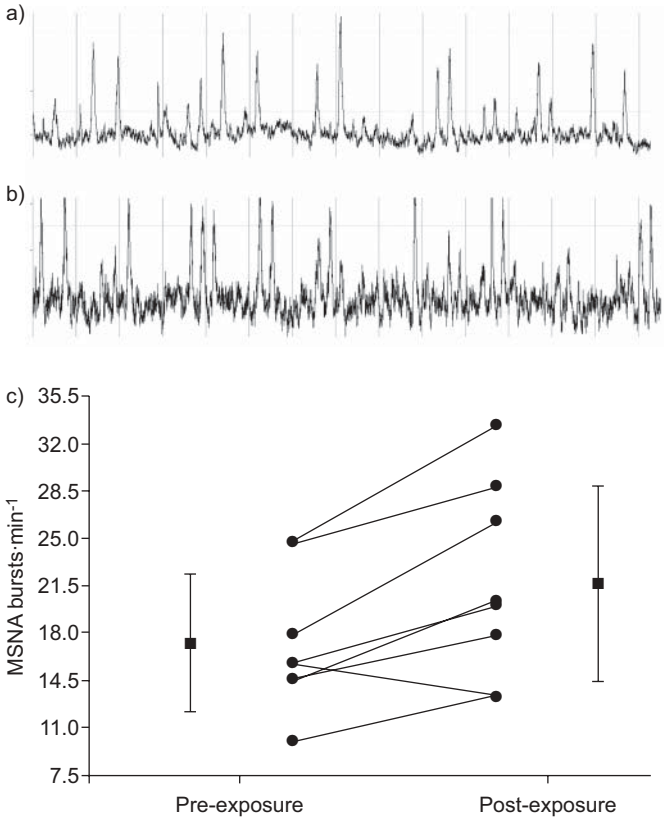

FIGURE 4. Representative neurograms of muscle sympathetic nerve activity (MSNA) during supine rest while breathing room air a) before and b) after 2 weeks intermittent hypoxia (IH) exposure. c) The mean±SE values before and after 2 weeks IH exposure in bursts·min^{-1} (n=8; p=0.008).

lesser sympathoinhibition; 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 maintaining 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 and after exposure, respectively). However, the magnitude of the sympathetic response in these five subjects with acute hypoxia appears to be diminished (pre-exposure 15.9±5.5 to 27.0±11.9 bursts·min^{-1}, post-exposure 21.0±7.5 to 25.0±10.1 bursts·min^{-1}). This does not fit with the fact that prolonged exposure to hypoxia results in ventilatory acclimatisation to hypoxia: greater increases in ventilation [34] are due to augmented peripheral chemosensitivity [35]. Moreover, inhalation of 100% F_{I,O_2} 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.

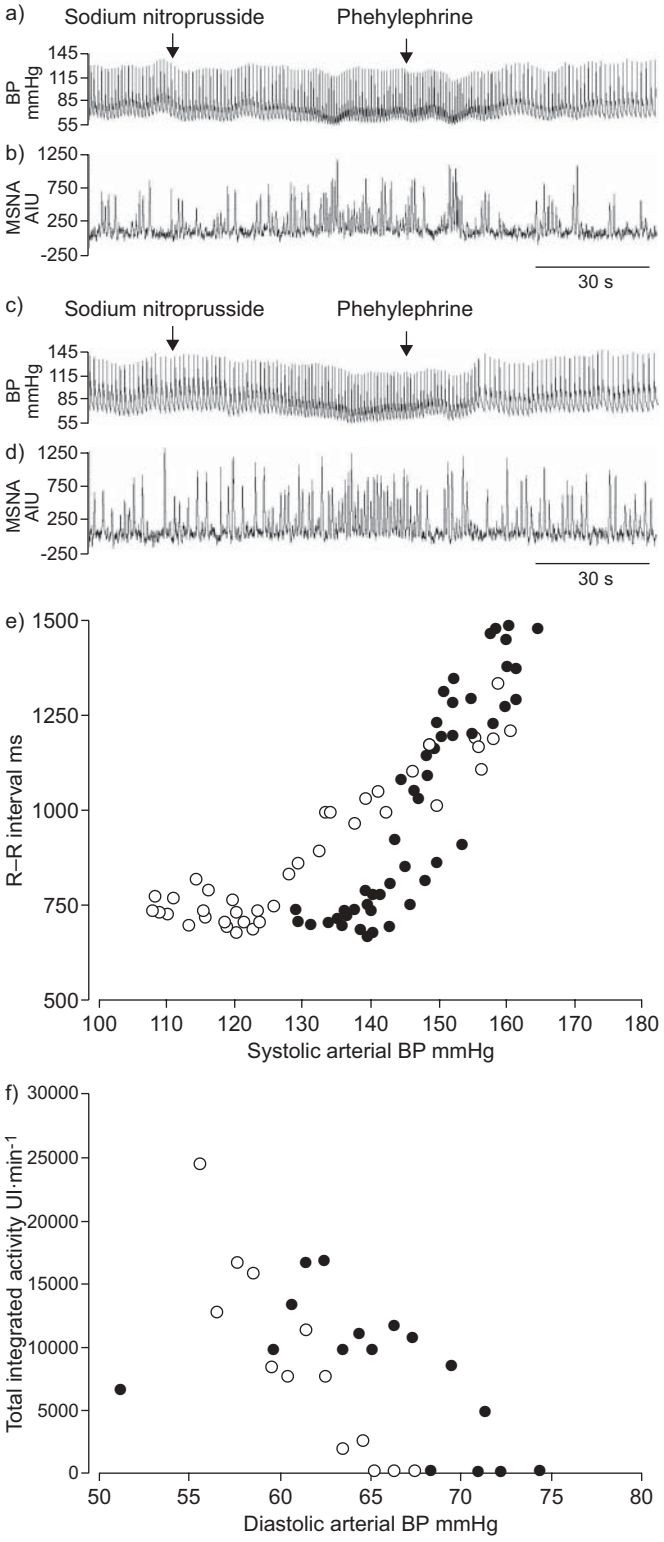

FIGURE 5. Representative sequences of arterial baroreflex testing a, b) before and c, d) after exposure and e, f) the relations derived from these time series. ○: before; ●: after. Analysis of these relations to obtain baroreflex gains is described in the text. BP: blood pressure; MSNA: muscle sympathetic nerve activity.

Hence, it remains unclear whether increased chemoreflex sensitivity plays a role in heightened sympathetic outflow after exposure to CIH in humans.

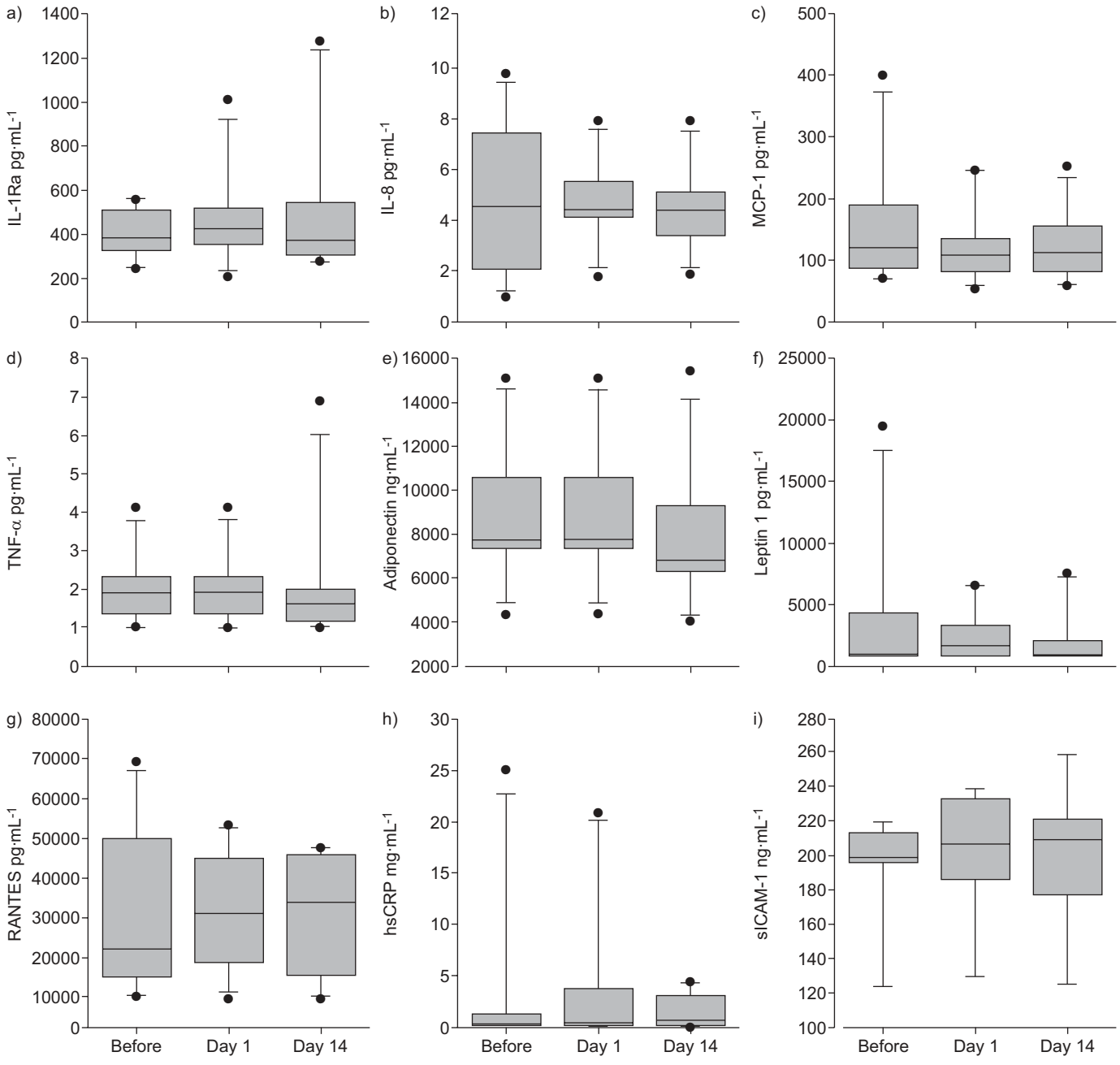

FIGURE 6. Box plots of circulating plasma levels of a) interleukin (IL)-1 receptor antagonist (Ra) (p=0.91), b) IL-8 (p=0.88), c) monocyte chemoattractant protein (MCP)-1 (p=0.06), d) tumour necrosis factor (TNF)-α (p=0.92), e) adiponectin (p=0.29), f) leptin (p=0.47), g) RANTES (regulated upon activation, normal T-cell expressed and secreted) (p=0.89), h) high-sensitivity C-reactive protein (hsCRP) (p=0.36) and i) soluble intercellular adhesion molecule (sICAM)-1 (p=0.44). Although MCP-1 tended to decrease during exposure, no significant change were found in other markers. The boxes represent interquartile range, and the whiskers represent the 5th and 95th percentiles. ——: mean; ●: extremes. a) p=0.91, b) p=0.88, c) p=0.06, d) p=0.92, e) p=0.29, f) p=0.47, g) p=0.89, h) p=0.36 and i) p=0.44.

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 and 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 hyperaemic stimulus. However, in contrast to previous studies in obstructive sleep apnoea patients [13, 37], we did not confirm an impairment in endothelially 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 (30 per hour), the stimulus we applied could be considered as clinically analogous to severe OSAS (fig. 1a and b); 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 OSAS (fig. 1a and b). Although the amount of desaturation is close to a typical sleep apnoea patient, the timeline of 2 min per cycle is longer than that usually exhibited by a patient (<1 min). Our exposure approximates IH paradigms applied to the rodent; however, it is closer to exposures in patients when breathing frequency is considered (about 16 breaths·min^{-1} in humans *versus* 80 breaths·min^{-1} in rats). Moreover, apnoeas produce asphyxia (*i.e.* hypoxia plus hypercapnia), whereas our model produces hypoxia with hypocapnia. This may underestimate the effect, since increased carbon dioxide enhances the cardiovascular responses to hypoxia in both healthy individuals [39, 40] and sleep apnoea 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 apnoea 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 yrs of age) and lean (BMI 22 kg·m^{-2}) cohort, did not produce increased nocturnal catecholamine excretion, decreased daytime vagal drive or surges in nocturnal blood pressure. These responses have previously been well illustrated in obese, middle-aged, borderline hypertensive individuals with established sleep apnoea of $\geqslant$10 yrs 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 over only a very short course of time. In addition, and as noted previously, the duration of exposure may not allow full resolution of other, potentially important pathophysiologic changes. 2 weeks of IH is likely a much shorter exposure than that experienced by individuals presenting with clinically significant OSAS. Finally, neither the 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 increase, such as the renin–angiotensin system or endothelin pathway. Unfortunately, we did not include in our design sampling that would allows us to run *a posteriori* renin and angiotensin activity assays. This should be considered in the design of future studies.

Future perspectives

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 5 days of recovery. We found that only 2 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.

SUPPORT STATEMENT

The study research was supported by grants from Direction de la Recherche Clinique (CHU Grenoble France), Agiradom and ANTADIR, and HL 74972 (J.W. Weiss and J.A. Taylor).

STATEMENT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

We would like to thank P. Roux-Lombard (CHU Geneve, Geneva, Swizterland) who performed the multiplex and ELISA assays.

REFERENCES

1 Young T, Palta M, Dempsey J, *et al.* The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* 1993; 328: 1230–1235.
2 Peppard PE, Young T, Palta M, *et al.* Prospective study of the association between sleep-disordered breathing and hypertension. *N Engl J Med* 2000; 342: 1378–1384.
3 Marin JM, Carrizo SJ, Vicente E, *et al.* Long-term cardiovascular outcomes in men with obstructive sleep apnoea–hypopnoea with or without treatment with continuous positive airway pressure: an observational study. *Lancet* 2005; 365: 1046–1053.
4 Gami AS, Howard DE, Olson EJ, *et al.* Day–night pattern of sudden death in obstructive sleep apnea. *N Engl J Med* 2005; 352: 1206–1214.
5 Yaggi HK, Concato J, Kernan WN, *et al.* Obstructive sleep apnea as a risk factor for stroke and death. *N Engl J Med* 2005; 353: 2034–2041.
6 Levy P, Pepin JL, Arnaud C, *et al.* Intermittent hypoxia and sleep-disordered breathing: current concepts and perspectives. *Eur Respir J* 2008; 32: 1082–1095.
7 Bradley TD, Floras JS. Obstructive sleep apnoea and its cardiovascular consequences. *Lancet* 2009; 373: 82–93.
8 Narkiewicz K, van de Borne PJ, Montano N, *et al.* Contribution of tonic chemoreflex activation to sympathetic activity and blood pressure in patients with obstructive sleep apnea. *Circulation* 1998; 97: 943–945.
9 Carlson JT, Hedner J, Elam M, *et al.* Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. *Chest* 1993; 103: 1763–1768.
10 Hedner J, Darpo B, Ejnell H, *et al.* Reduction in sympathetic activity after long-term CPAP treatment in sleep apnoea: cardiovascular implications. *Eur Respir J* 1995; 8: 222–229.
11 Hedner J, Ejnell H, Sellgren J, *et al.* Is high and fluctuating muscle nerve sympathetic activity in the sleep apnoea syndrome of pathogenetic importance for the development of hypertension? *J Hypertens Suppl* 1988; 6: S529–S531.
12 Imadojemu VA, Gleeson K, Quraishi SA, *et al.* Impaired vasodilator responses in obstructive sleep apnea are improved with continuous positive airway pressure therapy. *Am J Respir Crit Care Med* 2002; 165: 950–953.
13 Ip MSM, Tse H-F, Lam B, *et al.* Endothelial function in obstructive sleep apnea and response to treatment. *Am J Respir Crit Care Med* 2004; 169: 348–353.
14 Kato M, Roberts-Thomson P, Phillips BG, *et al.* Impairment of endothelium-dependent vasodilation of resistance vessels in patients with obstructive sleep apnea. *Circulation* 2000; 102: 2607–2610.
15 Jelic S, Padeletti M, Kawut SM, *et al.* Inflammation, oxidative stress, and repair capacity of the vascular endothelium in obstructive sleep apnea. *Circulation* 2008; 117: 2270–2278.
16 Fletcher EC, Lesske J, Qian W, *et al.* Repetitive, episodic hypoxia causes diurnal elevation of blood pressure in rats. *Hypertension* 1992; 19: 555–561.
17 Brooks D, Horner RL, Kozar LF, *et al.* Obstructive sleep apnea as a cause of systemic hypertension. Evidence from a canine model. *J Clin Invest* 1997; 99: 106–109.
18 Fletcher EC, Lesske J, Culman J, *et al.* Sympathetic denervation blocks blood pressure elevation in episodic hypoxia. *Hypertension* 1992; 20: 612–619.

19 Lesske J, Fletcher EC, Bao G, *et al.* Hypertension caused by chronic intermittent hypoxia: influence of chemoreceptors and sympathetic nervous system. *J Hypertens* 1997; 15: 1593–1603.
20 Greenberg HE, Sica A, Batson D, *et al.* Chronic intermittent hypoxia increases sympathetic responsiveness to hypoxia and hypercapnia. *J Appl Physiol* 1999; 86: 298–305.
21 Brooks D, Horner RL, Floras JS, *et al.* Baroreflex control of heart rate in a canine model of obstructive sleep apnea. *Am J Respir Crit Care Med* 1999; 159: 1293–1297.
22 Lai CJ, Yang CCH, Hsu YY, *et al.* Enhanced sympathetic outflow and decreased baroreflex sensitivity are associated with intermittent hypoxia-induced systemic hypertension in conscious rats. *J Appl Physiol* 2006; 100: 1974–1982.
23 Cook NR, Cohen J, Hebert PR, *et al.* Implications of small reductions in diastolic blood pressure for primary prevention. *Arch Intern Med* 1995; 155: 701–709.
24 Turnbull F. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. *Lancet* 2003; 362: 1527–1535.
25 Tamisier R, Gilmartin GS, Launois SH, *et al.* A new model of chronic intermittent hypoxia in humans: effect on ventilation, sleep, and blood pressure. *J Appl Physiol* 2009; 107: 17–24.
26 Pialoux V, Hanly PJ, Foster GE, *et al.* Effects of exposure to intermittent hypoxia on oxidative stress and acute hypoxic ventilatory response in humans. *Am J Respir Crit Care Med* 2009; 180: 1002–1009.
27 Foster GE, Brugniaux JV, Pialoux V, *et al.* Cardiovascular and cerebrovascular responses to acute hypoxia following exposure to intermittent hypoxia in healthy humans. *J Physiol* 2009; 587: 3287–3299.
28 Tamisier R, Hunt BE, Gilmartin GS, *et al.* Hemodynamics and muscle sympathetic nerve activity after 8 hours of sustained hypoxia in healthy humans. *Am J Physiol Heart Circ Physiol* 2007; 293: H3027–3035.
29 Davies CW, Crosby JH, Mullins RL, *et al.* Case–control study of 24 hour ambulatory blood pressure in patients with obstructive sleep apnoea and normal matched control subjects. *Thorax* 2000; 55: 736–740.
30 Hamner JW, Taylor JA. Automated quantification of sympathetic beat-by-beat activity, independent of signal quality. *J Appl Physiol* 2001; 91: 1199–1206.
31 Studinger P, Goldstein R, Taylor JA. Mechanical and neural contributions to hysteresis in the cardiac vagal limb of the arterial baroreflex. *J Physiol* 2007; 583: 1041–1048.
32 Studinger P, Goldstein R, Taylor JA. Age- and fitness-related alterations in vascular sympathetic control. *J Physiol* 2009; 587: 2049–2057.
33 Borel AL, Benhamou PY, Baguet JP, *et al.* Short sleep duration is associated with a blood pressure nondipping pattern in type 1 diabetes: the DIAPASOM study. *Diabetes Care* 2009; 32: 1713–1715.
34 Powell FL, Milsom WK, Mitchell GS. Time domains of the hypoxic ventilatory response. *Respir Physiol* 1998; 112: 123–134.
35 Bisgard GE. Carotid body mechanisms in acclimatization to hypoxia. *Respir Physiol* 2000; 121: 237–246.
36 Kraiczi H, Hedner J, Peker Y, *et al.* Increased vasoconstrictor sensitivity in obstructive sleep apnea. *J Appl Physiol* 2000; 89: 493–498.
37 Jelic S, Lederer DJ, Adams T, *et al.* Vascular inflammation in obesity and sleep apnea. *Circulation* 2010; 121: 1014–1021.
38 Dematteis M, Julien C, Guillermet C, *et al.* Intermittent hypoxia induces early functional cardiovascular remodeling in mice. *Am J Respir Crit Care Med* 2008; 177: 227–235.
39 Morgan BJ, Crabtree DC, Palta M, *et al.* Combined hypoxia and hypercapnia evokes long-lasting sympathetic activation in humans. *J Appl Physiol* 1995; 79: 205–213.
40 Tamisier R, Nieto L, Anand A, *et al.* Sustained muscle sympathetic activity after hypercapnic but not hypocapnic hypoxia in normal humans. *Respir Physiol Neurobiol* 2004; 141: 145–155.
41 Kara T, Narkiewicz K, Somers VK. Chemoreflexes: physiology and clinical implications. *Acta Physiol Scand* 2003; 177: 377–384.
42 Somers VK, Dyken ME, Clary MP, *et al.* Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest* 1995; 96: 1897–1904.
43 Katragadda S, Xie A, Puleo D, *et al.* Neural mechanism of the pressor response to obstructive and nonobstructive apnea. *J Appl Physiol* 1997; 83: 2048–2054.