Original Article

Continuous Positive Airway Pressure Improves Daytime Baroreflex Sensitivity and Nitric Oxide Production in Patients with Moderate to Severe Obstructive Sleep Apnea Syndrome

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Individuals with obstructive sleep apnea syndrome (OSAS) are at high risk for cardiovascular morbidity and mortality. The effects of OSAS severity and nocturnal continuous positive airway pressure (CPAP) on daytime baroreflex sensitivity (BRS) and nitric oxide (NO) production were investigated in OSAS patients. Fifty-one consecutive males with OSAS and 29 age-matched healthy men underwent the Valsalva test and standard polysomnography. Patients with an apnea-hypopnea index (AHI) of ≥20 episodes per hour were randomized to receive CPAP treatment for 3 months (*n*=14) or no such treatment (*n*=19). The BRS index measured from the overshoot phase (phase IV) of the Valsalva maneuver and plasma NO concentration were significantly lower, whereas the AHI, oxygen desaturation time, arousal index, percentage of sleep stage 1, and systolic blood pressure were significantly greater, in patients with an AHI of ≥20/h than in those with an AHI of <20/h or in controls. The 24-h urinary excretion of norepinephrine was significantly reduced and the plasma NO concentration was significantly increased after one night of CPAP. The BRS index for phase IV and the Valsalva ratio were significantly increased in the CPAP group after the 3-month treatment period but remained unchanged in the non-CPAP group of OSAS patients. The daytime BRS index and NO production were thus inversely related to the severity of OSAS, and successful CPAP treatment improved these parameters in patients with moderate to severe OSAS. CPAP may therefore reduce the risk of cardiovascular complications due to endothelial dysfunction or increased sympathetic activity. (*Hypertens Res* 2007; 30: 669–676)

***Key Words*: sleep apnea, autonomic nervous system, baroreflex sensitivity, nitric oxide, Valsalva maneuver**

Introduction

Patients with obstructive sleep apnea syndrome (OSAS) often experience diurnal as well as nocturnal hypertension, with OSAS being recognized as a strong risk factor for cardiovascular morbidity and mortality (*1–5*). The repeated episodes of both arousal from sleep and hypoxia as well as the futile respiratory effort due to upper airway obstruction in OSAS patients result in stimulation of the sympathetic nervous system and may adversely affect hemodynamics (*6–8*). Indeed, the autonomic responses to sleep apnea or hypopnea likely

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Table 1. Characteristics of Control Subjects as well as of OSAS Patients with an AHI of <20/h or of ≥20/h

Characteristic	Controls (n=29)	AHI of <20/h (n=11)	AHI of ≥20/h (n=40)
Age (years)	52.2±9.9	55.0±8.6	53.2±10.2
AHI (/h)	2.5±1.7	15.4±4.1	47.0±16.1*,†
Lowest oxygen saturation (%)	93.4±1.4	81.0±8.3*	72.1±9.2*,†
Oxygen desaturation time (min)	0.0±0.0	18.6±14.3	100.5±86.7*,†
Arousal index (/h)	14.8±5.3	20.6±7.7	46.2±13.0*,†
Stage 1 sleep (%)	16.8±9.0	17.0±9.9	38.8±17.0*,†
Stage 2 sleep (%)	55.3±7.9	59.3±8.5	43.6±18.5*
Stages 3 + 4 sleep (%)	7.9±6.0	1.1±1.9*	2.0±4.5*
REM sleep (%)	20.8±2.3	14.2±4.3	13.0±9.0*
Systolic blood pressure (mmHg)	122.5±10.3	128.0±13.9	138.3±17.8*,†
Diastolic blood pressure (mmHg)	70.9±9.0	81.4±9.8*	86.1±11.3*
BRSI phase IV (ms/mmHg)	6.8±3.1	5.0±1.6*	3.0±1.8*,†
Valsalva ratio	1.33±0.21	1.11±0.091*	1.02±0.094*
Plasma NO_x (μmol/L)	59.4±26.5	45.1±7.1*	31.3±8.9*,†

Data are means±SD. *p<0.05 *vs*. controls; †p<0.05 *vs*. OSAS patients with an AHI of <20/h. OSAS, obstructive sleep apnea syndrome; AHI, apnea-hypopnea index; REM, rapid eye movement; BRSI, baroreflex sensitivity index.

contribute to both the acute and chronic cardiovascular consequences of OSAS. We previously showed that repeated episodes of end-apneic arousal or hypoxia and consequent sleep fragmentation are associated with an increase in nocturnal blood pressure that can lead to sustained hypertension and left ventricular hypertrophy (*9–11*). Both hypertension and left ventricular hypertrophy are strong cardiovascular risk factors (*12*) and are associated with poor prognosis in patients with OSAS (*13*). However, the mechanisms by which recurrent nocturnal surges in blood pressure and sympathetic activity in OSAS patients lead to cardiovascular disease or abnormal autonomic control of the heart during the daytime are unclear.

Changes in the function of the cardiac autonomic nervous system are a powerful predictor of cardiac death in patients with cardiac disease (*14*, *15*), but little is known of the mechanisms by which this system is regulated. Recent studies have suggested that nitric oxide (NO) may play an important role in such regulation. Evidence indicates that NO may attenuate cardiovascular end-organ responses to sympathetic stimulation (*16*). In addition, central attenuation by NO of baroreflex-mediated vagal control has been described (*16*, *17*). OSAS patients experience endothelial dysfunction, a subclinical condition that is associated with atherosclerosis and which manifests both as an abnormal arterial reactivity to NO-dependent vasodilators (*18*) and as a reduced plasma level of NO in the morning (*19*).

In the present work, we investigated the relations of daytime baroreflex sensitivity (BRS) and the plasma level of NO to the severity of OSAS. We have also examined the effects of nocturnal therapy with continuous positive airway pressure (CPAP) both on baroreflex function during the daytime and on the plasma level of NO in patients with moderate to severe OSAS.

Methods

Subjects

Fifty-one consecutive male patients with OSAS, aged 28 to 71 years (mean±SD, 53.4±10.0 years), and 29 age-matched healthy males without OSAS (50.1±11.3 years) were included in the study. None of the subjects had diabetes mellitus, chronic obstructive lung disease, coronary or valvular heart disease, congestive heart failure, renal failure, or endocrine dysfunction. Moreover, none of them were taking antihypertensive agents at the time of enrollment in the study. The OSAS patients were divided into two subgroups: those with an apnea-hypopnea index (AHI) of ≥20 episodes per hour (n=40), and those with an AHI of <20/h (n=11). The control group was recruited from healthy males aged 40 to 60 years who were free from medication and had no history of cardiovascular disease or diabetes mellitus. The electrocardiograms, echocardiographic findings, and blood pressure of the control subjects were normal. The study protocol was approved by the appropriate institutional review committee, and subjects provided written consent to participation after being informed in detail of the purpose and methods of the study.

Sleep Analysis

All subjects underwent standard polysomnography (Alice III instrument; Respironics, Murrysville, USA) with pulse oximetry. Apnea, hypopnea, sleep stages, and electroencephalographic arousal were scored according to established criteria (*20–22*). OSAS was diagnosed when the AHI was ≥5/h (*20*).

After the initial polysomnographic evaluation, OSAS

Table 2. Multiple Regression Analysis Based on a Stepwise Forward Selection Method of Factors Contributing to the Decreased Valsalva Ratio and Baroreflex Sensitivity in 51 Patients with OSAS

Factor	*F*	β	*p*
Valsalva ratio			
Age	4.3	−0.004	<0.05
Arousal index	28.0	−0.002	<0.0001
BRSI phase IV			
Oxygen desaturation time	8.0	−0.009	<0.01

OSAS, obstructive sleep apnea syndrome; BRSI, baroreflex sensitivity index.

Table 3. Characteristics of OSAS Patients (n=14) before and during Treatment with CPAP

Characteristic	Before CPAP	During CPAP
AHI (/h)	53.0±11.7	9.5±3.5*
Lowest oxygen saturation (%)	69.4±7.6	88.0±3.5*
Oxygen desaturation time (min)	123.0±92.2	2.9±2.7*
Arousal index (/h)	48.5±8.8	14.1±5.8*
Stage 1 sleep (%)	36.3±13.1	20.9±11.5*
Stage 2 sleep (%)	47.5±15.2	50.2±20.2
Stages 3 + 4 sleep (%)	2.1±5.3	5.8±8.3
REM sleep (%)	9.7±5.1	19.7±7.4*

Data are means±SD. *p<0.05 *vs*. before CPAP. OSAS, obstructive sleep apnea syndrome; AHI, apnea-hypopnea index; REM, rapid eye movement; CPAP, continuous positive airway pressure.

patients with an AHI of ≥20/h, who have a poorer outcome compared with those with an AHI of <20/h (*23*), were randomized into groups that received CPAP (Respironics) for 3 months (CPAP group, n=21) or did not receive such treatment (non-CPAP group, n=19). During the 3-month treatment period, seven patients discontinued CPAP (five received treatment with an oral appliance and two underwent conventional tonsillectomy), with the result that 19 patients in the non-CPAP group and 14 patients in the CPAP group completed the trial. Six patients in the non-CPAP group were prescribed calcium antagonists during the 3-month treatment period.

On the night after the baseline sleep analysis, those patients assigned to receive CPAP underwent overnight titration of the pressure to adjust it so as either to abolish apnea, hypopnea, and oxygen desaturation or to determine the highest level tolerated. Urine samples were obtained on the two consecutive days involving overnight sleep analysis (both before and during treatment with CPAP), and 24-h urinary catecholamine excretion was measured. We also assessed the effects of CPAP treatment on pulse wave velocity (PWV) and plasma NO concentration. The concentration of NO in plasma was measured by a colorimetric method (Nitrate/Nitrite Colorimetric Assay Kit; Cayman Chemical, Ann Arbor, USA) that detects NO, NO_2^-, and NO_3^- (together designated NO_x) (*24*). Plasma NO_x was measured in the early morning on the days before and after the first night of CPAP treatment, and brachial-ankle PWV was measured with a volume-plethysmographic apparatus (PWV/ABI; Nihon Colin, Komaki, Japan) before and after 3 months of CPAP treatment. Patients in the CPAP group were sent home with a pressure device and instructed to use it for at least 4 h per night on at least 70% of nights. The patients visited their hospitals once a month, and compliance with the pressure treatment protocol was assessed each month from each individual's recorded usage of the pressure device.

Cardiovascular Autonomic Tests

The cardiovascular autonomic response of all subjects was evaluated with the Valsalva test (*25*), which was repeated in patients in the CPAP and non-CPAP groups after the 3-month treatment period. Beat-to-beat arterial blood pressure was measured noninvasively with a tonometric device (BP-508SP; Nihon Colin). With the subjects sitting upright and their nasal airflow stopped with a clip, the tonometric sensor was positioned on the radial artery at the same height as the heart. The subjects performed the Valsalva test by expiring into a rubber tube connected to a mouthpiece and maintaining a pressure of 40 mmHg, as revealed by a mercury manometer, for 10 s. Both blood pressure and the R–R interval on the electrocardiogram were continuously recorded until the hemodynamics of the subject had stabilized under the control condition. The BRS index (BRSI), defined as the slope of the linear relation between systolic blood pressure and the R–R interval on the electrocardiogram, was determined during the forced expiration and release (overshoot) phases (phases II and IV, respectively) of the Valsalva maneuver. We also calculated the ratio of the longest R–R interval in phase IV and the shortest R–R interval in phase II (Valsalva ratio). A regression line with a correlation coefficient of >0.8 and with a blood pressure change of >15 mmHg was used for analysis (*26*).

Statistical Analysis

Data are presented as the means±SD and were analyzed by analysis of variance followed by Scheffe's test. Parameters at baseline and after treatment with CPAP were compared with Student's paired *t*-test. Correlations were assessed by simple regression analysis and multiple regression analysis based on a stepwise forward selection method. All analyses were performed with the SPSS 12.0 software package (SPSS, Chicago, USA). A p value of <0.05 was considered statistically significant.

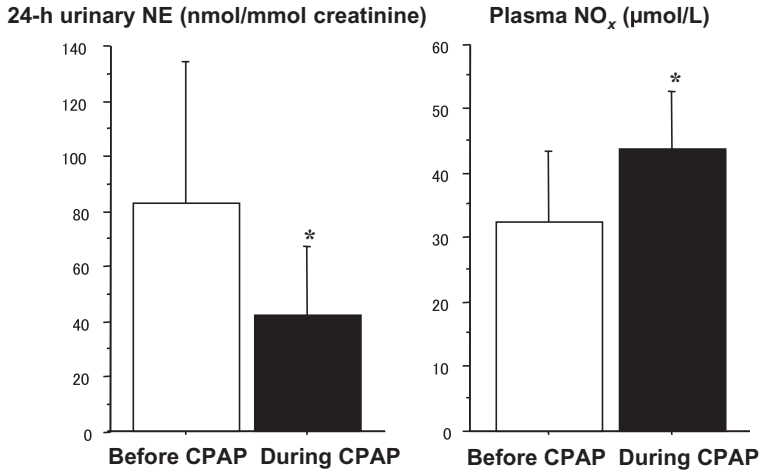

Fig. 1. *Effects of one night of CPAP treatment on 24-h urinary excretion and plasma concentration of NO_x in moderate to severe OSAS patients. *p < 0.05 vs. before CPAP. CPAP, continuous positive airway pressure; NE, norepinephrine; NO, nitric oxide.*

Results

The BRSI for phase IV of the Valsalva maneuver and plasma concentration of NO_x were significantly lower, whereas the AHI, oxygen desaturation time, arousal index, percentage of time spent in sleep stage 1, and systolic blood pressure were significantly greater, in OSAS patients with an AHI of ≥20/h than in control subjects or in patients with an AHI of <20/h (Table 1). The BRSI for phase IV of the Valsalva maneuver, Valsalva ratio, plasma concentration of NO_x, and the percentage of time spent in sleep stages 3 + 4 were significantly lower, whereas diastolic blood pressure was significantly greater, in patients with an AHI of ≥20/h or in those with an AHI of <20/h than in control subjects. The percentages of time spent in sleep stage 1, sleep stage 2, or rapid eye movement (REM) sleep did not differ significantly between patients with an AHI of <20/h and controls.

In patients with OSAS, the Valsalva ratio was significantly correlated with the AHI ($r=-0.45$, $p<0.001$), oxygen desaturation time ($r=-0.49$, $p<0.0005$), and the arousal index ($r=-0.57$, $p<0.0001$), whereas the BRSI for phase IV was correlated with oxygen desaturation time ($r=-0.37$, $p<0.01$) and the arousal index ($r=-0.37$, $p<0.01$) but not with the AHI. Multiple regression analysis, including the AHI, oxygen desaturation time, the arousal index, age, and systolic and diastolic blood pressures, revealed that oxygen desaturation time and the arousal index were the most significant contributing factors to the decreased BRSI for phase IV and the decreased Valsalva ratio, respectively, in OSAS patients (Table 2).

There were no significant differences in baseline sleep parameters or BRS values between the CPAP and non-CPAP groups. The percentages of sleep stage 1 and REM sleep were significantly decreased and increased, respectively, and the 24-h urinary excretion of norepinephrine was significantly reduced by treatment of OSAS patients with CPAP (Table 3; Fig. 1). The plasma concentration of NO_x was also significantly increased after one night of CPAP treatment (Fig. 1). Both the BRSI for phase IV and the Valsalva ratio were significantly increased, whereas systolic blood pressure and PWV were significantly decreased, in the CPAP group after the 3-month treatment period (Fig. 2). In contrast, these parameters remained unchanged in the non-CPAP group.

Discussion

We have shown that 1) the BRSI and plasma concentration of NO_x were significantly reduced in OSAS patients with an AHI of ≥20/h compared with those in healthy controls or in patients with an AHI of <20/h, whereas the Valsalva ratio was significantly lower in patients with an AHI of ≥20/h and in those with an AHI of <20/h than in control subjects; 2) the BRSI in OSAS patients was inversely related to the severity of sleep fragmentation and hypoxia; and 3) successful treatment of OSAS with CPAP was accompanied by an increase in both the BRSI and plasma NO_x concentration as well as by a decrease in 24-h urinary norepinephrine excretion. Our results suggest that a reduced BRSI and decreased NO production, possibly together with an increase in sympathetic activity, might play a role in the pathogenesis of cardiovascular complications in patients with OSAS.

Cardiovascular Autonomic Control in OSAS

We have now demonstrated a correlation between the severity of OSAS and the BRSI. In healthy individuals, sympathetic activity and average blood pressure are reduced during sleep

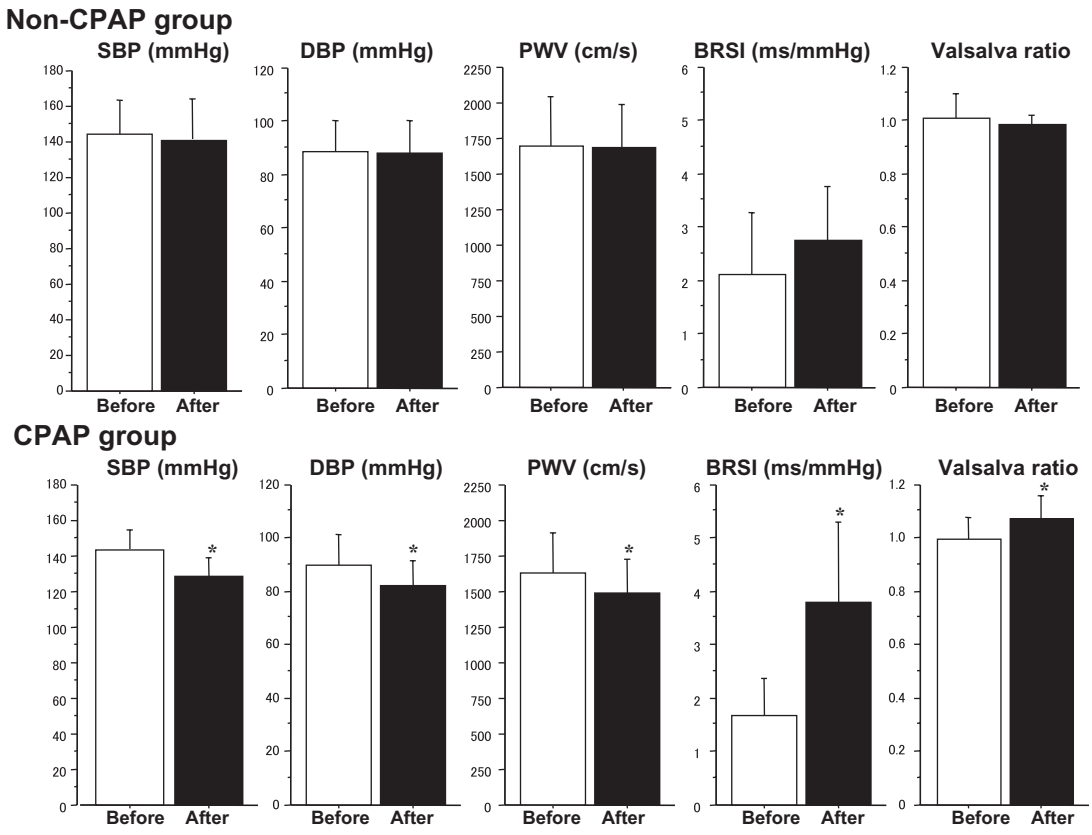

Fig. 2. *Effects of 3 months of CPAP treatment on blood pressure, brachial-ankle pulse wave velocity, and baroreflex sensitivity in moderate to severe OSAS patients. SBP, systolic blood pressure; DBP, diastolic blood pressure; PWV, pulse wave velocity; BRSI, baroreflex sensitivity index; CPAP, continuous positive airway pressure; CPAP group (n = 14), group that received CPAP for 3 months; non-CPAP group (n = 19), group that did not receive such treatment. *p < 0.05 vs. before CPAP.*

compared with those apparent during wakefulness (*9*, *27*). In patients with severe OSAS, however, these physiological changes do not occur. Moreover, such patients manifest increased blood pressure and sympathetic activity during wakefulness compared with healthy subjects (*8*, *28*). Clinical observations have linked sleep-disordered breathing, a condition characterized by repeated arousal and arterial oxygen desaturation, with hypertension (*29*). The reduction in the BRSI in OSAS patients may contribute to a further increase in blood pressure, leading to a vicious cycle that may have adverse clinical implications for the development of cardiovascular complications (*30*, *31*). The slope of the relation between heart period and arterial pressure has been shown to be similar when arterial pressure is increased pharmacologically (with phenylephrine) or physiologically (during phase IV of the Valsalva maneuver) (*32*, *33*). Given that a reduced BRS is a marker of high cardiovascular risk in several clinical conditions, including myocardial infarction (*15*) and congestive heart failure (*14*, *34*), our findings may explain the increased risk of cardiovascular complications in patients with OSAS.

Our present data show that oxygen desaturation time was inversely related to the Valsalva ratio in OSAS patients. This finding is similar to the results of our previous spectral analysis of heart rate variability on 24-h electrocardiograms recorded from OSAS patients (*35*). A reduction in heart rate variability in normotensive subjects has been shown to predict subsequent development of hypertension (*36*). The increase in blood pressure during phase IV of the Valsalva test stimulates baroreceptors, resulting in a reflex bradycardia secondary to an increased parasympathetic activity, all of which effects are abolished by administration of atropine (*37*). An altered parasympathetic activity may play a role in the development of cardiovascular disease, such as through daytime hypertension, in patients with OSAS (*38–40*).

Effect of CPAP Treatment on the BRSI in OSAS Patients

Treatment of OSAS patients with CPAP resulted in an increase in the daytime BRSI in addition to an improvement in sleep quality, normalization of nocturnal oxygenation, an increase in plasma NO_x concentration, and a decrease in 24-h urinary norepinephrine excretion. Acute application of CPAP

normalizes the augmented cardiovascular variability during sleep in patients with heart failure and OSAS (*41*). Moreover, long-term CPAP treatment normalized baroreflex dysfunction during sleep in normotensive patients with OSAS (*42*). Although the effects of CPAP on autonomic and cardiovascular alterations remain controversial, evidence suggests that appropriate CPAP treatment for OSAS ameliorates sympathetic hyperactivity as well as tonic activation of peripheral chemoreceptors and hyperresponsiveness to hypoxia (*43–45*).

The BRSI and plasma concentration of NO_x were significantly greater in control subjects or in patients with an AHI of <20/h than in patients with an AHI of ≥ 20/h. The increased sympathetic activity during sleep in patients with OSAS appears to carry over into the daytime (*26*). Increased sympathetic drive during wakefulness and repetitive surges in blood pressure during sleep may reduce BRS or reset the baroreflex function curve to higher levels of pressure (*40*, *46*). The reversible resetting and change in BRS associated with sleep are likely centrally mediated (*47*). NO plays a key role in regulation of vascular tone, the impairment of which is associated with the development of various pathological conditions such as hypertension and diabetes mellitus (*48–51*). NO is also implicated in the regulation of sympathetic outflow in the central nervous system (*33*). General inhibition of central NO synthesis or inhibition of NO synthesis in regions that are involved in modulation of sympathetic nerve activity results in increases in both arterial pressure and sympathetic outflow (*33*, *52*, *53*). NO may also act as an important mediator of cardiovascular autonomic control in OSAS (*16*, *17*). Furthermore, altered NO-mediated mechanisms in peripheral tissues and in the brain may contribute to the pathophysiology of several disease states, including heart failure (*54–56*). Our findings thus suggest that the BRSI of OSAS patients might be altered by sleep apnea itself or by such consequences as hypoxemia, sleep fragmentation, exaggerated sympathetic activity, a decreased plasma NO_x level, and increased catecholamine secretion. All of these sequelae may result in an impairment of baroreflex and cardiovascular reflex functions that carries over into the daytime. We have now shown that the blunted BRSI during the daytime in patients with moderate to severe OSAS is treatable by nocturnal CPAP.

Study Limitations

Six patients in the non-CPAP group were prescribed calcium antagonists and seven patients discontinued CPAP during the 3-month treatment period. The remaining randomized population of 33 OSAS patients in whom the effects of CPAP were evaluated in the present study may thus be somewhat biased. The effects of CPAP described in our study thus require verification with a large-scale placebo-controlled study.

Conclusions

We conclude that the daytime BRSI and NO production are impaired as a result of nocturnal oxygen desaturation, sleep fragmentation, and increased sympathetic activity in patients with moderate to severe OSAS. The alterations in the function of the cardiac autonomic nervous system and endothelial dysfunction likely contribute to the increased risk for cardiovascular morbidity and mortality in OSAS patients and are ameliorated by successful long-term treatment with CPAP.

References

1. Nieto FJ, Young TB, Lind BK, *et al*: Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. The Sleep Health Study. *JAMA* 2000; **283**: 1829–1836.
2. Shahar E, Whitney CW, Redline S, *et al*: Sleep-disordered breathing and cardiovascular disease. Cross-sectional results of the Sleep Heart Health Study. *Am J Respir Crit Care Med* 2001; **163**: 19–25.
3. Lavie P, Herer P, Peled R, *et al*: Mortality in sleep apnea patients: a multivariate analysis of risk factors. *Sleep* 1995; **18**: 149–157.
4. Martine JM, Carrizo SJ, Vicente E, Agusti AG: 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.
5. Tanigawa T, Tachibana N, Yamagishi K, *et al*: Relationship between sleep-disordered breathing and blood pressure levels in community-based samples of Japanese men. *Hypertens Res* 2004; **27**: 479–484.
6. Bradley TD, Floras JS: Sleep apnea and heart failure. Part I: Obstructive sleep apnea. *Circulation* 2003; **107**: 1671–1678.
7. Usui K, Bradley D, Spaak J, *et al*: Inhibition of awake sympathetic nerve activity of heart failure patients with obstructive sleep apnea by nocturnal continuous positive airway pressure. *J Am Coll Cardiol* 2005; **45**: 2008–2011.
8. Spaak J, Egri ZJ, Kubo T, *et al*: Muscle sympathetic nerve activity during wakefulness in heart failure patients with and without sleep apnea. *Hypertension* 2005; **46**: 1327–1332.
9. Noda A, Okada T, Hayashi H, *et al*: 24-hour ambulatory blood pressure variability in obstructive sleep apnea syndrome. *Chest* 1993; **103**: 1343–1347.
10. Noda A, Okada T, Yasuma F, *et al*: Cardiac hypertrophy in obstructive sleep apnea syndrome. *Chest* 1995; **107**: 1538–1544.
11. Noda A, Yasuma F, Okada T, *et al*: Influence of movement arousal on circadian rhythm of blood pressure in obstructive sleep apnea syndrome. *J Hypertens* 2000; **18**: 539–544.
12. Levy D, Garrison RJ, Savage DD, *et al*: Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990; **322**: 1561–1566.
13. Noda A, Okada T, Yasuma F, *et al*: Prognosis of the middle-aged and aged patients with obstructive sleep apnea syndrome. *Psychiatr Clin Neurosci* 1998; **52**: 79–85.
14. Mortara A, Rovere MTL, Pinna GD, *et al*: Arterial baroreflex modulation of heart rate in chronic heart failure. Clini-

cal and hemodynamic correlates and prognostic implications. *Circulation* 1997; **96**: 3450–3458.
15. La Rovere MT, Bigger JT, Marcus FI, *et al*: Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. *Lancet* 1998; **351**: 478–484.
16. Chowdhary S, Townend JN: Role of nitric oxide in the regulation of cardiovascular autonomic control. *Clin Sci* 1999; **97**: 5–17.
17. Liu J, Murakami H, Zucker IH: Effects of NO on baroreflex control of heart rate and renal nerve activity in conscious rabbits. *Am J Physiol* 1996; **270**: 1361–1370.
18. 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.
19. Ip MS, Lam B, Chan LY, *et al*: Circulating nitric oxide is suppressed in obstructive sleep apnea and is reversed by nasal continuous positive airway pressure. *Am J Respir Crit Care Med* 2000; **162**: 2166–2171.
20. The Report of an American Academy of Sleep Medicine Task Force. Sleep-related breathing disorders in adults: recommendation for syndrome definition and measurement techniques in clinical research. *Sleep* 1999; **22**: 667–689.
21. Rechtschaffen A, Kales A: A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stage of Human Subjects. Washington, DC, US Government Printing Office, 1968.
22. Sleep Disorders Atlas Task Force of the American Sleep Disorders Association: EEG arousals: scoring rules and examples. *Sleep* 1992; **15**: 173–184.
23. He J, Kryger MH, Zorick FJ: Mortality and apnea index in obstructive sleep apnea: experience in 385 male patients. *Chest* 1988; **94**: 9–14.
24. Kitaichi K, Wang L, Takagi K, *et al*: Decreased antipyrine clearance following endotoxin administration: *in vivo* evidence of the role of nitric oxide. *Antimicrob Agents Chemother* 1999; **43**: 2697–2701.
25. Benarroch EE, Sandoni P, Low PA: The Valsalva maneuver, in Low PA (ed): Clinical Autonomic Disorders. Boston, Toronto, and London, Little, Brown Co, 1993, pp 209–215.
26. Huikuri H, Pikkujamsa SM, Airaksinen J, *et al*: Sex-related differences in autonomic modulation of heart rate in middle-aged subjects. *Circulation* 1996; **94**: 122–125.
27. Somers VK, Dyken ME, Mark AL, Abboud FM: Sympathetic-nerve activity during sleep in normal subjects. *N Engl J Med* 1993; **328**: 303–307.
28. Somers VK, Dyken ME, Clary MP, *et al*: Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest* 1995; **96**: 1897–1904.
29. Young T, Peppard P, Palta M: Population-based study of sleep-disordered breathing as a risk factor for hypertension. *Arch Intern Med* 1997; **157**: 1746–1752.
30. Cortelli P, Parchi P, Sforza E, *et al*: Cardiovascular autonomic dysfunction in normotensive awake subjects with obstructive sleep apnea syndrome. *Clin Autonom Res* 1994; **4**: 57–62.
31. Sforza E, Parchi P, Contin M, *et al*: Do autonomic cardiovascular reflexes predict the nocturnal rise in blood pressure in obstructive sleep apnea syndrome? *Blood Pressure* 1994; **3**: 295–302.
32. Eckberg DL: Parasympathetic cardiovascular control in human disease: a critical review of methods and results. *Am J Physiol* 1980; **239**: H581–H593.
33. Palmero HA, Caeiro TF, Iosa DJ, Bas J: Baroreceptor reflex sensitivity index derived from phase 4 of the Valsalva maneuver. *Hypertension* 1981; **3** (Suppl II): II-134–II-137.
34. Wang W, Ma R: Cardiac sympathetic afferent reflexes in heart failure. *Heart Fail* 2000; **5**: 57–71.
35. Noda A, Yasuma F, Okada T, *et al*: Circadian rhythm of autonomic activity in patients with obstructive sleep apnea syndrome. *Clin Cardiol* 1998; **21**: 271–276.
36. Singh JP, Larson MG, Tsuji H, *et al*: Reduced heart rate variability and new-onset hypertension: insights into pathogenesis of hypertension: the Framingham Heart Study. *Hypertension* 1998; **32**: 293–297.
37. Eckberg D: Parasympathetic cardiovascular control in human disease: a critical review of methods and results. *Am J Physiol* 1980; **239**: H581–H593.
38. Nelson RA, Yu H, Ziegler MG, *et al*: Continuous positive airway pressure normalizes cardiac autonomic and hemodynamic responses to a laboratory stressor in apneic patients. *Chest* 2001; **119**: 1092–1101.
39. Khoo MC, Belozeroff V, Berry RB, *et al*: Cardiac autonomic control in obstructive sleep apnea. Long-term CPAP therapy. *Am J Respir Crit Care Med* 2001; **164**: 807–812.
40. Narkiewicz K, Pesek CA, Kato M, Phillips BG, Davison DE, Somers VK: Baroreflex control of sympathetic nerve activity and heart rate in obstructive sleep apnea. *Hypertension* 1998; **32**: 1039–1043.
41. Tkacova R, Dajani HR, Rankin F, *et al*: Continuous positive airway pressure improves nocturnal baroreflex sensitivity of patients with heart failure and obstructive sleep apnea. *J Hypertens* 2000; **18**: 1257–1262.
42. Bonsignore MR, Prati G, Insalaco G, *et al*: Continuous positive airway pressure treatment improves baroreflex control of heart rate during sleep in severe obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 2002; **166**: 279–286.
43. Waravdekar NV, Sinoway LI, Zwillich CW, *et al*: Influence of treatment on muscle sympathetic nerve activity in sleep apnea. *Am J Respir Crit Care Med* 1996; **153**: 1333–1338.
44. Narkiewicz K, Borne PJH, Pesek CA, *et al*: Selective potentiation of peripheral chemoreflex sensitivity in obstructive sleep apnea. *Circulation* 1999; **99**: 1183–1189.
45. Narkiewicz K, Kato M, Phillips BG, *et al*: Nocturnal continuous positive airway pressure decreases daytime sympathetic traffic in obstructive sleep apnea. *Circulation* 1999; **100**: 2332–2335.
46. Ogawa Y, Kanbayashi T, Saito Y, *et al*: Total sleep deprivation elevates blood pressure through arterial baroreflex resetting: a study with microneurographic technique. *Sleep* 2003; **26**: 986–989.
47. Bristow JR, Honour AJ, Pickring TG, *et al*: Cardiovascular and respiratory change during sleep in normal and hypertensive subjects. *Cardiovasc Res* 1969; **3**: 476–485.
48. Panza J, Quyyumi AA, Brush JE, Epstein SE: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Eng J Med* 1990; **323**: 22–27.
49. Lin KY, Asagami T, Tsao PS, *et al*: Impaired nitric oxide

synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. *Circulation* 2002; **106**: 987–992.
50. Hatta T, Nakata T, Harada S, *et al*: Lowering of blood pressure improves endothelial dysfunction by increase of nitric oxide production in hypertensive rats. *Hypertens Res* 2002; **25**: 455–460.
51. Furumoto T, Saito N, Dong J, *et al*: Association of cardiovascular risk factors and endothelial dysfunction in Japanese hypertensive patients: implications for early atherosclerosis. *Hypertens Res* 2002; **25**: 475–480.
52. Tseng CJ, Lin HY, Lin HC, *et al*: Cardiovascular effects of nitric oxide in the brain stem nuclei of rats. *Hypertension* 1996; **27**: 36–42.
53. Zhang K, Mayhan WG, Patel KP: Nitric oxide within the paraventricular nucleus mediates changes in renal sympathetic nerve activity. *Am J Physiol* 1997; **273**: 864–872.
54. Smith CJ, Sun D, Hoegler C, *et al*: Reduced gene expression of vascular endothelial NO synthase and cyclooxygenase-1 in heart failure. *Circ Res* 1996; **78**: 58–64.
55. Patel KP, Zhang K, Zucker IH, *et al*: Decreased gene expression of neuronal nitric oxide synthase in hypothalamus and brainstem of rats in heart failure. *Brain Res* 1996; **734**: 109–115.
56. Zhang K, Zucker IH, Patel KP: Altered number of diaphorase (NOS) positive neurons in the hypothalamus of rats with heart failure. *Brain Res* 1998; **786**: 219–225.