Basic Science Investigations

Respiration

Respiration 2009;77:85–90
DOI: 10.1159/000165630

Received: May 26, 2008
Accepted after revision: July 25, 2008
Published online: October 22, 2008

Increased Plasma Levels of Asymmetric Dimethylarginine and Soluble CD40 Ligand in Patients with Sleep Apnea

Antonia Barceló[a] Mónica de la Peña[b] Olga Ayllón[a] M. Victoria Vega-Agapito[e] Javier Piérola[c] Gerardo Pérez[a] Constancio González[e] Alberto Alonso[b] Alvar G.N. Agustí[b, d]

[a]Servei de Anàlisis Clíniques, [b]Servei de Pneumologia y [c]Unitat de Investigació, Hospital Universitari Son Dureta y [d]Fundació Caubet-Cimera, Palma de Mallorca, [e]Universidad de Valladolid, Valladolid, y Instituto de Salud Carlos III (CIBER de Enfermedades Respiratorias), Madrid, España

Key Words
Sleep apnea · Biomarkers · Endothelial dysfunction · Atherosclerosis · Obesity

Abstract
Background: Cardiovascular (CV) diseases are a leading cause of mortality and they are frequent in patients with the obstructive sleep apnea syndrome (OSAS). ***Objectives:*** In this study we investigated if OSAS influences CV function independently of other CV risk factors frequently present in these patients (e.g. obesity, high blood pressure). ***Methods:*** We compared plasma markers of endothelial dysfunction, asymmetric dimethylarginine (ADMA) and endothelin-1 (ET-1), and atherosclerosis progression (soluble fraction of the CD40 ligand, sCD40L) in OSAS patients with (n = 23) and without (n = 18) concurrent CV risk factors, as well as in healthy subjects (n = 23). ***Results:*** Plasma ADMA ($p < 0.01$) and sCD40L ($p < 0.05$) were abnormally increased in patients with OSAS versus healthy controls, but they were not influenced by the presence or absence of CV risk factors in OSAS. ET-1 levels were not different between the three groups of subjects studied. ***Conclusions:*** OSAS is associated with endothelial injury and atherosclerosis progression independently of other CV risk factors.

Introduction

Cardiovascular (CV) diseases are the leading cause of mortality in developed countries [1, 2]. Arterial hypertension, obesity, hypercholesterolemia and diabetes are well-established CV risk factors [3–5]. CV morbidity and mortality are increased in patients with obstructive sleep apnea syndrome (OSAS) [6–11]. However, the mechanisms underlying this association are unclear because most of the established CV risk factors (such as obesity, hypertension, hypercholesterolemia, diabetes and smoking) often coexist in patients with OSAS [12].

Recent reports have suggested that OSAS may be associated with early development of atherosclerosis [13, 14] and endothelial dysfunction [15–17].

The endothelium plays a pivotal role in the control of vascular tone by releasing several vasoactive substances such as nitric oxide (NO) or endothelin-1 (ET-1) [18, 19]. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO production [20]. There is evidence indicating that increased levels of ADMA can produce endothelial dysfunction and increase CV risk [21–24]. Several studies have shown that abnormalities in NO metabolism and endothelial dysfunction occur in OSAS [15, 25]. Furthermore, elevated ADMA plasma levels have

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0025–7931/09/0771–0085$26.00/0

Accessible online at:
www.karger.com/res

Dr. A. Barceló
Servei de Anàlisis Cliniques
Hospital Universitari Son Dureta, C/ Andrea Doria 55
ES–07014 Palma de Mallorca (Spain)
Tel. +34 971 175 490, Fax +34 971 175 490, E-Mail abarcelo@hsd.es

also been described in OSAS [26]. However, the potential role of the confounding factors mentioned above was not specifically considered. On the other hand, ET-1, a potent vasoconstrictor produced by endothelial cells, has also been implicated in the pathogenesis of endothelial dysfunction and CV disease [27, 28], but studies in patients with OSAS have yielded conflicting results [29, 30].

Increased plasma levels of the soluble fraction of the CD40 ligand (sCD40L) are considered a surrogate marker for atherosclerosis and increased CV risk [31]. This is because the CD40-CD40L system is a central signaling mechanism in the pathogenesis of atherosclerosis and a variety of cells associated with atheroma, including endothelial cells, smooth muscle cells, mononuclear phagocytes and platelets, express CD40L when activated [31–33]. Increased sCD40L plasma levels have been detected in OSAS [34], but the potential confounding effects of classical CV risk factors discussed above were not specifically considered in this study.

To distinguish the role of OSAS from that of these other established risk factors, we compared markers of endothelial dysfunction (ADMA and ET-1) and atherosclerosis progression (sCD40L) in patients with OSAS in the absence or presence of classical CV risk factors, as well as in healthy controls.

Methods

Subjects and Ethics

We studied 41 male patients with OSAS, 23 with and 18 without traditional CV risk factors (obesity, hypercholesterolemia, diabetes, hypertension and smoking). The diagnosis of OSAS was established by full polysomnography (E-Series Compumedics, Abbotsford, Australia) and included recording of oronasal flow, thoracoabdominal movements, electrocardiography, submental and pretibial electromyography, electrooculography, electroencephalography and transcutaneous measurement of arterial oxygen saturation. OSAS was defined when the apnea-hypopnea index was higher than 10. Apnea was defined by the absence of airflow for more than 10 s. Hypopnea was defined as any airflow reduction that lasted more than 10 s and resulted in arousal or oxygen desaturation. We considered desaturation a decrease in arterial oxygen saturation greater than 4%. The apnea-hypopnea index was defined as the sum of the number of apneas plus hypopneas per hour of sleep. Patients were considered obese when their body mass index (BMI) was higher than 30. Arterial hypertension was diagnosed if systolic blood pressure (SBP) was ≥140 mm Hg and/or diastolic pressure (DBP) was ≥90 mm Hg or the individual was under specific treatment.

As a control group, we also studied 23 healthy nonsmoking, nonobese males of similar age without a personal or familial history of CV disease or diabetes. In these subjects, the diagnosis of OSAS was excluded by a cardiorespiratory sleep study that recorded nasal flow, thoracic movements, heart rate, snoring, body position and transcutaneus oxyhemoglobin saturation (EdenTec Corp., Eden Prairie, Minn., USA).

No participant suffered from any chronic disease (chronic obstructive pulmonary disease, liver cirrhosis, thyroid dysfunction, rheumatoid arthritis, chronic renal failure and/or psychiatric disorders), or was taking any type of medication. The study was approved by the Ethics Committee of our institution, and all participants signed their consent after being fully informed of its goal and characteristics.

Blood Sampling

Venous blood samples were taken between 8 and 10 a.m. after an overnight fast and transferred into tubes (10 ml) containing (or not) EDTA. Samples were immediately centrifuged and stored at –80°C until analysis after separating serum and plasma aliquots.

Biochemical Analysis

Glucose and cholesterol concentrations were determined by standard enzymatic methods on a Hitachi 917 biochemical analyzer (Roche Diagnostics, Indianapolis, Ind., USA). HDL cholesterol was measured by a homogeneous, enzymatic colorimetric method using a commercial reagent set (Roche Diagnostics). LDL cholesterol was calculated using the Friedewald equation.

ADMA Assay

ADMA concentration was quantified by high-performance liquid chromatography with fluorescence detection. ADMA was previously extracted from plasma by Oasis MCX solid-phase extraction columns (Waters), evaporated under nitrogen and derivated with *o*-phthaldialdehyde reagent. After derivatization, ADMA was separated using a gradient method by reversed-phase chromatography on a 4.6 × 150-mm SunFire C_{18} 3.5-μm column (Waters). Mobil phase A consists of phosphate buffer, acetonitrile, methanol and tetrahydrofuran (96:2:2:0.2) and mobile phase B consists of phosphate buffer, acetonitrile, methanol and tetrahydrofuran (54:30:16:0.4).

We used a flow rate of 1 ml/min and a temperature of 34°C. Fluorescence detection was performed at an excitation and emission set at 330 and 450 nm, respectively. The inter-assay coefficient of variation for ADMA was 1.6% and the recuperation was 103%.

sCD40L and ET-1

sCD40L and ET-1 plasma levels were determined by enzyme-linked inmunosorbent assay using a commercially available kit (R & D Systems Inc., Minneapolis, Minn., USA, and BioMedica Diagnostic Systems, Vienna, Austria, respectively). Measurements were always done in duplicate, and mean values were used for analysis.

Statistical Analysis

Results are presented as means ± standard error of the mean (SEM). One-way analysis of variance followed by post-hoc contrast (least significant difference) if appropriate, and the χ^2 test for proportions were used to assess the statistical significance of differences between groups. Correlations between variables were explored using Spearman's rank test. Statistical significance was defined as $p < 0.05$.

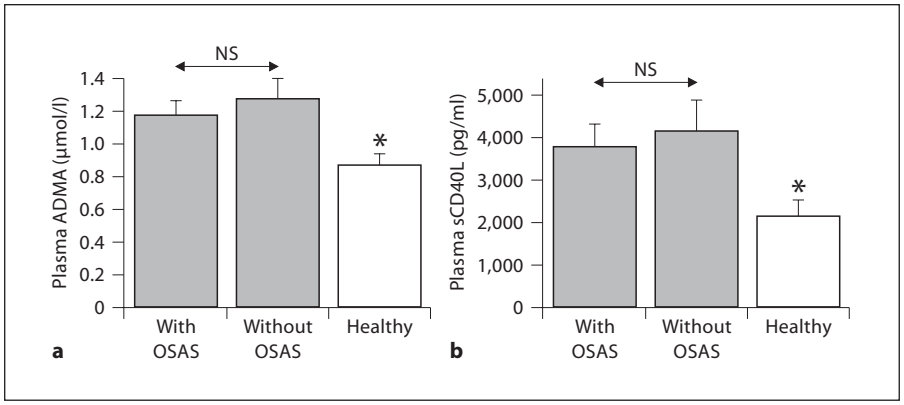

Fig. 1. Mean ± SEM values of ADMA (**a**) and sCD40L (**b**) in the three groups of subjects studied. * $p < 0.05$, NS = nonsignificant.

Results

We studied 23 patients with OSAS and CV risk factors, 18 with OSAS but without CV risk factors and 23 healthy subjects. OSAS patients with CV risk factors included 9 patients who had more than 1 risk factor and 2 patients with arterial hypertension alone, 2 with hypercholesterolemia, 6 obese and 4 smokers.

Table 1 shows the main clinical characteristics and biochemical parameters of the participants included in this study. OSAS patients with CV risk factors (n = 23) included 2 patients with arterial hypertension (SBP ≥140 mm Hg and/or DBP ≥90 mm Hg), 2 patients with hypercholesterolemia (cholesterol ≥220 mg/dl and/or LDL ≥140 mg/dl), 6 obese patients (BMI ≥30) and 4 smokers. Nine patients presented more than 1 of these risk factors or in combination with glucose >126 mg/dl as the threshold for diabetes.

Age was not significantly different between groups, although healthy subjects tended to be slightly younger than patients with OSAS. Apnea-hypopnea index, mean and minimal nocturnal oxygen saturation and daytime somnolence were similar in both groups of patients with OSAS (table 1). BMI, SBP and DBP values were significantly higher in patients with CV risk factors (vs. both healthy subjects and patients with OSAS and no other risk factor) (table 1).

Figure 1 shows the mean ± SEM values of the plasma levels of ADMA and sCD40L in the three groups of subjects studied. Compared to healthy controls (0.87 ± 0.07 μmol/l), the levels of ADMA were significantly increased in patients with OSAS, both with (1.17 ± 0.09 μmol/l) and without (1.27 ± 0.13 μmol/l) CV risk factors. By contrast, values were not significantly different between the two groups of patients with OSAS. A similar pattern was observed for sCD40L (fig. 1b) because OSAS patients, both with (3,776 ± 537 pg/ml) and without CV risk factors (4,137 ± 744 pg/ml), showed similar levels of sCD40L, but these were significantly higher than those detected in healthy controls (2,148 ± 392 pg/ml).

Finally, ET-1 levels were similar in the three groups of subjects studied: 0.96 ± 0.39 (OSAS with CV risk factors) versus 0.64 ± 0.04 (OSAS without CV risk factors) versus 0.76 ± 0.18 fmol/ml (healthy subjects).

Interestingly, when data from all participants were pooled, we found a significant relationship ($r = 0.498$, $p < 0.001$) between sCD40L and ADMA plasma concentrations (fig. 2).

Table 1. Clinical characteristics of subjects studied

	OSAS with traditional risk factors (n = 23)	OSAS without traditional risk factors (n = 18)	Healthy subjects (n = 23)
Age, years	49 ± 2	44 ± 2	42 ± 2
AHI, events/h	38 ± 6	46 ± 4	3 ± 1[a]
Mean SaO_2	92 ± 1	92 ± 1	95 ± 1[a]
Min. SaO_2	79 ± 1	73 ± 5	88 ± 1[a]
Epworth scale	11 ± 1	12 ± 1	6 ± 1[a]
BMI	31 ± 1	27 ± 1[b]	26 ± 1[b]
SBP, mm Hg	136 ± 4	119 ± 2[b]	113 ± 3[b]
DBP, mm Hg	84 ± 2	73 ± 2[b]	75 ± 3[b]
Glucose, mg/dl	107 ± 5	104 ± 3	94 ± 2[b]
Cholesterol total, mg/dl	210 ± 7	200 ± 9	183 ± 7[b]
LDL cholesterol, mg/dl	129 ± 7	126 ± 9	112 ± 7[b]
Smoking, pack-years	20 ± 5	0[b]	0[b]

AHI = Apnea-hypopnea index; SaO_2 = arterial oxygen saturation; LDL = low-density lipoprotein. [a] $p < 0.05$ versus both groups of OSAS; [b] $p < 0.05$ versus OSAS with traditional risk factors.

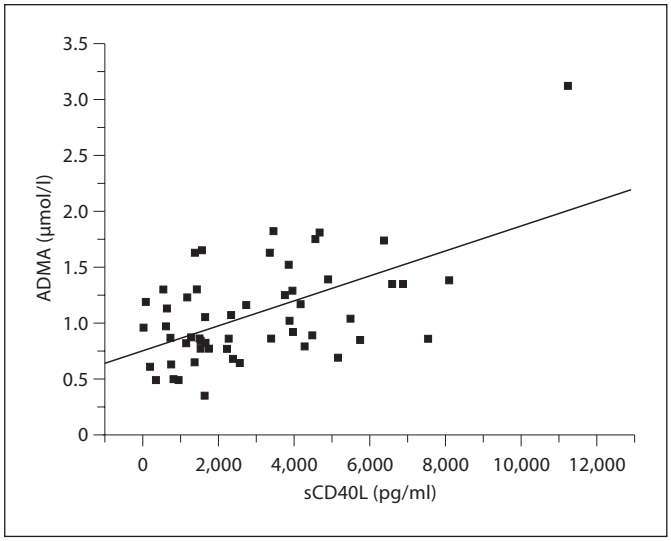

Fig. 2. Relationship between sCD40L and ADMA levels in all patients with OSAS.

Discussion

The main and novel finding of this study is that markers of endothelial dysfunction (ADMA) and atherosclerosis progression (sCD40L) were abnormal in patients with OSAS irrespectively of the presence or absence of other, more established CV risk factors (fig. 1). This indicates that OSAS is an independent CV risk factor.

The endothelium plays a pivotal role in the control of vascular tone by releasing several vasoactive substances such as NO or ET-1 [18, 19]. NO is synthesized from L-arginine by endothelial NO synthase [35–37] and is involved in a number of key CV regulatory mechanisms, including endothelium-dependent vasodilation, inhibition of platelet adhesion and aggregation, and inhibition of smooth muscle cell proliferation. ADMA is an endogenous inhibitor of NO production, and ADMA plasma levels are elevated in diseases characterized by endothelial dysfunction and impaired NO metabolism such as arterial hypertension, hypercholesterolemia and type 2 diabetes mellitus [22, 38]. The results of the multicenter CARDIAC study indicate that increased ADMA levels are an independent risk factor for coronary heart disease [24]. Abnormalities in NO metabolism and endothelial dysfunction occur in OSAS [15, 25]. In these patients, elevated ADMA plasma levels have also been described [26]. However, because other CV risk factors, including hypertension, hypercholesterolemia, diabetes and obesity, can also cause endothelial injury [39–41], it was unclear if the increased ADMA levels described in OSAS could be ascribed to the disease itself or not. Our results confirm that ADMA levels are increased in these patients and extend previous studies by showing that they can be elevated even in the absence of other CV risk factors, suggesting that OSAS is contributing to this abnormality. This interpretation is further supported by the observation that treatment of OSAS with continuous positive airway pressure reduces ADMA concentration in plasma [26].

By contrast, we found that ET-1 levels were not abnormal in patients with OSAS (irrespective of the presence or absence of other CV risk factors). Previous reports in these patients have yielded conflicting results [29, 30]. It is likely that the discrepancy between results is in part due to the differences in patient characteristics. Former studies that have reported elevated ET-1 levels studied severer OSAS [42, 43]. On the other hand, Jordan et al. [30] found increased plasma levels of the ET-1 precursor (but not of ET-1 itself), and suggested that, perhaps, cross-reactivity between different forms of endothelins explain the variable results of ET-1 published so far in OSAS.

The CD40-CD40L system plays a central role in the pathogenesis of atherosclerosis. A variety of cells associated with atheroma, including endothelial cells, smooth muscle cells, mononuclear phagocytes and platelets, express CD40L when activated [31–33]. An increased plasma level of sCD40L is considered a surrogate marker for atherosclerosis and increased CV risk [31]. Increased sCD40L plasma levels have been described in patients with OSAS, but the potential confounding effect of other CV risks was not considered [34]. Our results confirm that OSAS is associated with increased plasma levels of sCD40L and show that this effect occurs irrespective of other CV risk factors. This further supports the notion that OSAS is a CV risk factor in itself.

Finally, we found that the plasma levels of ADMA correlate with those of sCD40L. Recent studies have shown that OSAS is associated with early signs of atherosclerosis [13]. The mechanism(s) by which OSAS ultimately leads to atherosclerosis is (are) incompletely understood. Our observation of a relationship between a marker of endothelial dysfunction and a marker of atherosclerosis progression (fig. 2) further supports that OSAS damages the arterial wall and contributes to atherogenesis.

Potential Limitations

We did not measure the plasma levels of ADMA and sCD40L after continuous positive airway pressure treatment, and this may be a limitation in the assessment of

the independent effects of OSAS on these markers. Therefore, our results require confirmation in large populations to determine the impact of all these observations on CV risk of OSAS patients.

In summary, we found that plasma levels of ADMA and sCD40L were increased in patients with OSAS irrespective of the presence or absence of other CV risk factors. These observations support the notion that OSAS plays an independent role in endothelial injury and atherosclerosis progression.

Acknowledgments

We thank Margalida Bosch and Mónica Iglesias for their assistance in the coordination of the study. This study was supported in part by ABEMAR, SEPAR, Marató TV3 04/2410 and Fondo de Investigaciones Sanitarias 04/1593.

References

1 Heart disease and stroke statistics: Update. 2005. American Heart Association.
2 Murray CJ, Lopez AD: Mortality by cause for eight regions of the world: Global Burden of Disease Study. Lancet 1997;349:1269–1276.
3 Dzau VJ, Antman EM, Black HR, Hayes DL, Manson JE, Plutzky J, Popma JJ, Stevenson W: The cardiovascular disease continuum validated: clinical evidence of improved patient outcomes. I. Pathophysiology and clinical trial evidence (risk factors through stable coronary artery disease). Circulation 2006;114:2850–2870.
4 Zaninotto P, Mindell J, Hirani V: Prevalence of cardiovascular risk factors among ethnic groups: results from the Health Surveys for England. Atherosclerosis 2007;195:e48–e57.
5 Smith SC Jr: Multiple risk factors for cardiovascular disease and diabetes mellitus. Am J Med 2007;120(suppl 1):S3–S11.
6 Nieto FJ, Young TB, Lind BK, Shahar E, Samet JM, Redline S, D'Agostino R, Newman AB, Lebowitz MD, Pickering TG: Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. Sleep Heart Health Study. JAMA 2000;283:1829–1836.
7 Peker Y, Hedner J, Norum J, Kraiczi H, Carlson J: Increased incidence of cardiovascular disease in middle-aged men with obstructive sleep apnea. Am J Respir Crit Care Med 2005;166:159–165.
8 Shahar E, Whitney CW, Redline S, Lee ET, Newman AB, Nieto J, O´Connor GT, Boland LL, Schwartz JE, Samet JM: Sleep-disordered breathing and cardiovascular disease. Am J Respir Crit Care Med 2001;163:19–25.
9 Peppard P, Young T, Palta M, Skatrud J: Prospective study of the association between sleep-disordered breathing and hypertension. N Engl J Med 2000;342:1378–1384.
10 Marin JM, Carrizo SJ, Vicente E, Agusti AGN: Long-term cardiovascular outcomes in men with obstructive sleep apnea. Effects of treatment with CPAP. Lancet 2005;365:1046–1053.
11 Dincer HE, O'Neill W: Deleterious effects of sleep-disordered breathing on the heart and vascular system. Respiration 2006;73:124–130.
12 McNicholas WT, Bonsignore MR: Sleep apnoea as an independent risk factor for cardiovascular disease: current evidence, basic mechanisms and research priorities. Eur Respir J 2007;29:156–178.
13 Drager LF, Bortolotto LA, Lorenzi MC, Figueiredo AC, Krieger EM, Lorenzi-Filho G: Early signs of atherosclerosis in obstructive sleep apnea. Am J Respir Crit Care Med 2005;172:613–618.
14 de la Peña M, Barcelo A, Barbe F, Pierola J, Pons J, Rimbau E, Ayllon O, Agusti AG: Endothelial function and circulating endothelial progenitor cells in patients with sleep apnea syndrome. Respiration 2008;76:28–32.
15 Kato M, Roberts-Thomson P, Phillips BG, Haynes WG, Winnicki M, Accurso V, Somers VK: Impairment of endothelium-dependent vasodilation of resistance vessels in patients with obstructive sleep apnea. Circulation 2000;102:2607–2610.
16 Ip MSM, Tse HF, Lam B, Tsang KWT, Lam WK: Endothelial function in obstructive sleep apnea and response to treatment. Am J Respir Crit Care Med 2004;169:348–353.
17 Imadojemu VA, Gleeson K, Quraishi SA, Kunselman AR, Sinoway LI, Leuenberger UA: Impaired vasodilator responses in obstructive sleep apnea improved with continuous positive airway pressure therapy. Am J Respir Crit Care Med 2002;165:950–953.
18 Moncada S, Higgs A: The L-arginine-nitric oxide pathway. N Engl J Med 1993;329:2002–2012.
19 Cohen RA: The role of nitric oxide and other endothelium-derived vasoactive substances in vascular disease. Prog Cardiovasc Dis 1995;38:105–128.
20 Boger RH, Vallance P, Cooke JP: Asymmetric dimethylarginine (ADMA): a key regulator of nitric oxide synthase. Atheroscler Suppl 2003;4:1–3.
21 Vallance P, Leiper J: Cardiovascular biology of the asymmetric dimethylarginine: dimethylarginine dimethylaminohydrolase pathway. Arterioscler Thromb Vasc Biol 2004;24:1023–1030.
22 Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, Blaschke TF, Cooke JP: Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 1998;98:1842–1847.
23 Boger RH, Maas R, Schulze F, Schwedhelm E: Elevated levels of asymmetric dimethylarginine (ADMA) as a marker of cardiovascular disease and mortality. Clin Chem Lab Med 2005;43:1124–1129.
24 Schulze F, Lenzen H, Hanefeld C, Bartling A, Osterziel KJ, Goudeva L, Schmidt-Lucke C, Kusus M, Maas R, Schwedhelm E, Strodter D, Simon BC, Mugge A, Daniel WG, Tillmanns H, Maisch B, Streichert T, Boger RH: Asymmetric dimethylarginine is an independent risk factor for coronary heart disease: results from the multicenter Coronary Artery Risk Determination Investigating the Influence of ADMA Concentration (CARDIAC) study. Am Heart J 2006;152:493–498.
25 Ip MSM, Lam B, Chan LY, Zheng L, Tsang KWT, Fung PCW, Lam WK: 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.
26 Ohike Y, Kozaki K, Iijima K, Eto M, Kojima T, Ohga E, Santa T, Imai K, Hashimoto M, Yoshizumi M, Ouchi Y: Amelioration of vascular endothelial dysfunction in obstructive sleep apnea syndrome by nasal continuous positive airway pressure – possible involvement of nitric oxide and asymmetric N^G,N^G-dimethylarginine. Circ J 2005;69:221–226.
27 Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411–415.

28 Touyz RM, Schiffrin EL: Role of endothelin in human hypertension. Can J Physiol Pharmacol 2003;81:533–541.
29 Grimpen F, Kanne P, Schulz E, Hagenah G, Hasenfuss G, Andreas S: Endothelin-1 plasma levels are not elevated in patients with obstructive sleep apnoea. Eur Respir J 2000;15: 320–325.
30 Jordan W, Reinbacher A, Cohrs S, Grunewald RW, Mayer G, Ruther E, Rodenbeck A: Obstructive sleep apnea: plasma endothelin-1 precursor but not endothelin-1 levels are elevated and decline with nasal continuous positive airway pressure. Peptides 2005;26: 1654–1660.
31 Szmitko PE, Wang CH, Weisel RD, de Almeida JR, Anderson TJ, Verma S: New markers of inflammation and endothelial cell activation. Part I. Circulation 2003;108: 1917–1923.
32 Andre P, Nannizzi-Alaimo L, Prasad SK, Phillips DR: Platelet-derived CD40L: the switch-hitting player of cardiovascular disease. Circulation 2002;106:896–899.
33 Heeschen C, Dimmeler S, Hamm CW, van den Brand MJ, Boersma E, Zeiher AM, Simoons ML: Soluble CD40 ligand in acute coronary syndromes. N Engl J Med 2003; 348:1104–1111.
34 Kobayashi K, Nishimura Y, Shimada T, Yoshimura S, Funada Y, Satouchi M, Yokoyama M: Effect of continuous positive airway pressure on soluble CD40 ligand in patients with obstructive sleep apnea syndrome. Chest 2006;129:632–637.
35 Cooke JP, Dzau VJ: Derangements of the nitric oxide synthase pathway, L-arginine, and cardiovascular diseases. Circulation 1997; 96:379–382.
36 Cooke JP, Dzau VJ: Nitric oxide synthase: role in the genesis of vascular disease. Annu Rev Med 1997;48:489–509.
37 Busse R, Fleming I: Regulation and functional consequences of endothelial nitric oxide formation. Ann Med 1995;27:331–340.
38 Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, Imaizumi T: Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis. Circulation 1999;99: 1141–1146.
39 Stroes ES, Koomans HA, de Bruin TW, Rabelink TJ: Vascular function in the forearm of hypercholesterolaemic patients off and on lipid-lowering medication. Lancet 1995;346: 467–471.
40 Celermajer DS: Endothelial dysfunction: does it matter? Is it reversible? J Am Coll Cardiol 1997;30:325–333.
41 Arcaro G, Zamboni M, Rossi L, Turcato E, Covi G, Armellini F, Bosello O, Lechi A: Body fat distribution predicts the degree of endothelial dysfunction in uncomplicated obesity. Int J Obes Relat Metab Disord 1999; 23:936–942.
42 Phillips BG, Narkiewicz K, Pesek CA, Haynes WG, Dyken ME, Somers VK: Effects of obstructive sleep apnea on endothelin-1 and blood pressure. J Hypertens 1999;17:61–66.
43 Saarelainen S, Hasan J: Circulating endothelin-1 and obstructive sleep apnoea. Eur Respir J 2000;16:794–795.