Notice of Duplicate Publication

Hiroshi Yamamoto, Shinji Teramoto, Yasuhiro Yamaguchi, Yasuyoshi Ouchi: Effect of nasal continuous positive airway pressure treatment on plasma adrenomedullin levels in patients with obstructive sleep apnea syndrome: roles of nocturnal hypoxia and oxidant stress. *Hypertens Res* 2007; **30**: 1065–1076.

The above article published in Volume 30, No. 11 (November) is a duplicate publication published in *Sleep Medicine* as follows:

Hiroshi Yamamoto, Shinji Teramoto, Yasuhiro Yamaguchi, Yoko Hanaoka, Masaki Ishii, Shinichiro Hibi, Yasuyoshi Ouchi: Long-term oxygen administration reduces plasma adrenomedullin levels in patients with obstructive sleep apnea syndrome. *Sleep Med* 2007; **9**: 80–87.

With this notification, *Hypertension Research* is now withdrawing the article published in its 2007; **30** (11) issue.

Note from the Editor-in-Chief

Duplicate publication of essentially the same data constitutes a violation of copyright law and of the ethics of scientific publication. When there is doubt about the common source, overlapping, or coincidence of data in articles that are based on the same study, the author(s) must disclose any information about their contribution being under consideration or accepted by another journal at the time of submission of a manuscript.

Kazuyuki Shimada
Editor-in-Chief
Hypertension Research

Original Article

Effect of Nasal Continuous Positive Airway Pressure Treatment on Plasma Adrenomedullin Levels in Patients with Obstructive Sleep Apnea Syndrome: Roles of Nocturnal Hypoxia and Oxidant Stress

Hiroshi YAMAMOTO¹⁾, Shinji TERAMOTO¹⁾, Yasuhiro YAMAGUCHI¹⁾,
and Yasuyoshi OUCHI¹⁾

Obstructive sleep apnea syndrome (OSAS) is recognized as one of the risk factors of hypertension and cardiovascular disorders. In the current study, we hypothesized that the hypoxic stress and oxidative stress caused by obstructive sleep apnea would increase circulating adrenomedullin (ADM) levels in untreated OSAS patients as compared to an age and body mass index (BMI)-matched control group and an age-matched, but normal-BMI control group. We further hypothesized that nasal continuous positive airway pressure (nCPAP) treatment may decrease OSAS-induced hypoxic stress, oxidative stress and ADM levels. To examine these hypotheses, we measured circulating ADM and reactive oxygen species (ROS) from leukocytes before and after nCPAP therapy in OSAS patients. The circulating levels of ADM and amount of ROS in untreated OSAS patients were significantly greater than those in the controls. No differences in ADM levels were found between the increased-BMI controls and normal-BMI controls. We observed that nCPAP treatment decreased sleep apneas, nocturnal oxyhemoglobin desaturation, the circulating ADM, and ROS production by leukocytes in OSAS patients. The ADM levels were associated with the magnitude of oxyhemoglobin desaturation rather than the number of sleep apneas. These observations suggest that nCPAP therapy could reduce OSAS-induced nocturnal hypoxemia, generation of ROS, and ADM in patients with OSAS. (*Hypertens Res* 2007; 30: 1065–1076)

Key Words: adrenomedullin, sleep apnea, hypertension, oxyhemoglobin desaturation intensity, hypoxic stress

Introduction

It has been recognized that obstructive sleep apnea syndrome (OSAS) is one of the important risk factors of cardiovascular

disorders, including hypertension, ischemic heart disease and cerebrovascular diseases (1–5). Although obstructive sleep apnea (OSA) itself, OSA-related autonomic dysfunction and OSA-induced hypoxic stress may be dependently or independently involved in the development of cardiovascular disor-

From the ¹⁾Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

This work was supported in part by a Grant from the Mitsui Life Social Welfare Foundation Fund in Japan and by a research grant from the Mitsukoshi Health and Welfare Foundation Fund in Japan and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (17590781).

Address for Reprints: Shinji Teramoto, M.D., Department of Geriatric Medicine, Faculty of Medicine, The University of Tokyo, 7–3–1 Hongo, Bunkyo-ku, Tokyo 113–8655, Japan. E-mail: shinjit-ky@umin.ac.jp

Received December 8, 2006; Accepted in revised form June 29, 2007.

Table 1. The Characteristics of the Subjects

	Group		
	OSAS group	Control group (BMI<24)	Control group (BMI≥24)
Number of subjects	48	40	48
Age (years)	49.8±2.2	48.2±2.1	49.6±2.1
BMI	32.4±0.9	21.8±1.1	30.7±1.4
Systolic BP (mmHg)	145.1±8.7	138.1±6.7	142.1±10.7
Diastolic BP (mmHg)	84.1±3.2	78.1±2.8	82.1±2.9
HR (bpm)	74.1±3.2	70.1±2.6	72.1±2.9
Total cholesterol (mg/dL)	202.9±10.9	194.3±10.8	198.3±12.5
High-density lipoprotein cholesterol (mg/dL)	41.0±2.4	44.2±2.1	39.6±2.9
Low-density lipoprotein cholesterol (mg/dL)	128.7±5.2	121.1±5.8	130.1±5.8
Triglyceride (mg/dL)	150.1±12.7	128.0±10.9	148.0±11.8
Fasting plasma glucose (mg/dL)	98.6±1.3	94.3±1.2	97.3±1.2
HbA1c (%)	5.6±0.1	5.2±0.1	5.5±0.1
Total sleep time (min)	348.1±20.3*	444.3±20.9	424.3±22.2
ESS	14.6±0.9*	1.6±1.9	3.6±1.9

* $p<0.001$ vs. control group (BMI≥24). Data are presented as mean±SEM. OSAS, obstructive sleep apnea syndrome; BMI, body mass index; BP, blood pressure; HR, heart rate; %TST, percentage of total sleep time; HbA1c, hemoglobin A1c; ESS, Epworth sleepiness scale.

ders, the exact mechanism remains to be elucidated (6, 7).

One of the potential mechanisms is that OSAS-induced hypoxic stress and oxidative stress may increase circulating inflammatory mediators including adhesion molecules, inflammatory cytokines, and high sensitivity C-reactive protein (hsCRP), leading to hypertension and cardiovascular events (8–12). In addition, it has been reported that endothelium-dependent vasorelaxation is impaired in patients with OSA, with changes in the balance of endothelium-derived vasoactive factors favoring vasoconstriction (13). Whereas vasoconstrictors have been reported to be elevated in patients with OSAS, hypoxia, oxidative stress, inflammatory cytokines, endothelin, shear stress, and other changes induced by OSAS have been implicated in the production of adrenomedullin (ADM), which is a potent endothelial-derived vasodilator (14). Nocturnal chronic hypoxia caused by OSA, which is one of the major causes of pulmonary vascular remodeling, may induce the production of both reactive oxygen species (ROS) and ADM. Recent studies have indicated that hypoxia upregulates the expression of ADM, which is not only a potent vasodilator but also an antioxidant (15, 16). Because OSAS causes hypoxia, vasoconstriction, endothelin production, oxidative stress, shear stress, and the production of inflammatory cytokines, we hypothesized that circulating ADM is increased in patients with OSAS.

For the treatment of OSAS, nasal continuous positive airway pressure (nCPAP) has been reported to be effective (4, 17), reducing blood pressure (BP) during both the night and day. Although it is expected that nCPAP may ultimately improve the prognosis of cerebrovascular disorders associated with OSAS, its exact mechanism has not been clarified.

In the current study, we hypothesized that nCPAP may decrease OSAS-induced hypoxic stress and ROS production, and thereby decrease the counter effects of the vasorelaxant activity of ADM. To examine this hypothesis, we measured BP, plasma ADM and generation of ROS by leukocytes before and after nCPAP therapy in OSAS patients.

Methods

Subjects

Among patients diagnosed with OSAS in our department, 48 male subjects participated in the current study. As age and body mass index (BMI)-matched controls without OSAS, 48 male subjects were chosen and studied. Further, 40 age-matched, but normal-BMI subjects were studied as an additional control group. No subjects had any history of cardiovascular, pulmonary, metabolic or neuromuscular diseases, and all 48 patients were found to be free of each of the following: 1) renal and renovascular hypertension, 2) moderate hypertension, defined as a systolic BP (SBP) >160 mmHg and/or diastolic BP (DBP) >100 mmHg (18), 3) chronic renal and hepatic diseases, and 4) diabetes mellitus. No subject had an abnormal level of renin or aldosterone in plasma samples obtained in the supine position following a 15 min rest. Fasting plasma glucose (FPG) levels were less than 110 mg/dL, and hemoglobin A1c (HbA1c) levels were less than 6.0% in all participants in this study. Patients who smoked or had systemic infections at the time of the study or within 2 weeks before the study were excluded. No patients were treated with antihypertensive agents. All subjects were in a stable condi-

Table 2. Assessment of Apnea and Hypoxic Episodes in Both OSAS Group and Control Groups

	Group		
	OSAS group	Control group (BMI <24)	Control group (BMI ≥24)
AHI (events/h)	51.1±3.2*	2.1±0.4	4.1±0.4
SaO ₂ <90% (%TST)	34.1±6.2*	0±0	0±0
Lowest SaO ₂ (%)	68.9±3.0*	96.6±0.5	96.8±0.5
ODI	2.41±0.36*	0.01±0.01	0.02±0.01
SDS (2%) (events/h)	58.2±4.1*	2.6±1.5	8.6±2.4
SDS (4%) (events/h)	34.4±4.2*	0.1±0.1	1.1±0.2
SDS (6%) (events/h)	18.8±6.2*	0±0	0±0
Arousal index (/h)	41.9±3.2*	2.3±3.1	8.3±3.1

* $p < 0.001$ vs. control group. Data are presented as mean±SEM. OSAS, obstructive sleep apnea syndrome; BMI, body mass index; AHI, apnea-hypopnea index; SaO₂, arterial oxygen saturation; TST, total sleep time; ODI, oxyhemoglobin desaturation index; SDS, the number of significant oxyhemoglobin desaturation. SDS was determined by drops in oxyhemoglobin saturation of more than 2, 4, and 6%.

tion for 1 month prior to the study. These subjects were examined with polysomnography and classified as obese control subjects. All patients in the BMI-matched control group were examined by polysomnography and classified as obese. Although all the patients (*i.e.*, the experimental group) were obese, control subjects included obese and non-obese subjects for examining the effect of obesity on the plasma ADM and ROS production by peripheral leukocytes. All control subjects were examined by polysomnography and classified as obese or non-obese according to the data of the apnea-hypopnea index (AHI). The diagnosis of OSAS was also established with polysomnography. For the purpose of identifying the effect of nCPAP treatment on circulating ADM levels in OSAS patients, patients with mild OSAS were excluded because they tend to be less compliant with nCPAP therapy, and because the insurance system in Japan does not cover nCPAP therapy for mild OSAS patients. The study was approved by the Institutional Review Board of the Ethics Committee of Tokyo University Hospital, and all patients gave written informed consent.

The characteristics of the subjects in the OSAS and control groups are shown in Table 1. There were no significant differences in age and BMI between the two groups, while the AHI in the OSAS group was markedly greater than that in the controls. There were no significant differences in BP or metabolic indices. Following the polysomnography study, the patients with OSAS underwent therapeutic nCPAP treatment, and 48 subjects continued to receive nCPAP successfully for 6 months or longer. The Epworth Sleepiness Scale (ESS) was used to investigate changes in subjective daytime sleepiness. BP and heart rate (HR) were measured three times per day during the hospital stay. The values of these parameters were defined as the mean of the three measurements taken on the day before the initial polysomnography.

Polysomnography

The subjects underwent polysomnography for two consecutive nights. The results of the second night were analyzed to rule out the possible influence of any first-night effects on polysomnography. The polysomnography included an electroencephalogram (EEG), an electro-oculogram, an electromyogram of the chin, and an electrocardiogram (DG Compact32; Medelec, Woking, UK). Surface electrodes were used to record two channels of EEG (C3A2, C4A1), right and left electro-oculography, and submental electromyography. We monitored ventilation and airflow using inductive plethysmography (Respirace; Ambulatory Monitoring, Ardley, USA) and thermistors (Fukuda-Sangyo, Chiba, Japan) placed at the nostril and mouth. Arterial oxygen saturation (SaO₂) was continuously measured *via* pulse oxymeter (Datex, Helsinki, Finland). Data acquisition was performed overnight starting at 9:00 PM until 7:00 AM the next morning (19, 20). Apnea was defined as continuous cessation of airflow for >10 s, and hypopnea was defined as a reduction in airflow for >10 s with oxygen desaturation of 4% or an EEG arousal from sleep. Apneas were classified as obstructive, mixed, or central according to standard criteria by the American Academy of Sleep Medicine. The AHI was calculated as the total number of episodes of apnea and hypopnea per hour of sleep. An AHI of 5 was considered diagnostic of OSAS.

nCPAP Treatment

Before introduction of nCPAP, the patients with OSAS slept while connected to an automatic titration device (AutoSet; ResMed, North Ryde, Australia). The following night, these patients slept while connected to a conventional continuous positive airway pressure machine (S6 CPAP; ResMed) set at a fixed pressure determined from the results of the AutoSet. Thereafter, a nCPAP machine (REM star; Respiroics Inc., Murrysville, USA) commonly prescribed for home use was

prescribed for each patient. The duration of nCPAP treatment was 6 months or longer. Compliance was assessed with hourly meter readings, and the mean daily duration of nCPAP use was 5.8 ± 0.5 ($\pm$ SD) h in patients with OSAS. From 1 to 6 months after the introduction of nCPAP, the patients with OSAS were admitted, and polysomnography was re-performed as the patient received nCPAP. Samples of venous blood were obtained at 7:30 to 8:00 AM, and used for further analysis.

Assessment of Hypoxic Episodes and Arterial Oxygen Desaturation Magnitude

To assess OSAS-induced hypoxia quantitatively, we used several parameters, including the number of nocturnal apneas, and number of oxyhemoglobin desaturations. The number of significant oxyhemoglobin desaturations (SDS) was determined by drops in oxyhemoglobin saturation of more than 2%, 4%, and 6%. Drops in SaO_2 lasting more than 5 s were counted. We also applied the oxyhemoglobin desaturation index (ODI) in this study as previously described (8, 9). Desaturation episodes were defined as hypoxia of $SaO_2 \leq 90\%$. We defined ODI according to the formula

$$ODI = \sum (90 - SaO_2) \times t,$$

where t is the time of desaturation (h) (8, 9). As shown in the equation, ODI expresses the severity of hypoxic stress quantitatively.

Measurements of Circulating ADM

We obtained peripheral blood from the subjects at 7:30 to 8:00 AM before and after the 1 to 6 months of nCPAP treatment. Blood samples were placed in chilled polypropylene tubes containing ethylene-diamine-tetraacetic acid (EDTA) (1 mg/mL of blood) and aprotinin (Sigma Chemical Co., St. Louis, USA) (500 kU/mL of blood). The blood samples were then centrifuged at $250 \times g$ and 4°C for 10 min, and the plasma was separated and stored at -80°C until analysis. ADM was measured by a specific and sensitive radioimmunoassay using the kit from Phoenix Pharmaceuticals (Mountain View, USA). The range of the standard curve was 0.5 to 128 pg/mL. The interassay and intra-assay variabilities were $10.6 \pm 2.8\%$ and $10.1 \pm 1.9\%$, respectively.

Measurements of Spontaneous Production and Phorbol-12-Myristate-13-Acetate-Induced Generation of ROS by Peripheral Blood Leukocytes

We obtained peripheral blood from the subjects at 7:30 to 8:00 AM on the day before and the day after the nCPAP treatment. Leukocytes were obtained from the blood using the density gradient centrifugation method. Briefly, 5 mL of peripheral venous blood was stratified on 3 mL of a Polymorphprep cell separation medium (Axis-Shield, Oslo, Norway),

circulating
ADM levels
(pg/mL)

Fig. 1. The baseline levels of circulating adrenomedullin (ADM) levels in the control subjects (CTRL) and OSAS patients. There were two control groups, an age- and BMI-matched control group ($BMI \geq 24$) and an age-matched but normal-BMI control group ($BMI < 24$). The OSAS patients were divided into three groups based on AHI severity.

and the polymorphonuclear cells (PMNs) were separated by means of density gradient centrifugation. After centrifugation, the upper mononuclear cell band was discarded, and the lower PMN band was removed and washed in RPMI-1640 medium containing glutamine (Sigma Chemical Co.). Any residual erythrocytes in the granulocyte preparation were lysed using a 0.15 mol/L NH_4Cl solution (pH 7.4). After the aggregates were disrupted by being passed through a needle with an internal diameter of 150 μm , the PMNs were collected, washed in RPMI-1640, and tested for viability by means of trypan blue exclusion. The number of cells in the final cell suspension used for each test was adjusted by means of counting in a hemacytometer. ROS generation by peripheral blood leukocytes was measured by the lucigenin-dependent chemiluminescence method using a photon counter, Biolumat LB9505 (Berthold, FRG) (21, 22). The ROS generation by peripheral blood leukocytes was determined as the increase of chemiluminescence after stimulation with phorbol-12-myristate-13-acetate (PMA) (Sigma Chemical Co.). The 10^5 of peripheral blood leukocytes were suspended in 900 μL of HBSS containing Ca^{2+} and Mg^{2+} (pH 7.4) (Life Technologies, Inc., Grand Island, USA), and pre-warmed for 15 min at 37°C with 50 μL of lucigenin (2 mg/mL). Oxygen radical generation was measured for 90 min at 37°C with stimulation of 10 μL of PMA (0.1 mg/mL). Data were expressed as the peak counts of chemiluminescence for 90 min after stimulation, since previous studies indicated that

Fig. 2. The baseline levels of *a*: spontaneous production of reactive oxygen species (ROS) by 10⁵ peripheral blood leukocytes and *b*: the PMA-induced ROS generation by 10⁵ peripheral blood leukocytes in the control subjects (CTRL) and OSAS patients. There were two control groups, an age- and BMI-matched control group (BMI≥24) and an age-matched but normal-BMI control group (BMI<24). The OSAS patients were divided into three groups based on AHI severity.

Fig. 3. Correlation between circulating ADM and sleep apnea-associated parameters, i.e., *a*: apnea-hypopnea index (AHI) and *b*: oxygen desaturation magnitude as indicated by oxyhemoglobin desaturation index (ODI).

peak counts were always observed within 90 min and the results were reproducible (21, 22).

Data Analysis

The significance of differences within groups was analyzed with a Student's paired *t*-test, and the significance of differ-

Fig. 4. Correlation between spontaneous production of reactive oxygen species (ROS) and sleep apnea-associated parameters, i.e., a: apnea-hypopnea index (AHI) and b: oxygen desaturation magnitude as indicated by oxyhemoglobin desaturation index (ODI).

ences between groups was performed by analysis of variance first, followed by *t*-tests with Bonferroni correction. The correlation was analyzed with a Spearman rank correlation. Results are expressed as the mean $\pm$ SD, and values of $p<0.05$ were considered to indicate statistical significance.

Results

Assessment of Hypoxic Episodes

There were significant differences in baseline ODI between the OSAS and control groups (2.41 ± 0.36 and 0.02 ± 0.01 , respectively; $p<0.001$), suggesting that the OSAS patients were exposed to a significantly greater degree of hypoxia than the control subjects (Table 2).

Baseline Measurements of Circulating ADM and Production of ROS by Leukocytes

Figure 1 summarizes the ADM and ROS levels at baseline. The levels of both ADM and ROS in the OSAS group were significantly greater than those in the control group (Fig. 1). Baseline ADM levels in the OSAS group were significantly greater than those in the obese control (BMI $\geq$ 24) groups (49.1 ± 4.7 vs. 23.9 ± 5.2 pg/mL, respectively). There was no significant difference in ADM levels between normal-BMI controls and obese controls. The ADM levels in very severe OSAS patients (AHI $\geq$ 45) were greater than those in moderate OSAS patients ($15\leq$ AHI $<$ 30). The ROS production by

peripheral leukocytes in the OSAS group was significantly greater than that in obese control subjects without OSAS (Fig. 2a). There was a significant difference in ROS production between moderate OSAS patients and very severe OSAS patients. The PMA-induced generation of ROS by the leukocytes from OSAS patients was also greater than that from obese control subjects (Fig. 2b). The ROS production and generation were not different between normal-BMI controls and obese controls.

Relationships between ADM and Sleep Apnea-Related Variables

Figure 3 demonstrates the relationships between ADM and AHI or arterial oxygen desaturation magnitude as indicated by ODI. As shown, a significant correlation was observed between ADM and hypoxic episodes (ODI) rather than apnea episodes (AHI). Similarly, a significant correlation between ROS and ODI was detected, whereas a positive but insignificant association was suggested between ROS and AHI (Fig. 4).

Figure 5 shows the correlation between circulating ADM levels and spontaneous production of ROS or PMA-stimulated generation of ROS by 10^5 peripheral blood leukocytes. There was a fairly good correlation between circulating ADM levels and the amount of ROS production by peripheral blood leukocytes.

Table 3 shows the correlations between ADM and sleep apnea-related variables, including nocturnal hypoxia and oxi-

Fig. 5. Correlation between circulating ADM levels and a: spontaneous production of ROS or b: PMA-stimulated generation of ROS by 10^5 peripheral blood leukocytes.

Table 3. Correlations between ADM and Sleep Apnea-Related Variables

Variables	<i>r</i>	<i>p</i>
AHI (events/h)	0.378	<0.05
SaO ₂ <90% (%TST)	0.364	<0.05
Lowest SaO ₂ (%)	0.512	<0.05
ODI	0.598	<0.01
SDS (2%) (events/h)	0.312	0.09
SDS (4%) (events/h)	0.357	<0.05
SDS (6%) (events/h)	0.298	0.12
Arousal index (/h)	0.344	0.08
ROS production	0.618	<0.05
ROS generation	0.637	<0.01

ADM, adrenomedullin; AHI, apnea-hypopnea index; SaO₂, arterial oxygen saturation; TST, total sleep time; ODI, oxyhemoglobin desaturation index; SDS, the number of significant oxyhemoglobin desaturation; ROS, reactive oxygen species.

dant stress. The circulating ADM levels were significantly associated with the nocturnal hypoxic variables rather than with the apnea itself. The AHI, the ODI, the sleeping time spent below 90% SaO₂, and the lowest SaO₂t were correlated with circulating ADM levels. In terms of *p* values, the association between ODI and ADM was more significant than that between AHI and ADM. In addition, ROS production and generation were significantly correlated with ADM values, but the arousal index was not.

Effects of nCPAP on Physiological Indices and Circulating ADM and ROS Production

Following nCPAP, a decrease in sleepiness was observed in all of the OSAS patients that successfully received therapeutic nCPAP. Consequently, nCPAP significantly decreased apnea and oxyhemoglobin desaturation (Table 4).

Figure 6 summarizes the effects of long-term nCPAP on ADM and ROS levels. As shown, nCPAP treatment significantly decreased the levels of both ADM and ROS in the treated OSAS group. The effect was observed continuously over the 3–6 months of nCPAP treatment.

Figure 7 shows the relationships between the reduced magnitude of circulating ADM following nCPAP treatment and the reduced magnitude of spontaneous production of ROS or generation of ROS by peripheral blood leukocytes following nCPAP treatment in patients with OSAS. There was a significant correlation between the reduced magnitude of circulating ADM and the reduced magnitude of spontaneous production of ROS or generation of ROS by peripheral blood leukocytes in the patients.

Discussion

In the present study, circulating ADM levels were significantly increased in OSAS patients compared to control subjects. In addition, 6 months of treatment with nCPAP decreased apnea, oxyhemoglobin desaturation, ROS production by peripheral leukocytes and the circulating ADM in the OSAS patients. At baseline, the levels of both ADM and ROS

Table 4. Effects of nCPAP on Physiological Indices and Sleep Apnea-Related Variables

	Before	After treatment with nCPAP for		
		1 month	3 month	6 month
Systolic BP (mmHg)	145.1±8.7	141.1±7.7	140.5±7.4	140.1±7.1
Diastolic BP (mmHg)	84.1±3.2	82.4±2.8	82.6±2.9	82.1±2.7
HR (bpm)	74.1±3.2	72.7±2.9	73.1±2.8	71.9±2.6
Total sleep time (min)	348.1±20.3	396.3±21.2*	404.3±20.2*	411.3±21.9*
ESS	14.6±0.9	4.4±1.9*	3.2±1.6*	3.9±2.1*
NREM1+2 (%TST)	75.6±2.9	63.4±2.4*	61.9±2.6*	62.1±2.7*
NREM3+4 (%TST)	9.6±1.2	17.2±0.7*	17.9±0.8*	16.9±0.7*
REM (%TST)	15.6±1.9	19.4±1.5*	19.9±1.6*	21.0±1.7*
AHI (events/h)	51.1±3.2	6.1±1.4*	4.3±0.4*	4.6±0.8*
SaO ₂ <90% (%TST)	34.1±6.2	1.1±0.2*	0.9±0.3*	0.7±0.1*
Lowest SaO ₂ (%)	68.9±3.0	91.8±0.4*	92.8±0.5*	93.8±0.5*
ODI	2.41±0.36	0.12±0.06*	0.11±0.04*	0.08±0.02*
SDS (2%) (events/h)	58.2±4.1	4.6±2.1*	4.3±2.0*	3.6±1.4*
SDS (4%) (events/h)	34.4±4.2	1.0±0.2*	0.9±0.2*	0.8±0.2*
SDS (6%) (events/h)	18.8±6.2	0±0	0±0	0±0
Arousal index (/h)	41.9±3.2	12.3±2.1*	10.4±2.0*	8.8±2.2*

* $p < 0.05$ vs. the same value before treatment with nCPAP. Data are presented as mean±SEM. nCPAP, nasal continuous positive airway pressure; BP, blood pressure; HR, heart rate; ESS, Epworth sleepiness scale; NREM, non-rapid eye movement sleep; TST, total sleep time; REM, rapid eye movement sleep; AHI, apnea-hypopnea index; SaO₂, arterial oxygen saturation; ODI, oxyhemoglobin desaturation index; SDS, the number of significant oxyhemoglobin desaturation.

in the OSAS group were significantly greater than those in the control group. The ADM level was positively associated with the magnitude of ROS production in the patients. Both the ADM level and the amount of ROS were positively correlated with the severity of hypoxia as indexed by the ODI. These observations suggest that nCPAP therapy may have reduced OSAS-induced hypoxia and production of ROS, leading to the decreased levels of ADM.

Several issues warrant consideration before discussing the results. First, we measured circulating levels of ADM, but not tissue levels of ADM, in OSAS patients. Because the plasma levels of ADM and tissue and vascular endothelium levels of ADM may not be the same, we do not have real evidence of a significant relationship between the OSAS-induced hypoxic stress and the functional ADM levels in the endothelium. However, exogenous ADM has been reported to act as an effective vasodilator. Thus the increased levels of circulating ADM may have a significant effect on the vasodilation in the OSAS patients. Secondly, we measured ROS by leukocytes *in vitro*. These leukocytes were drawn from the patients, but we could not assess the exact ROS production *in vivo*. This approach—*i.e.*, the *in vitro* measurement of ROS generation—has been widely used (23, 24), but it remains unclear whether it precisely reflects the actual production of ROS by circulating leukocytes or other cells. However, previous studies have suggested that ROS production is increased in patients with OSAS both *in vivo* and *in vitro* (25, 26). Therefore, it may be reasonable to speculate, based on our data, that the ROS production *in vivo* was also increased in OSAS

patients before nCPAP treatment and decreased after nCPAP treatment. Thirdly, the mean value of BP in patients with OSAS was slightly greater than the control values. Therefore, this increase of BP may have affected the plasma ADM levels in the OSAS patients.

It has been recently reported that OSAS does not exert any significant acute or chronic effects on plasma ADM levels (27). However, the study sample was very small in this earlier report, and the magnitude of hypoxic stress was not examined. Therefore, while the authors can speculate that OSAS itself has an effect on the ADM levels, they cannot comment on the effects of OSAS-induced hypoxic stress and/or oxidative stress on the ADM levels in OSAS patients. At 2:00 AM, the levels of ADM in the OSAS group (28.2±5.9 pg/mL) were slightly greater than those in the control group (23.3±5.1 pg/mL) in their study (27). Thus, the results cannot completely rule out an increase in the ADM level in patients with OSAS and nocturnal hypoxemia. While they did indicate that there was no association between OSAS and the levels of ADM in their patients, they did not indicate a relationship between OSAS-induced oxyhemoglobin desaturation and the levels of ADM. In fact, our study indicated that the ADM levels were not correlated with BMI or the number of apneas, but were well correlated with the magnitude of hypoxia as indicated by ODI. These results were also supported by the recent study of Schulz and coworkers (28). They found that OSAS patients had markedly elevated ADM concentrations when compared to the controls. They also found that two nights of nCPAP therapy decreased ADM levels significantly (28).

Fig. 6. Effects of chronic treatment with nCPAP on a: circulating ADM levels and b: production of reactive oxygen species (ROS) (chemiluminescence counts/10⁵ peripheral blood leukocytes). *p < 0.05 vs. each baseline value before treatment with nCPAP.

Fig. 7. Correlation between the reduced magnitude of circulating ADM following nCPAP treatment and the a: reduced magnitude of spontaneous production of ROS or b: generation by peripheral blood leukocytes following nCPAP treatment.

It has been recently postulated that an inflammatory process plays a crucial role in the pathogenesis of atherosclerosis, which in turn can lead to various cardiovascular disorders (29–31). In a previous study, we reported that the circulating intercellular adhesion molecule-1 (ICAM-1) level in patients with OSAS was significantly increased compared to that in

the control group, suggesting that OSAS-induced hypoxia may induce the activation of ICAM-1 and the inflammation of the endothelium in patients with OSAS (8, 9). Further, we have also demonstrated that the circulating interleukin-8 (IL-8) and MCP-1 levels were increased in OSAS patients compared to control subjects. In that study, there was a significant

correlation between circulating ICAM-1 and IL-8 in the OSAS patients (9). In addition, we also found that the serum level of nitrite/nitrates (NO_x), which are stable metabolites of NO, was lower in patients with OSAS than in control subjects (32). There was a significant negative correlation between serum nitrites/nitrates and the magnitude of oxygen desaturation. Further, nocturnal oxygen supplementation increased the NO_x level but did not affect the apneas, suggesting that repeated episodes of nocturnal hypoxemia is one of the mechanisms responsible for the impaired NO production in patients with OSAS. Because the measurements of brachial artery diameter under baseline conditions, during reactive hyperemia and after sublingual administration of nitroglycerin (an endothelium-independent vasodilator) indicate that patients with OSAS have an impairment of resistance-vessel endothelium-dependent vasodilation, the endothelial function in OSAS may be disturbed (33, 34).

Because ADM is reported to induce cell surface expression of the adhesion molecules E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and ICAM-1 on human endothelial cells, an increase in the level of ADM is one of the mechanisms involved in the increase in ICAM-1 in OSAS patients (11, 35). It has also been reported that the ADM inhibits vascular endothelial growth factor (VEGF)-stimulated ICAM-1 and VCAM-1 expression through a phosphatidylinositol 3'-kinase/Akt pathway (36). Although circulating VEGF levels are elevated in OSAS patients, the ADM might have an anti-inflammatory role in controlling VEGF-induced adhesion molecule gene expression and adhesiveness toward leukocytes in endothelial cells (37).

To treat patients with OSAS, nCPAP therapy is widely used, since nCPAP reduces excessive daytime sleepiness and increases quality of life (4, 17). Based on recent studies, nCPAP is expected to have beneficial effects on the prognosis of OSAS-associated diseases, although further evidence will be needed to confirm this hypothesis. We therefore performed this study to address the question of whether nCPAP could affect physiologic phenomena and production of ROS and ADM. We observed that long-term nCPAP was effective to improve nocturnal apnea and oxyhemoglobin desaturation and that the levels of circulating mediators were reduced following nCPAP. It has been reported that patients on long-term CPAP treatment show complete normalization of plasma ADM concentrations (28). However, the mechanism of long-term CPAP efficacy on ADM normalization has not been elucidated. One of the possible explanations is that nCPAP decreases hypoxic episodes, resulting in the reduction of hypoxia-induced inflammation. Our results indicated that the reduced level of ADM was significantly correlated with the reduced degree of ROS production. The increased levels of ROS and oxidant stress may be responsible for the increased levels of ADM in OSAS. Although ADM is known as a potent vasodilator, the recent data suggest that ADM may also act as an antioxidant (15, 16). The increased levels of ADM may be an adaptive mechanism against the severe oxida-

tive stress due to intermittent hypoxia and increased production of ROS. There are the other possibilities. Because the OSAS patients have a higher sympathetic activity, a decrease in the sympathetic activity by nCPAP treatment may contribute to normalization of the ADM levels in OSAS patients (13). The decreased BP by nCPAP treatment may also decrease the ADM levels in OSAS patients (13).

To assess the severity of hypoxia induced by OSAS, we used the magnitude of the ODI. This parameter may reflect OSAS-induced hypoxic stress more quantitatively than AHI or nadir SaO_2 alone. The conventional way to assess the degree of OSAS is to count the number of apnea episodes alone, while ODI could reflect both decreases in SaO_2 and time spent below 90%. However, to accurately analyze the hypoxic stress, exploring other indices of hypoxic stress may be important and helpful. The ODI is significantly correlated with the levels of ADM and ROS. Although the AHI is also correlated with both parameters, the relationship is more significant with ODI than with AHI or nadir SaO_2 . We have previously reported that increased levels of pro-inflammatory cytokines and C-reactive protein in plasma are decreased by nCPAP in patients with OSAS (11, 12). The reduction of hypoxic stress and oxidant stress by nCPAP may contribute to a decrease in the inflammatory mediators. These results also support the notion that nCPAP has beneficial effects on circulating ADM and ROS production in patients with OSAS.

However, the role of ADM in the protective effect from vascular events in patients with OSAS remains to be elucidated. Although ADM is known to reduce ischemic brain injury after transient middle cerebral artery occlusion in animals, the protective effect of ADM on the brain injury severity following brain attacks in humans has not been determined (38). Similarly, ADM administration immediately after myocardial infarction has been shown to ameliorate the progression of heart failure in animals (39). However, the roles of ADM on the prevention of heart attacks in humans have not been elucidated. Further studies are needed to elucidate the role of ADM in prevention of vascular events in OSAS patients.

In summary, in the present study the levels of circulating ADM and ROS production were found to have increased in OSAS patients compared to those in obese control subjects. nCPAP therapy significantly decreased the levels of ADM and ROS production in patients with OSAS. Because the magnitude of ODI is significantly associated with the levels of ADM and ROS production, the amelioration of hypoxic stress and oxidant stress by nCPAP may be one of the mechanisms by which the circulating ADM level is reduced. Treatment of OSAS using nCPAP may be, therefore, a potential approach to decrease the risk of the progression of OSAS-associated vascular disorders.

References

1. Chhajed PN, Tamm M, Strobel W: Sleep apnea and heart

- disease. *N Engl J Med* 2006; **354**: 1086–1089.
2. Can M, Acikgoz S, Mungan G, et al: Serum cardiovascular risk factors in obstructive sleep apnea. *Chest* 2006; **129**: 233–237.
3. Suzuki YJ, Jain V, Park AM, Day RM: Oxidative stress and oxidant signaling in obstructive sleep apnea and associated cardiovascular diseases. *Free Radic Biol Med* 2006; **40**: 1683–1692.
4. Teramoto S, Kume H, Matsuse T: Ambulatory blood pressure after sleep apnoea treatment. *Lancet* 2002; **360**: 341–342.
5. Teramoto S, Ohga E, Ouchi Y: Obstructive sleep apnoea. *Lancet* 1999; **354**: 1213–1214.
6. Teramoto S, Kume H, Matsuse T, Fukuchi Y: The risk of future cardiovascular diseases in the patients with OSAS is dependently or independently associated with obstructive sleep apnoea. *Eur Respir J* 2001; **17**: 573–574.
7. Teramoto S, Yamamoto H, Yamaguchi Y, Namba R, Ouchi Y: Obstructive sleep apnea causes systemic inflammation and metabolic syndrome. *Chest* 2005; **127**: 1074–1075.
8. Ohga E, Nagase T, Tomita T, et al: Increased levels of circulating ICAM-1, VCAM-1, and L-selectin in obstructive sleep apnea syndrome. *J Appl Physiol* 1999; **87**: 10–14.
9. Ohga E, Tomita T, Wada H, Yamamoto H, Nagase T, Ouchi Y: Effects of obstructive sleep apnea on circulating ICAM-1, IL-8, and MCP-1. *J Appl Physiol* 2003; **94**: 179–184.
10. Caples SM, Gami AS, Somers VK: Obstructive sleep apnea. *Ann Intern Med* 2005; **142**: 187–197.
11. Teramoto S, Yamamoto H, Ouchi Y: Increased plasma interleukin-6 is associated with the pathogenesis of obstructive sleep apnea syndrome. *Chest* 2004; **125**: 1964–1965.
12. Teramoto S, Yamamoto H, Ouchi Y: Increased C-reactive protein and increased plasma interleukin-6 may synergistically affect the progression of coronary atherosclerosis in obstructive sleep apnea syndrome. *Circulation* 2003; **107**: E40.
13. Wolk R, Kara T, Somers VK: Sleep-disordered breathing and cardiovascular disease. *Circulation* 2003; **108**: 9–12.
14. Sugo S, Minamino N, Kangawa K, et al: Endothelial cells actively synthesize and secrete adrenomedullin. *Biochem Biophys Res Commun* 1994; **201**: 1160–1166.
15. Saito T, Itoh H, Chun TH, et al: Coordinate regulation of endothelin and adrenomedullin secretion by oxidative stress in endothelial cells. *Am J Physiol Heart Circ Physiol* 2001; **281**: H1364–H1371.
16. Yoshimoto T, Gochou N, Fukai N, Sugiyama T, Shichiri M, Hirata Y: Adrenomedullin inhibits angiotensin II-induced oxidative stress and gene expression in rat endothelial cells. *Hypertens Res* 2005; **28**: 165–172.
17. Crispin J, Robert JOD, Rebecca M, John RS: Comparison of therapeutic and subtherapeutic nasal continuous positive airway pressure for obstructive sleep apnoea: a randomised prospective parallel trial. *Lancet* 1999; **353**: 2100–2105.
18. Japanese Society of Hypertension: Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2004). *Hypertens Res* 2006; **29** (Suppl): S1–S105.
19. Teramoto S, Sudo E, Matsuse T, et al: Impaired swallowing reflex in patients with obstructive sleep apnea syndrome. *Chest* 1999; **116**: 17–21.
20. Teramoto S, Kume H, Yamamoto H, et al: Effects of oxygen administration on the circulating vascular endothelial growth factor (VEGF) levels in patients with obstructive sleep apnea syndrome. *Intern Med* 2003; **42**: 681–685.
21. Teramoto S, Suzuki M, Matsuse T, Ishii T, Fukuchi Y, Ouchi Y: Effects of angiotensin-converting enzyme inhibitors on spontaneous or stimulated generation of reactive oxygen species by bronchoalveolar lavage cells harvested from patients with or without chronic obstructive pulmonary disease. *Jpn J Pharmacol* 2000; **83**: 56–62.
22. Teramoto S, Matsuse T, Ohga E, Ishii T, Ouchi Y: Inhibitory effects of angiotensin-converting enzyme (ACE) inhibitors on oxygen radicals produced by bronchoalveolar lavage cells in young and aged guinea pigs. *Aging (Milano)* 2000; **12**: 22–28.
23. Maeda K, Yasunari K, Watanabe T, Nakamura M: Oxidative stress by peripheral blood mononuclear cells is increased in hypertensives with an extreme-dipper pattern and/or morning surge in blood pressure. *Hypertens Res* 2005; **28**: 755–761.
24. Yasunari K, Maeda K, Nakamura M, Watanabe T, Yoshikawa J: Benidipine, a long-acting calcium channel blocker, inhibits oxidative stress in polymorphonuclear cells in patients with essential hypertension. *Hypertens Res* 2005; **28**: 107–112.
25. Dyugovskaya L, Lavie P, Lavie L: Increased adhesion molecules expression and production of reactive oxygen species in leukocytes of sleep apnea patients. *Am J Respir Crit Care Med* 2002; **165**: 934–939.
26. Schulz R, Mahmoudi S, Hattar K, et al: Enhanced release of superoxide from polymorphonuclear neutrophils in obstructive sleep apnea. Impact of continuous positive airway pressure therapy. *Am J Respir Crit Care Med* 2000; **162**: 566–570.
27. Wolk R, Svatikova A, Otto ME, Hoffmann MS, Duenwald CJ, Somers VK: Plasma adrenomedullin and obstructive sleep apnea. *Am J Hypertens* 2004; **17**: 74–76.
28. Schulz R, Flototto C, Jahn A, et al: Circulating adrenomedullin in obstructive sleep apnoea. *J Sleep Res* 2006; **15**: 89–95.
29. Ferrario CM, Strawn WB: Role of the renin-angiotensin-aldosterone system and proinflammatory mediators in cardiovascular disease. *Am J Cardiol* 2006; **98**: 121–128.
30. Yoshii T, Iwai M, Li Z, et al: Regression of atherosclerosis by amlodipine via anti-inflammatory and anti-oxidative stress actions. *Hypertens Res* 2006; **29**: 457–466.
31. Toba H, Shimizu T, Miki S, et al: Calcium [corrected] channel blockers reduce angiotensin II-induced superoxide generation and inhibit lectin-like oxidized low-density lipoprotein receptor-1 expression in endothelial cells. *Hypertens Res* 2006; **29**: 105–116.
32. Teramoto S, Kume H, Matsuse T, et al: Oxygen administration improves the serum level of nitric oxide metabolites in patients with obstructive sleep apnea syndrome. *Sleep Med* 2003; **4**: 403–407.
33. 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.

34. Ip MS, Tse HF, Lam B, Tsang KW, Lam WK: Endothelial function in obstructive sleep apnea and response to treatment. *Am J Respir Crit Care Med* 2004; **169**: 348–353.
35. Hagi-Pavli E, Farthing PM, Kapas S: Stimulation of adhesion molecule expression in human endothelial cells (HUVEC) by adrenomedullin and corticotrophin. *Am J Physiol Cell Physiol* 2004; **286**: C239–C246.
36. Hagi-Pavli E, Farthing PM, Henshaw FN, Kapas S: Adrenomedullin reduces VEGF-induced endothelial adhesion molecules and adhesiveness through a phosphatidylinositol 3'-kinase pathway. *Arterioscler Thromb Vasc Biol* 2003; **23**: 1377–1383.
37. Lavie L, Kraiczi H, Hefetz A, *et al*: Plasma vascular endothelial growth factor in sleep apnea syndrome: effects of nasal continuous positive air pressure treatment. *Am J Respir Crit Care Med* 2002; **165**: 1624–1628.
38. Watanabe K, Takayasu M, Noda A, *et al*: Adrenomedullin reduces ischemic brain injury after transient middle cerebral artery occlusion in rats. *Acta Neurochir (Wien)* 2001; **143**: 1157–1161.
39. Nakamura R, Kato J, Kitamura K, *et al*: Adrenomedullin administration immediately after myocardial infarction ameliorates progression of heart failure in rats. *Circulation* 2004; **110**: 426–431.