



Association Between Endothelial Function (Assessed on Reactive Hyperemia Peripheral Arterial Tonometry) and Obstructive Sleep Apnea, Visceral Fat Accumulation, and Serum Adiponectin

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Background: Visceral obesity, low adiponectin, and severe obstructive sleep apnea (OSA) are associated with cardiovascular diseases, but the interactions among these factors on endothelial dysfunction are not well known.

Methods and Results: Endothelial function in 133 patients after polysomnography was evaluated as reactive hyperemia index (RHI) on reactive hyperemia peripheral arterial tonometry. Visceral obesity was defined as visceral fat area $\geq 100 \text{ cm}^2$ on computed tomography. RHI was significantly correlated with apnea hypopnea index (AHI), visceral fat area, and serum adiponectin ($r = -0.24$, $P = 0.0055$, $r = -0.19$, $P = 0.031$, and $r = 0.20$, $P = 0.019$, respectively). RHI in patients with visceral obesity was significantly decreased in the presence of severe OSA ($\text{AHI} \geq 30$; $P = 0.042$). On multivariate regression analysis, only severe OSA remained as an independent predictive factor of RHI ($P = 0.024$, $R^2 = 5.4\%$). RHI in patients with severe OSA ($n = 44$) was significantly improved after 3 months of continuous positive airway pressure (CPAP) treatment (1.78 ± 0.40 before CPAP vs. 2.00 ± 0.53 after CPAP, $P = 0.013$), similarly to those with $\text{AHI} < 30$ ($P = 0.45$).

Conclusions: Severe OSA, but not visceral fat area or serum adiponectin, was independently associated with endothelial function according to RHI. In addition, impaired endothelial function was reversible following 3 months of CPAP treatment. (*Circ J* 2015; **79**: 1381–1389)

Key Words: Adiponectin; Continuous positive airway pressure; Endothelial function; Obstructive sleep apnea; Visceral obesity

Obesity, in particular visceral obesity, is an important risk factor for cardiovascular disease (CVD).^{1,2} An interaction between visceral fat accumulation and endothelial dysfunction has been identified.^{3,4} Adiponectin is one of the adipokines secreted from adipocytes and has cardiovascular protective effects and anti-inflammatory effects.⁵ Low adiponectin has been associated with endothelial dysfunction⁶ and future CVD independently of body mass index (BMI) or waist circumference (WC).⁷ Obstructive sleep apnea (OSA), especially severe OSA, has also been associated with CVD^{8–10} and endothelial dysfunction.^{11,12}

Endothelial dysfunction results in functional and reversible changes in the arterial wall and is considered to precede the

development of atherosclerosis and CVD.^{13–16} There are 2 major methods for investigating endothelial function non-invasively: brachial artery ultrasound flow-mediated dilation (FMD) and reactive hyperemia peripheral arterial tonometry (RH-PAT). Both methods are considered to assess nitric oxide-dependent vascular function. Although FMD is dependent on operator skill and is difficult to use, RH-PAT is independent of operator skill and easy to use. It was recently shown that endothelial dysfunction evaluated on RH-PAT significantly correlated with future cardiovascular events.¹⁷

Although recent reports suggest that OSA exacerbates the cardiovascular risks attributed to obesity and metabolic syndrome (MetS),^{18,19} there are few reports on endothelial dys-

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function related to coexisting OSA and obesity.^{19,20} To our knowledge, there is no study using RH-PAT to investigate the additive effect of OSA on endothelial dysfunction due to visceral obesity.

We hypothesized that severe OSA exacerbated endothelial dysfunction in patients with visceral obesity. Thus, using RH-PAT, we investigated the association and interrelationships of endothelial function with visceral obesity, serum adiponectin, and OSA in addition to the effects of continuous positive airway pressure (CPAP) treatment.

Methods

Subjects

Study patients were consecutively recruited from the Sleep Unit of Kyoto University Hospital between September 2009 and February 2013. All had been referred to this sleep unit due to suspicion of OSA with symptoms such as habitual snoring or daytime sleepiness. None had been previously diagnosed with or treated for OSA. Patients with pulmonary diseases, chronic infection, or history of cancer or collagen disease were excluded. This study was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee (approval number E-2091), and written informed consent was obtained from all patients.

Polysomnography, CPAP and Follow-up

The diagnosis of OSA was confirmed on polysomnography (PSG; SomnoStar pro, Cardinal Health, Dublin, OH, USA or Alice 4, Philips Respironics, Murrysville, PA, USA), which was started at 22:00 hours and ended at 06:00 hours the following morning. Surface electrodes were attached using standard techniques to obtain an electro-oculogram, electromyogram of the chin, and 12-lead electroencephalograph. Sleep stages were defined according to the criteria of Rechtschaffen and Kales.²¹ Ventilation was monitored on inductive plethysmography (Respirace QDC, Viasys Healthcare, Palm Springs, CA, USA). Airflow was monitored with a nasal pressure transducer and supplemented by an oronasal thermal sensor. Arterial oxygen saturation (SpO₂) was monitored continuously with a pulse oximeter.

Apnea was defined as the complete cessation of airflow and hypopnea as a clear decrease in airflow $\geq 50\%$ lasting for ≥ 10 s accompanied by a decrease in SpO₂ of at least 3% and/or associated with arousal.²² Apnea-hypopnea index (AHI) was calculated as the number of episodes of apnea and hypopnea per hour over the total sleep time. Arousals were scored using the American Sleep Disorders Association's 3-s definition,²³ and the arousal index was calculated according to the number of arousals per h of sleep. Nocturnal oxygen desaturation was assessed as the minimum SpO₂ during sleep and SpO₂ $< 90\%$ time per total sleep time. Patients with predominantly central sleep apnea were excluded. OSA severity was defined as follows: none-mild OSA (AHI < 15), moderate OSA ($15 \leq \text{AHI} < 30$) and severe OSA (AHI ≥ 30). Those who agreed to CPAP underwent a second PSG with CPAP titration. We implemented CPAP with the autoadjusting positive airway pressure function for all patients. During the second sleep study, optimal minimum and maximum positive airway pressure were adjusted by the attending sleep laboratory physicians to abolish all respiratory events.

At the 3-month follow-up, we urged the patients to undergo a third sleep study to confirm whether an adjustment of the CPAP setting was necessary. To investigate the effect of CPAP treatment, at the third sleep study endothelial function data and blood samples were collected in the same way as at the first

sleep study.

RH-PAT

We evaluated endothelial dysfunction with RH-PAT in the morning following diagnostic and follow-up PSG. Previously, RH-PAT has been used to measure endothelial function in OSA patients.²⁴ With this method, specially designed finger probes (EndoPAT 2000, Itamar Medical, Caesarea, Israel) were placed on the index finger of each hand. Pulsatile arterial volume changes induced pressure alterations in the finger cuff, which were sensed by pressure transducers and recorded by the computer. The RH-PAT protocol consisted of a 5-min baseline measurement, after which a blood pressure cuff on the test arm was inflated to 60 mmHg above the baseline systolic blood pressure or at least 200 mmHg for 5 min. Occlusion of pulsatile arterial flow was confirmed by the reduction of the PAT tracing to zero. After 5 min, the cuff was deflated, and the PAT signal after cuff release compared with the baseline PAT signal was calculated via computer algorithm that automatically normalized for the baseline signal and was indexed to the control arm. As the index of endothelial function, the reactive hyperemia index (RHI) was calculated as the ratio of the average amplitude of PAT signal over 1 min starting 1.5 min after cuff deflation (control arm, A; occluded arm, C) divided by the average amplitude of the PAT signal of the 2.5-min time period before cuff inflation (baseline; control arm, B; occluded arm, D). $\text{RHI} = (\text{C/D})/(\text{A/B}) \times \text{baseline correction}$. Higher RHI represents a better preserved endothelial response to hyperemia.

Blood Sampling and Serum Adiponectin

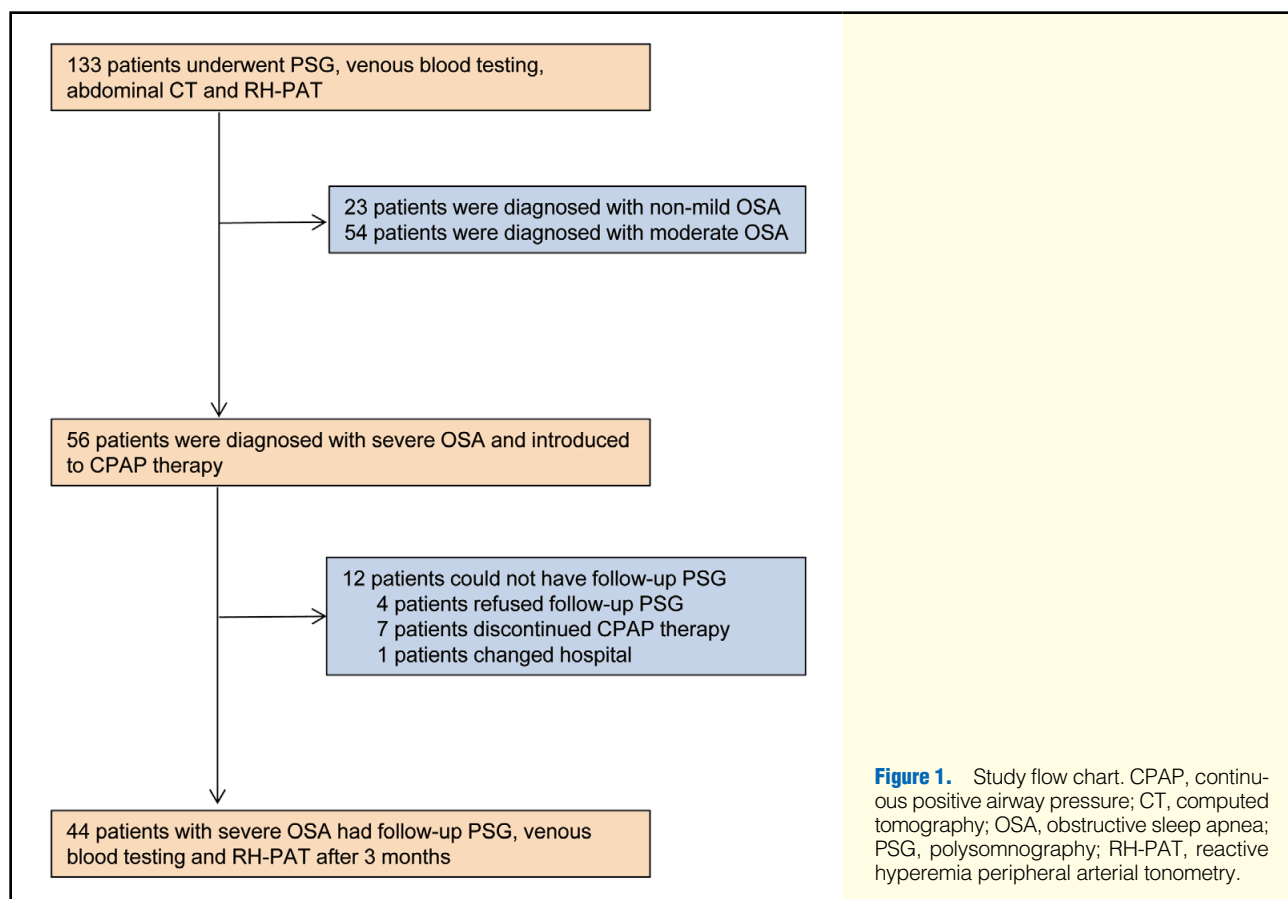
Blood samples were drawn at 07:00 hours after the subjects had fasted beginning at 20:00 hours the previous night. Blood samples were centrifuged immediately at $1,370 \times g$ at 4°C for 10 min. The separated samples were stored at -80°C until the assay. Serum adiponectin was measured using a latex agglutination kit (Otsuka Pharmaceutical, Tokyo, Japan). The minimum detection limit for adiponectin with this kit was $0.5 \mu\text{g/ml}$. The intra- and inter-assay coefficients of variation for adiponectin were 0.8–1.09% and 1.6–3.5%, respectively.

Visceral and Subcutaneous Fat Area

Visceral and subcutaneous fat area was assessed using an Aquilion 64 computed tomography (CT) system (Toshiba Medical Systems, Tochigi, Japan) running on 135 kVp, 440 mA, 0.5-s scan time and 10.0-mm slice thickness. We used a single CT scan obtained at the level of the umbilicus,²⁵ and the visceral and subcutaneous fat areas were quantified using a specialized image analysis program (AZE Virtual Place 99, AZE of America, Irvine, CA, USA).

Definition of Comorbidities

According to the criteria for Japanese subjects, we defined comorbidities as follows. BMI $\geq 25 \text{ kg/m}^2$ was defined as obesity.²⁶ Visceral obesity was determined to be positive at visceral fat area $\geq 100 \text{ cm}^2$ in both men and women.²⁶ WC was measured at the level of the navel with the patient standing, and abdominal obesity was determined to be positive at WC $\geq 85 \text{ cm}$ for men and $\geq 90 \text{ cm}$ for women.²⁶ A diagnosis of MetS required the subject to have abdominal obesity and 2 or 3 of the following: (1) dyslipidemia (triglycerides $\geq 150 \text{ mg/dl}$ and/or high-density lipoprotein cholesterol $< 40 \text{ mg/dl}$ or specific treatment for these lipid abnormalities); (2) hypertension (systolic blood pressure $\geq 130 \text{ mmHg}$ and/or diastolic blood pressure $\geq 85 \text{ mmHg}$ or treatment of previously diagnosed hypertension); and (3) hyperglycemia (fasting plasma glucose $\geq 110 \text{ mg/dl}$ or specific



treatment for diabetes mellitus).²⁷ Anthropometric parameters and blood pressure were measured immediately after PSG recording ended.

Statistical Analysis

Statistical analysis was done using JMP version 9.0.0 (SAS Institute, Cary, NC, USA). $P < 0.05$ was considered to be statistically significant. Results are expressed as mean $\pm$ SD. First, we classified the patients depending on the severity of OSA and compared their clinical backgrounds. The significance of intergroup differences was determined on analysis of variance. When a significant difference was found, we used Tukey's post-hoc test to identify where the difference was significant. Chi-squared test was used to compare categorical variables. Second, we evaluated the relationships among RHI and clinical variables using Pearson's or Spearman's correlation coefficients. Unpaired t-test was used for comparing RHI between 2 groups. Based on the results of these analyses, multiple regression analysis was done to determine the contribution of clinical variables to endothelial function. The variables entered into the multivariate analysis were those with $P < 0.10$ on univariate analysis. Third, we evaluated the impact on endothelial function of the coexistence of OSA and visceral obesity. In this subgroup analysis, the significance of intergroup differences was determined using Steel-Dwass test. Fourth, paired t-test was used to compare clinical variables before and after CPAP treatment.

Results

Subject Characteristics

For 133 patients, PSG, venous blood testing, abdominal CT and RH-PAT were performed. Fifty-six patients were diagnosed as having severe OSA and were indicated to undergo CPAP therapy. Seven patients, however, subsequently withdrew from CPAP therapy, 1 patient changed hospitals, and 4 patients refused follow-up PSG. As a result, 44 patients with severe OSA underwent follow-up PSG, venous blood testing, and RH-PAT at 3 months (Figure 1). There were no significant differences in characteristics (such as age, sex, blood pressure, BMI, Brinkman index, AHI) between patients who did and did not undergo follow-up PSG (Table S1). The baseline characteristics and PSG data of the total 133 patients are given in Tables 1,2. (Subject characteristics according to sex are given in Table S2.) Twenty-three had none-mild OSA, 54 had moderate OSA, and 56 had severe OSA. Those with severe OSA were characterized by a significantly higher WC, waist hip ratio, visceral fat area, and diastolic blood pressure than the other 2 groups. The percentages of patients who had fulfilled the criteria for visceral and abdominal obesity, hypertension, and MetS increased as the severity of OSA increased. There were no significant differences among the 3 groups in the other clinical background factors such as age, sex, dyslipidemia, hyperglycemia, and CVD.

Endothelial Function, Visceral Fat Accumulation, Serum Adiponectin and OSA

RHI was significantly correlated with AHI ($r = -0.24$, $P = 0.0055$), visceral fat area ($r = -0.19$, $P = 0.031$), and serum

Table 1. Baseline Subject Characteristics

	All patients	AHI <15	15≤AHI<30	30≤ AHI	P-value
No. subjects	133	23	54	56	
Age (years)	56.8±12.7	55.5±16.8	57.4±11.2	56.7±12.2	0.82
Sex (M/F)	105 (79)/28 (21)	14 (61)/9 (39)	44 (81)/10 (19)	47 (84)/9 (16)	0.062
SBP (mmHg)	128±19	122±17	125±16	132±21	0.041*
DBP (mmHg)	80±12	76±10	78±11	84±13	0.0070*
BMI (kg/m ²)	26.3±4.8	25.1±4.2	25.4±3.3	27.5±5.9	0.028*
BMI ≥25	73 (55)	12 (52)	27 (50)	34 (61)	0.51
WC (cm)	92±10.6	87.6±10.0	90.0±9.2	95.7±11.1	0.0014*
Abdominal obesity	97 (73)	13 (57)	37 (69)	47 (84)	0.029*
Waist hip ratio	0.97±0.049	0.95±0.056	0.96±0.048	0.99±0.040	0.0002*
Visceral fat area (cm ²)	121±69	80.6±53	120±70	139±67	0.0024*
Visceral obesity	80 (60)	9 (39)	29 (54)	42 (75)	0.0057*
Subcutaneous fat area (cm ²)	129±75	119±67	104±46	155±92	0.0010*
VFA/SFA ratio	1.14±0.81	0.91±0.90	1.28±0.71	1.10±0.85	0.17
Smoking status (current/ex/never) n	11/35/87	2/5/16	3/14/37	6/16/34	0.82
Brinkman index	141±281	80.7±160	143±305	164±296	0.49
MetS	50 (38)	4 (17)	18 (33)	28 (50)	0.018*
Hypertension	84 (63)	9 (39)	31 (57)	44 (79)	0.0023*
Dyslipidemia	74 (56)	9 (39)	32 (59)	33 (59)	0.22
Hyperglycemia	18 (14)	3 (13)	6 (11)	9 (16)	0.75
CVD	10 (7.5)	0 (0)	7 (13)	3 (5)	0.10
Total cholesterol (mg/dl)	197±40	186±39	193±36	205±43	0.10
LDL-C (mg/dl)	119±33	115±30	116±34	122±34	0.56
HDL-C (mg/dl)	51.6±13	55.0±14	52.4±12	49.5±14	0.20
Triglycerides (mg/dl)	140±103	117±73	125±60	164±138	0.065
Serum creatinine (mg/dl)	0.78±0.16	0.72±0.16	0.81±0.16	0.79±0.16	0.058
C-reactive protein (mg/dl)	0.11±0.19	0.13±0.32	0.063±0.12	0.15±0.17	0.049*
Serum adiponectin (μg/dl)	8.47±4.7	9.32±5.0	8.36±5.0	8.23±4.2	0.63

Data given as mean±SD or n (%). *P<0.05. Abdominal obesity was defined as WC ≥85 cm in male subjects, ≥90 cm in female subjects. Visceral obesity was defined as visceral fat area ≥100 cm². AHI, apnea hypopnea index; BMI, body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; SBP, systolic blood pressure; SFA, subcutaneous fat area; VFA, visceral fat area; WC, waist circumference.

Table 2. Polysomnography Data

	All patients (n=133)	AHI <15 (n=23)	15≤AHI<30 (n=54)	30≤ AHI (n=56)	P-value
AHI (/h)	33.2±21.8	8.72±4.4	23.0±3.9	53.2±18.9	<0.0001*
3% oxygen desaturation index (/h)	30.0±23.0	5.92±3.9	18.7±4.8	50.8±20.7	<0.0001*
Arousal index (/h)	30.9±16.8	20.8±11.0	23.2±11.4	42.4±16.5	<0.0001*
Sleep efficiency (%)	74.6±12.3	77.7±8.5	75.7±12.4	72.2±13.1	0.13
SpO ₂ <90% time per total sleep time (%)	13.6±21.3	0.96±1.5	3.51±3.7	28.5±26.1	<0.0001*
Minimum SpO ₂ (%)	78.6±11.6	87.6±4.3	81.0±9.2	72.5±12.4	<0.0001*

Data given as mean±SD. *P<0.05. SpO₂, arterial oxygen saturation. Other abbreviation as in Table 1.

adiponectin ($r=0.20$, $P=0.019$; **Figures 2A,C,E**). WC and subcutaneous fat area, however, were not significantly correlated with the RHI (**Figures 2B,D**). Serum adiponectin was not significantly correlated with AHI ($P=0.36$). RHI was significantly lower in male patients than in female patients (1.91 ± 0.47 vs. 2.20 ± 0.63 , $P=0.0074$). In male patients with severe OSA, endothelial function was significantly impaired (1.80 ± 0.42 AHI ≥30 vs. 2.00 ± 0.48 AHI <30, $P=0.025$). In female patients with even moderate OSA, endothelial function was significantly impaired (1.95 ± 0.37 AHI ≥15 vs. 2.74 ± 0.73 AHI <15, $P=0.0007$). The other clinical variables (hypertension, dyslipidemia, hyperglycemia, MetS, C-reactive protein, and serum creatinine) did not

significantly affect RHI (**Table 3**). On multivariate regression analysis, severe OSA remained as an independent predictive factor for RHI ($P=0.024$, $R^2=5.4\%$), but visceral fat area and serum adiponectin level did not (**Table 3**).

Additive Changes in RHI in Severe OSA Due to Visceral Obesity

On subgroup analysis, RHI in patients with visceral obesity significantly decreased in the presence of severe OSA ($P=0.042$). Patients with coexistent visceral obesity and severe OSA had significantly lower RHI than those with neither ($P=0.016$; **Figure 3**).

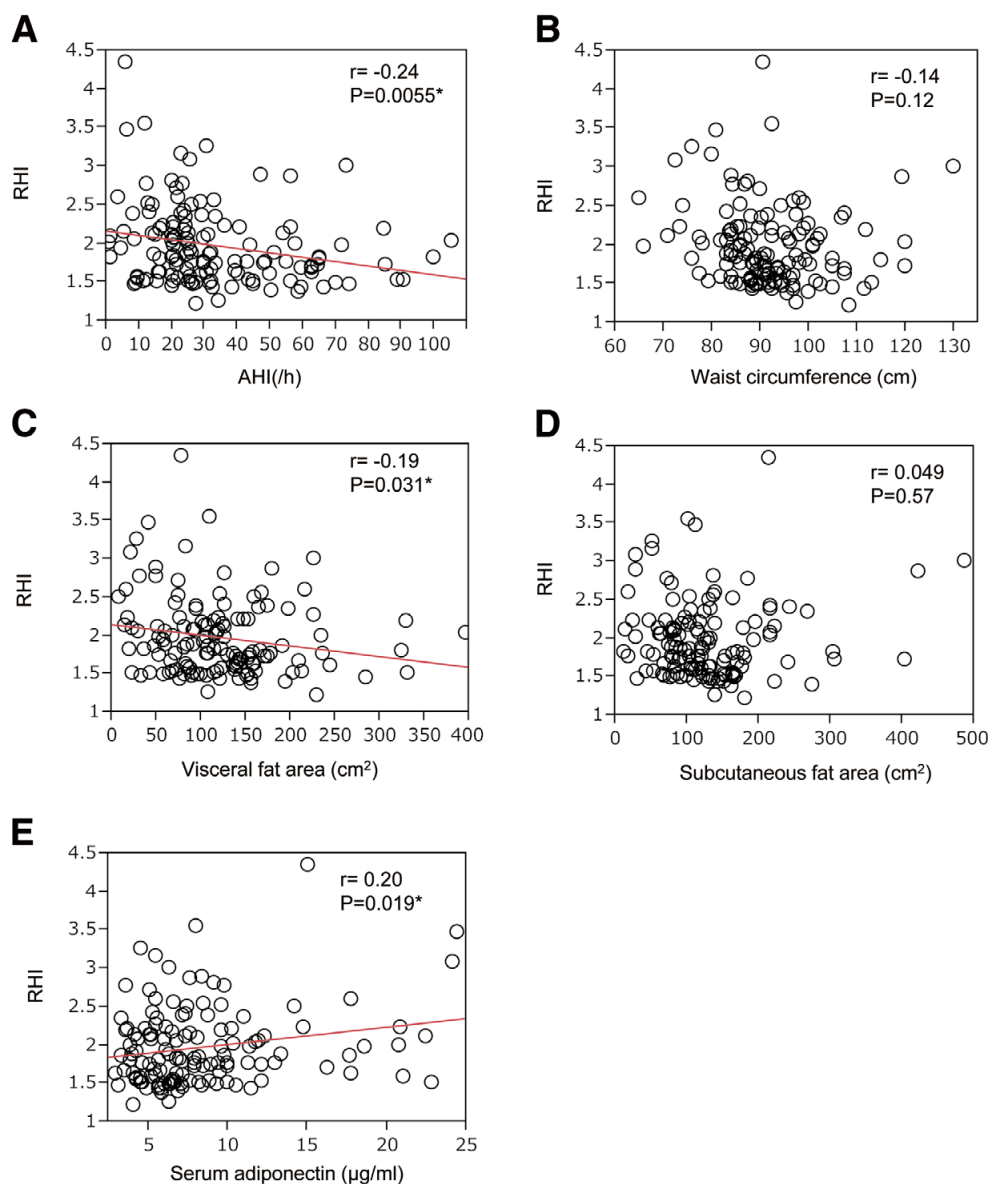


Figure 2. Reactive hyperemia index (RHI) vs. (A) apnea hypopnea index (AHI), (B) waist circumference, (C) visceral fat area, (D) subcutaneous fat area and (E) serum adiponectin. * $P < 0.05$. r , correlation coefficient.

Changes in Clinical Parameters After 3 Months of CPAP

The mean usage time for CPAP was 4.43 ± 1.8 h/day. After CPAP for 3 months, RHI in severe OSA patients improved significantly ($P = 0.013$; Table 4). RHI between patients with none-moderate OSA and those with severe OSA after CPAP treatment did not differ significantly (2.07 ± 0.55 vs. 2.00 ± 0.53 , $P = 0.45$), while the AHI following CPAP treatment significantly decreased ($P < 0.0001$). Serum adiponectin, WC, BMI, and waist hip ratio were not significantly improved (Table 4).

Discussion

This is the first study using RH-PAT to investigate the impact of OSA on endothelial function with or without visceral obesity. The main findings were as follows: (1) the coexistence of

severe OSA significantly impaired endothelial function in patients with visceral obesity; (2) severe OSA was associated with endothelial dysfunction independently of visceral fat area and serum adiponectin level; and (3) endothelial function in patients with severe OSA improved to that for none-moderate OSA following 3 months of CPAP treatment, although serum adiponectin and WC did not improve.

Endothelial Function in the Presence of Visceral Obesity and OSA

Visceral obesity is an important risk factor for CVD.² Visceral fat gain evaluated on abdominal CT is significantly correlated with impairment of endothelial function.⁴ The relationship between obesity and endothelial inflammation or increased vascular oxidative stress has also been reported.^{28,29} These reports,

Table 3. Independent Predictive Factors for RHI

	Univariate analysis		Multivariate analysis		
	r	P-value	β	P-value	R ² (%)
Age	0.13	0.14	—	—	
Male sex [†]	-0.23	0.0084*	-0.14	0.17	
Brinkman index	-0.15	0.081	-0.087	0.31	
BMI (kg/m ²)	-0.019	0.83	—	—	
Visceral fat area (cm ²)	-0.19	0.031*	-0.076	0.42	
Hypertension [†]	-0.026	0.76	—	—	
Dyslipidemia [†]	-0.13	0.15	—	—	
Hyperglycemia [†]	-0.10	0.23	—	—	
MetS [†]	-0.11	0.21	—	—	
AHI ≥ 30 /h [†]	-0.27	0.0020*	-0.20	0.024*	5.4
C-reactive protein (mg/dl)	0.040	0.65	—	—	
Serum adiponectin (μ g/ml)	0.20	0.019*	0.077	0.47	
Serum creatinine (mg/dl)	-0.081	0.36	—	—	

*P<0.05. [†]Spearman's correlation coefficient (the other correlations use Pearson's correlation coefficient). RHI, reactive hyperemia index. Other abbreviations as in Table 1.

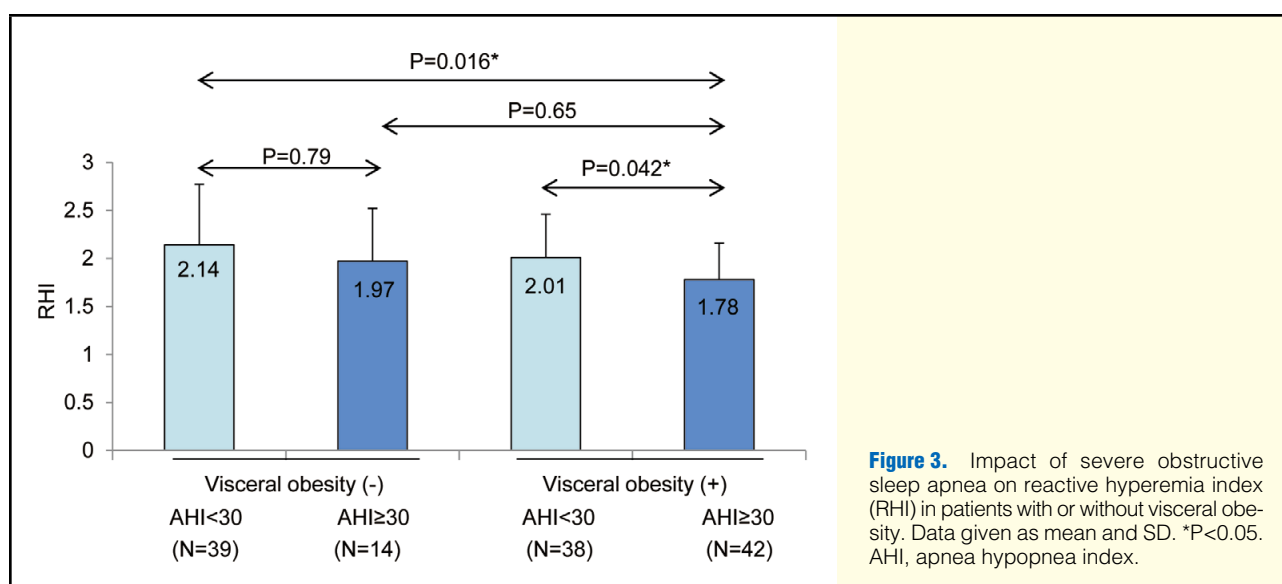


Figure 3. Impact of severe obstructive sleep apnea on reactive hyperemia index (RHI) in patients with or without visceral obesity. Data given as mean and SD. *P<0.05. AHI, apnea hypopnea index.

however, did not evaluate the coexistence of OSA with visceral obesity. A recent report showed that obesity does not significantly affect endothelial dysfunction in OSA patients, suggesting that the endothelial dysfunction in obesity patients is mainly due to OSA.¹⁹ Nuclear factor- κ B (NF- κ B) activation, in part via stimulation of oxidative stress, plays an important role in mediating vascular endothelial dysfunction in overweight and obese humans.²⁹ Therefore, given that it is a well known phenomenon that intermittent hypoxia mimicking OSA activates NF- κ B,³⁰ it might be reasonable that endothelial function as measured by RHI would worsen when patients had severe OSA and visceral obesity concomitantly. The present results support the idea that OSA exacerbates the cardiometabolic risk attributed to obesity or visceral obesity.¹⁸ In this study, CPAP treatment improved RHI in those with severe OSA to a level similar to those with none-moderate OSA, but WC did not change significantly during the observation period. From the present results, it can be considered that patients with severe OSA and visceral obesity should be treated with CPAP at least from the viewpoint

of endothelial function.

Endothelial Function, Serum Adiponectin and OSA

Adiponectin is secreted by adipocytes and has a multiplicity of actions affecting the cardiovascular system, such as insulin resistance, atherosclerosis, intimal hyperplasia, endothelial dysfunction, and pathological cardiac hypertrophy.³¹ Low adiponectin is one of the mechanisms explaining the association between obesity and CVD.³² Also, adiponectin was shown to be associated with endothelial dysfunction evaluated using FMD.⁶ Similarly, in this study, serum adiponectin was positively correlated with endothelial function evaluated on RH-PAT. On multivariate analysis, however, a relationship between serum adiponectin level and endothelial function was not shown. The present results suggest that severe OSA might have a greater association with endothelial dysfunction than adiponectin.

In this study, serum adiponectin was not associated with OSA. Lower adiponectin in patients with OSA than in control subjects has been reported.^{33,34} Adiponectin, however, was associ-

Table 4. Effect of 3-Month CPAP Treatment in Severe OSA (n=44)

	Baseline	After 3 months of CPAP	P-value
RHI	1.78±0.40	2.00±0.53	0.013*
Serum adiponectin (μg/ml)	8.74±4.1	8.73±4.4	0.95
AHI (/h)	53.2±17.8	4.18±4.0	<0.0001*
BMI (kg/m ²)	27.4±6.1	27.5±5.9	0.84
WC (cm)	95.9±10.6	95.0±11.7	0.18
Waist hip ratio	0.99±0.041	0.97±0.038	0.076
SBP (mmHg)	131±18	123±15	<0.0001*
DBP (mmHg)	83±11	76±12	<0.0001*
C-reactive protein (mg/dl)	0.14±0.18	0.082±0.099	0.018*
Total cholesterol (mg/dl)	199±42	191±47	0.046*
LDL-C (mg/dl)	117±32	111±29	0.12
HDL-C (mg/dl)	49.7±11	50.5±12	0.32
Triglycerides (mg/dl)	159±148	148±141	0.079

Data given as mean ± SD. *P<0.05. CPAP, continuous positive airway pressure; OSA, obstructive sleep apnea. Other abbreviations as in Tables 1,3.

ated with age, sex, and obesity, which are also major risk factors for OSA. In the present study, these confounding factors (age, sex, and obesity) were adequately evaluated. The present results support the negative reports on the association of OSA with adiponectin^{35,36} and suggest that severe OSA has an additive effect on endothelial dysfunction independently of serum adiponectin level.

Endothelial Dysfunction Evaluated With RH-PAT in OSA Patients

Several reports using FMD showed that OSA was associated with endothelial dysfunction and that treatment of OSA with CPAP significantly improved endothelial function.^{11,12,37,38} The present results were similar to that of these reports. FMD reflects endothelial function in larger vessels such as the brachial artery; in contrast, RH-PAT reflects endothelial function in the smaller vessels in the end of the finger. Using a forearm skin biopsy sample, it was suggested that patients with OSA with low cardiovascular risk had endothelial dysfunction in the microcirculation, which improved with CPAP.³⁹ The present results supported this finding that OSA patients had endothelial dysfunction in the microcirculation. A recent review suggested that microvascular endothelial function may be an earlier indicator of CVD risk than macrovascular endothelial function.⁴⁰ Using RH-PAT, we might be able to detect early cardiovascular risk in patients with OSA.

In 2 previous large epidemiological studies, including 1 that used RH-PAT, a significant association between OSA and endothelial function was shown in female subjects only.^{41,42} Although those studies had greater numbers of female participants than the present study, the prevalence of severe OSA was low. In addition to the present study, endothelial dysfunction was also seen in severe OSA patients in other studies, including 1 that used RH-PAT.^{43–45} In the present multivariate analysis, severe OSA was related to RHI independently of sex. Therefore, not only sex but also the severity of OSA might have a significant association with endothelial function as reflected by RH-PAT.

Numerous reports showed that endothelial function is impaired in patients with hypertension, dyslipidemia, or diabetes.^{46–48} These cardiovascular risk factors, however, were not associated with RH-PAT in the present study. Several mechanisms are possible. First, the previous reports usually have not

taken into account the presence of OSA in the cohort studied.^{46–48} Usually, the prevalence of OSA in patients with hypertension, dyslipidemia, or diabetes is higher than in those without these diseases.^{49–51} Therefore, in the previous reports, OSA might have influenced the data on patients with hypertension, hyperlipidemia or diabetes. Second, given that the present study was done in a sleep center, most of the patients (83%) had moderate or severe OSA. Indeed, most of the patients who did not have hypertension, dyslipidemia, or hyperglycemia had moderate or severe OSA (71%, 76%, and 83%, respectively). Therefore, in this cohort, it might be difficult to detect differences in endothelial function between patients with and without hypertension, dyslipidemia, or hyperglycemia independent of the effect of OSA. Third, similar to the present results, in the Framingham Heart Study, which is an epidemiological study using RH-PAT, an association between hypertension and endothelial function was not shown.⁵² Conversely, in the other report using FMD, this association was found.⁴⁶ Because RH-PAT and FMD focused on different vessels, these reports suggested that hypertension might have limited effects on the function of the small vessels evaluated using RH-PAT.⁵² Therefore, the difference in methodology might have influenced the present results. And fourth, the size of the present study cohort might not be sufficient to detect associations between endothelial dysfunction and hypertension, dyslipidemia or hyperglycemia. For reasons already noted, it was difficult to discuss fully the differences in results between the present study and the previous ones.^{46–48} Therefore, further studies with larger subject groups are needed to investigate these differences.

Study Limitations

The present study had a few limitations. First, CPAP was not allocated randomly. Unmeasured factors may have changed during CPAP therapy and could have contributed to the observed improvement in endothelial function. The reversal of endothelial dysfunction in the present study, however, suggested that OSA was mainly responsible for the change in endothelial function. We could not show causality or identify the mechanism because this was an observational study. We should confirm these results in a future randomized clinical trial. Second, we did not verify the RH-PAT results. The measurement of endothelial function as a clinical tool in daily practice is not well

established.⁴⁰ A further study would be needed with both RH-PAT and another method such as FMD to acquire comprehensive vascular information on OSA patients.

Conclusions

Severe OSA, but not visceral fat area or serum adiponectin level, was independently associated with endothelial function according to RHI. The coexistence of severe OSA impaired endothelial function in patients with visceral obesity. In addition, the disturbance in endothelial function improved significantly after 3 months of CPAP treatment.

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References

- Romero-Corral A, Montori VM, Somers VK, Korinek J, Thomas RJ, Allison TG, et al. Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: A systematic review of cohort studies. *Lancet* 2006; **368**: 666–678.
- Britton KA, Massaro JM, Murabito JM, Kreger BE, Hoffmann U, Fox CS. Body fat distribution, incident cardiovascular disease, cancer, and all-cause mortality. *J Am Coll Cardiol* 2013; **62**: 921–925.
- Matsuzawa Y, Sugiyama S, Sugamura K, Sumida H, Kurokawa H, Fujisue K, et al. Successful diet and exercise therapy as evaluated on self-assessment score significantly improves endothelial function in metabolic syndrome patients. *Circ J* 2013; **77**: 2807–2815.
- Romero-Corral A, Sert-Kunoyoshi FH, Sierra-Johnson J, Orban M, Gami A, Davison D, et al. Modest visceral fat gain causes endothelial dysfunction in healthy humans. *J Am Coll Cardiol* 2010; **56**: 662–666.
- Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation* 2000; **102**: 1296–1301.
- Torigoe M, Matsui H, Ogawa Y, Murakami H, Murakami R, Cheng XW, et al. Impact of the high-molecular-weight form of adiponectin on endothelial function in healthy young men. *Clin Endocrinol (Oxf)* 2007; **67**: 276–281.
- Frystyk J, Berne C, Berglund L, Jensen K, Flyvbjerg A, Zethelius B. Serum adiponectin is a predictor of coronary heart disease: A population-based 10-year follow-up study in elderly men. *J Clin Endocrinol Metab* 2007; **92**: 571–576.
- Yamada S, Suzuki H, Kamioka M, Suzuki S, Kamiyama Y, Yoshihisa A, et al. Sleep-disordered breathing increases risk for fatal ventricular arrhythmias in patients with chronic heart failure. *Circ J* 2013; **77**: 1466–1473.
- Floras JS. Sleep apnea and cardiovascular risk. *J Cardiol* 2014; **63**: 3–8.
- Jordan AS, McSharry DG, Malhotra A. Adult obstructive sleep apnoea. *Lancet* 2014; **383**: 736–747.
- Kraiczi H, Caidahl K, Samuelsson A, Peker Y, Hedner J. Impairment of vascular endothelial function and left ventricular filling: Association with the severity of apnea-induced hypoxemia during sleep. *Chest* 2001; **119**: 1085–1091.
- Chung S, Yoon IY, Shin YK, Lee CH, Kim JW, Lee T, et al. Endothelial dysfunction and C-reactive protein in relation with the severity of obstructive sleep apnea syndrome. *Sleep* 2007; **30**: 997–1001.
- Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 2000; **101**: 948–954.
- Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000; **101**: 1899–1906.
- Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, et al. Prognostic value of coronary vascular endothelial dysfunction. *Circulation* 2002; **106**: 653–658.
- Bugiardini R, Manfrini O, Pizzi C, Fontana F, Morgagni G. Endothelial function predicts future development of coronary artery disease: A study of women with chest pain and normal coronary angiograms. *Circulation* 2004; **109**: 2518–2523.
- Matsuzawa Y, Sugiyama S, Sumida H, Sugamura K, Nozaki T, Ohba K, et al. Peripheral endothelial function and cardiovascular events in high-risk patients. *J Am Heart Assoc* 2013; **2**: e000426, doi:10.1161/JAHA.113.000426.
- Drager LF, Togeiro SM, Polotsky VY, Lorenzi-Filho G. Obstructive sleep apnea: A cardiometabolic risk in obesity and the metabolic syndrome. *J Am Coll Cardiol* 2013; **62**: 569–576.
- Jelic S, Lederer DJ, Adams T, Padeletti M, Colombo PC, Factor PH, et al. Vascular inflammation in obesity and sleep apnea. *Circulation* 2010; **121**: 1014–1021.
- Namtded SK, Hissdal J, Randby A, Agewall S, Stranden E, Somers VK, et al. Impaired endothelial function in persons with obstructive sleep apnoea: Impact of obesity. *Heart* 2013; **99**: 30–34.
- Rechtschaffen A, Kales A. A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Washington, DC: National Institutes Health, 1968.
- Iber C, Ancoli-Israel S, Chesson A, Quan S. The AASM manual for the scoring of sleep and associated events: Rules, terminology and technical specifications. Westchester, IL: American Academy of Sleep Medicine, USA, 2007.
- Anonymous. EEG arousals: Scoring rules and examples: A preliminary report from the Sleep Disorders Atlas Task Force of the American Sleep Disorders Association. *Sleep* 1992; **15**: 173–184.
- Itzhaki S, Lavie L, Pillar G, Tal G, Lavie P. Endothelial dysfunction in obstructive sleep apnea measured by peripheral arterial tone response in the finger to reactive hyperemia. *Sleep* 2005; **28**: 594–600.
- Yoshizumi T, Nakamura T, Yamane M, Islam AH, Menju M, Yamasaki K, et al. Abdominal fat: Standardized technique for measurement at CT. *Radiology* 1999; **211**: 283–286.
- Examination Committee of Criteria for ‘Obesity Disease’ in Japan; Japan Society for the Study of Obesity. New criteria for ‘obesity disease’ in Japan. *Circ J* 2002; **66**: 987–992.
- Committee on Evaluation of Diagnosis Standards for Metabolic Syndrome. Definition and diagnostic standards for metabolic syndrome. *J Jpn Soc Int Med* 2005; **94**: 794–809.
- Silver AE, Beske SD, Christou DD, Donato AJ, Moreau KL, Eskurza I, et al. Overweight and obese humans demonstrate increased vascular endothelial NAD(P)H oxidase-p47(phox) expression and evidence of endothelial oxidative stress. *Circulation* 2007; **115**: 627–637.
- Pierce GL, Lesniewski LA, Lawson BR, Beske SD, Seals DR. Nuclear factor- κ B activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. *Circulation* 2009; **119**: 1284–1292.
- Ryan S, Taylor CT, McNicholas WT. Systemic inflammation: A key factor in the pathogenesis of cardiovascular complications in obstructive sleep apnoea syndrome? *Thorax* 2009; **64**: 631–636.
- Hopkins TA, Ouchi N, Shibata R, Walsh K. Adiponectin actions in the cardiovascular system. *Cardiovasc Res* 2007; **74**: 11–18.
- Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Walsh K. Obesity, adiponectin and vascular inflammatory disease. *Curr Opin Lipidol* 2003; **14**: 561–566.
- Zhang XL, Yin KS, Wang H, Su S. Serum adiponectin levels in adult male patients with obstructive sleep apnea hypopnea syndrome. *Respiration* 2006; **73**: 73–77.
- Yoshikawa M, Yamauchi M, Fujita Y, Koyama N, Fukuoka A, Tamaki S, et al. The impact of obstructive sleep apnea and nasal CPAP on circulating adiponectin levels. *Lung* 2014; **192**: 289–295.
- Kritikou I, Basta M, Vgontzas AN, Pejovic S, Liao D, Tsaousoglou M, et al. Sleep apnoea, sleepiness, inflammation and insulin resistance in middle-aged males and females. *Eur Respir J* 2014; **43**: 145–155.
- Sánchez-de-la-Torre M, Mediano O, Barceló A, Piérola J, de la Peña M, Esquinas C, et al. The influence of obesity and obstructive sleep apnea on metabolic hormones. *Sleep Breath* 2012; **16**: 649–656.
- 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.
- Bayram NA, Ciftci B, Keles T, Durmaz T, Turhan S, Bozkurt E, et al. Endothelial function in normotensive men with obstructive sleep apnea before and 6 months after CPAP treatment. *Sleep* 2009; **32**: 1257–1263.
- Patt BT, Jarjoura D, Haddad DN, Sen CK, Roy S, Flavahan NA, et

- al. Endothelial dysfunction in the microcirculation of patients with obstructive sleep apnea. *Am J Respir Crit Care Med* 2010; **182**: 1540–1545.
40. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, et al. The assessment of endothelial function: From research into clinical practice. *Circulation* 2012; **126**: 753–767.
 41. Randby A, Namtvedt SK, Hrubos-Strøm H, Einvik G, Somers VK, Omland T. Sex-dependent impact of OSA on digital vascular function. *Chest* 2013; **144**: 915–922.
 42. Faulx MD, Larkin EK, Hoit BD, Aylor JE, Wright AT, Redline S. Sex influences endothelial function in sleep-disordered breathing. *Sleep* 2004; **27**: 1113–1120.
 43. Seif F, Patel SR, Walia H, Rueschman M, Bhatt DL, Gottlieb DJ, et al. Association between obstructive sleep apnea severity and endothelial dysfunction in an increased background of cardiovascular burden. *J Sleep Res* 2013; **22**: 443–451.
 44. Chung S, Yoon IY, Lee CH, Kim JW. The association of nocturnal hypoxemia with arterial stiffness and endothelial dysfunction in male patients with obstructive sleep apnea syndrome. *Respiration* 2010; **79**: 363–369.
 45. Jelic S, Padeletti M, Kawut SM, Higgins C, Canfield SM, Onat D, et al. Inflammation, oxidative stress, and repair capacity of the vascular endothelium in obstructive sleep apnea. *Circulation* 2008; **117**: 2270–2278.
 46. Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF Jr, et al. Clinical correlates and heritability of flow-mediated dilation in the community: The Framingham Heart Study. *Circulation* 2004; **109**: 613–619.
 47. Creager MA, Cooke JP, Mendelsohn ME, Gallagher SJ, Coleman SM, Loscalzo J, et al. Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *J Clin Invest* 1990; **86**: 228–234.
 48. Clarkson P, Celermajer DS, Donald AE, Sampson M, Sorensen KE, Adams M, et al. Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. *J Am Coll Cardiol* 1996; **28**: 573–579.
 49. Silverberg DS, Oksenberg A. Essential hypertension and abnormal upper airway resistance during sleep. *Sleep* 1997; **20**: 794–806.
 50. Tishler PV, Larkin EK, Schluchter MD, Redline S. Incidence of sleep-disordered breathing in an urban adult population: The relative importance of risk factors in the development of sleep-disordered breathing. *JAMA* 2003; **289**: 2230–2237.
 51. West SD, Nicoll DJ, Stradling JR. Prevalence of obstructive sleep apnoea in men with type 2 diabetes. *Thorax* 2006; **61**: 945–950.
 52. Hamburg NM, Keyes MJ, Larson MG, Vasan RS, Schnabel R, Pryde MM, et al. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* 2008; **117**: 2467–2474.

Supplementary Files

Supplementary File 1

Table S1. Subject characteristics vs. follow-up PSG

Table S2. Subject characteristics vs. sex

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