

## Endothelial Dysfunction in Patients With Obstructive Sleep Apnoea Independent of Metabolic Syndrome

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### Abstract

**Introduction:** Obstructive sleep apnoea syndrome (OSAS), characterised by intermittent hypoxia/re-oxygenation, has been identified as an independent risk factor for cardiovascular diseases and endothelial dysfunction. Our aim was to investigate flow-mediated dilatation (FMD) in patients with obstructive sleep apnoea with and without metabolic syndrome. **Materials and Methods:** Fifty-two subjects with OSAS diagnosed by polysomnography were classified into 2 groups according to the presence and absence of the metabolic syndrome and also according to the severity: mild to moderate OSAS group and severe OSAS group. Endothelial function of the brachial artery was evaluated by using high-resolution vascular ultrasound. Endothelial-dependent dilatation (EDD) was assessed by establishing reactive hyperaemia and endothelial-independent dilatation (EID) was determined by using sublingual isosorbide dinitrate. Spearman correlation and regression analysis were performed. **Results:** EDD was not significantly different in patients with OSAS and metabolic syndrome as compared with OSAS without metabolic syndrome ( $4.62 \pm 0.69$  versus  $4.49 \pm 0.93$ ,  $P > 0.05$ ). **Conclusions:** Endothelial dysfunction in OSA may be independent of metabolic syndrome.

Ann Acad Med Singapore 2009;38:461-4

**Key words:** Endothelial function, Metabolic syndrome, Obstructive sleep apnoea syndrome

### Introduction

Obstructive sleep apnoea syndrome (OSAS) is a widely prevalent disorder characterised by recurrent partial or complete obstruction of upper airway during sleep. Compelling data from several large cross-sectional and longitudinal studies strongly suggest a role of OSAS in the development of cardiovascular disorders, including hypertension, coronary artery disease and stroke.<sup>1,2</sup> However, the patho-physiologic mechanism by which OSAS contributes to the cardiovascular pathology remains unclear and awaits further elucidation.

Several studies have suggested impaired endothelial function in patients with OSAS.<sup>3,4</sup> The sources of the endothelial injury are not well known, but potential aetiologies include hypoxemia with reactive oxygen species generation and systemic inflammation. OSAS is also associated with obesity, hypertension and metabolic dysregulation, which by themselves may contribute to adverse effects on endothelium. Endothelial injury results

in the alteration of endothelial hormones that are responsible for maintaining vascular tone and preventing abnormal cell proliferation, increased coagulability and altered leukocyte trafficking; exposes subendothelial structures to diverse growth factors in the circulation.<sup>5</sup> The resultant vasoconstriction, vascular smooth muscle proliferation, and hypercoagulability may lead to adverse cardiovascular consequences associated with OSAS, such as hypertension, coronary artery disease and cerebrovascular disease.<sup>1,6</sup> Treatment of obstructive sleep apnoea with continuous positive airway pressure (CPAP) therapy has been suggested to improve endothelial function in the systemic circulation.<sup>7</sup>

Metabolic syndrome has also been found to cause endothelial dysfunction.<sup>8</sup> Whether the endothelial dysfunction in OSAS is related to a possible co-existing metabolic syndrome or is an independent complication of OSAS has not been clarified. However, in a single report, endothelial dysfunction in 9 OSAS patients without metabolic syndrome has been reported.<sup>9</sup>

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Over the past decade, a non-invasive technique has evolved to evaluate flow-mediated dilatation (FMD), an endothelial-dependent function, in the brachial artery after occlusion.<sup>10,11</sup> This stimulus provokes the endothelium to release nitric oxide (NO), with subsequent vasodilatation that can be traced by ultrasonography and quantified as an index of vasomotor function.<sup>12</sup> The purpose of this study was to assess endothelial-dependent dilatation (EDD) and endothelial-independent dilatation (EID) in patients with OSAS and metabolic syndrome versus OSAS without metabolic syndrome. We hypothesised that endothelial dysfunction may be present in patients with OSAS without metabolic syndrome.

## Materials and Methods

### Patients

We enrolled all of the patients diagnosed with OSAS based on overnight polysomnography at the Bamdad Sleep Study Centre between March 2006 and September 2006.

The diagnosis of OSAS was based on the recognised criteria, including an apnoea-hypopnoea index (AHI)  $\geq 5$ /h and pathological daytime sleepiness.<sup>13</sup> The Epworth sleepiness scale was used to quantify subjective daytime sleepiness.<sup>14</sup> Patients with congestive heart failure, intrinsic pulmonary disease, and/or accompanying chronic renal or liver disease, or those scheduled for titration of nasal mask ventilation were excluded. As a result, there remained 52 patients with a definite diagnosis of OSAS. These patients were divided into 2 groups based on the presence or absence of the criteria for metabolic syndrome. None of the patients in either group were under CPAP therapy before the study.

Metabolic syndrome status was defined by criteria as defined in the third adult treatment panel of the national cholesterol education programme.<sup>15</sup> Presence of 3 of the following 5 criteria was assumed as a sufficient clue to be classified as metabolic syndrome: (i) fasting glucose  $>110$  mg/dL or being under treatment for diabetes, (ii) central obesity (waist circumference  $>102$  cm for men and  $>88$  cm for women), (iii) arterial pressure  $>130/85$  mmHg or being under treatment for hypertension, (iv) triglyceride levels  $>150$  mg/dL or current use of fibrates and (v) high-density lipoprotein (HDL) cholesterol  $<40$  mg/dL (men) and  $<50$  mg/dL (women).

Patients with OSAS were divided into 2 groups based on the severity of OSAS, i.e., a mild to moderate group (AHI: 5–29.9/h), and a severe group with (AH  $\geq 30$ /h).<sup>13</sup>

### Brachial Artery Measurements

Using ultrasonography, arterial endothelium and smooth muscle function were measured by examining brachial artery responses to endothelium-dependent (flow-mediated

dilatation) and endothelium-independent stimuli (sublingual nitroglycerine), respectively.

Ultrasonographic measurement was carried out according to the method described by Corretti et al.<sup>16</sup> The assessment was carried out after an overnight fast in a quiet, air-conditioned room (22 to 24°C) by an experienced cardiologist, who was blinded to diagnosis. The diameter of brachial artery was measured on B-mode ultrasound images, with use of a 7.5-MHz transducer. Ultrasound measurement was obtained using a high-resolution ultrasound machine (Power Vision 8000, Toshiba Shiomoishigami, Otawara-Shi, Japan). The right brachial artery was scanned in longitudinal sections 2 to 8 cm above the elbow. After the detection of the right transducer position, the skin surface was marked and the arm kept in the same position during the study. All scans were recorded on video recorder and analysed later. Arterial diameters were measured at rest, during reactive hyperaemia (FMD), again at rest, and after administration of 0.4 mg sublingual nitrate. Reactive hyperaemia was induced by inflation of a pneumatic cuff on the upper arm to suprasystolic pressure, followed by cuff deflation after 4.5 minutes. The diameter of the brachial artery was scanned and recorded after deflation. After 10 to 15 minutes rest, the second control scan diameter was recorded. Then sublingual nitrate was given, and 3.5 to 4 minutes later, a final scan of the diameter was recorded. The end diastolic arterial diameter was measured 3 times from one media-adventitia interface to the other at the clearest section: at baseline, every 20 seconds after reactive hyperaemia and after administration of nitrate. The maximum vessel diameter was defined as the average of the 3 consecutive maximum diameter measurements after hyperaemia and nitrate, respectively. Vasodilatation by reactive hyperaemia or nitrate was expressed as the per cent change in diameter, compared with baseline values.

### Statistical Analysis

Measurements between the 2 groups and their related sub-classes were compared using Mann-Whitney U and  $\chi^2$  tests. The Mann-Whitney U test was applied to the groups in pairs for all possible combinations.  $P < 0.05$  was considered to be statistically significant. All of the values were expressed as mean  $\pm$  SD.

## Results

Fifty-two patients with documented OSA were eligible for participation in the study. There were 14 men and 12 women in the metabolic syndrome group, while the non-metabolic syndrome group composed of 16 men and 10 women. The ages ranged from 18 to 70 years ( $49.03 \pm 12.98$ ) for the first group and 12 to 78 ( $49.5 \pm 17.69$ ) in the second. Four patients with metabolic syndrome were

smokers (15.4%), however, in the second group, 8 (30.5%) were smokers. Four of the patients with metabolic syndrome (15.4%) and 3 (11.5%) of the non-metabolic cases were using angiotensin converting enzyme inhibitor; also 3 (11.5%) versus 2 (7.7%) of the 2 groups received lipid lowering drugs (Table 1).

Table 1 depicts the baseline demographic, anthropomorphic, polysomnographic and vascular reactivity of the brachial artery data of patients with and without the metabolic syndrome. The patients with metabolic and without the metabolic syndrome group were comparable with respect to age, AHI, basal brachial diameter, EDD, EID, EDD/EID. Only the body mass index was higher in subjects with metabolic syndrome compared with the other group.

Furthermore, BMI, basal brachial diameter, EDD, EID and EDD/EID were significantly higher in the severe OSAS group than the mild to moderate OSAS group (Table 2).

## Discussion

Endothelial dysfunction, measured by FMD, was found to be associated with the degree of nocturnal hypoxaemia or AHI and hypoxaemia index in OSAS.<sup>17,18</sup> In our series, many cases with hypertension/and or hyperlipemia were under treatment for their conditions, so the results should be handled with caution. Therefore minimal differences in EDD and EID with a better profile in those with metabolic syndrome can be regarded as a possible effect of treatment. However, this observation was not statistically significant. Furthermore in cases with more severe OSAS, endothelial function was found to be better than those with a less severe form of disease, a finding that can also be attributed to treatment.

In our series, no association was found between endothelial function and metabolic syndrome in patients with OSAS. Since the time of study, none of the patients in either group was under treatment with CPAP and no data regarding the effects of CPAP therapy are available in this series. Also in previous studies, endothelial dysfunction has been reported to be improved after treatment with nasal continuous positive airway pressure.<sup>3</sup> This can be an explanation for our paradoxical results.

Endothelial nitric oxide plays a central role in endothelial function and endothelium-dependent flow mediated vasodilatation of the brachial artery.<sup>19,20</sup> Many pathogenetic mechanisms that may impair endothelium-dependent nitric oxide-mediated vasodilatation have been shown to be present in OSA. During recurrent apnoeas and hypopnoea, the vascular endothelium is conceivably subjected to recurrent shear stress and hypoxaemia-reoxygenation, which may result in decreased synthesis or enhanced

Table 1. Clinical Data of OSAS With Metabolic Syndrome and Without Metabolic Syndrome

Patient characteristic	OSAS with metabolic syndrome	OSAS without metabolic syndrome	P
Number of subjects	26	26	
Age (y)	49.03 ± 12.98	49.5 ± 17.69	0.91
Gender (M/F)	14/12	16/10	
AHI (number/h)	22.96 ± 24.86	16.51 ± 21.33	0.32
BBD (mm)	4.3 ± 0.65	4.08 ± 1.7	0.34
EDD(percent change)	4.62 ± 0.69	4.49 ± 0.93	0.56
EID (percent change)	5.05 ± 0.64	4.92 ± 0.88	0.54
EDD/EID	0.91 ± 5.30	0.90 ± 6.1	0.75
BMI (kg/m <sup>2</sup> )	34.38 ± 7.37	27.59 ± 5.93	0.001
ACE inhibitor use	4/26 (15.4%)	3/26 (11.5%)	0.5
Lipid lowering drugs	3/26 (11.5%)	2/26 (7.7%)	0.46

ACE: angiotensin converting enzyme; AHI: apnoea-hypopnoea index; BBD: basal brachial diameter; BMI: body mass index; EDD: endothelial dependent dilatation; EID: endothelial independent dilatation

Table 2. Clinical Data of OSA Based on Severity

Patient characteristic	OSAS with metabolic syndrome	OSAS without metabolic syndrome	P
Number of subjects	42	10	
Age (y)	48.26 ± 14.64	53.5 ± 18.36	0.17
BBD (mm)	4.08 ± 0.74	4.66 ± 0.88	0.03
EDD (percent change)	4.44 ± 0.77	5.07 ± 0.86	0.02
EID (percent change)	4.87 ± 0.75	5.47 ± 0.72	0.02
EDD/EID	0.91 ± 0.06	0.92 ± 0.06	0.47
BMI (kg/m <sup>2</sup> )	30.06 ± 6.85	34.91 ± 8.96	0.03

AHI: apnoea-hypopnoea index; BBD: basal brachial diameter; BMI: body mass index; EDD: endothelial dependent dilatation; EID: endothelial independent dilatation;

degradation of endothelial nitric oxide, and recent studies have demonstrated that circulating nitric oxide levels were decreased in subjects with OSA.<sup>19,21</sup> Other mechanisms for impaired nitric oxide-mediated endothelial function, including increased oxidant load, enhanced oxidation of low density lipoprotein, insulin resistance, and increased sympathetic activity, have been demonstrated to be present in OSA.<sup>19,21</sup> Nitroglycerin is used to determine the maximum obtainable vasodilator response and serve as a measure of EID reflecting vascular smooth muscle function. Thus, decreased vasodilator response to exogenous administration of NO donor suggests smooth muscle dysfunction in the arterial wall.

Patients with hypertension, hypercholesterolemia, congestive heart failure and diabetes mellitus have impaired endothelial function.<sup>9</sup> “Uncomplicated” central obesity has also been reported to be associated with impaired endothelial function.<sup>22</sup>

However, we included OSAS patients with hypertension, obesity and hypercholesterolemia and who were receiving ACE inhibitor and lipid lowering agents for their conditions. These drugs could not be discontinued due to ethical concerns and may affect the results of final analysis.

Another limitation of the present study is the subjects enrolled in this study were not evenly distributed in terms of a high proportion of patients with mild to moderate OSAS.

In conclusion, our results suggests that endothelial dysfunction in OSAS may be independent of metabolic syndrome. Further studies are warranted for confirmation.

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