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# Impairment of Endothelium-Dependent Vasodilation of Resistance Vessels in Patients With Obstructive Sleep Apnea

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**Background**—Patients with obstructive sleep apnea (OSA) experience repetitive episodic hypoxemia with consequent sympathetic activation and marked blood pressure surges, each of which may impair endothelial function. We tested the hypothesis that patients with OSA have impaired endothelium-dependent vasodilation, even in the absence of overt cardiovascular disease.

**Methods and Results**—We studied 8 patients with OSA (age  $44 \pm 4$  years) and 9 obese control subjects (age  $48 \pm 3$  years). Patients with OSA were newly diagnosed, never treated for OSA, on no medications, and free of any other known diseases. All obese control subjects underwent complete overnight polysomnographic studies to exclude occult OSA. Resistance-vessel function was tested by use of forearm blood flow responses to intra-arterial infusions of acetylcholine (a vasodilator that stimulates endothelial release of nitric oxide), sodium nitroprusside (an exogenous nitric oxide donor), and verapamil (a calcium channel blocker). Conduit-vessel function was also evaluated by ultrasonography. Brachial artery diameter was measured under baseline conditions, during reactive hyperemia (with flow increase causing endothelium-dependent dilatation), and after sublingual administration of nitroglycerin (an endothelium-independent vasodilator). Patients with OSA had a blunted vasodilation in response to acetylcholine ( $P < 0.007$ ), but responses to sodium nitroprusside and verapamil were not significantly different from those of control subjects. No significant difference in conduit-vessel dilation was evident between OSA patients and obese control subjects.

**Conclusions**—Patients with OSA have an impairment of resistance-vessel endothelium-dependent vasodilation. This may be implicated in the pathogenesis of hypertension and heart failure in this condition. (*Circulation*. 2000;102:2607-2610.)

**Key Words:** sleep ■ obesity ■ endothelium

Obstructive sleep apnea (OSA) has been linked to hypertension,<sup>1</sup> heart failure,<sup>2</sup> and stroke<sup>3</sup> and to increased mortality.<sup>4,5</sup> The mechanisms underlying the association between OSA and cardiovascular disease are not fully understood. Patients with OSA have a heightened sympathetic drive during wakefulness, which may increase even further during sleep. During sleep, these patients experience severe repetitive hypoxemic stress, with reflex sympathetic activation and consequent marked increases in blood pressure. Hypoxemia, sympathetic activation, and increased arterial pressure may impair endothelial function,<sup>6,7</sup> suggesting a possible mechanism for the development of cardiovascular disease in OSA patients.

Endothelial dysfunction is characterized by an imbalance of endothelium-derived relaxing and contracting factors. It may contribute to the progression and complications of hypertension, atherosclerosis, and chronic heart failure.<sup>8-13</sup>

We tested the hypothesis that patients with OSA have impaired endothelial function compared with closely matched normal obese subjects shown to be free of OSA and independent of the presence of other disease states. We studied endothelium-dependent and -independent vasodilation in both resistance and conduit vessels.

## Methods

### Subjects

We studied 8 male patients with OSA (age  $44 \pm 4$  years) and 9 male obese subjects without OSA (age  $48 \pm 3$  years). Patients with OSA were newly diagnosed, never treated for OSA, and free of any other known diseases. None of the subjects was taking medications. The studies were approved by the Institutional Review Board for Human Investigation, and written informed consent was obtained from all subjects before the study.

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**Figure 1.** Schematic of experimental approach showing timing of infusion of increasing doses of ACh ( $\mu\text{g}/\text{min}$ ), SNP ( $\mu\text{g}/\text{min}$ ), and VER ( $\mu\text{g}/\text{min}$ ). Order of ACh and SNP was randomized within each subject, with VER always administered last. Open bars indicate 3-minute period of FBF measurement at baseline and during last 3 minutes of infusion of each dose of ACh, SNP, and VER.

## Measurements

Heart rate was measured continuously by ECG. Forearm blood flow (FBF) was measured bilaterally by venous occlusion plethysmography (EC4, Hokanson). Blood pressure was measured on the noninfused arm with an automatic sphygmomanometer (Life Stat 200, PhysioControl) for 2 minutes after each FBF measurement. Respiration was monitored with a strain-gauge pneumotachometer.

## Protocol

### Resistance-Vessel Dilatation

The schematic of the randomized study design is shown in Figure 1. All subjects abstained from the use of alcohol or caffeine for  $\geq 24$  hours before the study. Subjects were studied in the supine position. A fine metal catheter was placed into the brachial artery for arterial infusion of acetylcholine (ACh; a vasodilator that stimulates endothelial release of nitric oxide [NO]), sodium nitroprusside (SNP; an exogenous NO donor), and verapamil (VER; a calcium channel blocker). Subjects then received intra-arterial infusions of ACh, SNP, and VER (Figure 1). The order of ACh and SNP was randomized between subjects. VER was always infused last because of its longer-lasting effect. Each infusion was preceded by a rest period of  $\geq 20$  minutes, during which time saline (0.9% NaCl) was infused intra-arterially at a constant rate of 1 mL/min.

Endothelium-dependent vasodilation was induced by infusing ACh intra-arterially in incremental doses of 3, 10, and 30  $\mu\text{g}/\text{min}$  for 6 minutes each. Endothelium-independent vasodilation was induced by infusing SNP intra-arterially in incremental doses of 1, 3, and 10  $\mu\text{g}/\text{min}$  for 6 minutes each. Calcium channel blocker vasodilation was induced by infusing VER intra-arterially in incremental doses of 30, 100, and 300  $\mu\text{g}/\text{min}$  for 6 minutes each. FBF was measured at baseline and during the last 3 minutes of infusion of each dose.

### Conduit-Vessel Dilatation

Conduit-vessel dilation was studied in 16 subjects, 8 with OSA and 8 matched control subjects. Fourteen of these subjects also underwent studies of resistance-vessel function. Subjects underwent ultrasound evaluation of vasodilator function of the brachial artery  $\geq 1$  hour after completion of resistance-vessel evaluation. The ultrasound method for measuring endothelium-dependent and endothelium-independent arterial dilation has been described previously.<sup>14</sup> Brachial artery diameter was measured by B-mode ultrasound images with a 5.0-MHz linear-array transducer (Ultramark 9, Advanced Technology Laboratories). The scans were obtained with the subject at rest and after sublingual administration of nitroglycerin. Subjects lay quietly for  $\geq 30$  minutes before the first scan. The brachial artery was scanned in longitudinal section 5 cm above the elbow, and the center of the artery was identified when the clearest picture of the anterior and posterior intimal layers was obtained. When a satisfactory transducer position was found, the skin was marked, and the arm remained in the same position throughout the study. A resting scan

**TABLE 1. Baseline Characteristics**

	OSA Patients (n=8)	Obese Control Subjects (n=9)
Age, y	44 $\pm$ 4	48 $\pm$ 3
Body mass index, kg/m <sup>2</sup>	35 $\pm$ 3	30 $\pm$ 1
Body fat, %	29 $\pm$ 2	27 $\pm$ 2
Apnea-hypopnea index, %	52 $\pm$ 22	1 $\pm$ 0
Total cholesterol, mg/dL	183 $\pm$ 6	213 $\pm$ 12
LDL cholesterol, mg/dL	109 $\pm$ 11	137 $\pm$ 12
HDL cholesterol, mg/dL	39 $\pm$ 2	47 $\pm$ 4
Triglycerides, mg/dL	177 $\pm$ 31	149 $\pm$ 28
Plasma glucose, mg/dL	92 $\pm$ 4	95 $\pm$ 4

Data are mean $\pm$ SEM.

was obtained, and the Doppler velocity of arterial flow was measured with a pulsed-Doppler signal at a 70° angle to the vessel in the center of the artery. Reactive hyperemia was then induced by the inflation of a blood pressure cuff placed around the forearm to a pressure of 220 mm Hg for 5 minutes, followed by release. The artery was scanned continuously for 1 minute before and 2 minutes after deflation of the cuff, including a repeated recording of flow velocity for the first 10 seconds after the cuff was released. Thereafter, 15 minutes was allowed for recovery of the vessel, after which an additional resting scan was performed. Sublingual nitroglycerin spray (400  $\mu\text{g}$ ) was then administered, and the vessel was scanned continuously for 5 minutes.

The diameter of the vessel was measured by 3 observers unaware of the intervention being used. Measurements were taken at end diastole (peak of R wave of the ECG). Five cardiac cycles and 5 points across the vessel in each frame were analyzed, and measurements were averaged. Flow-mediated dilatation was calculated as percent increase in arterial diameter during hyperemia compared with the corresponding resting value. Nitroglycerin-induced dilation was calculated similarly. Reactive hyperemia was calculated as maximum velocity during the first 10 seconds after cuff deflation divided by the corresponding rest velocity.

## Analyses

All analyses were conducted by observers unaware of whether or not subjects had OSA. ECG, FBF, and respiration were recorded simultaneously on a computerized data acquisition system (MacLab, AD Instruments Inc) in combination with a Macintosh Quadra 950 Computer (Apple Computer Inc). FBF was expressed as mL/min per 100 mL forearm volume. To avoid any systemic effects, changes in FBF were expressed as the percent change from baseline in the ratio between the infused-arm FBF and noninfused-arm FBF.

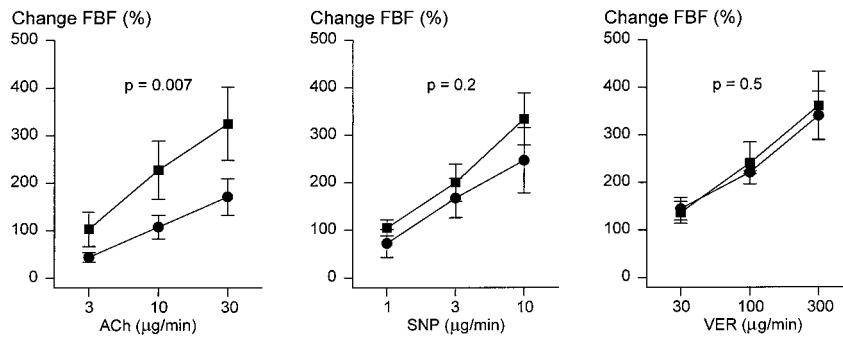
Results are expressed as mean $\pm$ SEM. Differences in resistance-vessel dilation in response to ACh, SNP, and VER infusions were determined by 2-way ANOVA with repeated measures (SAS Institute Inc). Differences in baseline characteristics and conduit-vessel parameters (diameter, vasodilation, and flow) between OSA and control subjects were determined by an unpaired Student's *t* test. Statistical significance was defined as  $P < 0.05$ .

## Results

### FBF Studies

#### Baseline Measurements

The clinical characteristics of the subjects in the 2 study groups are indicated in Table 1. There were no significant differences between the 2 groups in age, body mass index, percent body fat, plasma lipids, or plasma glucose. The apnea



**Figure 2.** Percent change in infused/non-infused FBF during ACh (left), SNP (middle), and VER (right) for patients with OSA (circles) and matched normal control subjects (squares). Data are mean±SEM.

hypopnea index was significantly different (OSA,  $52 \pm 22$ ; control subjects,  $1 \pm 0$ ;  $P=0.03$ ).

#### Vascular Responses to ACh, SNP, and VER

ACh infusion induced a dose-dependent increase in FBF in both groups (Figure 2). The percent change in infused/noninfused-arm FBF was significantly reduced in the patients with OSA compared with control subjects ( $P=0.007$ ). The impairment in responsiveness to ACh did not correlate with apnea hypopnea index or oxygen desaturation.

Administration of increasing doses of SNP and VER increased FBF in both groups, but there was no significant difference between patients with OSA and control subjects ( $P=0.2$  and  $P=0.5$ , respectively).

#### Brachial Artery Diameter Studies

##### Vascular Response to Reactive Hyperemia

The degree of reactive hyperemia produced by cuff inflation and release was similar in OSA patients and control subjects ( $P=0.7$ ) (Table 2). Arterial dilation increased by  $3.7 \pm 0.7\%$  in the patients with OSA and  $4.7 \pm 1.4\%$  in the control subjects after cuff release ( $P=0.54$ ). Nitrate-induced dilation was  $9.7 \pm 0.8\%$  in OSA and  $12.8 \pm 2.3\%$  in control subjects ( $P=0.22$ ) (Table 2).

##### Vascular Response to Sublingual Administration of Nitroglycerin

Nitroglycerin-induced dilation was  $9.7 \pm 0.8\%$  in patients with OSA and  $12.8 \pm 2.3\%$  in the control subjects (Table 2).

### Discussion

In the present study, the vasodilation in response to intra-arterial infusion of ACh (an endothelium-dependent vasodilator) was significantly blunted in patients with OSA compared with closely matched normal control subjects. By

contrast, the vasodilation responses to intra-arterial infusions of SNP (an endothelium-independent vasodilator) were not significantly different, and responses to VER (a calcium channel blocker) were very similar. Brachial artery conduit-vessel dilation, in response to both reactive hyperemia and nitroglycerin, was not significantly different in OSA patients and control subjects.

The important and novel finding in our study is therefore the attenuated endothelium-dependent vasodilation of resistance vessels demonstrated in patients with OSA compared with matched normal subjects. There is preservation of VER-mediated dilation, which is independent of NO bioactivity or smooth muscle responsiveness to NO. Thus, our finding cannot be explained by a nonspecific generalized vascular abnormality in OSA patients and is evident even in the absence of overt cardiovascular disease.

Patients with hypertension,<sup>8–11</sup> hypercholesterolemia,<sup>15,16</sup> congestive heart failure,<sup>12,13</sup> and diabetes mellitus<sup>17,18</sup> have impaired endothelial function. Our study suggests that OSA is associated with impaired resistance-vessel endothelial function, even in the absence of hypertension or other illnesses. OSA has been identified as an independent and additive risk factor for the development of systemic hypertension.<sup>19</sup> The mechanisms responsible for the development of sustained hypertension are unclear. Endothelial dysfunction may be implicated.

To the best of our knowledge, only one previous study has studied endothelial function in OSA patients. Carlson et al<sup>20</sup> showed abnormalities in both endothelium-dependent and -independent vasodilation of resistance vessels. Differences in our results are probably due to differences in subject selection and methodology. Impaired endothelium-dependent dilation was observed only at the highest doses of intra-arterial infusion of ACh in their patients with OSA, higher than doses used for the present study. Unlike our study, FBF was measured in only 1 arm, nonobese control subjects were used, some of the OSA patients had been treated with antihypertensive medications, and conduit-vessel endothelial function was not assessed.

#### Potential Mechanisms of Endothelial Dysfunction in Patients With OSA

Apnea-induced hypoxemia may contribute to endothelial damage.<sup>21</sup> Biosynthesis of NO from L-arginine is an oxygen-dependent process, and hypoxia might influence NO formation in vascular beds directly.<sup>22,23</sup> The endothelial cell re-

**TABLE 2. Brachial Artery Dilatation in Response to Hyperemia or Nitrates**

	OSA Patients (n=8)	Obese Control Subjects (n=8)
Vessel size at rest, mm	$4.7 \pm 0.1$	$4.4 \pm 0.1$
Mean velocity at rest, cm/s	$6.5 \pm 1$	$5.0 \pm 0.8$
Hyperemia, % change in velocity	$405 \pm 52$	$375 \pm 61$
Flow-mediated dilatation, %	$3.7 \pm 0.7$	$4.7 \pm 1.4$
Nitrate-induced dilatation, %	$9.7 \pm 0.8$	$12.8 \pm 2.3$

Data are mean±SEM.

sponse to hypoxic stress can result in 2 different consequences in surrounding tissues, depending on the duration of the exposure<sup>24</sup>: first, short-term exposure causes physiological and reversible modulation of vascular tone and blood flow; second, chronic hypoxic stress results in irreversible remodeling of the vasculature and surrounding tissues, with smooth muscle proliferation and fibrosis. The abnormality we describe is suggestive that the former mechanism may be involved, because structural vessel abnormalities would be likely to manifest as impaired endothelium-dependent and -independent dilation.

Important strengths of our study are, first, that all participants were free of medications. Second, control subjects were matched for age and body mass index, thus ruling out any potential confounding influence of age or obesity on our data. Third, all patients with OSA were free of other known diseases, were newly diagnosed, and had never been treated for sleep apnea. Fourth, the study design was randomized, and data analysis was blinded. Fifth, FBF was measured in both arms, ruling out confounding systemic effects of the vasodilators used, because the infused-arm flow was standardized by flow in the noninfused arm. Last, we obtained complete overnight polysomnographic recordings in patients with OSA and obese subjects, excluding any effects of occult sleep apnea<sup>25</sup> in our obese control subjects.

Limitations include the relatively small number of subjects studied, a consequence of the rigid and stringent selection criteria used for this study. It is possible that with substantially larger numbers of patients, significant differences might have emerged in the responses to SNP and/or conduit-vessel responsiveness to changes in flow or nitroglycerin. Thus, we cannot exclude a generalized impairment in responsiveness to NO, whether from the endothelium or from an NO donor, at the conduit artery and microcirculation level. Therefore, although our data demonstrate a clear impairment of endothelium-dependent resistance-vessel vasodilation, we cannot state with certainty that this impairment is selective. Nevertheless, our results imply that resistance-vessel endothelium may be more susceptible than conduit-vessel endothelium to damage by repetitive nocturnal hypoxia.

In conclusion, we observed a blunted resistance-vessel endothelium-dependent dilation in patients with OSA compared with matched control subjects. This is evident in the absence of other diseases, suggesting that OSA is an independent cause of endothelial dysfunction. We speculate that abnormalities in resistance-vessel endothelial function may precede, and possibly predispose to, hypertension and heart failure in sleep apnea patients.

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