# Antioxidant Vitamin C Improves Endothelial Function in Obstructive Sleep Apnea

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*Rationale:* **Obstructive sleep apnea (OSA) is associated with oxidative stress, endothelial dysfunction, and increased cardiovascular morbidity and mortality.**
*Objective:* **We tested the hypothesis that endothelial dysfunction in patients with OSA is linked to oxidative stress.**
*Methods:* **In the present study, we measured flow-mediated dilation (FMD) of the brachial artery by ultrasound in 10 otherwise healthy, untreated patients with OSA and 10 age-and sex-matched control subjects without sleep-disordered breathing before and after intravenous injection of the antioxidant vitamin C. The investigator performing the FMD measurements was blinded to the status of the patients.**
*Results:* **When compared with control subjects, baseline FMD was significantly reduced in the patients with OSA. After intravenous injection of 0.5 g vitamin C, vasoreactivity remained unchanged in the control subjects. In the patients with OSA, ascorbate led to an increase in FMD to a level comparable to that observed in the control group.**
*Conclusion:* **The reduced endothelial-dependent vasodilation in untreated patients with OSA acutely improves by the free radical scavenger vitamin C. These results are in favor of oxidative stress being responsible for the endothelial dysfunction in OSA. Antioxidant strategies should be explored for the treatment of OSA-related cardiovascular disease.**

**Keywords:** cardiovascular disease; endothelial dysfunction; obstructive sleep apnea; oxidative stress

Obstructive sleep apnea (OSA) is widely recognized as an important risk factor for the development of cardiovascular disorders such as arterial hypertension, atherosclerosis, and congestive heart failure (1, 2). One emerging pathophysiologic concept to explain this association is that the OSA-related chronic intermittent hypoxia specifically disturbs the vascular micromilieu (3). Apart from increased sympathetic activity and proinflammatory changes, there is accumulating evidence for an increased oxidative stress in OSA. This is supported by studies reporting enhanced *in vitro* release of free oxygen radicals from neutrophils and monocytes of patients with OSA (4, 5), reduced plasma levels of nitric oxide (NO) derivatives (6, 7), and increased lipid peroxidation (8).

Presumably, the disturbance of the vascular micromilieu in OSA gives rise to a reduction of endothelial-dependent vasodilation (i.e., endothelial dysfunction). This has recently been demonstrated in otherwise healthy patients with OSA by ultrasonographic measurements of flow-mediated dilation (FMD) of the brachial artery (9). FMD is a well-established marker of endothelial function that can be assessed by measuring arterial diameter responses to increased flow (10). It occurs mainly as the result of endothelial release of NO (11) and correlates with coronary endothelial function (12).

Up to now there has been no direct evidence linking the increased oxidative stress in OSA to endothelial dysfunction in these patients. In the present study, we tested this hypothesis by investigating vasoreactivity in otherwise healthy, untreated patients with OSA before and after intravenous injection of the antioxidant vitamin C. Vitamin C is a radical scavenger and has been demonstrated to ameliorate endothelial function in various diseases known to be associated with an increased oxidative stress (e.g., diabetes mellitus, hypercholesterolemia, essential hypertension, and congestive heart failure) (13–16). If the endothelial dysfunction characteristic of OSA is the result of oxidative stress, it should equally be reversed by vitamin C.

## METHODS

An expanded explanation of the methods can be found in the online supplement accompanying this article.

### Patient Recruitment

The patients with OSA were referred to our sleep laboratory for suspected sleep apnea. Only untreated patients who were otherwise healthy, nonsmoking, and not on medications known to influence vasoreactivity were allowed to enter the study. Healthy, nonsmoking control subjects without sleep-disordered breathing were recruited from an out-of-hospital–based medical check-up program.

### Patient Evaluation

To exclude comorbidities, the medical charts of the patients with OSA and the control subjects were analyzed with special reference to the occurrence of vascular and metabolic disease. Blood pressure values were measured at fixed time intervals and averaged to yield the mean daytime blood pressure. Total cholesterol, low-density lipoprotein and high-density lipoprotein cholesterol, and HbA1c values were determined in all patients.

### Sleep Studies

The patients with OSA were investigated by full-night attended polysomnography. In the control subjects, the presence of sleep-disordered breathing was excluded by ambulatory monitoring with a portable device.

### Vasoreactivity Testing Protocol

All measurements were performed at 4:00 P.M. on the day of completion of the sleep study. The patients were investigated in the supine position after a resting period of 15 min. FMD was measured by high-resolution ultrasonography of the brachial artery at the dominant arm.

The vessel diameter was measured under resting baseline conditions. Afterward, forearm blood ischemia was achieved for 5 min by inflating a cuff to suprasystolic pressures. The cuff was deflated, causing a dilation

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of the brachial artery, and arterial diameter was continuously monitored over a period of 3 min. The increase in vessel diameter observed 60 s after cuff release was determined and expressed in relative percent values as compared with baseline conditions. FMD in response to intravenous injection of 500 mg of vitamin C at the contralateral arm and sublingual application of 0.8 mg (i.e., two puffs) of nitroglycerine spray was evaluated. All measurements were performed by the same experienced investigator (M.G.), who was blinded to the status of the individual patient.

### Data Analysis

The data are reported as means ± SEM unless otherwise indicated. The characteristics of the patients with OSA and the control subjects were compared by one-way analysis of variance (ANOVA), unpaired *t* test (where appropriate), or a $\chi^2$ test for categoric variables. Within the OSA group, univariate analysis was applied to relate FMD to parameters of OSA severity (e.g., apnea–hypopnea index [AHI], $Sa_{O_2}$ indices) and other variables, such as body mass index (BMI). Differences in arterial diameter occurring in response to reactive hyperemia and after vitamin C/nitroglycerine were assessed by two-way ANOVA for repeated measurements, and a Student-Newman-Keuls test was applied if indicated. A p value of less than 0.05 was regarded as statistically significant.

## RESULTS

### Patient Characteristics

The anthropometric, blood pressure, and laboratory data of the OSA group and the control group (n = 10 in each group) are summarized in Table 1. The patients with OSA and the control subjects were matched for age and sex. On average, they were in the sixth decade of their life and, except for one patient, all were men. The patients with OSA had higher BMIs when compared with the control subjects (+4.6 kg/m$^2$); this difference was not statistically significant. None of the patients suffered from comorbidities. Polysomnography demonstrated severe sleep-disordered breathing in the OSA group, whereas nocturnal breathing as judged by ambulatory monitoring was normal in the control subjects (Table 2).

### FMD at Baseline, in Response to Vitamin C and after Sublingual Nitroglycerine

When compared with the control subjects, baseline FMD was significantly reduced in the patients with OSA (FMD, 5.3 ± 0.6 vs. 8.5 ± 0.6%; $p < 0.01$). Among the patients with OSA, FMD was neither related to the AHI/$Sa_{O_2}$ indices nor to the BMI (data not shown). After intravenous injection of 0.5 g vitamin C, vasoreactivity remained unchanged in the control subjects (FMD, 8.4 ± 0.7%; Figure 1). In the patients with OSA, vitamin C led to a highly significant increase in FMD to a level comparable to that observed in the control group (FMD, 7.4 ± 0.6%; $p < 0.01$ vs. baseline conditions; Figure 2). This increase was observed in all but one patient with OSA and averaged +40% in comparison to baseline values. After sublingual application of nitroglycerine, patients in both groups showed an increase of FMD to maximal values. These values did not significantly differ between patients with OSA and patients without OSA (FMD, 14.6 ± 1.1 vs. 16.2 ± 1.3%; p = not significant).

**TABLE 1. CHARACTERISTICS OF THE STUDY SUBJECTS**

| | Patients with Obstructive Sleep Apnea | Control Subjects | p Value |
|---|---|---|---|
| No. patients | 10 | 10 | |
| Age, yr | 56.4 ± 2.4 | 55.7 ± 1.5 | 0.81 |
| Sex, male/female | 9/1 | 9/1 | 0.46 |
| Body mass index, kg/m$^2$ | 31.5 ± 1.8 | 26.9 ± 1.8 | 0.09 |
| Blood pressure, mm Hg | | | |
| Systolic | 131 ± 3 | 130 ± 3 | 0.82 |
| Diastolic | 81 ± 5 | 77 ± 2 | 0.47 |
| HbA1c, % | 5.8 ± 0.1 | 5.5 ± 0.1 | 0.07 |
| Cholesterol, mmol/L | | | |
| Total | 5.3 ± 0.2 | 4.8 ± 0.2 | 0.25 |
| LDL | 3.3 ± 0.1 | 3.0 ± 0.1 | 0.29 |
| HDL | 1.4 ± 0.1 | 1.4 ± 0.1 | 0.84 |

*Definition of abbreviations*: HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Data are given as means ± SEM and as total numbers unless otherwise indicated.

## DISCUSSION

In the present study, we found that the endothelial dysfunction known to occur in patients with OSA can be acutely reversed by intravenous injection of the antioxidant vitamin C. Numerous factors might affect vasoreactivity, such as concomitant metabolic disease (e.g., diabetes mellitus, hypercholesterolemia) and smoking. All of these possible confounders were carefully excluded in the patients studied. Thus, all of them were otherwise healthy nonsmokers and not on medications known to influence vascular reactivity.

Another important aspect of the study design was that the investigator performing the FMD measurements was blinded to the status of the individual patient (i.e., he was not aware if the patient had a diagnosis of OSA).

Because obesity has been reported to cause blunted vasodilation (17), one point of criticism may be that the OSA and the control groups were not completely matched with regard to their body weight. In our series of 10 patients, FMD was neither related to the AHI nor the BMI; however, data from the large scale Sleep Heart Health Study have shown that measures of OSA severity are associated with the percentage of FMD even after adjustment for BMI and other confounders (18). Furthermore, FMD in OSA has been found to improve after several weeks of continuous positive airway pressure therapy without any changes in BMI occurring during these time intervals (9). In addition, the studies investigating the influence of obesity on vascular reactivity did not evaluate if the patients enrolled suffered from OSA. Finally, it seems that the impact of obesity on FMD is less relevant than previously thought. In this context, the Framingham Heart Study has found that each 5-kg/m$^2$ increment in BMI is associated with a negligible reduction of 0.16% in FMD (19). We think that the difference in BMI between patients with OSA and control subjects in our study did not significantly contribute to the lower FMD in the OSA group.

A further possible limitation of our study is that we used a screening device to exclude the presence of OSA in the control subjects. It has to be considered, however, that this device was equipped with the same channels for the detection of sleep-disordered breathing as the computerized system used for polysomnography and that all polygraphic data were visually analyzed. In addition, none of the control subjects reported sleep-related symptoms, such as snoring, witnessed apneas, or daytime sleepiness.

Therefore, it is unlikely that any of these patients had significant OSA. A final drawback may be that the results might be applicable only to male subjects. However, we speculate that female patients with OSA might show equal improvements of endothelial function after vitamin C because they have been reported to suffer from a similar or even more pronounced reduction in endothelial-dependent vasodilation than their male counterparts (20).

The fact that vitamin C led to an increase in FMD in the patients with OSA but not in the control subjects is in favor of

**TABLE 2. SLEEP STUDY RESULTS**

| | Patients with Obstructive Sleep Apnea (polysomnography) | Control Subjects (polygraphy) | p Value |
|---|---|---|---|
| No. patients | 10 | 10 | |
| AHI, n/h | 45 ± 9 | 1 ± 0.3 | < 0.01 |
| $Sa_{O_2}$, % | 91.5 ± 1.7 | 95.8 ± 0.3 | < 0.05 |
| Lowest $Sa_{O_2}$, % | 68.2 ± 4.9 | 91.8 ± 0.3 | < 0.01 |
| $Sa_{O_2}$ < 90%, % of TST or TIB | 19.1 ± 8.1 | 0 | < 0.01 |
| Non-REM 1 + 2, % of TST | 71 ± 3 | NA | |
| Non-REM 3 + 4, % of TST | 14 ± 3 | NA | |
| REM, % of TST | 15 ± 4 | NA | |
| Sleep efficiency, % of TIB | 73 ± 3 | NA | |
| Arousal index, n/h | 38 ± 6 | NA | |

*Definition of abbreviations*: AHI = apnea–hypopnea index; NA = not available; TIB, time in bed; TST, total sleep time.
Data are given as means ± SEM unless otherwise indicated.

oxidative stress being responsible for endothelial dysfunction in these patients. Vitamin C is a radical scavenger. Presumably, the intravenous injection of ascorbate decreased the amount of circulating free oxygen radicals, restored NO levels, and thereby improved vascular reactivity. This is also supported by the recent observation that FMD in OSA is positively related to the plasma levels of the NO derivatives nitrite and nitrate (21).

Scavenging reactive oxygen species by vitamin C may enhance NO bioavailability (and endothelial function) through various mechanisms. First, the reaction of superoxide with NO resulting in the formation of peroxynitrite is prevented. Second, the oxidative degradation of the endothelial NO synthase cofactor tetrahydrobiopterin is inhibited. Third, the activity of the enzyme dimethylarginine dimethylaminohydrolase is increased, leading to decreased levels of the endothelial NO synthase inhibitor asymmetric dimethylarginine. The last of these mechanisms is likely to be involved because a negative correlation between % FMD and plasma asymmetric dimethylarginine concentrations has been found in OSA (21).

If the improvement of FMD observed after vitamin C were due to the antioxidant properties of this substance, it is critical to evaluate if the dose and regimen of ascorbate used in our study (i.e., 0.5 g bolus injection) achieved plasma concentrations sufficiently high to exert antioxidant effects. To address this issue, we measured vitamin C plasma levels after its intravenous injection in a separate group of healthy volunteers. The concentrations reached within the first few minutes of this experiment were in the range of 1 mmol/L, which has been reported to be associated with significant antioxidant activity (22). After vitamin C administration, the FMD values in the patients with OSA rose to a level that was comparable to that of the control subjects without sleep-disordered breathing. In our opinion, this suggests that increased oxidative stress is likely to play a predominant role in the emergence of OSA-related endothelial dysfunction. Other mediator systems that might be related to a loss of endothelial function and are known to be upregulated in OSA are the catecholamines and endothelin (23, 24). However, in view of the presently observed marked effect of vitamin C on FMD, we presume that the changes in these vasoactive substances are less important in this context.

Our study was not designed to investigate the long-term effects of vitamin C on vascular reactivity in OSA. To answer this question, it is necessary to give vitamin C as an oral substitute and to follow the patients by serial vasoreactivity measurements. On the basis of our study, we are not able to clarify if there is a dose–response relationship between the amount of vitamin C

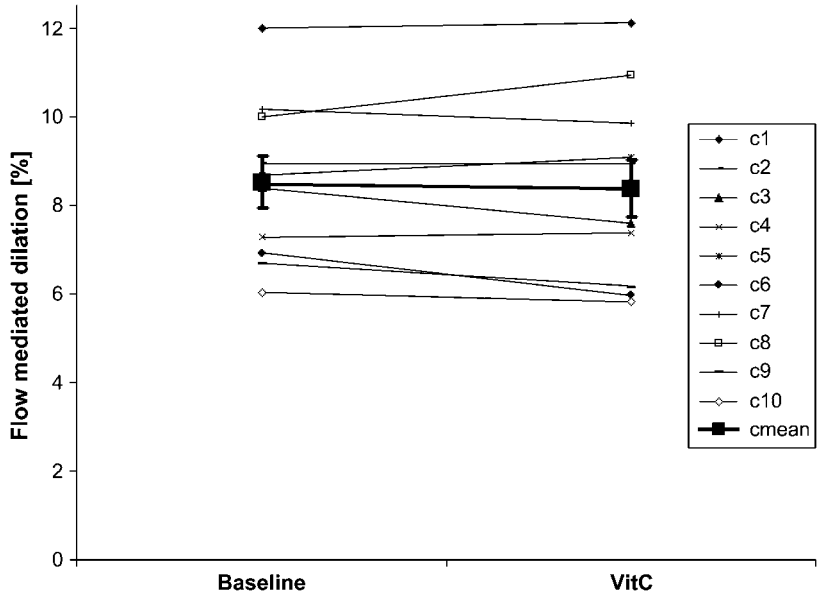


***Figure 1.*** Flow-mediated dilation of the brachial artery at baseline and after intravenous administration of 0.5 g vitamin C in the control group (n = 10; p = not significant).

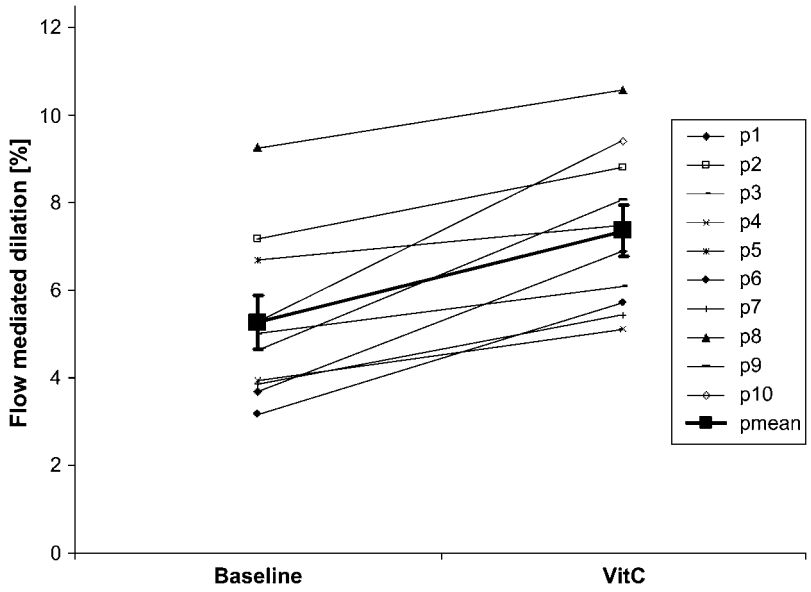


***Figure 2.*** Flow-mediated dilation of the brachial artery at baseline and after intravenous administration of 0.5 g vitamin C in the obstructive sleep apnea group ($n = 10$; $p < 0.01$).

given intravenously and the improvement of FMD. All patients received the same dose of vitamin C regardless of their body weight. This means, however, that the patients with OSA were exposed to lower weight-adjusted vitamin C doses when compared with the control subjects. Given these considerations, we speculate that the increase in FMD in the patients with OSA would have been even greater if we had administered the same relative dose of the vitamin as in the control subjects.

The results of the present study may have important clinical implications. Because vitamin C acutely improved vascular function, future strategies to treat OSA-related cardiovascular disease might include antioxidant medications. One might argue that the majority of trials of antioxidant vitamin supplementation in patients at risk for or with established cardiovascular disease have yielded negative results (25, 26). However, because these studies did not differentiate between patients with OSA and without OSA, we believe that the issue of cardioprotective antioxidant therapy in OSA remains to be addressed. In our opinion, such an approach may be of special value in patients with OSA who are not compliant with continuous positive airway pressure therapy or primarily refuse this form of treatment.

In summary, we present the first direct evidence that increased oxidative stress is the likely cause of endothelial dysfunction in OSA. Future studies must show if similar observations can be made under long-term substitution of vitamin C (or other antioxidants) and if this may translate into a beneficial influence on cardiovascular endpoints in these patients.

***Conflict of Interest Statement***: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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